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Official journal
of the Spanish Society
of Chemotherapy

Francisco Javier Candel

Introduction to XI Updating Course of Antimicrobials and Infectious Diseases

Department of Clínical Microbiology and Infectious Diseases. IdISSC and IML Health Research Institutes. Hospital Clínico Universitario San Carlos. Madrid.

Last february, the XI Updating Course of Antimicrobials and Infectious Diseases was held at the Hospital Clínico San Carlos in Madrid. It is a scientific activity accredited by the Community of Madrid (Commission for Continuing Education of Health Professions at the Community of Madrid, file number 07-AFOC-00080.7/2021, 1 credit) and endorsed by the Spanish Society of Clinical Microbiology and Infectious Diseases (SEIMC), the Spanish Society of Chemotherapy (SEQ) and the Madrid Society of Clinical Microbiology (SMMC). This year, the course was online edited and reached peaks of more than 1,500 conexions with continous mean over 750. The audience consisted of multidisciplinar proffesionals of all specialties related to infection, the teachers made an update of the most relevant aspects on bacteriology, mycology and virology.

Current issue of the magazine includes summaries of the lectures given in the presential course. It also includes the questionnaire with the evaluations made by the students and a sheet of correct answers to being able to contrast the results. The supplement is divided into three headings. The first include a infectious diseases update with the most relevant information related to diagnostic techniques and management of infections presented during 2020, beyond COVID-19. It also includes an update on the Utility of level 2 laboratories in the National Antimicrobial Resistance Plan, an update of bioinformatics applied to the study of bacterial resistance and another on the treatment of multi-resistant microorganisms in hospital at home units. The second section provides a brief update on current antimicrobial pharmacotherapy and the third is a specific review of SARS-COV-2 infection, the value of diagnostic techniques, the integral management of infection, its complications and superinfections.

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Update in infection diseases 2020

Emilia Cercenado

What happened to microbiological diagnosis in 2020 beyond COVID-19?

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ABSTRACT

The global pandemic of COVID-19 has had negative repercussions on the activities and research in clinical microbiology laboratories other than those related to SARS-CoV-2. Nonetheless, the research activity has also continued in other fields. In this brief review, some of the recent publications related to new diagnostic tests, methods for rapid antimicrobial susceptibility testing and for the detection of resistance genes, new diagnostic technologies, and some aspects related to old and emergent pathogens (*Candida auris, Elizabethkingia* spp. *Streptococcus pyogenes*) are summarized.

Keywords: microbiological diagnosis, new diagnostic technologies, resistance to antimicrobials, *Candida auris*, *Streptococcus pyogenes*, Elizabethkingia spp.

INTRODUCTION

The global pandemic of COVID-19 has had negative repercussions on the entire global activities, and these include the activities and research in clinical microbiology laboratories other than those related to SARS-CoV-2 and COVID-19. However, the activity of clinical microbiologists has continued, among others, with the development of new diagnostic tests, new methods for rapid antimicrobial susceptibility testing and for the detection of resistance genes, new diagnostic technologies, and with the study of old and emergent pathogens. This minireview includes a brief summary of the publications presented as a lecture at the XI Updating Course of Antimicrobials and Infectious Diseases last February 2021 in Madrid (Spain).

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DIAGNOSTIC MICROBIOLOGICAL TECHNIQUES

In recent years *Candida auris* has emerged as an opportunistic yeast of clinical importance because it causes infections in at risk populations including critically ill and immunosuppressed patients. In addition, it is resistant to many antifungal treatments and persists in hospital environments causing hospital outbreaks difficult to control and to eradicate. Early and accurate diagnosis of *C. auris* infections is crucial, however this organism is difficult to identify by commonly used identification systems, and misidentifications as other *Candida* species is frequent. Alvarado et al. [1] describe the development of conventional and real-time PCR methods for accurate and rapid identification of *C. auris* and its discrimination from closely related species by exploiting the uniqueness of certain glycosylphosphatidylinositol (GPI)-modified protein-encoding genes. They designed species-specific primers for two unique putative GPI protein-encoding genes per species, including *C. auris, C. haemulonii, C. pseudohaemulonii, C. duobushaemulonii, C. lusitaniae* and *C. albicans*. In addition, the efficiency of the *C. auris* primers was validated using a panel of 155 *C. auris* isolates, including all known genetically diverse clades. All primers combinations showed excellent species specificity and in real-time multiplex PCR, *C. auris* was easily differentiated from other related species. *C. auris* limit of detection was 5 CFU/reaction with a threshold value of 32, and the method was also able to detect *C. auris* in spiked blood and serum. The authors conclude that this PCR identification based on unique GPI protein-encoding genes allows for accurate and rapid species identification of *C. auris* and related species.

Since the availability of molecular methods is limited in many routine diagnostic laboratories, Das et al. [2] developed a selective medium in order to significantly reduce the time and cost associated with the identification of *C. auris* even in low-resource health care settings. By using 18 *C. auris* isolates and 30 non-*C. auris* yeasts they standardized a selective medium with yeast extract-peptone-dextrose (YPD) agar, including

various combinations of sodium chloride and ferrous sulphate, followed by incubation at different temperatures and times. For validation, they used 579 additional yeast isolates and 40 signal-positive blood culture broths. The named Selective Auris Medium (SAM) comprising YPD agar with 12.5% NaCl and 9 mM ferrous sulphate incubated at 42°C for 48 h, allowed selective growth of *C. auris*. A total of 95% (127/133) of *C. auris* isolates tested grew on this media within 48 h, and the remaining 6 isolates grew after 72 h, whereas the growth of 446 non-*C. auris* yeast isolates was completely inhibited. The specificity, sensitivity, positive and negative predictive values of the test medium were 100% after 72 h of incubation. The authors indicate that this medium is inexpensive, can easily be prepared, and can be used as an alternative to molecular methods.

The microbiological diagnosis of infections caused by difficult to grow bacterial organisms is challenging since they require enriched media and long incubation times. Moreover, in many of these cases prompt and accurate diagnosis is important for treatment and control of disease transmission. *Bordetella pertussis* and *Helicobacter pylori* are among these difficult to grow organisms that require rapid alternatives, like PCR or other molecular methods, for their detection and identification. The article by Chow et al. [3] describes the evaluation of a commercialized PCR-based kit for the detection of *Bordetella pertussis* and *Bordetella parapertussis* in nasopharyngeal swab specimens. In this multicenter study, a total of 1,103 fresh and residual frozen specimens from eight clinical sites were tested. Combining the data from individual clinical sites using different comparative assays, the overall positive percent agreement (PPA) and negative percent agreement (NPA) for *B. pertussis* were 98.7% and 97.3%, respectively. The overall PPA and NPA for *B. parapertussis* were 96.7% and 100%, respectively. For prospective fresh specimens, the overall PPA and NPA for both targets were 97.7% and 99.3%, respectively. For retrospective frozen specimens, the overall PPA and NPA for both targets were 92.6% and 93.2%, respectively. The kit was 100% specific, and the limits of detection were 150 CFU/ml or 3 fg/µl of DNA for *B. pertussis* and 1,500 CFU/ml or 10 fg/µl of DNA for *B. parapertussis*. The hands-on time of the kit for one sample was 2 min and the total assay run time per 8 samples was 78 min. In conclusion, this study shows that this kit (Simplexa Bordetella Direct kit) was non-inferior to the molecular assays currently available on the market or developed in-house for the detection and differentiation of *B. pertussis* and *B. parapertussis*, being a rapid and accurate approach for better diagnosis of pertussis.

Concerning *H. pylori,* its non-invasive detection and its resistance to clarithromycin is essential for the rapid management of *H. pylori* infection. Pichon et al. [4] conducted a prospective, multicenter study to evaluate the performance of a commercialized real-time PCR based assay (Amplidiag *H. pylori*+ClariR) on DNA from stools from 1,200 adult patients who were addressed for gastroduodenal endoscopy with gastric biopsies and who were naive for eradication treatment. The results were compared with those of culture/Etest and quadruplex real-time PCRs performed on two gastric biopsy samples (from the antrum and corpus) to detect the *H. pylori glmM* gene and mutations in the 23S rRNA genes conferring clarithromycin resistance. In this cohort, 160 patients (14.7%) were found to be infected (positive by culture and/or PCR). The sensitivity and specificity of the detection of *H. pylori* were 96.3% and 98.7%, respectively. The positive and negative predictive values were 92.2% and 99.3%, respectively. The sensitivity and specificity for detecting resistance to clarithromycin were 100% and 98.4%, respectively. The feasibility of the assay was very good and minimally time-consuming (5 min for extraction and 10 min for amplification, with a complete turnaround time of 3 h 45 min), and the total price per patient was less than €30. The very good performances of this non-invasive test for the detection of *H. pylori* and clarithromycin resistance in stools makes it highly recommended for use in all cases where histological study of the gastric mucosa is not necessary. In addition, patients had very good compliance with auto-sampling.

The microbiological diagnosis of fungal meningitis is difficult since testing accuracy varies with each etiological agent, and delay in diagnosis and treatment leads to poor outcomes. Since (1,3)-beta-D-glucan (BDG) measurement in cerebrospinal fluid (CSF) is not specific for any particular cause of fungal meningitis, the study by Davis et al. [5] starts with the hypothesis that this measurement could have some utility as a marker of fungal disease, particularly in cases of subacute meningitis without clear etiologies. In this line, they performed a systematic review in order to characterize the evidence regarding CSF (1,3)-beta-D-glucan measurement to detect fungal meningitis. Fourteen studies were included and a variety of fungi, including species of *Candida*, *Aspergillus, Exserohilum, Cryptococcus, Histoplasma*, and *Coccidioides*, were studied, although most were case reports. Diagnostic accuracy was examined in 5 studies. The analysis revealed that CSF BDG measurement showed >95% sensitivity in one corticosteroid injection-related outbreak of *Exserohilum rostratum*, one study in *Histoplasma* spp. meningitis found 53% (53/87) sensitivity and 87% (133/153) specificity, while another study of *Cryptococcus* spp. meningitis found 89% (69/78) sensitivity and 85% (33/39) specificity. They conclude that CSF BDG testing may be useful, primarily as a non-specific marker of fungal meningitis, and should be used in conjunction eith organism-specific testing. Although the FDA black box warning states that *Cryptococcus* spp. do not make BDG, this review shows that BDG is detectable in cryptococcal meningitis.

