REVISTA ESPAÑOLA DE Quimioterapia

SPANISH JOURNAL
OF CHEMOTHERAPY
ISSN: 1988-9518
Volumen 37
Número 6
Diciembre 2024
Páginas: 438-526

Publicación Oficial
de la Sociedad Española
de Quimioterapia

Imagen portada: María Teresa Corcuera

REVISTA ESPAÑOLA DE
Quimioterapia

Revista Española de Quimioterapia tiene un carácter multidisciplinar y está dirigida a todos aquellos profesionales involucrados en la epidemiología, diagnóstico, clínica y tratamiento de las enfermedades infecciosas

Fundada en 1988 por la Sociedad Española de Quimioterapia

Indexada en
Science Citation Index Expanded (SCI),
Index Medicus (MEDLINE),
Excerpta Medica/EMBASE,
Índice Médico Español (IME),
Índice Bibliográfico en Ciencias de la Salud (IBECS)

Secretaría técnica
Dpto. de Microbiología
Facultad de Medicina
Avda. Complutense, s/n
28040 Madrid
revista@seq.es
Disponible en Internet:
www.seq.es

Sociedad Española de Quimioterapia

Publicidad y Suscripciones
Sociedad Española de Quimioterapia
Dpto. de Microbiología
Facultad de Medicina
Avda. Complutense, s/n
28040 Madrid

Atención al cliente
Teléfono 91 394 15 12
Correo electrónico
info@seq.es

Consulte nuestra página web
www.seq.es

Publicación que cumple los requisitos de soporte válido

ISSN
0214-3429

e-ISSN
1988-9518

Depósito Legal
M-32320-2012

Maquetación
VicPG

Impresión
España

Esta publicación se imprime en papel no ácido.
This publication is printed in acid free paper.

REVISTA ESPAÑOLA DE
Quimioterapia

Sumario

REVISTA ESPAÑOLA DE Quimioterapia

Volumen 37
Número 6
Diciembre 2024

Revisión

Microbioma humano intestinal: Papel en la salud y en la enfermedad 438
Rafael Cantón, Pilar De Lucas Ramos, Alejandra García-Botella, Alberto García-Lledó, Teresa Hernández-Sampelayo, Javier Gómez-Pavón, Juan González Del Castillo, Mari Cruz Martín-Delgado, Francisco Javier Martín Sánchez, Manuel Martínez-Sellés, José María Molero García, Santiago Moreno Guillén, Fernando Rodríguez-Artalejo, Elena Reigadas, Rosa Del Campo, Sergio Serrano, Julián Ruiz-Galiana, Emilio Bouza

Infección invasora por estreptococo del grupo A (*Streptococcus pyogenes*): situación actual en España 454
Mari Cruz Martín-Delgado, Pilar De Lucas Ramos, Alejandra García-Botella, Rafael Cantón, Alberto García-Lledó, Teresa Hernández-Sampelayo, Javier Gómez-Pavón, Juan González Del Castillo, Francisco Javier Martín Sánchez, Manuel Martínez-Sellés, José María Molero García, Santiago Moreno Guillén, Fernando Rodríguez-Artalejo, Julián Ruiz-Galiana, Almudena Burillo, Patricia Muñoz, Cristina Calvo Rey, Mercedes Catalán-González, Emilio Cendejas-Bueno, Ana Verónica Halperin-Benito, Raúl Recio, Carmen Viñuela-Benítez, Emilio Bouza

Originales

Estudio de 5 años de infecciones del torrente sanguíneo producidas por bacilos gramnegativos productores de carbapenemasas en el sur de España 472
Fernando Cobo, Juan Antonio Reguera-Márquez, José Antonio Marín-Rodríguez, Francisco José Martín-Pérez, Patricia Pérez-Palacios, Esther Recacha, José María Navarro-Marí

Resistencia a los inhibidores de integrasa en embarazadas con VIH 479
Diego Cecchini, Javier Sfalcin, Inés Zapiola, Alan Gomez, Silvina Fernandez-Giuliano, Claudia Rodriguez, Lilia Mammana, Analía Seravalle, Fabián Fay, María Cecilia Torroija Gabriela Bugarín, María Belén Bouzas

Poder diagnóstico de infección bacteriana de LIAISON MeMed BV® en los pacientes adultos atendidos en urgencias por sospecha de infección 486
Javier Cabañas Morafraile, Elena De Rafael González, Laura Serrano Martín, Rafael Rubio Díaz, María Torres Fernández, Eva Heredero Gálvez, William Esneider López Forero, Laura Morell Jurado, Raúl Canabal Berlanga, M. Francisca Calafell Mas, Elia Chaves Prieto, M. Remedios Asensio Nieto, Álvaro Thomas-Balaguer Cordero, María Carmen Lorenzo Lozano, Agustín Julián-Jiménez, En Nombre Del Grupo Urgen-Labqmic Del Chut (Idiscam).

Originales breves

Efectividad de nirsevimab en la prevención de los ingresos por bronquiolitis por virus respiratorio sincitial en lactantes 498
Rosa Rodríguez-Fernández, Felipe González-Martínez, Inés Ojeda Velázquez, Marta Rodríguez Díaz, María Victoria Capozzi Bucciol, María Isabel González-Sánchez, Jimena Pérez-Moreno, Blanca Toledo Del Castillo

Uso de antibióticos en Barcelona en 2023 en atención primaria y reducción potencial ajustando el tamaño de los envases a las guías actuales 504
Carl Llor, Cristina Rodríguez-Bernuz, Amelia Troncoso-Mariño

Sumario

REVISTA ESPAÑOLA DE Quimioterapia

Volumen 37
Número 6
Diciembre 2024

Cartas al Director

La importancia de un diagnóstico precoz. *Tinea capitis* producida por *Trichophyton tonsurans* en un adolescente 509
Laura Eva Franco-Fobe, María Luisa Monforte, María Victoria Fuentelsaz Del Barrio, Rocío Cebollada, Concepción López-Gómez, Carmen Aspiroz

Impacto en la población adulta de la inmunización con nirsevimab en los menores de 6 meses frente al virus respiratorio sincitial 512
Jordi Reina, Julia Viana-Ramirez

Amenaza de parto prematuro y nacimiento pretérmino en presencia de *Lachnoanaerobaculum gingivalis* 515
Lucía Iglesias-Linares, Ana Román-De-La-Torre, María Aguilera-Franco, Alberto Puertas-Prieto, María José Medina-Pascual, José Gutiérrez-Fernández

Erupción mucocutánea infecciosa reactiva (RIME) en pacientes pediátricos: afectación extrapulmonar de *Mycoplasma pneumoniae* 518
Cristina González, Borja Croche, Emilio Gómez

Lesiones en diana y fiebre, un microorganismo inesperado 521
Patricia Andres-Ibarrola, Laura Guío Carrión

Coinfección por *Mucor* y *Nocardia* en receptores de trasplante de corazón 523
Marina Fayos, Andrea Severo, María Dolores García-Cosío, Carlos Prados, Marina Alonso, Francisco López-Medrano

Contents

REVISTA ESPAÑOLA DE Quimioterapia

Volume 37
Number 6
December 2024

Review

Human intestinal microbiome: Role in health and disease 438
Rafael Cantón, Pilar De Lucas Ramos, Alejandra García-Botella, Alberto García-Lledó, Teresa Hernández-Sampelayo, Javier Gómez-Pavón, Juan González del Castillo, Mari Cruz Martín-Delgado, Francisco Javier Martín Sánchez, Manuel Martínez-Sellés, José María Molero García, Santiago Moreno Guillén, Fernando Rodríguez-Artalejo, Elena Reigadas, Rosa del Campo, Sergio Serrano, Julián Ruiz-Galiana, Emilio Bouza

Invasive group A *Streptococcus* infection (*Streptococcus pyogenes*): Current situation in Spain 454
Mari Cruz Martín-Delgado, Pilar De Lucas Ramos, Alejandra García-Botella, Rafael Cantón, Alberto García-Lledó, Teresa Hernández-Sampelayo, Javier Gómez-Pavón, Juan González del Castillo, Francisco Javier Martín Sánchez, Manuel Martínez-Sellés, José María Molero García, Santiago Moreno Guillén, Fernando Rodríguez-Artalejo, Julián Ruiz-Galiana, Almudena Burillo, Patricia Muñoz, Cristina Calvo Rey, Mercedes Catalán-González, Emilio Cendejas-Bueno, Ana Verónica Halperin-Benito, Raúl Recio, Carmen Viñuela-Benítez, Emilio Bouza

Originals

A 5-year study of bloodstream infections caused by carbapenemase-producing Gram-negative bacilli in southern Spain 472
Fernando Cobo, Juan Antonio Reguera-Márquez, José Antonio Marín-Rodríguez, Francisco José Martín-Pérez, Patricia Pérez-Palacios, Esther Recacha, José María Navarro-Marí

Integrase strand transfer inhibitors resistance-associated mutations in HIV-infected pregnant women 479
Diego Cecchini, Javier Sfalcin, Inés Zapiola, Alan Gomez, Silvina Fernandez-Giuliano, Claudia Rodriguez, Lilia Mammana, Analía Seravalle, Fabián Fay, María Cecilia Torroija Gabriela Bugarín, María Belén Bouzas

Diagnostic power of LIAISON MeMed VB® for bacterial infection in adults patients seen in Emergency Departments due to infections 486
Javier Cabañas Morafraile, Elena de Rafael González, Laura Serrano Martín, Rafael Rubio Díaz, María Torres Fernández, Eva Heredero Gálvez, William Esneider López Forero, Laura Morell Jurado, Raúl Canabal Berlanga, M. Francisca Calafell Mas, Elia Chaves Prieto,M. Remedios Asensio Nieto, Álvaro Thomas-Balaguer Cordero, María Carmen Lorenzo Lozano, Agustín Julián-Jiménez, En nombre del grupo URGEN-LABQMIC del CHUT (IDISCAM).

Brief Report

Nirsevimab effectiveness against hospital admission for respiratory syncytial virus bronchiolitis in infants 498
Rosa Rodríguez-Fernández, Felipe González-Martínez, Inés Ojeda Velázquez, Marta Rodríguez Díaz, María Victoria Capozzi Bucciol, María Isabel González-Sánchez, Jimena Pérez-Moreno, Blanca Toledo del Castillo

Antibiotic use in Barcelona in 2023 in primary care and the potential reduction by adjusting box sizes to current guidelines 504
Carl Llor, Cristina Rodríguez-Bernuz, Amelia Troncoso-Mariño

Contents

REVISTA ESPAÑOLA DE Quimioterapia

Volume 37
Number 6
December 2024

Letters to the editor

The importance of early diagnosis. *Tinea capitis* caused by *Trichophyton tonsurans* in an adolescent 509
Laura Eva Franco-Fobe, María Luisa Monforte, María Victoria Fuentelsaz del Barrio, Rocío Cebollada, Concepción López-Gómez, Carmen Aspiroz

Impact in the adult population of immunization with nirsevimab in children under 6 months of age against respiratory syncytial virus 512
Jordi Reina, Julia Viana-Ramirez

Threat of preterm labor and preterm birth in the presence of *Lachnoanaerobaculum gingivalis* 515
Lucía Iglesias-Linares, Ana Román-de-la-Torre, María Aguilera-Franco, Alberto Puertas-Prieto, María José Medina-Pascual, José Gutiérrez-Fernández

Reactive infectious mucocutaneous eruption (RIME) in paediatric patients: extrapulmonary *Mycoplasma pneumoniae* involvement 518
Cristina González, Borja Croche, Emilio Gómez

Target lesions and fever, an unexpected microorganism 521
Patricia Andres-Ibarrola, Laura Guío Carrión

***Nocardia* and mucoral co-infection in heart transplant recipient** 523
Marina Fayos, Andrea Severo, María Dolores García-Cosío, Carlos Prados, Marina Alonso, Francisco López-Medrano

Official journal of the Spanish Society of Chemotherapy

Review

Revista Española de Quimioterapia
doi:10.37201/req/056.2024

Rafael Cantón[1]
Pilar De Lucas Ramos[2]
Alejandra García-Botella[3]
Alberto García-Lledó[4]
Teresa Hernández-Sampelayo[5]
Javier Gómez-Pavón[6]
Juan González del Castillo[7]
Mari Cruz Martín-Delgado[8]
Francisco Javier Martín Sánchez[9]
Manuel Martínez-Sellés[10]
José María Molero García[11]
Santiago Moreno Guillén[12]
Fernando Rodríguez-Artalejo[13]
Elena Reigadas[14]
Rosa del Campo[1]
Sergio Serrano[12]
Julián Ruiz-Galiana[15]
Emilio Bouza[16]

Human intestinal microbiome: Role in health and disease

[1]Servicio de Microbiología. Hospital Ramón y Cajal e Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS). CIBER de Enfermedades Infecciosas (CIBERINFEC). Madrid. Spain.
[2]Emérito. Servicio de Neumología. Hospital General Universitario Gregorio Marañón. Universidad Complutense. Madrid. Spain.
[3]Servicio de Cirugía General. Hospital Clínico Universitario de San Carlos. Universidad Complutense. Madrid. Spain.
[4]Servicio de Cardiología. Hospital Universitario Príncipe de Asturias. Universidad de Alcalá de Henares. Madrid. Spain.
[5]Emérito. Servicio de Pediatría y ACES. Hospital General Universitario Gregorio Marañón, Universidad Complutense. Madrid. Spain.
[6]Servicio de Geriatría. Hospital Central de la Cruz-Roja. Universidad Alfonso X el Sabio. Madrid. Spain.
[7]Servicio de Urgencias. Hospital Clínico Universitario de San Carlos. Universidad Complutense. Madrid. Spain.
[8]Servicio de Medicina Intensiva. Hospital Doce de Octubre. Universidad Complutense. Madrid. Spain.
[9]Hospital de Emergencias Enfermera Isabel Zendal. Madrid. Spain.
[10]Servicio de Cardiología. Hospital General Universitario Gregorio Marañón, Universidad Europea. Madrid. Spain.
[11]Medicina de Familia. Enfermedades Infecciosas. Madrid. Spain.
[12]Servicio de Enfermedades Infecciosas. Hospital Universitario Ramón y Cajal e Instituto de Investigación Sanitaria Ramón y Cajal (IRICYS). Universidad de Alcalá. CIBER de Enfermedades Infecciosas (CIBERINFEC). Madrid, Spain.
[13]Departamento de Medicina Preventiva y Salud Pública. Universidad Autónoma. Madrid. Spain.
[14]Servicio de Microbiología Clínica y E. Infecciosas. Hospital General Universitario Gregorio Marañón, Universidad Complutense. CIBER de Enfermedades Respiratorias (CIBERES). Madrid. Spain.
[15]Servicio de Medicina Interna. Hospital Ruber Internacional. Madrid. Spain.
[16]Emérito asistencial, Comunidad de Madrid. Servicio de Microbiología Clínica y E. Infecciosas. Hospital General Universitario Gregorio Marañón, Universidad Complutense. CIBER de Enfermedades Respiratorias (CIBERES). Madrid. Spain.

Article history
Received: 21 June 2024; Accepted: 1 July 2024; Published: 9 July 2024

ABSTRACT

The study of the microbiota and the microbiome, and specifically the intestinal one, has determined great interest due to the possible association of their alterations with numerous diseases. These include entities as diverse as Crohn's disease, autism, diabetes, cancer or situations as prevalent today as obesity. In view of this situation, different recommendations have been performed regarding the use of probiotics, prebiotics, and postbiotics as modulators of the microbiota and the microbiome, seeking both preventive and therapeutic effects, and faecal material transfer (FMT) is proposed as an alternative. The latter has emerged as the only proven beneficial intervention on the intestinal microbiome, specifically in the treatment of recurrent colitis associated with *Clostridioides difficile* (R-CDI). In the rest of the entities, the lowering of laboratory costs has favored the study of the microbiome, which is resolved by delivering reports with catalogs of microorganisms, metabolites or supposed biomarkers without consensus on their composition associated with healthy or diseased microbiota and the disease. There is still insufficient evidence in any disease for interventions on the microbiome beyond FMT and R-CDI. Multi- and multi-disciplinary work with extensive research and the application of artificial intelligence in this field may shed light on the questions raised currently. Ethical issues must also be resolved in light of possible interventions within the umbrella of personalized medicine.

Keywords: microbiota; microbiome; *Clostridioides difficile*; faecal transfer; postbiotics; probiotics; prebiotics.

Correspondence:
Rafael Cantón.
Servicio de Microbiología. Hospital Universitario Ramón y Cajal and Instituto Ramón y Cajal de Investigación Sanitaria. CIBER de Enfermedades Infecciosas (CIBERINFEC). Madrid.
E-mail: rafael.canton@salud.madrid.org

All authors belong to the Scientific Committee on COVID-19 of the Madrid College of Physicians (ICOMEM).

Microbioma humano intestinal: Papel en la salud y en la enfermedad

RESUMEN

El estudio de la microbiota y el microbioma, y en concreto el intestinal, ha despertado gran interés ante la posible asociación de sus alteraciones con numerosas enfermedades. Estas abarcan entidades tan diversas como la enfermedad de Crohn, el autismo, la diabetes, el cáncer o situaciones tan prevalentes en la actualidad como la obesidad. Ante ello, han surgido diferentes recomendaciones en el uso de probióticos, prebióticos

y postbióticos como moduladores de la microbiota y el microbioma, buscando tanto efectos preventivos como terapéuticos y se propone la trasferencia de materia fecal (TMF) como alternativa. Esta última, se ha erigido como intervención con beneficio demostrado en el tratamiento de la colitis recurrente asociada a *Clostridioides difficile* (R-CDI). En el resto de las entidades, el abaratamiento de los costes de laboratorio ha favorecido el estudio del microbioma que se resuelve con la emisión de informes con catálogos de microorganismos, metabolitos o supuestos biomarcadores sin que existan consensos sobre su composición que se asocien a microbiotas "sanas" o "patológicas" con enfermedad humana. No existen aún evidencias suficientes en ninguna enfermedad para la intervención sobre el microbioma más allá del TMF y la R-CDI. El trabajo multi y pluridisciplinar con investigaciones amplias y la aplicación de la inteligencia artificial en este terreno pueden arrojar luz a los interrogantes hoy planteados. También deben resolverse cuestiones éticas a la luz de las posibles intervenciones dentro del paraguas de la medicina personalizada.

Palabras clave: microbiota; microbioma; *Clostridioides difficile*; trasferencia fecal; postbióticos; probióticos; prebióticos

INTRODUCTION

The relationship between our health and the set of microorganisms that reside in different territories of our body (microbiota or colloquially "flora") has been the subject of study for years. The concept of microbiota has been expanded into that of microbiome, which includes the ecological niche in which the microbiota is found and its interaction with the host [1]. There are currently numerous research lines with essentially three main objectives: 1) to determine whether alterations in the microbiota cause disease; 2) to discern whether their analysis has diagnostic utility in these diseases; and 3) to evaluate the possibility of modifying an altered microbiota as a form of treatment. The latter includes the possibility of administering substances that modify the microbiota (prebiotics), elements of the microbiota (probiotics) or potentially beneficial substances synthesized by the microbiota (postbiotics).

The interest the study of this field is enormous, but so is the confusion in it, a fact that motivates this review promoted by the COVID-19 and Emerging Pathogens Committee of the Illustrious Official College of Physicians of Madrid (ICOMEM). We understand that this confusion has three main causes. The first is due to the extensiveness of the fields of study, ranging from allergies to autism or cancer. The second is due to the economic interests underlying the offer of non-standardized diagnostic techniques with as yet unproven usefulness (studies of the microbiota), and of products aimed at improving its composition (pre, pro and postbiotics). They are often developed and marketing with lack of strict regulation, which facilitates their entry into the market without evidence of clear benefit, with a multitude of products and diverse posology. Finally, the multidisciplinary approach, in the great majority with disconnection between professionals, and the absence of criteria and consensus in the relevance of the studies and analysis of the results.

From the literature reviewed, this Committee only appreciates sufficient evidence on the therapeutic use of the intestinal microbiota for the treatment of recurrent colitis due to *Clostridioides difficile* (R-CDI) and believes it is necessary to consider the need to regulate not only the research of products intended to modify the microbiota, but also their advertising. The following lines summarize the outcome of the deliberations of the Committee of Experts convened by ICOMEM in response to a series of questions posed by the Committee.

WHAT IS THE MICROBIOME? HOW DOES IT DIFFER FROM THE MICROBIOTA?

The term microbiota is defined as the set of microorganisms (bacteria, fungi, archaea, viruses, and parasites) that reside in our body in different mucous membranes and other territories. Colloquially it is also known as flora. Microbiome refers to a broader concept as it also includes the habitat or ecological niche and the interaction with the host. It refers to the microbial communities found in an ecological niche, their genes and metabolites, as well as the environmental conditions surrounding them. It was first used by Whipps et al. in 1988 [2], referring to the set of microbial communities, the characteristics of their interrelationship, and the properties of the habitat where they are found.

The microbiota is therefore part of the microbiome in defined ecological niches or locations. Among all these locations, the intestinal microbiome stands out for its complexity and diversity, which is the most studied so far. The genitourinary, the oral cavity, the nasopharynx, the respiratory tract, and the skin microbiomes, among others, also stand out. Each of them has its own particular niche and fingerprint in its composition. The microbial communities that make up the microbiome have a symbiotic and mutualistic behavior with human cells and maintain an important dialogue with the immune system [1]. It is therefore considered as an "organ" essential for life and with a clear influence on health and disease. The microbiome has its own particularities and characteristics inherent in each individual, which may vary depending on the genetic substrate, diet, early exposure, geography, interaction with the environment, or simply the age of the individual.

The vast majority of the microorganisms that make up the microbiome are not able to be cultured in the traditional culture media and conditions in which they are grown. The introduction of massive sequencing techniques and bioinformatics tools for massive data analysis (metaomic techniques) and, more recently, artificial intelligence (AI), have brought about a revolution and a great advance in the knowledge of the microbiota and in the catalogs of its composition [3]. However, it is more difficult to characterize the conditions of the microbiome and the effects derived from its composition. Recent studies suggest that, more than microbial composition, the importance of the microbiome lies in its functionality,

since different microbial species can perform equivalent metabolic functions and the same species can perform different functions. Figure 1 shows the main groups of microorganisms found in the intestinal microbiota and their taxonomic update.

WHAT IS THE FUNCTION OF THE GUT MICROBIOME?

The intestinal microbiome represents a complex ecosystem that, in a symbiotic manner, contributes to numerous functions of the host physiology. To date, its participation in the absorption and regulation of nutrient metabolism, the synthesis of essential vitamins, the elimination of toxic compounds, the strengthening of the intestinal barrier and protection against pathogenic microorganisms and the development of the immune system is well known. However, the mechanisms of this contribution are far from being sufficiently elucidated [4]. The participation of the intestinal microbiota in these functions is synthesized below.

Nutrition and metabolism. The intestinal microbiota provides energy and nutrients to the organism, intervening in the absorption and metabolism mainly of carbohydrates, but also of lipids and proteins, besides being able to interfere in the metabolism of some drugs. It has been demonstrated that commensal species of *Bifidobacterium* can synthesize vitamin K and water-soluble B vitamins. Other groups such as *Bacteroides* can generate short-chain fatty acids (SCFA) by fermentation of non-digestible carbohydrates, also collaborating with *Bifidobacterium* species in the fermentation of oligosaccharides. The fatty acids generated, in the form of acetate, propionate and butyrate, constitute a primary energy source for the colonic epithelium and can also reach systemic availability. In acetate form they become substrate for lipogenesis and gluconeogenesis [5,6]. In butyrate form they can prevent lactic acid accumulation. Some microorganisms such as those of the genus *Oxalobacter* and certain *Bifidobacterium* prevent the formation of oxalates. In relation to lipid metabolism, they enhance lipid hydrolysis by regulation of the colipase required by pancreatic lipases for lipid digestion. Finally, it affects protein metabolism through proteinases and peptidases that potentiate those of the host [7].

Immunomodulation. The intestinal microbiota also works in conjunction with the innate and adaptive immune system of the host. It contributes to the maintenance of intestinal lymphoid tissue (GALT, gut-associated lymphoid tissue), modulates the expression of T cells, mainly T-helper (Th) and T-regulatory (Treg) cells, and influences innate lymphoid cells (ILC). It also interacts with IL10-producing macrophages [6].

Phylum	Class	Order	Genus	Expected %	Main characteristics
Firmicutes (Bacillota)*	*Clostridia*	*Clostridiales*	*Faecalibacterium* *Clostridium* *Roseburia* *Ruminococcus*	**50-80%**	Mainly Gram-positives Secretion of vitamins Spore production Carbohydrates fermentation/SCFAs**
	Negativicutes	*Veillonellales*	*Dialister*		
	Bacilli	*Lactobacillales*	*Lactobacillus* *Enterococcus*		
		Bacillales	*Staphylococcus*		
Bacteroides (Bacteroidota)*	*Bacteroides*	*Bacteroidales*	*Bacteroides* *Tannerella* *Parabacteroides* *Alistipes* *Prevotella*	**20-40%**	Mainly Gram-negatives Production of SCFAs** Biliary acids metabolisms
	Sphingobacteria	*Sphingobacteriales*	*Sphingobacterium*		
Proteobacteria (Pseudomonadota)*	*Y-proteobacteria*	*Enterobacterales*	*Escherichia* *Shigella*	**1-10%**	Gram-negatives Stimulation of the immune system
	Δ-proteobacteria	*Desulfovibrionales*	*Desulfovibrio* *Bilophila*		
	ϵ-proteobacteria	*Campylobacterales*	*Helicobacter*		
Actinobacteria (Actinomycetota)*	*Actinobacteria*	*Actinomycetales*	*Corynebacterium*	**1-10%**	Gram-positives Haemostasis maintenance
		Bifidobacteriales	*Bifidobacterium*		
	Coriobacteria	*Coriobacteriales*	*Atopobium*		
Fusobacteria	*Fusobacteria*	*Fusobacterales*	*Fusobacterium*	**1-5%**	Gram-negatives
Verrucomicrobia	*Verrucomicrobia*	*Verrucomicrobiales*	*Akkermansia*	**<1%**	Gram-negatives Mucous regulation

Figure 1 Main groups of microorganisms found in the intestinal microbiota and their taxonomic update

*updated taxonomic designation; ** Short-chain fatty acids

Protection against infection. This is a multifactorial action in which the direct action of the microbiota and the interaction with the host's defense mechanisms must be considered. In the former, the intestinal microbiota enters into competition for nutrients against possible pathogenic microorganisms. In addition, some microorganisms have the function of synthesizing proteins with antimicrobial activity. However, interaction with host defense mechanisms is essential. The microbiota induces the production of IgA, improving the barrier function exerted by the intestinal mucous layer. Also the production of lactic acid during metabolism, enhances the activation of host lysozyme which acts as an antimicrobial agent.

However, it should not be forgotten that the microbiome constitutes a dynamic ecosystem, rapidly changing according to changes in diet or exposure to chemical agents, which can cause not only positive but also negative actions [8].

WHAT EXTRINSIC AND INTRINSIC FACTORS OF THE INDIVIDUAL INFLUENCE THE GUT MICROBIOME?

Several factors have been identified that influence the composition of the intestinal microbiota. It is not well understood, however, which are the ultimate determinants affecting its stability and the fluctuations that occur throughout life. The factors most frequently implicated include the following:

- Diet. The basic composition of the human microbiota is affected by diet over the long term, especially diets with a high proportion of fat and protein versus a high-fibre diet. Marked differences in gut microbiota have been found to be associated with these dietary patterns [9].
- Host genetics. In particular, the genetic determinants of the immune system, which plays a key role in selecting the bacteria that reside in the body [10].
- Use of antimicrobials. They significantly alter the intestinal microbiota, affecting its diversity and composition. This effect appears both in the short and long term [11].
- Lifestyle and environment. Factors related to lifestyle, diet and medications in general have a profound impact on the gut microbiota [11].
- This is another crucial factor affecting the diversity of the microbiome. Its composition has been observed to vary with age, possibly reflecting changes in the immune system, diet and general health [12].
- Stress. Both chronic and acute, can influence the gut microbiota. Although the exact mechanism is not fully elucidated, stress is thought to affect the intestinal barrier and the interactions of the microbiota with the nervous system [13].
- Geographic factors. Geographic location and local dietary habits account for a significant part of the variation in gut microbiota [14].
- Sex. Sex differences exist in the composition of the gut microbiota, although studies show mixed results and the underlying mechanism is still under investigation [15].

CAN THE HEALTH OF THE INTESTINAL MICROBIOME BE IMPROVED? HOW?

Based on the factors that modify the microbiota and the microbiome, some interventions have been postulated to improve their "health". However, their effect on overall health is still far from being well defined. Some of these interventions are summarized in Table 1. Although these strategies are promising, further research is essential to fully understand their impact on global health and disease prevention. Individualization of these interventions, taking into account the uniqueness of each person's microbiota, could be key in the quest for optimal health and lasting well-being [16-21].

IS THERE A LINK BETWEEN THE GUT MICROBIOME AND DISEASE? WHAT IS THE EVIDENCE BASE?

The involvement of the microbiota in health or disease is postulated on three basic pillars: the influence on the construction, maintenance and modulation of immunity. Its action is positive in the digestive process and in the generation of energetic and vitamin resources essential for life or, on the contrary, negative in the production of potentially toxic substances that can alter its normal functionalism. The biological aggressiveness of many microorganisms is manifested when they colonize or when they undergo translocation and colonize extraintestinal tissues. When studying the relationship between the microbiota and health or disease, methodological problems arise in the study of a microbiome of a changing nature and its possible influence on future disease. Its follow-up over time would show greater certainty, and always including large numbers of study subjects and their controls to assert the relationship.

The ultimate example of a pathogenic dysbiosis and its eradication by manipulation of the microbiota is R-CDI. From this immediate evidence, we can jump to the already tested scenario of stool transplantation (or transfer) for the improvement of autism [22]. Between these two extremes, the relationship between the microbiota and almost every type of disease is raised.

The lack of antigenic stimuli due to decreased diversity and the alteration of what would be a "healthy" microbiome in the early stages of life, including gestation, have been linked to reduced immune efficiency and increased risk of disease or earlier onset of disease. These include atopic eczema [23,24], food allergies [25], asthma [26-28] and metabolic diseases as important as obesity [29,30] and type-1 diabetes [31-33], in the genesis of which, in addition to genetic susceptibility, there is a background of inflammation [34,35] or autoimmune aggression [36]. These "dysbioses" seem to be strongly influenced by the type of delivery (natural or cesarea) [37,38], the lack of

Table 1 Interventions on the microbiome to improve your health

Intervention	Comments	Ref.
Prebiotics	Non-digestible compounds, present in the diet, that stimulate the growth or activity of microorganisms of the microbiota, resulting in a possible health benefit. Generally, plant fibres that we do not digest and that are food for bacteria. Examples: inulin (fructooligosaccharide) and oligosaccharides from breast milk.	16
Probiotics	A live microorganism that, when administered in adequate amounts, is intended to improve health problems such as obesity. Examples: There are many types of probiotics, generally they are of the species *Lactobacillus* or *Bifidobacterium* or related such as *Lactecaseibacillus rhamnosus* GG. The *Escherichia coli* Nissle 1917 strain is also used.	18
Postbiotics	Inactivated microorganisms and their metabolites that confer a potential beneficial effect on intestinal health by providing possible anti-inflammatory, immunomodulatory and intestinal barrier protective effects. Examples: Current focus is on gut-derived bacteria such as *Akkermansia municiphila*, a strict anaerobic bacterium that releases metabolites with potentially health-promoting activity, including short-chain fatty acids	21
Dietary fibre	Its consumption through fibre-rich foods may improve glucose metabolism. It is associated with an increase in bacteria to which beneficial effects are attributed.	17
Dietary components and dietary habits	Healthy dietary habits, such as a diet rich in plant foods, can promote a diverse and health-promoting microbial ecosystem. Vegetarian and vegan diets are associated with an increase in certain potentially beneficial bacteria (such as *Bacteroidetes*) and may promote the production of short-chain fatty acids, which are considered beneficial.	19, 20

maternal exposure during gestation to domestic animals, receiving antibiotics during this period or in the first months of life [39,40] and, of course, an artificial neonatal diet. What we would understand as a more "healthy" state would favor the development of "allergies" [28,41]. On the other hand, some of the relationships that have been established between infant dysbiosis and diseases have not been confirmed in observational studies in real life, as is the case of the use of antibiotics and obesity [42].

There are direct pathogenic aspects of the microbiota in the newborn and especially in the premature infant that predispose to necrotizing enterocolitis, characterized by a pathological predominance of Enterobacterales and *Staphylococcus* with respect to *Bifidobacterium* and *Bacteroidetes* [43-45], contrary to what occurs in healthy children. Immune dysfunction against this dysbiosis is suspected as the pathogenesis [46]. Late-onset sepsis, which sometimes occurs, would be an example of bacterial translocation. The same bacteria isolated during sepsis have been found in the pre-sepsis intestine of these children [47,48].

Inflammatory bowel disease (Crohn's disease and ulcerative colitis) shares the same suspected pathogenesis with the interaction of microbiota, genetic susceptibility and inadequate immune-inflammatory response [49-51]. Dysbiosis due to an increase of proteobacterias and decrease of *Bacteroidetes* and *Firmicutes* [52] is pointed to in its genesis, as well as the interaction between diet and microbiota and its consequences on intestinal permeability [50]. Irritable bowel syndrome, with such a high prevalence, is a permanent focus of studies looking for a direct relationship with the microbiota, without evidence that allows definitive treatment actions [53].

Obesity deserves a separate mention. At the moment, it is not clear what the profile of the microbiota attributed to obesity is, although in animal models it seems to be related to a higher proportion of *Firmicutes*, to the detriment of *Bacteroidetes*. On the other hand, bariatric/metabolic surgery changes the ratio between these two groups of bacteria, with predominance of *Bacteroidetes* [54-56]. These changes may be long-lasting and determinant in weight loss and metabolic comorbidity control. However, there is little evidence to support a causal or associative relationship between the microbiome and obesity. Although there is evidence that the gut microbiome may affect the risk of adiposity-related comorbidities, such as type-2 diabetes and inflammation, there is no evidence that manipulation of the gut microbiome is an effective treatment for obesity [57-58].

Another field that opens a huge range of studies is the influence of the microbiota with neurogenesis, behavior and diseases of the nervous system through the so-called "gut-brain microbiome axis" and again the invitation to modify this "environmental" part of the individual to control the alterations of its genetic disposition [59]. Depression, autism, attention deficit disorder, schizophrenia, Parkinson's disease and neurodegenerative disorders, including Alzheimer's disease, do not escape this relationship.

Evidence has shown bidirectional communication between the gut microbiome and the central nervous system, making it a target for ameliorating the development and progression of neurodegenerative diseases [60,61]. This communication would be mediated by the immune system, vagus nerve, enteric nervous system, neuroendocrine and circulatory systems [60]. In mouse models it has been observed that bacterial metabolites generate responses in neurotransmitters

(serotonin, dopamine, noradrenaline, etc.) and in the immune system, regulating neurophysiological function and cognition [61]. Likewise, alterations in the gut microbiota have been associated with autism spectrum disorders, anxiety, depressive behaviors, impaired physical performance and motivation, as well as neurodegenerative diseases such as Alzheimer's disease and patients with Parkinson's disease) [62]. Aging, a key player in neurodegenerative diseases, shows that the gut microbiome of centenarians has a great similarity with those of young individuals.

On the other hand, the relationship of microorganisms and cancer has a long history focused on treatment. Today, the role that microbiota can play in direct or inducing and especially modulating causation through the immune system and the production of functional metabolites is recognized [63,64]. The concept of the immune-oncology-microbiota axis is created. Much of this relationship is still maintained with the intestinal microbiota (the colon harbors 97% of the bacterial microbiota and is the organ with the highest production of biologically active substances with immunomodulatory activity). The rest corresponds to bacterial microbiota of other organs.

Once again, a strictly intratumoral microbiome appears, found in more than thirty types of tumors [65,66] whose pathogenic role remains to be defined both in oncogenesis and in the interference in the response to chemotherapy or immunotherapy, and therefore of a possible therapeutic interventionism. The specificity of the intratumoral microbiome is postulated as a localization or body mapping tool when investigating this microbiota in blood.

There are very few microorganisms recognized as directly oncogenic, also called oncomicrobes: Epstein Barr virus; HBV; HCV; Kaposi's herpesvirus; HIV-1; human papillomavirus; HTL-1 virus; *Opistorchis viverrini* and *Clonorchis sinensis*, *Schistosoma haematobium* and *Helicobacter pylori* [67], but there are many others that could act as facilitators through their active metabolites or by immunomodulation [68-71]. In any case, we would be at a point that opens the way for the future, but still without clinically validated tools for therapeutic interventionism [65,66,72-77].

CAN THE MICROBIOME HELP DIAGNOSE DISEASE OR BE USEFUL IN DISEASE MONITORING? WHAT ARE THE INDICATIONS FOR MICROBIOME STUDIES IN THE CLINIC?

Microbiome analysis has the potential to revolutionize many aspects of healthcare, from diagnosis and treatment to prevention. However, it is still at a very early stage and further clinical research and technological developments are needed for full implementation. Therefore, at present, microbiome analysis should not be recommended in clinical practice as we lack the necessary knowledge to translate the results of its analysis into clinical decisions. Some relevant findings of recent research are schematically presented below:

- Inflammatory Bowel Disease (IBD). The microbiome plays a role in the pathogenesis of IBD. Microbial biomarkers in blood and other body fluids are emerging as promising tools for the diagnosis and prognosis of IBD, although their clinical application is still under development [78].
- Diagnosis of precancerous lesions. This is a hot area. There are numerous groups interested in developing microbiota-based diagnostic tools for colon cancer. For example, it has been observed that the abundance of *Fusobacterium nucleatum* is higher in patients with colorectal cancer than in controls [79]. Similarly, it has been observed that the determination of metabolites produced by anal bacteria can help identify subjects with precancerous lesions [80].
- Predictor of response to immunotherapy in cancer. The role of the microbiome in response to immunotherapy is an area of growing interest in oncology. Studies in preclinical, observational models, and pilot clinical trials suggest that it is possible to predict the response to immunotherapy based on the composition of the microbiome, and to modify this response by microbiota transplantation. Further studies are needed to introduce this tool into the clinic [81,82].
- Personalized Medicine. Host gene-microbiome interactions appear to influence the development of chronic multifactorial diseases such as type-1 diabetes, ulcerative colitis, and Crohn's disease. Analysis of the microbiome could help identify subjects at risk for developing these diseases and become a tool for diagnosis and personalized medicine [83].

HOW IS THE INTESTINAL MICROBIOME STUDIED? WHAT LABORATORY REPORTS ARE ISSUED AND HOW SHOULD THEY BE INTERPRETED?

The study of the microbiome can be approached with different techniques. Typically, mass sequencing is performed after amplification of the gene encoding the 16S ribosomal subunit (16S rDNA for bacteria), or of the ITS1-2 intergenic space (for fungi) [84]. First, the total DNA of microorganisms from a clinical sample is obtained, then the chosen target is amplified by PCR, and finally mass sequencing is performed. After passing the quality filters of the process and discarding incomplete or chimeric sequences, each amplicon (DNA fragment amplified by PCR, approximately 300 base pairs – bp –) is assigned to a specific microorganism after matching it to databases with bioinformatics tools. The final step consists of assigning values of alpha diversity (total number of different taxa and balance in their abundance) and beta diversity (statistical differences in groups of subjects). This is the most common technique because of its low cost (approx. 50 €/sample in reagents) and relative simplicity, but in most cases it cannot discern the complete taxonomy, and generally only reaches genus without descending to species. The new massive sequencing platforms, such as PacBio®, perform sequences up to 1,500 bp, allowing a more accurate taxonomic assignment as they can read the complete 16S rDNA gene and reach the species level with high confidence.

There is a more complex approach called "shotgun" in which all the DNA is sequenced without a previous amplification step, but again with short sequences of 300 bp [84]. With this strategy we can identify all microorganisms, including viruses, and metabolism, virulence, or antibiotic resistance genes. However, it has as a disadvantage the difficulty in the bioinformatic reconstruction of the individual genomes of each microorganism.

Both with the amplification of 16RNA sequences and with a "shotgun" approach we can know the composition and abundance of each taxon in a sample in which we want to characterize the microbiome, with the limitation that the process has not been standardized.

Other study techniques collect the expression of genes (transcriptomics), the proteins synthesized (proteomics), the metabolites of the entire ecosystem (metabolome) and finally the connections between them (interactome). Current interest in the study of the gut microbiome is focused on metabolites of exclusive microbial production that also have an impact on health and disease such as SCFAs and trimethylamine N-oxide (TMAO)

It is important to emphasize that, despite technical advances, there is no consensus on the normality of the microbiota in its composition, and this prevents the preparation of a report to grant the degree of "normality". There are diagnostic laboratories that make reports on the composition of the microbiota based on internal criteria of normality, but in no case do they have, for the moment, any validity for therapeutic decisions. These reports are only based on the abundance of each bacterial taxon, attributing to them a functionality that has not been proven, for example, they refer to the abundance of proteolytic bacteria only with the abundance of certain taxa. The problem with these reports is that they often have therapeutic implications, in many cases with antimicrobials, without prior consensus or evidence of their usefulness.

CAN THE MODULATON OF INTESTINAL MICROBIOME BE USED TO TREAT DISEASES? WHAT IS A FECAL TRANSPLANT/TRANSFER AND WHAT ARE ITS INDICATIONS?

Modulation of the intestinal microbiome has become a potential therapeutic target to treat putative diseases associated with its dysbiosis. Among the therapeutic interventions targeting the gut microbiome, fecal microbiota transplantation (FMT) has attracted the interest of the scientific and clinical communities. Its use has been documented in at least 85 diseases, with heterogeneous results [85].

FMT can be defined as the transfer of fecal matter from a healthy donor to a sick person, with the aim of restoring or modifying his or her microbiota. The procedure is performed by introducing the processed product from the feces of the healthy donor into the gastrointestinal tract of the recipient, either through colonoscopy [86], enema [87], nasoduodenal tube [88] or more recently, through orally administered capsules [89-91].

FMT is believed to have originated in 4th century China, where fecal material was administered orally to treat patients with diarrhea [92]. In the current medical literature, FMT was first used to treat pseudomembranous colitis in 1958 in Denver, USA [93]. Most of the clinical experience of FMT has originated in the treatment of R-CDI, where it has demonstrated high success rates (around 90%) in both randomized clinical trials and long case series [94,95]. The European Society for Clinical Microbiology and Infectious Diseases (ECCMID) treatment guidelines recommend fecal transplantation for R-CDI [96,97]. The Infectious Diseases Society of America (IDSA) and the Society for Healthcare Epidemiology of America (SHEA) guidelines [98] also include a strong recommendation for its use in second and subsequent episodes of R-CDI. R-CDI nonresponsive to standard therapies is the only indication approved by the US Food and Drug Administration (FDA) for FMT since 2013 [99].

Despite its high efficacy, the exact mechanisms by which FMT acts on R-CDI are not well understood. Some of the proposed mechanisms include direct competition of C. difficile with the administered microbiota, reconstitution of alpha diversity that helps prevent *C. difficile* colonization, changes in bile acid metabolism, and repair of the intestinal barrier by stimulating the mucosal immune system [100-102]. FMT in R-CDI has been limited by practical barriers such as donor selection, product preparation, storage, delivery, and in some cases, patient reluctance [88,103,104]. Stool donors should undergo periodic thorough controls in which the presence of pathogenic microorganisms is ruled out both in the stool and by serology.

Due to increasing clinical demand, the FMT has gradually evolved. The use of frozen material versus fresh stool was the first step in its modernization, allowing the creation of stool banks [94]. There are several ways of processing stool depending on the route of administration. For administration by colonoscopy, the stool is mixed with a solvent (water or saline), whereas nowadays it is most commonly administered in capsules. For this purpose, the stool is dissolved with a cryoprotectant, then freeze-dried and finally encapsulated with the resulting powder. The stool or its derivatives have a viability of 6 months but there are currently protocols that will enlarge this period.

The development of FMT in oral capsules has represented a great advance in the treatment and prevention of R-CDI, overcoming the main disadvantages of other routes of administration. Several studies have shown that encapsulated TFM has similar efficacy (around 90%) and produces fewer adverse events [89,91,95,105-108].

Current data suggest that FMT is a safe therapeutic method with few adverse effects, although its long-term outcomes have not been fully elucidated [109]. In a systematic review collecting FMT-related adverse effects reported over 20 years (2000-2020) and including 5,688 procedures [110], 19% of ad-

verse events were found, of which the most frequent were diarrhea (10%), abdominal discomfort, pain, and cramping (7%). Serious adverse events related to FMT were reported in 1.4% of patients undergoing FMT. The mortality rate was low (0.13%), mostly as a result of aspiration pneumonia related to the route of FMT administration. This suggests that most FMT-related deaths could be avoided by paying attention to risks related to the route of administration. Of note, serious adverse effects related to FMT occurred in patients with mucosal barrier injury. Another recent review and meta-analysis with 5,099 patients showed that serious adverse events developed in less than 1% of patients [111].

In conclusion, FMT is proposed as a therapeutic action with great potential in the prevention or treatment of many diseases, although the only current clinical indication is the treatment of R-CDI. However, much effort is still needed to unravel the mechanisms of action and to further update the guidelines for FMT on an international scale. It is hoped that with advances in technology and knowledge, FMT will become a standardized treatment in which the current regimen will be replaced by well-defined microbial consortia, metabolites or laboratory-synthesized compounds that can be easily administered.

WHAT ARE PROBIOTICS, PREBIOTICS AND POSTBIOTICS AND WHAT ARE THEIR CLINICAL INDICATIONS?

Prebiotics are non-digestible dietary ingredients that facilitate intestinal health by stimulating the growth of beneficial bacteria such as bifidobacteria and lactobacilli. An example of these would be human milk oligosaccharides, unique for their high concentration in the human species, synthesized in the mammary gland, and the first prebiotics ingested by the newborn and infant. Like probiotics, their real benefit is controversial, although by favoring intestinal colonization by bifidobacteria, they could improve infant colic, prevent early constipation and intestinal infections. For this reason, the companies that manufacture them are incorporating them into milk formulas and as food supplements. Prebiotics are also present in foods rich in fibre.

Probiotics are live microorganisms that, when administered in adequate amounts, may offer significant health benefits to the host. They are found in fermented foods and can be purchased as dietary supplements. Although the actual benefits are controversial, they would contribute to the stability of the microbiota, strengthen the integrity of the intestinal barrier and enhance immune function. *Lactecaseibacillus rhamnosus* GG is the strain with the most evidence of therapeutic and/or preventive benefit for different types of diarrhea (community, traveler's and post-antibiotic), functional digestive disorders, and immune boosting (against allergies and respiratory infections).

Postbiotics are metabolic products or cellular components of inactive microorganisms of the intestinal microbiota. An example is SCFA, metabolites formed in the colon after enzymatic degradation by the microbiota (Table 1). Their beneficial effects would be observed both at the intestinal level (inhibition of pathogens, barrier effect, increased intestinal transit) and as anti-inflammatory, immunomodulators and protectors of the intestinal barrier. Their use in the management of gastrointestinal disorders and their potential in improving general health are being increasingly investigated [21].

Research in this area is difficult due to the absence of strict regulation of their use. Facilitating their entry into the market without clear evidence of benefit is the multitude of different products and posologies. They have been studied in multiple indications, such as prevention of recurrences of IBD [112], recurrences of bacterial vaginosis [113], prevention of *C. difficile* diarrhea [114] or prevention of recurrent urinary tract infection [115]. There is great heterogeneity in the results and products investigated, resulting in a low quality of evidence, although the most recent investigations show promising data on the use of probiotics for the prevention of bacterial vaginosis [113] and recurrent urinary tract infection [115].

There is no firm evidence to recommend the use of prebiotics, probiotics or postbiotics in clinical practice. Even so, the prescription of probiotics has increased a lot and it is important to know their safety. Although adverse reactions have rarely been demonstrated, there are doubts as to whether their administration is safe. In theory, due to their composition, probiotics could be implicated in five types of adverse effects: 1) infectivity and/or pathogenicity as they are live products; 2) production of undesirable metabolites; 3) possibility of transmission of genes that confer resistance to antibiotics; 4) excessive immunostimulation or immunosuppression in sensitized individuals; and 5) adverse reaction associated with their excipients. In case of doubt, it should be taken into account which are the potential subjects at risk of suffering complications (Table 2).

WHAT IS THE INTEREST OF THE MICROBIOTA AND THE USE OF PROBIOTICS AND PREBIOTICS IN PEDIATRICS?

Microbial colonization of the digestive tract during infancy is an essential process for our life. It is very important that, in the immediate neonatal period, a healthy and healthy microbiota is established, which will maintain its beneficial effect in the child's life, protecting him/her from intestinal infections and favoring his/her immune development [116]. The intestinal microbiome would have an important role in the maintenance of a healthy gut-brain axis.

The intestinal microbiota is fundamentally established in the perinatal period (between 22 weeks of gestation and 8 days of life), when the most important intestinal colonization of the individual occurs, especially during delivery. Although for years it seemed that the fetus developed in a sterile en-

Table 2 Patients potentially at risk of complications derived from the use of probiotics [116].

Immunocompromised (including severely malnourished and oncology patients)
Premature infants (*)
Neonates with severe pathology
Cardiopathies (valvular alterations and their replacement, history of endocarditis)
Pregnant women (*)
Patients in ICU (severe pathologies and central catheter carriers)
Patients undergoing surgery (*)
Severe risk of intestinal translocation (acute abdomen, intestinal fistula, neutropenia or severe risk of doing so due to chemotherapy or radiotherapy)
Administration of probiotics through jejunostomy
Concomitant administration of broad-spectrum antibiotics to which they are resistant (*) (Lactobacilli often have natural resistance to vancomycin)
Probiotics with high intestinal mucosal binding capacity or known pathogenicity

(*) Relative risk. In general, their use is considered safe in the following groups.

vironment, DNA from different bacterial species has recently been detected in amniotic fluid, placenta, umbilical cord blood and meconium. This suggests that the microbiota may be initiated in gestation. The neonatal microbiome is very dynamic and changes from the initial intrauterine colonization until 3 years of life, when it becomes similar to that of the adult.

Several protective factors favor the establishment of a healthy microbiome. Thus, while vaginal delivery, maternal contact, and breastfeeding favor this physiological process, birth by cesarean section, artificial breastfeeding, and early exposure to antibiotics hinder it [117]. The following are some proven findings:

- In vaginal delivery, the newborn is colonized by microorganisms from the maternal vaginal, fecal and cutaneous microbiota. It incorporates *Escherichia coli*, *Staphylococcus, Streptococcus,* and anaerobic bacteria of the *Bacteroides* and *Bifidobacterium* spp. type.
- The continuous contact of the newborn with its mother and breastfeeding expose it to a large number of microorganisms that are also incorporated into its initial microbiota: *Staphylococcus, Streptococcus, Serratia, Pseudomonas, Corynebacterium,* and *Propionobacterium.*
- Breast milk contains prebiotics that promote the growth of *Bifidobacterium* in the infant's intestine.
- In children born by cesarean section, intestinal colonization by the bacteria that appear to be most beneficial (*Lactobacillus, Bifidobacterium,* and *Bacteroides*) is delayed.
- Artificially breastfed infants, compared to breastfed infants, have less *Bifidobacterium* which facilitates intestinal colonization by enterobacteria and the acquisition of antibiotic resistance genes.
- Early exposure of the newborn to antibiotics alters the intestinal microbiota and can have permanent effects on the microbiome, especially on *Bifidobacterium* which sometimes persists for the first 12 months of life.

There is increasing interest in the use of probiotics and prebiotics in pediatrics, including in neonates and premature infants [118,119]. Many studies have shown some benefit in the prevention and/or treatment of digestive and nutritional diseases in children [120]. Recently, the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) [121,122] has published Clinical Practice Guidelines on the use of probiotics to unify criteria for the appropriate management of acute diarrhea, antibiotic-associated diarrhea, prevention and treatment of necrotizing enterocolitis of prematurity, *H. pylori* infection, and functional digestive disorders such as infant colic, functional abdominal pain, and functional constipation. Although there is a high level of agreement among the clinical practice recommendations of the Scientific Societies, there are also differences. Comparison of the studies is difficult due to their heterogeneity in terms of indications, doses, duration, and composition of the probiotics used, which makes it difficult to recommend them routinely.

In older children and adolescents, the use of probiotics is being evaluated, as in adults, for many other pathologies: allergic (allergic rhinitis), cutaneous (acne), nephrotic syndrome, endocrinological (obesity and diabetes), neurological diseases (TDH, autism), etc. In some cases, the results are encouraging, but for the moment there is insufficient information for its recommendation and routine use.

It is important to insist that the use of probiotics as treatment and/or prevention of specific diseases should be done in a personalized way, knowing well the product, its doses, benefits and risks, and the maximum time of administration. This is the only way to better understand the expected benefit of these products and others that will probably be available in the future.

WHAT ARE LIVE BIOTHERAPEUTIC AGENTS OR SYNTHETIC MICROBIOTAS?

Synthetic microbiota (SynCom) is a laboratory-created, structurally defined and/or controlled microbial community consisting of relatively few microorganisms that have been cultured and that acts as a substitution for the original functions and structure of the microbiome. These communities when created for therapeutic purposes are also known as "biotherapeutic agents".

The SynCom approach has the great advantage that we can manipulate this community by simply adding, deleting or substituting one or a few strains to achieve the desired functions. Furthermore, these manipulations can affect the genomes of the strains; for example, certain functions can be eliminated or enhanced by gene silencing or expression enhancement, respectively. Because SynCom member microorganisms can be cultured, the strains that comprise SynCom are ideal for dissecting the structural complexity and associated functions of the microbiota using reductionist approaches. Although many strains exist, the most commonly used synthetic microbiotas are of murine (ASF, OMM, GM15) or human (SIHUMI, SIM, MET-1, or B4PC2) origin [123, 124]. All of them need first to grow the microorganisms, stabilize them, and co-culture them so that all of them are represented and in previously established proportions.

The FDA authorized in April 2023 a product (VOWST®) containing encapsulated *Firmicutes* spores (between 1x106 and 3x107 CFU/ml) to prevent R-CDI in individuals 18 years of age and older after failure of antibacterial treatment [125]. Ingestion of the capsules (4 per day for 3 consecutive days) should be done 2-4 days after completing antimicrobial treatment for R-CDI. Their efficacy in clinical trials was demonstrated by lower R-CDI [12,45] compared to placebo (39.8%) (https://www.fda.gov/vaccines-blood-biologics/vowst). Earlier (November 2022) the FDA cleared a stool suspension (Rebyota®) from qualified donors to prevent R-CDI in individuals 18 years and older [124]. It is prepared for rectal administration, 150 ml containing between 1x108 and 5x1010 UCF/ml of fecal microorganisms including more than 1x105 CFU/mL of *Bacteroides*. It should be administered 24 to 72 hours after the last dose of antibiotic for R-CDI. Its efficacy in clinical trials was 70.6% versus 57.5% in the placebo group (https://www.fda.gov/vaccines-blood-biologics/vaccines/rebyota). The estimated price in the United States for one dose of VOWST® is $17,000 and that of Rebyota® is $9,000 [126].