Diagnosis of *Mycoplasma pneumoniae* infection in patients with community-acquired pneumonia (CAP) is challenging. Current diagnostic tests include *M. pneumoniae* specific IgM serology and PCR of respiratory specimens, but these tests are also positive in asymptomatic carriers of *M. pneumoniae* in the upper respiratory tract. In addition, IgM serology may lead to false-negative results early in disease course and after reinfection. The measurement of *M. pneumoniae*-specific IgM antibody secreting cells (ASC) by enzyme-linked immunospot (ELISpot) assay differentiates between *M. pneumoniae* infection and carriage, but this assay cannot be performed at bedside

and no point-of-care (POC) test is available for direct *M. pneumoniae* detection. Meyer-Sauteur et al. [6] assessed a new immunochromatographic POC IgM lateral flow assay (LFA) as an on-site screening tool for the detection of *M. pneumoniae* CAP in children. A set of 239 pediatric serum samples from 94 CAP patients and 145 healthy controls was used from a previous study. CAP patient samples were collected median 7.0 days after symptom onset. The IgM-LFA was performed, and results were visually read after 10 min. The results were identical for both fingertip blood and serum samples. Compared to IgM-ELISA, IgM-LFA-negative results were true negative in 97.8% (n=178/182), and IgM-LFA-positive results were true positive in 87.7% (n=50/57). The IgM-LFA also was positive for all individuals who tested positive with IgM-ELISA+PCR (n=41) and IgM-ASC-ELISpot (n=29). In summary, IgM-LFA results are predictive for *M. pneumoniae* infection, despite the possibility of false-positive results, however, this test cannot currently replace other diagnostic tests and results need to be confirmed with *M. pneumoniae*-specific PCR, IgM-ELISA, and/or IgM-ASC-ELISpot assay.

The microbiological diagnosis of *Pneumocystis jirovecii* pneumonia (PCP) is difficult since the *P. jirovecii* load in the lungs is, in general, low in non-HIV-positive patients. Currently, the laboratory gold standard for the detection of *P. jirovecii* is a real-time quantitative PCR (qPCR) assay, however, this is laborious, require skilled personnel, and execution outside regular working hours of the molecular biology laboratory is limited. The commercialized eazyplex *P. jirovecii* assay (PJA) uses loop-mediated isothermal amplification (LAMP) for detection of *P. jirovecii*. This assay is performed directly with respiratory specimens, without the need for special skills, and delivers a result within 3 to 25 min, requiring a hands-on time of 2 min 45 s. The study of Scharmann et al. [7] compared the performance of this assay with that of *P. jirovecii* qPCR assays. Forty-nine patients with proven PCP and 126 patients without PCP were included. The sensitivity and specificity of the assay (95.7% and 96.5%, respectively) were comparable to those for three different *P. jirovecii* qPCR assays. The detection limit was 10 to 20 *P. jirovecii* cells and the eazyplex PJA reliably discriminated patients with PCP from patients with *P. jirovecii* colonization. This study demonstrates identical performance of the LAMP assay for the diagnosis of PCP, compared to qPCR assays, with the advantages of its practicability, allowing for around-the-clock molecular testing.

Catheter-related bloodstream infections (CRBSI) account for 15%-30% of all nosocomial bloodstream infections (BSI) and several microbiological diagnostic procedures not requiring catheter removal have been devised. Among them, the differential time to positivity (DTP) of ≥2 hours between peripheral-blood and catheter-blood cultures has been described as an excellent test with high sensitivity and specificity values and has been included in different guidelines as a criterium for the definitive diagnosis of CRBSI. The study by Orihuela-Martín et al. [8] assessed the performance of this method over a period of 15 years in their institution. A total of 512 BSI were included, of which 302 (59%) were CRBSI. Discrimination ability of DTP was low for *Staphylococcus aureus* (AUC 0.656 ± 0.06), coagulase-negative staphylococci (AUC 0.618 ± 0.081), enterococci (AUC 0.554 ± 0.117) and non-AmpC-producing *Enterobacteriaceae* (AUC 0.653 ± 0.053); moderate for *Pseudomonas aeruginosa* (AUC 0.841 ± 0.073), and high for AmpC-producing *Enterobacteriaceae* (AUC 0.944 ± 0.039). For the entire sample, DTP had a low-to-moderate discrimination ability (AUC 0.698 ± 0.024). A DTP ≥2 h had a low sensitivity for coagulase-negative staphylococci (60%) and very low for *S. aureus* (34%), enterococci (40%) and non-AmpC-producing *Enterobacteriaceae* (42%). A DTP cut-off of 1 h improved sensitivity (90%) for AmpC-producing *Enterobacteriaceae*. This long experience indicates that DTP used on a routine basis has adequate discrimination ability and performance characteristics for CRBSI diagnosis only when AmpC-producing *Enterobacteriaceae* and *P. aeruginosa* are involved. For other organisms, a negative test should not be used to rule out CRBSI.

DETECTION OF RESISTANCE TO ANTIMICROBIALS

Extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales constitute a global burden for hospital infection, and the identification of carriers by screening patients at risk is recommended by several European guidelines. Blanc et al. [9] evaluated the impact of rapid ESBL tests on the turnaround time (TAT) of screening. Rectal swabs were analysed by culture and synergism tests for identification of non-*Escherichia coli* Enterobacterales that produce ESBLs (NEcESBL). The Rapid ESBL NP (colorimetric test) and NG CTX-M MULTI test (immunoassay) were performed on colonies grown on chromogenic media. PCR and sequencing of ESBL genes were used as the gold standard. Among 473 rectal swabs, 75 (15.9%) grew NEcESBL. ESBL screening using the synergism, Rapid ESBL NP and NG CTX-M MULTI showed sensitivities of 0.96, 0.81, and 0.91; specificities of 1.00, 0.99, and 1.00; positive predictive values of 0.96, 0.94, and 1.00; and negative predictive values of 1.00, 0.98, and 0.99, respectively. When no-NEcESBL were observed, the mean TAT was 30 h. When NEcESBL were detected, the mean TATs were 74.7, 38.0 and 36.7 h for the synergism, Rapid ESBL NP and NG CTX-M MULTI tests, respectively. This study shows that the two rapid ESBL tests evaluated (colorimetric and immunoassay) showed good performances and significantly reduced the TAT of the screening protocol to identify ESBL-producing Enterobacterales. This study underlines the importance and impact of rapid tests to identify emerging antibiotic resistant pathogens.

Carbapenemase-producing Gram-negative bacteria are a public health concern, and methods for rapid detection and characterization of the different carbapenemase subtypes are necessary. Among carbapenem-resistant *Pseudomonas aeruginosa*, phenotypic detection of carbapenemases SPM, IMP and GES is challenging and genotypic tests are not commercially available. In the study of Gill et al. [10] the authors evaluate the performance of the commercially

available Xpert Carba-R (Carba-R) and the research-use-only Xpert Carba-R NxG (Carba-R NxG) in a global collection of 123 *P. aeruginosa* from 12 countries previously categorized via PCR or whole-genome sequencing. Carbapenemase classes tested included VIM, IMP, NDM, SPM, KPC, and GES. Non-carbapenemase (non-CP)-harboring isolates were also tested (negative control). Both assays gave negative results for all non-CP isolates and positive results for all VIM, NDM, and KPC isolates. An improvement in IMP detection among isolates was observed (100% detection by Carba-R NxG versus 58% by Carba-R). All SPM and GES isolates, targets not present in commercially available Carba-R, were positive by Carba-R NxG. Two isolates harbored both VIM and GES, while a third isolate contained VIM and NDM. The Carba-R NxG identified both targets in all 3 isolates, while the Carba-R was negative for both GES-containing isolates. Overall, the Carba-R NxG successfully categorized 100% of isolates tested compared with 68% for its predecessor. The authors conclude that this new Carba-R NxG test expands the detection spectrum of the current Carba-R assay to include SPM, GES, and expanded IMP variants, and increases the global utility of the test. Continuous expansion of genotypic detection assays is very important due to the growing diversity of enzyme subtypes.

Over the last years, identification of staphylococci other than *Staphylococcus aureus* (SOSA) has become more frequent, due to a better understanding of their role as opportunistic pathogens and to the availability of MALDI-TOF MS in clinical laboratories. However, testing of SOSA for *mecA*-mediated resistance is challenging. In the study of Humphries et al. [11] isolates of *Staphylococcus capitis* (n=50), *Staphylococcus haemolyticus* (n=50), *Staphylococcus hominis* (n=50), and *Staphylococcus warneri* (n=48), were evaluated by cefoxitin and oxacillin broth microdilution (BMD), disk diffusion (DD), and PBP2a immunoassay, and the results were compared to *mecA* PCR results. No phenotypic susceptibility test correlated well with PCR results across all species, although the PBP2a immunoassay yielded 100% correlation. Oxacillin BMD testing by current Clinical and Laboratory Standards Institute (CLSI) SOSA breakpoints led to 2.1% very major errors (VMEs) and 7.1% major errors (ME). Oxacillin DD yielded high ME rates (20.7 to 21.7%) using CLSI or European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints with VMEs ranging from 0 to 5.3%. Cefoxitin BMD led to 4.9% VMEs and 1.6% MEs, and cefoxitin DD led to 1.0% VMEs and 2.9% MEs. The results of this study indicate that laboratories should be aware that no individual phenotypic test correlates well across all species of SOSA with *mecA* PCR results. Molecular testing for *mecA* or evaluation for PBP2a is the preferred approach for the adequate detection of methicillin-resistance among these staphylococcal species.