WHAT ROLE DOES THE GUT MICROBIOME PLAY IN DRUG EFFICACY?

The microbiome can condition the pharmacokinetics and metabolism of drugs, especially those with an enterohepatic route of elimination, affecting their effectiveness, but also causing unexpected side effects [127]. The gut hosts the most abundant microbiota in our body, however, effects may also be at the local level following interaction with vaginal, skin, or respiratory tract microbiota. Altered pharmacokinetics of tacrolimus and other immunosuppressants have been reported [128], highlighting that these products are also of microbial origin. Although microorganisms can metabolize drugs, the main mechanism by which their action is affected is sequestration inside the microbial cell.

Another aspect to consider is the opposite, the impact of drugs on the microbiota. Many compounds, beyond antimicrobials, may have inhibitory activity by causing death or exerting a decelerating effect on metabolism, while others may be metabolic accelerators. In recent years, the study of omeprazole or metformin has focused on the intestinal microbial ecosystem [128,129]. Finally, the major focus of current research is how the microbiota conditions the response to oncological treatment, particularly immunosuppressive treatment. Thus, there are proposals for fecal transfer from immunomodulator responders to non-responders. In some cases, adequate responses have been achieved with this strategy.

WHAT IS THE FUTURE OF THE APPLICATION OF ARTIFICIAL INTELLIGENCE IN MICROBIOME RESEARCH AND ITS POTENTIAL ROLE IN THE CLINIC? ETHICAL CONSIDERATIONS

Research in relation to the microbiota, beyond FMT, is in full swing. Metabolites associated with the intestinal microbiota, such as TMAO or SCFAs, may have a potential therapeutic impact on the future of some diseases. Other potential strategies would come from the use of micro RNA (miRNA) that would allow the manipulation of the intestinal microbiome, hyaluronan, nanomedicine or extracellular vesicles [130]. Much of the knowledge about microbiota-host interactions comes from animal models. There are methodological complexities and manifest limitations inherent in translating reductionist animal models to complex human disease. Although it is a gateway to research and future treatments, at present there are no robust conclusions in either the diagnosis or treatment of diseases that have been linked to alterations in the microbiota. Therefore, great expectation has been created in the possible use of AI to facilitate the knowledge of the microbiome and its relationship with health and disease.

AI and in particular machine learning (machine-learning) and deep learning (deep-learning), is opening new frontiers in microbiota research. The use of these tools can improve the understanding, diagnosis and treatment of diseases related to the human microbiome [131]. AI allows the analysis of high volume and complex datasets by identifying patterns and associations limited to other traditional statistical methods, as well as assisting in the sequencing of metagenomic data by allowing the identification and classification of microbial species. These tools can be used to design predictive models and improve the diagnostic accuracy of some microbiota-related diseases. In addition, they can contribute to personalized treatments by improving knowledge of individual microbiome profiles. There are international experiences and networks to promote research and work on the identification of predictive

and discriminatory "omics" features, the improvement of repeatability and comparability, the development of automation procedures, and the definition of priority areas for the development of novel machine learning methods targeting the microbiome [132].

Despite the potential of AI application in microbiota research, there are important challenges related to data quality and standardization, improving algorithms into applicable models that better handle the variability and complexity of microbiome data, combining them with other data such as genomic, proteomic and metabolomic data, and ensuring their ethical use in research. It would be important to start identifying potential ethical issues early on rather than waiting for problems to arise. In this sense, this new technology leads to try to answer the following points that can be generalized to the study of the microbiome [133,134]:

- Personal identity: The microbiome of each individual is unique. If this microbiome, together with genes, conditions our susceptibility to disease and response to treatment, it can be considered part of our identity.
- Privacy: The guarantee of confidentiality is fundamental to the practice of medicine and research. Therefore, in both clinical and research settings, everything related to confidentiality is fully applicable to the microbiome. Patients reasonably expect that their information will not be disclosed.
- Ownership: If someone's microbiome is unique and valuable, to whom does it belong? Do we have ownership rights over our microbiome because our body is its host? When knowledge from a microbiome study has commercial value, who should get the benefits?
- Research with human subjects, creation of biobanks: Having treatments to prevent diseases requires advances in basic science and translational research including biobanks, sample banks and research with human subjects. In this respect, autonomy gives primacy to informed consent and privacy in the current ethics of all research, including therefore all microbiome research. But aside from the above basic consideration, questions arise about the rules and governance of biobanks and sample banks. Who should be given access to biological samples, what information should be shared, what projects should the samples support, and who should make these decisions?

The biomedical research community will need to answer these questions as microbiome research progresses, avoiding both exaggeration of the risks and ignorance of the real problems.

CONCLUSIONS

Although there is evidence of the possible association between the composition of the gut microbiota and numerous diseases, beyond those affecting the digestive tract, and the possible benefit on its modulation, it is still too early to establish general recommendations for action. Only FMT in the treatment of R-CDI has demonstrated unequivocal benefits, and in the rest of the diseases it is necessary to carry out extensive studies with ambitious designs that allow conclusions to be drawn. Part of the difficulty lies in the definition of what is a healthy microbiota (or state of eubiosis) or a diseased microbiota (state of dysbiosis), which is covered by the standardized application of laboratory techniques for its study and interpretation. Around this, decision making has arisen in the face of reports with catalogs of microorganisms that are part of the microbiota that in many cases favor the use of probiotics, prebiotics and postbiotics as a medicine of complacency with the patient. Efforts should be made by scientific societies and regulatory agencies in the analysis of the evidence and the promotion of research by funding agencies. The application of AI, the reduction in the cost of laboratory techniques, and work in multi- and multidisciplinary teams can facilitate this work. Also, and given that action on the microbiome can be considered part of personalized medicine, it is necessary to clarify numerous questions that arise from the ethical point of view. On this depends a better knowledge of the microbiome and the role of prevention and treatment of possible diseases associated with the composition of the microbiota, without falling into a pseudo-medicine that does not add value to scientific progress.

FUNDING

Nothing to declare

CONFLICTS OF INTEREST

The authors declare the absence of conflicts of interest.

REFERENCES

1. Graham DB, Xavier RJ. Conditioning of the immune system by the microbiome. Trends Immunol. 2023 Jul;44(7):499-511. doi: 10.1016/j.it.2023.05.002.
2. Whipps JM, Lewis K, Cooke RC. Mycoparasitism and plant disease control 161–187. In: Burge, NM (editor), Fungi in Biological Control Systems. Manchester University Press; 1988. P. 176.
3. Malakar S, Sutaoney P, Madhyastha H, Shah K, Chauhan NS, Banerjee P. Understanding gut microbiome-based machine learning platforms: A review on therapeutic approaches using deep learning. Chem Biol Drug Des. 2024 Mar;103(3):e14505. doi: 10.1111/cbdd.14505.
4. Heintz-Buschart A, Wilmes P. Human gut microbiome: function matters. Trends in microbiology 2018; 26:563-574. https://doi.org/10.1016/j.tim.2017.11.002.
5. Nishida A, Inoue R, Inatomi O, Bamba S, Naito Y, Andoh A. Gut microbiota in the pathogenesis of inflammatory bowel disease. Clin J Gastroenterol. 2018;11:1-10. doi: 10.1007/s12328-017-0813-5.
6. Marchesi JR, Adams DH, Fava F, Hermes GDA, Hirschfield GM, Hold G, et al. The gut microbiota and host health: a new clinical frontier. Gut 2016; 65:330-339. doi:10.1136/gutjnl-2015-309990.

7. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Reddy DN. Role of the normal gut microbiota. World J Gastroenterol 2015; 21(29): 8787-8803 doi: 10.3748/wjg.v21.i29.8787.
8. Koh A, Bäckhed F. From association to causality: the role of the gut microbiota and its functional products on host metabolism. Mol cell 2020; 78:584-596. doi:10.1016/j.molcel.2020.03.005.
9. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science. 2011 Oct 7;334(6052):105-8. doi: 10.1126/science.1208344.
10. Kurilshikov A, Wijmenga C, Fu J, Zhernakova A. Host Genetics and Gut Microbiome: Challenges and Perspectives. Trends Immunol. 2017;38:633-647. doi: 10.1016/j.it.2017.06.003.
11. Hills RD Jr, Pontefract BA, Mishcon HR, Black CA, Sutton SC, Theberge CR. Gut Microbiome: Profound Implications for Diet and Disease. Nutrients.2019; 11:1613. doi: 10.3390/nu11071613.
12. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. Nature. 2012;489:220-30. doi: 10.1038/nature11550.
13. Clemente JC, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. Cell. 2012;148:1258-70. doi: 10.1016/j.cell.2012.01.035.
14. Tandon D, Haque MM, Shaikh S, Dubey AK, Mande SS. A snapshot of gut microbiota of an adult urban population from Western region of India. PLoS One. 2018;13:e0195643. doi: 10.1371/journal.pone.0195643.
15. Kim YS, Unno T, Kim BY, Park MS. Sex Differences in Gut Microbiota. World J Mens Health. 2020;38:48-60. doi: 10.5534/wjmh.190009.
16. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr. 1995;125:1401-12. doi: 10.1093/jn/125.6.1401.
17. Kovatcheva-Datchary P, Nilsson A, Akrami R, Lee YS, De Vadder F, Arora T, et al. Dietary Fiber-Induced Improvement in Glucose Metabolism Is Associated with Increased Abundance of Prevotella. Cell Metab. 2015; 22:971-82. doi: 10.1016/j.cmet.2015.10.001.
18. Kong C, Gao R, Yan X, Huang L, Qin H. Probiotics improve gut microbiota dysbiosis in obese mice fed a high-fat or high-sucrose diet. Nutrition. 2019;60:175-184. doi: 10.1016/j.nut.2018.10.002.
19. Rinninella E, Cintoni M, Raoul P, Lopetuso LR, Scaldaferri F, Pulcini G, et al. Food Components and Dietary Habits: Keys for a Healthy Gut Microbiota Composition. Nutrients. 2019 Oct 7;11(10):2393. doi: 10.3390/nu11102393.
20. Tomova A, Bukovsky I, Rembert E, Yonas W, Alwarith J, Barnard ND, et al. The Effects of Vegetarian and Vegan Diets on Gut Microbiota. Front Nutr. 2019;6:47. doi: 10.3389/fnut.2019.00047.
21. Cunningham M, Azcarate-Peril MA, Barnard A, Benoit V, Grimaldi R, Guyonnet D, et al. Shaping the Future of Probiotics and Prebiotics. Trends Microbiol. 2021 Aug;29(8):667-685. doi: 10.1016/j.tim.2021.01.003. Epub 2021 Feb 4. PMID: 33551269.
22. Kang DW, Adams JB, Gregory AC, Borody T, Chittick L, Fasano A, et al. Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. Microbiome. 2017 Jan 23;5(1):10. doi: 10.1186/s40168-016-0225-7.
23. Wang M, Karlsson C, Olsson C, Adlerberth I, Wold AE, Strachan DP, et al. Reduced diversity in the early fecal microbiota of infants with atopic eczema. J Allergy Clin Immunol. 2008 Jan;121(1):129-34. doi: 10.1016/j.jaci.2007.09.011.
24. Forno E, Onderdonk AB, McCracken J, Litonjua AA, Laskey D, Delaney ML, et al. Diversity of the gut microbiota and eczema in early life. Clin Mol Allergy. 2008 Sep 22;6:11. doi: 10.1186/1476-7961-6-11.
25. Hua X, Goedert JJ, Pu A, Yu G, Shi J. Allergy associations with the adult fecal microbiota: Analysis of the American Gut Project. EBioMedicine. 2015 Nov 27;3:172-179. doi: 10.1016/j.ebiom.2015.11.038.
26. Fujimura KE, Sitarik AR, Havstad S, Lin DL, Levan S, Fadrosh D, et al. Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation. Nat Med. 2016 Oct;22(10):1187-1191. doi: 10.1038/nm.4176. Epub 2016 Sep 12.
27. Thorburn AN, McKenzie CI, Shen S, Stanley D, Macia L, Mason LJ, et al. Evidence that asthma is a developmental origin disease influenced by maternal diet and bacterial metabolites. Nat Commun. 2015 Jun 23;6:7320. doi: 10.1038/ncomms8320.
28. Kemter AM, Nagler CR. Influences on allergic mechanisms through gut, lung, and skin microbiome exposures. J Clin Invest. 2019 Feb 25;129(4):1483-1492. doi: 10.1172/JCI124610.
29. Kalliomäki M, Collado MC, Salminen S, Isolauri E. Early differences in fecal microbiota composition in children may predict overweight. Am J Clin Nutr. 2008 Mar;87(3):534-8. doi: 10.1093/ajcn/87.3.534.
30. Scheepers LE, Penders J, Mbakwa CA, Thijs C, Mommers M, Arts IC. The intestinal microbiota composition and weight development in children: the KOALA Birth Cohort Study. Int J Obes (Lond). 2015 Jan;39(1):16-25. doi: 10.1038/ijo.2014.178.
31. Brown CT, Davis-Richardson AG, Giongo A, Gano KA, Crabb DB, Mukherjee N, et al. Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. PLoS One. 2011;6(10):e25792. doi: 10.1371/journal.pone.0025792.
32. Giongo A, Gano KA, Crabb DB, Mukherjee N, Novelo LL, Casella G, et al. Toward defining the autoimmune microbiome for type 1 diabetes. ISME J. 2011 Jan;5(1):82-91. doi: 10.1038/ismej.2010.92.
33. Dunne JL, Triplett EW, Gevers D, Xavier R, Insel R, Danska J, et al. The intestinal microbiome in type 1 diabetes. Clin Exp Immunol. 2014 Jul;177(1):30-7. doi: 10.1111/cei.12321.
34. Kimura I, Ozawa K, Inoue D, Imamura T, Kimura K, Maeda T, et al. The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. Nat Commun. 2013;4:1829. doi: 10.1038/ncomms2852.
35. Agustí A, García-Pardo MP, López-Almela I, Campillo I, Maes M, Romaní-Pérez M, et al. Interplay Between the Gut-Brain Axis, Obesity and Cognitive Function. Front Neurosci. 2018 Mar 16;12:155. doi: 10.3389/fnins.2018.00155.

36. Knip M, Honkanen J. Modulation of Type 1 Diabetes Risk by the Intestinal Microbiome. Curr Diab Rep. 2017 Sep 23;17(11):105. doi: 10.1007/s11892-017-0933-9.
37. Khashan AS, Kenny LC, Lundholm C, Kearney PM, Gong T, Almqvist C. Mode of obstetrical delivery and type 1 diabetes: a sibling design study. Pediatrics. 2014 Sep;134(3):e806-13. doi: 10.1542/peds.2014-0819.
38. Pallasmaa Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. Nature. 2011 Jun 15;474(7351):307-17. doi: 10.1038/nature10209.
39. Livanos AE, Greiner TU, Vangay P, Pathmasiri W, Stewart D, McRitchie S, et al. Antibiotic-mediated gut microbiome perturbation accelerates development of type 1 diabetes in mice. Nat Microbiol. 2016 Aug 22;1(11):16140. doi: 10.1038/nmicrobiol.2016.140.
40. Raymond SL, Rincon JC, Wynn JL, Moldawer LL, Larson SD. Impact of Early-Life Exposures to Infections, Antibiotics, and Vaccines on Perinatal and Long-term Health and Disease. Front Immunol. 2017 Jun 23;8:729. doi: 10.3389/fimmu.2017.00729.
41. Martinez FD, Vercelli D. Asthma. Lancet. 2013 Oct 19;382(9901):1360-72. doi: 10.1016/S0140-6736(13)61536-6. Epub 2013 Sep 13.
42. Gerber JS, Bryan M, Ross RK, Daymont C, Parks EP, Localio AR, et al. Antibiotic Exposure During the First 6 Months of Life and Weight Gain During Childhood. JAMA. 2016 Mar 22-29;315(12):1258-65. doi: 10.1001/jama.2016.2395.
43. Madan JC, Salari RC, Saxena D, Davidson L, O'Toole GA, Moore JH, et al. Gut microbial colonisation in premature neonates predicts neonatal sepsis. Arch Dis Child Fetal Neonatal Ed. 2012 Nov;97(6):F456-62. doi: 10.1136/fetalneonatal-2011-301373
44. Mai V, Torrazza RM, Ukhanova M, Wang X, Sun Y, Li N, et al. Distortions in development of intestinal microbiota associated with late onset sepsis in preterm infants. PLoS One. 2013;8(1):e52876. doi: 10.1371/journal.pone.0052876.
45. Cernada M, Bäuerl C, Serna E, Collado MC, Martínez GP, Vento M. et al. Sepsis in preterm infants causes alterations in mucosal gene expression and microbiota profiles compared to non-septic twins. Sci Rep. 2016 May 16;6:25497. doi: 10.1038/srep25497.
46. Collado MC, Cernada M, Neu J, Collado MC, Cernada M, Neu J, Pérez-Martínez G. Factors influencing gastrointestinal tract and microbiota immune interaction in preterm infants. Pediatr Res. 2015 Jun;77(6):726-31. doi: 10.1038/pr.2015.54.
47. Carl MA, Ndao IM, Springman AC, et al. Sepsis from the gut: the enteric habitat of bacteria that cause late-onset neonatal bloodstream infections. Clin Infect Dis. 2014 May;58(9):1211-8. doi: 10.1093/cid/ciu084.
48. Stewart CJ, Embleton ND, Marrs ECL, mith DP, Fofanova T, Nelson A, et al. Longitudinal development of the gut microbiome and metabolome in preterm neonates with late onset sepsis and healthy controls. Microbiome. 2017 Jul 12;5(1):75. doi: 10.1186/s40168-017-0295-1.
49. Podolsky DK. Inflammatory bowel disease. N Engl J Med. 2002 Aug 8;347(6):417-29. doi: 10.1056/NEJMra020831
50. Llewellyn SR, Britton GJ, Contijoch EJ, Vennaro OH, Mortha A, Colombel JF, et al. Interactions Between Diet and the Intestinal Microbiota Alter Intestinal Permeability and Colitis Severity in Mice. Gastroenterology. 2018 Mar;154(4):1037-1046.e2. doi: 10.1053/j.gastro.2017.11.030.
51. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. Nature. 2011 Jun 15;474(7351):307-17. doi: 10.1038/nature10209.
52. Huttenhower C, Kostic AD, Xavier RJ. Inflammatory bowel disease as a model for translating the microbiome. Immunity. 2014 Jun 19;40(6):843-54. doi: 10.1016/j.immuni.2014.05.013.
53. Milani C, Duranti S, Bottacini F, Casey E, Turroni F, Mahony J, et al. The First Microbial Colonizers of the Human Gut: Composition, Activities, and Health Implications of the Infant Gut Microbiota. Microbiol Mol Biol Rev. 2017 Nov 8;81(4):e00036-17. doi: 10.1128/MMBR.00036-17.
54. Hjorth MF, Blædel T, Bendtsen LQ, Lorenzen JK, Holm JB, Kiilerich P, et al. Prevotella-to-Bacteroides ratio predicts body weight and fat loss success on 24-week diets varying in macronutrient composition and dietary fiber: results from a post-hoc analysis. Int J Obes (Lond). 2019 Jan;43(1):149-157. doi: 10.1038/s41366-018-0093-2.
55. Murphy R, Tsai P, Jüllig M, Liu A, Plank L, Booth M. Differential Changes in Gut Microbiota After Gastric Bypass and Sleeve Gastrectomy Bariatric Surgery Vary According to Diabetes Remission. Obes Surg. 2017 Apr;27(4):917-925. doi: 10.1007/s11695-016-2399-2.
56. Wang FG, Bai RX, Yan WM, Yan M, Dong LY, Song MM. Differential composition of gut microbiota among healthy volunteers, morbidly obese patients and post-bariatric surgery patients. Exp Ther Med. 2019 Mar;17(3):2268-2278. doi: 10.3892/etm.2019.7200.
57. Mathur R, Barlow GM. Obesity and the microbiome. Expert Rev Gastroenterol Hepatol. 2015;9(8):1087-99. doi: 10.1586/17474124.2015.1051029.
58. Rosenbaum M, Knight R, Leibel RL. The gut microbiota in human energy homeostasis and obesity. Trends Endocrinol Metab. 2015 Sep;26(9):493-501. doi: 10.1016/j.tem.2015.07.002..
59. Cenit MC, Sanz Y, Codoñer-Franch P. Influence of gut microbiota on neuropsychiatric disorders. World J Gastroenterol. 2017 Aug 14;23(30):5486-5498. doi: 10.3748/wjg.v23.i30.5486
60. Loh JS, Mak WQ, Tan LKS, Ng CX, Chan HH, Yeow SH, et al. Microbiota-gut-brain axis and its therapeutic applications in neurodegenerative diseases. Signal Transduct Target Ther. 2024 Feb 16;9(1):37. doi: 10.1038/s41392-024-01743-1.
61. Sittipo P, Choi J, Lee S, Lee YK. The function of gut microbiota in immune-related neurological disorders: a review. J Neuroinflammation. 2022 Jun 15;19(1):154. doi: 10.1186/s12974-022-02510-1.
62. Socała K, Doboszewska U, Szopa A, Serefko A, Włodarczyk M, Zielińska A, et al. The role of microbiota-gut-brain axis in neuropsychiatric and neurological disorders. Pharmacol Res. 2021 Oct;172:105840. doi: 10.1016/j.phrs.2021.105840.
63. Sepich-Poore GD, Zitvogel L, Straussman R, Hasty J, Wargo JA, Knight R. The microbiome and human cancer. Science. 2021 Mar 26;371(6536):eabc4552. doi: 10.1126/science.abc4552.

64. Garrett WS. Cancer and the microbiota. Science. 2015 Apr 3;348(6230):80-6. doi: 10.1126/science.aaa4972.

65. Nejman D, Livyatan I, Fuks G, Gavert N, Zwang Y, Geller LT, et al. The human tumor microbiome is composed of tumor type-specific intracellular bacteria. Science. 2020 May 29;368(6494):973-980. doi: 10.1126/science.aay9189.

66. Poore GD, Kopylova E, Zhu Q, Carpenter C, Fraraccio S, Wandro S, et al. Microbiome analyses of blood and tissues suggest cancer diagnostic approach. Nature. 2020 Mar;579(7800):567-574. doi: 10.1038/s41586-020-2095-1.

67. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Biological agents. IARC Monogr Eval Carcinog Risks Hum. 2012;100(Pt B):1-441. PMID: 23189750.

68. Pleguezuelos-Manzano C, Puschhof J, Rosendahl Huber A, van Hoeck A, Wood HM, Nomburg J, et al. Mutational signature in colorectal cancer caused by genotoxic pks+ E. coli. Nature. 2020 Apr;580(7802):269-273. doi: 10.1038/s41586-020-2080-8.

69. Wilson MR, Jiang Y, Villalta PW, Stornetta A, Boudreau PD, Carrá A, et al. The human gut bacterial genotoxin colibactin alkylates DNA. Science. 2019 Feb 15;363(6428):eaar7785. doi: 10.1126/science.aar7785.

70. Barrett M, Hand CK, Shanahan F, Murphy T, O'Toole PW. Mutagenesis by Microbe: the Role of the Microbiota in Shaping the Cancer Genome. Trends Cancer. 2020 Apr;6(4):277-287. doi: 10.1016/j.trecan.2020.01.019.

71. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000 Jan 7;100(1):57-70. doi: 10.1016/s0092-8674(00)81683-9.

72. Jin C, Lagoudas GK, Zhao C, Bullman S, Bhutkar A, Hu B, et al. Commensal Microbiota Promote Lung Cancer Development via γδ T Cells. Cell. 2019 Feb 21;176(5):998-1013.e16. doi: 10.1016/j.cell.2018.12.040.

73. Geller LT, Barzily-Rokni M, Danino T, Jonas OH, Shental N, Nejman D, et al. Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. Science. 2017 Sep 15;357(6356):1156-1160. doi: 10.1126/science.aah5043.

74. Bullman S, Pedamallu CS, Sicinska E, Clancy TE, Zhang X, Cai D, et al. Analysis of *Fusobacterium* persistence and antibiotic response in colorectal cancer. Science. 2017 Dec 15;358(6369):1443-1448. doi: 10.1126/science.aal5240.

75. Aykut B, Pushalkar S, Chen R, Li Q, Abengozar R, Kim JI, et al. The fungal mycobiome promotes pancreatic oncogenesis via activation of MBL. Nature. 2019 Oct;574(7777):264-267. doi: 10.1038/s41586-019-1608-2.

76. Pushalkar S, Hundeyin M, Daley D, Zambirinis CP, Kurz E, Mishra A, et al. The Pancreatic Cancer Microbiome Promotes Oncogenesis by Induction of Innate and Adaptive Immune Suppression. Cancer Discov. 2018 Apr;8(4):403-416. doi: 10.1158/2159-8290.CD-17-1134.

77. Parhi L, Alon-Maimon T, Sol A, Nejman D, Shhadeh A, Fainsod-Levi T, et al. Breast cancer colonization by Fusobacterium nucleatum accelerates tumor growth and metastatic progression. Nat Commun. 2020 Jun 26;11(1):3259. doi: 10.1038/s41467-020-16967-2.

78. Dubinsky M, Braun J. Diagnostic and Prognostic Microbial Biomarkers in Inflammatory Bowel Diseases. Gastroenterology. 2015 Oct;149(5):1265-1274.e3. doi: 10.1053/j.gastro.2015.08.006. Epub 2015 Aug 15.

79. Dai Z, Coker OO, Nakatsu G, Wu WKK, Zhao L, Chen Z, et al. Multi-cohort analysis of colorectal cancer metagenome identified altered bacteria across populations and universal bacterial markers. Microbiome. 2018 Apr 11;6(1):70. doi: 10.1186/s40168-018-0451-2.

80. Serrano-Villar S, Tincati C, Raju SC, Sáenz JS, Moreno E, Bargiela R, et al. Microbiome-derived cobalamin and succinyl-CoA as biomarkers for improved screening of anal cancer. Nat Med. 2023 Jul;29(7):1738-1749. doi: 10.1038/s41591-023-02407-3. Epub 2023 Jul 18. Erratum in: Nat Med. 2024 Jan;30(1):303. PMID: 37464040.

81. McQuade JL, Daniel CR, Helmink BA, Wargo JA. Modulating the microbiome to improve therapeutic response in cancer. Lancet Oncol. 2019 Feb;20(2):e77-e91. doi: 10.1016/S1470-2045(18)30952-5.

82. Baruch EN, Youngster I, Ben-Betzalel G, Ortenberg R, Lahat A, Katz L, et al. Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. Science. 2021 Feb 5;371(6529):602-609. doi: 10.1126/science.abb5920.

83. Virgin HW, Todd JA. Metagenomics and personalized medicine. Cell. 2011 Sep 30;147(1):44-56. doi: 10.1016/j.cell.2011.09.009.

84. Alarcón Cavero T, D'Auria G, Delgado Palacio S, Del Campo Moreno, R, Ferrer Martínez, M. Microbiota. 59. Del Campo Moreno R (coordinadora). Procedimientos en Microbiología Clínica. Cercenado E, Cantón R (editores). Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC). 2016.

85. Wang Y, Zhang S, Borody TJ, Zhang F. Encyclopedia of fecal microbiota transplantation: a review of effectiveness in the treatment of 85 diseases. Chin Med J (Engl). 2022;135(16):1927-39. doi: 10.1097/CM9.0000000000002339

86. Kao D, Roach B, Silva M, Beck P, Rioux K, Kaplan GG, et al. Effect of Oral Capsule- vs Colonoscopy-Delivered Fecal Microbiota Transplantation on Recurrent *Clostridium difficile* Infection: A Randomized Clinical Trial. JAMA. 2017;318(20):1985-93. doi: 10.1001/jama.2017.17077.

87. Lee CH, Steiner T, Petrof EO, Smieja M, Roscoe D, Nematallah A, et al. Frozen vs Fresh Fecal Microbiota Transplantation and Clinical Resolution of Diarrhea in Patients With Recurrent *Clostridium difficile* Infection: A Randomized Clinical Trial. JAMA. 2016;315(2):142-9. doi: 10.1001/jama.2015.18098.

88. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, et al. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. N Engl J Med. 2013 Jan 31;368(5):407-15. doi: 10.1056/NEJMoa1205037.

89. Reigadas E, Bouza E, Olmedo M, Vazquez-Cuesta S, Villar-Gomara L, Alcala L, et al. Faecal Microbiota Transplantation for Recurrent *Clostridioides difficile* Infection: Experience with Lyophilized Oral Capsules. J Hosp Infect. 2020 Jun;105(2):319-324. doi: 10.1016/j.jhin.2019.12.022.

90. Youngster I, Russell GH, Pindar C, Ziv-Baran T, Sauk J, Hohmann EL. Oral, capsulized, frozen fecal microbiota transplantation for relapsing *Clostridium difficile* infection. JAMA. 2014;312(17):1772-8. doi: 10.1001/jama.2014.13875.

91. Staley C, Hamilton MJ, Vaughn BP, Graiziger CT, Newman KM, Kabage AJ, et al. Successful Resolution of Recurrent *Clostridium difficile* Infection using Freeze-Dried, Encapsulated Fecal Microbiota; Pragmatic Cohort Study. Am J Gastroenterol. 2017;112(6):940-7. doi: 10.1038/ajg.2017.6.

92. Zhang F, Luo W, Shi Y, Fan Z, Ji G. Should we standardize the 1,700-year-old fecal microbiota transplantation? Am J Gastroenterol. 2012;107(11):1755. doi: 10.1038/ajg.2012.251

93. Eiseman B, Silen W, Bascom GS, Kauvar AJ. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. Surgery. 1958;44(5):854-9. PMID: 13592638

94. Quraishi MN, Widlak M, Bhala N, Moore D, Price M, Sharma N, et al. Systematic review with meta-analysis: the efficacy of faecal microbiota transplantation for the treatment of recurrent and refractory *Clostridium difficile* infection. Aliment Pharmacol Ther. 2017;46(5):479-93. doi: 10.1111/apt.14201.

95. Ramai D, Zakhia K, Fields PJ, Ofosu A, Patel G, Shahnazarian V, Lai JK, Dhaliwal A, Reddy M, Chang S. Fecal Microbiota Transplantation (FMT) with Colonoscopy Is Superior to Enema and Nasogastric Tube While Comparable to Capsule for the Treatment of Recurrent *Clostridioides difficile* Infection: A Systematic Review and Meta-Analysis. Dig Dis Sci. 2021 Feb;66(2):369-380. doi: 10.1007/s10620-020-06185-7.

96. Debast SB, Bauer MP, Kuijper EJ. European Society of Clinical Microbiology and Infectious Diseases: update of the treatment guidance document for *Clostridium difficile* infection. Clin Microbiol Infect. 2014;20 Suppl 2:1-26. doi: 10.1111/1469-0691.12418.

97. van Prehn J, Reigadas E, Vogelzang EH, Bouza E, Hristea A, Guery B, et al. European Society of Clinical Microbiology and Infectious Diseases: 2021 update on the treatment guidance document for *Clostridioides difficile* infection in adults. Clin Microbiol Infect. 2021;27 Suppl 2:S1-s21. doi: 10.1016/j.cmi.2021.09.038.

98. McDonald LC, Gerding DN, Johnson S, Bakken JS, Carroll KC, Coffin SE, et al. Clinical Practice Guidelines for *Clostridium difficile* Infection in Adults and Children: 2017 Update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clin Infect Dis. 2018;66(7):987-94. doi: 10.1093/cid/ciy149.

99. U.S. Food and Drug Administration. Enforcement Policy Regarding Investigational New Drug Requirements for Use of Fecal Microbiota for Transplantation to Treat *Clostridium difficile* Infection. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/enforcement-policy-regarding-investigational-new-drug-requirements-use-fecal-microbiota.

100. Khoruts A, Sadowsky MJ. Understanding the mechanisms of faecal microbiota transplantation. Nat Rev Gastroenterol Hepatol. 2016;13(9):508-16. doi: 10.1038/nrgastro.2016.98.

101. Seekatz AM, Aas J, Gessert CE, Rubin TA, Saman DM, Bakken JS, et al. Recovery of the gut microbiome following fecal microbiota transplantation. MBio. 2014;5(3):e00893-14. doi: 10.1128/mBio.00893-14.

102. Seekatz AM, Rao K, Santhosh K, Young VB. Dynamics of the fecal microbiome in patients with recurrent and nonrecurrent *Clostridium difficile* infection. Genome Med. 2016;8(1):47. doi: 10.1186/s13073-016-0298-8.

103. Youngster I, Sauk J, Pindar C, Wilson RG, Kaplan JL, Smith MB, et al. Fecal microbiota transplant for relapsing *Clostridium difficile* infection using a frozen inoculum from unrelated donors: a randomized, open-label, controlled pilot study. Clin Infect Dis. 2014;58(11):1515-22. doi: 10.1093/cid/ciu135

104. Moayyedi P, Yuan Y, Baharith H, Ford AC. Faecal microbiota transplantation for *Clostridium difficile*-associated diarrhoea: a systematic review of randomised controlled trials. Med J Aust. 2017;207(4):166-72. doi: 10.5694/mja17.00295.

105. Postigo R, Kim JH. Colonoscopic versus nasogastric fecal transplantation for the treatment of *Clostridium difficile* infection: a review and pooled analysis. Infection. 2012;40(6):643-8.

106. Baxter M, Colville A. Adverse events in faecal microbiota transplant: a review of the literature. J Hosp Infect. 2016;92(2):117-27. doi: 10.1007/s15010-012-0307-9.

107. Wang S, Xu M, Wang W, Cao X, Piao M, Khan S, et al. Systematic Review: Adverse Events of Fecal Microbiota Transplantation. PLoS One. 2016;11(8):e0161174. doi: 10.1371/journal.pone.0161174.

108. Iqbal U, Anwar H, Karim MA. Safety and efficacy of encapsulated fecal microbiota transplantation for recurrent *Clostridium difficil*e infection: a systematic review. Eur J Gastroenterol Hepatol. 2018;30(7):730-4. doi: 10.1097/MEG.0000000000001147.

109. Ooijevaar RE, van Nood E, Goorhuis A, Terveer EM, van Prehn J, Verspaget HW, et al. Ten-Year Follow-Up of Patients Treated with Fecal Microbiota Transplantation for Recurrent *Clostridioides difficile* Infection from a Randomized Controlled Trial and Review of the Literature. Microorganisms. 2021;9(3). doi: 10.3390/microorganisms9030548.

110. Marcella C, Cui B, Kelly CR, Ianiro G, Cammarota G, Zhang F. Systematic review: the global incidence of faecal microbiota transplantation-related adverse events from 2000 to 2020. Aliment Pharmacol Ther. 2021;53(1):33-42. doi: 10.1111/apt.16148.

111. Rapoport EA, Baig M, Puli SR. Adverse events in fecal microbiota transplantation: a systematic review and meta-analysis. Ann Gastroenterol. 2022;35(2):150-63. doi: 10.1111/apt.16148.

112. Wang Y, Chen N, Niu F, Li Y, Guo K, Shang X, et al. Probiotics therapy for adults with diarrhea-predominant irritable bowel syndrome: a systematic review and meta-analysis of 10 RCTs. Int J Colorectal Dis. 2022 Nov;37(11):2263-2276. doi: 10.1007/s00384-022-04261-0.

113. Chieng WK, Abdul Jalal MI, Bedi JS, Zainuddin AA, Mokhtar MH, Abu MA, et al. Probiotics, a promising therapy to reduce the recurrence of bacterial vaginosis in women? a systematic review and meta-analysis of randomized controlled trials. Front Nutr. 2022 Sep 20;9:938838. doi: 10.3389/fnut.2022.938838.

114. Valdés-Varela L, Gueimonde M, Ruas-Madiedo P. Probiotics for Prevention and Treatment of *Clostridium difficile* Infection. Adv Exp Med Biol. 2018;1050:161-176. doi: 10.1007/978-3-319-72799-8_10.

115. Gupta V, Mastromarino P, Garg R. Effectiveness of Prophylactic Oral and/or Vaginal Probiotic Supplementation in the Prevention of Recurrent Urinary Tract Infections: A Randomized, Double-Blind,

Placebo-Controlled Trial. Clin Infect Dis. 2024 May 15;78(5):1154-1161. doi: 10.1093/cid/ciad766. PMID: 38084984.

116. Alvarez Calatayud G, Leis Trabazo R, Diaz Martin JJ. Modulación de la microbiota intestinal. Uso de probióticos y prebióticos en Pediatría. Protocolos. Asociación Española de Pediatria. 2022 Available at: https://www.aeped.es/sites/default/files/documentos/39_microbiota.pdf
117. Diez-Delgado J. Probióticos y Prebióticos en el periodo perinatal. Evidencia científica. An Microbiota Probióticos Prebióticos.2024;5(1):153-157.
118. De Veaux A, Ryou J, Dantas G, Warner BB, Tarr PI. Microbiome-targeting therapies in the neonatal intensive care unit: safety and efficacy. Gut Microbes.2023;15(1):2221758. doi:10.1080/19490976.2023.2221758.
119. Chiruvolu A, Hendrikson H, Hanson R, Reedy A, Reis J, Desai S, et al. Effects of prophylactic probiotics supplementation on infants born very preterm or very low birth weight. J Perinatol.2023;43(5): 635-41.doi:10.1038/ s41372-023-01657-w.
120. Diaz Martin J. Evidencia científica sobre el empleo de probióticos en Gastroenterología Pediátrica. Guias de práctica clínica. An Microbiota Probióticos Prebióticos 2024;5(1):79-82.
121. Szajewska H, Berni Canani R, Domelloof M , el al; ESPGHAN Special Interest Group on Gut Microbiota and Modifications. Probiotics for the management of pediatric gastrointestinal disorders: Position Paper of the ESPGHAN Special Interest Group on Gut Microbiota end Modifications. J Pediatr Gstroenterol Nutr. 2023; 76(2):232-247. doi: 10.1097/MPG.0000000000003633.
122. Directrices mundiales de la organización Mundial de Gastroenterología Probióticos y prebióticos. Avalaible at: https://www.worldgastroenterology.org/guidelines/probiotics-and-prebiotics-prebiotics-and-prebiotics-spanish. Feb 2023.
123. Bolsega S, Bleich A, Basic M. Synthetic Microbiomes on the Rise-Application in Deciphering the Role of Microbes in Host Health and Disease. Nutrients. 2021 Nov 21;13(11):4173. doi: 10.3390/nu13114173.
124. Torres-Carrillo N, Martínez-López E, Torres-Carrillo NM, López-Quintero A, Moreno-Ortiz JM, González-Mercado A, et al. Pharmacomicrobiomics and Drug-Infection Interactions: The Impact of Commensal, Symbiotic and Pathogenic Microorganisms on a Host Response to Drug Therapy. Int J Mol Sci. 2023 Dec 4;24(23):17100. doi: 10.3390/ijms242317100.
125. Anonimous. Live fecal microbiota (Rebyota) for prevention of CDI recurrence. Med Lett Drugs Ther. 2023 Mar 6;65(1671):35-36. doi: 10.58347/tml.2023.1671b.
126. Anonimous. Live fecal microbiota oral capsules (Vowst) for prevention of CDI recurrence. Med Lett Drugs Ther. 2023; 65:81-82. doi: 10.58347/tml.2023.1677a.
127. Manes A, Di Renzo T, Dodani L, Reale A, Gautiero C, Di Lauro M, et al. Pharmacomicrobiomics of Classical Immunosuppressant Drugs: A Systematic Review. Biomedicines. 2023 Sep 18;11(9):2562. doi: 10.3390/biomedicines11092562.
128. Weersma RK, Zhernakova A, Fu J. Interaction between drugs and the gut microbiome. Gut. 2020 Aug;69(8):1510-1519. doi: 10.1136/gutjnl-2019-320204.
129. Yang Y, An Y, Dong Y, Chu Q, Wei J, Wang B, et al. Fecal microbiota transplantation: no longer cinderella in tumour immunotherapy. EBioMedicine. 2024 Feb;100:104967. doi: 10.1016/j.ebiom.2024.104967.
130. Gebrayel P, Nicco C, Al Khodor S, Bilinski J, Caselli E, Comelli EM, et al. Microbiota medicine: towards clinical revolution. J Transl Med. 2022;20(1):111. doi:10.1186/s12967-022-03296-9.
131. Hernández Medina R, Kutuzova S, Nielsen KN, Johansen J, Hansen LH, Nielsen M, et al. Machine learning and deep learning applications in microbiome research. ISME Commun. 2022;2(1):98. doi:10.1038/s43705-022-00182-9.
132. D'Elia D, Truu J, Lahti L, Berland M, Papoutsoglou G, Ceci M, et al. Advancing microbiome research with machine learning: key findings from the ML4Microbiome COST action. Front Microbiol. 2023 Sep 25;14:1257002. doi: 10.3389/fmicb.2023.1257002.
133. Rhodes R. Ethical issues in microbiome research and medicine. BMC Med. 2016 Oct 12;14(1):156. doi: 10.1186/s12916-016-0702-7.
134. Ejtahed HS, Parsa M, Larijani B. Ethical challenges in conducting and the clinical application of human microbiome research. J Med Ethics Hist Med. 2023 Jul 7;16:5. doi: 10.18502/jmehm.v16i5.13313.

Official journal of the Spanish Society of Chemotherapy

Original

Revista Española de Quimioterapia
doi:10.37201/req/067.2024

Invasive group A *Streptococcus* infection (*Streptococcus pyogenes*): Current situation in Spain

Mari Cruz Martín-Delgado[1]
Pilar De Lucas Ramos[2]
Alejandra García-Botella[3]
Rafael Cantón[4]
Alberto García-Lledó[5]
Teresa Hernández-Sampelayo[6]
Javier Gómez-Pavón[7]
Juan González del Castillo[8]
Francisco Javier Martín Sánchez[8]
Manuel Martínez-Sellés[9]
José María Molero García[10]
Santiago Moreno Guillén[11]
Fernando Rodríguez-Artalejo[12]
Julián Ruiz-Galiana[13]
Almudena Burillo[14]
Patricia Muñoz[15]
Cristina Calvo Rey[16]
Mercedes Catalán-González[17]
Emilio Cendejas-Bueno[18]
Ana Verónica Halperin-Benito[19]
Raúl Recio[20]
Carmen Viñuela-Benítez[21]
Emilio Bouza[22]

[1]Department of Intensive Care Medicine. Hospital Doce de Octubre. Universidad Complutense. Madrid.
[2]Pneumology Department. Hospital General Universitario Gregorio Marañón. Universidad Complutense. Madrid.
[3]General Surgery Department. Hospital Clínico Universitario de San Carlos. Universidad Complutense. Madrid.
[4]Microbiology Department. Ramón y Cajal Hospital and Ramón y Cajal Institute for Health Research (IRYCIS). CIBER of Infectious Diseases (CIBERINFEC). Madrid.
[5]Cardiology Department. Hospital Universitario Príncipe de Asturias. University de Alcalá de Henares. Madrid.
[6]Paediatrics Department and ACES. Hospital General Universitario Gregorio Marañón, Universidad Complutense. Madrid.
[7]Geriatrics Service. Hospital Central de la Cruz-Roja. Universidad Alfonso X el Sabio. Madrid.
[8]Emergency Department. Hospital Clínico Universitario de San Carlos. Universidad Complutense. Madrid.
[9]Service of Cardiology. Hospital General Universitario Gregorio Marañón. Universidad Europea. Madrid.
[10]Family Medicine. Infectious Diseases. Madrid.
[11]Infectious Diseases Service. Ramón y Cajal Hospital. University de Alcalá de Henares. CIBER of Infectious Diseases (CIBERINFEC). Madrid.
[12]Department of Preventive Medicine and Public Health. Universidad Autónoma. Madrid.
[13]Internal Medicine Department. Hospital Ruber Internacional. Madrid.
[14]Clinical Microbiology and Infectious Diseases Department. Hospital General Universitario Gregorio Marañón. Gregorio Marañón Institute of Health Research. Universidad Complutense. Madrid.
[15]Clinical Microbiology and Infectious Diseases Department. Hospital General Universitario Gregorio Marañón, Universidad Complutense. Gregorio Marañón Institute of Health Research. CIBER of Respiratory Diseases (CIBERES). Madrid.
[16]Department of Paediatrics and Infectious Diseases. Hospital Universitario La Paz. IdiPaz Foundation. Universidad Autónoma. Madrid. CIBERINFEC. Translational Research Network in Paediatric Infectious Diseases (RITIP). Madrid.
[17]Intensive Care Department. Hospital Universitario Doce de Octubre. Universidad Complutense. Madrid.
[18]Clinical Microbiology Department. Hospital Universitario La Paz. Madrid. CIBER of Infectious Diseases (CIBERINFEC). Madrid.
[19]Clinical Microbiology Department. Hospital Ramón y Cajal. Ramón y Cajal Institute of Health Research. Madrid.
[20]Clinical Microbiology Department. Hospital Universitario Doce de Octubre. Hospital Universitario Doce de Octubre Institute of Health Research. Madrid.
[21]Obstetrics and Gynaecology Department. Hospital General Universitario Gregorio Marañón. Department of Maternal and Child Public Health, Universidad Complutense. Gregorio Marañón Hospital Research Institute. Madrid.
[22]Emeritous in care, Comunidad de Madrid. Clinical Microbiology and Infectious Diseases Department. Hospital General Universitario Gregorio Marañón. Universidad Complutense. CIBER of Respiratory Diseases (CIBERES). Madrid.

Article history
Received: 18 July 2024; Accepted: 23 July 2024; Published: 30 July 2024

ABSTRACT

Group A ß-hemolytic *Streptococcus* (*S. pyogenes*), also known as GAS, is a Gram-positive bacterium. It can be easily identified in the microbiology laboratory by its ability to hemolyse blood in culture media. This bacterium is highly virulent due to its production of enzymes and toxins, and its ability to cause immunologically mediated diseases such as rheumatic fever and post-streptococcal glomerulonephritis.

GAS is the primary cause of bacterial pharyngotonsillitis, although it is typically a benign and non-invasive disease. However, it also has the potential to cause severe skin and soft tissue infections, necrotising fasciitis, bacteraemia and endocarditis, pneumonia and empyema, and streptococcal toxic shock syndrome, without any age or predisposition limits. The term invasive GAS disease (iGAS) is used to refer to this group of conditions.

In more developed countries, iGAS disease has declined thanks to improved hygiene and the availability of antibiotics. For example, rheumatic fever has practically disappeared in

Correspondence:
Emilio Bouza.
Servicio de Microbiología Clínica y Enfermedades Infecciosas. Hospital General Universitario Gregorio Marañón, Universidad Complutense. CIBER de Enfermedades Respiratorias (CIBERES). Madrid.
E-mail: emilio.bouza@gmail.com

All authors belong to the Scientific Committee on COVID-19 of the Madrid College of Physicians (ICOMEM).

countries such as Spain. However, recent data suggests a potential increase in some iGAS diseases, although the accuracy of this data is not consistent.

Because of this, the COVID and Emerging Pathogens Committee of the Illustrious Official College of Physicians of Madrid (ICOMEM) has posed several questions about invasive GAS infection, especially its current situation in Spain. The committee has enlisted the help of several experts in the field to answer these questions. The following lines contain the answers that we have collaboratively produced, aiming to assist not only the members of ICOMEM but also anyone interested in this topic.

Keywords: *Streptococcus pyogenes*, **group A *streptococcus*, pharyngotonsillitis, bacteraemia, endocarditis, skin and soft tissue infection, necrotising fasciitis, pneumonia, explosive pleuritis, empyema, streptococcal toxic shock syndrome, SSTS, rheumatic fever. glomerulonephritis.**

Infección invasora por estreptococo del grupo A (*Streptococcus pyogenes*): situación actual en España

RESUMEN

Streptococcus ß-hemolítico del grupo A (*S. pyogenes*) (SGA) es una bacteria Gram positiva fácil de identificar en el laboratorio de microbiología por muchos procedimientos, pero particularmente por su capacidad de hemolizar la sangre en los medios de cultivo. Su virulencia está bien acreditada por la producción de enzimas y toxinas, pero también por la capacidad de inducir enfermedades inmunológicamente mediadas tales como la fiebre reumática o la glomerulonefritis postestreptocócica.

Es el agente causal de la mayoría de las faringoamigdalitis bacterianas que en general se comportan como enfermedades benignas y no invasoras. Al mismo tiempo, ha demostrado su capacidad de producir infecciones graves en piel y tejidos blandos, fascitis necrotizantes, bacteriemia y endocarditis, neumonías y empiemas, síndrome del shock tóxico estreptocócico y otras muchas sin respetar límites de edad ni de predisposición. Para este último conjunto de cuadros utilizamos el término de enfermedad invasora por SGA (iSGA).

La iSGA había disminuido en los países más desarrollados al amparo de la mejor calidad de la higiene y de la disponibilidad de antibióticos al punto de una práctica desaparición de la fiebre reumática en países como España. Sin embargo, datos recientes, aunque no siempre precisos, hablan de un aumento de algunas enfermedades iSGA.

Por este motivo, el Comité de COVID y patógenos emergentes, del Ilustre Colegio Oficial de Médicos de Madrid (ICOMEM) se ha formulado una serie de preguntas sobre la infección iSGA y particularmente su situación en España. El Comité ha convocado a algunos expertos en el tema recabando su ayuda para responder a dichas preguntas. Las líneas que siguen son las respuestas que hemos producido entre todos, tratando de ser útiles no solo a los colegiados de Madrid si no a todos los interesados en el tema.

Palabras clave: *Streptococcus pyogenes*, ***Streptococcus* grupo A, faringoamigdalitis, bacteriemia, endocarditis, infección de piel y partes blandas, fascitis necrotizante, neumonía, pleuritis explosiva, empiema, síndrome del shock tóxico estreptocócico, SSTS, fiebre reumática, glomerulonefritis**

INTRODUCTION

The disease caused by Lancefield's group A *Streptococcus* (*Streptococcus pyogenes*) has a long history as a cause of infection in humans. Unlike less severe forms of the disease, such as pharyngitis, invasive Group A *Streptococcus* infections (iGAS) can lead to systemic and often fatal manifestations. Additionally, Group A *Streptococcus* can trigger immune responses that result in tissue damage, such as rheumatic fever and post-streptococcal glomerulonephritis. The pathogen's ability to produce toxins with multiple targets makes it one of the most feared infectious diseases in history. The availability of antimicrobials, particularly penicillin and its derivatives, has helped alleviate some of the fear associated with this pathogen.

During the pandemic years, there was a documented decrease in iGAS infections. However, there have been warnings of an increase in iGAS diseases in the aftermath, particularly in the last two years and especially in children [1]. Since the pathogen is not notifiable in most nations, records may not always be accurate, but many data point to a resurgence of iGAS as a cause of disease in all age groups.

Given these uncertainties and a desire to understand the situation in Spain, the COVID-19 and Emerging Pathogens Committee of the Official College of Physicians of Madrid (ICOMEM) has posed a series of questions on this issue and sought answers from experts. The following are the answers to these questions, discussed and agreed upon by the working group.

WHAT DEFINES A GROUP A *STREPTOCOCCUS* (*S. PYOGENES*) AS A MICROORGANISM, AND HOW CAN ITS TOXIC CAPACITY AND MECHANISMS BE DESCRIBED?

S. pyogenes, also known as group A ß-hemolytic *Streptococcus* (GAS), is a highly virulent bacterium that can cause a wide range of infections with varying severity. These infections can range from mild, such as acute pharyngitis or erysipelas, to very aggressive forms like necrotising skin and soft tissue infections or streptococcal toxic shock syndrome (SSTS). These severe cases are referred to as invasive GAS infections (iGAS). Additionally, GAS can lead to autoimmune or post-infectious diseases such as rheumatic fever (RF) and acute glomerulonephritis.

GAS is a human-exclusive pathogen, and the skin and mucous membranes of colonised individuals (healthy carriers) serve as the natural reservoir.

In terms of its essential microbiological characteristics, it is a Gram-positive bacterium that appears as cocci in chains when viewed under Gram staining.

GAS is an aerobic and facultative anaerobic, non-motile, non-spore-forming bacterium. It thrives well on standard culture media at 37°C, preferably in a 10% CO_2 environment. It produces complete hemolysis in blood-enriched culture media (Figure 1). Identification in the laboratory is straightforward.

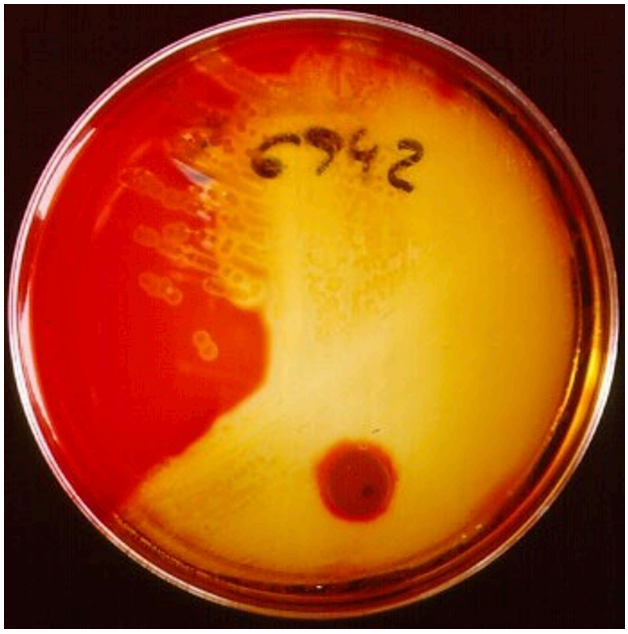


Figure 1 | **Complete haemolysis in a culture medium enriched with blood from *S. pyogenes***

Like other streptococci, it is catalase negative, which sets it apart from the *Staphylococcus* genus, and belongs to group A according to the Lancefield classification. It can ferment some carbohydrates, producing lactic acid, and can be readily identified by MALDI-TOF (matrix-assisted laser desorption/ionisation time-of-flight) and other procedures.

The microorganism has various virulence factors involved in the adhesion and colonisation process (lipoteichoic acid), in evading the immune system (hyaluronic acid capsule, C5a peptidase, M protein, streptolysin O, streptococcal pyrogenic exotoxin B), and in facilitating the spread of the bacterium in the host's soft tissues (streptokinase, hyaluronidase, streptolysin S) [2-9]. Some of their toxins and their effects are presented in Table 1.

HOW IS THE HUMAN DISEASE CAUSED BY GAS CLASSIFIED? WHAT ARE THE LEADING CAUSES? WHAT ARE THE PRIMARY FORMS OF INVASIVE DISEASE?

GAS can cause a wide range of symptoms, from no symptoms to severe and life-threatening illnesses [10]. Diseases caused by GAS can be categorised based on how they are produced: through direct invasion of tissues by the bacteria or by toxins, immunological mechanisms, or inflammation (Table 2).