The need for rapid antimicrobial susceptibility testing (RAST) in bloodstream infections is important for adjustment of therapy and many attempts have been made to shorten the time required for reporting antimicrobial susceptibility testing results. Recently, the EUCAST developed a disc diffusion RAST method directly from positive blood cultures delivering reliable AST results within 4-8 h of positivity of blood culture bottles. Akerlund et al. [12] validate this method in 55 European laboratories including clinical blood cultures positive for *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus* or *Streptococcus pneumoniae*. Categorical results at 4, 6 and 8 h of incubation were compared with results for EUCAST standard 16-20 h disc diffusion. After analysing 1151 isolates, the number of zone diameters that could be read (88%, 96% and 99%) and interpreted (70%, 81% and 85%) increased with incubation time (4, 6 and 8 h). The categorical agreement was acceptable, with total error rates of 3% at 4 h, 2.1% at 6 h and 2.2% at 8 h. The percentages of false susceptibility ranged from <0.3% to 1.1% and the corresponding percentages for false resistance ranged from <1.9% to 2.8%.

These results support that the EUCAST RAST method can be implemented in routine laboratories providing reliable AST results. This method is easy to use, cheap, flexible, and can be adapted to new antimicrobials without major investments.

NEW DIAGNOSTIC TECHNOLOGIES

The development and use of rapid tests for bacterial detection and AST is one of the priorities for the adequate management of patients and the appropriate use of antimicrobials. Recent phenotypic assays (imaging, microfluidic culture) and molecular methods (PCR, nanoparticle-based assays, microfluidic-based capture and enrichment, electrochemical sensors, CRISPR, sequencing, etc) can reduce assay time to hours but are often not sensitive enough to detect bacteria at low concentrations (<1 to 100 CFU/ml) and require expensive equipment and lengthy, complex sample processing. Abram et al. [13] developed a rapid bacterial detection and AST method in whole blood using one-step, high throughput blood digital PCR. This technology prototype provides a high sensitivity (10 CFU/ml) and a rapid assay time (one hour) and is applicable for the detection of a wide range of antimicrobial resistance genes without requiring blood culture or sample processing. This new diagnostic technology holds great potential for the rapid diagnosis of BSI directly in blood samples.

The CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats-CRISPR-associated protein-9 nuclease) system, has been engineered to create site-specific double-strand breaks for genome editing and provides a new tool and approach to eradicate carbapenem-resistant (CR) genes and plasmids. Hao et al. [14] conducted a proof-of-concept study (pCasCure) to demonstrate that CRISPR-Cas9-mediated resistance gene and plasmid curing can effectively resensitize CR *Enterobacteriaceae* to carbapenems. The results showed that pCasCure effectively cured bla_{KPC}, bla_{NDM}, and bla_{OXA-48} in various clinical isolates of *Enterobacteriaceae* species with a >94% curing efficiency. In addition, the pCasCure efficiently eliminated, with a few exceptions, several epidemic CR plasmids, successfully restoring the susceptibility to carbapenems, with a >8-fold reduction of MIC values in all tested isolates. In the next future, the integration of pCasCure

in an optimal deliver system will make it applicable for clinical intervention and may serve as a potential tool to control the dissemination of carbapenem resistance in clinical pathogens.

OLD AND EMERGENT BACTERIAL PATHOGENS

Emerging Gram-negative bacterial pathogens have gained global attention in recent years as a cause of nosocomial infections. Among them, the intrinsically multidrug resistant *Elizabethkingia* genus is one example of a worldwide pathogen, primarily infecting immunocompromised individuals and associated with high mortality (20%-40%). Burnard et al. [15] describe a series of 22 clinical and 6 hospital environmental *Elizabethkingia* spp. isolates obtained in a hospital in Australia over a 16-year period. They performed whole-genome sequencing and identified 22 *E. anophelis*, 3 *E. miricola*, 2 *E. meningoseptica*, and 1 *E. bruuniana* isolates, most of which branched as unique lineages. Global analysis revealed that some Australian *E. anophelis* isolates were genetically closely related to strains from the United States, England, and Asia. They also demonstrated evidence of nosocomial transmission in patients. Furthermore, AST against 39 antimicrobials revealed almost ubiquitous resistance to aminoglycosides, carbapenems, cephalosporins, and penicillins, and susceptibility to minocycline and levofloxacin, and less commonly to trimethoprim-sulfamethoxazole. This study demonstrates important new insights into the antimicrobial resistance, genetic diversity, environmental persistence, and transmission of this emerging pathogen.

For many years, we have been taught that *Streptococcus pyogenes* was universally susceptible to beta-lactams. However, in 2019 two related *S. pyogenes* strains with reduced susceptibility to ampicillin, amoxicillin, and cefotaxime were reported. The two strains had the same mutation in the *pbp2x* gene, encoding penicillin binding protein 2X. Musser et al. [16] investigated a library of 7,025 genome sequences of *S. pyogenes* clinical strains recovered from intercontinental sources for mutations in *pbp2x*, and they identified 137 strains that had mutations in this gene, observing that these strains had decreased susceptibility *in vitro* to multiple beta-lactam antibiotics.

Phylogenetic analysis showed that, with one exception, strains of the same *emm* type with the same amino acid replacement were clonally related. These results indicate that clinical isolates of *S. pyogenes* with *pbp2x* mutations associated with small decreases in beta-lactam susceptibility are more widespread worldwide than appreciated. Probably, clinical microbiology laboratories not routinely performing beta-lactam susceptibility testing of *S. pyogenes*, reasonably must consider to do it.

CONFLICT OF INTEREST

The author declares that there is no conflicts of interest

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Update in infection diseases 2020

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What happened to infectious diseases and anti-infective therapy in 2020 beyond COVID-19?

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ABSTRACT

The year 2020 was the year of infectious diseases with the arrival of SARS-CoV-2, which represented a profound change in the world we knew. However, we present a brief description of some of the top infectious diseases articles from 2020 not related with SARS-CoV-2. We reviewed a selection of the most important and relevant achievements in diagnosis and therapy related to bacteremia, nosocomial pneumonia, skin and soft tissue infections, infections by *Clostridioides difficile*, mycobacterial infections and invasive fungal infections. This year entailed a significant step forward in the indisputable value of the health care stewardship programs.

Keywords: Infectious diseases non-COVID, antimicrobial therapy, nosocomial infections

The year 2020 was the year of infectious diseases with the arrival of SARS-CoV-2, which represented a profound change in the world we knew. However, we present a brief description of some of the top infectious diseases articles from 2020 not related with SARS-CoV-2.

BACTEREMIA

Sepsis is life-threatening organ dysfunction, it is considered a major cause of health loss, but data for the global burden of sepsis are limited. In the study of Rudd *et al.*, data about global sepsis incidence and mortality from 1990 to 2017 are analysed. In 2017, an estimated 48.9 million incident cases of sepsis were recorded worldwide and 11 million sepsis-related deaths were reported, representing 19.7% of all global deaths. Age-standardised sepsis incidence fell by 37.0% and mortality decreased by 52.8% from 1990 to 2017. However, sepsis incidence and mortality varied substantially across regions, with the highest burden in sub-Saharan Africa, Oceania, South Asia, East Asia, and Southeast Asia [1].

Methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia is associated with mortality of more than 20% so the use of appropriate treatment is under continuous study. In the study of Tong *et al.*, participants of 27 hospitals with MRSA bacteremia were randomized to standard therapy (intravenous vancomycin or daptomycin) plus an antistaphylococcal β-lactam (intravenous flucloxacillin, cloxacillin, or cefazolin) or standard therapy alone. They conclude that addition of an antistaphylococcal β-lactam to standard antibiotic therapy with vancomycin or daptomycin did not result in significant improvement in the primary composite end of mortality, persistent bacteremia, relapse, or treatment failure [2].

Pujol *et al.* designed a multicentre trial to test the hypothesis that daptomycin plus fosfomycin achieves higher treatment success than daptomycin alone in hospitalized adults with MRSA bacteremia and native valve endocarditis. Daptomycin plus fosfomycin provided a 12% higher rate of treatment success than daptomycin alone, but this difference did not reach statistical significance and it was more often associated with adverse events. They suggest that this antibiotic combination could be more effective in younger patients and those with more severe disease [3].

NOSOCOMIAL PNEUMONIA

The relatively high incidence, rising rates of antimicrobial resistance, and suboptimal clinical outcomes of patients with nosocomial pneumonia provide the impetus to optimize the use of existing antibiotics. Meropenem is a licensed agent for the treatment of nosocomial pneumonia. The pharmacodynamics is optimized with the use of prolonged infusions, especially continuous infusion (CI).

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In the study of Benitez-Cano *et al*., critically ill patients with nosocomial pneumonia were enrolled to received 1 g/8 h or 2 g/8 h by CI (8 h infusion). Although, the administration of meropenem by CI improves drug exposure in the epithelial lining fluid, only the highest dose of meropenem allowed achieving an optimal probability of target attainment (PTA) for all isolates with a MIC < 4 mg/L. However, in intermediate strains (MIC between 2 and 8 mg/L), the meropenem dose by CI needed to achieve an optimal PTA would have to be as high as 8 g/8 h, a dose that is four times higher than the highest approved meropenem dose [4].

SKIN AND SOFT TISSUE INFECTIONS

Skin and soft tissue infections are a common chief complaint in the Emergency Department. Research has shown that clinical examination alone can be unreliable in distinguishing between cellulitis and abscesses, a distinction that is important because each one require different treatments. Point-of-care ultrasonography has been demonstrated as a good tool to improve the diagnostic accuracy for these skin and soft tissue infections [5].