GAS can directly invade specific tissues, leading to localised infections, or enter the bloodstream and spread to other organs. The most common localised infections associated with GAS are throat and tonsil infections and skin and soft tissue infections, which significantly impact global health [11,12]. Throat and tonsil infections can lead to local suppurative infections like suppurative adenitis, cellulitis, and abscesses or spread to nearby sinuses and the central nervous system. Skin

Table 1 | **Main virulence factors of *S. pyogenes* and their effects**

Virulence factor	Effects
Lipoteichoic acid	It forms a complex with the M-protein and contributes to the adherence to epithelial cells.
Hyaluronic acid capsule	It confers antiphagocytic properties by preventing the opsonisation of the bacteria [5].
Peptidase C5a	An enzyme that degrades the C5a component of complement (essential in chemotaxis), reducing the attraction of complement to the phagocytes.
M protein	Antigen that inhibits phagocytosis by polymorphonuclear cells and prevents intracellular killing of the bacteria. The increased prevalence of M1 and M3 types has been associated with increased invasive infections by this microorganism [6].
Haemolysins or streptolysins O and S	Streptolysin O is an antigenic cytotoxin that forms transmembrane pores in leukocytes, tissue cells and platelets, leading to their lysis [7]. Streptolysin S is non-antigenic but also produces pores in various cells, especially toxic to leukocytes that phagocytose streptococci [8]. Both are responsible for the erythrocyte lysis that can be observed in the blood agar cultures mentioned above.
Streptokinase	Transformation of plasminogen into plasmin that destroys fibrin and contributes to the dissemination of the infection.
Hyaluronidase	Hydrolyses the hyaluronic acid in connective tissue, conferring the ability to disseminate it in tissues.
Streptodornases or deoxyribonucleases	They promote dissemination by depolymerising tissue DNA.
Streptococcal pyrogenic or erythrogenic toxin (Spe)	There are four types: A, B, C, and D. They behave as superantigens, causing fever, rash (scarlet fever), T-lymphocyte proliferation, B-lymphocyte suppression, and increased susceptibility to endotoxins [9] The production of types A and C depends on the presence of an early gene carried by a bacteriophage. A chromosomal gene produces B.
Protein F and LT	Surface proteins that bind to fibronectin and interfere with opsonisation.

Table 2 Diseases caused by *S. pyogenes* according to location and mechanism of production

Through direct invasion by GAS
Focal infections
Tonsillopharyngitis
Skin and soft tissue infections
Impetigo
Erysipelas
Cellulitis
Necrotising fasciitis (myonecrosis, streptococcal gangrene)
Scarlet fever*
Puerperal sepsis
Others (pneumonia, empyema, endocarditis, meningitis, arthritis, osteomyelitis)
Bacteraemia
By other mechanisms
Inflammatory: Streptococcal toxic shock syndrome
Immunological:
Glomerulonephritis
Rheumatic fever

* Produced by toxin

Table 3 Diseases caused by *S. pyogenes* according to their degree of invasion

Superficial infections
Tonsillopharyngitis
Impetigo
Scarlet fever
Invasive infections
Erysipelas
Cellulitis
Necrotising fasciitis
Bacteraemia
Streptococcal toxic shock syndrome
Puerperal sepsis
Others (pneumonia, empyema, endocarditis, meningitis, arthritis, osteomyelitis)
Non-suppurative sequelae
Glomerulonephritis
Rheumatic fever

and soft tissue infections can affect all layers, from the surface to the muscle, and can lead to severe necrotising fasciitis (NF) [13]. Additionally, GAS can affect the skin through the pyrogenic (erythrogenic) toxin, causing scarlet fever and resulting in recent outbreaks in multiple countries following its near disappearance at the end of the 20th century [14]. GAS can also lead to rare conditions such as puerperal sepsis.

Any localised infection can lead to GAS entering the bloodstream, causing bacteraemia and potentially leading to distant metastasis. Although rare, GAS can also cause other localised infections through the bloodstream (such as pneumonia, endocarditis, meningitis, and osteoarticular infections). Streptococcal toxic shock syndrome (STSS) can occur in association with any streptococcal infection, with or without bacteraemia, and is also triggered by pyogenic toxins.

In addition to direct invasion or toxin-based diseases, some GAS infections can result in non-suppurative complications through immunological mechanisms, such as developing autoantibodies and immune complexes. Post-streptococcal glomerulonephritis can develop after skin and throat infections. In contrast, rheumatic fever (RF), which typically follows a throat infection, can lead to secondary valvular heart disease and remains a common cause of early cardiovascular morbidity and mortality in young people worldwide [15] (Table 2).

Another way to classify diseases caused by GAS is by considering the level of invasion they cause (Table 3).

Based on the information above, it can be inferred that GAS infections can be divided into superficial, relatively mild cases (such as pharyngotonsillitis, superficial foot infections, and scarlet fever) and invasive, often severe infections (such as deep skin infections, bacteraemia, distant organ metastases, SSTS), which are increasingly being observed even in high-income countries [16].

WHAT IS THE CURRENT STATUS OF RHEUMATIC FEVER, BOTH GLOBALLY AND IN SPAIN?

Rheumatic fever (RF) is an inflammatory disease caused by an autoimmune response to a GAS infection. The autoimmune response is due to a similarity between the components of the *Streptococcus* and those of the affected tissues. Both humoral antibody-mediated and cellular T-cell-specific responses are involved in tissue damage. The characteristic Aschoff nodules found in RF histology account for this cellular response. Reactive arthritis, although clinically different, is considered to be part of the spectrum of RF.

The autoimmune response to GAS also contributes to post-streptococcal glomerulonephritis, which has a different immune pathogenesis from RF.

In addition to chorea, a cardinal symptom of rheumatic fever, other neurological disorders such as certain behavioral issues and stuttering have been epidemiologically linked to streptococcal infection.

RF manifests in the second to third week after GAS infection with rapidly migrating polyarthritis (60-80% of cases) -monoarthritis may occur- and is highly responsive to anti-inflammatory drugs; pancarditis (50-80%); chorea (10-30%), a markedly later symptom; skin involvement in the form of ery-

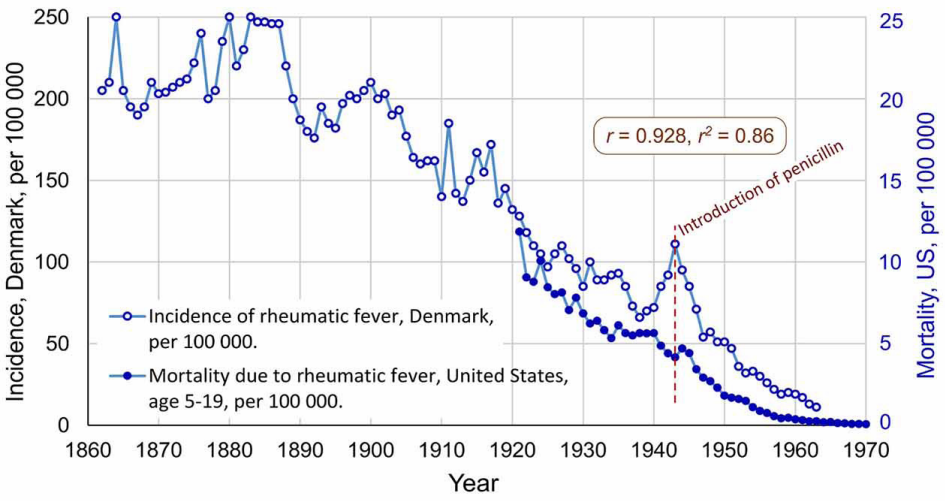


Figure 2 | **Incidence of rheumatic fever in Denmark and mortality of rheumatic fever in the United States. Taken from Alm PA [24] CC-BY license, version 4.0**

thema marginatum, a rare but characteristic lesion (less than 6%) and nodules in the subcutaneous tissue (0-10%). These symptoms and signs, notably defining the entity, are accompanied by other more frequent but non-specific symptoms and signs: fever, increase in acute phase reactants (ESR and RHDP), and prolongation of the PR interval of the electrocardiogram.

Diagnosis is primarily clinical, considering the history of pharyngitis, if present. The diagnostic criteria established by Thomas Ducktt Jones in 1944 [17] are universally accepted; they have undergone revisions considering the sensitivity and specificity of the symptoms in the different compilation series, the epidemiological risk of the population where it occurs, and the influence of the diagnostic techniques incorporated in the confirmation of previous GAS infection and the diagnosis of carditis. The latest globally accepted update was established by the American Heart Association in 2015 [18].

The disease primarily but not exclusively affects children aged 5-14 and can and often does recur. RF is a self-limiting disease, although chorea can last for months. Endocardial injury and the resulting valvulitis are responsible for the chronicity and severity and are enhanced by recurrences. Mortality in acute attacks is very low, and if it occurs, it is always due to carditis.

So important is it that in geographic areas where it is still prevalent, rheumatic heart disease (RHD) remains the most common cause of death in the 5-18 age group [19], as it was in Western countries and our environment well into the 20th century [20] and the leading cause of cardiovascular death in those under 50 years of age [21].

GAS transmission is a determinant for endemic maintenance and RF and RHD epidemic outbreaks, with overcrowding being the main risk factor [22,23].

It was previously believed that certain types of throat infections and specific strains of *Streptococcus* posed a higher risk of causing rheumatic fever (referred to as "rheumatogenic strains"). However, the current understanding of rheumatic fever epidemiology challenges this concept, as a wide range of strains are now implicated in the disease. In areas where rheumatic fever is common, skin infections such as impetigo are considered another possible initial trigger for the body's sensitisation to the disease.

Genetic susceptibility, similar to other autoimmune processes, also plays a role in rheumatic fever, although specific genetic markers have not been identified. There is a higher incidence of rheumatic fever in identical twins.

The improvement in living conditions in Europe, the United States of America, and other high-income Western countries over the last century has been a significant factor in the decline of rheumatic fever. This decline accelerated from the mid-20th century with the availability of antibiotics effective against GAS. The graph depicting this decline in Denmark applies to all the mentioned regions [24] (Figure 2).

The global incidence of rheumatic fever (RF) is estimated at 470,000 new cases annually, with 282,000 of those cases leading to rheumatic heart disease (RHD). It affects more than 33 million people in areas where it is still prevalent, and 220,000 people in the Western world. Seventy-three percent of cases are concentrated in the world's most populous countries. South Asia, Oceania, and sub-Saharan Africa bear the highest burden of the disease [25,26]. The incidence varies widely, ranging from 150-380 cases per 100,000 school-aged children among Indigenous people in Australia and New Zealand (this has been consistent since the 1980s) [27] to less than 2 per 10^5 in the same age group and less than 1 per 10^5 in the gener-

al population in the Western world. This results in 230,000 to 320,000 annual deaths from RHD worldwide.

Globally, there is a downward trend in the incidence of the disease, although occasional resurgences are reported in various regions, including the USA, Utah [28], Italy [29], Slovenia [30], South Asia, Central Africa, and Sub-Saharan Africa. The burden of the disease is higher in areas with poor epidemiological data collection, so the figures used are estimates.

In Spain, reporting of the disease ceased to be mandatory at the national level in 1996, although some regions continued to require it. The incidence declined in most countries, with a slight delay lasting until the 1970s. However, its cardiac consequences and the need for surgery remained significant until the 1980s. The most recent data in the literature is an update by Cortina et al., which covers up to the mid-1980s [31].

Today, the disease is practically non-existent in Spain, but it should always be considered [32,33], especially given the immigrant population from areas with active rheumatic fever. It's important to remember that early treatment can positively impact the course of heart disease.

WHAT IS THE TREND IN GAS BACTERAEMIA EVOLUTION IN RECENT YEARS IN SPAIN AND AROUND THE WORLD?

The occurrence of GAS bacteraemia can occur with or without focal pictures as its portal of entry. The current understanding of the incidence of GAS bacteraemia and its recent evolution is still not fully clarified. Not all data indicates a recent increase in episodes, let alone bacteraemic episodes.

It is estimated that there are between 10,649 and 13,434 cases of iGAS infections annually in the US, causing between 1,136 and 1,607 deaths [34]. In 2016, the US Centers for Disease Control and Prevention (CDC) published surveillance results for iGAS infections between 2005 and 2012 in 10 US areas with a population of 32.8 million. A total of 9,557 cases (3.8 cases per 100,000 persons per year) with 1,116 deaths (case fatality rate, 11.7%) were identified, with isolation of the organism from blood in 7,837 cases (82%). The study did not show an increase in invasive infection rates during the study period [34].

In contrast, in subsequent years, several publications have emerged warning of a recent increase in iGAS infection [35,36]. Incidence increased from 1.04 to 4.76 cases per 100,000 persons from 2008 to 2019 in Idaho [35], with SSTS numbers evolving from 0 to 6.4% of cases. In the USA, recent outbreaks associated with an increase in GAS bacteraemia in drug-addicted patients [37] have been reported in association with the presence of xylazine. Xylazine, often referred to as "tranq," is an adulterant in an increasing number of illicit drug mixtures. Among other effects, it produces vasoconstriction and necrosis, and users experience effects similar to those of opioids.

In Alberta, Canada, out of 3,551 cases, there has been an increase in the incidence of iGAS infections from 4.24 per 100,000 population in 2003 to 10.24 in 2017, with half of the cases being bacteraemic [36].

In Europe, the incidence of iGAS infections in Finland has shown a fluctuating but increasing trend. The incidence of bacteraemic cases has been estimated at 3.52 episodes/100,000 population per year, reaching 7.93 episodes/100,000 in 2018 [38]. In England, there has been an apparent increase in the incidence of GAS infections, especially respiratory tract infections in children [39], but not clearly in bacteraemic episodes.

In the Netherlands, a study involving seven hospitals showed that the incidence of GAS infections doubled after the pandemic compared to pre-pandemic data in paediatric patients [40].

In France, GAS infections requiring intensive care unit (ICU) admission have increased more than 4-fold in equivalent periods before and after the pandemic [41], as have paediatric episodes in patients with previous viral infections [42]. Data from Belgium show an increase in bacteraemia, including in adults [43].

Among the Danish population of 1,152,000 children and adolescents aged 0-17, a significant increase in iGAS infection episodes greater than 9-fold was demonstrated. The incidence of iGAS infection increased in 2022-23 compared to the three pre-COVID-19 seasons of 2016-17, 2017-18, and 2018-19 without increasing severity [44].

Other papers suggesting an increased incidence of iGAS infections in paediatrics come from Australia [45], Portugal [46], and British Columbia, Canada [47].

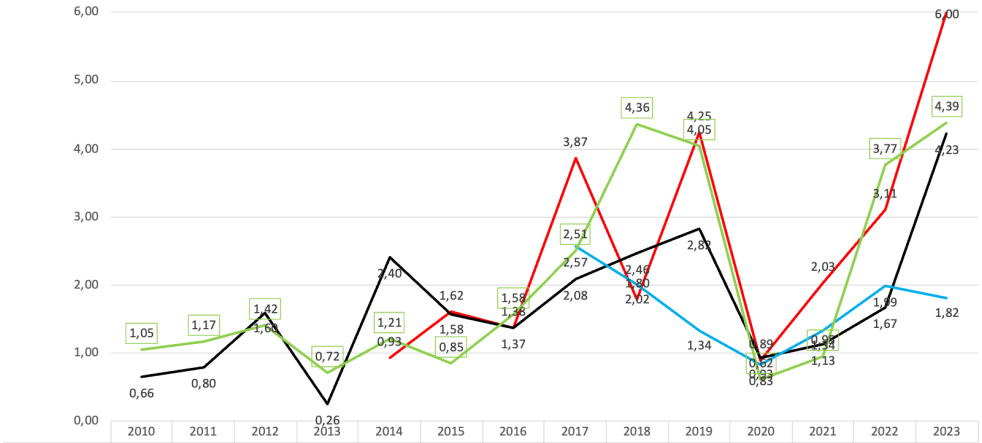
In Spain, the Gregorio Marañón Hospital published a prospective series of 100 episodes of GAS bacteraemia in 1997. At that time, 62% of the cases occurred in patients addicted to injecting drugs and had an origin in skin infections in most cases [48].

In a 2006 publication covering practically the previous decade, the Hospital La Fe in Valencia, Spain, published 42 cases of GAS bacteraemia, amounting to 1.01 cases per 100,000 inhabitants. The origin of the bacteraemia was determined in 38 patients (90.5%), with skin and soft tissue infection being the main foci [49].

Recently, a retrospective study on GAS bacteraemia in 16 hospitals in Madrid in children under 16 years of age included 109 cases, with an incidence rate of 4.3 episodes/100,000 children/year. The incidence was compared between two periods (June 2005 to June 2011 versus July 2011 to July 2017), and a non-significant increase in cases was observed over the study period. Twenty-two percent of cases required admission to the paediatric ICU (PICU) and two of the children died [50].

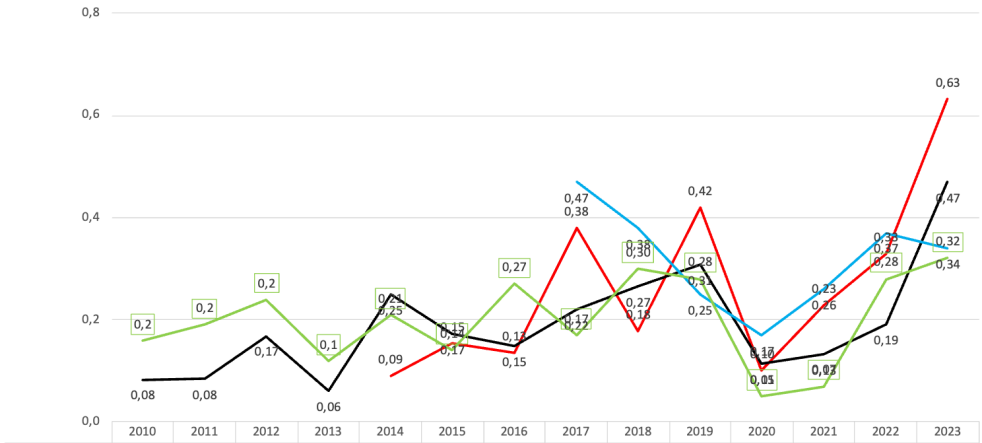
Ramírez de Arellano et al. have described the clinical, microbiological, and molecular characteristics of iGAS infections in children between September 2022 and March 2023 [51] in Spain. Ninety-three cases were included, of which 46 (49.5%) required admission to the PICU. These findings suggest but do not confirm an increase in the incidence of episodes shown in another Spanish study [52].

A high proportion of patients with GAS bacteraemia have some prior underlying disease [53], including malignant



	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Doce Octubre					0,93	1,62	1,38	3,87	1,80	4,25	0,89	2,03	3,11	6,00
La Paz	0,66	0,80	1,60	0,26	2,40	1,58	1,37	2,08	2,46	2,82	0,93	1,13	1,67	4,23
Ramón Cajal								2,57	2,02	1,34	0,83	1,34	1,99	1,82
G. Marañón	1,05	1,17	1,42	0,72	1,21	0,85	1,58	2,51	4,36	4,05	0,62	0,95	3,77	4,39

Figure 3 Evolution of bacteraemia episodes per 100,000 inhabitants of GAS in four large hospitals in Madrid



	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Doce Octubre					0,09	0,15	0,13	0,38	0,18	0,42	0,10	0,23	0,33	0,63
La Paz	0,08	0,08	0,17	0,06	0,25	0,17	0,15	0,22	0,27	0,31	0,11	0,13	0,19	0,47
Ramón Cajal								0,47	0,38	0,25	0,17	0,26	0,37	0,34
G. Marañón	0,2	0,2	0,2	0,1	0,21	0,14	0,27	0,17	0,30	0,28	0,05	0,07	0,28	0,32

Figure 4 Evolution of episodes of GAS bacteraemia per 1000 hospital admissions in 4 large hospitals in Madrid

diseases, diabetes mellitus, chronic obstructive pulmonary disease, congestive heart failure, respiratory viral infections, drug addiction, and immunosuppression from various causes [54,55].

Skin and soft tissue infections are common entry points for GAS bacteraemia, with factors such as advanced age, residence in a nursing home, recent surgery, septic shock, meningitis, pneumonia, and underlying chronic diseases being associated with poor outcomes. GAS bacteraemia has a mortality rate ranging from 5.6% to 32% [56,57].

WHAT DATA ON GAS BACTERAEMIA HAVE BEEN AVAILABLE IN FOUR LARGE HOSPITALS IN MADRID SINCE 2010?

To obtain a clear understanding of the evolution of GAS bacteraemia, four large hospitals in Madrid were asked to provide the number of episodes per 100,000 population and 1,000 hospital admissions in recent years.

Figures 3 and 4 in the accompanying report illustrate the evolution of GAS bacteraemia in these four centres using data

collected from their annual reports. The impact of the COVID-19 pandemic is evident in the data, with a significant reduction in cases across all centres in 2020 and 2021. The incidence of GAS bacteraemia episodes has ranged from 0.7 to 6 episodes per 100,000 population and from 0.08 to 0.63 cases per 1,000 hospital admissions, in those centers.

WHAT METHODS ARE AVAILABLE FOR RAPIDLY DIAGNOSING INVASIVE GAS DISEASE?

The gold standard for diagnosing GAS infection is microbiological culture, particularly in cases of invasive disease. This involves culturing blood and affected tissue samples such as deep tissue for cellulitis, necrotising fasciitis, bone samples for osteomyelitis, joint fluid for arthritis, respiratory samples for pneumonia, pleural fluid for empyema, and cerebrospinal fluid for meningitis. Culturing these samples usually yields high results and is recommended for suspected invasive infections. Blood cultures should be taken regardless of the location of the infection.

Microbiological culture is also crucial for non-invasive infections like streptococcal pharyngitis, with pharyngeal exudate being the most common culture. However, rapid point-of-care techniques have become more common for ruling out GAS pharyngitis and avoiding unnecessary antibiotic use [58-60]. Molecular techniques, such as rapid tests based on the detection of genetic material, have increased the sensitivity of detection. While rapid antigen detection testing (RADT) is licensed for detecting GAS in pharyngeal samples, its off-label use has had varying success in samples from patients with invasive infections. Studies have compared the performance of RADTs, culture, GAS PCR, and 16S rRNA gene PCR assays with a composite gold standard (GAS-PCR assay or culture) for diagnosing severe GAS infection. A total of 192 specimens from deep-tissue sites enriched for 75 GAS-positive samples were enrolled in the study. The three evaluated RADTs showed sensitivities ranging from 88.0% to 94.7% versus 98.7% for GAS PCR, 84% for 16S rRNA gene PCR, and 77.3% for culture. Antigen detection has even been used in surgical procedures to assess the extent of soft tissue necrotising lesions and debridement decision-making [61]. Point-of-care systems based on molecular diagnostic techniques have also been evaluated in non-invasive skin and soft tissue infections with 100% sensitivity and 99.5% specificity compared to culture [62].

Syndromic molecular panels are a new addition to microbiological diagnostics. These panels detect microorganisms associated with a specific type of infection and identify genes linked to resistance mechanisms. Some approved panels target bacteraemia (performed on a positive blood culture) and lower respiratory tract infections, such as community-acquired pneumonia, hospital-acquired pneumonia, and those linked to mechanical ventilation. Some panels even include GAS as a detection target [63]. Although they are suitable for bacteraemia and respiratory infections [64], they have shown promising results when used off-label for conditions like joint infection, empyema, or brain abscesses [65,66]. However, when used in these cases, the panels should be used under control, and the results should be discussed with the clinical decision-makers. It's important to note that a negative result does not necessarily mean the absence of GAS (false negatives). Additionally, there are no case series or multicentre evaluations to determine their sensitivity and specificity in these situations.

WHAT ARE THE MECHANISMS OF ANTIBIOTIC RESISTANCE PRESENT IN GAS?

The latest World Health Organisation (WHO) report of 2024, revising the list of priority pathogens for developing new antimicrobials, introduces GAS. It classifies it as a medium-priority pathogen, essentially because of its resistance to macrolides, especially in low- and middle-income countries [1].

GAS has traditionally been considered universally susceptible to penicillin, although isolates that lose sensitivity to penicillin have been described. Moreover, they may be associated with some therapeutic failure. Initially recognised in Japan, such isolates have been reported from different parts of the world, which, although considered sensitive to penicillin, have somewhat higher minimum inhibitory concentrations (MICs) than most isolates [67-69]. These sporadic strains have mutations in some penicillin-binding proteins (such as PBP2x and PBP1a), sites of action of ß-lactam antibiotics. They also have higher MIC values for ampicillin and cefotaxime in the antibiogram, which can be used for phenotypic recognition [70]. Exceptionally, isolates for which penicillin has a MIC value of 2 mg/L have been isolated in Japan [71]. In Spain, although there is a lack of epidemiological surveillance studies over time to ensure categorically that they are not present in our environment, the studies carried out with invasive isolates of GAS do not demonstrate their presence [51,72].

More worrying is macrolide resistance. Since the first description of erythromycin-resistant GAS in 1968 in the USA, macrolide resistance has been progressively increasing. In some countries, it reaches percentages of up to 40%, being lower in Spain in both upper respiratory tract isolates (10-15%) and invasive isolates (3%) [51,72]. Resistance of GAS to macrolides is due to different mechanisms. The most prevalent is produced by post-transcriptional modification of the target of action by the production of rRNA methylases associated with erm genes. This mechanism can be constitutive or inducible, leading to resistance to macrolides, lincosamides (clindamycin), and streptogramin B (MLSB phenotype). In Spain, it is the most important mechanism and is present in 80% of the resistant isolates. It is also produced by mutations in the 23S rRNA subunit of the ribosome or in ribosomal proteins (L4 and L22) that confer a variable phenotype and mechanisms associated with expulsion pumps (*mef* genes) that determine resistance to macrolides, but susceptibility to clindamycin (phenotype M) [73]. The latter would be present in 25-30% of isolates). Overall, clindamycin resistance would be 8% in non-invasive isolates and 3% in invasive isolates [51,72,73].

Resistance to fluoroquinolones is rare (3-5%) and is due to mutations in the quinolone resistance-determining region

(QRDR) of gyrase (*gyrA* mutations) and topoisomerase IV (*parC* mutations). More than one mutation is required for a significant increase in MICs of fluoroquinolones (ciprofloxacin, levofloxacin, and moxifloxacin) [73]. No resistance mechanisms to fluoroquinolones associated with expulsion pumps have been described in this pathogen.

Finally, GAS resistance to tetracyclines is produced by enzymatic inactivation, ribosomal protection, or efflux mechanisms, the latter being the most prevalent worldwide. In Spain, GAS resistance to tetracycline reported is 12% [72].

WHAT ARE THE RISK FACTORS FOR INVASIVE GAS INFECTION?

Risk factors that increase the likelihood of severe iGAS infection in adults include age, comorbidities, dermatological diseases, history of trauma resulting in hematomas, surgical wounds, immunosuppression, and treatments such as corticosteroids [43,74,75].

The incidence of iGAS is higher in patients with pre-existing chronic conditions such as cancer, diabetes, chronic renal failure, chronic suppurative respiratory disease, and immunodeficiency diseases, particularly HIV infection [76,77].

Previous viral skin diseases, especially varicella and herpes zoster, may lead to impetigo and subsequent development of iGAS [43].

Even blunt trauma leading to small hematomas can result in severe and high-mortality necrotising fasciitis (NF) caused by GAS [78]. The factors contributing to the development of NF without skin breakdown or cellulitis are poorly understood. However, they appear to be related to microorganism-dependent factors, high-pathogenicity strains, and host factors such as low body mass index [78,79]. NF can also occur in connection with abscesses resulting from injections and surgical wounds [43,78]. iGAS associated with injecting drug use has been well-documented [43,80,81].

Other factors linked to an increased risk of iGAS include malnutrition with a low body mass index, NF, smoking, and alcohol consumption [43,74,75].

Additionally, older individuals in institutional care are at a higher risk of iGAS compared to those with similar characteristics in the general community [82], as well as older individuals receiving care from external caregivers [83]. This heightened risk, as reported in England due to its home care organisation, should be considered in our setting, especially as home care aids are becoming increasingly common.

WHAT IS THE CURRENT REALITY OF STREPTOCOCCAL TOXIC SHOCK SYNDROME?

SSTS is a complication of iGAS disease characterised by shock and multi-organ failure. It occurs due to capillary leakage and tissue damage caused by inflammatory cytokines released by streptococcal toxins [84].

Between 8% and 22% of patients with severe GAS infection and 40-50% of patients with NF will develop SSTS. SSTS can occur in all age groups, and most patients with SSTS are not immunosuppressed. Confirmatory diagnosis requires the presence of hypotension, multi-organ dysfunction, and isolation of GAS in usually sterile tissues. The main focus of infection is the vagina, pharyngeal mucosa, skin, and soft tissues. In 45% of patients with SSTS, no clear entry point is identified, and blood cultures are positive in 60-86% of cases.

The main superantigenic exotoxins described in GAS are the streptococcal pyrogenic exotoxins (SpE) A, B, and C, and the streptococcal superantigen A (SSA). The mortality rate of SSTS is high, estimated to be between 14% and 64%, and can exceed 25% within the first 24 hours. It is also associated with high morbidity, requiring admission to the ICU.

Treatment is based on early diagnosis, adequate resuscitation of shock, combined antibiotic treatment with clindamycin and ß-lactams, drainage of the focus of infection, and support of organ dysfunction. Intravenous immunoglobulins (IVIG) may reduce mortality [85]. In a recent meta-analysis, the factors associated with prognosis in SSTS were clindamycin treatment and, within this subgroup, IVIG treatment, albeit with a low level of evidence [86].

Different series in Spain have been published showing an increase in incidence [87]. One of the most significant case series is that of Vall D'Hebron Hospital in Barcelona, which includes 13 patients with iGAS infection and sepsis code criteria admitted to the ICU from November 2022 to March 2023. The study identified three distinct phenotypic profiles: hyperinflammatory with high levels of cytokines and endotoxemia; with low perfusion, the presence of cardiomyopathy (54%), and need for extracorporeal venous, arterial support techniques (38.4%); and hypogammaglobulinemia, which could guide personalised therapeutic approaches [88]. In the paediatric setting, an increase in incidence has also been observed without correlation with an increase in antibiotic resistance or a shift in M-protein types (emm) [51].

In the ISTRE (Infections invasives à Streptocoque du groupe A en Réanimation) study in 37 French ICUs, considering the pre- and post-pandemic period for COVID-19, the case rate and frequency of SSTS was higher in the post-pandemic period (205 vs 949/100,000 ICU admissions and 61% vs 45%), with no increase in ICU mortality (14% vs 22%). Mechanical ventilation was required in 61%, and vasoactive support in 74%. The causes of this increase in incidence are proposed to be more virulent strains, their relationship with respiratory viral infections such as influenza, favouring co-infection and superinfection, or the loss of immunity following the restrictive measures of the pandemic [41].

WHAT IS THE EXTENT OF NECROTISING SKIN AND SOFT TISSUE INFECTION CAUSED BY GAS?

It's important to remember that iGAS infection can cause a condition called necrotising fasciitis (NF). NF is characterised by

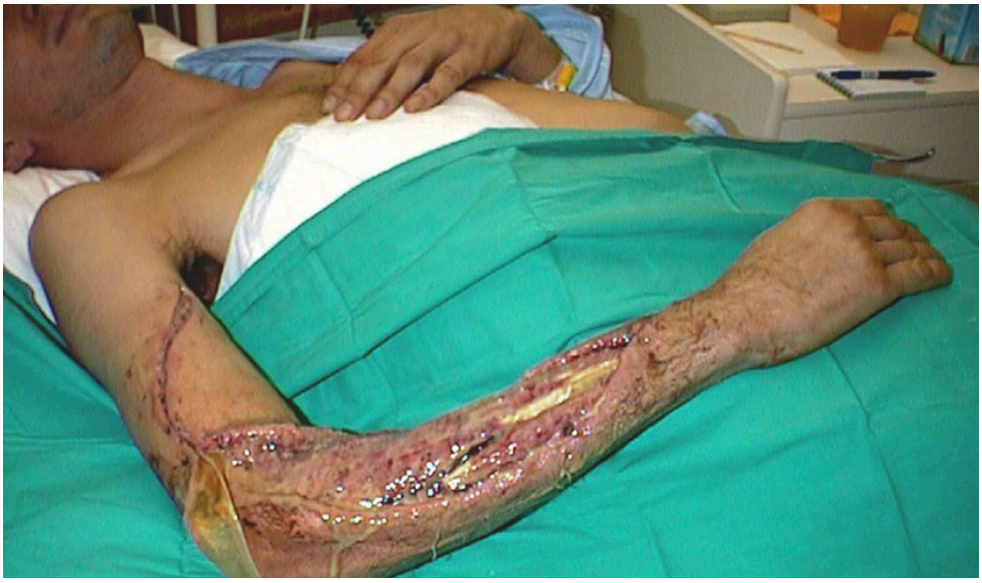


Figure 5 | ***S. pyogenes* necrotising fasciitis**

rapidly progressing soft tissue damage and can lead to sepsis, systemic toxicity, multiple organ failure, and potentially fatal outcomes. The infection rapidly spreads along tissue planes, causing blockages in small blood vessels, tissue necrosis, and affecting multiple tissue layers. Initially, the skin may look normal, but it can become hot, red, and tender after a few days. Early detection is crucial because only prompt surgical removal of affected tissue can reduce the risk of death. However, accurate diagnosis at the time of presentation is only achieved in 15% to 34% of cases. The most commonly affected areas are the extremities (58%), followed by the trunk (26%) and perineum (40%). This also includes specific subgroups like Fournier gangrene, as well as the head and neck, periorbital region, and hands (Figure 5).

The most common clinical signs of necrotising fasciitis (NF) include local inflammation, pain, fever, and symptoms of systemic toxicity, often disproportionate to the original lesion [89-92].

In established NF, the affected fascia is usually not attached to adjacent layers, allowing the surgeon to quickly dissect with the finger along the fascial plane (finger test). If there is a high suspicion of NF, and the imaging results are negative or the necessary means are not available, the finger test can confirm the diagnosis. Local scanning also allows examination of the underlying fat and muscle [90,92]. Although the loss of tissue adherence is a sensitive sign for NF, it may not be present early. It does not apply to all entities within the classification of necrotising soft tissue infections. It is essential to follow the evolution until the symptoms improve, as even if initially no NF is present, there may be an evolution towards NF in the following hours/days.

In 2004, Wong et al. introduced the concept of the LRINEC (Laboratory Risk Indicator for Necrotizing Fasciitis) score based on six analytical parameters as a tool to distinguish NF from other soft tissue infections [93]. In 2021 [94], this scale (m-LRINEC) was modified to include renal disease and diabetes, suggesting a high sensitivity for early diagnosis of NF, but validation in more extensive studies is needed.

WHEN AND WHY SHOULD SURGERY BE PERFORMED?

If necrotizing fasciitis (severe infection, rapid deterioration, crepitus, necrosis, blistering) is present or highly suspected, early surgical debridement is essential. This helps stop the infection from getting worse, reduces tissue loss, lowers the likelihood of amputation, and decreases the risk of death. The initial debridement should be thorough, and samples need to be taken from the edge of the wound for microbiological and histological examination. Even tissue that looks normal can have extensive blood clotting when examined under a microscope. That's why it's important to remove tissue down to where it's well-vascularized and bleeding [95-96].

A second necessary surgical procedure should be scheduled within 24 hours of the initial debridement unless the patient's condition worsens quickly. In that case, the surgical revision should happen sooner. It may be necessary to perform multiple operations, with an average of 3 to 4 debridements.

After removing the dead tissue, the exposed area needs to be treated with negative pressure wound therapy, also known as vacuum-assisted wound closure (VAC). This therapy involves applying continuous or intermittent sub-atmospheric pressure

to a filler substance (such as foam or gauze) on the surface of the wound. VAC therapy helps prepare the exposed tissue for subsequent reconstruction by maintaining a moist and closed environment, controlling excess exudate, reducing the need for frequent dressings, minimizing pain, and promoting the early formation of granulation tissue [97].

Once the tissue is healed, the infection is under control, and no further surgical debridement is needed, reconstruction and coverage of the exposed tissues should be carried out.

HOW IMPORTANT IS GAS AS A CAUSE OF INFECTION IN PAEDIATRICS?

On December 2, 2022, a UK alert reported an unusual increase in the incidence of GAS infections, mainly tonsillitis and scarlet fever, along with a significant number of deaths of children under ten years of age in a short period [98]. Several European countries quickly reported a similar rise in streptococcal infections [99-101], and cases of pneumonia may have been the clinical condition that increased the most during this epidemic outbreak [102].

This increase in incidence had already been observed in the years before the pandemic, as described by other authors [40,102], and also observed in Spain [52].

During the COVID-19 pandemic, viral and bacterial infections were significantly reduced. However, in the winter of 2022-23, a resurgence of infections in children was observed, leading to the concept of "immune debt" [103]. In Spain, cases of GAS infection, including the invasive forms, are not officially reported, so accurate data is not available. Nonetheless, the paediatric network "PedGAS-net," supported by the Spanish Society of Paediatric Infectious Diseases, has been collecting data on invasive infections since 2019 from a network of 51 national public hospitals. Initially, some national publications suggested a return to pre-pandemic normality. However, a group from Madrid later calculated the incidence of paediatric GAS infections seen in the emergency department (ED). In the first half of 2023, the incidence was 22.85 per 1000 ED visits, which is double the rates found in 2022 (10.2 per 1000 visits) and 2019 (12.38 per 1000 visits). Similarly, the rate of invasive infections also increased during this period, nearly doubling from 0.2 per 1000 visits in 2022 and 0.38 per 1000 ED visits in 2019 to 0.58 per 1000 visits in 2023. Analysis by PedGAS-Net showed a significant increase in invasive GAS infections in late 2022 and early 2023, surpassing the frequency and severity observed in the pre-pandemic years [104-105]. This surge in cases, which are often associated with a significant number of pneumonias, has coincided with an increase in cases of respiratory infections caused by RSV and influenza in both the United Kingdom and Spain, often involving virus-bacteria co-infections.

In terms of circulating strains, the same pre-pandemic strains appear to be detected, particularly in invasive infections [106], with a predominance of serotype M1 and a variant M1UK, especially in pneumonia. However, a study conducted in Spain in collaboration with Centro Nacional de Microbiología (CNM), PedGAS-net, and the CIBER de Enfermedades Infecciosas (CIBERINFEC) did not find any evidence of new strains or significant microbiological differences between mild and severe cases that could explain the recent epidemic. It also seems that resistance to GAS to different antibiotics was not the cause of the severe cases. No resistance to penicillin or clindamycin has been identified, which could explain more severe cases of invasive infections, often treated with these antibiotics as an adjuvant [51].

It is also believed that the recognized virus-bacteria co-infection could have been the perfect breeding ground for the emergence of this significant outbreak, which, although it may still be too early to tell, does not seem to be recurring with the same intensity in the current season.

IS GAS A DREADED PATHOGEN IN PREGNANCY AND THE POSTPARTUM PERIOD?

The significance of GAS infection during pregnancy, childbirth, and the postpartum period is primarily due to its potential role in causing puerperal infection, leading to early endometritis shortly after childbirth or cellulitis in the surgical wound. In rare cases, it can progress to sepsis or even more rarely, invasive diseases such as necrotizing fasciitis or streptococcal toxic shock syndrome. Among cases of puerperal sepsis, invasive GAS infection still contributes to at least 45-50% of deaths. Prepartum GAS infection is uncommon, accounting for 7-15% of all pregnancy-related GAS infections, and may also occur in the context of septic abortion. A systematic review of 9 studies in high- and middle-income countries reported that the incidence of invasive GAS infection during pregnancy and the postpartum period was 0.12 per 1000 live births (95% CI 0.11 to 0.14) [107-112].

During childbirth or a caesarean section, GAS can cross the vaginal-skin mucosal barrier, increasing the risk of infection in the postpartum period. Other suggested risk factors for infection include immunosuppression during pregnancy, genetic susceptibility, virulence of the bacterial strains, or the presence of superantigens. GAS colonization in the vagina can occur through contact with carriers of GAS from the throat or skin, respiratory secretions, or contact with skin exudates and infected wounds. Although vaginal GAS identification is rare in the general population (0.03% to 0.37%), hospital isolates of GAS are associated with infections related to pregnancy or the postpartum period. The rupture of membranes can alter vaginal pH and facilitate the growth and ascension of microorganisms. Given the low vaginal colonization by GAS in the general population, it is suggested that the oropharynx is a possible route of entry, and symptomatic maternal pharyngitis is considered a risk factor for pregnancy-related GAS infection.

Maternal infection usually occurs in the first two days after childbirth (0-5 days). However, a significant percentage of women with puerperal sepsis acquire the infection from their other children at home or from other contacts. Symptoms

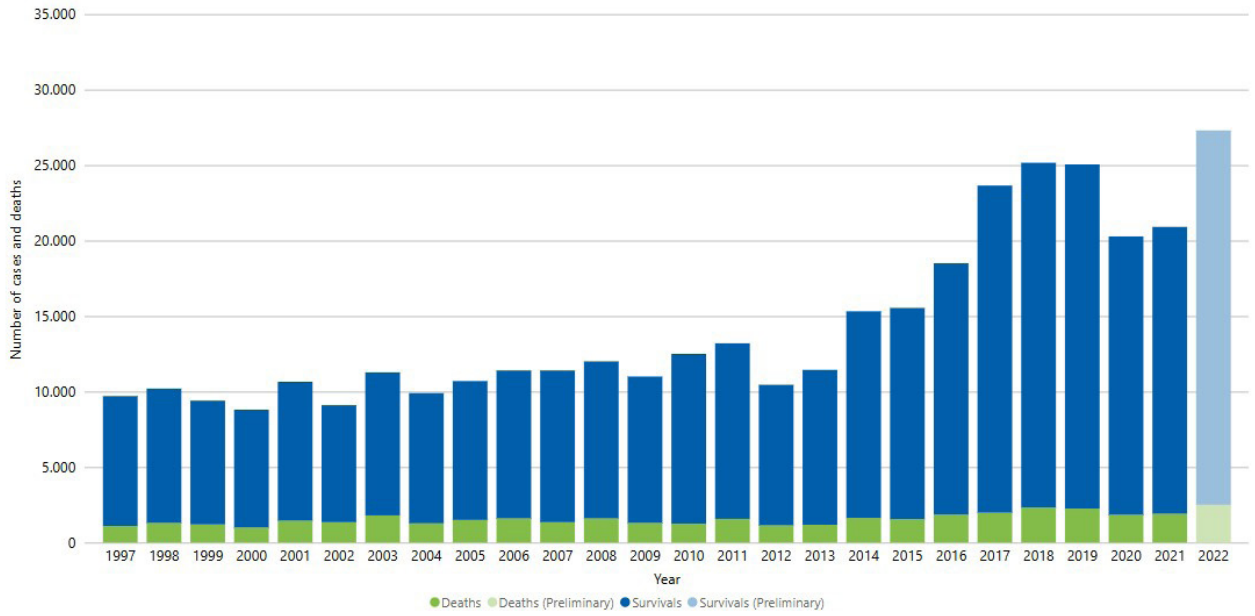


Figure 6 | **Estimated number of cases and deaths due to invasive GAS disease in USA [117,118].**

typically include high fever, abdominal pain, purulent vaginal discharge, uterine tenderness, chills, and gastrointestinal symptoms. Uncommon symptoms such as leukocytosis, tachycardia, and hypotension may indicate a more severe course of infection. Upon examination, inflammation and infection may be found at the episiotomy, perineal tear, or caesarean section wound, and in severe cases, this can progress to necrotizing forms.

To prevent a potentially deadly iGAS infection in pregnant or postpartum women, it's crucial to consider this condition in a patient with suspicious symptoms or disproportionate pain during physical examination. Early diagnosis, antibiotic treatment, and timely surgical debridement if necessary are essential [113].

There are national and international protocols for managing and preventing puerperal sepsis [114]. Prevention measures include proper hygiene practices for the patient, her family, and healthcare workers (such as handwashing and avoiding contact with contaminated items), cleaning and disinfecting wounds, and using appropriate dressings. Standard precautions should be followed in healthcare settings.

WHAT ARE THE MOST RECENT OUTBREAKS OF GAS DISEASE DESCRIBED?

Since the COVID-19 pandemic, there has been a noticeable increase in iGAS disease cases in Spain, other European countries, Asia, Canada, the USA, and Latin America, especially since 2022. This increase could be related to changes in the strains of the bacteria, an overall rise in GAS infections, co-infection with respiratory viruses like influenza and respiratory syncytial virus, and improved surveillance measures. The disease can occur in outbreaks among children sharing school and leisure time or in older adults' homes.

The CNM reported an epidemic of iGAS infections starting in October 2022, which was also observed globally [52]. The initial surge was seen in the emm12 serotype, followed by an increase in the emm1 serotype. This epidemic situation continued for several months in 2023. Furthermore, resistance rates are higher, particularly for tetracycline, which is double the average from 2007 to 2019. The presence of uncommon tetracycline-resistant serotypes like emm81, emm94, emm102, emm118, emm119, and emm183, along with the low incidence of usually susceptible emm1, emm3, and emm89, could explain the increase in resistance rates [52].

In Europe, there has been a reported increase in the number of iGAS cases among children under ten in countries such as Ireland, France, the Netherlands, Sweden, and the United Kingdom since September 2022 [1,115]. Portugal [56] and Denmark [116] have also subsequently reported increased cases. Meanwhile, in the US, there were more cases and deaths from invasive disease in 2022 than during the COVID-19 pandemic [117]; however, the 2022 figures were quite similar to 2018 and 2019 (Figure 6). Preliminary results from 2023 indicate that the number of cases of invasive disease is the highest in 20 years [118].

Public health authorities recommend improved surveillance of the problem, increased vaccination coverage against viruses (influenza, syncytial virus) that cause co-infection to

reduce severity, increased hand and respiratory hygiene to reduce transmission, and early diagnosis and treatment of cases to improve prognosis [115,118].

WHAT IS THE CURRENT MEDICAL TREATMENT OF INVASIVE GAS INFECTION?

Treating iGAS infection involves collaboration between infectious disease specialists, microbiologists, intensivists, and surgeons. This usually requires a multidisciplinary approach based on the clinical condition of the patient. In this section, we will focus only on medical treatment and disregard surgical treatment and the management of septic shock, which have already been addressed.

Empirical antibiotics should be administered immediately while awaiting confirmatory results. Generally, the antimicrobial regimen includes a ß-lactam agent (which inhibits cell wall synthesis) in combination with clindamycin or other agents that inhibit protein synthesis.

It is recommended to use a combination of agents with different targets because using ß-lactam alone has been linked to higher morbidity and mortality, particularly in severe invasive infections [119-121]. Research indicates that penicillin monotherapy is ineffective when the bacterial inoculum is high and growth rates decrease [122]. In such cases, the availability of PBPs to bind ß-lactams may decrease [123]. Clindamycin, on the other hand, remains effective regardless of inoculum size or growth stage, inhibits bacterial toxin production, and has a more prolonged post-antibiotic effect compared to ß-lactams [124].

Observational studies have shown that adding clindamycin to ß-lactams is associated with lower mortality, even in patients without shock and necrotizing fasciitis [125].

The recommended dose of clindamycin for adults is 900 mg IV every eight hours. For children, the recommended dosage is 30-40 mg/kg IV per day, administered every six to eight hours.

In cases where patients have clindamycin-resistant isolates, linezolid can be used as an alternative, as resistance to oxazolidinones is very low [126-128].

For patients with hypersusceptibility to ß-lactams and a history of anaphylaxis, vancomycin or other glycolipopeptides may be used.

Combination therapy with penicillin and clindamycin should be continued until clinical and hemodynamic stability is achieved, typically for 48-72 hours. After this period, penicillin monotherapy can be considered.

Combination therapy with penicillin G and clindamycin is recommended for the initial treatment of GAS bacteraemia or pneumonia in the absence of shock, organ failure, or necrotising infection. However, in these circumstances, penicillin G monotherapy is a reasonable alternative.

For patients with bacteraemia and those with complicated deep infections, it is advisable to continue treatment for at least 14 days, and sometimes longer.

Non-antibiotic treatments for SSTS (streptococcal toxic shock syndrome) include intravenous immunoglobulin (IVIG), hyperbaric oxygen, and anti-tumor necrosis factor (TNF) antibodies.

In patients with invasive SSTS, intravenous immunoglobulins (IVIG) are recommended for treating patients at a dosage of 1 g/kg on the first day, followed by 0.5 g/kg over the next 2-3 days. A meta-analysis conducted by Parks et al. [85] in 2018, which included five studies of patients with SSTS, concluded that the use of IVIG was associated with a reduction in 30-day mortality (33.7% to 15.7%). However, subsequent studies do not confirm the efficacy of IVIG [129,130]. A Spanish study group also found no confirmation of IVIG's efficacy in patients with GAS bacteraemia requiring ICU admission. This retrospective multicenter study, conducted in nine ICUs in southern Spain, included 57 patients, and it was observed that clindamycin but not IVIG behaved as a protective factor for mortality [130].

The use of hyperbaric oxygen has been proposed for a small number of patients with SSTS [131,132], but no controlled trials have been conducted to affirm the efficacy of this treatment.

Since TNF levels are elevated in patients with SSTS [133], the use of TNF blockers has been occasionally studied in experimental animals [134,135], but no clinical data justifies their use in humans.

HOW CAN GAS INFECTION BE PREVENTED THROUGH VACCINES?

Preventing and controlling GAS infection requires a comprehensive approach [136]. This approach includes promoting hygiene, educating the community, promptly diagnosing and treating infections, and implementing specific strategies to manage outbreaks [83,137]. Health professionals, educators, and the community need to collaborate to reduce the impact of these infections and prevent serious complications. The following preventive measures are highlighted [136, 38]:

Educational campaigns should emphasize training programs, particularly for high-risk populations such as children, the elderly, and institutionalized individuals. The focus should be on promoting handwashing, especially after coughing or sneezing, before meals, or after using the toilet. Additionally, individuals should be encouraged to cover their nose and mouth with their elbow when coughing or sneezing, or to use a disposable handkerchief, while following mask recommendations for respiratory diseases.

It's important to maintain good control of indoor environments by disinfecting surfaces and ensuring proper ventilation to reduce the spread of respiratory droplets. To control outbreaks, it's important to use rapid antigen detection tests and throat cultures for early diagnosis. Infected individuals should

be temporarily isolated and should avoid close contact with others until at least 24 hours after starting antibiotic treatment. Surveillance systems should be implemented to detect and monitor cases of GAS infections. Close contacts of confirmed cases should be identified and assessed, and prophylactic treatment should be provided when necessary.

Regarding vaccination, there is currently no commercially available vaccine against GAS, but research in this area is a major focus.

The development of an effective and safe vaccine against GAS presents several challenges due to the genetic diversity of the pathogen, potential autoimmune epitopes, and issues with animal models [136]. Currently, only four candidates have progressed to early clinical trials [136,138,139]. These candidates primarily target the M protein of GAS, excluding autoepitopes and utilizing N-terminal fragments from different serotypes. For instance, StreptAnova® has demonstrated immunogenicity and good tolerance in initial trials, while MJ8CombiVax® has been reformulated to include epitopes that protect against highly virulent variants. Other vaccines are based on non-M proteins, with examples from GlaxoSmithKline® and Vaxcyte® showing efficacy in animal models.

Recent efforts led by the WHO, which have recognized GAS vaccine research and development as a global priority, are resulting in significant progress in vaccine formulation and delivery, including the potential use of microarray patches. However, greater investment is necessary in the formulation and delivery of vaccines, along with coordinated efforts to achieve comprehensive global vaccine coverage and substantially reduce the disease burden caused by GAS [140].

FUNDING

Nothing to declare

CONFLICTS OF INTEREST

The authors declare the absence of conflicts of interest.

REFERENCES

1. Cunningham C, Fisher L, Wood C, et al. Incidence and treatment of group A streptococcal infections during covid-19 pandemic and 2022 outbreak: retrospective cohort study in England using OpenSAFELY-TPP. BMJ Med 2024; 3:e000791.
2. Alamiri F, Andre O, De S, et al. Role of serotype and virulence determinants of *Streptococcus pyogenes* biofilm bacteria in internalization and persistence in epithelial cells in vitro. Front Cell Infect Microbiol 2023; 13:1146431.
3. Bogiel T, Domian A, Dobrzyńska Z, et al. Assessment of the Relationship between Clinical Manifestation and Pathogenic Potential of *Streptococcus pyogenes* Strains-Distribution of Genes and Genotypes of Toxins. Biomedicines 2022; 10(4):799.
4. Kozińska A, Sitkiewicz I. Detection of *Streptococcus pyogenes* Virulence Factors. Methods Mol Biol 2020; 2136:3-16.
5. Hurst JR, Shannon BA, Craig HC, et al. The *Streptococcus pyogenes* hyaluronic acid capsule promotes experimental nasal and skin infection by preventing neutrophil-mediated clearance. PLoS Pathog 2022; 18:e1011013.
6. González-Abad MJ, Alonso Sanz M. [Invasive *Streptococcus pyogenes* infections (2011-2018): EMM-type and clinical presentation]. An Pediatr (Engl Ed) 2020; 92:351-8.
7. Langshaw EL, Reynolds S, Ozberk V, et al. Streptolysin O Deficiency in *Streptococcus pyogenes* M1T1 covR/S Mutant Strain Attenuates Virulence in In Vitro and In Vivo Infection Models. mBio 2023; 14:e0348822.
8. Shannon BA, Hurst JR, Flannagan RS, et al. Streptolysin S is required for *Streptococcus pyogenes* nasopharyngeal and skin infection in HLA-transgenic mice. PLoS Pathog 2024; 20:e1012072.
9. Veselá R, Vohrnová S, Kozáková J. Detection of superantigens in *Streptococcus pyogenes* isolates based on whole genome sequencing data. Epidemiol Mikrobiol Imunol 2023; 72:191-4.
10. Walker MJ, Barnett TC, McArthur JD, et al. Disease manifestations and pathogenic mechanisms of Group A *Streptococcus*. Clin Microbiol Rev 2014; 27:264-301.
11. Miller KM, Carapetis JR, Van Beneden CA, et al. The global burden of sore throat and group A *Streptococcus* pharyngitis: A systematic review and meta-analysis. EClinicalMedicine 2022; 48:101458.
12. Cannon JW, Zhung J, Bennett J, et al. The economic and health burdens of diseases caused by group A *Streptococcus* in New Zealand. Int J Infect Dis 2021; 103:176-81
13. Allaw F, Wehbe S, Kanj SS. Necrotizing fasciitis: an update on epidemiology, diagnostic methods, and treatment. Curr Opin Infect Dis 2024; 37:105-11.
14. Turner CE, Pyzio M, Song B, et al. Scarlet Fever Upsurge in England and Molecular-Genetic Analysis in North-West London, 2014. Emerg Infect Dis 2016; 22:1075-8.
15. Dougherty S, Okello E, Mwangi J, Kumar RK. Rheumatic Heart Disease: JACC Focus Seminar 2/4. J Am Coll Cardiol 2023; 81:81-94.
16. Tyrrell GJ, Bell C, Bill L, Fathima S. Increasing Incidence of Invasive Group A *Streptococcus* Disease in First Nations Population, Alberta, Canada, 2003-2017. Emerg Infect Dis 2021; 27:443-51.
17. Jones TD. The diagnosis of rheumatic fever. JAMA 1944; 126.:481–4. .
18. Gewitz MH, Baltimore RS, Tani LY, et al. Revision of the Jones Criteria for the diagnosis of acute rheumatic fever in the era of Doppler echocardiography: a scientific statement from the American Heart Association. Circulation 2015; 131:1806-18.
19. Yokchoo N, Patanarapeelert N, Patanarapeelert K. The effect of group A streptococcal carrier on the epidemic model of acute rheumatic fever. Theor Biol Med Model 2019; 16:14.
20. Hajar R. Rheumatic Fever and Rheumatic Heart Disease a Historical Perspective. Heart Views 2016; 17:120-6.
21. Watkins DA, Johnson CO, Colquhoun SM, et al. Global, Regional, and National Burden of Rheumatic Heart Disease, 1990-2015. N Engl J Med 2017; 377:713-22.