In patients with diabetic foot osteomyelitis who underwent surgical debridement, the duration of systemic antibiotic treatment with a short antibiotic regimen (3 weeks) compared with a long regimen (6 weeks) is associated with non-inferior results for clinical remission and adverse events [6].

Rates of cardiac implantable electronic device (CIED) related infections have increased and been associated with increased morbidity, mortality and financial burden on healthcare systems. The utilization of an antibiotic envelope at the time of device implantation or upgrade reduces major CIED infections, especially if used in patients to be at higher risk for infection [7].

Clostridioides difficile

Clostridioides difficile has been a significant enteric pathogen of humans. Previously, it was thought that *C. difficile* was primarily a hospital-acquired infection; however, with the emergence of community-associated cases, and whole-genome sequencing suggesting the majority of the hospital *C. difficile* infection (CDI) cases are genetically distinct from one another, there is compelling evidence that sources/reservoirs of *C. difficile* outside hospitals play a significant role in the transmission of CDI [8].

Regarding CDI treatment, a systematic review of the literature and network meta-analysis, compare the relative effectiveness of vancomycin (VCM), metronidazole (MTZ) and fidaxomicin (FDX). The meta-analysis suggests that FDX and VCM, but not MTZ, are effective first-line treatments for CDI, and that FDX may be more effective at preventing CDI recurrence than VCM [9].

NON-TUBERCULOUS MYCOBACTERIA

Non-tuberculous mycobacteria (NTM) represent over 190 species and subspecies, some of which can produce disease in humans of all ages and can affect both pulmonary and extrapulmonary sites. In the guideline of Daley *et al*., a revision of the treatment of pulmonary disease in adults (without cystic fibrosis or human immunodeficiency virus infection) caused by the most common NTM pathogens such as *Mycobacterium avium* complex, *Mycobacterium kansasii*, *Mycobacterium xenopi* and *Mycobacterium abscessus* was done [10].

FUNGAL INFECTIONS

Aspergillus fumigatus sensu lato encompasses a number of difficult-to-distinguish species, the highest percentage being *A. fumigatus sensu stricto* and the so-called cryptic species accounting for 10–15% of the isolates. Cryptic species commonly show intrinsic resistance to amphotericin B and azoles. In contrast, *A. fumigatus sensu stricto* isolates may acquire resistance following azole exposure. Tackling resistance is a challenge since azole-resistant patients present up to 31% higher mortality than azole-susceptible cases.

In the study of Escribano *et a*l., 30 hospitals from Spain and the Spanish Mycology Reference Laboratory (SMRL) were enrolled. Eight hundred and forty-seven isolates [*A. fumigatus sensu stricto* (*n* = 828) and cryptic species (*n* = 19)] were included. Only cryptic species were amphotericin B resistant. *A. fumigatus* clinical isolates proved that 7.4% of isolates were azole resistant. Resistance was commonly found in cryptic species or in isolates carrying TR_{34}-L98H *cyp51A* gene substitutions, the latter restricted to some cities located in the northern and mediterranean areas of Spain [11].

Candida auris is a recently emerging nosocomial pathogen, which was initially described in Japan in 2009 and then reported in over 30 countries worldwide afterwards. *C. auris* is usually resistant to several drugs, such as fluconazole, voriconazole, and amphotericin B.

In the study of Chen *et al.,* a systematic review and meta-analysis was done. More than 4733 cases of *C. auris* were reported in over 33 countries, with more cases in South Africa, United States of America, India, Spain, United Kingdom, South Korea, Colombia and Pakistan. *C. auris* exhibited a decrease in case count after 2016. Resistance to fluconazole, amphotericin B, caspofungin, micafungin and anidulafungin in *C. auris* were 91, 12, 12.1, 0.8 and 1.1%. The overall mortality of *C. auris* infection was 39%.

In recent years, the global public health community has increasingly recognized the importance of antimicrobial stewardship (AMS). However, the subject of antifungal stewardship (AFS) has received less attention. While the principles of AMS guidelines likely apply to stewarding of antifungal agents, there are additional considerations unique to AFS. AFS activities are outlined in Table 1 [12].

Table 1 **Essential, achievable, and aspirational antifungal stewardship activities. Adapted from [12].**

Stewardship Activity Level	Description
Essential	Development of institutional treatment pathways or bundles for antifungal prophylaxis and empiric therapy
	Development of targeted education programs for appropriate diagnosis and treatment
	Antifungal prescription review for drug-drug interactions
	Handshake rounds or postprescription review and feedback
	Intravenous to oral transition program
	Local surveillance and reporting of IFD to prescribers
Achievable	Rapid non-culture-based diagnostic tests for *Candida* and *Aspergillus* spp communicated to AFS team/clinicians
	Provide timely antifungal susceptibility testing results provided and communicated in a timely manner to AFS team/clinicians
	Specific comments to guide therapy and antifungal dosing recommendations are provided on microbiology reports
	Cumulative antifungal susceptibility reports reported to prescribers
	Timely TDM reported to AFS team and clinicians
	Review of autopsy reports and patient outcomes systematically to assess for undiagnosed IFDs and/or underutilization of antifungal agents
Aspirational	Participate in regional or national surveillance systems
	Individualized patient risk assessment (eg, institutional risk model, genetic risk factor screening)
	Optimize use of point-of-care microbiological tests, when available
	Utilize personalized TDM-dose adaptation (such as Bayesian methods) for antifungal therapy
	Incorporate advanced radiologic approaches for invasive aspergillosis (CT pulmonary angiography, FDG PET/CT)

AFS: antifungal stewardship; CT: computad tomography; FDG PET/CT: fluorodeoxy glucose positron emission tomography/computed tomography; TDM: therapeutic drug monitoring.

CONFLICT OF INTEREST

The authors declare no conflicts of interest

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Update in infection diseases 2020

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RedLabRA; a Spanish Network of Microbiology Laboratories for the Surveillance of Antibiotic Resistant Microorganisms

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ABSTRACT

There is an urgent need to control the clinical and public health impact that antibiotic resistance (AR) causes worldwide. Any measure for its control must be based on an up-to-date and comprehensive knowledge of the situation. However, it is difficult to determine the current dimension of AR because a large part of the available information is based on heterogeneous, insufficiently unified and retrospective data. The integration of genomic information in the surveillance of AR is another important factor for improvement. The Spanish Network of Laboratories for the Surveillance of Resistant Microorganisms (RedLabRA) is a structured network of interconnected microbiology laboratories developed within the Spanish National Plan against Antibiotic Resistance. Its main objective is to support the diagnosis of resistance to antibiotics, integrating molecular characterization in the surveillance.

Keywords: Antibiotic resistance, antibiotic resistance mechanisms, surveillance, RedLabRA.

INTRODUCTION

Multiple factors such as inaccurate antibiotic treatment regimens, self-medication, low adherence to the treatment, use of antibiotics in food industry, inability to access to clean water, and bad hygiene habits, among others, contribute to the selection and dissemination of microorganisms presenting antibiotic resistance (AR) to commonly used antibiotics [1,2].

An estimation based on the European Antimicrobial Resistance Surveillance Network data, showed that the clinical impact of selected antibiotic resistant bacteria in Europe could be quantified in generated 671,689 infections, 33,110 deaths, and 874,541 disability-adjusted life-years (DALYs) (which is a composite health measure estimating years lived with disabilities following the onset of a disease and of years of life lost due to pre-mature mortality compared to a standardized life expectancy) [3]. These infections generated an additional health expenditure of €1,500 million. It is estimated that more than 4 million people in Spain are infected annually, causing around 2,800 deaths [4], implying an expense of €150 million [5].

Current therapeutic options are being compromised by the emergence of novel resistance mechanisms such as, for instance, *optr*A and *poxt*A linezolide resistance genes [6] and *mcr* colistin resistance genes [7]. Changes in dissemination patterns of already known resistance mechanisms and high risk multidrug resistant clones, such as the *Escherichia coli* ST131/CTX-M-15 [8], *Klebsiella pneumoniae* ST258-512/KPC [9] and *K. pneumoniae* ST307/OXA-48 [10] are of great concern.

Resistance to carbapenems in *K. pneumoniae* and *E coli* has been increasing during the last years [11], enhancing the emergence of nosocomial infections that do not have optimal treatment options [2].

It is difficult to determine the current dimension of RA due to many available data come from heterogeneous, fragmented, and retrospective reports, showing issues of concern in the implementation of an agile and effective surveillance. An active genomic surveillance allows a better understanding of bacterial dynamics and to stablish strain/mechanism associations [12], facts that are crucial to carry out fast interventions in public health [13].

If measures are not implemented, effectiveness of antibiotic treatments against bacterial infections will decrease, and medical procedures such as organ transplants, chemotherapy and great surgeries could be also compromised [5]. In 2050, about 10 million deaths are expected to occur worldwide due to AR, becoming the leading cause of death [14], what could deeply compromise global economy, as World Bank estimated in 2017 [15].