22. Jaine R, Baker M, Venugopal K. Acute rheumatic fever associated with household crowding in a developed country. Pediatr Infect Dis J 2011; 30:315-9.

23. de Crombrugghe G, Baroux N, Botteaux A, et al. The Limitations of the Rheumatogenic Concept for Group A *Streptococcus*: Systematic Review and Genetic Analysis. Clin Infect Dis 2020; 70:1453-60.

24. Alm PA. Streptococcal Infection as a Major Historical Cause of Stuttering: Data, Mechanisms, and Current Importance. Front Hum Neurosci 2020; 14:569519.

25. Ou Z, Yu D, Liang Y, et al. Global burden of rheumatic heart disease: trends from 1990 to 2019. Arthritis Res Ther 2022; 24:138.

26. Ghamari SH, Abbasi-Kangevari M, Saeedi Moghaddam S, et al. Rheumatic Heart Disease Is a Neglected Disease Relative to Its Burden Worldwide: Findings From Global Burden of Disease 2019. J Am Heart Assoc 2022; 11:e025284.

27. Jaine R, Baker M, Venugopal K. Epidemiology of acute rheumatic fever in New Zealand 1996-2005. J Paediatr Child Health 2008; 44:564-71.

28. Veasy LG, Wiedmeier SE, Orsmond GS, et al. Resurgence of acute rheumatic fever in the intermountain area of the United States. N Engl J Med 1987; 316:421-7.

29. Munteanu V, Petaccia A, Contecaru N, et al. Paediatric acute rheumatic fever in developed countries: Neglected or negligible disease? Results from an observational study in Lombardy (Italy). AIMS Public Health 2018; 5:135-43.

30. Kočevar U, Toplak N, Kosmač B, et al. Acute rheumatic fever outbreak in southern central European country. Eur J Pediatr 2017; 176:23-9.

31. Cortina Greus P, Alfonso Sánchez JL, Cortés Vizcaíno C, et al. [Epidemiological course of rheumatic fever and rheumatic heart disease in Spain (1951-1986)]. Rev Sanid Hig Publica (Madr) 1991; 65:17-24.

32. Feinstein AR, Spagnuolo M. The clinical patterns of acute rheumatic fever: a reapraisal. Medicine (Baltimore) 1962; 41:279-305.

33. Noonan S, Zurynski YA, Currie BJ, et al. A national prospective surveillance study of acute rheumatic fever in Australian children. Pediatr Infect Dis J 2013; 32:e26-32.

34. Nelson GE, Pondo T, Toews KA, et al. Epidemiology of Invasive Group A Streptococcal Infections in the United States, 2005-2012. Clin Infect Dis 2016; 63:478-86.

35. Dunne EM, Hutton S, Peterson E, et al. Increasing Incidence of Invasive Group A *Streptococcus* Disease, Idaho, USA, 2008-2019. Emerg Infect Dis 2022; 28:1785-95.

36. Tyrrell GJ, Fathima S, Kakulphimp J, Bell C. Increasing Rates of Invasive Group A Streptococcal Disease in Alberta, Canada; 2003-2017. Open Forum Infect Dis 2018; 5:ofy177.

37. Raymond MJ, Wolfe TR, Smith LM. Notes from the Field: Group A *Streptococcus* Bacteremia in Persons Who Inject Drugs - Northern Vermont, January 2020-October 2023. MMWR Morb Mortal Wkly Rep 2024; 73:382-4.

38. Vilhonen J, Vuopio J, Vahlberg T, et al. Group A streptococcal bacteremias in Southwest Finland 2007-2018: epidemiology and role of infectious diseases consultation in antibiotic treatment selection. Eur J Clin Microbiol Infect Dis 2020; 39:1339-48.

39. Guy R, Henderson KL, Coelho J, et al. Increase in invasive group A streptococcal infection notifications, England, 2022. Euro Surveill 2023; 28.

40. van Kempen EB, Bruijning-Verhagen PCJ, Borensztajn D, et al. Increase in Invasive Group a Streptococcal Infections in Children in the Netherlands, A Survey Among 7 Hospitals in 2022. Pediatr Infect Dis J 2023; 42:e122-e4.

41. Orieux A, Prevel R, Dumery M, et al. Invasive group A streptococcal infections requiring admission to ICU: a nationwide, multicenter, retrospective study (ISTRE study). Crit Care 2024; 28:4.

42. Lassoued Y, Assad Z, Ouldali N, et al. Unexpected Increase in Invasive Group A Streptococcal Infections in Children After Respiratory Viruses Outbreak in France: A 15-Year Time-Series Analysis. Open Forum Infect Dis 2023; 10:ofad188.

43. Zangarini L, Martiny D, Miendje Deyi VY, et al. Incidence and clinical and microbiological features of invasive and probable invasive streptococcal group A infections in children and adults in the Brussels-Capital Region, 2005-2020. Eur J Clin Microbiol Infect Dis 2023; 42:555-67.

44. Nygaard U, Hartling UB, Munkstrup C, et al. Invasive group A streptococcal infections in children and adolescents in Denmark during 2022-23 compared with 2016-17 to 2021-22: a nationwide, multicentre, population-based cohort study. Lancet Child Adolesc Health 2024; 8:112-21.

45. Abo YN, Oliver J, McMinn A, et al. Increase in invasive group A streptococcal disease among Australian children coinciding with northern hemisphere surges. Lancet Reg Health West Pac 2023; 41:100873.

46. Gouveia C, Bajanca-Lavado MP, Mamede R, et al. Sustained increase of paediatric invasive *Streptococcus pyogenes* infections dominated by M1(UK) and diverse emm12 isolates, Portugal, September 2022 to May 2023. Euro Surveill 2023; 28.

47. Nwosu A, Schut A, Wood CA, et al. Invasive group A streptococcal (iGAS) surveillance in Island Health, British Columbia, 2022. Can Commun Dis Rep 2023; 49:342-50.

48. Bernaldo de Quirós JC, Moreno S, Cercenado E, et al. Group A streptococcal bacteremia. A 10-year prospective study. Medicine (Baltimore) 1997; 76:238-48.

49. Vallalta Morales M, Soriano Navarro CJ, Salavert Lletí M, et al. Group A streptococcal bacteremia: outcome and prognostic factors. Rev Esp Quimioter 2006; 19:367-75.

50. Cobo-Vázquez E, Aguilera-Alonso D, Carbayo T, et al. Epidemiology and clinical features of *Streptococcus pyogenes* bloodstream infections in children in Madrid, Spain. Eur J Pediatr 2023; 182:3057-62.

51. Ramírez de Arellano E, Saavedra-Lozano J, Villalón P, et al. Clinical, microbiological, and molecular characterization of pediatric invasive infections by *Streptococcus pyogenes* in Spain in a context of global outbreak. mSphere 2024; 9:e0072923.

52. Villalón P, Medina-Pascual MJ, Garrido N M, et al. Enfermedad invasiva por *Streptococcus pyogenes* o estreptococo del grupo A. In: En: Echevarria Mayo J E OIJ, Jado Garcia I. (editores). editor. Progra-

mas de Vigilancia Microbiológica Centro Nacional de Microbiología Volumen 2 2021-2022 Majadahonda (Madrid); Instituto de Salud Carlos III, Centro Nacional de Microbiología. Available at: https://www.isciii.es/QuienesSomos/CentrosPropios/CNM/PublishingImages/Paginas/default/PV_CNM_2022_V2.pdf

53. Nguyen ADK, Smith S, Davis TJ, et al. The efficacy and safety of a shortened duration of antimicrobial therapy for group A *Streptococcus* bacteremia. Int J Infect Dis 2023; 128:11-9.
54. Linder KA, Alkhouli L, Ramesh M, et al. Effect of underlying immune compromise on the manifestations and outcomes of group A streptococcal bacteremia. J Infect 2017; 74:450-5.
55. Ho EC, Cataldi JR, Silveira LJ, et al. Outbreak of Invasive Group A *Streptococcus* in Children-Colorado, October 2022-April 2023. J Pediatric Infect Dis Soc 2023; 12:540-8.
56. Huang YC, Huang YC, Chiu CH, et al. Characteristics of group A streptococcal bacteremia with comparison between children and adults. J Microbiol Immunol Infect 2001; 34:195-200.
57. Livni G, Ashkenazi S, Leibovici L, et al. Incidence and severity of group A streptococcal bacteremia in a medical center in Israel, 1981-1994. Scand J Infect Dis 1996; 28:139-42.
58. Cohen JF, Tanz RR, Shulman ST. Group A *Streptococcus* pharyngitis in Children: New Perspectives on Rapid Diagnostic Testing and Antimicrobial Stewardship. J Pediatric Infect Dis Soc 2024; 13:250-6.
59. Dubois C, Smeesters PR, Refes Y, et al. Diagnostic accuracy of rapid nucleic acid tests for group A streptococcal pharyngitis: systematic review and meta-analysis. Clin Microbiol Infect 2021; 27:1736-45.
60. Gazzano V, Berger A, Benito Y, et al. Reassessment of the Role of Rapid Antigen Detection Tests in Diagnosis of Invasive Group A Streptococcal Infections. J Clin Microbiol 2016; 54:994-9.
61. Tsuge I, Matsui M, Takeda T, et al. Less-invasive Treatment for Group A Streptococcal Fasciitis with Rapid Antigen Detection Test and Collagen/Gelatin Sponge. Plast Reconstr Surg Glob Open 2021; 9:e3793.
62. Close RM, Sutcliffe CG, Galdun P, et al. Point-of-care molecular diagnostics for the detection of group A *Streptococcus* in non-invasive skin and soft tissue infections: a validation study. Diagn Microbiol Infect Dis 2022; 103:115729.
63. Miller JM, Binnicker MJ, Campbell S, et al. Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2024 Update by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM). Clin Infect Dis 2024:ciae104.
64. Bagge K, Pedersen L, Lisby JG. Streptococcal toxic shock syndrome in a patient with community-acquired pneumonia. Impact of rapid diagnostics on patient management. Access Microbiol 2020; 2:acmi000144.
65. Michos A, Palili A, Koutouzis EI, et al. Detection of bacterial pathogens in synovial and pleural fluid with the FilmArray Blood Culture Identification System. IDCases 2016; 5:27-8.
66. Escudero D, Forcelledo L, Leoz B, et al. [Utility of multiplex PCR (FilmArray Blood Culture Identification) in other biological liquids. Detection of *Streptococcus pyogenes* in brain abscess and synovial fluid]. Rev Esp Quimioter 2019; 32:194-7.
67. Beres SB, Zhu L, Pruitt L, et al. Integrative Reverse Genetic Analysis Identifies Polymorphisms Contributing to Decreased Antimicrobial Agent Susceptibility in *Streptococcus pyogenes*. mBio 2022; 13:e0361821.
68. Yu D, Zheng Y, Yang Y. Is There Emergence of β-Lactam Antibiotic-Resistant *Streptococcus pyogenes* in China? Infect Drug Resist 2020; 13:2323-7.
69. Kebede D, Admas A, Mekonnen D. Prevalence and antibiotics susceptibility profiles of *Streptococcus pyogenes* among pediatric patients with acute pharyngitis at Felege Hiwot Comprehensive Specialized Hospital, Northwest Ethiopia. BMC Microbiol 2021; 21:135.
70. Vannice KS, Ricaldi J, Nanduri S, et al. *Streptococcus pyogenes* pbp2x Mutation Confers Reduced Susceptibility to β-Lactam Antibiotics. Clin Infect Dis 2020; 71:201-4.
71. Yu D, Guo D, Zheng Y, Yang Y. A review of penicillin binding protein and group A *Streptococcus* with reduced-β-lactam susceptibility. Front Cell Infect Microbiol 2023; 13:1117160.
72. Villalón P, Bárcena M, Medina-Pascual MJ, et al. National Surveillance of Tetracycline, Erythromycin, and Clindamycin Resistance in Invasive *Streptococcus pyogenes*: A Retrospective Study of the Situation in Spain, 2007-2020. Antibiotics (Basel) 2023; 12.
73. Cattoir V. Mechanisms of *Streptococcus pyogenes* Antibiotic Resistance. In: Ferretti JJ, Stevens DL, Fischetti VA, editors.: Basic Biology to Clinical Manifestations. Oklahoma City (OK): University of Oklahoma Health Sciences Center. The University of Oklahoma Health Sciences Center 2022.
74. Factor SH, Levine OS, Schwartz B, et al. Invasive group A streptococcal disease: risk factors for adults. Emerg Infect Dis 2003; 9:970-7.
75. Bartolomé-Álvarez J, Galán-Ros J, Ferrer-Amate F, Escribano Garaizábal E. [Community-acquired invasive soft tissue infections due to *Streptococcus pyogenes*]. Enferm Infecc Microbiol Clin 2014; 32:691-2.
76. Davies HD, McGeer A, Schwartz B, et al. Invasive group A streptococcal infections in Ontario, Canada. Ontario Group A Streptococcal Study Group. N Engl J Med 1996; 335:547-54.
77. Langley G, Hao Y, Pondo T, et al. The Impact of Obesity and Diabetes on the Risk of Disease and Death due to Invasive Group A *Streptococcus* Infections in Adults. Clin Infect Dis 2016; 62:845-52.
78. Nuwayhid ZB, Aronoff DM, Mulla ZD. Blunt trauma as a risk factor for group A streptococcal necrotizing fasciitis. Ann Epidemiol 2007; 17:878-81.
79. Stevens DL, Bryant AE. Necrotizing Soft-Tissue Infections. N Engl J Med 2017; 377:2253-65.
80. Bruun T, Rath E, Madsen MB, et al. Risk Factors and Predictors of Mortality in Streptococcal Necrotizing Soft-tissue Infections: A Multicenter Prospective Study. Clin Infect Dis 2021; 72:293-300.
81. Kwiatkowska RM, Manley P, Sims B, et al. Outbreak of group A *Streptococcus* emm94.0 affecting people who inject drugs in southwest England, April 2017. Am J Infect Control 2018; 46:238-40.
82. Saavedra-Campos M, Simone B, Balasegaram S, et al. Estimating the risk of invasive group A *Streptococcus* infection in care home residents in England, 2009-2010. Epidemiol Infect 2017; 145:2759-65.

83. Nabarro LE, Brown CS, Balasegaram S, et al. Invasive Group A *Streptococcus* Outbreaks Associated with Home Healthcare, England, 2018-2019. Emerg Infect Dis 2022; 28:915-23.
84. Atchade E, De Tymowski C, Grall N, et al. Toxic Shock Syndrome: A Literature Review. Antibiotics (Basel) 2024; 13.
85. Parks T, Wilson C, Curtis N, et al. Polyspecific Intravenous Immunoglobulin in Clindamycin-treated Patients With Streptococcal Toxic Shock Syndrome: A Systematic Review and Meta-analysis. Clin Infect Dis 2018; 67:1434-6.
86. Bartoszko JJ, Elias Z, Rudziak P, et al. Prognostic factors for streptococcal toxic shock syndrome: systematic review and meta-analysis. BMJ Open 2022; 12:e063023.
87. Talavera M, Martínez A, Vicent C, et al. Four cases of unexpected severe community-acquired pneumonia aetiology: Group A *Streptococcus pyogenes* disruption. Med Intensiva (Engl Ed) 2023; 47:475-7.
88. Ruiz-Rodríguez JC, Chiscano-Camón L, Maldonado C, et al. Catastrophic *Streptococcus pyogenes* Disease: A Personalized Approach Based on Phenotypes and Treatable Traits. Antibiotics (Basel) 2024; 13.
89. Salati SA. Necrotizing fasciitis a review. Pol Przegl Chir 2022; 95:1-8.
90. Sartelli M, Guirao X, Hardcastle TC, et al. 2018 WSES/SIS-E consensus conference: recommendations for the management of skin and soft-tissue infections. World J Emerg Surg 2018; 13:58.
91. Glass GE, Sheil F, Ruston JC, Butler PE. Necrotising soft tissue infection in a UK metropolitan population. Ann R Coll Surg Engl 2015; 97:46-51.
92. Oruç E, Turunç T, Beyaz S, et al. Necrotizing Fasciitis: Evaluation of 85 Cases and Usage of LRINEC Score. Infect Dis Clin Microbiol 2022; 4:81-6.
93. Wong CH, Khin LW, Heng KS, et al. The LRINEC (Laboratory Risk Indicator for Necrotizing Fasciitis) score: a tool for distinguishing necrotizing fasciitis from other soft tissue infections. Crit Care Med 2004; 32:1535-41.
94. Wu H, Liu S, Li C, Song Z. Modified Laboratory Risk Indicator for Necrotizing Fasciitis (m-LRINEC) Score System in Diagnosing Necrotizing Fasciitis: A Nested Case-Control Study. Infect Drug Resist 2021; 14:2105-12.
95. Carlin PS, Botella AG, Valladares LD, et al. Necrotizing fasciitis caused by *Streptococcus pyogenes* in immunocompetent patient. Surg Infect (Larchmt) 2014; 15:859-60.
96. Hakkarainen TW, Kopari NM, Pham TN, Evans HL. Necrotizing soft tissue infections: review and current concepts in treatment, systems of care, and outcomes. Curr Probl Surg 2014; 51:344-62.
97. Malmsjö M, Gustafsson L, Lindstedt S, et al. The effects of variable, intermittent, and continuous negative pressure wound therapy, using foam or gauze, on wound contraction, granulation tissue formation, and ingrowth into the wound filler. Eplasty 2012; 12:e5.
98. Alcolea-Medina A, Snell LB, Alder C, et al. The ongoing *Streptococcus pyogenes* (Group A *Streptococcus*) outbreak in London, United Kingdom, in December 2022: a molecular epidemiology study. Clin Microbiol Infect 2023; 29:887-90.
99. Villalón P, Sáez-Nieto JA, Rubio-López V, et al. Invasive *Streptococcus pyogenes* disease in Spain: a microbiological and epidemiological study covering the period 2007-2019. Eur J Clin Microbiol Infect Dis 2021; 40:2295-303.
100. UK Health Security Agency, UKHSA. Update on scarlet fever and invasive group A strep. Latest data from the UK Health Security Agency (UKHSA) on scarlet fever and invasive group A strep cases. From: UK Health Security Agency. Published 2 December 2022. Last updated 15 December 2022. Available at: https://www.gov.uk/government/news/ukhsa-update-on-scarlet-fever-and-invasive-group-a-strep-1
101. Ladhani SN, Guy R, Bhopal SS, et al. Paediatric group A streptococcal disease in England from October to December, 2022. Lancet Child Adolesc Health 2023; 7:e2-e4.
102. Holdstock V, Twynam-Perkins J, Bradnock T, et al. National case series of group A *Streptococcus* pleural empyema in children: clinical and microbiological features. Lancet Infect Dis 2023; 23:154-6.
103. Calvo C. Changes in the epidemiology of infections in children. Is there an immune debt? Only for respiratory viruses? An Pediatr (Engl Ed) 2023; 98:155-6.
104. de Ceano-Vivas M, Molina Gutiérrez M, Mellado-Sola I, et al. *Streptococcus pyogenes* infections in Spanish children before and after the COVID pandemic. Coming back to the previous incidence. Enferm Infecc Microbiol Clin (Engl Ed) 2024; 42:88-92.
105. de Ceano-Vivas M, Molina Gutiérrez M, López López R, Calvo C. Comments to the article "*Streptococcus pyogenes* infections in Spanish children before and after the COVID pandemic. Coming back to the previous incidence". Enferm Infecc Microbiol Clin (Engl Ed) 2024; 42:114-5.
106. Zhi X, Li HK, Li H, et al. Emerging Invasive Group A *Streptococcus* M1(UK) Lineage Detected by Allele-Specific PCR, England, 2020(1). Emerg Infect Dis 2023; 29:1007-10.
107. Hasegawa J, Ikeda T, Sekizawa A, et al. Recommendations for saving mothers' lives in Japan: Report from the Maternal Death Exploratory Committee (2010-2014). J Obstet Gynaecol Res 2016; 42:1637-43.
108. Instituto de Salud Carlos III. Enfermedad invasiva por *Streptococcus* del Grupo A en España. Sistema de Información Microbiológica. Años 1995-2008. Boletín Epidemiológico Semanal 2009; 17:253–6. https://revista.isciii.es/index.php/bes/article/view/141/140
109. Tanaka H, Katsuragi S, Hasegawa J, et al. The most common causative bacteria in maternal sepsis-related deaths in Japan were group A *Streptococcus*: A nationwide survey. J Infect Chemother 2019; 25:41-4.
110. Harris K, Proctor LK, Shinar S, et al. Outcomes and management of pregnancy and puerperal group A streptococcal infections: A systematic review. Acta Obstet Gynecol Scand 2023; 102:138-57.
111. Gustafson LW, Blaakær J, Helmig RB. Group A streptococci infection. A systematic clinical review exemplified by cases from an obstetric department. Eur J Obstet Gynecol Reprod Biol 2017; 215:33-40.
112. Upton A, Taylor S. Observational study of *Streptococcus pyogenes* isolated from vaginal swabs of adult women in a hospital and community laboratory. Pathology 2013; 45:678-80.

113. Hamilton SM, Stevens DL, Bryant AE. Pregnancy-related group a streptococcal infections: temporal relationships between bacterial acquisition, infection onset, clinical findings, and outcome. Clin Infect Dis 2013; 57:870-6.
114. UK Health Security Agency. UK guidelines for the management of contacts of invasive group A *Streptococcus* (iGAS) infection in community settings. Version 2.0. March 2023. Available at: https://assets.publishing.service.gov.uk/media/64071ec5d3bf7f25fa417a91/Management-of-contacts-of-invasive-group-a-*Streptococcus*.pdf
115. European Centre for Disease Prevention and Control. Increase in Invasive Group A streptococcal infections among children in Europe, including fatalities. Dec 2022. Available at: https://www.ecdc.europa.eu/en/news-events/increase-invasive-group-streptococcal-infections-among-children-europe-including
116. Johannesen TB, Munkstrup C, Edslev SM, et al. Increase in invasive group A streptococcal infections and emergence of novel, rapidly expanding sub-lineage of the virulent *Streptococcus pyogenes* M1 clone, Denmark, 2023. Euro Surveill 2023; 28.
117. Centers for Disease Control and Prevention. ABCs Bact facts Interactive Data Dashboard. Available at: https://www.cdc.gov/abcs/bact-facts/data-dashboard.html
118. US Centers for Disease Control and prevention Group A Streptococcal (GAS) Disease. Current Group A Strep Activity. Available at: https://www.cdc.gov/group-a-strep/php/surveillance/index.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fgroup-a-strep%2Fdata-research%2Ffacts-stats%2Findex.html
119. Burillo A, Bouza E. The eternal dilemma of antitoxin antibiotics for skin and soft tissue infection. Curr Opin Infect Dis 2021; 34:80-8.
120. Stevens DL. Invasive group A *Streptococcus* infections. Clin Infect Dis 1992; 14:2-11.
121. Stevens DL, Tanner MH, Winship J, et al. Severe group A streptococcal infections associated with a toxic shock-like syndrome and scarlet fever toxin A. N Engl J Med 1989; 321:1-7.
122. Eagle H. Experimental approach to the problem of treatment failure with penicillin. I. Group A streptococcal infection in mice. Am J Med 1952; 13:389-99.
123. Stevens DL, Bryant AE, Yan S. Invasive group A streptococcal infection: New concepts in antibiotic treatment. Int J Antimicrob Agents 1994; 4:297-301.
124. Mascini EM, Jansze M, Schouls LM, et al. Penicillin and clindamycin differentially inhibit the production of pyrogenic exotoxins A and B by group A streptococci. Int J Antimicrob Agents 2001; 18:395-8.
125. Babiker A, Li X, Lai YL, et al. Effectiveness of adjunctive clindamycin in β-lactam antibiotic-treated patients with invasive β-haemolytic streptococcal infections in US hospitals: a retrospective multicentre cohort study. Lancet Infect Dis 2021; 21:697-710.
126. Rezahosseini O, Roed C, Gitz Holler J, et al. Adjunctive antibiotic therapy with clindamycin or linezolid in patients with group A *Streptococcus* (GAS) meningitis. Infect Dis (Lond) 2023; 55:751-3.
127. Heil EL, Kaur H, Atalla A, et al. Comparison of Adjuvant Clindamycin vs Linezolid for Severe Invasive Group A *Streptococcus* Skin and Soft Tissue Infections. Open Forum Infect Dis 2023; 10:ofad588.
128. Cortés-Penfield N, Ryder JH. Should Linezolid Replace Clindamycin as the Adjunctive Antimicrobial of Choice in Group A Streptococcal Necrotizing Soft Tissue Infection and Toxic Shock Syndrome? A Focused Debate. Clin Infect Dis 2023; 76:346-50.
129. Senda A, Endo A, Fushimi K, Otomo Y. Effectiveness of intravenous immunoglobulin therapy for invasive group A *Streptococcus* infection: A Japanese nationwide observational study. Int J Infect Dis 2023; 135:84-90.
130. Fernández-Galilea A, Estella A, García-Garmendia JL, et al. Clindamycin but not Intravenous Immunoglobulins reduces mortality in a retrospective cohort of critically ill patients with bacteremic Group A Streptococcal infections. Rev Esp Quimioter 2022; 35:475-81.
131. Bisno AL, Stevens DL. Streptococcal infections of skin and soft tissues. N Engl J Med 1996; 334:240-5.
132. Lazzeri D, Lazzeri S, Figus M, et al. Hyperbaric oxygen therapy as further adjunctive therapy in the treatment of periorbital necrotizing fasciitis caused by group A *Streptococcus*. Ophthalmic Plast Reconstr Surg 2010; 26:504-5; author reply 5.
133. Norrby-Teglund A, Pauksens K, Norgren M, Holm SE. Correlation between serum TNF alpha and IL6 levels and severity of group A streptococcal infections. Scand J Infect Dis 1995; 27:125-30.
134. Stevens DL, Bryant AE. A critical role of tumor necrosis factor (TNF) alpha in experimental group A streptococcal (GAS) bacteremia. Adv Exp Med Biol 1997; 418:797-800.
135. Liu YH, Wu PH, Kang CC, et al. Group A *Streptococcus* Subcutaneous Infection-Induced Central Nervous System Inflammation Is Attenuated by Blocking Peripheral TNF. Front Microbiol 2019; 10:265.
136. Brouwer S, Rivera-Hernandez T, Curren BF, et al. Pathogenesis, epidemiology and control of Group A *Streptococcus* infection. Nat Rev Microbiol 2023; 21:431-47.
137. Degala S, Puleston R, Bates R, et al. A protracted iGAS outbreak in a long-term care facility 2014-2015: control measures and the use of whole-genome sequencing. J Hosp Infect 2020; 105:70-7.
138. Bouza E, García Navarro JA, Alonso S, et al. Infection control in long term care institutions for the elderly: A reflection document on the situation in Spain. Rev Esp Quimioter 2023; 36:346-79.
139. Burns K, Dorfmueller HC, Wren BW, et al. Progress towards a glycoconjugate vaccine against Group A *Streptococcus*. NPJ Vaccines 2023; 8:48.
140. Bloom DE, Carapetis J. Strep A: challenges, opportunities, vaccine-based solutions, and economics. NPJ Vaccines 2024; 9:80.

Official journal of the Spanish Society of Chemotherapy

Original

Revista Española de Quimioterapia
doi:10.37201/req/045.2024

Fernando Cobo[1]
Juan Antonio Reguera-Márquez[1]
José Antonio Marín-Rodríguez[2]
Francisco José Martín-Pérez[2]
Patricia Pérez-Palacios[3,4,5]
Esther Recacha[3,4,5]
José María Navarro-Marí[1]

A 5-year study of bloodstream infections caused by carbapenemase-producing Gram-negative bacilli in southern Spain

[1]Department of Microbiology and Instituto de Investigación Biosanitaria Ibs.GRANADA, University Hospital Virgen de las Nieves. Granada, Spain.
[2]Department of Preventive Medicine. University Hospital Virgen de las Nieves. Granada, Spain.
[3]Unidad Clínica de Enfermedades Infecciosas y Microbiología, Hospital Universitario Virgen Macarena, Seville, Spain.
[4]Instituto de Biomedicina de Sevilla IBIS, Hospital Universitario Virgen Macarena/CSIC/Universidad de Sevilla, Sevilla, Spain.
[5]Centro de Investigación Biomédica en Red en Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, Madrid, Spain

Article history
Received: 4 May 2024; Revision Requested: 8 July 2024; Revision Received: 25 July 2024; Accepted: 30 July 2024; Published: 19 September 2024

ABSTRACT

Introduction. The aim of this study was to evaluate the microbiological epidemiology of carbapenemase-producing Gram-negative bacilli (CPGNB) isolated from blood during a 5-year period.

Methods. A total of 80 isolates from 78 patients were finally included; fifty-five (70.5%) were men and the mean age was 60 years. Detection of carbapenemase production was performed by immunocromatography (IC) and polymerase chain reaction (PCR). Genotyping was carried-out by pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST), and characterization of carbapenemase-producing isolates was performed by whole genome sequencing (WGS).

Results. The main microorganisms isolated were *K. pneumoniae* (29.4%), *E. cloacae* (28.2%), *A. baumannii* (17.9%) and *P. aeruginosa* (15.3%). Overall, the most common carbapenemase in Enterobacterales was OXA-48 group (57.7%). The most common carbapenemase in non-fermenting bacilli was OXA-23 (60.8%). The most common ST in *K. pneumoniae* producing OXA-48 types was ST45 and in *E. cloacae* ST114, while in *E. cloacae* producing VIM types was ST78. In OXA-23 types, the most common clone in *A. baumannii* was ST2, whereas in *P. aeruginosa* producing IMP types *was* ST253.

Conclusions. There was an increase in cases recorded in the years of highest incidence and severity of the SARS-CoV-2 pandemic, with a significant number of cases in patients admitted to the ICU. All bacteremias caused by A. baumannii were caused by the same clone, and 12 of the 14 cases caused by A. baumannii were part of outbreaks in the ICU.

Keywords: Carbapenemase; Enterobacterales; non-fermenting bacilli; OXA-48 type; OXA-23 type; ST-clones

Correspondece:
Dr. Fernando Cobo
Department of Microbiology and Instituto de Investigación Biosanitaria Ibs.GRANADA
University Hospital Virgen de las Nieves, Avda Fuerzas Armadas, 2. 18014 Granada, Spain
E-mail: fernando.cobo.sspa@juntadeandalucia.es

Estudio de 5 años de infecciones del torrente sanguíneo producidas por bacilos gramnegativos productores de carbapenemasas en el sur de España

RESUMEN

Introducción. El objetivo de este estudio fue evaluar la epidemiología microbiológica de los bacilos Gram negativos productores de carbapenemasas (CPGNB) aislados de sangre durante un período de 5 años.

Métodos. Se incluyeron finalmente 80 aislamientos de 78 pacientes; cincuenta y cinco (70,5%) eran hombres y la edad media fue de 60 años. La detección de la producción de carbapenemasas se realizó mediante inmunocromatografía (IC) y reacción en cadena de la polimerasa (PCR). El genotipado se llevó a cabo mediante electroforesis en gel de campo pulsado (PFGE) y tipificación de secuencia multilocus (MLST), y la caracterización de los aislados productores de carbapenemasas se realizó mediante secuenciación del genoma completo (WGS).

Resultados. Los principales microorganismos aislados fueron *K. pneumoniae* (29,4%), *E. cloacae* (28,2%), *A. baumannii* (17,9%) y *P. aeruginosa* (15,3%). En general, la carbapenemasa más común en Enterobacterales fue el grupo OXA-48 (57,7%). La carbapenemasa más común en los bacilos no fermentadores fue la OXA-23 (60,8%). El ST más común en *K. pneumoniae* que produce tipos OXA-48 fue ST45 y en *E. cloacae* ST114, mientras que en *E. cloacae* que produce tipos VIM fue ST78. En los tipos OXA-23, el clon más común en *A. baumannii* fue ST2, mientras que en *P. aeruginosa* tipo IMP fue ST253.

Conclusiones. Hubo un incremento de casos registrados en los años de mayor incidencia y gravedad de la pandemia por SARS-CoV-2, con un número importante de casos en pacientes ingresados en UCI. Todas las bacteriemias producidas por *A. baumannii* lo fueron por el mismo clon, y 12 de los 14 casos producidos por *A. baumannii* formaron parte de brotes en la UCI.

Palabras clave: Carbapenemasa; Enterobacterales; bacilos no fermentadores; OXA-48 type; OXA-23 type; clones ST

INTRODUCTION

The increasing prevalence of carbapenemase-producing gram-negative bacilli (CPGNB) is a major public health threat worldwide, causing significant morbidity and mortality [1]. This trend is particularly, but not limited, to Enterobacterales, especially *Klebsiella pneumoniae* and extensively drug-resistant strains of *Pseudomonas aeruginosa*. In addition, the increasing prevalence of nosocomial infections caused by multidrug-resistant bacteria complicates the choice of appropriate treatment [2].

The importance of monitoring carbapenem-resistant gram-negative bacteria is due to their potential for widespread transmission of resistance through mobile genetic elements. In fact, WHO considers these microorganisms as a priority 1 (critical) in the list of antibiotic-resistant "priority pathogens", that responds to urgent public health needs (https://who.int). Particularly serious are the bloodstream infections caused by these bacteria due to their potential higher risk of morbidity and mortality. Mortality and clinical outcomes associated with carbapenem-resistant gram-negative bacteria can be severe. It is important to understand factors that may help guide earlier detection and therefore earlier treatment of infections caused by these pathogens [3,4].

Moreover, the economic impact could be higher than the annual cost of many chronic diseases and many acute diseases. Costs rise proportionally with the incidence of these infections, so prevention and control strategies should be considered.

Currently, carbapenemases are classified into three groups and the most frequently isolated enzymes are as follows: class A enzymes that includes KPC, class B metallo-β-lactamases (MBLs) represented by the groups VIM, IMP and NDM, and class D enzymes such as OXA-48-like [3]. In European countries, current data on these enzymes are diverse and the distribution of different types of carbapenemases varies in different countries of this world region [4]. In a recent report of carbapenem-resistant *K. pneumoniae* in Europe, KPC was predominant in some countries such as Italy, Greece, Portugal and Israel, while OXA-48 was the main carbapenemase enzyme in Romania, Turkey and Spain [5]. OXA-48 is by far the most common carbapenemase in *K. pneumoniae* circulating in Spain, followed by MBLs and KPCs [6]. Regarding carbapenemase-producing *P. aeruginosa* in Spain, the prevalence was low, accounting for approximately 0.4% of carbapenem-resistant isolates [7]. Due to the great diversity, it is important to know the patterns of CPGNBs especially in bloodstream infections, because early detection may allow timely approaches to adopt preventive measures and controlling possible outbreaks. This manuscript addresses the assessment of the burden of CPGNBs isolated from blood in our hospital over a 5-year period.

METHODS

Setting and demographic data. This study was conducted in the Department of Microbiology of the University Hospital Virgen de las Nieves located in Granada, southeastern Spain. This center is a tertiary care facility with approximately 1,100 beds and serves as a primary care facility for nearly 550,000 inhabitants. Only samples obtained from blood of hospitalized patients were included and analyzed. Data about age, sex, ward of admission, infections in other location, colonization before or after the infection, risk factors for carbapenemase acquisition, presence of septic shock, antimicrobial treatment applied, and outcome were recorded.

Bacterial isolates: identification and antimicrobial susceptibility testing. Eighty CPGNBs isolates, from January 2017 to February 2022, were included from 78 patients with bacteremia due to CPGNB. All isolates were initially identified by MALDI-TOF MS (Bruker Biotyper, Bellerica, MA, USA) according to the manufacturer's recommendations. Antimicrobial susceptibility was determined using the panel N96 of MicroScan WalkAway system (Beckman Coulter, USA). The isolates with a MIC to meropenem > 0,12 mg/L were initially selected for the study. Phenotypic detection of carbapenemase production was performed using the βCarba assay (BioRad, Spain) and commercial combined disk test (Rosco Diagnostica A/S, Denmark), containing meropenem and inhibitors (phenyl-boronic acid, dipicolinic acid and cloxacillin) as well as a disk of temocillin for Enterobacterales, and imipenem and inhibitors (phenyl-boronic acid, dipicolinic acid, EDTA and cloxacillin) for *P. aeruginosa*. The NG test Carba5 (NG Biotech, Guipry, France) was used to detect the carbapenemase group (KPC, VIM, IMP, NDM and OXA-48). All isolates were sent to the Reference Laboratory of Andalusia (located in Hospital Universitario Virgen Macarena, Seville, Spain) for phenotypic and molecular characterization, as part of PIRASOA programme, an antimicrobial stewardship programme. The PIRASOA programme was developed to prevent and control healthcare-associated infections and promote the appropriate use of antimicrobials throughout the Andalusian public health

Pulsed-field electrophoresis (PFGE). Genotyping was assessed by pulsed field gel electrophoresis (PFGE) after digestion of total chromosomal DNA with *XbaI* (Enterobacterales), *SpeI* (*Pseudomonas* spp) and *ApaI* (*Acinetobacter* spp.). DNA fragments were separated using a CHIEF DR-II system (Biorad, Madrid, Spain). Normalization strain used were *E. coli* ATCC25922 for *K. pneumoniae*, *Salmonella* braenderup for the other Enterobacterales species and *P.aeruginosa* ATCC27853 for *Pseudomonas* spp. Banding patterns of the gel were analyzed using Bionumerics 8.1 software (AppliedMaths, Austin, TX, USA) using Dice's coefficient (1%) to measure the genetic similarity between the isolates.

Molecular detection of carbapenemase and MLST. The presence of carbapenemases (NDM, VIM, KPC, IMP and OXA-48 types) was assessed by PCR with gene-specific primers and direct sequencing by Sanger until 2017. From October 2017, molecular detection and identification of carbapenemase genes was performed using whole genome sequencing (WGS) with MiSeq system (Illumina®, San Diego, CA, USA). DNA library was prepared

Table 1 Demographic data of cases of bacteremia with carbapenemase-producing Gram-negative bacilli (2017-2022).

Demographic data	n	%
Average age (years)	60	
Sex		
Men	55	70.5
Women	23	29.5
Ward of admission		
Medical ward	43	55.1
Surgical ward	3	3.8
Intensive care unit	32	41
Microorganisms		
K. pneumoniae	23	29.5
E. cloacae	22	28.2
A. baumannii	14	17.9
P. aeruginosa	12	15.3
E. coli	3	3.8
P. putida	2	2.5
K. oxytoca	1	1.2
C. freundii	1	1.2
Colonized patients		
Before infection	14	17.9
After infection	10	12.8
Without colonization	54	69.2
Septic shock		
No	55	70.5
Yes	23	29.5
Risk factors for infection		
Use of invasive procedures	47	60.2
ICU admission	39	50
Immunosuppression	35	44.8
Other (surgery, long antibiotic treatment)	7	8.9
Infection in other location	35	44.8
Empirical therapy*		
Correct	52	66.6
Incorrect	26	33.3
Outcome		
Successful	42	53.8
Death	36	46.1

*** Empirical therapy was considered correct when the antibiotic applied was susceptible to this pathogen.**

with the Nextera DNA flex (Illumina®). Raw reads were assembled *de novo* using the CLC Genomic Workbench v9 (Qiagen). Antimicrobial resistance genes were determined using ResFinder4.1 database [8-10]. MLST was determined by PCR and sequencing or by WGS data (from 2017) using MLSTfinder 2.0 [11, 12].

Outbreaks' study. During the period of study, the outbreaks declared were analyzed. An outbreak was considered when two or more cases of an infection were caused by the same microorganism with a time-space association and suspicion of an epidemiologic link [13]. Initially, isolates of the same species with similar resistance pattern were studied with different molecular techniques to consider them belonging to an outbreak.

RESULTS

Demographic data. Seventy-eight patients with bacteremia due to CPGNB were finally included in the study; 55 (70.5%) isolates belonged to male patients with an average age of 60 years (range 19-94). Most of samples came from medical units (n=43; 55.1%) and intensive care unit (ICU) (n=32; 41%). Moreover, 35 (44.8%) patients had an infection for the same microorganism in other location. Twenty-three (29.5%) patients had septic shock and 36 (46.1%) patients died as a consequence of this infection. The main risk factor for infection due to microorganisms carbapenemase-producing was the use of invasive procedures (n=47; 60.2%) followed by ICU admission (n=39; 50%). An incorrect empirical therapy was applied in 26 (33.3%) patients and in 17 (65.3%) of them the outcome was unsuccessful (Table 1).

Characteristics of carbapenemase-producing isolates. During the period of study, a total of 80 strains were included in the study (two patients had one isolate producing two types of carbapenemases each one). From these isolates, Enterobacterales represented 64% (n=52) of them while non-fermenting Gram-negative bacilli represented 36% (n=28). Among Enterobacterales, *K. pneumoniae* was the microorganism most common isolated (n=23; 29.4%), followed by *E. cloacae* (n=22; 28.2%). Among non-fermenting Gram-negative bacilli, *A. baumannii* was the bacterium most common isolated (n=14; 17.9%) followed by *P. aeruginosa* (n=12; 15.3%) (Table 1). Overall, the most common carbapenemases detected were OXA-48 (n=26; 32.5%) and VIM (n=25; 31.2%), followed by OXA-23 (n=14; 17.5%). In Enterobacterales, regarding to the prevalence through the years, VIM-like had the highest prevalence in 2017 (8 cases), meanwhile OXA-48-like had the highest prevalence in 2020 (12 cases) and OXA-23 had the highest prevalence in 2021 (11 cases). The carbapenemase less frequent through all period of study was NDM-like (only 2 cases). In non-fermenting Gram-negative bacilli, the most common carbapenemase was OXA-23 (n=14; 17.5%) followed by IMP-like (n=6; 26.1%). Only in 9 isolates, co-production of an additional associated resistance mechanism was detected, especially penicillinases in 8 strains of *K. pneumoniae* (TEM-1). One isolate of *K. pneumoniae* harbored two carbapenemases: OXA-48 and NDM-5, and other isolate of *C. freundii* produced also two carbapenemases: OXA-48 and VIM-63 (Table 3).

Regarding to clonal lineages of carbapenemase-producing Enterobacterales, *K. pneumoniae* ST45/OXA-48 and *E. cloacae* ST114/OXA-48 were the most prevalent (n= 6, each one),

Table 2 Types of carbapenemases in Gram-negative bacilli causing bacteremia (2017-2022).

Type of carbapenemase	2017	2018	2019	2020	2021	2022	TOTAL
VIM	8	4	4	3	5	1	25
OXA-48	-	-	1	12	10	3	26
OXA-23	-	-	1	2	11	-	14
IMP	-	5	1	1	2	-	9
KPC	3	-	-	-	1	-	4
NDM	-	-	1	1	-	-	2
TOTAL	11	9	8	19	29	4	80

followed by *E. cloacae* ST78/VIM-1 (n=5). Moreover, two isolates of *K. pneumoniae* ST258/KPC-1 and KPC-3 were detected. In non-fermenting Gram-negative bacilli, the most frequent variant was *A. baumannii* ST2/OXA-23 (n=14) followed by *P. aeruginosa* ST253/IMP-16 (n=5) (Table 4).

Outbreaks detected. The data obtained through PFGE allowed us to establish which strains were part of the different outbreaks. During the period of study, four outbreaks were declared; the first of them was caused by *P. aeruginosa* ST253/IMP-16 in March 2018 in the ICU (three cases); the other three outbreaks were caused by *A. baumannii* ST2/OXA-23 in the ICU in December/January 2020, at the beginning of February 2021 and at the end of March and beginning of April 2021 (four cases each one).

DISCUSSION

This is a report of the bacteremias due to CPGNB isolated in a tertiary-care hospital in southeast Spain in a 5-year period. It was performed from routine testing of 80 Gram-negative bacilli isolates belonging to Enterobacterales and non-fermenting Gram-negative bacilli species isolated from blood cultures during this period.

CPGNB represent a global threat to healthcare systems, especially when they are causing severe infections such as bloodstream infection. Regarding to the prevalence of CPGNB, there is currently considerable heterogeneity across different countries and regions. Several surveillance studies focused on epidemiological situation of CPGNB are available in the scientific literature and, recently, a report about the epidemiological status in carbapenemase-producing Enterobacterales in 37 European countries has been recently published [1]. Overall, four countries such as Greece, Italy, Malta and Turkey were in "endemic" situation and other countries, including Spain, were in an "inter-regional spread" stage. The result of this report indicates that 11 countries reported a worsened epidemiological situation compared with the previous report [5]. These trends are highlighted in Enterobacterales, especially in *K. pneumoniae*; in these bacteria, carbapenemases are the main contributing factor to extensive drug resistance.

Regarding to the type of carbapenemase, different reports showed different distribution in Europe [3]; while KPC-like is predominant in Italy, Greece, Portugal and Israel, VIM-like is more frequent in Hungary and OXA-48-like in the main carbapenemase in Romania, Turkey and Spain [3].

From the point of view of CPGNB that cause bacteremia, there are some studies reporting this item. A multicenter study conducted in the USA demonstrated that the majority of cases were due to *K. pneumoniae* KPC-2 and KPC-3, and the ST258 predominated among KPC-producing *K. pneumoniae* [14]. Other study showed that in cases of bacteremia caused by carbapenemase-producing *K. pneumoniae* (CPKP), 61.1% of these bacteria were KPC [15]. In Korea, in 131 cases of bacteremia due to carbapenemase-producing Enterobacterales, 69% were caused by *K. pneumoniae*, followed by *Enterobacter* spp (10%) and *E coli* (8%); regarding to the type of carbapenemase, KPC was the most commonly observed (66%) followed by NDM (20%) [16]. Moreover, other study stated that from 146 carbapenem-resistant *K. pneumoniae* strains, KPC-2 was the main mechanism of carbapenem resistance (n=127) [17]. Finally, a report recently published of bacteremia with carbapenemase-producing Enterobacterales in immunocompromised patients previously colonized, 12.7% (28 from 221) developed bloodstream infections of carbapenemase-producing Enterobacterales [18]. Most of these infections were caused by *K. pneumoniae*, and KPC was the most frequent type of carbapenemase, followed by NDM.

A multicenter study of CPKP and carbapenemase-producing *E. coli* conducted in Spain in 2019, revealed that CPKP was the most frequent carbapenemase-producing bacteria found in blood (50 out of 52 isolates), although in this work only 52 isolates were collected from bacteremia [19]. The main limitation of this study to know the true prevalence of CPGNB in blood is that only isolates of *K. pneumoniae* and *E. coli* were included. Regarding to the type of carbapenemase, almost 70% of isolates expressed OXA-48, although no data of carbapenemases from type of samples were reported. However, in this study, the distribution of carbapenemase-producing

Table 3 Main variants and clonal lineages of carbapenemase-producing Enterobacterales (n=45).

Carbapenemase (n/%)	Variants (n)	Species and clones (n)
KPC (2/4.4)	KPC-1 (1)	*K. pneumoniae*, ST258/KPC-1 (1)
	KPC-3 (1)	*K. pneumoniae*, ST258/KPC-3 (1)
VIM (13/28.8)	VIM-1 (12)	*K. pneumoniae*, ST25/VIM-1 (1)
		K. pneumoniae, ST469/VIM-1 (1)
		E. cloacae, ST78/VIM-1 (5)
		E. cloacae, ST111/VIM-1 (1)
		E. cloacae, ST90/VIM-1 (2)
		E. cloacae, ST311/VIM-1 (1)
		K. oxytoca, ST36/VIM-1 (1)
	VIM-63 (1)	*E. cloacae*, ST22/VIM-63 (1)
OXA (24/53.3)	OXA-48 (24)	*K. pneumoniae*, ST307/OXA-48 (4)
		K. pneumoniae, ST45/OXA-48 (6)
		K. pneumoniae, ST405/OXA-48 (2)
		K. pneumoniae, ST6446/OXA-48 (1)
		E. cloacae, ST114/OXA-48 (6)
		E. cloacae, ST307/OXA-48 (1)
		E. cloacae, ST110/OXA-48 (1)
		E. coli, ST405/OXA-48 (2)
		E. coli, ST307/OXA-48 (1)
NDM (2/4.4)	NDM-5 (2)	*K. pneumoniae*, ST15/NDM-5 (2)
NDM + OXA (2/4.4)	NDM-5 + OXA-48	*K. pneumoniae*
		ST15/NDM
		ST307/OXA-48
VIM + OXA (2/4.4)	VIM-63 + OXA-48	*C. freundii*
		ST22/VIM-63
		ST405/OXA-48

bacteria was different depending on the geographical location; founding in southern Spain more KPC-producing isolates. Moreover, in this report 8 high-risk CPKP clones were detected, especially ST307/OXA-48 (16.4%) and ST11/OXA-48 (16.4%), although in bacteremias ST512-258/KPC and ST15/OXA-48-like were the most frequent bacteremia-producing clones (24 and 16%, respectively). In our study, *K. pneumoniae* was also the most frequent carbapenemase-producing isolated bacteria (29.5%) followed by *E. cloacae* (28.2%) and *A. baumannii* (17.9%). However, the most frequent type of carbapenemase was OXA-48, followed by VIM-like, and, in *A. baumannii*, the main type of carbapenemase was OXA-23. These data contrast with those previously mentioned, and show the differences between countries or regions respect to the prevalence of carbapenemases in bloodstream infections and the different measures to be taken to prevent its spread, reinforcing the need for epidemiological control at regional level.

Respect to the high-risk bacteremia-producing clones, in our study the most frequent association between high-risk clone and carbapenemase-producing *K. pneumoniae* was ST307/OXA-48 (4 cases), followed by *K. pneumoniae* ST258/KPC-1 (1 case).

Regarding to non-fermenting Gram-negative bacilli (NF-GNB), data about carbapenemase-producing NFGNB in bloodstream infections are scarce. An Italian nationwide survey on *P. aeruginosa* from invasive infections included 935 clinical isolates from bloodstream infections and lower respiratory tract infections collected 20 centres between 2014 and 2023; they showed that 12 and 32 isolates were positive for carbapenemase types VIM and IMP, respectively [20]. However, the authors do not specify the type of samples for each group of carbapenemases. In *A. baumannii*, a study found that the acquired carbapenemase detected most frequently was OXA-23,

Table 4 Main variants and clonal lineages of carbapenemase-producing non Enterobacterales (n=23).

Carbapenemase (n/%)	Variants (n)	Species and clones (n)
IMP (6/26.1)	IMP-16 (5)	*P. aeruginosa*, ST253/IMP-16 (5)
	IMP-23 (1)	*P. aeruginosa*, ST175/IMP-23 (1)
VIM (3/13.1)	VIM-1 (2)	*P. aeruginosa*, ST115/VIM-1 (1)
		P. putida, ST78/VIM-1 (1)
	VIM-2 (1)	*P. aeruginosa*, ST277/VIM-2 (1)
OXA (14/60.8)	OXA-23 (14)	*A. baumannii*, ST2/OXA-23 (14)

which was present in 69% of the isolates, but the type of sample was not specified either [21].

A Spanish nationwide survey on *P. aeruginosa* showed that 2.1% of isolates produced carbapenemases, especially GES type; bloodstream samples only represented 5.9% of all isolated tested, but no more details were shown [7]. However, in our bloodstream isolates, the main type of carbapenemase was IMP-like, particularly IMP-16, and all strains belonged to ST253 clone (Table 4). Respect to this, three of five cases belonged to the same outbreak. These data demonstrated that epidemiological studies should be performed in all health areas, in order to know the differences in the prevalence of carbapenemases in each region.

The rapid detection of carbapenemases is an important factor in order to establish a rapid and adequate empirical therapy, especially in bloodstream infections and other severe infections. In our patients, an incorrect empirical therapy was applied in 26 (33.3%) patients and in 17 (65.3%) of them the outcome was unsuccessful. Thus, taking into account the epidemiology of carbapenemase-producing Gram-negative bacilli in our area, an improvement in the initiation of empirical treatment should occur in patients with risk factors for acquiring carbapenemases, although this improvement may be due to a rapid microbiological diagnosis.

In our study, the great increase in cases registered during the years 2020 and 2021 is striking, which were the years with the highest incidence and severity of the pandemic produced by SARS-CoV-2. It is also striking that a very significant number of cases occurred in patients admitted to the ICU, a service that was especially pressured during this period. All bacteremias caused by *A. baumannii* were caused by the same clone and occurred in these three years (2019, 1 case and 2020-21, thirteen cases). Twelve of the fourteen cases caused by *A. baumannii* were from outbreaks (two or three) that occurred in the ICU in 2020 and 2021.

The main limitations of the present study is that the data are limited to one health area; further studies including a great number of hospitals will be necessary to corroborate these data.

In summary, respect to the prevalence of CPGNB, our results add information to several studies published last years in Spain and in other countries, emphasizing the need for epidemiological surveillance both at regional and local level. A rapid detection of carbapenemases in bloodstream infections can help physicians to establish the best therapeutic strategy as well as to implement control measures to prevent the spread of these strains in order to reduce the mortality of these patients.

FUNDING

None to declare

CONFLICT OF INTEREST

Authors declare no conflict of interest.

REFERENCES

1. Brolund A, Lagerqvist N, Byfors S, et al. Worsening epidemiological situation of carbapenemase-producing Enterobacteriaceae in Europe, assessment by national experts from 37 countries, July 2018. Euro Surveill 2019; 24: pII=1900123. doi: 10.2807/1560-7917.ES.2019.24.9.1900123.
2. Livermore M.D. Has the era of untreatable infections arrived? J Antimicrob Chemother 2009; 64 (Suppl 1): 29-36. doi: 10.1093/jac/dkp255.
3. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tasios PT, Daikos G.L. Carbapenemases in *Klebsiella pneumoniae* and other Enterobacteriaceae: an evolving crisis of global dimensions. Clin Microbiol Rev 2012; 25: 682-707. doi: 10.1128/CMR.05035-11.
4. Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. J Infect Dis 2017; 215 (Suppl 1): S28-S36. doi: 10.1093/infdis/jiw282.