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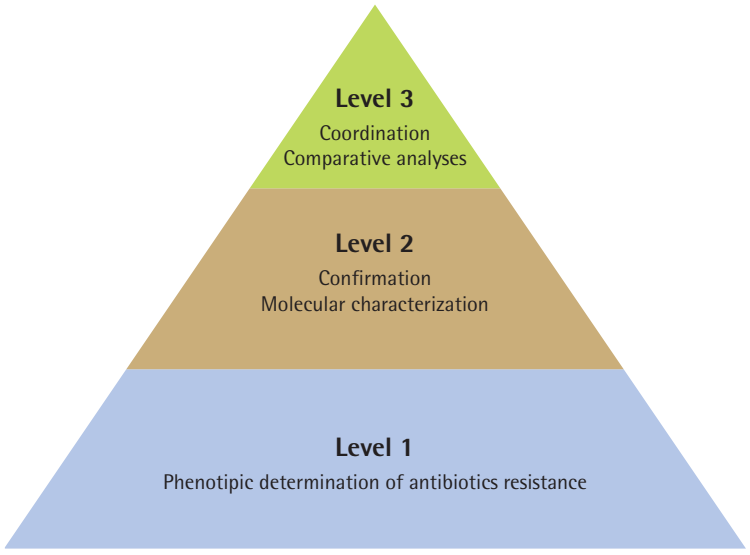

Figure 1 | **Network structure of RedLabRA.**

PRAN

The Spanish National Plan against Antibiotic Resistance (PRAN) is a strategic and action plan aimed to reduce the risk of selection and spread of AR and, consequently, to reduce its clinical and public health impact. The ultimate goal is to sustainably preserve the efficacy of antibiotics [5]. The PRAN is coordinated by the Agencia Española del Medicamento y Productos Sanitarios (AEMPS) and has the participation of all the autonomous communities, nine Spanish Ministries, and more than 70 scientific societies, collegiate organizations, professional associations and universities.

The PRAN addresses in a multidisciplinary way the AR threat from six strategic lines for action: 1) surveillance of consumption and resistance to antibiotics; 2) control of the emergence and spread of antibiotic resistant microorganisms; 3) prevention measures to reduce infections and promote the use of tools for an early diagnosis; 4) research to improve the knowledge of causes and consequences of AR and development of new therapeutic and diagnostic alternatives; 5) training of health professionals; and 6) awareness of the population about a prudent use of antibiotics through communication campaigns [5].

The PRAN considers a priority the implementation of a national network of laboratories that allows early and accurate diagnosis of healthcare-related infections (HAI) caused by multi-resistant microorganisms. This fact contributes to the establishment of early and effective treatments, as well as to the application of control measures [16].

REDLABRA

The Spanish Network of Laboratories for the Surveillance of Resistant Microorganisms (RedLabRA) has been created as a network of microbiology laboratories, coordinated and interconnected at a national level, to work together in the diagnosis and molecular study of infectious diseases caused by antibiotic resistant microorganisms. After being approved by the Interterritorial Board and the Public Health Commission of the Spanish National Health System, this network is working led by a Coordinating Committee dependent on the Ministry of Health, Consumer Affairs and Social Welfare (MSCBS) and the Instituto de Salud Carlos III (ISCIII) [16].

The general aim of RedLabRA is to achieve a complete and quality diagnosis of every case of infection or colonization by resistant microorganisms included in the epidemiological surveillance of the National Health System. This network aims to carry out a molecular study of the infectious diseases, including the next generation genome sequencing as a gold standard, allowing the better understanding of bacterial evolution, outbreaks, and transmission events avoiding the current temporal gaps. For its full implementation, there is a remarkable need of shared databases, agile informatics systems and political implication to acquire resources and health professionals.

RedLabRA is structured in three levels of action (Figure 1). Level 1 laboratories include all clinical microbiology laboratories of the National Health System, both public and private, being a key element of the Network. These laboratories must have the capability to phenotypically detect resistant pathogens and resistance mechanisms. Level 2 laboratories must have the capacity to support level 1 laboratories by performing an agile molecular characterization of resistance mechanisms and clones, as well as being able to address the study of outbreaks. Level 2 laboratories are designated by each autonomous community; the Centro Nacional de Microbiología

(CNM) can provide support in the performance of level 2 functions in those cases deemed necessary.

The third level will be restricted to the CNM (ISCIII) and certain laboratories designated by the Network for specific diagnoses. They must have the same capabilities and functions as level 2 laboratories, but their specific functions are to coordinate the Network, to carry out quality controls, to elaborate protocols and to perform comparative and evolutionary national studies with representative strains of circulating clones by whole genome sequencing.

In RedLabRA, a continuous feedback of information between laboratories of all levels is required; as well as the communication of the obtained results to the National Epidemiological Surveillance Network (RENAVE).

No individual, agency, region, or country will be able to control AR on their own. RedLabRA is a tool that arises with the purpose of helping to unify, coordinate and jointly analyze the AR information generated by microbiology laboratories, which will facilitate the implementation of early measures to reduce its impact and dissemination.

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Update in infection diseases 2020

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ABSTRACT

Detection and monitoring of antimicrobial resistance are two pillars on which clinical microbiology will be based in the coming decades. The implementation of certain technologies such as whole genome sequencing (WGS) or mass spectrometry and the creation of national and international databases that include and gather data on antimicrobial resistance from around the world has allowed the application of bioinformatics in the study of antimicrobial resistance in microorganisms involved in human pathology.

Keywords: bioinformatics, antimicrobial resistance, whole genome sequencing.

INTRODUCTION

The development of antimicrobial resistance (AMR), intrinsic to the use of antibiotics, has growth over the last few decades, spreading fast due to their overuse and misuse in both humans and animals. AMR has become the main challenge facing global health. For some pathogens frequently involved in human infection, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* or the *Mycobacterium tuberculosis complex*, among others, high rates of resistance against antibiotics have been observed world-wide. Infections caused by these microorganisms are associated with high morbidity and mortality rates; in fact, a recent WHO report estimates that drug-resistant microorganisms will cause up to 10 million deaths each year by 2050 with its consequent social, political, and economic impact [1]. In this context, the WHO and several other institutions are developing action plans in order to improve the understanding of AMR, reduce the incidence of infectious diseases through implementing infection prevention measures, optimize the use of antimicrobial medicines and develop new drugs and treatment strategies. Despite huge efforts have been made within the framework of these plans to combat AMR, this is still an unavoidable problem that reduces the effectiveness of antibiotics for treating infections. A practical and feasible solution to this issue might be use of certain bioinformatic tools that permit healthcare systems worldwide to have a greater control on AMR. To this end, certain techniques such as MALDI-TOF mass spectrometry and whole genome sequencing, that are slowly becoming part of the daily routine of most microbiology laboratories, can help us not only to detect different mechanisms of antimicrobial resistance in bacteria and other pathogens but guiding us to a better understanding of those mechanisms from an epidemiological and molecular point of view. Moreover, with the aid of bioinformatics, we can analyze the vast amount of information generated, in order to implement certain strategies, tailored to a specific context, to improve prevention, control and treatment measures for infections caused by antimicrobial resistant microorganisms.

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BIOINFORMATIC TOOLS FOR WHOLE GENOME SEQUENCING ANALYSIS IN AMR

Phenotypic antimicrobial susceptibility testing (AST) is the classic method to detect AMR, but in the last few years Whole Genome Sequencing-based AST (WGS-AST) has emerged as a fast and accurate method for AMR detection. In some cases, there is a direct connection between genotype and phenotype, allowing the determination of certain AMR genes by applying molecular tests available in clinical practice, as for example occurs in the detection of the *mecA* gene, responsible for beta-lactam antibiotics resistance in *S. aureus*. However, most of the mechanisms of antimicrobial resistance involve multiple genes and cellular signaling pathways or simply are not well known yet, and therefore WGS presents as an alternative to understand these mechanisms by obtaining the whole genome of pathogens isolated in clinical

practice, and enabling comprehensive AMR detection. Nevertheless, there is one big problem concerning WGS: it provides large amounts of data, making difficult to predict the presence of unknown antibiotic resistance genes or gene variants related to antibiotic resistance. Fortunately, these data sets obtained from applying WGS to clinical isolates can be analyzed through free bioinformatic tools such as Resfinder (the first pipeline addressed to non-trained users), AMRfinder or the Comprehensive Antibiotic Resistance Database (CARD) among others, that are actively curated and can be employed to identify genetic elements involved in AMR (single-nucleotide polymorphism mutations, horizontal gene transfer, inversions, etc) without having in-depth bioinformatics skills. To identify and predict the presence of AMR genes or other AMR mechanisms in a certain clinical isolate, these tools rely on large, high-quality AMR gene databases. The differences between these tools are based on the algorithm these databases use and their data composition. Despite the fact that some studies have shown the utility of these bioinformatic resources for AMR detection, a recent inter-laboratory study by Doyle et al [2] found that there is high variability in the results between laboratories depending on what software and analytical pipeline are used for detecting and predicting the presence or absence of AMR genes when studying the same clinical isolates, resulting in poor concordance with phenotypic AST results. These discordant results may be due not only to the bioinformatic tool used but also to the quality of the sequences obtained by WGS (samples have to be sequenced to a sufficient depth) and to the interpretation of the data obtained after the bioinformatic analysis. We must also note that some authors, including a European Committee on Antimicrobial Susceptibility Testing (EUCAST) subcommittee comprising experts on WGS [3], have concluded that even though there are large amounts of data from studies focused on phenotypic-genotypic AST concordance, WGS is still a poor tool to accurately predict antimicrobial susceptibility. Furthermore, the EUCAST subcommittee recommends using epidemiological cut-off values (ECOFF) as comparators for WGS-based prediction of AST instead of clinical breakpoints. As of today, WGS-AST techniques are not routinely performed in most clinical microbiology laboratories, because of their high cost, the need of skilled personnel, the poor quality of the data obtained and the difficulty in interpreting those data. In the same way, and as previously discussed, it is understandable the urgent need for standardized, comprehensive resistance sequence databases, accessible online for free, as well as standardized recommendations on sequence data quality. Even so, in the next few years the development of new bioinformatic pipelines and their application for studying AMR will facilitate the translation of large amounts of data into apprehensible and indictable insights, allowing the detection of AMR genes in WGS data, which was unimaginable a few years ago. In our opinion, WGS will be the method of choice for the detection of AMR in clinical practice, as the cost of WGS continues to decrease and experience is gained in data analysis and interpretation.

BIOINFORMATIC TOOLS FOR MONITORING AMR

Monitoring of antimicrobial resistance is as important as its detection. In this era of globalization, AMR determinants can easily spread around the world, transcending geographical barriers and increasing the global prevalence of AMR. In the past few years, for example, there has been a rapid increase in the detection of carbapenemase-resistant Enterobacterales (CPE) [4], both in hospital and community settings. Likewise, the use of antibiotics in animals has grown substantially in the last decades, leading to the propagation of antibiotics in the environment and increasing the burden of AMR in zoonotic agents, which means that AMR surveillance using only clinical samples is not useful as an early warning system for detecting resistance mechanisms. Thus, the development of tools for tracking global AMR has gained importance, since these tools may provide relevant information to guide healthcare professionals in the development of stewardship programs and public health actions. Building a network and infrastructure to support this amount of information is necessary too. The largest AMR surveillance system in Europe, the European Antimicrobial Resistance Surveillance Network (EARS-Net), collects comparable, representative and accurate AMR data, analyzing temporal and spatial trends of AMR, providing evidence of unfavorable events and encouraging immediate countermeasures. The main problem regarding EARS-Net is the fact that it is based on routine clinical antimicrobial susceptibility data, obtained only form invasive clinical isolates (blood and cerebrospinal fluid) [5]. With that in mind, it is obvious that we need to improve ARM surveillance systems capable of gather information not only from clinical but environmental isolates. As we have seen, AMR detection is possible by applying WGS, which generates huge amounts of data that will be subsequently analyzed using bioinformatic pipelines, helping to define the microbial populations and establishing trends in antimicrobial resistance. WGS is being integrated into the clinical and public health settings, though the use of WGS has been centred primarily on outbreak identification and monitoring. In a recent survey on the current epidemic of carbapenem-resistant *Klebsiella pneumoniae* (CRKp) in Europe, David et al [6] demonstrated (by analyzing the genomic sequences and geographical distribution of 1,717 *K. pneumoniae* clinical isolates) that carbapenemase acquisition is the major cause of carbapenem resistance in *K. pneumoniae*, and that almost 70% of the CRKp isolates were concentrated in just four clonal lineages; the analysis of the genomic sequences even showed that the highest transmissibility in hospital settings is related with the degree of carbapenem resistance. Another example of applying WGS in AMR monitoring is the observational study carried out by Harris et al [7] on clinical isolates of *Neisseira gonorrhoeae* from the European Gonococcal Antimicrobial Surveillance Programme. They found that WGS is an optimal tool for molecular epidemiology, since it identifies mixed infections, predicts antimicrobial resistance, and allows rapid analysis of genomic-phylogenetic relationships, giving a realistic picture of the circulating *N. gonorrhoeae* strains. But the largest achievement of this study was

the creation of a database including epidemiological, phenotypic, and genotypic data, that can be used for AMR prediction, molecular typing and phylogenetic clustering. The creation of these kind of databases should be a priority for the control of the dissemination of AMR, as they allow the storage of multiple resistance profiles and genomic information from microorganisms of high epidemiological interest like methicillin-resistant *Staphylococcus aureus*, MDR and XDR *Pseudomonas aeruginosa* or *Acinetobacter baumannii*.

PROTEOMIC TOOLS IN AMR

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is a rapid, accurate method to routinely identify microorganisms from both clinical isolates and clinical samples, and it has recently emerged as a reliable method to detect antimicrobial resistance. Detection of antimicrobial resistance via MALDI-TOF MS can be done in one of three ways: detection of AMR by analysis of the peak patterns of pathogens, measuring antibiotic modifications due to the enzymatic activity of bacteria (e.g. detecting β-lactamases by measuring mass changes in the antibiotic), and quantification of bacterial growth in the presence of an antibiotic [8]. Although MALDI-TOF MS has showed in several studies its capacity to detect AMR from clinical isolates, there are two main limitations to its routinely use: a limited number of AMR mechanisms that can be identified by this method and its low sensitivity for direct AMR detection from clinical samples. However, machine learning methods have been recently used for detection of drug resistant pathogens using the profile spectra obtained via MALDI-TOF MS [9]. Despite the fact that some advances have been made, the analysis of hundreds of peaks is still a challenge when applying machine learning methods. In spite of being a promising method for AMR detection, more studies on MALDI-TOF MS are required before it is integrated into the normal workflow of microbiology laboratories.

CONCLUSION

Antimicrobial resistance is the biggest public health challenge of our time. Fighting it requires global strategies, focused on preventing AMR emergence by implementing infection prevention, improving antibiotic use and the development of new drugs, implementing surveillance systems to track resistance, and improving laboratories capacity to identify resistant pathogens. Microbiology laboratories play a key role in detecting and monitoring AMR. In this sense, we have moved forward towards new ways to detect AMR in clinical settings. Progress in mass spectrometry and whole genome sequencing technologies has allowed their application not only in pathogen identification but also in AMR detection, becoming potent systems that may change the paradigms of antimicrobial susceptibility testing. Since these methods provides a huge amount of data, sophisticated bioinformatic tools are needed to interpret the results. There is an urgent need for comprehensive, open access databases that include all known resistance genes/mutations to facilitate comparison between methods and bioinformatics tools; although some progress has been made, lack of standardization hold back their potential. In our opinion, the development and implementation of these technologies in microbiology laboratories will permit fast and accurate identification of AMR, facilitating personalized medicine and simplifying AMR surveillance.

CONFLICTS OF INTEREST

The authors declare no conflict of interests.

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Update in infection diseases 2020

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Treatment of infections caused by multi-resistant microorganisms in hospital at home units

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ABSTRACT

Hospital at home units allow the treatment of moderate and severe infections by administering intravenous antibiotics to patients who would otherwise have to remain hospitalised. Increasing antibiotic resistance adds an element of difficulty to outpatient treatment of infections because multiple daily doses of antimicrobials or combinations of antimicrobials are sometimes required. This manuscript discusses some of the challenges of outpatient management of infections with multidrug-resistant microorganisms and shows the main antibiotic resistances and the outcomes of treatment of these infections in Spanish home hospitalisation units.

Keywords: multidrug-resistant microorganisms, hospital at home, OPAT, intravenous antibiotic treatment.

Infections caused by multi-resistant microorganisms represent a challenge for healthcare organisations, not only because of the risk to patients, but also because of the consumption of resources. In hospitals, multidrug-resistant infections force beds to become unusable to ensure isolation measures, and the frequent need to treat these infections with intravenous antibiotics results in prolonged hospital stays.

Outpatient treatment of these infections can help relieve hospital pressure and, in turn, reduce the risk of nosocomial infections. However, treating infections caused by multidrug-resistant microorganisms at home presents a new challenge. The lack of room temperature stability of some antimicrobials that are administered several times a day intravenously or the need to combine antibiotics to broaden the spectrum or achieve synergies can make home therapy difficult.

For this reason, the choice of antibiotic for outpatient treatment is sometimes made on the basis of ease of administration regardless of clinical practice guidelines and recommendations. In one study, outpatient therapy with antibiotics administered as a single daily dose was found to be inadequate in 28% of patients [1]. Therefore, the choice of antimicrobial should not be based on the ease of administration of the drug, as this may lead to further resistance or therapeutic failure; this decision should rather be based on its activity, efficacy and safety profile.

The appropriateness of the use of parenteral antibiotics in the outpatient setting is related to the organisation, resources and competence of the team treating and monitoring patients with infectious conditions. These circumstances may also influence treatment outcomes. Salles *et al.* observed that a lack of medical visits was a predictor of readmission and mortality for patients receiving outpatient antimicrobial therapy [2].

The home hospitalisation model is staffed by healthcare professionals with hospital dependency and training who monitor and control patients on a daily basis. This model allows early identification of complications and control of the evolution of the infectious process, and may explain why the therapeutic success rate is over 90% and the return rate to hospital is less than 8% in large series of cases [3]. Even so, the risk of treatment inadequacy remains [4].

Regardless of the care model, outpatient parenteral antimicrobial therapy cannot be considered as an isolated procedure, but as an organised process that includes criteria for the selection of patient, drug, venous access route and infusion modality, daily monitoring of infection evolution, indication and interpretation of complementary tests, de-escalation and sequencing of antibiotics when indicated, duration of treatment, discharge planning and subsequent follow-up [5]. The need to ensure safe and effective outcomes has recently prompted the development of quality indicators for the practice of outpatient parenteral antimicrobial therapy [6].

In addition, in the case of multidrug-resistant microor-

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Table 1 Microbiological isolates (all samples) and antibiotic resistance in Spanish home hospitalisation units (source: TADE Registry)

Microorganism	Total isolates	Isolates with antibiotic resistance, n (%)					
		Amoxicillin-clavulanate	Ampicillin	Ceftriaxone	Ciprofloxacin	Ertapenem	Gentamicin
Escherichia coli	2,657	932 (35.1)	1710 (64.4)	856 (32.2)	1325 (49.9)	18 (0.7)	479 (18.0)
Proteus mirabilis	229	67 (29.3)	122 (53.3)	47 (20.5)	116 (50.7)	11 (4.8)	60 (26.2)
Klebsiella pneumoniae	685	327 (47.7)	518(75.6)	347 (50.7)	373 (54.5)	29 (4.2)	190 (27.7)
Enterobacter cloacae	168	135 (80.4)	124 (73.8)	72 (42.9)	55 (32.7)	6 (3.6)	27 (16.1)
		Amikacin	Ceftazidime	Ciprofloxacin	Gentamicin	Imipenem-cilastatin	Piperacillin-tazobactam
Pseudomonas aeruginosa	1650	144 (8.7)	307 (18.6)	951 (57.6)	519 (31.5)	426 (25.8)	342 (20.7)
		Ciprofloxacin	Cloxacillin	Trimethoprim-sulfamethoxazole	Daptomycin	Penicillin	Vancomycin
Staphylococcus aureus	559	138 (24.7)	171 (30.6)	30 (5.4)	8 (1.4)	361 (64.6)	18 (3.2)
Staphylococcus epidermidis	177	92 (52.0)	103 (58.2)	71 (40.1)	3 (1.7)	108 (61.0)	6 (3.4)
		Ampicillin	Daptomycin	Gentamicin	Linezolid	Penicillin	Vancomycin
Enterococcus faecalis	328	15 (4.6)	6 (1.8)	83 (25.3)	3 (0.9)	20 (6.1)	3 (0.9)

ganisms, the choice of antimicrobials should take into account risk factors for resistance. Carrier states, high local incidence, recent hospitalisation, recent and repeated use of antibiotics, instrumental manipulations and health care are all risk factors for antibiotic resistance. For each microorganism there are, in addition, some specific risk factors [7,8].