5. David S, Reuter S, Harris SR, et al. Epidemic of carbapenem-resistant *Klebsiella pneumoniae* in Europe is driven by nosocomial spread. Nat Microbiol 2019; 4: 1919-1929. doi: 10.1038/s41564-019-0492-8.
6. Palacios-Baena ZR, Oteo J, Conejo C, et al. Comprehensive clinical and epidemiological assessment of colonisation and infection due to carbapenemase-producing Enterobacteriaceae in Spain. J Infect 2016; 72: 152-160. doi: 10.1016/j.jinf.2015.10.008.
7. Sastre-Femenia MA, Fernández-Muñoz A, Gomis-Font MA, et al. *Pseudomonas aeruginosa* antibiotic susceptibility profiles, genomic epidemiology and resistance mechanisms: a nation-wide five-year time lapse analysis. Lancet Reg Health Eur 2023; 34: 100736. doi: 10.1016/j.lanepe.2023.100736.
8. Bortolaia V, Kaas RS, Ruppe E, et al. ResFinder 4.0 for predictions of phenotypes from genotypes. J Antimicrob Chemother 2020; 75: 3491-3500. doi: 10.1093/jac/dkaa345.
9. Zankari E, Allesøe R, Joensen KG, Cavaco LM, Lund O, Aarestrup FM. PointFinder: a novel web tool for WGS-based detection of antimicrobial resistance associated with chromosomal point mutations in bacterial pathogens. J Antimicrob Chemother 2017; 72: 2764-2768. doi: 10.1093/jac/dkx217.
10. Camacho C, Coulouris G, Avagyan V, et al. BLAST +: architecture and applications. BMC Bioinformatics; 2009; 10: 421. doi: 10.1186/1471-2105-10-421.
11. Larsen MV, Cosentino S, Rasmussen S, et al. Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol 2012; 50: 1355-1361.doi: 10.1128/JCM.06094-11.
12. Bartual SG, Seifert H, Hippler C, Luzon MA, Wisplinghoff H, Rodríguez-Valera F. Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. J Clin Microbiol 2005; 43: 4382-4390. doi: 10.1128/JCM.43.9.4382-4390.2005.
13. Surveillance Definitions for Specific Types of Infections. CDC/NHSN, January 2016. Available in: http://www.cdc.gov/nhsn/PDFs/pscManual/17pscNosInfDef_current.pdf.
14. Satlin MJ, Chen L, Patel G, et al. Multicenter clinical and molecular epidemiological analysis of bacteremia due to carbapenem-resistant Enterobacteriaceae (CRE) in the CRE epicenter of the United States. Antimicrob Agents Chemother 2017; 61: e02349-16. doi: 10.1128/AAC.02349-16.
15. Hsu JY, Chuang YC, Wang JT, Chen YC, Hsieh SM. Healthcare-associated carbapenem-resistant *Klebsiella pneumoniae* bloodstream infections: risk factors, mortality, and antimicrobial susceptibility, 2017-2019. J Formos Med Assoc 2021; 120: 1994-2002. doi: 10.1016/j.jfma.2021.04.014.
16. Park JW, Lee H, Park SY, Kim TH. Epidemiological, clinical, and microbiological characteristics of carbapenemase-producing Enterobacteriaceae bloodstream infection in the Republic of Korea. Antimicrob Resist Infect Control 2019; 8: 48. doi: 10.1186/s13756-019-0497-3.
17. Zhang N, Qi L, Liu X, et al. Clinical and molecular characterizations of carbapenem-resistant *Klebsiella pneumoniae* causing bloodstream infection in a Chinese Hospital. Microbiol Spectrum 2022; 10: 1-11.doi: 10.1128/spectrum.01690-22.
18. Averbuch D, Moshkovitz L, Llan S, Abu Ahmad W, Temper V, Strahilevitz J. Bacteremia with carbapenemase-producing Enterobacterales in immunocompromised patients colonized with these bacteria. Microb Drug Resist 2022; 28: 593-600. doi: 10.1089/mdr.2021.0253.
19. Cañada-García JE, Moure Z, Sola-Campoy PJ, et al. CARB-ES-19 multicenter study of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* from all Spanish provinces reveals interregional spread of high-risk clones such as ST307/OXA-48 and ST512/KPC-3. Front Microbiol 2022; 13: 918362. doi: 10.3389/fmicb.2022.918362.
20. Giani T, Arena F, Pollini S, et al. Italian nationwide survey on *Pseudomonas aeruginosa* from invasive infections: activity of ceftolozane/tazobactam and comparators, and molecular epidemiology of carbapenamase producers. J Antimicrob Chemother 2018; 73: 664-671. doi: 10.1093/jac/dkx453.
21. Iovleva A, Mustapha MM, Griffith MP, et al. Carbapenem-resistatn *Acinetobacter baumanii* in U.S. hospitals: diversification of circulating lineages and antimicrobial resistance. MBio 2022; 13: 1-16. doi: 10.1128/mbio.02759-21.

Official journal of the Spanish Society of Chemotherapy

Original

Revista Española de Quimioterapia
doi:10.37201/req/074.2024

Diego Cecchini[1]
Javier Sfalcin[2]
Inés Zapiola[3]
Alan Gomez[2]
Silvina Fernandez-Giuliano[3]
Claudia Rodriguez[1]
Lilia Mammana[3]
Analía Seravalle[2]
Fabián Fay[2]
María Cecilia Torroija[4]
Gabriela Bugarín[4]
María Belén Bouzas[3]

Integrase strand transfer inhibitors resistance-associated mutations in HIV-infected pregnant women

[1]Infectious Diseases Unit, Hospital Argerich, Buenos Aires, Argentina.
[2]CIBIC, Rosario, Argentina.
[3]Virology Unit, Clinical Analysis Division, Hospital Francisco J. Muñiz, Buenos Aires, Argentina.
[4]MSD, Munro, Argentina

Article history
Received: 19 July 2024; Revision Requested: 12 September 2024; Revision Received: 13 September 2024; Accepted: 20 September 2024; Published: 4 October 2024

ABSTRACT

Objective. To date, no data exist regarding the prevalence of integrase inhibitor (INSTI) resistance-associated mutations (HIVDRM) in HIV-infected pregnant women (HPW) in Latin America. We describe the prevalence and transmissibility of integrase HIVDRM in a historical cohort of INSTI-naïve HPW from Argentina (n=56) with Next Generation Sequencing (NGS).

Material and methods. Bioinformatics analysis was performed by HyDRA software for 20%, 10%, 5%, 2%, and 1% sensitivity thresholds. We calculated the mutational viral load for each INSTI-HIVDRM, considering those with >1000 c/mL as of high risk of transmissibility.

Results. The predominant HIV subtype was BF (78.5%). Major HIVDRM were not detected with the population sequencing 20% filter. With a 1% threshold, the prevalence increased to 8.9%; Y143C/S, E92G, E138K, and T66I mutations were found. The median (range) mutational load (expressed in c/mL) was: 355 (50.2-11705); with only 1 case >1000 c/mL Accessory mutations (G163R/K, T97A) were detected mostly with a 20% sensitivity threshold with an overall prevalence of 23.2%; the median (IQR) mutational load was: 23929 (4009-63158) c/mL; all of them above 1000 c/mL.

Conclusion. Our results show evidence of the presence of major INSTI-HIVDRM as aleatory mutations and a high frequency of accessory mutations with potential transmissibility in HPW.

Keywords: mutations; prevalence; pregnant women; HIV; integrase inhibitors

Correspondence:
Diego Cecchini
Infectious Diseases Unit, Hospital Cosme Argerich
Almirante Brown 240, Buenos Aires 1155AHD, Argentina
E-mail: diegocec@gmail.com

Resistencia a los inhibidores de integrasa en embarazadas con VIH

RESUMEN

Objetivo. Hasta la fecha, no existen datos sobre la prevalencia de mutaciones asociadas a la resistencia (HIVDRM) a los inhibidores de la integrasa (INSTI) en mujeres embarazadas infectadas por VIH (HPW) en América Latina. Describimos la prevalencia y la transmisibilidad de las HIVDRM de la integrasa en una cohorte histórica de HPW naive de INSTI de Argentina (n=56) mediante Next Generation Sequencing (NGS).

Material y métodos. Se realizó un análisis bioinformático mediante el software HyDRA para umbrales de sensibilidad del 20%, 10%, 5%, 2% y 1%. Calculamos la carga viral mutacional para cada INSTI-HIVDRM, considerando aquellas con >1000 c/mL como de alto riesgo de transmisibilidad.

Resultados. El subtipo de VIH predominante fue BF (78,5%). No se detectaron HIVDRM principales con el filtro de secuenciación poblacional del 20%. Con un umbral del 1%, la prevalencia aumentó al 8,9%; se encontraron las mutaciones Y143C/S, E92G, E138K y T66I. La mediana (rango) de la carga mutacional (expresada en c/mL) fue: 355 (50,2-11705); con solo 1 caso >1000 c/mL. Las mutaciones accesorias (G163R/K, T97A) se detectaron principalmente con un umbral de sensibilidad del 20%, con una prevalencia general del 23,2%; la mediana (RIQ) de la carga mutacional fue: 23929 (4009-63158) c/mL; todas ellas por encima de 1000 c/mL.

Conclusión. Nuestros resultados muestran evidencia de la presencia de INSTI-HIVDRM principales como mutaciones aleatorias y una alta frecuencia de mutaciones accesorias con potencial transmisibilidad en HPW.

Palabras clave: mutaciones; prevalencia; embarazadas; VIH; inhibidores de integrasa

INTRODUCTION

Despite massive public health efforts, rates of mother-to-child transmission of HIV remains high in Argentina and in several countries in Latin America [1]. Antiretroviral therapy (ART) is mandatory for pregnant women to suppress HIV replication to prevent perinatal transmission. However, perinatal transmission is mostly driven by late-presenting pregnant women and those with a high peripartum viral load due to lack of access and HIV testing, nonadherence to ART, and virologic failure [2].

The emergence of primary mutations in the viral genome is a significant cause of drug resistance, which can lead to treatment failure [3]. Thus, drug resistance- associated mutations (HIVDRM) in HIV-infected pregnant women (HPW) may increase the risk of perinatal transmission with impact in women and children´s future treatment options [4]. According to published reports, levels of transmitted drug resistance are high in Argentina in the general population, HPW, and newborns, mostly for first-generation non-nucleoside reverse transcriptase inhibitors (NNRTI) such as efavirenz and nevirapine (NVP) [5-7].

Local and international guidelines currently recommend an integrase-inhibitor (INSTI) based ART for HPW [4]. Dolutegravir (DTG) or, alternatively, raltegravir (RAL) as anchor drugs are recommended with a backbone of two nucleoside analogs (emtricitabine/tenofovir; lamivudine/tenofovir; abacavir/lamivudine). For those mothers with a high risk of transmission (those with peripartum viral loads >1000 c/mL) there is indication of zidovudine infusion during delivery and performance of cesarean section when possible. In addition, neonatal prophylaxis is an essential component of prevention for those high-risk newborns. DHHS guidelines recommend presumptive HIV therapy with lamivudine, zidovudine plus either RAL or NVP in this clinical scenario [4]. Guidelines from Argentina prefer RAL to NVP in this setting, due to the high levels of NNRTI resistance reported [8].

Despite the fundamental role of INSTI in maternal and neonatal therapy, there is no data regarding the prevalence of primary resistance to INSTI in Argentina in general or in pregnant women populations. In addition, INSTI HIVDRM transmissibility in the mother-child pair is unknown. This study constitutes a baseline epidemiological survey for INSTI resistance in pregnant women in Argentina using Next Generation Sequencing (NGS).

MATERIAL AND METHODS

Study design. Cross-sectional investigation. We included stored baseline plasma samples from a previous study that evaluated transmitted and acquired drug resistance in a cohort of HIV-infected pregnant women in Argentina [6].

Study population. HPW were enrolled in a reference public Hospital in Buenos Aires City from 2008 until 2014. All women enrolled had their blood drawn, processed, and stored for the determination of HIV resistance. For the present analysis, 89 plasma samples were available: 40 were from treatment-naïve and 49 from treatment-experienced patients. None of them had exposure to INSTI, as these drugs were not routinely available and not recommended in local guidelines during the collection period. The clinical and epidemiological profile of this cohort has been described previously [6].

Sample amplification. RT-PCR was performed on 10 µl of each extract of clinical samples using Superscript™ III One-Step RT-PCR Platinum® Hi-Fidelity Taq System (Thermo Fisher Scientific, Canada), and the primers IN-out-F1 5'- CACAYAARGGRATTGGAGGAAATG-3' (HXB2 loci 4162-4185) and IN-out-R1 5'- TARTGGRATGTGTACTTCTGAAC-3' (HXB2 loci 5195-5217). RT-PCR conditions were as follows: 50 °C for 30 minutes, 94 °C for 2 minutes, 40 cycles of 94 °C for 20 seconds, 54 °C for 30 seconds, and 68 °C for 60 seconds, and a final extension at 68 °C for 5 minutes. Following RT-PCR, a 5 µl aliquot was transferred to a nested- PCR reaction, with Phusion® Hot Start II Hi-Fidelity DNA Polymerase (Thermo Fisher Scientific, Canada) and the primers IN-nest-F1 5'- AACARGTAGATAAATTAGTHAGT-3' (HXB2 loci 4186 - 4208) and IN-nest-R1 5'-ATACATATGRTGYTTTACTARACT-3' (HXB2 loci 5107 - 5130). Nested-PCR conditions were performed as follows: 95 °C for 10 minutes, 40 cycles at 94 °C for 20 seconds, 52 °C for 30 seconds, and 72 °C for 60 seconds, followed by a final extension at 72 °C for 5 minutes.

MiSeq library prep and ultra-deep sequencing. Samples were sequenced for the integrase (IN) region using an ultra-deep sequencing protocol published by Taylor et al for genotyping of protease and retrotranscriptase region [9]. Libraries were prepared using Nextera® XT DNA Library Preparation Kit (Illumina, USA) and amplicon final concentration 0.2 ng/ul. Tagmentation, indexing, and purification were all performed according to the manufacturer's detailed Reference Guide. Libraries normalization was performed using the Quant-iT™ PicoGreen™ dsDNA Assay Kit (Invitrogen). Libraries were pooled in equal fractions and the mixture was diluted to 2 nM. PhiX control library (20 pM, v3, Illumina, USA) was spiked in at 10%, to measure quality and to elevate the diversity of the amplicons libraries. Finally, the product was sequenced on the MiSeq using a v2 300-cycle MiSeq reagent kit (Illumina, USA) [9-10].

MiSeq data analysis. Reference-based mapping and variant calling against the HXB2 pol gene (loci 2253– 5096, GenBank Accession number: K03455) was performed with HyDRA Web (*http://hydra.canada.ca*) with the default settings. Drug resistance mutations detected at 1%, 2%, 5%, 10%, and 20% thresholds of sensitivity were identified based on the current Stanford list. In addition, the list of INSTI-mutations for the surveillance of transmitted drug resistance according to Tzou et al was considered [11-12].

Mutational viral load. Mutational load was estimated from maternal viral load and the frequency of each mutation within viral quasispecies. The baseline viral load per patient was the starting point to calculate the mutational load (for exam-

ple, a mutational load was 100 if a patient has a viral load of 10% corresponding to a specific mutation over a baseline viral load of 1000 c/mL). Those HIVDRM with mutational load >1000 c/mL were considered at high risk of being transmitted perinatally considering the reference value for cesarean section indication [13].

RESULTS

RT-PCR was performed on 89 samples from INSTI-naïve HPW. Of these, 56 (38 ART naïve; 18 exposed to other drug classes) were satisfactorily amplified and sequenced as shown in Figure 1. The predominant HIV subtype was BF (78.5%). Median (interquartile range, IQR) viral load was 15545 (5228-47688) c/mL.

Major mutations were not detected with the population sequencing 20% threshold neither in the overall sample (0/56) nor ART-naive population (0/38). With a 5% threshold, the prevalence was 1.7% (1/56) in the overall population and 2.6% (1/38) in the naive population. The 2% threshold increased the prevalence to 7.1% (4/56) in the overall population and 7.9% (3/38) in naive. With a 1% threshold, the overall prevalence was 8.9% (5/56) and 10.5% (4/38) in the naive population. Accessory mutations were detected mostly with a 20% sensitivity threshold with a prevalence of 23.2% (13/56). Such prevalence did not increase significantly with the 5, 2, and 1% thresholds. The viral quasispecies harboring major HIVDRM were not predominant within the overall viral population. In contrast, those harboring accessory mutations, were. A summary of these findings is described in Table 1. The proportion of quasispecies harboring HIVDRM among individual samples of experienced and naive patients is shown in Tables 2 and 3, respectively.

Considering major mutations, the median (range) mutational load was 355 c/mL (50.2-11705); with only 1 case >1000 c/mL (1/56; 1.7%), at expenses of a high baseline maternal viral load (487732 c/mL). Mutational load for Y143C, Y143S, E92G, E138K, and T66I mutations were: 63.5, 11705, 50.2, 761.7, and 355 c/mL, respectively. Considering accessory mutations T97A and G163K/R median (IQR) mutational load was 23929 (4009-63158) c/mL, all of them above the 1000 c/mL threshold.

DISCUSSION

To our knowledge, this study is the first report of primary resistance to INSTI in HIV– infected pregnant women in Latin America. We describe baseline data before its general use in our region, which may allow a better future understanding of evolving patterns of INSTI-resistance. Recently, Brazil reported

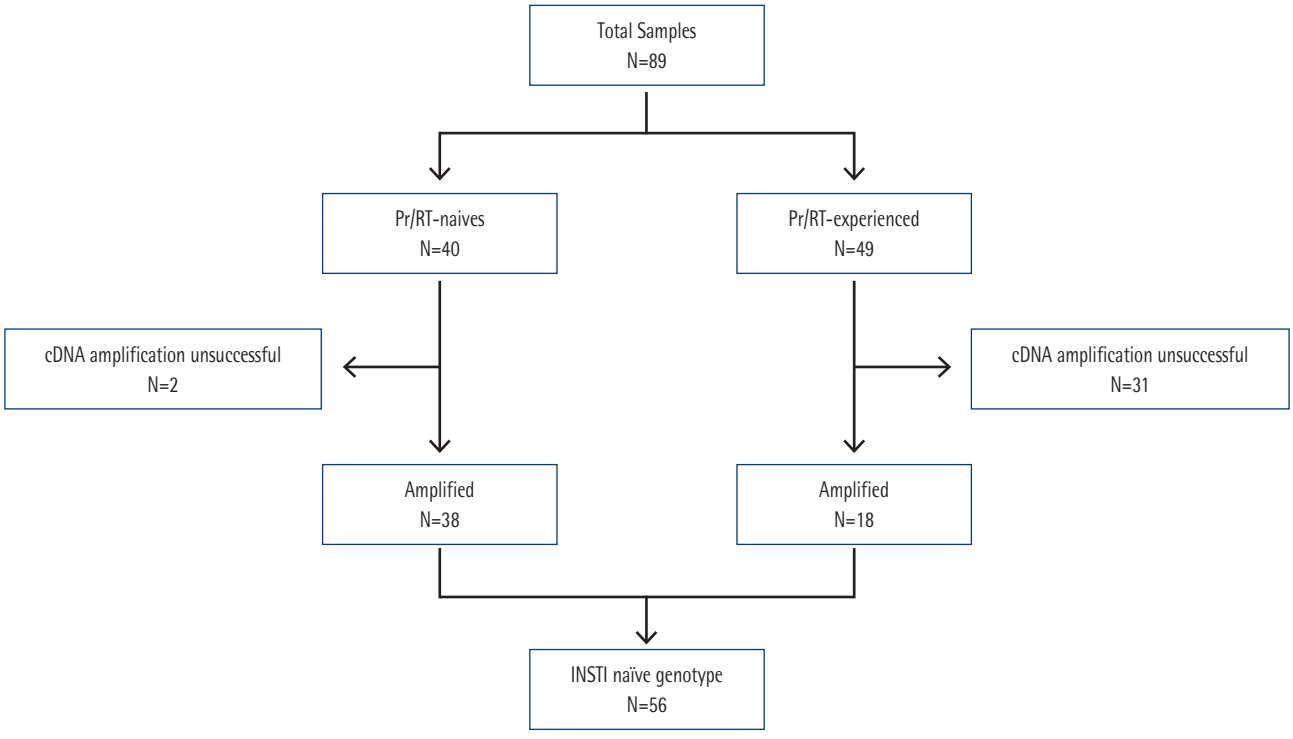


Figure 1 **Flowchart describing the inclusion of stored samples (period 2008-2014) from HIV-infected pregnant women for a baseline survey of resistance to integrase inhibitors (INSTI) by next-generation sequencing in Argentina.**

Pr/RT: protease inhibitor/reverse transcriptase inhibitor; cDNA: complementary DNA

Table 1 Prevalence of major, accessory, and other mutations (Stanford algorithm) in the integrase coding region of INSTI-naive pregnant women living with HIV in Argentina.

NGS threshold	20%	10%	5%	2%	1%
Major mutations *					
Y143C	--	--	1.7%	1.7%	1.7%
Y143S	--	--	--	1.7%	1.7%
T66I	--	--	--	1.7%	1.7%
E138K	--	--	--	--	1.7%
E92G	--	--	--	1.7%	1.7%
Accessory mutations					
T97A	3.5%	3.5%	3.5%	3.5%	3.5%
G163R	12.5%	12.5%	12.5%	12.5%	12.5%
G163K	7.1%	7.1%	8.9%	8.9%	8.9%
Other					
V151I	5.3%	7.1%	8.9%	8.9%	10.7%
L74I	1.7%	1.7%	1.7%	1.7%	1.7%

NGS: Next-generation sequencing; INSTI: integrase strand transfer inhibitor; *mutations included in the list of INSTI-mutations for the surveillance of transmitted drug resistance

Table 2 INSTI resistance-associated mutations detected in individual baseline samples of ART-experienced/INSTI-naive pregnant women in Argentina by Next Generation Sequencing (NGS) considering different thresholds. Each line represents a unique patient sample; the percentage in brackets represents the prevalence of viral quasispecies harboring each mutation and the mutational load. The first detection (threshold) of a major mutation appears in bold.

VL (cop/ml)	NGS 20%	NGS 10%	NGS 5%	NGS 2%	NGS 1%	Subtype
2018	-	-	V151I (5.33%, 107)	V151I (5.33%, 107)	V151I (5.33%, 107)	BF
60507	-	-	G163K (5.5%, 3327)	G163K (5.5%, 3327)	G163K (5.5%, 3327)	B
45184	G163R (99.9%, 45138)	G163R (99.9%, 45138)	G163R (99.9%, 45138)	G163R (99.9%, 45138)	G163R (99.9%, 45138)	BF
5529	V151I (41.1%, 2272)	V151I (41.1%, 2272)	V151I (41.1%, 2272)	V151I (41.1%, 2272)	V151I (41.1%, 2272)	B
55617	G163R (99.9%, 55561)	G163R (99.9%, 55561)	G163R (99.9%, 55561)	G163R (99.9%, 55561)	G163R (99.9%, 55561)	BF
70826	G163K (99.9%, 70755)	G163K (99.9%, 70755)	G163K (99.9%, 70755)	G163K (99.9%, 70755)	G163K (99.9%, 70755)	BF
12103	G163R (99.9%,12090)	G163R (99.9%, 12090)	G163R (99.9%, 12090)	G163R (99.9%, 12090)	G163R (99.9%, 12090)	BF
487732	S230N, (99.5%,485293)	S230N (99.5%, 485293)	S230N (99.5%, 485293)	**Y143S (2,4%, 11705),** S230N (99.5%, 485293)	Y143S (2.4%, 11705), S230N (99.5%, 485293)	B
4706	G163K (99.7%,4691)	G163K (99.7%, 4691)	G163K (99.7%, 4691)	G163K (99.76%,4691)	G163K (99.76%, 4691)	BF
33147	L74I (99.2%, 32881), S230N (99.3%, 32914)	L74I (99.2%,32881), S230N (99.3%, 32914)	L74I (99.2%, 32881), S230N (99.3%, 32914)	L74I (99.21%, 32881), S230N (99.31%,32914)	L74I (99.2%, 32881), S230N (99.3%, 32914)	BF

INSTI: integrase strand transfer inhibitor; PR/TR: protease/reverse transcriptase viral subtype; VL: viral load

increasing INSTI resistance in naive populations in the context of the use of these drugs in the general population [14].

In our historical cohort, major INSTI mutations were detected only by thresholds <2%. This is in concordance with other publications in which these mutations were infrequent or not reported at all [15-17]. Therefore, the 8.9% prevalence of HIVDRM to INSTI detected at the 1% threshold warrants careful interpretation. It´s important to note that this preva-

Table 3 **INSTI resistance-associated mutations detected in individual baseline samples of ART-naive pregnant women in Argentina by Next Generation Sequencing (NGS) considering different thresholds. Each line represents a unique patient sample; the percentage in brackets represents the prevalence of viral quasispecies harboring each mutation and the mutational load. The first detection (threshold) of a major mutation appears in bold.**

VL (cop/ml)	NGS 20%	NGS 10%	NGS 5%	NGS 2%	NGS 1%	Subtype
214738	T97A (99,48%, 213621)	T97A (99,48%, 213621)	T97A (99.4%, 213449)	T97A (99.4%, 213449)	T97A (99.4%, 213449)	BF
13851	-	V151I (14%, 1939)	V151I (14%, 1939)	V151I (14%, 1939)	V151I (14%, 1939)	BF
69941	V151I (100%, 69941)	V151I (100%, 69941)	V151I (100%, 69941)	V151I (100%, 69941)	V151I (100%, 69941)	B
1095	-	-	**Y143C (5.8%, 63)**	**E92G (4.5%, 49),** Y143C (5.8%, 63)	E92G (4.5%, 49), Y143C (5.8%, 63)	BF
18700	T97A (93.3%, 17447)	T97A (93.3%, 17447)	T97A (93.3%, 17447)	T97A (93.3%, 17447)	T97A (93.3%, 17447)	BF
111000	G163R (99.7%, 110667)	G163R (99.7%, 110667)	G163R (99.7%, 110667)	G163R (99.7%, 110667)	G163R (99.7%, 110667)	BF
3152	G163R (99.6%, 3139)	G163R (99.6%, 3139)	G163R (99.6%, 3139)	G163R (99.6%, 3139)	G163R (99.6%, 3139), Q164P (1.8%, 56)	BF
242473	G163R (63.8%, 154697)	G163R (63.8%, 54697)	G163R (63.8%, 154697)	G163R (63.8%, 154697)	G163R (63.8%, 154697), V151I (1.1%, 2667)	BF
3036	G163R (43.4%, 1317)	G163R (43.4%, 1317)	G163R (43.4%, 1317)	G163R (43,4%, 1317)	G163R (43.4%, 1317)	BF
26967	V151I (99.5%, 26832)	V151I (99.5%, 26832)	V151I (99.5%, 26832)	V151I (99,5%, 26832)	V151I (99.5%, 26832)	BF
24074	G163K (99.4%, 23929)	G163K (99.4%, 23929)	G163K (99.4%, 23929)	G163K (99.4%, 23929)	G163K (99.4%, 23929)	BF
17000	-	-	-	**T66I (2.09%, 355)**	T66I (2.09%, 355)	BF
55200	G163K (99.8%, 55089)	G163K (99.8%,55089)	G163K (99.8%,55089)	G163K (99.8%, 55089)	G163K (98.2%, 55089), **E138K (1.38%, 761)**	BF

INSTI: integrase strand transfer inhibitor; PR/TR: protease/reverse transcriptase viral subtype

lence was observed at very low thresholds (1-2%) and not detected at the clinically relevant 20% threshold typically used in population sequencing. These low-frequency mutations likely represent random mutations rather than true transmitted drug resistance, especially considering the study period (2008-2014) when INSTI use was not widespread in our country. Detection of major HIVDRM with the highest sensitivity filters shows evidence of their existence as aleatory mutations, with minimal representation within viral quasispecies. In consequence, they have a low mutational load and risk of transmissibility in the absence of selective pressure. Therefore, the high sensitivity of NGS allows detection of viral quasispecies that may not be clinically relevant. Considering the current recommendations and the widespread use of INSTI in people living with HIV, including pregnant women, further research is needed with current samples to detect signals of increasing trends in INSTI resistance in our country. This finding underscores the importance of interpreting low-frequency mutations cautiously and in the context of their true potential clinical impact.

Conversely, we found a high overall prevalence of accessory HIVDRM in the integrase coding gene, mostly due to G163K/R mutations. Such mutations have a potential impact on susceptibility ("low-level resistance") to first-generation INSTIs (raltegravir, elvitegravir) according to the Stanford algorithm. These results were congruent with previous reports [18-19] and may be attributable to polymorphisms of circulating subtypes, with high representation within viral quasispecies. These quasispecies can be transmitted perinatally because they exceed the threshold of 1000 c/mL.

Neither Latin America nor Argentina have achieved the programmatic objective of HIV perinatal transmission <2% expected for non-breastfeeding populations, being >15% for Latin America and >4% for Argentina [1]. Despite the late diagnosis and limited access to ART remain significant determinants of mother-to-child transmission, drug-resistance is a cause of maternal virologic failure potentially contributing to neonatal infection [2-4]. The INSTIs are, to date, the most effective therapeutic strategy to reduce the viral load in pregnant women to prevent perinatal HIV transmission, even in late diagnoses [8]. Considering the recommendations of INSTI- based therapy in local and international perinatal guidelines (as well as for the general population), resistance surveillance remains crucial.

While our study reports findings at various detection thresholds, including 1%, it's crucial to emphasize the limit-

ed clinical significance of mutations detected at such low frequencies. The 20% threshold is generally considered clinically relevant for population sequencing, as it represents the limit of detection for Sanger sequencing and is more likely to impact treatment outcomes [20-21]. Therefore, mutations detected at the 1% threshold, while interesting from a research perspective, may not have clinical implications. These low-frequency variants may not persist over time due to lack of selective pressure or fitness costs, are unlikely to impact the virological response to INSTI-based regimens and may represent natural aleatory or random mutations rather than true drug resistance. Therefore, while NGS allows us to detect these low-frequency variants, their clinical relevance remains uncertain [1]. Treatment decisions should primarily be based on mutations detected at higher, clinically validated thresholds. The low-frequency mutations reported in this study should be interpreted as providing insight into viral diversity and potential resistance pathways rather than as direct predictors of potential treatment failure

Despite the intrinsic limitations and limited external validity inherent to the retrospective and local nature of our study, this first report on HIVDRM in pregnant women supports the need for surveillance of transmitted and acquired drug resistance in Argentina and other Latin American countries.

ACKNOWLEDGMENTS

Portions of this research were presented at the 24th International AIDS Conference, Montreal, Canada, 29 July - 2 August 2022 (poster EPB235) and HIV Drug Therapy Conference, Glasgow, UK 23 – 26 October 2022 (poster P226)

FUNDING

This study was funded by MSD Argentina.

CONFLICTS OF INTEREST

DC participated in educational activities organized by MSD (travel grant, speaker fees). Rest of authors declare no conflict of interest.

REFERENCES

1. Epidemia del VIH y respuesta en América Latina y el Caribe - OPS/OMS | Organización Panamericana de la Salud [cited May 31, 2023]. Available at: https://www.paho.org/es/documentos/epidemia-vih-respuesta-america-latina- caribe.
2. Luzuriaga K, Mofenson LM. Challenges in the Elimination of Pediatric HIV-1 Infection. N Engl J Med. 2016;374:761–770.doi:10.1056/NEJMra1505256
3. Hamers RL, Rinke de Wit TF, Holmes CB. HIV drug resistance in low-income and middle-income countries. The Lancet HIV. 2018;5(10):e588–e596. doi:10.1016/s2352-3018(18)30173-5
4. Antiretroviral Drug Resistance and Resistance Testing in Pregnancy | NIH [cited May 31, 2023]. Available at: https://clinicalinfo.hiv.gov/en/guidelines/perinatal/antepartum-care-arv-drug-resistance-testing.
5. Bissio E, Barbás MG, Bouzas MB, Cudolá A, Salomón H, Espínola L, et al. Pretreatment HIV-1 drug resistance in Argentina: results from a surveillance study performed according to WHO-proposed new methodology in 2014-15. J Antimicrob Chemother. 2017;72:504–510.doi:10.1093/jac/dkw445
6. Zapiola I, Cecchini D, Fernández GS, Martínez M, Rodríguez C, Bouzas MB. [HIV-1 resistance to antiretroviral drugs in pregnant women from Buenos Aires metropolitan area]. Medicina *(B Aires)*. 2016;76:349–354.
7. Aulicino PC, Zapiola I, Kademian S, Valle MM, Fernandez Giuliano S, Toro R, et al. Pre-treatment drug resistance and HIV- 1 subtypes in infants from Argentina with and without exposure to antiretroviral drugs for prevention of mother-to-child transmission. J Antimicrob Chemother. 2019;74:722–730.doi:10.1093/jac/dky486
8. SADI - Sociedad Argentina de Infectología - Actualización 2023 del VIII Consenso Argentino de Terapia Antirretroviral [cited September 12, 2024]. Available at: https://www.sadi.org.ar/publicaciones/item/1733-consenso-argentino-de-terapia-antirretroviral
9. Taylor T, Lee ER, Nykoluk M, Enns E, Liang B, Capina R, et al. A MiSeq-HyDRA platform for enhanced HIV drug resistance genotyping and surveillance. Sci Rep. 2019;9:8970.doi:10.1038/s41598-019-45328-3
10. Arias A, López P, Sánchez R, Yamamura Y, Rivera-Amill V. Sanger and Next Generation Sequencing Approaches to Evaluate HIV-1 Virus in Blood Compartments. Int J Environ Res Public Health. 2018;15.doi:10.3390/ijerph15081697
11. Tzou PL, Rhee SY, Descamps D, Clutter DS, Hare B, Mor O, et al. Integrase strand transfer inhibitor (INSTI)-resistance mutations for the surveillance of transmitted HIV-1 drug resistance. J Antimicrob Chemother. 2020;75:170–182.doi:10.1093/jac/dkz417
12. HIV Drug Resistance Database [cited May 31, 2023]. Available at https://hivdb.stanford.edu/dr- summary/resistance-notes/INSTI/.
13. Cecchini D, Sfalcin J, Zapiola I, Gómez A, Fernández Giuliano S, Mammana L, et al. Reverse transcriptase and protease inhibitors mutational viral load in HIV infected pregnant women with transmitted drug resistance in Argentina. Rev Esp Quimioter. 2021;34:371–375.doi:10.37201/req/022.2021
14. Caldeira D, Vergara T, Santos E, Lopes C, Alves C, Caseiro M et al. High transmitted drug resistance in Brazil: unprecedented levels of INSTI resistance [cited May 31, 2023]. Available at: https://programme.aids2022.org/Abstract/Abstract/?abstractid=11802
15. Ji H, Patterson A, Taylor T, Rank C, Halverson J, Capina R, et al. Prevalence of Primary Drug Resistance Against HIV-1 Integrase Inhibitors in Canada. J Acquir Immune Defic Syndr. 2018;78:e1–e3.doi:10.1097/QAI.0000000000001649
16. Bailey AJ, Rhee S-Y, Shafer RW. Integrase Strand Transfer Inhibitor Resistance in Integrase Strand Transfer Inhibitor-Naive Persons. AIDS Research and Human Retroviruses. 2021;37(10):736–743. doi:10.1089/aid.2020.0261

17. Yang Z, Yang X, Deng X, Wei S, Liu J, Ma J, et al. Prevalence of integrase strand transfer inhibitor (INSTIs) resistance mutations in Henan Province China (2018-2020). Infection. 2021;49(6):1195–1202. doi:10.1007/s15010-021-01668-9

18. Casadellà M, Santos JR, Noguera-Julian M, Micán-Rivera R, Domingo P, Antela A, et al. Primary resistance to integrase strand transfer inhibitors in Spain using ultrasensitive HIV-1 genotyping. J Antimicrob Chemother. 2020;75:3517–3524.doi:10.1093/jac/dkaa349

19. Inzaule SC, Hamers RL, Noguera-Julian M, Casadellà M, Parera M, Rinke de Wit TF, et al. Primary resistance to integrase strand transfer inhibitors in patients infected with diverse HIV-1 subtypes in sub- Saharan Africa. J Antimicrob Chemother. 2018;73:1167–1172.doi:10.1093/jac/dky005

20. de Salazar A, Viñuela L, Fuentes A, Teyssou E, Charpentier C, Lambert-Niclot S, et al. Transmitted Drug Resistance to Integrase-Based First-Line Human Immunodeficiency Virus Antiretroviral Regimens in Mediterranean Europe. Clin Infect Dis. 2023;76:1628-1635. doi:10.1093/cid/ciac972

21. Salmona M, Lascoux-Combe C, Nere ML, Rubenstein E, Molina JM, Delaugerre C, et al. Transmission of S230R integrase drug resistance mutation affecting second-generation integrase inhibitors in a French primary HIV-1 infected man. *J Antimicrob Chemother.* 2023;78:1802-1803. doi:10.1093/jac/dkad135

Official journal of the Spanish Society of Chemotherapy

Original

Revista Española de Quimioterapia
doi:10.37201/req/078.2024

Javier Cabañas Morafraile[1]
Elena de Rafael González[2]
Laura Serrano Martín[3]
Rafael Rubio Díaz[1]
María Torres Fernández[2]
Eva Heredero Gálvez[3]
William Esneider López Forero[1]
Laura Morell Jurado[2]
Raúl Canabal Berlanga[1]
M. Francisca Calafell Mas[2]
Elia Chaves Prieto[1]
M. Remedios Asensio Nieto[2]
Álvaro Thomas-Balaguer Cordero[1]
María Carmen Lorenzo Lozano[2]
Agustín Julián-Jiménez[1,4]
En nombre del grupo URGEN-LABQMIC del CHUT (IDISCAM).

Poder diagnóstico de infección bacteriana de LIAISON MeMed BV® en los pacientes adultos atendidos en urgencias por sospecha de infección

[1]Servicio de Urgencias, Complejo Hospitalario Universitario de Toledo, IDISCAM, Toledo, España.
[2]Servicio de Análisis Clínicos y Bioquímica, Complejo Hospitalario Universitario de Toledo, IDISCAM, Toledo, España.
[3]Servicio de Microbiología y Parasitología, Complejo Hospitalario Universitario de Toledo, IDISCAM, Toledo, España.
[4]Universidad de Castilla La Mancha.

Article history
Received: 24 July 2024; Revision Requested: 12 September 2024; Revision Received: 23 September 2024; Accepted: 23 September 2024; Published: 16 October 2024

RESUMEN

Objetivos. Analizar la precisión diagnóstica de la nueva prueba MeMed® para predecir infección bacteriana en los pacientes adultos atendidos con sospecha clínica de infección en el servicio de urgencias hospitalario (SUH), así como comparar su rendimiento con otros biomarcadores de uso habitual (proteína C reactiva-PCR-, procalcitonina -PCT-).

Métodos. Estudio observacional, de cohortes, prospectivo y analítico de pacientes adultos atendidos en un SUH con el diagnóstico clínico de un proceso infeccioso. Se realizó un seguimiento durante 30 días. Como variable dependiente se consideró el diagnóstico de infección bacteriana (IB). Se analizó la capacidad predictiva con el área bajo la curva (ABC) de la característica operativa del receptor (COR) y los valores de sensibilidad (Se), especificidad (Es), valor predictivo positivo (VPP) y negativo (VPN) de la PCR, PCT, recuento de leucocitos y el test LIAISON® MeMed®.

Resultados. Se incluyó a 258 pacientes, de los que 36 (14%) habían fallecido a los 30 días tras su consulta en el SUH. La edad media fue 68,28 (DE 19,53) años, el 57,4% (148) eran hombres. A los 30 días el grupo con el diagnóstico IB tenía 137 pacientes, el grupo infección viral 68 casos y 17 en el grupo indeterminado. El ABC-COR que consigue MeMed® en el grupo que analiza todos los pacientes es de 0,920 (IC 95%: 0,877-0,962) y la PCT de 0,811 (IC 95%: 0,754-0,867). Con un punto de corte (PC) > 65 puntos del test MeMed® se obtiene una Se:79,2% y Es:91,2% y con PC > 90 puntos una Se: 57% y Es:95,9%. Aplicando el índice de Youden el PC > 50 puntos consigue una Se:84,1% y Es:88,2%.

Conclusiones. En los pacientes adultos atendidos con sospecha clínica de infección en el SUH, la prueba de LIAISON MeMed® presenta una gran capacidad para diagnosticar su origen bacteriano y obtiene un mejor rendimiento que la PCT, la PCR y el recuento de leucocitos.

Palabras clave: Biomarcadores, Servicio de Urgencias, Diagnóstico, Infección bacteriana.

Diagnostic power of LIAISON MeMed VB® for bacterial infection in adults patients seen in Emergency Departments due to infections

ABSTRACT

Objectives. To analyze the diagnostic accuracy of the new MeMed® test to predict bacterial infection in adult patients seen in emergency departments (ED) with clinical suspicion of infection, as well as to compare its performance with other commonly used biomarkers (protein C reactive-PCR-, procalcitonin -PCT-).

Methods. A prospective, observational and analytical study was carried out on adult patients who were treated in an ED with the clinical diagnosis of an infectious process. Follow-up was carried out for 30 days. The diagnosis of bacterial infection (BI) was considered as the dependent variable. The predictive ability was analyzed with the area under the curve (AUC) of the receiver operating characteristic (COR) and the values of sensitivity (Se), specificity (Es), positive predictive value (PPV) and negative predictive value (NPV) of the PCR, PCT, leukocyte count and the LIAISON® MeMed® test.

Results. The study included 258 patients, 54 (15.6%) of whom died within 30 days of visiting the ED. The mean age was 68.28 (SD 19.53) years, 57.4% (148) were men. At 30 days, the group with the IB diagnosis had 137 patients, the viral infection group 68 cases and 17 in the indeterminate group. The AUC-COR achieved by MeMed® in the group that analyzes all patients was 0.920 (95% CI: 0.877-0.962) and the PCT was 0.811 (95% CI: 0.754-0.867). With a cut-off point (PC) > 65

Correspondencia:
Dr. Agustín Julián-Jiménez
Servicio de Urgencias-Coordinador de Docencia, Formación, Investigación y Calidad.
Complejo Hospitalario Universitario de Toledo, Avda. Río Guadiana s/n. 45071. Toledo, España.
E-mail: agustinj@sescam.jccm.es

points of the MeMed® test, achieves a Se: 79.2% and Es: 91.2% and with PC > 90 points a Se: 57% and Es: 95.9%. Applying the Youden index, the PC > 50 points achieves Se:84.1% and Es:88.2%.

Conclusions. In adult patients treated with clinical suspicion of infection in the ED, the LIAISON MeMed® test has a great ability to diagnose its bacterial origin and achieves better performance than PCT, PCR and leukocyte count.

Keywords: Biomarkers, Emergency Department, Diagnostic, Bacterial Infection

INTRODUCCIÓN

La presencia de pacientes con sospecha de un proceso infeccioso en los servicios de urgencias hospitalarios (SUH) se ha incrementado significativamente en los últimos años, hasta suponer estos entre un 15%-20% de todos los atendidos diariamente en estas áreas asistenciales en España [1,2]. Asimismo, la gravedad de su presentación clínica y la mortalidad tanto intrahospitalaria como a corto plazo (30 días), también han aumentado en los últimos años, especialmente en los pacientes que cumplen criterios de sepsis, presentan comorbilidades relevantes, inmunodeprimidos, ancianos o con bacteriemia significativa confirmada desde el SUH [3,4].

En este escenario, la administración precoz y adecuada del tratamiento antibiótico (AB), junto con el control y drenaje del foco de infección, así como la toma inmediata de otras decisiones diagnóstico-terapéuticas (como solicitar pruebas complementarias, obtener hemocultivos y otras muestras microbiológicas, la intensidad del soporte hemodinámico, o la necesidad de ingreso, entre otras), repercuten directamente en la supervivencia de los enfermos con infección bacteriana grave [1,2,5,6].

Por otro lado, es conocido que las manifestaciones clínicas de los procesos infecciosos son a menudo inespecíficas y variables, lo que dificulta el reconocimiento precoz de estos enfermos y de su posible etiología bacteriana o viral [1,2]. Todo ello favorece que se prescriban AB innecesarios sin la certeza de que el cuadro tenga un origen infeccioso bacteriano, sobre todo en aquellos pacientes con síndrome febril de origen desconocido en el SUH (en los que del 30 al 50% de las ocasiones su etiología es no infecciosa), con la consiguiente presión antibiótica y la posibilidad de aumento de las resistencias y de la virulencia bacterianas [2,7,8]. Esta realidad representa un verdadero problema, al conllevar un incremento de las pruebas diagnósticas realizadas, de la estancia hospitalaria, de los costes y de la administración en ocasiones de tratamientos antibióticos innecesarios o, en otros casos, de la no administración precoz [1,2,7,8].

En los últimos años, se ha acentuado la búsqueda de herramientas objetivas de ayuda para intentar predecir, desde la primera valoración del paciente con sospecha de infección grave, un diagnóstico precoz, el pronóstico, la gravedad y junto con la posible etiología bacteriana la sospecha de bacteriemia (al ser estos factores claramente determinantes del pronóstico y la mortalidad de los procesos infecciosos) [2,5,8-11]. En esta línea, los biomarcadores de respuesta inflamatoria e infección (BMRIeI) se han posicionado como herramientas de gran ayuda para el clínico a la hora de mejorar el diagnóstico y el tratamiento de la infección, ya que podrían facilitar y adelantar la toma de decisiones vitales en el SUH [2,5,8-12]. Entre todos ellos, la procalcitonina (PCT) sigue destacando como un BMRIeI sensible y específico para predecir infección bacteriana, guiar hacia el patógeno causante de la infección, así como prever su evolución clínica (hacia sepsis grave y shock séptico) y la mortalidad asociada [2,9,10,12].

Recientemente, se ha descrito la utilidad de una nueva prueba basada en la alteración de las concentraciones de distintas proteínas que se producen en la respuesta inmunológica. Este test, denominado LIAISON®MeMed®, calcula una puntuación en función de un modelo que correlaciona los resultados de tres proteínas solubles del huésped, lo que permite diferenciar entre el origen bacteriano frente al viral de la infección [13,14]. Hasta ahora, se han publicado pocos estudios que evalúen esta capacidad predictiva de infección bacteriana de LIAISON®MeMed®, la mayoría en pacientes pediátricos [15-20], aunque también alguno en pacientes adultos [21-27]. Se trata de la primera prueba de diagnóstico que incluye una puntuación basada en la combinación de la concentración de 3 proteínas circulantes en sangre de BMRIeI inducidos tanto por virus como por bacterias [13-27]: 1.- El ligando inductor de apoptosis relacionado con el factor de necrosis tumoral (TRAIL) que se eleva como expresión de infección viral y disminuye en infección bacteriana, 2.- la proteína 10 inducida por el interferón gamma (IP-10) que se incrementa en mayor medida en infecciones virales y en menor medida en bacterianas, y 3.- la proteína C reactiva (PCR) que muestra un patrón opuesto a la IP-10. De esta manera, la presencia de proteínas del huésped no relacionadas entre sí que participan en diferentes vías puede mejorar la precisión del diagnóstico. En particular, la inclusión de nuevas proteínas del huésped que están reguladas positivamente en infecciones virales puede ser un complemento innovador a las proteínas inducidas por bacterias en el uso clínico actual, como la PCR o la PCT [13-27].

El objetivo principal de este estudio es investigar la precisión diagnóstica de la prueba MeMed® para predecir infección bacteriana en los pacientes adultos atendidos con sospecha clínica de infección en el SUH, así como comparar su rendimiento con otros BMRIeI de uso habitual (PCR, PCT).

PACIENTES Y MÉTODOS

Diseño y sitio. Estudio observacional, de cohortes, prospectivo y analítico de pacientes adultos (con 18 o más años) atendidos en un SUH con el diagnóstico clínico de un proceso infeccioso. Se realizó un seguimiento durante 30 días y tras este periodo el diagnóstico de infección fue mantenido. El estudio fue elaborado en un hospital universitario de tercer nivel de 786 camas perteneciente al Servicio de Salud de Castilla La Mancha.

Tabla 1 **Interpretación de los resultados de la fórmula LIAISON® MeMed®**

Puntuación MeMed®	Interpretación
0 a 10	Muy alta probabilidad de IV (u otra etiología no bacteriana)
11 a 34	Probabilidad moderada de IV (u otra etiología no bacteriana)
35 a 65	Resultado dudoso en relación a la etiología (IV frente IB o coinfección)
66 a 89	Probabilidad moderada de IB (o coinfección IB-IV)
90-100	Muy alta probabilidad de IB (o coinfección IB-IV)

IV: infección de etiología viral,IB: infección de etiología bacteriana. Adaptado de las indicaciones del fabricante y referencias 13, 21 y 22

Periodos de estudio y población incluida. Desde el 1 de julio de 2023 hasta el 7 de febrero de 2024 se incluyeron mediante un muestreo por oportunidad (cuando los investigadores estuvieron de guardia) a los pacientes con 18 o más años que fueron diagnosticados clínicamente de un proceso infeccioso en el SUH y en los que, por sus características clínicas y epidemiológicas, los médicos responsables indicaron la obtención de muestras sanguíneas para realizar pruebas complementarias analíticas (hemograma, bioquímica y biomarcadores como PCR, PCT, lactato y el test LIAISON® MeMed®), así como pruebas para el diagnóstico microbiológico (hemocultivos -HC-, otros cultivos, pruebas de detección rápida, serologías, antigenuria, etc.).

Se excluyeron los pacientes de pediatría y obstetricia-ginecología.

Variables recogidas. Como variable dependiente se consideró el diagnóstico de infección bacteriana (IB). Se diseñaron varios grupos entre los pacientes en función del mantenimiento del diagnóstico de sospecha o confirmado microbiológicamente a los 30 días: 1.- Diagnóstico de IB sospechado en el SUH y confirmado microbiológicamente; 2.- Diagnóstico de infección viral (IV) sospechado en el SUH y confirmado microbiológicamente; 3.- Diagnóstico de IB sospechado en el SUH y mantenido a los 30 días pero no confirmado microbiológicamente (diagnóstico y decisión tomada por unanimidad por un grupo formado por un/a médico de urgencias, un/a de microbiología, un/a del laboratorio de análisis clínicos y un/a de medicina interna); 4.- Diagnóstico de IV sospechado en el SUH y mantenido a los 30 días pero no confirmado microbiológicamente (decisión por el grupo de seguimiento); 5.- Sospecha indeterminada (el grupo de seguimiento no consensuó una decisión).

Como variables independientes se recogieron aquellas que se consideraron interesantes y que pudieran influir en el pronóstico y evolución del paciente durante los 30 días posteriores a la visita al SUH: A.-Demográficas y epidemiológicas: edad, sexo, institucionalización, toma de AB previos, ingreso en el último mes, de comorbilidad (índice de Charson [28]). B.-Clínicas: temperatura, alteración de la consciencia definida con ≤14 puntos en la escala del coma de Glasgow, existencia de náuseas/vómitos, escalofríos/tiritona, presión arterial sistólica (PAS), criterios de sepsis y las variables que los definen según la conferencia de expertos de sepsis de 2001 [1], definición de sepsis según un qSOFA ≥2 y de shock séptico y las variables que la constituyen según la tercera conferencia de consenso de sepsis(Sepsis-3)1. C.-De evolución y destino: días desde el inicio de la clínica y estancia hospitalaria, destino de los pacientes, reconsulta-reingreso en el SUH y mortalidad en 30 días tras la atención en el SUH. D.-De laboratorio de bioquímica con la analítica habitual y determinaciones de los BM incluidos (PCR en mg/L, PCT en ng/ml, lactato sérico en mmol/L); y E.- Del Servicio de Microbiología.

Definiciones, técnicas y métodos establecidos para las muestras. El test LIAISON® MeMed® es una fórmula matemática que integra un modelo que combina las concentraciones sanguíneas de tres proteínas del sistema inmunológico y con ellas genera una puntuación final cualitativa numérica (de 0 a 100 puntos) que indica la probabilidad de una respuesta inmune bacteriana o coinfección frente a una probable respuesta inmune viral. Utiliza tecnología de inmunoensayo de electroquimioluminiscencia ECLIA para las tres proteínas (TRAIL de 15-300 pg/ml, IP-10 de 100-2000 pg/ml y PCR 1-250 mg/ml). Inicialmente se estudió y comparó el rendimiento diagnóstico de IB en todos los pacientes de nuestra muestra y, posteriormente, de forma individual en distintos grupos con interés en los SUH (pacientes con el diagnóstico microbiológico confirmado, en los mayores de 65 años y en los diagnosticados como infección respiratoria de vías bajas -IVRB-). Además, según indicaciones del fabricante (DiaSorin) y debido a la posible respuesta inmunológica artefactada o no controlada, también se realizó la comparación eliminando a los pacientes que LIAISON® MeMed® incluye como criterios de exclusión ("grupo exclusión LIAISON-MeMed"): aquellos con sintomatología y fiebre constatada de más de 7 días, sospecha de gastroenteritis/colitis infecciosa, enfermedad inflamatoria activa, inmunodeficiencia congénita o adquirida, infección por virus de la inmunodeficiencia humana (VIH), de la hepatitis B (VHB), de la hepatitis C (VHC), tuberculosis activa, infección fúngica o parasitaria crónica, embarazadas, con neoplasia maligna activa y aquellos con traumatismos o quemaduras importantes o intervenidos de cirugía mayor en los últimos 7 días.

La interpretación de los resultados de LIAISON® MeMed® se realizó, inicialmente, según las indicaciones del fabricante y los primeros estudios publicados [13,21,22] (Tabla 1).

Tabla 2 Pruebas microbiológicas realizadas

Dos muestras de hemocultivos compuestas por dos frascos de cultivo (medios BD BACTEC™ Plus Aerobic y Lytic Anaerobic) incubados durante 5 días en el sistema BACTEC de Becton Dickinson. En los casos de sospecha de endocarditis se prolongó hasta 30 días.
PCR múltiple del sistema FilmArray® de Biomereux para la detección de bacterias, virus, levaduras y parásitos mediante diferentes paneles sindrómicos.
Estudio bacteriológico aerobio/anaerobio y fúngico de muestras del tracto respiratorio superior e inferior con un periodo de incubación entre 3-5 días.
Antigenuria de *S. pneumoniae* y *L. pneumophila* (serogrupo 1) en muestras de orina mediante inmunocromatografía de flujo lateral ImmuView® de SSI Diagnostica.
Detección de virus respiratorios (SARS-CoV-2, influenza A, influenza B y virus respiratorio sincitial) en muestras de exudado nasofaríngeo mediante los sistemas de PCR en tiempo real LIAISON MDX de Diasorin, GeneXpert de Cepheid y Vircell en el equipo Equipo CFX96 de Werfen.
Serología para la detección de patógenos implicados en infección respiratoria. Detección de clase IgG/IgM mediante técnica de inmunoanálisis quimioluminiscente en equipo Virclia (Vircell).
Estudio bacteriológico aerobio y fúngico en muestras de orina con periodo de incubación entre 24-48 horas.
Estudio bacteriológico aerobio/anaerobio y fúngico de muestras de piel y partes blandas, líquidos estériles y otros exudados con periodo de incubación entre 3-5 días.
Estudio bacteriológico de muestras del tracto gastrointestinal con un periodo de incubación entre 2-5 días.
La identificación de los microorganismos en los estudios bacteriológicos se realizó mediante espectrometría de masas MALDI-TOF de BrukerDaltonics.
Detección de antígeno GDH de *Clostridioides difficile* en muestras de heces mediante inmunoensayo enzimático cualitativo Immunocard® *C. difficile* GDH de Meridian Bioscience y confirmación de cepas toxigénicas mediante método LAMP en el sistema Illumipro-10™ de Meridian Bioscience.
Determinación de marcadores relacionados con otras infecciones víricas (virus de hepatitis, VIH) mediante técnica de inmunoanálisis quimioluminiscente en equipo Alinity (Abbott).
Detección de micobacterias: Tinción de Ziehl-Neelsen y cultivo en medio de cultivo líquido MGIT (Beckton Dickinson) con incubación en sistema MGIT960 (Becton Dickinson®) durante 40 días y medio sólido Lowenstein con un periodo de incubación de 60 días. La identificación de las micobacterias se realizó mediante espectrometría de masas MALDI-TOF de BrukerDaltonics. PCR de *Mycobacterium tuberculosis* mediante sistema GeneXpert de Cepheid en muestra directa.