One aspect not yet fully resolved is the stability of some antimicrobials at room temperature. This property of drugs is crucial as lack of stability limits the home use of antibiotics that are administered more than once a day. When nursing shifts allow, antimicrobials can be administered every 12 hours, even if they are not stable. For the rest, the solution is refrigeration in a refrigerator and self-administration, or infusion via systems that allow the dilution to be kept refrigerated. The latter is not sufficiently widespread and has the disadvantage of the need to incorporate a temperature control system and direct infusion of a cold dilution into the vein, which can be uncomfortable for the patient.

Meropenem is an example of an antibiotic for which there is a discrepancy between laboratory stability and clinical use. While most sources do not give this drug a room temperature stability of more than 12 hours at different concentrations and with different diluents, some authors have successfully used it in continuous infusion without refrigeration [9,10].

For drugs that are not stable at room temperature for 24 hours, self-administration of antibiotics intravenously is a safe procedure. It requires that the patient or caregiver is trained, properly instructed and supervised by health care personnel [11,12]. Today, self-administration is used even for stable drugs or antibiotics administered as a single daily dose.

Another aspect that has not been sufficiently analysed in outpatient antimicrobial therapy is the application of PK/PD principles [13]. In the presence of microorganisms with high MIC, the same recommendations should be followed as for any other patient, as well as for cases where the spectrum of activity or synergistic effect needs to be broadened by combining drugs. However, the benefit of strategies such as extended or continuous infusion of antibiotics in patients in the defervesce stage of infection, as in many of the patients seen in hospital at home, is less clear. Therefore, these strategies, and their efficacy compared to intermittent infusion in the outpatient setting, require further study to recommend their use [14].

Despite the rise of multidrug-resistant infections and the increasing activity of outpatient parenteral therapy, there are still few studies that specifically analyse the results of this practice in this type of infections. However, the available data suggest that the efficacy of treatment, both in targeted and empirical therapy, is comparable to those caused by sensitive microorganisms.

The registry of intravenous home antimicrobial therapy (TADE Registry) records infection treatment activity in Spanish home hospitalisation units. From July 2011 to December 2020, more than 12,000 episodes of infections treated at home were included. The registry analyses the microorganisms causing the infection and the pattern of antibiotic sensitivity of some of them to the main groups of antibiotics. Table 1 shows the main Gram-positive and Gram-negative microorganisms causing infections and the proportion of resistance to various antibiotics. Table 2 shows the percentage of cure or improvement of infections for each of them and the 30-day hospital readmission rate.

Table 2 **Cure/improvement and 30-day readmission[a] rates for microbiological isolates with antibiotic resistance (R) in home hospitalisation (source: TADE Registry).**

Microorganism	Outcome	Antibiotic resistance (R)					
		Amoxicillin-clavulanate R	Ampicillin R	Ceftriaxone R	Ciprofloxacin R	Ertapenem R	Gentamicin R
Escherichia coli	Cure/improvement	94.1%	95.2%	94.3%	94.4%	86.7%	94.1%
	30-day readmission	8.7%	6.4%	9.0%	7.7%	5.56%	6.5%
Proteus mirabilis	Cure/improvement	91.9%	93.0%	93.0%	91.9%	77.8%	91.2%
	30-day readmission	13.4%	10.7%	14.9%	11.2%	18.2%	15.0%
Klebsiella pneumoniae	Cure/improvement	92.6%	93.4%	91.8%	92.0%	85.2%	94.4%
	30-day readmission	8.9%	8.5%	10.1%	8.3%	0.0%	6.8%
Enterobacter cloacae	Cure/improvement	90.6%	91.5%	93.0%	88.9%	100.0%	92.6%
	30-day readmission	5.9%	5.6%	8.3%	7.3%	16.7%	11.1%
		Amikacin R	Ceftazidime R	Ciprofloxacin R	Gentamicin R	Imipenem-cilastatin R	Piperacillin-tazobactam R
Pseudomonas aeruginosa	Cure/improvement	87.2%	84.2%	88.4%	88.8%	88.1%	83.7%
	30-day readmission	16.0%	14.3%	11.6%	11.6%	13.1%	14.9%
		Ciprofloxacin R	Cloxacillin R	Trimethoprim-sulfamethoxazole R	Daptomycin R	Penicillin R	Vancomycin R
Staphylococcus aureus	Cure/improvement	91.5%	86.8%	85.7%	100.0%	90.4%	93.3%
	30-day readmission	9.4%	7.6%	16.7%	0.0%	8.3%	5.6%
Staphylococcus epidermidis	Cure/improvement	84.9%	87.2%	81.8%	66.7%	86.7%	80.0%
	30-day readmission	6.5%	3.9%	4.2%	33.3%	3.7%	16.7%
		Ampicillin R	Daptomycin R	Gentamicin R	Linezolid R	Penicillin R	Vancomycin R
Enterococcus faecalis	Cure/improvement	85.7%	75.0%	89.5%	100.0%	80.0%	50.0%
	30-day readmission	20.0%	0.0%	9.6%	0.0%	5.0%	33.3%

[a]Relative to the total number of isolates with resistance to each antibiotic

These data suggest that treatment of infections caused by multidrug-resistant microorganisms in hospital at home is common and effective, although with differences depending on the microorganism and the antibiotic(s) to which it is resistant. The organisation of these units with doctors and nurses following up patients on a daily basis may explain these good results. However, more information is needed on the appropriateness of antimicrobial treatment to clinical practice guidelines and recommendations in the home setting.

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CONFLICTS OF INTEREST

The authors declare no conflict of interests.

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Tedizolid: new data and experiences for clinical practice

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ABSTRACT

The most relevant information on the clinical uses of tedizolid from studies published in the last 18 months is presented in this brief review. The most important data indicate better tolerance and safety profile of long-term therapeutic regimes in off-label indications, such as osteoarticular infections and those caused by mycobacteria. Its lower risk of hazardous interactions compared to linezolid should be emphasized. Furthermore, tedizolid in its combination with rifampicin shows a more favourable way of acting as demonstrated *in vitro* and *in vivo* studies. A recent trial also opens the door for its potential use in nosocomial pneumonia caused by Gram-positive bacteria.

Keywords: Tedizolid phosphate, Gram-positive, osteoarticular infections, pneumonia, safety

Tedizolid phosphate is an expanded-spectrum oxazolidinone with activity against Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* and *cfr*-mediated linezolid-resistant *S. aureus*. Currently, it is indicated for the treatment of acute bacterial skin and skin structure infections (ABSSSI) in adults (Figure 1). Two phase III randomized, double-blind clinical trials -ESTABLISH-1 and ESTABLISH-2- demonstrated the non-inferiority of tedizolid for 6 days (200 mg per day) versus linezolid for 10 days (600 mg every 12 h) in patients with ABSSSI. Gastrointestinal disorders (nausea, diarrhoea, and vomiting) and myelotoxicity were less frequent in the tedizolid group than in the linezolid group [1]. A recent meta-analysis of four randomized clinical trials involving 2,056 patients, comparing the efficacy of linezolid versus tedizolid for the treatment of ABSSSI, reconfirmed the previous results [2]. Its potent activity against Gram-positive cocci, high oral bioavailability, improved dosage profile (once daily), as well as the expected lower risk of drug-drug interactions with better safety profile at 6 days of treatment compared to linezolid makes tedizolid an attractive alternative for infections requiring long therapeutic regimens, such as bone and joint infections or mycobacterial infections, among others. New interesting evidence on tedizolid has appeared in the last year and a half, somewhat hidden by the overwhelming COVID19 pandemic we are still suffering, which will be summarized in this brief review.

Despite there is scarce information about the tolerability of tedizolid for treatments lasting more than 6 days, new data have recently come to light. A few years ago, Kim et al. evaluated the safety and tolerability of tedizolid in 25 patients with nontuberculous mycobacterial infections who received tedizolid for a median of 91 days [3]. They suggested that long-term tedizolid therapy might have a safety profile comparable to linezolid. Tedizolid was approved in Spain in 2015, and it has been used off-label for more than 6 days in different clinical situations. A recent study carried out by a Spanish group has evaluated these indications and described the long-term safety profile of tedizolid. A multicentric retrospective study of patients who received tedizolid for more than 6 days was conducted [4]. Eighty-one patients, treated with tedizolid 200 mg once daily for a median duration of 28 (14 to 59) days, were included; 36 (44.4%) had previously received linezolid. The main reasons for choosing tedizolid were to avoid potential linezolid toxicity or interactions (53.1%) or previously documented linezolid-related adverse effects (27.2%). The most common indications were off-label, such as osteomyelitis, prosthetic joint infections (PJI), and respiratory infections (77.8%). A favourable clinical outcome was documented in 75.3% of the patients, with clinical or microbiological failure in 19.8% during the follow-up. Overall, only 9/81 patients (11.1%) experienced a probably associated adverse event: 2 patients (2.5%) developed gastrointestinal disorders, 1 (1.2%) developed anae-