Para los BMRIeI se adoptaron como valores de referencia los de nuestro laboratorio. Así, para la PCT por ECLIA en el equipo cobas e801 de Roche (R), medido en plasma con heparina de litio (con rango de detección de 0,02-100 ng/ml). Para el lactato amperometría en gasómetro Gem 5000 de Werfen (R) en sangre total con heparina de litio (con rango de detección de 0,3-17 mmol/l o 3-153 mg/dl). Y para la PCR por inmunoturbidimetría en cobas c702 de Roche en plasma con heparina de litio (con rango de detección 0,6-305 mg/L).

En relación con las pruebas microbiológicas realizadas, en la tabla 2 se detallan las utilizadas para la confirmación de la etiología bacteriana o viral. A todos los pacientes se les realizaron hemocultivos (por protocolo del centro al ser consideramos como pacientes con una potencial infección grave) y, además, una o varias de las pruebas microbiológicas recogidas en la tabla 2 (en función de la sospecha diagnóstica decidida por el médico de urgencias).

La extracción de los hemocultivos (HC) se realizó por la técnica estándar por venopunción cutánea. En cada paciente se realizaron dos extracciones separadas entre sí en el tiempo (y asegurando que los sitios de venopunción eran diferentes). La técnica de extracción, el tiempo de inoculación y las definiciones de bacteriemia verdadera y hemocultivos contaminados fueron las mismas que las utilizadas en un reciente estudio del grupo INFURG-SEMES [29].

Análisis estadístico. Para el análisis de la asociación entre la IB y las variables independientes se utilizaron medias y sus desviaciones estándar (DE) para variables cuantitativas y porcentajes para las cualitativas. Se utilizaron las pruebas de Ji al cuadrado o exacta de Fisher, la t de Student y la U de Mann-Whitney, según fueran aplicables, para investigar la relación entre el diagnóstico de IB y las variables independientes (y aquellas que se dicotomizaron). Se consideró como significativo un valor de $p < 0,05$ y todos los contrastes fueron bilaterales.

Se realizó un análisis descriptivo (números absolutos y porcentajes) de ambos grupos de pacientes (en función del diagnóstico final de IB o IV).

La eficacia para la predicción de IB a los 30 días de los distintos BMRIeI, recuento de leucocitos y la fórmula LIAISON® MeMed® se estudió mediante el análisis de las curvas de la característica operativa del receptor (COR) con el IC 95% del área bajo la curva (ABC) y se comparó frente al valor neutro (0,5). Los errores estándar de las ABC se calcularon por métodos no paramétricos.

Se utilizaron los puntos de corte (PC) en los valores de los BMRIeI (según las publicaciones recientes del grupo INFURG-SEMES) [9,10] y del test LIAISON® MeMed® según recomendaciones del fabricante y publicaciones previas [13-25]. Asimismo, para el test LIAISON® MeMed® se buscó el PC con mayor capacidad diagnóstica que maximizaba la diferencia entre la tasa de verdaderos positivos y falsos positivos mediante el índice de Youden. Se halló la sensibilidad (Se), la especificidad (Es), el valor predictivo positivo (VPP) y el valor predictivo negativo (VPN), el coeficiente de probabilidad positivo (CP+) y negativo

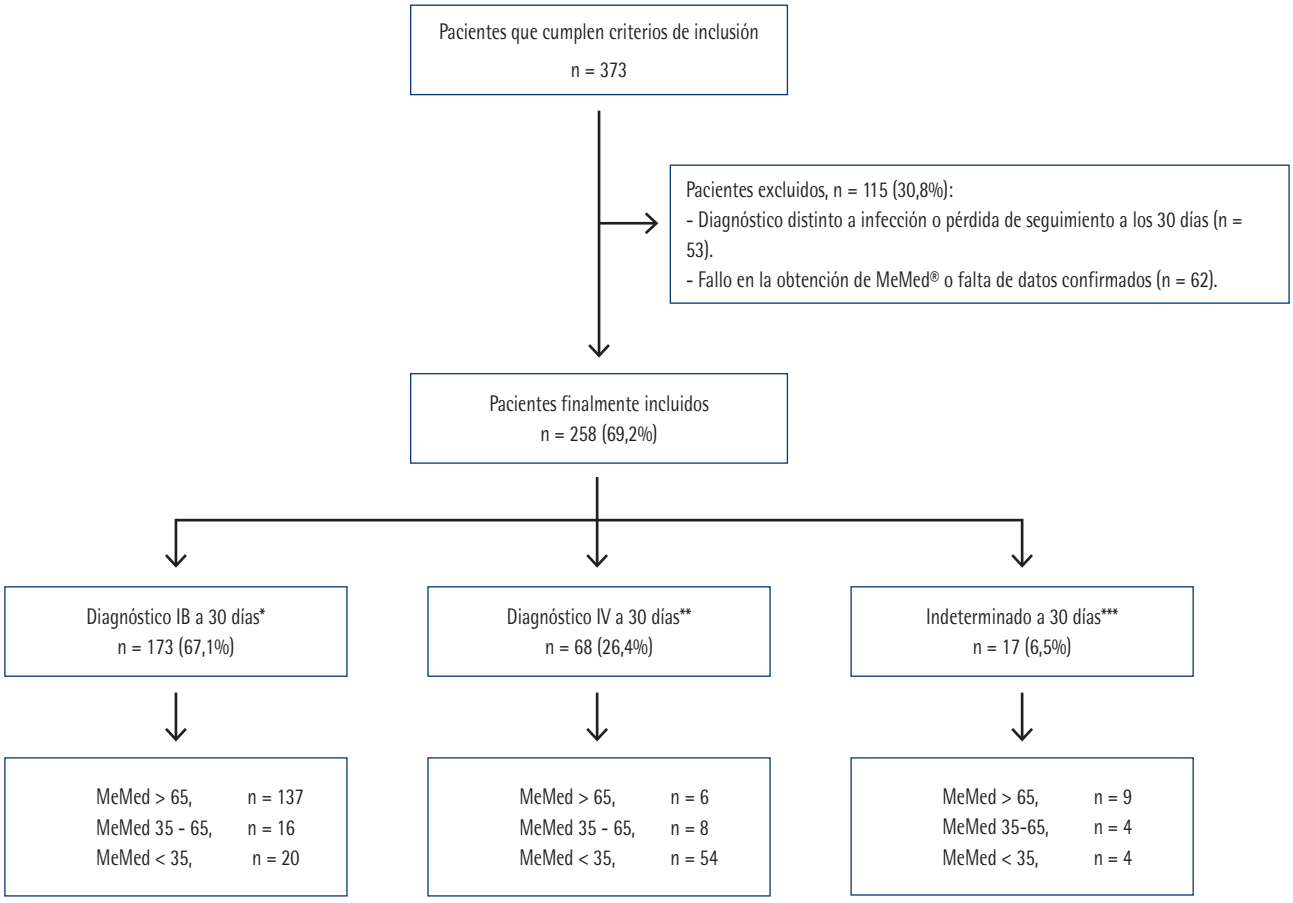


Figura 1 | **Diagrama de flujo de la inclusión de casos**

IB: infección bacteriana; IV: infección viral

***: Incluye a los pacientes con diagnóstico de IB confirmado microbiológicamente y aquellos con la sospecha de IB en urgencias que se mantuvo a los 30 días (por unanimidad por el grupo formado por un/a médico de urgencias, un/a de microbiología, un/a del laboratorio de análisis clínicos y un/a de medicina interna).**

****: Incluye a los pacientes con diagnóstico de IV confirmado microbiológicamente y aquellos con la sospecha de IV en urgencias que se mantuvo a los 30 días (por unanimidad por el grupo evaluador descrito antes).**

*****: Pacientes con diagnóstico de IB o IV sospechados en urgencias sin confirmación microbiológica y en los que el grupo evaluador no llegó a un consenso por unanimidad o se planteó posibilidad de coinfección (IB + IV).**

(CP–) para cada resultado estudiado, así como sus IC 95% por métodos binomiales exactos y por el de Taylor para los CP.

El análisis estadístico se realizó con los programas IBM-SPSS® Statistics 29 para Windows y STATA 17.0.

Consideraciones éticas. El estudio ha seguido todos los protocolos y normas de nuestro centro e internacionales (Declaración de Helsinki) para la utilización de los datos de los pacientes que se codificaron para asegurar la confidencialidad de los mismos. Se revisó la historia clínica informatizada y de atención primaria cuando se requirió. El estudio fue aprobado por el Comité Ético de Investigación Clínica con medicamentos (CEIm) del Hospital Universitario de Toledo (nº: 1075/2023). Se informó oralmente y por escrito al paciente o sus familiares y se solicitó consentimiento informado previo a la inclusión. El estudio no supuso ninguna intervención terapéutica ni tuvo ninguna implicación clínica.

RESULTADOS

Durante el periodo de estudio se seleccionaron por oportunidad 373 pacientes que cumplían con los criterios de inclusión inicialmente. De estos, se excluyeron 115 (30,8%) por perderse en el seguimiento de 30 días o cambiar o añadir otro diagnóstico distinto a un proceso infeccioso por un lado, o

Tabla 3 **Características clínico-epidemiológicas, de comorbilidad, de evolución y analíticas estudiadas en la primera valoración del paciente en el SUH (análisis univariable)**

	Total n=258	Diagnóstico indeterminado n=17 (6,5%)	Diagnóstico infección bacteriana n= 173 (67,1%)	Diagnóstico infección viral n= 68 (26,4%)	Valor p*
DATOS DEMOGRÁFICOS-EPIDEMIOLÓGICOS					
Edad (años), media (DE)	68,28 (19,53)	70,47 (17,05)	69,03 (19,33)	65,79 (20,61)	0,252
Edad >65 años, (%)	162 (62,8)	10 (58,8)	112 (64,7)	40 (58,8)	0,652
Género masculino, (%)	148 (57,4)	10 (58,8)	99 (57,2)	39 (57,4)	0,952
Institucionalizado, (%)	42 (16,3)	4 (23,5)	29 (16,8)	9 (13,2)	0,302
Toma de AB en mes previo, (%)	101 (39,1)	8 (47,1)	66 (38,2)	27 (39,7)	0,768
Ingreso en el último mes previo, (%)	48 (18,6)	3 (17,6)	31 (17,9)	14 (20,6)	0,654
COMORBILIDADES					
Índice de Charlson[a] [media (DE)]	4,78 (3,16)	5,65 (3,98)	4,83 (3,15)	4,83 (3,15)	0,352
Índice de Charlson ≥ 3, (%)	188 (72,86)	13 (76,47)	129 (74,56)	129 (74,56)	0,084
DATOS CLÍNICOS Y DE GRAVEDAD					
Temperatura en grados centígrados [media (DE)]	36,86 (0,94)	36,42 (0,56)	36,80 (0,81)	37,13 (1,23)	**0,015**
Temperatura > 38,3°C, (%)	25 (9,7)	1 (5,9)	15 (8,7)	9 (13,2)	0,233
FC en lpm [media (DE)]	96,22 (21,24)	95,47 (17,77)	97,67 (21,76)	93,96 (20,50)	0,216
FC > 90 lpm, (%)	153 (59,3)	7 (41,2)	108 (62,4)	38 (55,9)	0,188
FR en rpm [media (DE)]	23,59 (6,5)	24,14 (6,46)	23,86 (6,70)	22,85 (6,30)	0,417
FR ≥ 22 rpm, (%)	145 (56,6)	11 (68,8)	94 (54,7)	40 (58,8)	0,505
Alteración de la consciencia ECG ≤ 14, (%)	69 (19,9)	2 (0,6)	41 (14,0)	28 (51,9)	**0,008**
PAS en mmHg [media (DE)]	118,66 (27,06)	121,18 (32,64)	115,76 (26,28)	125,41 (26,70)	**0,011**
PAS < 100 mmHg, (%)	75 (29,1)	5 (29,4)	57 (32,9)	13 (19,1)	0,083
Criterios de sepsis (SRIS ≥2), (%)	167 (64,7)	7 (41,2)	123 (71,1)	37 (54,4)	**0,006**
qSOFA ≥2, (%)	72 (27,9)	6 (35,3)	56 (32,4)	10 (14,7)	**0,018**
Criterios Shock séptico (Sepsis-3), (%)	17 (6,6)	1 (5,9)	16 (9,2)	0 (0,0)	**0,004**
Náuseas/vómitos, (%)	57 (22,1)	8 (47,1)	38 (22,0)	11 (16,2)	**0,023**
Escalofríos/Tiritona, (%)	108 (41,9)	4 (23,5)	73 (42,2)	31 (45,6)	0,254
DATOS DE EVOLUCIÓN Y DESTINO					
Días desde inicio de la clínica [media (DE)]	3,55 (3,23)	3,88 (3,60)	3,65 (3,36)	3,22 (2,79)	0,348
Destino inicial de los pacientes					**<0,001**
Alta	53 (20,5)	5 (29,4)	17 (9,8)	31 (45,6)	
Observación-Unidad corta estancia	26 (10,1)	1 (5,9)	16 (9,2)	9 (13,2)	
Planta de hospitalización convencional	152 (58,9)	9 (52,9)	116 (67,1)	27 (39,7)	
Unidad de cuidados intensivos	16 (6,2)	1 (5,9)	14 (8,1)	1 (1,5)	
Quirófano	10 (3,9)	1 (5,9)	9 (5,2)	0 (0,0)	
Éxitus en urgencias	1 (0,4)	0 (0,0)	1 (0,6)	0 (0,0)	
Reingreso en 30 días tras atención en SUH, n (%)	33 (12,8)	4 (23,5)	21 (12,4)	8 (11,8)	0,729
Estancia hospitalaria en días [media (DE)]	7,72 (8,47)	10,71 (18,64)	8,65 (7,37)	4,60 (6,18)	**<0,001**
Mortalidad durante 30 días tras atención en SUH	36 (14,0)	3 (17,6)	28 (16,2)	5 (7,4)	**0,048**
HALLAZGOS ANALÍTICOS Y MICROBIOLÓGICOS					
Bacteriemia verdadera[b], n (%)	47 (18,2)	2 (11,7)	45 (26,1)	0 (0,0)	**<0,001**
Creatinina en mg/dl [media (DE)]	1,38 (1,04)	1,52 (1,41)	1,47 (1,14)	1,13 (0,54)	**0,023**
Leucocitos por mm^3 [media (DE)]	12.603 (6.544)	12.606 (7.886)	14.112 (6.662)	8.538 (3.795)	**<0,001**
Neutrófilos (% de los leucocitos) [media (DE)]	80,22 (14,03)	72,80 (21,15)	82,21 (13,79)	77,01 (11,21)	**0,006**
Monocitos (% de los leucocitos) [media (DE)]	7,34 (5,48)	9,81 (11,55)	6,57 (4,91)	8,68 (4,10)	**0,002**
Linfocitos (% de los leucocitos) [media (DE)]	11,50 (11,28)	15,15 (11,87)	10,49 (12,05)	13,16 (8,53)	**0,096**
Plaquetas por mm^3 [media (DE)]	230.291 (101.369)	240.118 (108.662)	241.486 (105.615)	199.353 (81.623)	**0,003**
Lactato sérico en mmol/l ([media (DE)]	19,34 (14,24)	19,46 (17,40)	20,52 (15,17)	15,18 (7,62)	**0,029**
Proteína C reactiva en mg/L [media (DE)]	118,6 (116,6)	79,8 (102,3)	152,1 (122,2)	43,2 (48,1)	**<0,001**
Procalcitonina en ng/ml [media (DE)]	4,14 (13,40)	0,86 (1,73)	5,84 (16,01)	0,57 (2,23)	**<0,001**
Procalcitonina ≥ 0,25 ng/ml, n (%)	137 (54,6)	7 (43,8)	116 (68,6)	14 (21,2)	**<0,001**
Procalcitonina ≥ 0,5 ng/ml, n (%)	106 (42,4)	5 (31,3)	94 (55,6)	7 (10,6)	**<0,001**
Puntuación MeMed® [media (DE)]	62 (39)	65 (35)	79 (29)	18 (27)	**<0,001**
MeMed® < 35, n (%)	78 (30,23)	4 (23,52)	20 (11,56)	54 (79,41)	**<0,001**
MeMed® 35 - 65, n (%)	28 (10,85)	4 (23,52)	16 (9,24)	8 (11,76)	**<0,001**
MeMed® > 65, n (%)	152 (58,91)	9 (52,94)	137 (79,19)	6 (8,82)	**<0,001**

SUH: servicio de urgencias hospitalario; DE: desviación estándar; n: número de casos; AB: antibióticos; C: centígrados; FC: frecuencia cardiaca; lpm: latidos por minuto; FR: frecuencia respiratoria; rpm: respiraciones por minuto; máx: máximo; ECG: escala del coma de Glasgow; PAS: presión arterial sistólica; SRIS: síndrome de respuesta inflamatoria sistémica; qSOFA: quick Sepsis-related Organ Failure Assessment. *contraste bilaterales entre los grupos infección bacteriana frente a infección viral. [a]Índice de Charlson: ponderado por la edad (referencia 28). [b] Bacteriemia verdadera: definida según criterios de anteriores estudios de INFURG-SEMES (referencia 29). Criterios de sepsis (SRIS ≥ 2) según conferencia de Consenso de 2001 (referencia 1). Criterios de sepsis (qSOFA ≥ 2) según la tercera conferencia de consenso (Sepsis-3) (referencia 1)

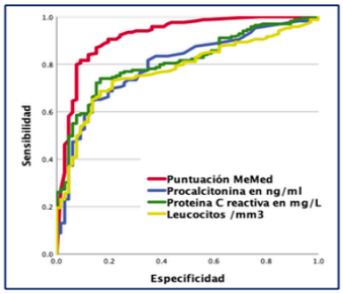


Figura 2a	ABC-COR (IC 95%)	Valor de p
MeMed® (puntuación)	0,920 (0,877-0,962)	< 0,001
Procalcitonina (ng/ml)	0,811 (0,754-0,867)	< 0,001
PCR (mg/L)	0,796 (0,735-0,857)	< 0,001
Leucocitos (mm^3)	0,778 (0,717-0,839)	< 0,001

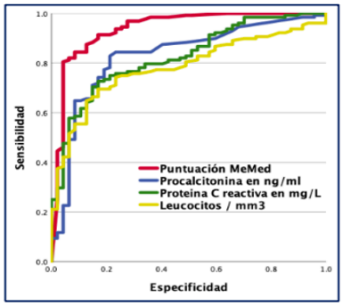


Figura 2b	ABC-COR (IC 95%)	Valor de p
MeMed® (puntuación)	0,938 (0,892-0,984)	< 0,001
Procalcitonina (ng/ml)	0,829 (0,758-0,900)	< 0,001
PCR (mg/L)	0,824 (0,759-0,888)	< 0,001
Leucocitos (mm^3)	0,782 (0,713-0,851	< 0,001

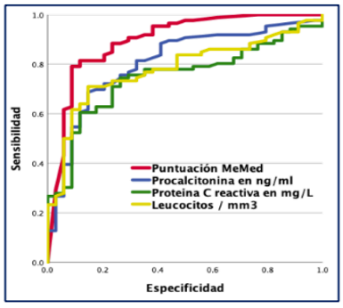


Figura 2c	ABC-COR (IC 95%)	Valor de p
MeMed® (puntuación)	0,896 (0,827-0,964)	< 0,001
Procalcitonina (ng/ml)	0,806 (0,721-0,892)	< 0,001
PCR (mg/L)	0,747 (0,657-0,837)	< 0,001
Leucocitos (mm^3)	0,780 (0,695-0,865	< 0,001

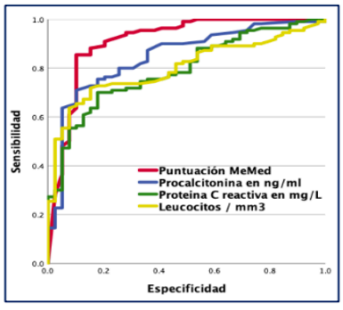


Figura 2d	ABC-COR (IC 95%)	Valor de p
MeMed® (puntuación)	0,910 (0,847-0,972)	< 0,001
Procalcitonina (ng/ml)	0,847 (0,776-0,917)	< 0,001
PCR (mg/L)	0,792 (0,717-0,868)	< 0,001
Leucocitos (mm^3)	0,800 (0,729-0,871)	< 0,001

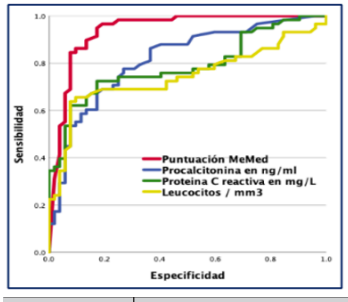


Figura 2e	ABC-COR (IC 95%)	Valor de p
MeMed® (puntuación)	0,938 (0,891-0,986)	< 0,001
Procalcitonina (ng/ml)	0,815 (0,735-0,895)	< 0,001
PCR (mg/L)	0,787 (0,701-0,873)	< 0,001
Leucocitos (mm^3)	0,744 (0,647-0,840)	< 0,001

Figura 2 Capacidad diagnóstica de infección bacteriana en pacientes atendidos en el servicio de urgencias por infección

2a : todos los pacientes; 2b : grupo exclusión LIAISON-MeMed; 2c : grupo confirmación microbiológica; 2d: grupo ≥ 65 años; 2e: grupo pacientes con infección respiratoria de vías bajas; El valor de p indica el riesgo de error tipo I en el contraste de la hipótesis nula de que el ABC-ROC es igual a 0,5.; ABC-COR: área bajo la curva de la capacidad operativa del receptor; IC 95%: intervalo de confianza del 95%; PCR: proteína C reactiva

Tabla 4 **Posibles focos/diagnósticos clínico en el servicio de urgencias en los grupos según sospecha etiológica**

Foco/diagnóstico clínico en el Servicio de Urgencias	Total n = 258	Diagnóstico indeterminado n=17 (6,5%)	Diagnóstico infección bacteriana n= 173 (67,1%)	Diagnóstico infección viral n= 68 (26,4%)
Infección respiratoria de vías bajas, n (%)	115 (44,6)	4 (23,5)	58 (33,5)	53 (77,9)
Infección del tracto urinario, n (%)	60 (23,3)	4 (23,5)	56 (32,4)	0 (0,0)
Infección abdominal[a],n (%)	34 (13,2)	1 (5,9)	28 (16,2)	5 (7,3)
Fiebre o sospecha de infección de origen desconocido, n (%)	29 (11,2)	3 (17,6)	20 (11,6)	6 (8,8)
Infección de piel y partes blandas, n (%)	14 (5,4)	4 (23,5)	6 (3,5)	4 (5,9)
Otros focos[b], n (%)	6 (2,3)	1 (5,9)	5 (2,9)	0 (0,0)

[a]**Gastroenteritis, colitis infecciosa, colangitis o colecistitis, hepatitis, apendicitis**

[b]**Otorrinolaringológico, sospecha de endocarditis, infección de dispositivos vasculares, etc.**

bien por no obtenerse adecuadamente y de forma validada el resultado de MeMed®. Finalmente, se incluyeron 258 pacientes (Figura 1). De éstos, 36 pacientes (14,0%) fallecieron durante los 30 días posteriores a su consulta en el SUH. La edad media fue 68,28 (DE 19,53), el 57,4% (148) eran hombres.

Las características demográficas, epidemiológicas, de comorbilidad, datos clínicos (signos y síntomas) y de gravedad, destino y evolución durante 30 días, así como las pruebas analíticas, resultados de los hemocultivos y del test MeMed® se muestran en la tabla 3.

Se encontraron diferencias significativas al comparar los pacientes en función del diagnóstico final de IB con el resto en las siguientes variables: temperatura, alteración del nivel de consciencia, PAS <100 mmHg, qSOFA ≥2, SRIS ≥ 2, criterios de shock séptico (sepsis-3), existencia de náuseas/vómitos, destino del paciente, estancia hospitalaria, mortalidad a los 30 días, bacteriemia verdadera y en distintos resultados del laboratorio (creatinina ≥2 mg/dl, lactacidemia, recuento de plaquetas, recuento de leucocitos y proporciones de monocitos y neutrófilos, así como en las concentraciones de PCR, PCT (y dicotomizado para ≥ 0,25 y ≥ 0,5 ng/ml) y MeMed® (y dicotomizado para <35, 35-65 y >65). En 45 (26,1%) de las IB y 2 (11,7%) del grupo indeterminado, hubo aislamiento significativo en los hemocultivos.

El posible foco u origen clínico de presunción en el SUH en relación a los pacientes con diagnóstico final de IB o IV a los 30 días se muestra en la tabla 4.

En la figura 2 se describen los valores de ABC-COR del recuento leucocitos, de los BMRIel estudiados (PCR, PCT) y del test MeMed® para la capacidad de diagnóstico de IB en todos los pacientes atendidos en el SUH por un proceso infeccioso (Figura 2a), así como en el grupo exclusión LIAISON-MeMed (Figura 2b) con 237 pacientes (una vez excluidos 21 que cumplían alguno de los criterios de exclusión), en los pacientes en los que se consiguió confirmación microbiológica (155) (Figura 2c), en los 162 pacientes con ≥ 65 años (Figura 2d) y en los 115 pacientes con IRVB (neumonías, EPOC agudizado, bronquitis) (Figura 2e). Para el grupo con bacteriemia verdadera (47 aislamientos) el ABC-COR es de 0,936 (0,859-1,000), p <0,001.

Los mejores resultados en todos los grupos (2a, 2b, 2c, 2d y 2d) por orden de rendimiento los obtiene el test MeMed®, seguido de la PCT. El ABC-COR que consigue MeMed® en el grupo que analiza todos los pacientes es de 0,920 (IC 95%: 0,877-0,962) y la PCT de 0,811 (IC 95%: 0,754-0,867). En la tabla 5 se pueden consultar todos los valores de rendimiento diagnóstico de los PC definidos para MeMed® previamente y el encontrado por el índice de Youden, así como para la PCT, en el grupo que incluye todos los pacientes.

Finalmente, por su interés, se analizaron y compararon los resultados de rendimiento diagnóstico de IB de la PCT, la PCR y MeMed® en el grupo de los 28 pacientes con resultado dudoso (rango 35-65 puntos de MeMed®). Se obtuvieron los siguientes resultados: Para la PCT un ABC-COR de 0,622 (IC 95%: 0,376-0,869), p=0,371; para la PCR un ABC de 0,469 (IC 95%: 0,210-0,729), p=0,823; y para MeMed® un ABC-COR de 0,500 (IC 95%: 0,213-0,787), p=1.

DISCUSIÓN

Los resultados del presente estudio nos permiten confirmar la gran capacidad diagnóstica de infección bacteriana que ofrecen algunos BMRIel, como la PCT [9,10,12] y sobre todo el nuevo test MeMed®, para los pacientes atendidos en los SUH con la sospecha de sufrir un proceso infeccioso [13-26]. En este estudio, LIAISON-MeMed® se presenta como una prueba novedosa que obtiene el mejor rendimiento diagnóstico de infección bacteriana frente a aquellos pacientes con infección de origen viral y los no infecciosos [13,14]. Este hecho ya había sido señalado por otros autores para los pacientes adultos [21-27], así como para los pacientes pediátricos [15-20].

Se trata de la primera prueba de diagnóstico que incluye una puntuación de 0 a 100 basada en la combinación de la concentración de 3 proteínas circulantes en sangre (por una fórmula matemática surgida de un modelo de regresión). Di-

Tabla 5 **Puntos de corte y rendimiento para el rendimiento diagnóstico de infección bacteriana**

TODOS LOS PACIENTES n = 258	ABC-COR (IC 95%)	Se % (IC 95%)	Es % (IC 95%)	VPP % (IC 95%)	VPN % (IC 95%)	CP+ (IC 95%)	CP- (IC 95%)
Puntuación MeMed > 65	0,857 (0,803-0,911)	79,2 (72,2-84,8)	91,2 (81,1-96,49)	95,8 (90,7-98,3)	63,3 (52,9-72,6)	8,97 (4,21-19,32)	0,23 (0,17-0,31)
Puntuación MeMed > 90	0,801 (0,741-0,861)	57,0 (49,3-64,5)	95,9 (87,2-99,2)	97,4 (91,3-99,5)	46,8 (38,4-55,4)	13,24 (4,61-39,42)	0,45 (0,38-0,54)
Puntuación MeMed > 50*	0,852 (0,797-0,907	84,1 (77,4-89,3)	88,2 (77,6-94,4)	94,7 (89,4-97,5)	65,9 (55,2-75,3)	6,98 (3,63-13,43)	0,20 (0,15-0,28)
Procalcitonina ≥ 0,25 ng/ml	0,787 (0,707-0,858)	70,7 (62,9-77,4)	80,8 (68,7-89,6)	91,2 (84,3-95,8)	51,5 (41,7-61,4)	3,24 (2,01-5,21)	0,40 (0,31-0,51)
Procalcitonina ≥ 0,50 ng/ml	0,725 (0,659-0,791)	57,6 (49,8-65,2)	89,4 (78,8-95,3)	93,1 (85,8-96,9)	46,1 (37,6-54,9)	5,44 (2,77-10,9)	0,50 (0,41-0,60)

***: punto de corte obtenido por el índice de Youden**

Se: sensibilidad; Es: especificidad; VPP: valor predictivo positivo; VPN: valor predictivo negativo;

CP+: cociente de probabilidad positivo; CP-: cociente de probabilidad negativo; IC 95%: intervalo de confianza al 95%

chos BMRIel son inducidos tanto por virus como por bacterias [13-27]: el TRAIL que se eleva como expresión de IV y disminuye en IB, la IP-10 que se incrementa en mayor medida en IV y en menor medida en las bacterianas, y la PCR que muestra un patrón opuesto a la IP-10 [13,14]. Este modelo representa un claro ejemplo de la "sinergia de los BMRIel" que aumenta y supera el rendimiento diagnóstico de cada uno de ellos individualmente [12].

LIAISON-MeMed® puede convertirse en una nueva herramienta de ayuda cuando las manifestaciones clínicas de los procesos infecciosos son inespecíficas y variables, lo que dificulta el reconocimiento precoz de estos enfermos y, asimismo, de su posible etiología bacteriana o viral [1,2]. De forma, que se puede mejorar la adecuación de la indicación de antibioterapia desde los SUH en todos los pacientes, pero sobre todo en los más vulnerables [2,5,30]. El test puede tener sus resultados al mismo tiempo que el resto de la analítica urgente (y otros BMRIel) utilizando los aparatos de inmunoensayo automatizado que habitualmente disponen los centros hospitalarios para su analítica urgente [13-15]. En este sentido, con los datos comunicados de nuestro laboratorio y de los fabricantes, se estima que en este momento en España la realización de la prueba urgente tendría un coste de 30-40€, según si se realiza individualmente o junto con otras determinaciones urgentes. Por lo que serían necesarios estudios de coste-efectividad donde se pueda valorar una reducción y adecuación del uso de antibióticos y/o del destino del paciente que demuestren la rentabilidad y eficiencia del uso de la prueba.

Los resultados de nuestro estudio, en todos los pacientes evaluados (figura 2a), son muy relevantes, MeMed® consigue un ABC-COR excelente de 0,920 que no difiere del grupo que excluye a los pacientes indicados por el fabricante (figura 2b) con ABC-COR de 0,938 o la de los diagnosticados de un cuadro de IVRB (figura 2e), también con 0,938. Y son ligeramente superiores de los del grupo de pacientes con ≥ 65 años (figura 2d) con un ABC-COR de 0,910 y del grupo que tuvo una confirmación microbiológica (figura 2c) con un ABC-COR de 0,896 (aunque estos siguen siendo muy buenos). En cuanto a los resultados de los PC evaluados como referencia por artículos previos y el fabricante [13-26], cabe destacar que con PC > 65 puntos del test MeMed® en nuestra muestra obtiene una Se de 79,2% y Es de 91,2% y con PC > 90 puntos se pierde bastante Se (57%), pero se gana en Es (95,9%). Pero, aplicando el índice de Youden el PC > 50 puntos consigue una interesante Se de 84,1% y Es 88,2%. Por lo que creemos que en futuros estudios habrá que seguir explorando y analizando distintos PC para valorar y validar estos en los distintos subgrupos. Por su parte, la PCT tanto con el PC ≥ 0,25 ng/ml, señalado por una reciente revisión sistemática como el más indicado para predecir el diagnóstico de IB [10], como el PC ≥ 0,50 ng/ml como el más adecuado para predecir bacteriemia [9], en nuestro estudio confirma un menor rendimiento con diferencias significativas respecto a los PC de 65 y 90 puntos de MeMed®. Y, aunque no ha sido publicado en otros estudios, el ABC-COR de 0,936 en el grupo de pacientes con bacteriemia verdadera, abre otra línea interesante para explorar en un futuro con el test MeMed®.

En uno de los primeros estudios publicados, Oved et al [13], consiguen un ABC de 0,94 (IC 95%: 0,92-0,96) para todos los pacientes y, también, en los subgrupos de adultos, de confirmación microbiológica e IRVB. Y, como en nuestro estudio, con un significativo mejor rendimiento que la PCT, la PCR y el recuento de leucocitos. Asimismo, Ashkenazi-Hoffnung et al [22], también comunican unos resultados excelentes para MeMed® con un ABC superior a 0,92 para pacientes adultos, con una Se del 92,6% y una Es de 95,7%, muy superiores a los conseguidos por

la PCT y la PCR para distinguir entre etiologías bacterianas y virales en pacientes con infecciones respiratorias y fiebre sin foco conocido. De forma que estos autores estimaron que se podría reducir el uso de los AB innecesarios en un 88% de los casos [22]. En esta misma línea, Stein et al [21], en una muestra de 124 casos de IRVB en adultos y niños, consigue una Se del 93% y Es del 91%, superando a la PCR y leucocitos. Más recientemente, Halibi et al [23], en una muestra de 415 IRVB en adultos, publican unos resultados muy relevantes para proponer el test MeMed® como una prueba de cribado en el SUH para diferenciar las IB de las IV e indicar adecuadamente la administración de antibioterapia, al conseguir una Se del 98,1%, Es de 88,4% y en VPN de 98,8%.

Finalmente, en un estudio publicado en el último año, Bachur et al [27], en un trabajo prospectivo, multicéntrico y ciego sobre 314 adultos y 162 niños, tras conseguir una Se de 90%, Es de 92,8% y un VPN de 98,8%, concluyen que el test MeMed® es útil en adultos y niños atendidos en el SUH para distinguir el origen bacteriano del viral en distintos procesos infecciosos y para decidir la administración de antibióticos o no.

Aunque, como hemos comentado, sí se ha comparado en otros estudios los resultados en pacientes con la sospecha clínica de infección bacteriana (consensuada por paneles de expertos) y aquellos con confirmación microbiológica o, específicamente, en pacientes con IRVB, es la primera vez que se analiza el test MeMed® en el subgrupo de pacientes ≥ 65 años y con bacteriemia, específicamente.

Nuestro estudio tiene distintas limitaciones. Las principales son, por una parte, el carácter unicéntrico del estudio y, por otra, el haber reclutado los casos por oportunidad (cuando los investigadores estaban de guardia) en una muestra limitada (258 casos). Por ello, existe la posibilidad de un sesgo de selección al depender la inclusión de los pacientes de la presencia de uno de los investigadores, aunque esta fuera consecutiva durante esos periodos de tiempo. Por otro lado, se trata de una muestra heterogénea con distintos procesos (IRBV, ITU, infecciones abdominales, fiebre sin foco, etc.) y múltiples patógenos bacterianos y virales y no se han analizado las características diferenciales entre estos grupos.

A pesar de estas limitaciones, creemos que el estudio es un reflejo de la realidad clínica de nuestros SUH y de los pacientes que se atienden habitualmente. Y es que, a pesar de los avances en el diagnóstico de enfermedades infecciosas, la identificación oportuna de infecciones bacterianas sigue siendo un desafío, así como mejorar la adecuación de la administración de la antibioterapia precoz en los SUH.

Por todo ello, es necesario que se realicen estudios prospectivos, multicéntricos y con potencia que puedan validar o matizar estos resultados sobre una prueba novedosa pero prometedora como es MeMed®. En la actualidad, son muchos los grupos como el nuestro que están estudiando nuevos BMRIeI y modelos combinados para proporcionar el mejor rendimiento diagnóstico y pronóstico en la primera evaluación del paciente en el SUH que mejoren y superen las limitaciones de los que se utilizan habitualmente [31-35]. Asimismo, junto con otros BMRIeI, estos futuros estudios deberán evaluar para MeMed® junto al rendimiento diagnóstico de IB, el predictivo de bacteriemia y el pronóstico (gravedad, ingreso en UCI, mortalidad) con el objetivo de encontrar el BMRIeI o combinación de estos que sea eficaz y eficiente para la práctica clínica habitual en los SUH [12,32,33].

Como conclusiones de este estudio podemos señalar que la prueba de LIAISON MeMed® podría ser útil para diagnosticar el origen bacteriano en los pacientes adultos atendidos con sospecha clínica de infección en el SUH, así como que obtiene un mejor rendimiento que la PCT, la PCR y el recuento de leucocitos.

FINANCIACIÓN

Los reactivos para medir LIAISON®MeMed®fueron cedidos por Diasorin.

Nadie de la compañía participó en el diseño del estudio ni en la evaluación de sus resultados ni condicionó ninguna fase de su elaboración. Este manuscrito no ha recibido ninguna financiación por parte de ningún organismo ni público ni privado.

CONFLICTO DE INTERESES

Los autores declaran la ausencia de conflictos de intereses en relación con el presente artículo.

AJJ ha participado en reuniones científicas organizadas por Roche, Thermo Scientific Biomarkers, B.R.A.H.M.S. AG, ViroGates y Biomerieux. Ningún autor ha recibido compensación económica por participar en este trabajo.

BIBLIOGRAFÍA

1. Julián-Jiménez A, Supino M, López Tapia JD, Ulloa González C, Vargas Téllez LE, González del Castillo J, et al. Puntos clave y controversias sobre la sepsis en los servicios de urgencias: propuestas de mejora para Latinoamérica. Emergencias 2019;31:123-135. PMID: 30963741.
2. Julián-Jiménez A, Gorordo-Delsol LA, Merinos-Sánchez G, Santillán-Santos DA, Rosas Romero FA, Sánchez Arreola D, et al. Declaración de Guadalajara: una visión constructiva desde el servicio de urgencias a partir de la Surviving Sepsis Campaign 2021. Emergencias. 2023;35:53-64. PMID: 36756917.
3. Rudd KE, Johnson SC, Agesa KM, Shackelford KA, Tsoi D, Kievlan DR, et al. Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. Lancet. 2020; 395(10219): 200-11. doi: 10.1016/S0140-6736(19)32989-7.
4. Bauer M, Gerlach H, Vogelmann T, Preissing F, Stiefel J, Adam D. Mortality in sepsis and septic shock in Europe, North America and Australia between 2009 and 2019–results from a systematic review and meta-analysis. Crit Care. 2020;24:239. doi: 10.1186/s13054-020-02950-2.

5. Evans L, Rhodes A, Alhazzani W, Antonelli M, Coopersmith CM, French C, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. Intensive Care Med. 2021;47:1181-247. doi: 10.1007/s00134-021-06506-y. .

6. Carbó Díez M, Osorio Quispe G, Fresco Quindós L, Miota Hernández N, Perea Gainza M, Ortega Romero MM. Cumplimiento del paquete de medidas en las primeras tres horas en urgencias y su relación con el pronóstico de los pacientes mayores de 65 años con sepsis. Emergencias. 2024;36:179-87 doi: 10.55633/s3me/009.2024. PMID: 38818983.

7. Martínez-de la Cruz P, Moreno-Núñez L, Álvarez-Atienza S, Sanz-Márquez S, Valverde-Canovas JF, Losa- García JE. Antibiotic discontinuation through an antibiotic treatment optimization program in emergency department patients with low suspicion of infection. Enferm Infecc Microbiol Clin (Engl Ed). 2023;41:423-425. doi: 10.1016/j.eimce.2022.10.004. .

8. Rodríguez M.R, Llopis Roca F, Rubio Díaz R, García DE, Julián-Jiménez A. Administración precoz de la antibioterapia empírica en los pacientes atendidos con sospecha de sepsis en el servicio de urgencias: una revisión sistemática. Emergencias. 2024; doi: 10.55633/s3me/092.2024

9. Julián-Jiménez A, Eduardo García D, Merinos-Sánchez G, García de Guadiana-Romualdo L, González Del Castillo J. Precisión diagnóstica de la procalcitonina para la bacteriemia en el servicio de urgencias: una revisión sistemática. Rev Esp Quimioter. 2024;37:29-42. doi: 10.37201/req/099.2023.

10. Julián-Jiménez A, García de Guadiana-Romualdo L, Merinos-Sánchez G, Eduardo García D. Precisión diagnóstica de la procalcitonina para la infección bacteriana en el servicio de urgencias: una revisión sistemática. Rev Clin Esp. 2024;224:400-16. doi: 10.37201/req/099.2023.

11. Cancella de Abreu M, Freund Y. Conjunto de medidas para la sepsis y consideraciones pragmáticas desde el servicio de urgencias. Emergencias. 2024;36:164-5 doi: 10.55633/s3me/015.2024.

12. Julián-Jiménez A, Candel-González FJ, González del Castillo J. Utilidad de los biomarcadores de inflamación e infección en los servicios de urgencias. Enferm Infecc Microbiol Clin. 2014;32:177–190. doi: 10.1016/j.eimc.2013.01.005.

13. Oved K, Cohen A, Boico O, Navon R, Friedman T, Etshtein L, et al. A novel host-proteome signature for distinguishing between acute bacterial and viral infections. PLoS One. 2015;10:e0120012. doi: 10.1371/journal.pone.0120012.

14. Eden E, Srugo I, Gottlieb T, Navon R, Boico O, Cohen A, et al. Diagnostic accuracy of a TRAIL, IP-10 and CRP combination for discriminating bacterial and viral etiologies at the Emergency Department. J Infect. 2016;73:177-80. doi: 10.1016/j.jinf.2016.05.002.

15. Papan C, Argentiero A, Porwoll M, Hakim U, Farinelli E, Testa I, et al. A host signature based on TRAIL, IP-10, and CRP for reducing antibiotic overuse in children by differentiating bacterial from viral infections: a prospective, multicentre cohort study. Clin Microbiol Infect. 2022;28:723-730. doi: 10.1016/j.cmi.2021.10.019. .

16. van Houten CB, de Groot JAH, Klein A, Srugo I, Chistyakov I, de Waal W, et al. A host-protein based assay to differentiate between bacterial and viral infections in preschool children (OPPORTUNITY): a double-blind, multicentre, validation study. Lancet Infect Dis. 2017;17:431-440. doi: 10.1016/S1473-3099(16)30519-9.

17. Chokkalla AK, Tam E, Liang R, Cruz AT, Devaraj S. Validation of a multi-analyte immunoassay for distinguishing bacterial vs. viral infections in a pediatric cohort. Clin Chim Acta. 2023;546:117387. doi: 10.1016/j.cca.2023.117387. Erratum in: Clin Chim Acta. 2023;548:117468. doi: 10.1016/j.cca.2023.117468.

18. Ibrahim RB, Ona H, Chokkalla AK, Tam E, Devaraj S. Validation of a whole blood machine learning strategy for distinguishing between bacterial and viral infection in a pediatric hospital setting. Pract Lab Med. 2024;39:e00387. doi: 10.1016/j.plabm.2024.e00387.

19. Lacroix L, Papis S, Mardegan C, Luterbacher F, L'Huillier A, Sahyoun C, et al. Host biomarkers and combinatorial scores for the detection of serious and invasive bacterial infection in pediatric patients with fever without source. PLoS One. 2023;18:e0294032. doi: 10.1371/journal.pone.0294032.

20. Klein A, Shapira M, Lipman-Arens S, Bamberger E, Srugo I, Chistyakov I, et al. Diagnostic Accuracy of a Real-Time Host-Protein Test for Infection. Pediatrics. 2023;152:e2022060441. doi: 10.1542/peds.2022-060441.

21. Stein M, Lipman-Arens S, Oved K, Cohen A, Bamberger E, Navon R, et al. A novel host-protein assay outperforms routine parameters for distinguishing between bacterial and viral lower respiratory tract infections. Diagn Microbiol Infect Dis. 2018;90:206-213. doi: 10.1016/j.diagmicrobio.2017.11.011.

22. Ashkenazi-Hoffnung L, Oved K, Navon R, Friedman T, Boico O, Paz M, et al. A host-protein signature is superior to other biomarkers for differentiating between bacterial and viral disease in patients with respiratory infection and fever without source: a prospective observational study. Eur J Clin Microbiol Infect Dis. 2018;37:1361-1371. doi: 10.1007/s10096-018-3261-3.

23. Halabi S, Shiber S, Paz M, Gottlieb TM, Barash E, Navon R, et al. Host test based on tumor necrosis factor-related apoptosis-inducing ligand, interferon gamma-induced protein-10 and C-reactive protein for differentiating bacterial and viral respiratory tract infections in adults: diagnostic accuracy study. Clin Microbiol Infect. 2023;29:1159-1165. doi: 10.1016/j.cmi.2023.05.033.

24. Novak D, Masoudi A, Shaukat B, Kabariti S, Drapkin J, Christian M, et al. MeMed BV testing in emergency department patients presenting with febrile illness concerning for respiratory tract infection. Am J Emerg Med. 2023;65:195-199. doi: 10.1016/j.ajem.2022.11.022.

25. Schneider JE, Cooper JT. Cost impact analysis of novel host-response diagnostic for patients with community-acquired pneumonia in the emergency department. J Med Econ. 2022;25:138-151. doi: 10.1080/13696998.2022.2026686.

26. Langedijk AC, Rengerink KO, Harding E, Wensing A, van Slooten R, Israeli Y, et al. Natural variability of TRAIL, IP-10, and CRP in healthy adults - The "HERACLES" study. Cytokine. 2024;176:156530. doi: 10.1016/j.cyto.2024.156530.

27. Bachur RG, Kaplan SL, Arias CA, Ballard N, Carroll KC, Cruz AT, et al. A rapid host-protein test for differentiating bacterial from viral

infection: Apollo diagnostic accuracy study. J Am Coll Emerg Physicians Open. 2024;5:e13167. doi: 10.1002/emp2.13167.

28. Charlson M, Pompei P, Ales KL, McKenzie CR. A new method of classyfing pronostic comorbidity in longitudinal studies: Development and validation. J Chron Dis. 1987;40:373–83. doi: 10.1016/0021-9681(87)90171-8.
29. Julián-Jiménez A, García-Lamberechts EJ, González del Castillo J, Navarro Bustos C, LLopis-Roca F, Martínez-Ortiz de Zárate M, et al. Validación del modelo predictivo de bacteriemia (5MPB-Toledo) en los pacientes atendidos en el servicio de urgencias por infección. Enferm Infecc Microbiol Clin. 2021. doi: 10.1016/j.eimc.2020.12.007.
30. Aguiló Mir S. Identificar al paciente frágil en un servicio de urgencias: un esfuerzo urgente y necesario. Emergencias. 2023;35:165-6. doi: 10.55633/s3me/E058.2023.
31. Bachur RG, Kaplan SL, Arias CA, Ballard N, Carroll KC, Cruz AT, et al. A rapid host-protein test for differentiating bacterial from viral infection: Apollo diagnostic accuracy study. J Am Coll Emerg Physicians Open. 2024;5:e13167. doi: 10.1002/emp2.13167.
32. Clemente C, Fuentes Ferrer ME, Ortega Heredia D, Julián Jiménez A, Martín-Sánchez FJ, González Del Castillo J. Utilidad de la combinación de biomarcadores de respuesta inflamatoria y escalas clínicas para la estratificación del riesgo en pacientes atendidos en urgencias por sospecha de infección. Emergencias. 2024;36:9-16. doi: 10.55633/s3me/04.2023.
33. Yañez Palma MC, Roman F, Llopis-Roca F, Fragiel M, Julián Jiménez A, Martín-Sánchez FJ, et al. Utilidad de la región medial de la pro-adrenomodulina para la detección de bacteriemia verdadera en pacientes mayores atendidos en urgencias por sospecha de infección. Rev Esp Quimioter. 2024;37:78-87. doi: 10.37201/req/110.2023.
34. Castro-Delgado R, Pardo Ríos M. Artificial intelligence and emergency services: We need to take a step forward. Emergencias. 2024;36:145-7. doi: 10.55633/s3me/002.2024.
35. Leticia Fernandez-Carballo B, Escadafal C, MacLean E, Kapasi AJ, Dittrich S. Distinguishing bacterial versus non-bacterial causes of febrile illness - A systematic review of host biomarkers. J Infect. 2021;82:1-10. doi: 10.1016/j.jinf.2021.01.028.

Official journal of the Spanish Society of Chemotherapy

Original breve

Revista Española de Quimioterapia
doi:10.37201/req/026.2024

Rosa Rodríguez-Fernández[1,2]
Felipe González-Martínez[1,2]
Inés Ojeda Velázquez[1]
Marta Rodríguez Díaz[1]
María Victoria Capozzi Bucciol[1]
María Isabel González-Sánchez[1,2]
Jimena Pérez-Moreno[1,2]
Blanca Toledo del Castillo[1,2]

Efectividad de nirsevimab en la prevención de los ingresos por bronquiolitis por virus respiratorio sincitial en lactantes

[1]Servicio de Pediatría, Hospital Infantil Gregorio Marañón
[2]Instituto de Investigación Sanitaria Gregorio Marañón (IISGM), Madrid, España

Article history
Received: 24 February 2024; Revision Requested: 1 April 2024; Revision Received: 27 April 2024; Accepted: 2 September 2024; Published: 23 September 2024

RESÚMEN

Introducción. La bronquiolitis por virus respiratorio sincitial (VRS) es la primera causa de hospitalización en lactantes. Esta temporada disponemos de un anticuerpo monoclonal de vida media prolongada (Nirsevimab) para prevenir esta enfermedad en los lactantes nacidos entre el 1 abril-30 de septiembre 2023, y los nacidos durante la epidemia (octubre 2023-marzo 2024). El objetivo de este estudio fue evaluar el impacto de la implementación de este anticuerpo en los ingresos por VRS en un hospital de tercer nivel.

Material y métodos. Estudio observacional, retrospectivo y analítico. Se incluyeron todos los pacientes <6 meses al inicio de la epidemia en el mes de octubre ingresados por bronquiolitis en 2 tiempos: T1 o Tiempo Pre-nirsevimab: 1 septiembre 2015-30 septiembre 2023 y T2 o Tiempo nirsevimab: 1 octubre-31 diciembre 2023. La población de referencia fueron los ingresos totales debidos a cualquier causa de lactantes <6 meses. Se calculó la reducción del porcentaje de ingresos (RPI) debidos a VRS respecto al total de ingresos en ambos periodos, y se calculo el test doble negativo para calcular la efectividad de la intervención (1-Odds ratio) x 100.

Resultados. En menores de 6 meses de edad encontramos disminución en el numero de ingresos por bronquiolitis VRS entre la ultima temporada y las 7 previas [574/1195 (48%) vs 6/138 (4,3%); p<0,01, RPI: 91%). En la temporada 2023/2024 la efectividad de nirsevimab en menores de 6 meses fue del 85% (IC 95%:32-97%).

Conclusiones. La implementación de nirsevimab ha tenido un importante impacto en el numero de ingresos hospitalarios por bronquiolitis VRS. No hubo diferencias en la gravedad de la bronquiolitis.

Palabras clave: Bronquiolitis, VRS, prevención, anticuerpos monoclonales, nirsevimab.

Correspondencia:
Rosa Rodriguez-Fernández.
Sección de Pediatría Interna Hospitalaria. Servicio de Pediatría. Hospital Gregorio Marañón.
Calle O'Donnell 50, 28007, Madrid, España.
E-mail: rrfernandez@salud.madrid.org

Nirsevimab effectiveness against hospital admission for respiratory syncytial virus bronchiolitis in infants

ABSTRACT

Introduction. Respiratory syncytial virus (RSV) bronchiolitis is the leading cause of hospitalization in infants. This season, a long half-life monoclonal antibody (Nirsevimab) is available to prevent this disease for all infants born from 1 April-30 September to 2023 and all those born during RSV season (October2023- March 2024). The aim of this study was to evaluate the impact of the implementation of this antibody on RSV admissions in a tertiary hospital.

Material and methods. Observational, retrospective and analytical study. All patients <6 months in October admitted for bronchiolitis at 2 time points were included: T1 or Pre-nirsevimab time: 1 September 2015-30 September 2023 and T2 or Nirsevimab time: 1 October-31 December 2023. Total admissions due to any cause of infants <6 months in the same period were used as the reference population. To assess the impact of the implementation of nirsevimab, we calculated the reduction in the percentage of admissions due to RSV with respect to total admissions in both periods, and also in the 2023-2024 season we calculated the double negative test to calculate the effectiveness of the intervention (1-Odds ratio) x 100.

Results. In infants under 6 months of age, we found significant differences in the number of admissions for RSV bronchiolitis between the last season and the previous 7 seasons [574/1195 (48%) vs 6/138 (4.3%); p<0.01, RPI: 91%). In the 2023/2024 season, the effectiveness of nirsevimab in preventing admission for RSV bronchiolitis in children under 6 months of age was 85% (CI 95%: 32-97%).

Conclusions. The implementation of nirsevimab has had an important impact on the number of hospital admissions for RSV bronchiolitis. There were no differences in the severity of bronchiolitis.

Keywords: Bronchiolitis, RSV, prevention, monoclonal antibodies, nirsevimab

INTRODUCCIÓN

La bronquiolitis por virus respiratorio sincitial (VRS) es la primera causa de hospitalización en lactantes. Es responsable de 3,4 millones de ingresos de lactantes en todo el mundo y es la segunda causa de muerte en niños menores de 12 meses, especialmente en países con bajos recursos socioeconómicos [1-4].

Es una enfermedad epidémica y estacional que ocurre en nuestra latitud entre octubre y marzo. Cada temporada de VRS supone un reto para el sistema sanitario debido al elevado numero de lactantes afectados y al gran numero de ingresos, que ocasiona falta de camas hospitalarias convencionales y también en las unidades de cuidados intensivos [5,6].

A pesar de que VRS se descubrió en 1956 y que se trata de un importante reto de salud global, hasta ahora no disponíamos de terapias especificas ni de estrategias de prevención para todos los lactantes. En la década de los '60 se desarrolló una vacuna inactivada para prevenir la infección por VRS en niños, que resultó un fracaso. Este desafortunado hecho ha lastrado durante mas de 50 años el desarrollo de estrategias preventivas frente a VRS [7-9].

En el año 2013, se describió que la proteína de superficie de fusión (F) del VRS tenía dos configuraciones [10, 11]: la forma post-fusión alargada, muy estable, la única conocida hasta ese momento, es la forma en que la proteína F se encuentra una vez que ha entrado en el organismo; y una nueva configuración, la forma pre-fusión, redondeada, inestable, es la forma en que se encuentra la proteína F antes de entrar en el organismo. Destaca que en su forma pre-fusión, la proteína F muestra sitios antigénicos con mayor potencia neutralizante, que no existen en su forma post-fusión; por ejemplo el sitio Ø.

Este hallazgo impulsó el desarrollo de una nueva generación de anticuerpos monoclonales de vida media prolongada (nirsevimab) que actúan sobre el sitio Ø de la proteína Pre-F de VRS para prevenir esta enfermedad en todos los lactantes menores de 6 meses [8].

A finales de septiembre de 2023, nuestro país incluyó la profilaxis universal contra el VRS en su calendario de inmunización. [12] La población diana fueron los lactantes nacidos entre el 1 abril y el 30 de septiembre de 2023 (menores de 6 meses al inicio de la epidemia en octubre 2023), los recién nacidos durante la temporada y los grupos de riesgo.

El objetivo de este estudio fue evaluar el impacto de la implementación de nirsevimab sobre los ingresos VRS en lactantes menores de 6 meses de edad al inicio de la epidemia VRS, en un hospital de tercer nivel, comparando la actual temporada 2023-2024 con las 7 temporadas anteriores desde 2015.

MATERIAL Y MÉTODOS

Se diseñó un estudio observacional y analítico. Los análisis descriptivos incluyeron todos los < 2 años de edad diagnosticados de bronquiolitis VRS en el periodo comprendido entre 1 septiembre 2015- 31 diciembre 2023. Para el impacto de la administración de nirsevimab se analizaron los ingresos por bronquiolitis VRS en los lactantes <6 meses al inicio de la epidemia en cada temporada.

Se describen 2 tiempos:

T1: incluye a todos los menores de 6 meses ingresados por bronquiolitis VRS entre el 1 de septiembre de 2015-30 de septiembre de 2023 antes de la implementación de nirsevimab.

T2: incluye prospectivamente todos los menores de 6 meses ingresados por bronquiolitis VRS entre el 1 octubre de 2023-31 de diciembre de 2023 tras la implementación de nirsevimab.

En este estudio se incluyeron los datos de la primera parte de las epidemias de VRS (octubre a diciembre) ya que en el momento de realizar este análisis la epidemia de VRS 2023-2024 aún estaba activa. Se excluyeron para los análisis de impacto de la administración de nirsevimab las epidemias coincidentes con la pandemia COVID 19 ya que las características clínicas y epidemiológicas de estas dos temporadas fueron diferentes [13,14]. Se utilizó como población de referencia para establecer el denominador en los análisis el número de ingresos totales en <6 meses en ese mismo periodo.

El diagnostico microbiológico se realizó mediante test rápido antigénico entre 2015-2019 y mediante PCR en muestras nasofaríngeas entre 2020-2023. La sensibilidad de los test de antígenos oscila entre el 80-90% y la especificidad alrededor del 95% [15]

La identificación de los pacientes se realizó con los códigos del CIE- 10: J21.0, bronquiolitis aguda VRS; J12.1, neumonía VRS; y J21.9 bronquiolitis no especificada. El estado de inmunización con nirsevimab se recogió de la historia clínica.

El análisis estadístico se realizó mediante el software R versión 3.5.3 [16] y GraphPad Prism versión 9.5.1. Para valorar el impacto de la implementación de nirsevimab se realizaron dos aproximaciones:

1. Se calculó la reducción del porcentaje de ingreso de bronquiolitis VRS (RPI) comparando T2 con T1 en menores de 6 meses utilizando como denominador el total de ingresos de lactantes de la misma edad por cualquier causa.

2. En T2, se utilizó un diseño de casos y controles mediante el "test doble negativo" y la efectividad se calculó como (1-odds ratio) × 100%. En este análisis los casos fueron lactantes < 6 meses al inicio de la epidemia ingresados por bronquiolitis que dieron positivo en las pruebas del VRS y los controles eran lactantes < 6 meses con resultados negativos en las pruebas del VRS teniendo en cuenta en ambos casos si recibieron o no nirsevimab [17].

Se han realizado análisis de sensibilidad comparando la temporada 2023-2024 en la que se administró nirsevimab con las temporadas en las que hubo menor numero de ingresos por bronquiolitis por VRS, se ha analizado también el impacto de nirsevimab en los no candidatos a inmunización y además un análisis de sensibilidad utilizando como denominador el numero total de ingresos debidos a bronquiolitis de cualquier etiología. Se consideraron significativos valores de p<0,05.

Tabla 1 **Características clínicas y epidemiológicas de los ingresos por bronquiolitis por VRS en menores de 6 meses al inicio de la epidemia según temporada de administración de nirsevimab (incluye solo ingresos octubre-diciembre)**

VARIABLE	T_1 (n=574)	T_2 (n=21)	p
Sexo varón	302/574 (52,6%)	12/21 (57,1%)	0,82
Antecedentes personales	107/574 (18,6%)	3/21 (14,3%)	0,77
Prematuridad	77/574 (13,4%)	2/21 (9,5%)	0,90
<28 semanas EG	3/574 (0,5%)	0/21 (0%)	
28- 32 semanas EG	2/574 (0,3%)	0/21 (0%)	0,90
32-36 semanas EG	74/574 (12,9%)	2/21 (9,5%)	
No prematuro	495/574 (86,3%)	19/21 (90,5%)	
Score gravedad			
Leve	43/574 (7,5%)	3/21 (14,2%)	
Moderado	278/574 (48,4%)	9/21 (42,9%)	0,42
Grave	253/574 (44,1%)	9/21 (42,9%)	
Estancia media (días)	5 (4-8)	5 (3-7)	0,41
PRUEBAS COMPLEMENTARIAS			
Leucocitos	10.300 (7.800-13.475)	11.700 (8.200-16.500)	0,43
Neutrofilos	3.800 (2.228-6.450)	5.800 (2200-6.000)	0,96
Linfocitos	4.500 (3.100-6.200)	5.100 (4.600-8.300)	0,45
PCR	2,5 (0,7-7,2)	1,7 (0,4-4,2)	0,80
TRATAMIENTO			
O_2	549/574 (95,6%)	18/21 (85,7%)	0,10
OAF	267/574 (46,5%)	9/21 (42,9%)	0,82
Aerosoles			
No aerosoles	304/574 (53%)	20/21 (95,2%)	
Adrenalina	240/574 (41,8%)	1/21(4,8%)	**<0,01***
Salbutamol	22/574 (3,8%)	0/21 (0%)	
Suero hipertónico solo	8/574 (1,4%)	0/21 (0%)	
Corticoides	5/574 (0,8%)	0/21 (0%)	0,98
Antibiótico	122/574 (21,3%)	2/21 (9,5%)	0,27
Nutrición enteral	194/574 (33,8%)	7/21 (33,3%)	0,96
Ingreso en UCIP	172/574 (29,8%)	6/21 (28,6%)	0,98
VMNI	158/574 (27,5%)	6/21 (28,6%)	0,92
VMI	25/574 (4,4%)	0/21 (0%)	0,90

OAF: oxigenoterapia de alto flujo. O_2: oxigeno, PCR: proteína C reactiva, UCIP: Unidad de cuidados intensivos pediátricos, VMNI: Ventilación mecánica no invasiva, VMI: Ventilación mecánica invasiva. T_1: Temporadas desde 2015-2023 incluye solo primera mitad de las epidemias (octubre-diciembre). T_2: Temporada 2023-2024 incluye solo la primera mitad de la epidemia (octubre-diciembre)

RESULTADOS

Se incluyeron 2.109 lactantes menores de 2 años de edad ingresados por bronquiolitis entre el 1 septiembre 2015-31 de diciembre de 2023. De ellos, 1.333 ingresaron por bronquiolitis VRS, con una edad de 3,5 (1,5-8) meses y una estancia media de 5 (3-7) días. El 18% de bronquiolitis VRS presentaron neumonía en la radiografía de tórax, el 34% precisaron oxigenoterapia de alto flujo (OAF) y 19,5% ingresaron en UCIP. Analizando todos los lactantes ingresados por bronquiolitis por VRS encontramos que la edad fue mayor en T2: 7,2 (2-12) meses que en T1: 3 (1,5-7) meses; p<0,01.

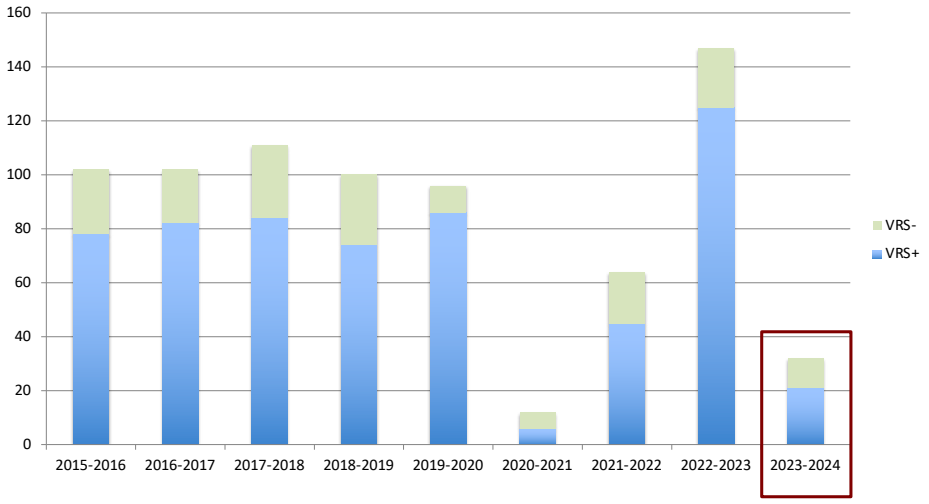


Figura 1 **Distribución de las hospitalizaciones por bronquiolitis por VRS y otros virus desde 2015 hasta 2023. El eje horizontal (eje x) representa todas estaciones analizadas; el eje vertical (eje y) muestra el número total de casos de bronquiolitis VRS positivo y negativo ingresados por mes en el periodo de estudio. Destaca la ultima estación 2023-2024 (en recuadro rojo) con la importante disminución del numero de ingresos tras la implementación de nirsevimab. Destaca asimismo la temporada 2020-2021 con importante descenso del numero de hospitalizaciones por VRS debido a la pandemia COVID-19.**

En T1, ingresaron en nuestro hospital entre los meses de octubre a diciembre un total de 1.195 niños <6 meses por cualquier causa, 718 ingresaron por bronquiolitis (574 de ellos fueron bronquiolitis por VRS) y en T2, ingresaron 138 lactantes <6 meses por cualquier causa, de ellos 32 ingresaron por bronquiolitis (21 fueron bronquiolitis por VRS).

Impacto de la estrategia de inmunización con nirsevimab en menores de 6 meses

1.- Primera aproximación: Cuando comparamos la ultima epidemia con las 7 epidemias previas existían importantes diferencias en el numero de ingresos por bronquiolitis por VRS [574/1195 (48%) en T1 vs 21/138 (15,2%) en T2; p<0,01, RPI: 68,7%]. Si excluimos del análisis aquellos lactantes candidatos a nirsevimab que no lo recibieron: [574/1195 (48%) vs 6/138 (4,3%); p<0,01, RPI: 91%)].

No encontramos diferencias en cuanto a estancia media, necesidad de OAF e ingreso en UCIP entre ambos periodos (Tabla 1).

2.- Segunda aproximación. En T2 ingresaron 21 lactantes < 6 meses al inicio de la epidemia por bronquiolitis por VRS, 6 (28%) habían recibido nirsevimab y 15 no; en el mismo periodo ingresaron 11 lactantes por bronquiolitis debida a otras causas de los cuales 8 recibieron nirsevimab (72%) y 3 no; (p=0,02); la efectividad del nirsevimab frente a la hospitalización por VRS fue del 85% (IC 95%: 32-97%) (Figura 1).

Los análisis de sensibilidad comparando con las temporadas con menor numero de ingresos por VRS (2018/19-2021/22) confirman los resultados (p=0,01). Se realizaron los mismos análisis utilizando como denominador el numero total de bronquiolitis de cualquier etiología [574/718 (79,9%) vs 21/32 (65,6%) p= 0,06] y también en los niños no candidatos a nirsevimab (> de 6 meses el 1 de octubre de cada año) demostrando que nirsevimab no tuvo impacto en este grupo de edad (p=0,45)

DISCUSIÓN

Este estudio proporciona datos de efectividad de los primeros tres meses tras la implantación de la estrategia de inmunización con nirsevimab a todos los lactantes menores de 6 meses de edad al inicio de la epidemia y a todos los recién nacidos durante la primera temporada.

En España se implementó la estrategia universal de administración de nirsevimab a partir de la primera semana de octubre de 2023 y se han alcanzado unas tasas de cobertura de inmunización que varían entre el 83-94% en todo el territorio nacional [5,18].

Los ensayos clínicos proporcionaron datos de reducción del riesgo relativo de hospitalización por VRS del 76,8%-83,2% [19-21]. Recientemente, el CDC estableció un 90% la efectivi-

dad de nirsevimab en vida real, para la prevención de las hospitalizaciones por bronquiolitis por VRS en USA [17]. Los datos españoles describen una efectividad global mayor del 70% [5,22] y en el caso de Cataluña una efectividad en la prevención de la hospitalización de 87,6% y de ingresos en UCIP del 90,1% [22,23]. En nuestro estudio la efectividad fue del 85% (IC 95%: 32-97%), en concordancia con los primeros ensayos clínicos y con los datos publicados hasta este momento. Se ha especulado con la posibilidad de un aumento de gravedad en la segunda estación respiratoria en los niños que recibieron nirsevimab en su primeros meses de vida sin embargo un estudio reciente demuestra que no hay mayor numero de episodios de VRS ni de mayor gravedad en la segunda temporada [24].

Durante la pandemia COVID la edad de lactantes con bronquiolitis VRS aumentó, en relación con las medidas de confinamiento y la interferencia viral [13]. Con la implementación de nirsevimab, la edad de los lactantes ingresados por bronquiolitis por VRS ha aumentado probablemente porque en el grupo de no intervención los ingresos han continuado igual que en temporadas previas.

Entre las limitaciones de este estudio destaca que se trata de un estudio unicéntrico, si bien la casuística es amplia debida al gran número de lactantes incluidos ya que se recogen datos de las últimas 7 temporadas.

Es la primera vez que disponemos de un producto para la prevención de la infección por VRS en toda la cohorte de nacimiento y en los lactantes menores de 6 meses lo que sin duda contribuirá a disminuir la presión asistencial durante la temporada de invierno [5, 18]. Se necesitan estudios de vigilancia epidemiológica y secuenciación del VRS en las próximas temporadas

AGRADECIMIENTOS

Agradecemos a los pacientes y a sus familias y al equipo de enfermería de nuestro hospital

FINANCIACIÓN

Este estudio ha sido parcialmente financiado por la beca FIS PI21/00840

CONFLICTO DE INTERESES

RRF declara haber impartido charlas relacionadas con la bronquiolitis por VRS financiadas por Pfizer, Sanofi, Astra Zeneca, y MSD. Ha participado en Advisory boards financiados por Pfizer y Sanofi. Ha recibido becas para asistencia a conferencias y congresos financiado por Pfizer y Sanofi

BIBLIOGRAFÍA

1. Hall CB, Simões EA, Anderson LJ. Clinical and epidemiologic features of respiratory syncytial virus. Curr Top Microbiol Immunol. 2013;372:39-57. doi: 10.1007/978-3-642-38919-1_2
2. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380(9859):2095-128. doi: 10.1016/s0140-6736(12)61728-0
3. Bont L, Checchia PA, Fauroux B, Figueras-Aloy J, Manzoni P, Paes B, et al. Defining the Epidemiology and Burden of Severe Respiratory Syncytial Virus Infection Among Infants and Children in Western Countries. Infect Dis Ther. 2016;5(3):271-98. doi: 10.1007/s40121-016-0123-0
4. Löwensteyn YN, Willemsen JE, Mazur NI, Scheltema NM, van Haastregt NCJ, Buuren A, et al. Nosocomial RSV-related In-hospital Mortality in Children <5 Years: A Global Case Series. Pediatr Infect Dis J. 2023;42(1):1-7. doi: 10.1097/inf.0000000000003747
5. López-Lacort M, Muñoz-Quiles C, Mira-Iglesias A, López-Labrador FX, Mengual-Chuliá B, Fernández-García C, et al. Early estimates of nirsevimab immunoprophylaxis effectiveness against hospital admission for respiratory syncytial virus lower respiratory tract infections in infants, Spain, October 2023 to January 2024. Euro Surveill. 2024;29(6). doi: 10.2807/1560-7917.es.2024.29.6.2400046
6. Rodriguez-Fernandez R, González-Sánchez MI, Perez-Moreno J, González-Martínez F, de la Mata Navazo S, Mejias A, et al. Age and respiratory syncytial virus etiology in bronchiolitis clinical outcomes. J Allergy Clin Immunol Glob. 2022;1(3):91-8. doi: 10.1016/j.jacig.2022.05.005
7. Mejias A, Rodríguez-Fernández R, Oliva S, Peeples ME, Ramilo O. The journey to a respiratory syncytial virus vaccine. Ann Allergy Asthma Immunol. 2020;125(1):36-46. doi: 10.1016/j.anai.2020.03.017
8. Rodriguez-Fernandez R, Mejias A, Ramilo O. Monoclonal Antibodies for Prevention of Respiratory Syncytial Virus Infection. Pediatr Infect Dis J. 2021;40(5s):S35-s9. doi: 10.1097/inf.0000000000003121
9. Ramilo O, Rodriguez-Fernandez R, Mejias A. Respiratory Syncytial Virus Infection: Old Challenges and New Approaches. J Infect Dis. 2023;228(1):4-7. doi: 10.1093/infdis/jiad010
10. McLellan JS, Chen M, Joyce MG, Sastry M, Stewart-Jones GB, Yang Y, et al. Structure-based design of a fusion glycoprotein vaccine for respiratory syncytial virus. Science. 2013;342(6158):592-8. doi: 10.1126/science.1243283
11. McLellan JS, Chen M, Leung S, Graepel KW, Du X, Yang Y, et al. Structure of RSV fusion glycoprotein trimer bound to a prefusion-specific neutralizing antibody. Science. 2013;340(6136):1113-7. doi: 10.1126/science.1234914
12. Grupo de Trabajo utilización de nirsevimab frente a infección por virus respiratorio sincitial de la Ponencia de Programa y Registro de Vacunaciones. Comisión de Salud Pública del Consejo Interterritorial del Sistema Nacional de Salud. Ministerio de Sanidad, julio 2023.
13. Rodríguez-Fernández R, González-Martínez F, Perez-Moreno J, González-Sánchez MI, de la Mata Navazo S, Toledo Del Castillo B, et al. Clinical Phenotype of Respiratory Syncytial Virus Bronchiolitis before and during the Coronavirus Disease 2019 Pandemic. Am J Perinatol. 2022. doi: 10.1055/s-0042-1759602

14. Agha R, Avner JR. Delayed Seasonal RSV Surge Observed During the COVID-19 Pandemic. Pediatrics. 2021;148(3). doi: 10.1542/peds.2021-052089

15. Echávarri Olavarría F MAC. VRS. Pruebas para la detección rápida. En Guía-ABE. Infecciones en Pediatría. Guía rápida para la selección del tratamiento antimicrobiano empírico. 2022. Available at: http://www.guia-abe.es

16. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2018. Available at: https://www.R-project.org/)

17. Moline HL, Tannis A, Toepfer AP, Williams JV, Boom JA, Englund JA, et al. Early Estimate of Nirsevimab Effectiveness for Prevention of Respiratory Syncytial Virus-Associated Hospitalization Among Infants Entering Their First Respiratory Syncytial Virus Season New Vaccine Surveillance Network, October 2023-February 2024. MMWR Morb Mortal Wkly Rep. 2024;73(9):209-14. doi: 10.15585/mmwr.mm7309a4

18. Martinón-Torres F, Mirás-Carballal S, Durán-Parrondo C. Early lessons from the implementation of universal respiratory syncytial virus prophylaxis in infants with long-acting monoclonal antibodies, Galicia, Spain, September and October 2023. Euro Surveill. 2023;28(49). doi: 10.2807/1560-7917.es.2023.28.49.2300606

19. Hammitt LL, Dagan R, Yuan Y, Baca Cots M, Bosheva M, Madhi SA, et al. Nirsevimab for Prevention of RSV in Healthy Late-Preterm and Term Infants. N Engl J Med. 2022;386(9):837-46. doi: 10.1056/NEJMoa2110275

20. Drysdale SB, Cathie K, Flamein F, Knuf M, Collins AM, Hill HC, et al. Nirsevimab for Prevention of Hospitalizations Due to RSV in Infants. N Engl J Med. 2023;389(26):2425-35. doi: 10.1056/NEJMoa2309189

21. Muller WJ, Madhi SA, Seoane Nuñez B, Baca Cots M, Bosheva M, Dagan R, et al. Nirsevimab for Prevention of RSV in Term and Late-Preterm Infants. N Engl J Med. 2023;388(16):1533-4. doi: 10.1056/NEJMc2214773

22. Resultados de implementación de Nirsevimab. Campaña iniciada en la semana 13. Sesión VRS iniciada en la semana 45-46 https://www.nirsegal.es/informe-es/ultimo-informe2024 [

23. Coma E, Martinez-Marcos M, Hermosilla E, Mendioroz J, Reñé A, Fina F, et al. Effectiveness of nirsevimab immunoprophylaxis against respiratory syncytial virus-related outcomes in hospital and primary care settings: a retrospective cohort study in infants in Catalonia (Spain). Arch Dis Child. 2024 Aug 16;109(9):736-741. doi: 10.1136/archdischild-2024-327153.

24. Dagan R, Hammitt LL, Seoane Nuñez B, Baca Cots M, Bosheva M, Madhi SA, et al. Infants Receiving a Single Dose of Nirsevimab to Prevent RSV Do Not Have Evidence of Enhanced Disease in their Second RSV Season. J Pediatric Infect Dis Soc. 2024. doi: 10.1093/jpids/piad113

Official journal of the Spanish Society of Chemotherapy

Brief report

Revista Española de Quimioterapia
doi:10.37201/req/054.2024

Carl Llor[1,2,3,4]
Cristina Rodríguez-Bernuz[5]
Amelia Troncoso-Mariño[5]

Antibiotic use in Barcelona in 2023 in primary care and the potential reduction by adjusting box sizes to current guidelines

[1]Via Roma Health Centre, Catalonian Institute of Health, Barcelona, Spain
[2]University Institute in Primary Care Research Jordi Gol, Barcelona, Spain
[3]CIBER Enfermedades Infecciosas, Instituto de Salud Carlos III, Spain
[4]Research Unit of General Practice, Department of Public Health, University of Southern Denmark, Odense, Denmark
[5]Primary Care Pharmacy Unit, Catalonian Institute of Health, Barcelona, Spain

Article history
Received: 12 June 2024; Revision Requested: 2 September 2024; Revision Received: 7 September 2024; Accepted: 20 September 2024; Published: 1 October 2024

ABSTRACT

Introduction. Prior research has not examined the size of antibiotic packages prescribed. We assessed 2023 prescription rates in Barcelona and the most prescribed presentations in pharmacies, while evaluating potential reductions if all amoxicillin and amoxicillin/clavulanate containers had 20 doses.

Methods. Antibiotics prescribed by primary care doctors working for the Catalonian Health Institute in Barcelona in 2023 were analysed by calculating the defined daily doses per 1,000 inhabitants and day (DID).

Results. The observed prescribing rate was 8 DID, with penicillins accounting for 4.6 DID (57.2%). The most frequently prescribed antibiotics were amoxicillin and amoxicillin/clavulanate, making up 4.4 DID. If all the 30-dose presentations of amoxicillin and amoxicillin/clavulanate had been 20-dose containers, the total number of DIDs would have been 3.3, resulting in a reduction of 1.1 DID (25.4% less).

Conclusions. Antibiotic prescribing rate in Barcelona was low. Aligning the dosage of antibiotics with established guidelines could further reduce antibiotic consumption.

Key words: Antibacterial Agents; Primary Healthcare; Antimicrobial Stewardship; Package Size; Prescribing guidelines.

Uso de antibióticos en Barcelona en 2023 en atención primaria y reducción potencial ajustando el tamaño de los envases a las guías actuales

RESUMEN

Introducción. No se ha analizado hasta ahora el tamaño de los envases antibióticos. Evaluamos el uso de antibióticos recetados en Barcelona en 2023. Además, evaluamos la posible reducción en su uso si los envases de amoxicilina y amoxicilina + ácido clavulánico prescritos contuvieran 20 dosis.

Métodos. Se analizaron los antibióticos prescritos por los médicos de atención primaria en Barcelona pertenecientes al ICS en el año 2023, calculando la dosis diaria definida por 1000 habitantes y día (DHD).

Resultados. El uso total fue de 8 DHD, correspondiendo a penicilinas 4,6 DHD (57,2%). Los antibióticos más prescritos fueron amoxicilina y amoxicilina + ácido clavulánico, representando 4,4 DHD. Si todas las presentaciones de 30 dosis hubieran sido de 20 dosis, el número total de DHD habría sido de 3,3, con una reducción de 1,1 DHD (25,4% menos).

Conclusiones. El uso antibiótico en Barcelona fue bajo. Ajustar las dosis de antibióticos con las pautas establecidas podría reducir aún más su uso.

Palabras clave: Antibióticos; Atención Primaria; Programa de Optimización de Antibióticos; Tamaño de Envases; Guías de Prescripción.

INTRODUCTION

The prescription of antibiotics in Spain has been declining over the last years, with a significant drop when COVID-19 pandemic broke out and with a slight increase in the subsequent years. However, the amount of antibiotics prescribed in 2023 was lower compared to the last years of the prior decade [1]. Spain is currently the eighth country when it comes to the number of antibiotics prescribed [2]. This is the result of many antimicrobial stewardship programmes that have been running in the last years throughout the country and a deeper sensibilization of health care professionals regarding the global threat of antimicrobial resistance. Our group has been implementing antimicrobial stewardship programmes with a focus on promoting the use of first-line antibiotics for uncomplicated urinary tract infections, advocating for the use of 3g-single doses of fosfomycin, and we have started implementing pro-

Correspondence:
Carl Llor
Via Roma Health Centre. c. Manso, 19, 3rd floor. 08015 Barcelona.
Email: carles.llor@gmail.com

grammes for respiratory tract infections, promoting the use of narrow spectrum antimicrobials and shorter courses [3].

Primary care is responsible for about 80% of antibiotic prescriptions, and both respiratory and urinary tract infections account for more than two thirds of all antibiotic prescriptions globally [4]. Antimicrobial stewardship programmes are usually more focused on the choice of antibiotics and when these drugs could be averted, with less implementation of duration when these antibiotics are necessary. In our country, the two most common antibiotics considered first-line treatments for respiratory tract infections, amoxicillin and amoxicillin/clavulanate, are only available in boxes of 20 and 30 units, based on a 2012 resolution by the Spanish Agency of Medicines and Health Products [5]. However, there is a growing recommendation for shorter treatment courses for also respiratory tract infections, and current guidelines advocate for five to seven days of antibiotic treatment [6]. Despite this, no studies have assessed the prescription patterns of the various presentations of the most prescribed antibiotics in our country. This study aimed to assess the current prescription rates in Barcelona in 2023 and the presentations most frequently dispensed in community pharmacies. Additionally, we examined the potential reduction in prescription rates if all containers of amoxicillin and amoxicillin/clavulanate contained only 20 doses.

METHODS

Cross-sectional study carried out during the year 2023. We present the antibiotic prescriptions in the city of Barcelona, including all the 51 centres, encompassing primary care centres, primary care out-of-hours services, primary care sexual and reproductive services, and the home visit service, belonging to the Catalonian Institute of Health, which covers 75.5% of the population in Barcelona, based on data in 2023 [7]. Of the population living in Barcelona assigned to the Catalonian Institute of Health in December 2023, 52.2% were women, 20.3% were older than 65, and 11.5% were children.

We calculated the daily defined doses (DDD) and the DDD per 1,000 inhabitants and day (DID). The DID was calculated using the formula: DID = DDD * 1000 / (365 * 1,302,574) [8], where 1,302,574 represents the population living in Barcelona assigned to the Catalonian Institute of Health. We also determined how much the reduction in the DID would have been if all and 90% of the containers containing 30 doses of amoxicillin and amoxicillin/clavulanate were instead containers of 20 doses.

RESULTS

The observed prescribing rate was 7.999 DIDs. Among these, 4.576 doses were penicillins, representing 57.2% of the total. Macrolides were the second most frequently prescribed antibiotic family, with 1.063 daily defined doses, followed by quinolones and cephalosporins at 0.643 and 0.566 daily defined doses, respectively (Table 1).

The most prescribed antibiotics were amoxicillin and amoxicillin/clavulanate (2.487 and 1.887 DID, respectively), which in total accounted for 54.7% of all the antibiotics given. A total of 194,750 containers of amoxicillin and amoxicillin/clavulanate were prescribed, with 150,080 boxes designated for adults. Out of these, 119,866 presentations contained 30 doses (79.9%), while only 20.1% of the prescriptions were for boxes with 20 doses. As illustrated in Table 2, the combined DID for both antibiotics was 4.374. However, if all the 30-dose presentations had been 20-dose containers, the total number of DIDs would have been 3.260, resulting in a reduction of 1.114 DID (25.4% less). Considering a scenario where 90% of these 30-dose containers are replaced with 20-dose containers, allowing 10% of the two β-lactam presentations to be dispensed in 30-dose containers, the reduction would have been 1.035 DID. The majority of azithromycin presentations consisted of boxes containing three doses (64.5% of the total presentations), while fosfomycin prescriptions primarily involved single 3g doses of the antibiotic (58.7%).

DISCUSSION

The results of this study clearly indicate a low antibiotic prescribing rate in primary care in 2023—less than 8 DID—which is below the average antibiotic consumption in both Europe and other Autonomous Communities in Spain [2,9]. However, antibiotic use would have been significantly lower if all the prescribed box sizes of both amoxicillin and amoxicillin/clavulanate were containing twenty doses each. In addition, the antibiotic more commonly prescribed, amoxicillin, is the first-choice antibiotic in most of the common infections in our country [6,10]. This good choice is also shown with the most frequent prescription of 3g single presentations of fosfomycin, which is also considered as the first-line antibiotic for uncomplicated urinary tract infections.

Recent guidelines advise against prescribing amoxicillin and amoxicillin/clavulanate for respiratory tract infections for a duration of ten days, except in the case of streptococcal pharyngitis [6]. However, a ten-day course of narrow-spectrum antibiotics like penicillin V is recommended for this specific infection. Consequently, the availability of containers containing both amoxicillin and amoxicillin/clavulanate is not justified for any of the infections typically managed by a general practitioner. Some recent clinical guidelines, like the WHO AWaRe antibiotic book, advocate for even shorter durations, like five-day courses of antibiotics for acute rhinosinusitis, acute exacerbations of chronic obstructive pulmonary disease, and community-acquired pneumonia [10]. Despite this evidence, most clinicians still use standard or longer courses [11]. To adhere to these guidelines effectively, one approach is to reduce the number of doses in antibiotic boxes. Firstly, by discontinuing the availability of boxes containing 30 doses, and secondly, by aligning the number of doses with these updated guidelines, allowing a maximum of 15 doses per container of amoxicillin and amoxicillin/clavulanate. Policymakers play a crucial role in promoting the rational use of antibiotics, and it is vital to im-

Table 1 Antibiotics prescribed in Barcelona in 2023 in primary care.

Anatomical Therapeutic Chemical Classification antibiotics	Number of boxes	DID*
J01A. Tetracyclines	11,284	0.506
J01C. β-lactam antibacterials, penicillins	211,750	4.576
J01CA. Broad-spectrum penicillins	124,786	2.488
J01CE. β-lactamase susceptible penicillins	9,572	0.111
J01CF. β-lactamase resistant penicillins	7,334	0.090
J01CR. Combinations of penicillins	70,058	1.887
J01D. Other β-lactams antibacterials, cephalosporins	5,849	0.566
J01DB. First generation cephalosporins	10,542	0.063
J01DC. Second generation cephalosporins	17,252	0.417
J01DD. Third generation cephalosporins	4,303	0.086
J01DE. Fourth generation cephalosporins	12	0.000
J01E. Sulphonamides and trimethoprim	6,223	0.197
J01EA. Trimethoprim	57	0.000
J01EC. Sulfadiazine	40	0.001
J01EE. Cotrimoxazole	6,126	0.196
J01F. Macrolides, lincosamides and streptogramins	90,673	1.165
J01FA. Macrolides	82,171	1.063
J01FF. Lincosamides	8,502	0.102
J01G. Aminoglycoside antibacterials	1,165	0.001
J01M. Quinolones antibacterials	40,647	0.643
J01X. Other antibacterials	78,888	0.345
J01XC. Fusidic acid	175	0.001
J01XE. Nitrofurantoin	7,946	0.131
J01XX. Fosfomycin	70,767	0.213
TOTAL	474,285	7.999

DID=Daily defined doses per 1,000 inhabitants per day.

plement these changes. Our study shows that simply adjusting the prescription of β-lactams to containers with a maximum of 20 pills would be effective in reducing the DIDs to less than 7. Community pharmacies can exchange antibiotics prescribed as long as they have the same number of tablets per package but cannot give containers with a different number of doses. Another aspect that could be influencing this is that when searching for an antibiotic in the electronic medical history in primary care, the drop-down menu displays the different presentations of the antibiotics randomly, instead of first displaying presentations with fewer units.

This study has some limitations. This study consisted of a large population, including a whole city and all prescriptions dispensed by general practitioners, professionals working in out-of-hours centres, sexual and reproductive services, and a home visit service of a public healthcare service, thus providing a complete picture of overall antibiotic prescribing in primary care in the whole city. However, prescription of doctors belonging to other healthcare providers and private doctors were not considered in this study. We have focused our analysis only on the estimated savings of the two most common antibiotics, rather than all of them, allowing us to specifically evaluate the savings that can be achieved with first-line antibiotics for common infectious diseases in primary care. While we acknowledge the limitations of extrapolating the findings of this study to other regions in Spain, we do not consider the results would have been much different.

Although there may be bias in extrapolating findings from a single city to the broader Spanish context, our results also indicate an improvement in antibiotic prescribing practices over recent years. This improvement is characterized by a reduction in both inappropriate and excessive antibiotic prescriptions. However, to further enhance these positive trends, regulatory measures aimed at reducing the number of doses per antibiot-

Table 2 **Number of amoxicillin and amoxicillin/clavulanate presentations prescribed, along with the resulting DID that would have been observed if the 30-dose boxes prescribed had instead been 20-dose boxes.**

Antibiotic presentation	Number of boxes dispensed	Sum of DDD	Sum of DID	Sum of DDD* adjusted to boxes with 20 doses in all cases	Sum of DID* adjusted to boxes with 20 doses in all cases	Sum of DDD** adjusted to boxes with 20 doses in 90% of cases	Sum of DID** adjusted to boxes with 20 doses in 90% of cases
Amoxicillin, 1,000 mg	15,905.00	283,233.33	0.596	212,066.67	0.446	219,183.33	0.461
Amoxicillin, 125 mg susp.	171.00	342.00	0.001	342.00	0.001	342.00	0.001
Amoxicillin, 250 mg	55.00	150.00	0.001	275,00	0.001	275.00	0.001
Amoxicillin, 250 mg susp.	39,370.00	154,800.00	0.326	154,800.00	0.326	154,800.00	0.326
Amoxicillin, 500 mg	42,127.00	392,980.00	0.827	280,846.67	0.591	292,060.00	0.614
Amoxicillin, 750 mg	27,064.00	350,645.00	0.738	270,640.00	0.569	278,640.50	0.586
Amoxicillin/clavulanate, 100 mg susp.	4,716.00	33,797.33	0.071	33,797.33	0.071	33,797.33	0.071
Amoxicillin/clavulanate, 125 mg susp.	116.00	232.00	0.000	232.00	0.000	232.00	0.000
Amoxicillin/clavulanate, 250 mg susp.	341.00	1,408.00	0.003	1,334.67	0.003	1,334.67	0.003
Amoxicillin/clavulanate, 500/125 mg	32,264.00	322,640.00	0.679	215,093.33	0.452	225,848.00	0.475
Amoxicillin/clavulanate, 875/125 mg	32,621.00	539,175.00	1.134	380,578.33	0.800	396,438.00	0.800
TOTAL presentations of both antibiotics	194,750.00	2,079,402.67	4.374	1,550,006.17	3.260	1,602,950.83	3.339

DDD=daily defined dose. DID=DDD per 1,000 inhabitants and day.

***These columns represent the potential DDD and DID if all the containers of amoxicillin and amoxicillin/clavulanate, originally prescribed with 30 doses, had been in presentations with 20 doses instead. For amoxicillin/clavulanate, the reduction in DDD would have been 266,216.67 DDD, and for amoxicillin, it would have been 263,179.99 DDD.**

****These columns represent the potential DDD and DID if 90% the containers of amoxicillin and amoxicillin/clavulanate, originally prescribed with 30 doses, had been in presentations with 20 doses instead.**

ic container or a move to an exact pill-count system should be implemented, as suggested by other studies [12,13].

FUNDING

None to declare.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest

REFERENCES

1. Plan Nacional Resistencia Antibióticos. Consumos Antibióticos Sector Comunitario, 2023. https://www.resistenciaantibioticos.es/es/lineas-de-accion/vigilancia/mapas-de-consumo/consumo-antibioticos-humana/consumos-antibioticos-en-atencion-primaria
2. European Centre for Disease Prevention and Control. Antimicrobial consumption in the EU/EEA (ESAC-Net) - Annual Epidemiological Report for 2022. Nov 2023. https://www.ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-consumption-europe-2022
3. Gallardo S, Troncoso-Mariño A, Nadal-Braqué N, Amado-Guirado E, Mallecot YH, Llor C; PROA-ICS Barcelona Study Group. Improved management of cystitis in primary care following the implementation of a simple multifaceted intervention. Aten Primaria. 2022;54(11):102493. doi: 10.1016/j.aprim.2022.102493.
4. Gulliford MC, Dregan A, Moore MV, *et al.* Continued high rates of antibiotic prescribing to adults with respiratory tract infection: survey of 568 UK general practices. BMJ Open. 2014;4(10):e006245.
5. Resolución por la que se modifica la Resolución de la Agencia Española de Medicamentos y Productos Sanitarios de fecha 31 ø de julio de 2012, sobre la adecuación de los formatos de los medicamentos de los grupos terapéuticos J01 y J02. Ministerio de Sanidad, servicios sociales e igualdad. July 2012.
6. Patología infecciosa. (eds.) Martín Zurro A, Cano Pérez JF, Gené Badia J, Atención Primaria, Principios, organización y métodos en Medicina de Familia, Barcelona: Elsevier España, 2024; pp. 856–892.
7. Institut Català de la Salut. Gerència Territorial de Barcelona Memòria 2023. Barcelona; 2023. https://ics.gencat.cat/web/.content/memories-2023/Memoria-ICS-2023.pdf
8. Leung V, Langford BJ, Ha R, Schwartz KL. Metrics for evaluating antibiotic use and prescribing in outpatient settings. JAC Antimicrob Resist. 2021;3(3):dlab098. doi: 10.1093/jacamr/dlab098.
9. Plan Nacional Resistencia Antibióticos. Consumos Antibióticos Sector Comunitario por Comunidades Autónomas, 2023. https://www.resistenciaantibioticos.es/es/lineas-de-accion/vigilancia/

mapas-de-consumo/consumo-antibioticos-humana/consumos-antibioticos-extrahospitalarios-por-comunidades

10. World Health Organization. The WHO AWaRe (Access, Watch, Reserve) antibiotic book. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO.
11. Lee RA, Centor RM, Humphrey LL, *et al.* Appropriate use of short-course antibiotics in common infections: Best practice advice from the American College of Physicians. Ann Intern Med. 2021;174(6):822–7. doi: 10.7326/M20-7355.
12. Füri J, Widmer A, Bornand D, Berger C, Huttner N, Bielicki JA. The potential negative impact of antibiotic pack on antibiotic stewardship in primary care in Switzerland: a modelling study. Antimicrob Resist Infect Control. 2020;9(1):60. doi: 10.1186/s13756-020-00724-7.
13. McGuire TM. Does size matter? Addressing pack size and antibiotic duration. Aust Prescr. 2019;42(1):2–3. doi: 10.18773/austprescr.2019.005.

Official journal of the Spanish Society of Chemotherapy

Carta al Director

Revista Española de Quimioterapia
doi:10.37201/req/007.2024

Laura Eva Franco-Fobe[1,3,4]
María Luisa Monforte[1]
María Victoria Fuentelsaz del Barrio[2]
Rocío Cebollada[1]
Concepción López-Gómez[3]
Carmen Aspiroz[1]

La importancia de un diagnóstico precoz. *Tinea capitis* producida por *Trichophyton tonsurans* en un adolescente

[1]Servicio de Microbiología, Hospital Universitario Royo Villanova
[2]Servicio de Dermatología, Centro Médico de Especialidades Grande Covián, Zaragoza
[3]Servicio de Microbiología, Hospital Universitario Miguel Servet
[4]Instituto de Investigación Sanitaria de Aragón

Article history
Received: 10 January2024; Revision Requested: 23 July 2024; Revision Received: 4 October 2024; Accepted: 7 October 2024; Published: 15 October 2024

Estimado Editor:

La *tinea capitis* (TC) es una infección fúngica del cuero cabelludo y los folículos pilosos causada comúnmente por hongos dermatofitos del género *Microsporum* y *Trichophyton*. En España hasta comienzos del siglo XXI el dermatofito más aislado en los casos de TC fue *Microsporum canis*. En la actualidad parece que se está incrementando el número de las tiñas antropofílicas, y entre sus agentes causales se encuentra *Trichophyton tonsurans* [1], siendo este el dermatofito más frecuentemente implicado en brotes familiares e institucionales [2,3].

T. tonsurans se propaga a través del contacto directo con personas infectadas o mediante el uso compartido de objetos contaminantes [4]. Las manifestaciones clínicas afectan fundamentalmente al cuero cabelludo y a los folículos pilosos causando picor, descamación de la zona junto con placas alopécicas, y en casos más avanzados inflamación, supuración y dolor. En ocasiones puede causar *kerion* (lesión inflamatoria del cuero cabelludo con supuración de los folículos pilosos).

En los últimos años ha habido un incremento de casos de TC entre la población masculina adolescente contraídos en peluquerías, en los que el hongo aislado con mayor frecuencia ha sido *T. tonsurans*. Esto guarda relación con una moda internacional consistente en acudir a la peluquería con frecuencia semanal o bisemanal para cortarse el pelo de las zonas occipital y temporal, mediante degradado o rasurado. Esto puede haber favorecido el contagio de la tiña a través de las maquinillas eléctricas infectadas [5,6]. Las lesiones tienden a aparecer predominantemente en la nuca y el área temporal, que son las zonas donde se apura más el rasurado. A continuación se presenta el caso de un paciente con una TC por *T. tonsurans*.

Varón de 21 años de edad que acude a su centro de salud en julio de 2023 con lesiones eritematosas de 6 meses de evolución alguna de ellas sobreinfectadas de predominio en la región cervical y alguna aislada en cuero cabelludo pruriginosas especialmente por la noche (Figura 1A). El paciente refería haberse aplicado una solución cutánea con betametasona dipropionato 0,5mg/g. Su médico le recomendó una crema con betametasona 0,5mg/g y gentamicina 1mg/g, y realizó una interconsulta con Dermatología para la evaluación de las lesiones. Dermatología contestó la interconsulta al día siguiente valorando la lesión como una posible micosis cutánea recomendando cultivo de hongos hasta la espera de la citación y evitar la aplicación de la crema con betametasona 0,5mg/g y gentamicina 1mg/g. Citó al paciente con prioridad preferente a las dos semanas. Durante ese periodo de tiempo no se recogieron muestras de las lesiones ni se suspendió el uso de la pomada. El paciente no acudió a la primera citación.

Finalmente, a mediados de septiembre de 2023 el paciente fue visto por Dermatología. La lesión fue catalogada como una posible *tinea incognito* y se solicitó cultivo micológico de la zona.

Se tomaron muestras en Microbiología de las lesiones de piel de cuello y nuca y de folículos del cuero cabelludo y nuca. Las lesiones que se observaron en septiembre estaban más evolucionadas que las que se observaron por primera vez en julio (Figura 1B).

A las 24h de la toma de muestras, el servicio de Microbiología informó de la observación de estructuras fúngicas en la visión directa de la muestra y en la tinción de Gram de la extensión de material de los folículos (Figura 2A). Al cabo de 3 semanas se informó del crecimiento de *T. tonsurans*. El microorganismo tardó en crecer y su identificación fue dificultosa, posiblemente debido al tratamiento tópico a base de corticoesteroides que había recibido el paciente. El aislamiento en cultivo se realizó en medios habituales como agar Sabouraud-dextrosa con cicloheximida y DTM-Taplin (Dermatophyte Test Medium). Macroscópicamente las colonias tienen aspecto pulverulento de color blanco a grisáceo, a veces con centro rosado (Figura 2B). La identificación de la especie se realizó por sus características micro y macroscópicas en cultivo y mediante MALDI-TOF.

Correspondencia:
Laura Eva Franco Fobe
Hospital Universitario Miguel Servet, Zaragoza, España
E-mail: laueva f@gmail.com

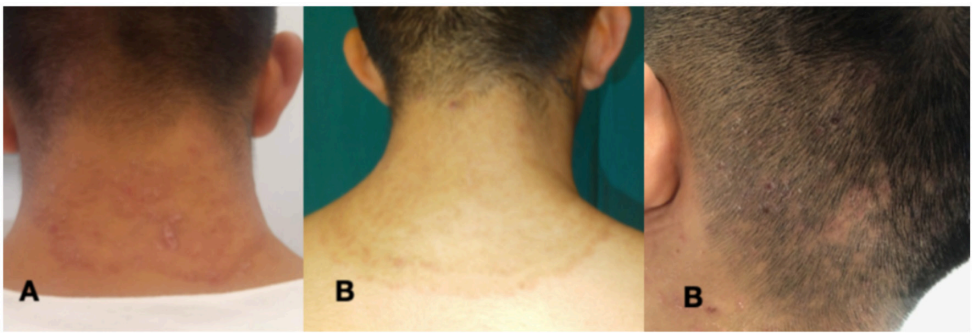


Figura 1 | **A) Lesiones eritematosas de julio 2023. B) Lesiones eritematosas junto con placa alopécica secundaria a tiña inflamatoria en septiembre 2023.**

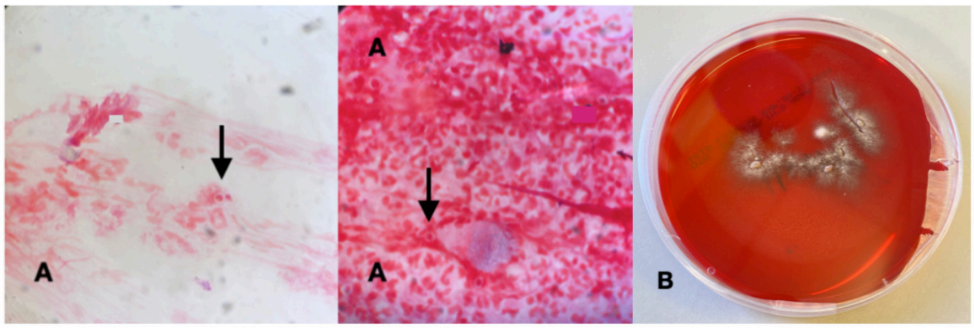


Figura 2 | **A) Estructuras fúngicas en la tinción de Gram. B) Crecimiento de *T. tonsurans* en DTM-Taplin.**

El paciente se trató tras la toma del cultivo con terbinafina oral 250mg 1 cápsula/día durante 4 semanas, terbinafina 1% crema 2 veces/día durante 3 semanas y ketoconazol 2% champú 1 vez/ semana para él y sus convivientes. La evolución se presume satisfactoria porque el paciente no ha consultado ni a su médico de atención primaria ni a las numerosas llamadas de Dermatología para controlar su curación definitiva y *ad integrum*.

El término de *tinea incognito* hace referencia a la infección dermatofítica subdiagnosticada por alteración de la apariencia clínica debido al uso de esteroides tópicos o otras cremas polivalentes. La aplicación inapropiada de corticoides tópicos en infecciones micóticas de la piel puede originar una modificación de la presentación clínica [7].

Como conclusiones queremos destacar la importancia que tiene un diagnóstico precoz en este tipo de infecciones, que muchas veces pasan desapercibidas, ya que en poco tiempo la evolución de las lesiones puede ser rápida, progresando a fases inflamatorias en las que, además del perjuicio ligado a la progresión de las lesiones, se puede seguir propagando la infección a través del contacto directo. Es importante que todos los especialistas se familiaricen con este tipo de lesiones para así poder hacer un diagnóstico precoz y evitar el uso inadecuado de las cremas con corticoides.

FINANCIACIÓN

Los autores declaran que no han recibido financiación para la realización de este trabajo.

CONFLICTOS DE INTERES

Los autores declaran que no existe ningún conflicto de interés.

BIBLIOGRAFÍA

1. Del Boz-González, J. Tendencias en la *Tinea capitis* en España. Actas Dermosifiliográficas. 2011;102(10):731-738.
2. Durán-Valle MT, Regodón-Domínguez M, Velasco-Rodríguez MJ, Aragón A, Gómez-Garcés JL. Epidemia de tiña por *Trichophyton tonsurans* en un área sanitaria de la Comunidad de Madrid (España). Rev Iberoam Micol. 2016;33:126–8.
3. Moreno-Morales E, Valdez-Landrum P, García-Valdés *et al.* Brote epidémico intrafamiliar de tiña de la cabeza por *Trichophyton tonsurans*: informe de cuatro casos en tres generaciones. Med Cutan Iber Lat Am 2015; 43: 217-221.

4. Monzón A, Luis J, Tudela R. *Trichophyton tonsurans*. Control Calidad SEIMC. 2001.
5. Bascón L, Galvañ JI, López-Riquelme I, Navarro-Guillamón, *et al*. Brote de dermatofitos en egión de cabeza y cuello asociadas al rasurado en peluquerías: estudio descriptivo multicéntrico de una serie de casos. Actas Dermo-sifilogr. 2023;114:371–6.
6. Müller VL, Kappa-Markovi K, Hyun J, Georgas D, Silberfarb G, Paasch U, et al. *Tinea capitis* et barbae caused by *Trichophyton tonsurans*: A retrospective cohort study of an infection chain after shavings in barber shops. Mycoses. 2021;64:428–36.
7. Hernández IM, Sanahuja i Juncadella J. Tiña incógnita. FMC - Form Médica Contin Aten Primaria. 2010;17:33–4.

Official journal of the Spanish Society of Chemotherapy

Carta al Director

Revista Española de Quimioterapia
doi:10.37201/req/049.2024

Jordi Reina
Julia Viana-Ramirez

Impacto en la población adulta de la inmunización con nirsevimab en los menores de 6 meses frente al virus respiratorio sincitial

Unidad de Virología. Servicio de Microbiología. Hospital Universitario Son Espases, Palma de Mallorca.

Article history
Received: 14 May 2024; Revision Requested: 28 May 2024; Revision Received: 12 June 2024; Accepted: 8 July 2024; Published: 16 July 2024

Estimado Editor:

Las infecciones respiratorias agudas (IRAs) de etiología viral son una entidad que afectan a la población general. De los diferentes virus implicados en ellas, el virus respiratorio sincitial (VRS) se presenta de forma epidémica durante los meses invernales. Este virus afecta preferentemente a la población infantil, aunque en los últimos años se ha observado un incremento en el número de casos en la población adulta [1,2], probablemente, fruto del incremento en el diagnóstico microbiológico y la sensibilización de los médicos para solicitar pruebas virológicas en casos de infección respiratoria aguda grave.

En 1970 se pudo confirmar la participación directa de este virus en las infecciones respiratorias agudas de la población adulta y anciana [1-4] presentando en ellas una elevada morbilidad y mortalidad. En la población de mayor edad (>60 años) se calcula que del 3-10% de las IRAs están causadas por el VRS, lo cual representaría unas 250.000 admisiones y 17.000 fallecimientos anuales por neumonía o sus complicaciones [4,5]. La inmunosenescencia y las patologías de base favorecerían la predisposición a las infecciones respiratorias, incluidas el VRS [6,7].

Hasta hace un año no se disponía de ninguna medida preventiva para hacer frente a las infecciones por VRS, de modo que la mayoría de los niños padecían la infección de forma natural. Sin embargo en 2023 se comercializó en España en anticuerpo monoclonal (niservimab) dirigido específicamente frente al "sitio cero (Φ)" (altamente conservado) de la forma prefusión de la proteína F del VRS [8]. Los diferentes ensayos clínicos han demostrado una eficacia cercana al 85% en la prevención del ingreso hospitalario y en la UCI de los pacientes infectados por el VRS, así como un descenso significativo de la carga global de enfermedad (asistencia a centros de salud y urgencias hospitalarias) [9]. En España se han publicado varios estudios que han demostrado el impacto en vida real de la inmunización con nirsevimab de la población lactante [10,11].

Diversos datos epidemiológicos parecen indicar que la población lactante es la que transmite preferentemente el VRS a los adultos [2-5]; aunque también se postula que la trasmisión del virus puede realizarse desde los niños en edad escolar al adulto joven y al de edad avanzada [12]. Por ello hemos creído interesante observar prospectivamente el posible impacto de esta inmunización en la carga de enfermedad por VRS en la población adulta comunitaria.

El estudio se realizó en el Hospital Universitario Son Espases (Palma de Mallorca), centro de referencia para la Comunidad de las Islas Baleares. Desde el inicio de la inmunización hasta el 31 de marzo de 2024 (temporada 2023-2024) se estudiaron prospectivamente todos los adultos (>15 años) que acudían a urgencias con una IRA; a cada uno se les tomaba un frotis nasofaríngeo y se remitía al laboratorio en un medio de transporte. La detección de los diferentes virus respiratorios se realizó mediante una RT-PCR en tiempo real comercial (Allplex Respiratory Assay, Seegene) que detecta de forma simultánea y diferencia 18 virus respiratorios distintos. Se compararon los resultados con los obtenidos en la temporada previa.

Durante el período de estudio se detectaron 480 casos de IRAs causados por el VRS, correspondiendo 280 (58,4%) a la población infantil y 200 (41,6%) a la población adulta (>15 años). Como se observa en la Figura 1, en la actual temporada se ha producido un aumento en el porcentaje de casos detectados en adultos (30,2% en la temporada previa). Sin embargo el número de casos detectados fue de 200 lo que representa un descenso del 7,9% con respecto a la temporada anterior (217 casos). Por edades los casos de VRS se distribuyeron de la siguiente forma: 15-50 años 29 casos (15,5%), de 51-60 años 32 casos (16%), de 61-70 años 39 casos (19,5%), de 71-80 años 42 casos (21%), de 81-90 45 casos (22,5%) y de más de 90 años 13 casos (6,5%). De este modo los mayores de 61 años representaron el 69,5% de todos los casos.

Correspondencia:
Jordi Reina
Unidad de Virología. Servicio de Microbiología. Hospital Universitario Son Espases. Carretera de Valldemossa 79. 07120 Palma de Mallorca.
E-mail: jordi.reina.pmi@gmail.com

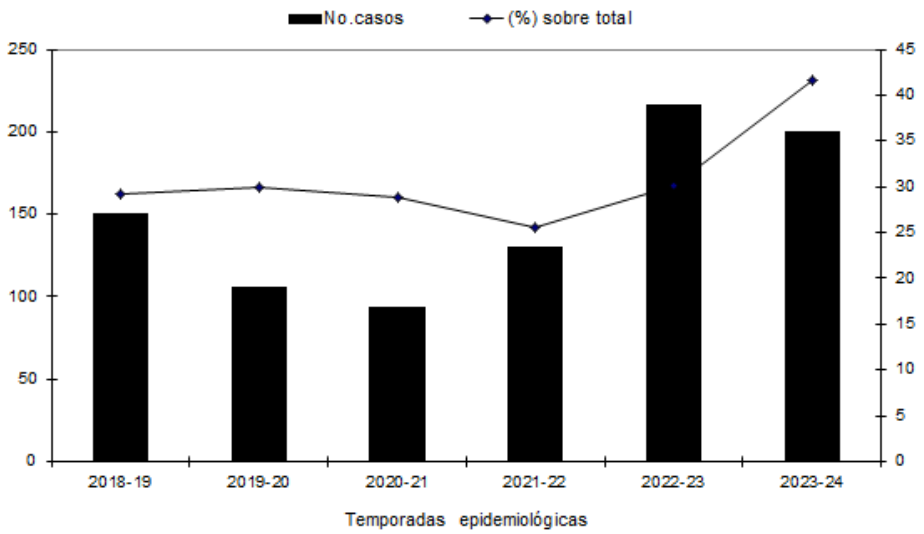


Figura 1 **Evolución del número de casos de VRS en adultos y el porcentaje sobre el total de casos detectados en las últimas temporadas.**

Las IRAs causadas por el VRS en el adulto empiezan a ser una entidad con una incidencia no despreciable (2-39%), con un porcentaje de ingresos hospitalarios cercano al 70% y patologías respiratorias graves como neumonía (26.8%), traqueobronquitis (22.9%); aunque entre el 20-25% de los pacientes tan solo presentan un síndrome gripal, afectando preferentemente a pacientes con patologías cardiorrespiratorias crónicas [1-5].

En Baleares la inmunización de los menores de 6 meses con nirsevimab ha comportado un descenso en el número de casos de VRS detectados en la población infantil (61,3% menos) que ha determinado un ligero descenso asimismo de las infecciones detectadas en la población adulta (7,9%). Sin embargo este descenso no puede atribuirse directamente al proceso de inmunización ya cada temporada de VRS es distinta dependiendo del tipo de VRS que circule, la intensidad de la misma y de otros factores epidemiológicos. Aunque el porcentaje de casos en adultos se ha incrementado hasta el 41,6%, este aumento no es real en cuanto al número de casos detectados sino a su distribución, debido a la disminución del número de casos infantiles.

En una reciente revisión [12] se establece que el impacto de la inmunización infantil sobre la población de edad avanzada es, por ahora, de baja intensidad, coincidiendo con nuestros datos, ya que no se sabe con seguridad si esta inmunización disminuye la carga viral en la orofaringe de los lactantes. Esta revisión es tan sólo hipotética y basada en artículos referenciados, pero se desconoce la situación actual, de modo que los datos publicados recientemente [11,12] podrán sentar las bases de futuros estudios en diferentes temporadas en otros grupos de edad más allá de los lactantes.

Evidentemente la magnitud epidemiológica de la epidemia de VRS del adulto es mucho menor, al menos por ahora, y su impacto en salud pública no comparable a la infantil, aunque el porcentaje de ingresos hospitalarios y fallecimientos podría ser superior a los detectados en la población infantil [4,5]. Parece observarse, por ahora, un ligero impacto del nirsevimab sobre la carga de enfermedad del VRS en adultos, que junto con la posibilidad de utilizar una vacuna específica frente a este virus en la población de >60 años, permitiría el mejor control de esta infección que parece incrementarse con el paso de los años.

FINANCIACIÓN

Los autores declaran no haber recibido financiación para la realización de este estudio.

CONFLICTO DE INTERESES

Los autores declaran no tener ningún conflicto de intereses.

BIBLIOGRAFIA

1. Johnson KM, Bloom HH, Mufson MA, Chanock RM. Natural infection of adults by respiratory syncytial virus: possible relation to mild upper respiratory disease. N Engl J Med 1962; 267:68-72. https://doi.org/ 10.1056/NEJM196207122670204.
2. Hart RJ. An outbreak of respiratory syncytial virus infection in an old people´s home. J Infect Dis 1984; 8:259-61. https://doi.org/10.1016/s0163-4453(84)94075-1.
3. Vikerfors T, Grandien M, Olcen P. Respiratory syncytial virus infection in adults. Am Rev Respir Dis 1987; 136:561-4. https://doi.org/10.1164/ajrccm/136.3.561.

4. Colosia AD, Yang J, Hillson E, Mauskopf J, Copley-Marriman C, Shinde V, Stoddard J. The epidemiology of medically atended respiratory syncytial virus in older adults in the United States: a systematic review. PLoS ONE 2017; 12(8):e0182321. https://doi.org/10.1371/journal.pone.0182321.

5. Thompson WW, Shay DK, Weintraub E. Mortality associated with influenza and respiratory syncytial virus in the United States. JAMA 2003; 289:179-86. https://doi.org/10.1001/jama.289.2.179.

6. Reina J, López C. Infecciones respiratorias causadas por el Virus Respiratorio Sincitial en la población adulta: descripción de una serie de 16 casos. Med Clin (Barc.) 2013; 141:163-6. https://doi.org/10.1016/j.medcli.2013.05.012.

7. Reina J, Iñigo A, Rubio R, López-Causapé C. El Virus Respiratorio Sincitial como causante de infecciones respiratorias agudas en el adulto ¿Una enfermedad emergente?. Rev Clin Esp 2015; 215:418-9. https://doi.org/10.1016/j.rce.2015.03.002.

8. Reina J, Iglesias C. Nirsevimab: hacia la inmunización universal de la población infantil frente al virus respiratorio sincitial. Vacunas 2023; 24:68-73. https://doi.org/10.1016/j.vacun.2022.10.10.002.

9. Recomendaciones de utilización de nirsevimab frente a virus respiratorio sincitial para la temporada 2023-2024. Grupo de trabajo: utilización de nirsevimab frente a virus respiratorio sincitial de la Ponencia de Programa y Registro de Vacunaciones. Comisión de Salud Pública del Consejo Interterritorial del Sistema Nacional de Salud. Ministerio de Sanidad, julio 2023.Disponible en: https://www.sanidad.gob.es/areas/promocionPrevencion/vacunaciones/comoTrabajamos/sincitial.htm.

10. López-Lacort M, Muñoz-Qiles C, Mira-Iglesias A, López-Labrador FX, Mengual-Chulia B, Fernández-García C et al. Early estimates of nirsevimab immunoprophylaxis effectiveness against hospital admission for respiratory syncytial virus lower respiratory tract infections in infants, Spain. October 2023 to January 2024. Euro Surveill 2024; 29(6):pii=2400046. https://doi.org/10.2807/1560-7917.ES.2024.29.6.2400046.

11. Ares Gómez S, Mallah N, Santiago-Pérez MI, Pardo-Seco J, Pérez-Martinez O, Oteri-Barrós MT et al. Effectiveness and impact of universal prophylaxis with nirsevimab in infants against hospitalization for respiratory syncytial virus in Galicia, Spain: initial results of a population-based longitudinal study. https://doi.org/10.1016/S1473-3099(24)00215-9.

12. Sanz-Muñoz I, Castrodeza-Sanz J, Eiros JM. Potential effects on elderly people from nirsevimab use in infants. Open Respir Arch 2024; 6:100320. https://doi.org/10.1016/j.opresp.2024.100320.

Official journal of the Spanish Society of Chemotherapy

Letter to the Editor

Revista Española de Quimioterapia
doi:10.37201/req/050.2024

Lucía Iglesias-Linares[1]
Ana Román-de-la-Torre[2]
María Aguilera-Franco[2]
Alberto Puertas-Prieto[1,3]
María José Medina-Pascual[4]
José Gutiérrez-Fernández[2,3,5]

Threat of preterm labor and preterm birth in the presence of *Lachnoanaerobaculum gingivalis*

[1]Servicio de Obstetricia y Ginecología, Hospital Universitario Virgen de las Nieves. Granada, España.
[2]Servicio de Microbiología, Hospital Universitario Virgen de las Nieves. Granada, España.
[3]Instituto de Investigación Biosanitaria Granada. Ibs. Granada.
[4]Laboratorio de Referencia e Investigación en Taxonomía. Centro Nacional de Microbiología (Instituto de Salud Carlos III)
[5]Departamento de Microbiología, Facultad de Medicina y Programa de Doctorado en Medicina Clínica y Salud Pública, Universidad de Granada.

Article history
Received: 16 May 2024; Revision Requested: 8 July 2024; Revision Received: 6 August 2024;
Accepted: 4 September 2024; Published: 30 September 2024

Sir,

Bacteria of the *Lachnoanaerobaculum* genus are obligate Gram-positive, spore-forming, filamentous bacilli that may appear Gram-negative due to their easy decolorization [1]. The genus contains four species, *L. orale*, *L. saburreum*, *L. umeaense*, and *L. gingivalis*, found in the oral cavity, saliva, and small intestine of humans. Their presence has been associated with gingival disease and bacteremia in patients with hematological malignancies [1-5]. We present the first report of an association between this genus and an episode of chorioamnionitis. We examine the clinical, diagnostic, and therapeutic data and other factors that may elucidate the pathogenesis.

A 25-year-old woman with no medical history was referred to our Regional Hospital at 29+1 weeks of gestation for the diagnosis of threatened preterm labor. She was asymptomatic at arrival, and her vital signs were within normal ranges. She reported low-intensity uterine contractions and exhibited a shortened cervical length of 6 mm. In the emergency department, premature membrane rupture was ruled out, vagino-rectal swabs were taken to detect *Streptococcus agalactiae*, and vaginal discharge and urine samples were gathered for routine microbiological cultures. Analytic findings included leukocytosis (13,000/µL; normal range: 3,500-10,500/µL), neutrophilia (86%; 42-77%), and C-reactive protein (CRP) level of 28.4 mg/L (0.1-5 mg/L). Treatment commenced with intravenous tocolysis and the administration of corticosteroids for lung maturation. The patient remained clinically stable, but an increase in CRP levels was observed.

Intravenous antibiotic prophylaxis was initiated with clindamycin (900 mg/8 h) and gentamicin (240 mg/24 h), due to a penicillin allergy. Amniocentesis revealed the presence of elongated, slender Gram-negative bacilli (Figure 1) with reduced glucose (8 mg/dL) and elevated interleukin 6 (160,834 pg/mL) levels, prompting the clinical diagnosis of chorioamnionitis. Polymerase chain reaction (PCR) analysis of the amniotic fluid was positive for *Ureaplasma urealyticum*. Tocolytic treatment was discontinued, and magnesium sulfate infusion was initiated for fetal neuroprotection, followed by labor induction.

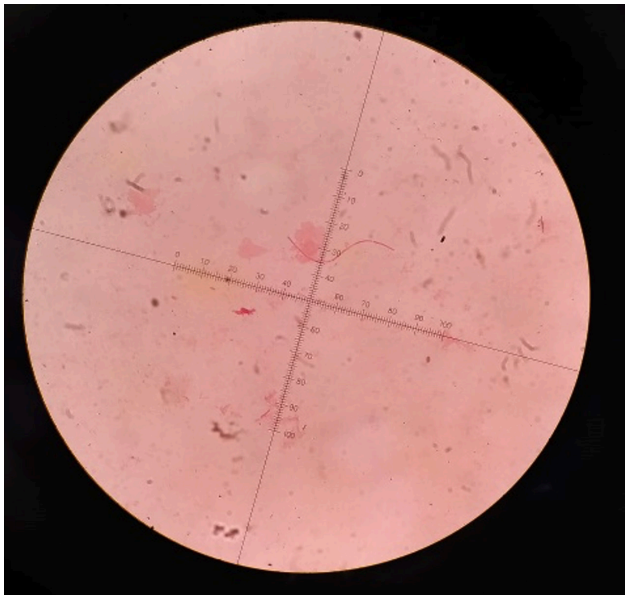


Figure 1 | **Gram staining of *Lachnoanaerobaculum gingivalis***

After eight hours of induction, she delivered a 1,350 g female infant with an Apgar score of 6 at one minute and 9 at five minutes, umbilical artery pH of 7.35, and venous pH of 7.37. The antibiotic treatment was continued for 24 h postpartum.

L. orale colonies alone were isolated within the first 48 h of anaerobic incubation on sheep blood agar plates (Bi-

Correspondence:
Lucía Iglesias Linares
Servicio de Obstetricia y Ginecología, Hospital Universitario Virgen de las Nieves. Granada, España.
E-mail: iglesiaslinaresl@gmail.com

Table 1 **Antibiotic susceptibility of *Lachnoanaerobaculum gingivalis* according to the E-test.**

Antibiotics	MIC (mg/L)	Clinical category
Ampicillin	<0.016	Susceptible
Piperacillin-tazobactam	<0.016	Susceptible
Cefotaxime	0.12	Susceptible
Imipenem	0.008	Susceptible
Clindamycin	24	Resistant
Tetracycline	8	Intermediate
Metronidazole	0.125	Susceptible

oMerieux, France), presenting as gray, shiny, spread-out, non-hemolytic colonies with soft consistency and irregular edges. The colonies were identified (score 2.103) as *L. orale* by MALDI-TOF spectrometry (Bruker Biotyper, Germany) and were then genetically identified as *L. gingivalis* at the National Center of Microbiology (Majadahonda, Madrid, Spain) by 16S rRNA gene sequencing, following a previously described protocol and using E781 and U1115 as primers [6,7] and the Applied Biosystems 3730xl DNA Analyzer for sequencing. A sequence of 1331pb was obtained and compared with strain sequences in the GenBank data bank (https://blast.ncbi.nlm.nih.gov/Blast.cgi), observing 99.2% identity with L. gingivalis (ChDC B114), described in a previous study [8]

Antimicrobial susceptibility testing was performed using gradient strips (Liofilchem, Italy) according to 2023 CLSI criteria for anaerobic Gram-negative bacilli (CMI value in mg/L) on Brucella blood agar supplemented with hemin (5 µg/mL), vitamin K1 (1 µg/mL), and laked sheep blood (5% v/v) (Becton Dickinson, BD, Franklin Lakes, NJ, USA) anaerobically cultured at 36 °C±1 °C for 48 h. Table 1 exhibits the results.

L. gingivalis is rarely isolated in clinical samples [4] due to its easy decolorization in Gram staining and the resulting mis-assignment to other Gram-negative bacilli in this rapid test. Its correct identification is also hampered by current Maldi-TOF databases, with frequent confusion between *L. orale* and *L. gingivalis*.

Acute or subacute chorioamnionitis is defined by placental membrane inflammation of infectious origin. It has been proposed that microorganisms can gain access to the amniotic cavity by four routes: *via* ascent from the lower genital tract, hematogenous spread, invasive procedures, and/or seeding from the peritoneal cavity [9].

Bacteria involved in periodontal disease can reach the amniotic cavity, depending on patient-related and/or microorganism-related factors [10,11]. *L. gingivalis* was isolated from amniotic fluid in the present patient; however, its significance appears to be limited given that the therapy was not effective, as shown by the antibiogram.

Membrane rupture is not a prerequisite for the entry of microorganisms into the amniotic cavity. Bacteria can penetrate intact membranes and cause subclinical infections that are often undetected when the amniotic fluid is not analyzed.

L. gingivalis is more frequently detected in saliva and dental plaque and is associated with the development of gingival diseases [5]; hence, the oral cavity is a probable source of the infection in the present patient. *Lachnoanaerobaculum* has also been isolated from blood, causing bacteremia in patients under chemotherapy for acute myeloid leukemia [2-4].

The absence of data on this bacterium in amniotic fluid can be attributed to the lack of comprehensive microbiological studies in these patients, explained by the subacute progression of the infection, with no evident symptoms of acute chorioamnionitis.

The antibiogram indicated *in vitro* activity against ampicillin, piperacillin/tazobactam, cefotaxime, imipenem, and metronidazole and resistance to clindamycin. Despite this resistance, the patient remained stable and showed a favorable clinical progression, suggesting that the infection was successfully resolved by the completion of gestation.

In conclusion, this is the first reported case of the isolation from amniotic fluid of *L. gingivalis*, a potential cause of intrauterine infection and threatened preterm birth.

FUNDING

None to declare

CONFLICT OF INTEREST

Authors declare no conflict of interest

REFERENCES

1. Hedberg M, Moore E, Svensson-Stadler L, Hörstedt P, Baranov V, Hernell O, et al. *Lachnoanaerobaculum* gen. nov., a new genus in the Lachnospiraceae: characterization of *Lachnoanaerobaculum umeaense* gen. nov., sp. nov., isolated from the human small intestine, and *Lachnoanaerobaculum orale* sp. nov., isolated from saliva, and reclassification of *Eubacterium saburreum* (Prevot 1966) Holdeman and Moore 1970 as *Lachnoanaerobaculum saburreum* comb. nov. Int J Syst Evol Microbiol. 2012;62(11):2685-2690. doi: 10.1099/ijs.0.033613-0.
2. Sabrie N, Halani S, Maguire F, Aftanas P, Kozak R, Andany N. *Lachnoanaerobaculum orale* bacteremia in a patient with acute myeloid leukemia and stomatitis: An emerging pathogen. IDCases. 2023 Jul;33:e01837. doi: 10.1016/j.idcr.2023.e01837.
3. Ida Y, Okuyama T, Araki K, Sekiguchi K, Watanabe T, Ohnishi H. First description of *Lachnoanaerobaculum orale* as a possible cause of human bacteremia. Anaerobe. 2022;73:102506. doi: 10.1016/j.anaerobe.2021.102506.
4. Okada N, Murakami A, Sato M, Nakamura S, Fujii S, Sogabe K, et al. First reported case of *Lachnoanaerobaculum gingivalis* bacteremia

in an acute myeloid leukemia patient with oral mucositis during high dose chemotherapy. Anaerobe. 2022 Aug;76:102610. doi: 10.1016/j.anaerobe.2022.102610.

5. Lim YK, Park SN, Jo E, Shin JH, Chan YH, Shin Y, et al. *Lachnoanaerobaculum gingivalis* sp. nov., Isolated from Human Subgingival Dental Plaque of a Gingivitis Lesion. Curr Microbiol. 2019;76(10):1147-1151. doi: 10.1007/s00284-019-01747-z.
6. Baker GC, Smith JJ, Cowan DA. Review and re-analysis of domain-specific 16S primers. J Microbiol Methods 2003;55: 541–555. doi: 10.1016/j.mimet.2003.08.009.
7. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 1991; 173:697-703. doi: 10.1128/jb.173.2.697-703.199
8. Lim YK, Park SN, Jo E, Shin JH, Chang YH, Shin Y, et al. *Lachnoanaerobaculum gingivalis* sp. nov., isolated from human subgingival dental plaque of a gingivitis lesion. Curr Microbiol 2019; 76:1147-1151. doi: 10.1007/s00284-019-01747-z.
9. Conde-Agudelo A, Romero R, Jung EJ, Garcia-Sánchez ÁJ. Management of clinical chorioamnionitis: an evidence-based approach. Am J Obstet Gynecol. 2020 Dec;223(6):848-869. doi: 10.1016/j.ajog.2020.09.044.
10. Cardona-Benavides I, Puertas-Prieto A, Pinilla-Martín FJ, Navarro-Marí JM, Gutiérrez-Fernández J. *Alloscardovia omnicolens* emerging presence in premature rupture of membranes. New Microbiol. 2019 Oct;42(4):237-239.
11. Hidalgo-Chicharro A, Abad-Torreblanca R, Navarro-Marí JM, Gutiérrez-Fernández J. 32-week premature rupture of membranes caused by oropharyngeal microbiota. JMM Case Rep. 2017 Oct 12;4(10):e005121. doi: 10.1099/jmmcr.0.005121.

Official journal
of the Spanish Society
of Chemotherapy

Carta al Director

Revista Española de Quimioterapia
doi:10.37201/req/061.2024

Cristina González
Borja Croche
Emilio Gómez

Erupción mucocutánea infecciosa reactiva (RIME) en pacientes pediátricos: afectación extrapulmonar de *Mycoplasma pneumoniae*

Servicio de Pediatría General, Hospital Universitario Virgen del Rocío, Sevilla, España

Article history
Received: 24 June 2024; Revision Requested: 30 July 2024; Revision Received: 16 August 2024;
Accepted: 4 September 2024; Published: 8 October 2024

Estimado Editor:

La infección por *Mycoplasma pneumoniae* tiene un espectro clínico de presentación amplio, que va desde formas asintomáticas a cuadros respiratorios de diversa gravedad o manifestaciones extrapulmonares [1]. Afecta predominantemente a pacientes en edad escolar, siendo los cuadros más frecuentes la traqueobronquitis y la neumonía atípica. Sin embargo, existen manifestaciones menos frecuentes que conviene saber reconocer como son la mucositis y/o el exantema mucocutáneo [2]. La erupción mucocutánea infecciosa reactiva (RIME) engloba a este exantema y mucositis inducidos por *M. pneumoniae* (conocido por las siglas MIRM *"Mycoplasma pneumoniae induced rash and mucositis"*) y a otros producidos en menor frecuencia por *C. pneumoniae*, metapneumovirus, parainfluenza tipo 2, influenza B, enterovirus, adenovirus y SARS-CoV-2 [3].

Presentamos 4 casos de RIME por *M. pneumoniae* identificados en nuestro centro hospitalario en un periodo inferior a un mes (Tabla 1). Realizamos una revisión sobre el diagnóstico y tratamiento de esta entidad.

El RIME se caracteriza por la presencia de mucositis intensa en dos o más localizaciones, con afectación preferente en boca produciendo la aparición de costras hemorrágicas en labios, erosiones y úlceras en mucosa (Figura 1). Suele afectar a la zona genitourinaria y, menos frecuentemente, a los ojos causando conjuntivitis bilateral purulenta o seromucosa y edema de párpados. La afectación cutánea puede ser en forma de lesiones vesículo-bullosas (más frecuentes), anulares en diana, morbilliformes o pápulo-maculosas. De forma característica afectan a menos del 10% de la superficie corporal. La aparición de esta sintomatología mucocutánea suele producirse tras un cuadro previo de siete a diez días de evolución caracterizado por fiebre, tos no productiva, astenia, cefalea u otros síntomas respiratorios [4].

Correspondencia:
Cristina González Vargas
Servicio de Pediatría General, Hospital Universitario Virgen del Rocío, Sevilla, España
E-mail: cristinaglezvargas@gmail.com

El diagnóstico es clínico, basándose en el reconocimiento de estas lesiones en un contexto compatible con infección por *M. pneumoniae* o por los otros gérmenes asociados a esta entidad. Es imprescindible descartar la ingesta reciente de fármacos que pudieran inducir el desarrollo de cuadros similares como son el Síndrome de Stevens-Johnson/necrólisis epidérmica tóxica (SJS/NET). Se recomienda realizar radiografía de tórax, analítica, PCR en exudado nasofaríngeo para *M. pneumoniae*, virus respiratorios y virus herpes simple [2]. Además, dada la dificultad de interpretación que a veces conllevan las pruebas moleculares para diferenciar infección de colonización puede ser conveniente realizar serología. La biopsia cutánea no es necesaria realizarla de forma sistemática, reservándose para casos dudosos o evoluciones inusuales. El diagnóstico diferencial se establece principalmente con el eritema multiforme (EM) y con el SJS/NET. El EM es producido en su mayoría por virus herpes. Se distingue clínicamente por la morfología de las lesiones en forma de diana que pueden confluir y por presentar escasa afectación mucosa [5].

El SJS/NET es típicamente producido por fármacos, presentando lesiones más extensas, Nikolsky positivo y síntomas constitucionales. Otras entidades que considerar en el diagnóstico diferencial son la gingivoestomatitis herpética, el pénfigo, la enfermedad mano-boca-pie y la estomatitis aftosa [5].

El tratamiento se basa fundamentalmente en medidas de soporte. Se debe garantizar una adecuada analgesia dado el intenso dolor que producen las lesiones en mucosas oral. Es fundamental asegurar el correcto estado de hidratación y nutrición, siendo necesario en ocasiones la sueroterapia endovenosa. Respecto al manejo local de las lesiones mucocutáneas se recomienda realizar curas evitando la acumulación de costras, emplear vaselina como mantenimiento de la barrera cutánea, corticoides tópicos si existe intensa inflamación y pomadas antibióticas si hay datos de sobreinfección por otros gérmenes. En caso de afectación ocular puede ser necesario el uso de lubricantes oculares y/o corticoides oftálmicos. No está claro el papel de la antibioterapia dirigida frente a *M. pneumoniae* en

Tabla 1 **Descripción de casos de RIME. Revisión de diagnóstico y tratamiento**

Paciente	Presentación clínica	Diagnostico microbiológico	Tratamiento	Evolución
Mujer 8 años	Fiebre de 7 días, tos y mucosidad. Edema de labios con lesiones costrosas negruzcas, aftas bucales friables, inyección conjuntival bilateral, úlceras genitales y perianales. Lesiones pápulo-costrosas no confluentes en cara, tronco y miembros (Figura 1).	PCR nasofaríngea positiva a *M. pneumoniae*. Serología IgG e IgM positiva a *M. pneumoniae*.	Azitromicina IV 5 días. Amoxicilina-clavulánico IV 7 días. Metrilprednisolona IV 1 mg/Kg día 5 días. Dexametasona tópica conjuntival. Betametasona /gentamicina tópica sobre las lesiones genitourinarias.	Ingreso hospitalario 7 días. Lesiones hipocrómicas residuales a las 3 semanas.
Varón 5 años	Fiebre de 7 días y síntomas respiratorios. Lesiones en mucosa yugal, edema de encías y labios. Lesiones cutáneas vesiculosas sobre base eritematosa que se decapitan, bordes costrosos en diana.	PCR nasofaríngea positiva a *M. pneumoniae*. Serología IgM e IgG positiva a *M. pneumoniae*.	Aciclovir VO (hasta resultado PCR VHS). Azitromicina VO 3 días. Prednisolona VO 1 mg/Kg 3 días. Ácido fusídico /betametasona tópica sobre lesiones cutáneas.	Manejo ambulatorio. Evolución favorable. Seguimiento por dermatología.
Varón 4 años	Fiebre 4 días, malestar general, síntomas respiratorios. Queilitis y lesiones costrosas en labios. En piel maculo-pápulas con vesícula central y bordes costrosos en los cuatro miembros.	PCR nasofaríngea positiva a *M. pneumoniae*.	Azitromicina VO 3 días. Mupirocina tópica en lesiones dérmicas. Gel lidocaína 1% sobre lesiones mucosa oral.	Manejo ambulatorio. Evolución favorable sin lesiones residuales.
Varón 10 años	Fiebre de 4 días, tos y disnea. Labios agrietados, lesiones ampollosas y úlceras en mucosa yugal. No lesiones cutáneas. Hiperemia conjuntival bilateral.	PCR nasofaríngea negativa a *M. pneumoniae*. Serología IgM positiva a *M. pneumoniae*.	Azitromicina VO 3 días. Dexametasona tópica conjuntival. Enjuague oral de triamcinolona + Lidocaína oral.	Ingreso hospitalario 3 días. Evolución favorable de lesiones mucosas.

IV: intravenoso; VO: vía oral.

la evolución de las lesiones, aunque se recomienda su administración. Para ello empleamos azitromicina oral preferentemente, o intravenosa en caso de imposibilidad oral o casos graves en ciclos de 3 -5 días. Respecto al uso de corticoides sistémicos se recomiendan en casos graves con afectación extensa de piel y mucosas con el objetivo de disminuir la inflamación y el dolor. Habitualmente se emplean ciclos de 5-7 días de prednisona o metilprednisolona a 1 mg/kg/día [6].

El pronóstico del RIME es habitualmente bueno, siendo lo más frecuente la curación completa y sin secuelas. Ocasionalmente se han descrito casos en los que se han identificado tras el cuadro alteraciones pigmentarias postinflamatorias en piel, sinequias o cicatrices genitales, ulceraciones corneales, ojos secos, ceguera o caídas de pestañas [6].

A modo de conclusión subrayamos la importancia del reconocimiento del RIME ante cuadros clínicos compatibles ya que ello nos va a permitir llevar a cabo su diagnóstico precoz, evitar la realización de pruebas invasivas como la biopsia y establecer el tratamiento necesario que conduzca a una curación sin secuelas.

Figura 1 **Afectación mucosa oral con edema y lesiones costrosas en labios. Afectación cutánea con lesiones pápulo-vesiculosas sobre base eritematosa.**

FINANCIACIÓN

Los autores declaran no haber recibido financiación para la realización de este estudi

CONFLICTO DE INTERESES

Los autores declaran no tener ningún conflicto de intereses

BIBLIOGRAFÍA

1. Xing Y, Wang D, Sheng K, Xiao X, Wei H, Liu L, et al. Dynamic change of Mycoplasma pneumoniae pneumonia in hospitalized children in a general hospital: a 3-year retrospective analysis. Transl Pediatr. 2020; 9(4):522-531. doi: 10.21037/tp-20-149
2. Ramien ML, Bruckner AL. Erupciones mucocutáneas en pacientes pediátricos con enfermedades agudas: piense primero en Mycoplasma pneumoniae (y otras infecciones). JAMA Dermatol. 2020;156(2):124-125. doi: 10.1001/jamadermatol.2019.3589
3. Ramien ML. Reactive infectious mucocutaneous eruption: Mycoplasma pneumoniae-induced rash and mucositis and other parainfectious eruptions. Clin Exp Dermatol, 2021];46(3):420-429. doi.org/10.1111/ced.14404
4. Canavan TN, Mathes EF, Frieden I, Shinkai K. Mycoplasma pneumoniae-induced rash and mucositis as a syndrome distinct from Stevens-Johnson syndrome and erythema multiforme: a systematic review. J Am Acad Dermatol. 2015;72(2):239-45. doi: 10.1016/j.jaad.2014.06.026
5. Amode R, Ingen-Housz-Oro S, Ortonne N, Bounfour T, Pereyre S, Schlemmer F, et al. Clinical and histologic features of Mycoplasma pneumoniae-related erythema multiforme: A single-center series of 33 cases compared with 100 cases induced by other causes. J Am Acad Dermatol. 2018;79(1):110-117. doi: 10.1016/j.jaad.2018.03.013
6. Mathes E, Kittler N. Reactive infectious mucocutaneous eruption (RIME). UpToDate.

Official journal of the Spanish Society of Chemotherapy

Carta al Director

Revista Española de Quimioterapia
doi:10.37201/req/065.2024

Patricia Andres-Ibarrola[1]
Laura Guío Carrión[2]

Lesiones en diana y fiebre, un microorganismo inesperado

[1]Departamento de Dermatología del Hospital Universitario de Cruces, Bizkaia, España.
[2]Departamento de Enfermedades Infecciosas del Hospital Universitario de Cruces, Barakaldo, Bizkaia, España.

Article history
Received: 8 June 2024; Accepted: 12 September 2024; Published: 27 September 2024

Estimado Editor:

Exponemos un caso clínico de una paciente que presentó un eritema multiforme asociado a una infección respiratoria atípica por *Chlamydophila pneumoniae*.

Caso: Mujer de 77 años que acudió al servicio de urgencias por presentar fiebre de 39ºC, tiritona, astenia, dolor articular, y disnea de cuatro días de evolución. El día previo la paciente comenzó a presentar un exantema maculopapuloso, de predominio en tronco y raíces de miembros, no pruriginoso. La paciente relataba que las lesiones habían ido aumentando de tamaño y se habían extendido a palmas y plantas. Negaba contacto con animales, ingesta de productos sin pasteurizar, picaduras recientes, contacto con niños pequeños e introducción de nuevos fármacos.

En la exploración física destacó fiebre de 38ºC y la presencia de lesiones maculares confluyentes distribuidas por toda la superficie corporal respetando cabeza y cuello, con predominio en tórax, abdomen y extremidades. Estas lesiones presentaban morfología dianiforme, con borde sobreelevado y eritematoso, y el centro más blanquecino (Figura 1). En la auscultación pulmonar se objetivaron crepitantes generalizados en ambas bases pulmonares.

En las pruebas complementarias realizadas en la urgencia, se observó una marcada elevación de parámetros inflamatorios en la analítica. Las PCRs para virus de la gripe y SARS-CoV-2 resultaron negativas, así como la determinación de antígenos de *Streptococcus pneumoniae* y *Legionella sp.* en orina. Se realizó una radiografía de tórax que mostró un pinzamiento de ambos senos costofrénicos sin áreas de aumento de densidad (Figura 2).

Se decidió su ingreso en la Unidad de Enfermedades Infecciosas, donde se inició tratamiento con ceftriaxona y azitromicina ante la sospecha de una infección respiratoria atípica. Durante el seguimiento, se solicitaron serologías de *C. pneumoniae*, *Mycoplasma pneumoniae*, VIH, VHS-1, VHS-2, *Parvovirus B19* y *Coxiella burnetii*. Pendiente de los resultados serológicos, tras varios días de estabilización clínica y mejoría de la sintomatología, se recomendó el alta de la paciente con seguimiento en consultas externas.

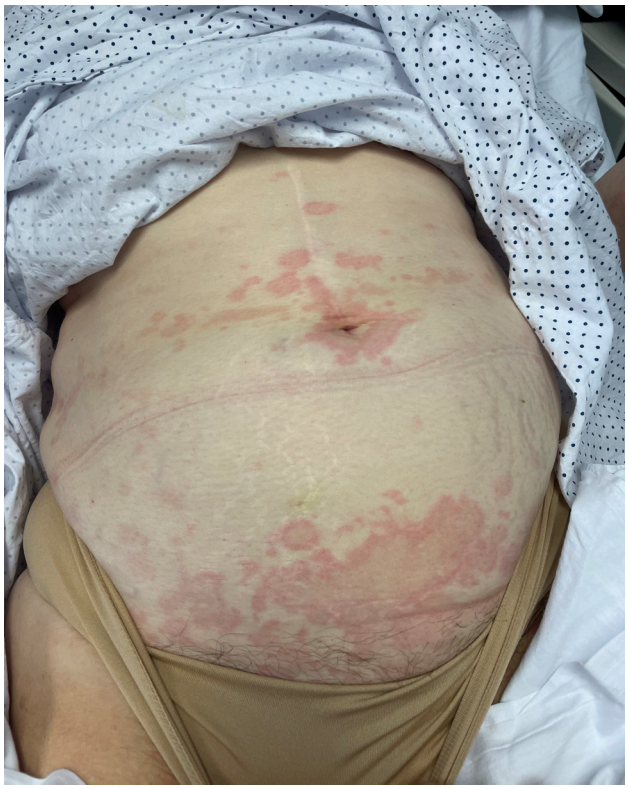


Figura 1 Exantema maculopapular en abdomen con lesiones dianiformes concluyentes.

Correspondencia:
Patricia Andrés Ibarrola
Hospital Universitario de Cruces, Cruces Plaza, s/n, 48903 Barakaldo, Bizkaia, España.
E-mail: patricia.andresibarrola@osakidetza.eus

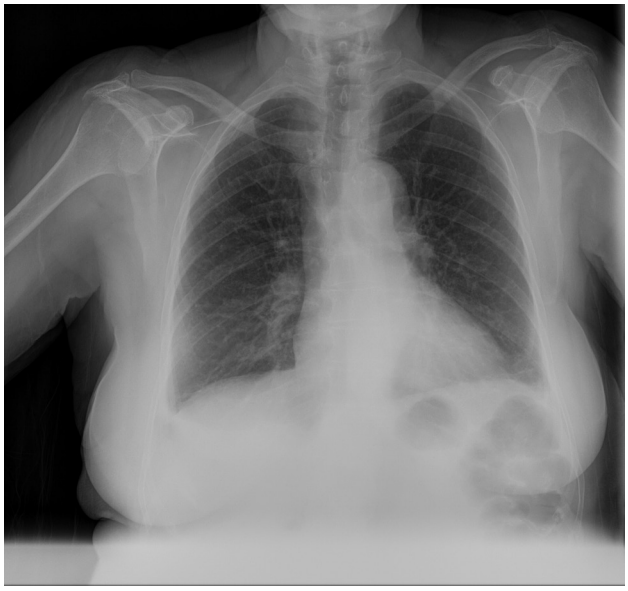


Figura 2 | **Radiografía de tórax que muestra un pinzamiento de ambos senos costofrénicos sin áreas de aumento de densidad.**

Un mes después, la paciente presentó mejoría de su sintomatología con resolución completa del exantema y normalización de los parámetros inflamatorios en la analítica de control. Se recibió finalmente el resultado de las serologías solicitadas, destacando una IgG elevada (1/256) para *C. pneumoniae*, aunque con IgM negativa. En el control realizado una semana después, la serología resultó IgM positiva por técnica CLIA, y el título de IgG se había duplicado (1/512). Las serologías restantes fueron negativas.

La paciente fue finalmente diagnosticada de infección respiratoria atípica por *C. pneumoniae* asociada a eritema multiforme.

El eritema multiforme es un exantema agudo autolimitado caracterizado por la presencia de lesiones maculopapulosas en forma de diana de predominio acral. Típicamente se asocia a la infección por Virus Herpes Simple y por *M. pneumoniae*, así como a la exposición a diversos fármacos [1]. Si bien clásicamente la infección por *C. pneumoniae* se ha correlacionado con la presencia de lesiones cutáneas tipo urticaria o eritema nodoso, no se describe habitualmente asociada a esta clase de exantema [2]. No obstante, se han descrito en la literatura hasta 28 casos de eritema multiforme asociados a la infección producida por este microorganismo [3-6].

El caso descrito junto con la evidencia científica disponible orienta a que es posible que exista una asociación entre la infección por *C. pneumoniae* y la presencia de eritema multiforme, pero que ésta esté siendo infradiagnosticada. De cara a evitar este infradiagnóstico y con el fin de lograr un mejor manejo terapéutico de estos pacientes, consideramos que podría ser recomendable investigar la presencia no sólo de *M. pneumoniae* sino también de *C. pneumoniae* en todo paciente con eritema multiforme y clínica respiratoria atípica aguda, a pesar de la ausencia de infiltrados neumónicos en la radiografía de tórax.

FINANCIACIÓN

Los autores declaran no haber recibido financiación para la realización de este estudio.

CONFLICTO DE INTERESES

Los autores declaran no tener ningún conflicto de intereses.

BIBLIOGRAFÍA

1. Lerch M, Mainetti C, Terziroli Beretta-Piccoli B, Harr T. Current Perspectives on Erythema Multiforme. Clin Rev Allergy Immunol. 2018;54(1):177-84. https://doi.org/10.1007/s12016-017-8667-7
2. Burillo A, Bouza E. *Chlamydophila pneumoniae*. Infect Dis Clin North Am. 2010;24(1):61-71. https://doi.org/10.1016/j.idc.2009.10.002
3. De Luigi G, Zgraggen L, Kottanattu L, Simonetti GD, Terraneo L, Vanoni F, et al. Skin and Mucous Membrane Eruptions Associated with *Chlamydophila Pneumoniae* Respiratory Infections: Literature Review. Dermatology. 2021;237(2):230-5. https://doi.org/10.1159/000506460
4. Imashuku S, Kudo N. *Chlamydia pneumoniae* infection-associated erythema multiforme. Pediatr Rep. 2013;5(2):35-7. https://doi.org/10.4081/pr.2013.e9
5. Sawada T, Suehiro M. Erythema multiforme associated with *Chlamydophila pneumoniae* infection: a report of two cases and a mini-literature review. J Dermatol. 2015;42(3):336-7. https://doi.org/10.1111/1346-8138.12769
6. Hosokawa R, Kobayashi T, Higashino T, Asano C, Ono K, Fujimoto N, et al. Two cases of erythema exsudativum multiforme associated with *Chlamydia pneumoniae* infection. J Dermatol. 2012;39(3):306-8. https://doi.org/10.1111/j.1346-8138.2011.01319.x

Official journal of the Spanish Society of Chemotherapy

Letter to the Editor

Revista Española de Quimioterapia
doi:10.37201/req/075.2024

Marina Fayos[1,2]
Andrea Severo[3]
María Dolores García-Cosío[3]
Carlos Prados[4]
Marina Alonso[4]
Francisco López-Medrano[1,2]

Nocardia and mucoral co-infection in heart transplant recipient

[1]Unit of Infectious Diseases, Hospital Universitario "12 de Octubre", Instituto de Investigación Sanitaria Hospital "12 de Octubre" (imas12), Madrid, Spain.
[2]Centro de Investigación Biomédica en Red de Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III (ISCIII), Madrid, Spain.
[3]Department of Cardiology, Hospital Universitario "12 de Octubre". Madrid, Spain.
[4]Department of Pathological anatomy. 12 de Octubre University Hospital. Madrid, Spain.

Article history
Received: 19 July 2024; Revision Requested: 12 September 2024; Revision Received: 13 September 2024; Accepted: 20 September 2024; Published: 10 October 2024

Sir,

Bacterial and fungal coinfection presents a significant diagnostic and therapeutic challenge in solid organ transplant recipients. This case involves a heart transplant recipient who developed pulmonary consolidation and multiple brain lesions. *Nocardia farcinica* was isolated from a subcarinal adenopathy, but despite targeted treatment, new lesions appeared in the lungs, brain, and stomach. Additionally, *Cunninghamella* spp. was detected in a bronchoalveolar lavage, leading to a complicated clinical course.

A 64-year-old male underwent heart transplantation in March 2022 for ischemic cardiomyopathy. The patient received induction therapy with basiliximab (2 doses) and was on immunosuppressive therapy with tacrolimus 1,5 mg every 12 hours (last levels 9.7 ng/ml), everolimus 0,5 mg every 12 hours (last levels < 0.30 ng/ml), and prednisone 15 mg per day. In October 2023, he was admitted for Ogilvie syndrome following a hernioplasty, where he received meropenem due to respiratory symptoms and radiological findings (Figure 1A). A follow-up computed tomography (CT) revealed a decrease in consolidation after treatment, along with a subcarinal adenopathy (Figure 1B). Despite initial improvement, the patient's condition deteriorated, leading to readmission and further diagnostic challenges.

A transbronchial biopsy of the subcarinal adenopathy performed on day +7 of admission revealed the presence of *N. farcinica* in the culture, leading to targeted therapy with cotrimoxazole 1600/320 mg every 8 hours and meropenem 1 g every 8 hours. However, the patient's clinical condition continued to deteriorate, with the development of multiple abscesses detected on brain magnetic resonance imaging (MRI) performed on day +21 of admission (Figure 1C). Suspecting disseminated nocardiosis, the dosage of meropenem was increased to 1,5 g every 8 hours, but the patient's condition worsened further. On day +24, the patient exhibited a reactivation of cytomegalovirus (CMV) infection with 8800 copies/ml. A follow-up chest CT scan on day +35 showed worsening consolidation in the left upper lobe with cavitation and involvement of the right upper lobe. The serum levels of Wako beta-D-glucan were 37.84 pg/ml, and galactomannan was negative. Suspecting an invasive fungal superinfection, liposomal amphotericin B was started at a dose of 5 mg/kg/24h. Additionally, moxifloxacin was added to the meropenem and cotrimoxazole regimen due to concerns about Nocardia progression.

Further complications arose, and the patient required admission to the Intensive Care Unit (ICU) on day +39. Complications included gastrointestinal bleeding, respiratory distress, and neurological deficits. A cranial CT scan revealed a subacute ischemic lesion newly appearing in the left parietal area, with improvement of the previous lesions observed on the MRI. Additionally, an endoscopy showed multiple necrotic gastric ulcers. Due to the progression of the condition, the patient continued to receive an antibiotic regimen for *Nocardia* consisting of cotrimoxazole, imipenem, and amikacin. The antifungal treatment was changed to isavuconazole after *Aspergillus fumigatus* was isolated from a sputum culture taken on day +35. A subsequent bronchoalveolar lavage on day +41 revealed non-septate mycelia consistent with mucorales and the isolation of *Cunninghamella* spp. in the culture. Surgical intervention for suspected disseminated mucormycosis was ruled out by the thoracic surgery department at that time. Despite intensive medical support and treatment in the ICU, the patient deteriorated rapidly and passed away on day +45 of hospitalization.

A clinical autopsy confirmed the presence of mucormycosis in the lungs, gastric ulcers, spleen, and central nervous system, as well as *Nocardia* infection in lymph nodes and the central nervous system (Figure 2). The cause of death

Correspondence:
Marina Fayos
Hospital Universitario 12 de Octubre. Madrid.
Av. Cordoba, s/n, Usera, 28041. Madrid, Spain.
E-mail: marina.fayos.perez@gmail.com.

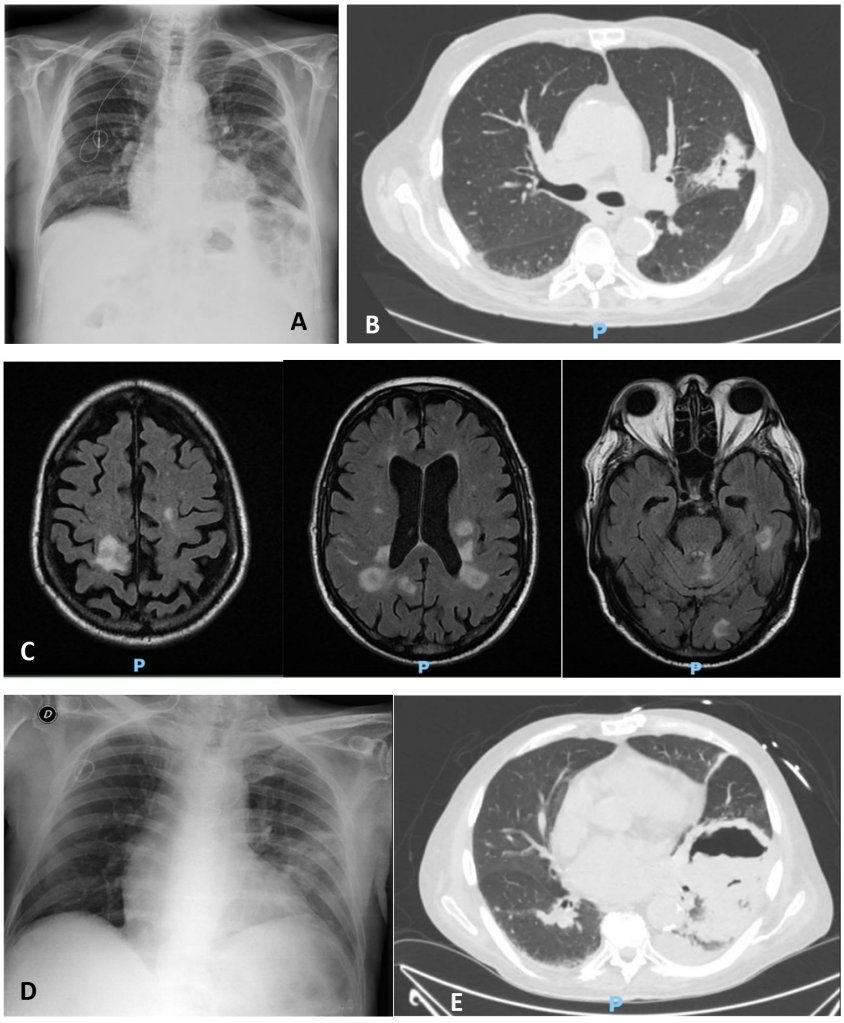


Figure 1 Image A presents a chest X-ray performed on day +4 of the previous admission, due to dry cough and fever. It shows a subsegmental parenchymal consolidation with air bronchogram in the left upper lobe (LUL). Image B presents a CT scan performed on day +10 of the last admission, due to dry cough and fever. The CT scan reveals a decrease in consolidation after 5 days of antibiotic treatment, along with a subcarinal adenopathy cluster. Image C: Brain MRI performed on day 21 of admission, showing 2 infratentorial and 20 supratentorial lesions. These lesions are ring-shaped, subcentimetric, and associated with vasogenic edema. The FLAIR sequence depicts signal hyperintensity in some right convexity sulci, suggestive of meningitis. Image D shows the patient's chest X-ray 7 days after discharge, revealing progression of pulmonary consolidation in the left upper lobe. Image E shows a control chest CT on day +35 of the new admission, depicting worsening lung lesions. Extensive consolidation in LSI with a large area of cavitation, cavitating satellite nodules, and contralateral involvement of the RUL, all consistent with necrotizing pneumonia.

was attributed to invasive fungal infection by mucormycosis, highlighting the challenges of managing co-infections in transplant recipients. This case underscores the complexities involved in diagnosing and treating concurrent mucormycosis and *Nocardia* infections. Differential diagnosis considerations include opportunistic infections, neoplasms, and interstitial lung disease [1]. Nocardia, a member of actinomycetes, presents diagnostic challenges, emphasizing the need for comprehensive diagnostic approaches such as fibrobronchoscopy [2,3]. Mucormycosis, known for its aggressive nature, requires prompt diagnosis through biopsy for direct microscopy, culture, and histopathological examination [4]. This patient exhib-

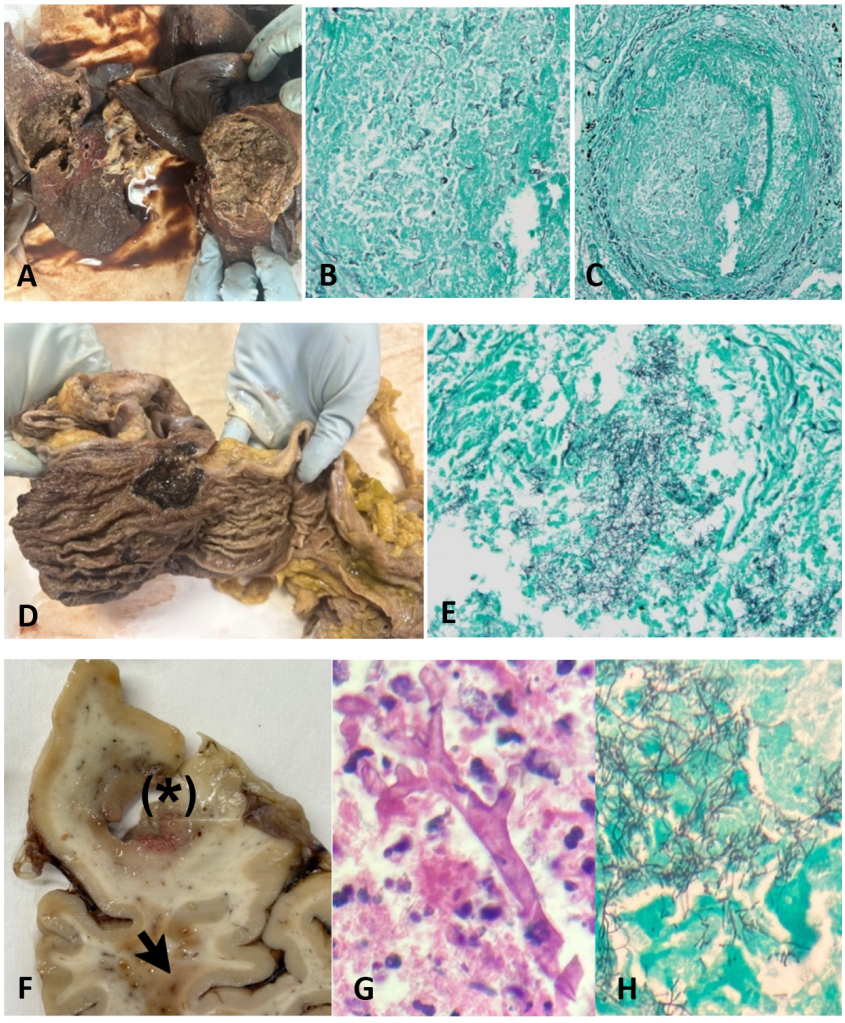


Figure 2 Macroscopic and histopathological study of clinical autopsy. Image A shows areas of necrosis and abscess formation in the left lung, consistent with mucormycosis. Image B and C shows areas of necrosis and abscess formation in the left lung, with thick, sparsely septate hyphae within vessels as observed in Grocott stain, with mucoral (scale bar 100 µm and 200 µm). Image D shows a gastric ulcer with transmural necrosis, consistent with mucoral Image E presents a cavitated lesion with areas of necrosis in a lymph node, Grocott stain positive for positive filamentous bacilli, consistent with Nocardia (scale bar 100 µm). Image F shows the macroscopic piece of central nervous system study, in which an acute necrotic lesion consistent with mucoral is observed (asterisk) upon a previous lesion in remission consistent with Nocardia (arrow). Image G shows the microscopic findings from the nervous system study, revealing sparsely septate hyphae at 90° in hematoxylin-eosin stain, consistent with mucoral (scale bar 50 µm). Image H reveals positive Grocott stain lesions with positive filamentous bacilli, consistent with *Nocardia* (scale bar 100 µm).

ited multiple risk factors for invasive fungal infection, including CMV infection, prolonged hospitalization, and subsequent admission to the ICU. Treatment involves a multidisciplinary approach combining antifungals and surgical interventions due to the rapid progression of mucormycosis [4].

Solid organ transplant recipients are particularly susceptible to opportunistic infections like nocardiosis and mucorales. These infections can occur in both post-transplant patients and those with normal immune function, often mimicking other diseases clinically and radiologically. Unfortunately, ac-

curate diagnosis may only occur when these diseases have already spread extensively. Therefore, it is crucial to remain vigilant for signs and symptoms to identify and treat these conditions early on. Highly immunosuppressed patients may develop multiple simultaneous opportunistic infections, posing a challenge for diagnosis and treatment when superinfections arise during ongoing treatment for a previous infection.

FUNDING

None to declare

CONFLICTS OF INTEREST

The authors declare the absence of conflicts of interest.

REFERENCES

1. Kanne JP, Yandow DR, Mohammed TL, Meyer CA. CT findings of pulmonary nocardiosis. AJR Am J Roentgenol. 2011;197(2):W266-272. doi: 10.2214/AJR.10.6208
2. Wilson JW. Nocardiosis: updates and clinical overview. Mayo Clin Proc. 2012;87(4):403-407. doi: 10.1016/j.mayocp.2011.11.016
3. Hardak E, Yigla M, Berger G, Sprecher H, Oren I. Clinical spectrum and outcome of Nocardia infection: experience of 15-year period from a single tertiary medical center. Am J Med Sci. 2012;343(4):286-290. doi: 10.1097/MAJ.0b013e31822cb5dc
4. Cornely OA, Arikan-Akdagli S, Dannaoui E, Groll AH, Lagrou K, Chakrabarti A, et al. ESCMID and ECMM joint clinical guidelines for the diagnosis and management of mucormycosis 2013. Clin Microbiol Infect. 2014;20 Suppl 3:5-26. doi: 10.1111/1469-0691.12371.