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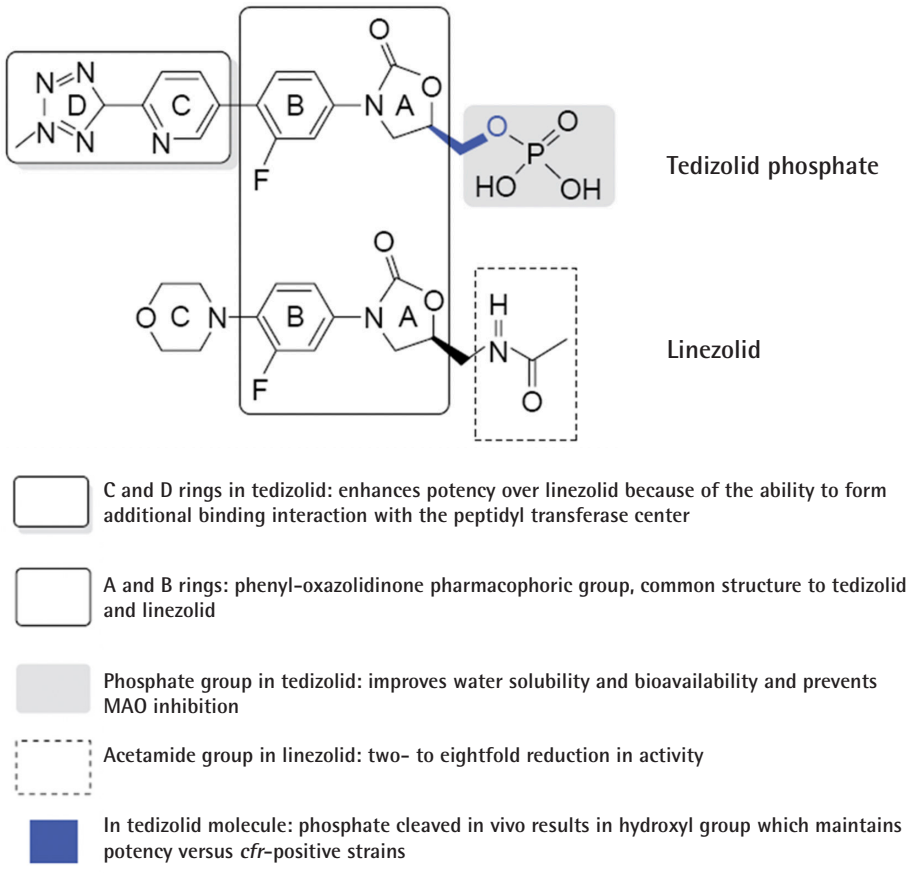

Figure 1 | **Structure–activity differences between tedizolid and linezolid**

Drug reference Company; MSD

mia, and 6 developed thrombocytopenia (7.4%) after a median duration of treatment of 26.5 (17 to 58.5) days. Four (5%) patients discontinued tedizolid due to adverse events. The rate of myelotoxicity among 23 patients with chronic renal failure (CRF) was 17.4%. Only 8.7% had to stop tedizolid and 20 out of 22 patients with previous linezolid-associated toxicity had no adverse events. Long-term tedizolid treatments showed good tolerance, with lower rates of gastrointestinal and haematological toxicity than those reported with linezolid, especially in patients with CRF or a history of linezolid-associated toxicity.

A similar experience of more than 6 days tedizolid therapy and other indications beyond ABSSSI, has been reported by a research group from the United Kingdom [5]. Sixty patients received tedizolid (from May 2016 to November 2018) mainly after documenting adverse effects with linezolid. Bone and joint infections were the most frequent indications. Despite the mean length of tedizolid therapy was 27 days, haematological adverse effects were infrequent. Most patients (72%) finished the antibiotic course and their clinical condition improved during treatment (72%). Adverse events were common, but often not thought to be tedizolid-related. The authors concluded that tedizolid appears to be safe in prolonged regimes. Hence, it could be suitable as long-term antibiotic therapy in the context of complex outpatient oral and parenteral antibiotic treatments. Patients who do not tolerate linezolid can be safely switched to tedizolid if appropriate.

The experience of long-term use of tedizolid in osteoarticular infections has been described by a research group from Barcelona (Catalonia, Spain) in a multicentric retrospective study [6]. Cases (n = 51) included patients with osteoarthritis (53%), prosthetic joint infection (33.3%), and diabetic foot infections (18%), 59% of which were orthopaedic device-related. The most frequent isolates were *Staphylococcus* spp. (65%, n = 47; *S. aureus*, 48%). The reasons for choosing tedizolid were potential drug-drug interactions (63%) and cytopenia (55%). The median treatment duration was 29 days. Twenty-four per cent received rifampicin concomitantly, with scarce adverse effects (3 cases). Long-term use of tedizolid was effective, showing a better safety profile with less myelotoxicity and lower drug-drug interactions than linezolid. For the

Table 1 Summary of new evidence for long-term treatments with tedizolid

Author (year, N)	Age (median, in years)	Linezolid (previous use,%)	BJI (%)	Duration of tedizolid therapy (days, interval)	Adverse events (%)	Discontinuation (%)	Cure or improvement (%)
Mensa et al., 2020; N=81	66	44%	47%	28 (14-59)	11%	5%	80%
York et al., 2020; N=60	62	82%	85%	27 (22-32)	GI: 15% fatigue: 12% anaemia: 2%	18%	72%
Benavent et al., 2021; N=51	65	16%	100%	29 (15-44)	5.8% (only GI)	0	83%
Senneville et al., 2020; N=33	73	9%	100% (PJI)	56 (42-84)	60% anaemia: 12% pruritus:12%	12%	82%

BJI: bone and joint infections; GI: gastrointestinal; N: number of patients /cases; PJI: prosthetic joint infections

authors, further confirmation of these advantages could make tedizolid the oxazolidinone of choice for most osteoarticular infections. The debate on the adequacy of combining oxazolidinones with rifampicin has progressed after a study in an *in vitro* model of *S. aureus* mature biofilm showed that the combination of tedizolid-rifampicin prevented the appearance of rifampicin resistance [7]. These effects were similar to those obtained with the well-known and widely used combination of daptomycin plus rifampicin.

Also recently, the results of a French prospective multicentre study reassured the good tolerance of prolonged oral tedizolid therapy for PJI. This study included patients with PJI who were treated for at least 6 weeks but not more than 12 weeks [8]. Thirty-three adult patients with PJI [hip (n = 19), knee (n = 13) and shoulder (n = 1)] were included. All patients underwent surgery, with retention of the infected implants and one/two stage replacements in 11 (33.3%) and 17/5 (51.5/15.2%), respectively. Staphylococci and enterococci were the most prevalent identified bacteria. The mean duration of tedizolid therapy was 8.0 ± 3.27 weeks. Tedizolid was associated with another antibiotic in 18 patients (54.5%), including rifampicin in 16 cases (48.5). Six patients (18.2%) had to prematurely stop tedizolid therapy because of potentially tedizolid-attributable intolerance (n = 2), early failure of PJI treatment (n = 2) or severe anaemia due to bleeding (n = 2). Regarding therapeutic compliance with tedizolid, two or more omissions of drug intake were not recorded during the whole treatment duration. These results suggest good compliance and a favourable safety profile of tedizolid, providing evidence of the potential benefit of its use in PJI. A summary of the main data and most relevant results of the reviewed studies are presented in Table 1.

Lastly, important information has been provided in the field of the treatment of Gram-positive nosocomial pneumonia. A recent randomized, non-inferiority, double-blind, phase 3 clinical trial has evaluated the efficacy and safety of tedizolid for the treatment of Gram-positive hospital-acquired bacterial pneumonia (HABP) and ventilator-associated bacterial pneumonia (VABP) [9]. Patients were randomized 1:1 to receive intravenous tedizolid phosphate 200 mg once daily for 7 days or intravenous linezolid 600 mg every 12 hours for 10 days. Treatment duration was 14 days in patients with concurrent Gram-positive bacteraemia. Overall, 726 patients were randomized. Their baseline characteristics, including the incidence of methicillin-resistant *Staphylococcus aureus* (31.3% overall), were well balanced. Tedizolid was non-inferior to linezolid for day 28 all-cause mortality rate (28.1% and 26.4%, respectively) in the treatment of Gram-positive VABP. Non-inferiority of tedizolid was not demonstrated for investigator-assessed clinical cure at test of cure (TOC) in the intention-to-treat population. The difference in the clinical response of both groups was not determined by any single factor according to the post hoc analyses. Approximately 12% and 8% of the patients presented adverse effects with linezolid and tedizolid, respectively. Regardless of whether this trial would allow expanding the indications in the technical data sheet for tedizolid, it represents a great advance in reinforcing the potential clinical use of this safer drug in the treatment of nosocomial pneumonia involving Gram-positive microorganisms.

CONFLICTS OF INTEREST

The authors declare no conflict of interests.

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Update on antimicrobial pharmacotherapy

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ABSTRACT

Dalbavancin is a long-acting antimicrobial agent with an excellent *in vitro* activity against Gram-positive pathogens, including staphylococcal biofilms. The unusually long terminal half-life ranging from 149 to 250 hours in human subjects, allows a weekly dose. Currently is indicated in acute bacterial skin and skin structure infections (ABSSSIs), but in real-life clinical practice it has already been used successfully and safely in other infections, especially as consolidation therapy.

Key words: Dalbavancin, Gram-positive pathogens, ABSSSIs, bone and joint infections, endocarditis, bacteremia

INTRODUCTION

Dalbavancin is a semisynthetic lipoglycopeptide with a long lipophilic side chain that confers it two new determining properties: 1) a faster and more potent bactericidal activity than vancomycin or teicoplanin, and 2) a long terminal half-life ranging from 149 to 250 hours in human subjects, allowing for a weekly dose. Dalbavancin also possesses an amidated carboxyl side group that increases the agent's anti-staphylococcal activity (Figure 1). It was approved by both the FDA in May 2014 and the EMA in February 2015 for the treatment of adult patients with acute bacterial skin and skin structure infections (ABSSSIs). Recently, dalbavancin has also received the FDA approval to treat ABSSSIs in pediatric patient (July 2021) [1].

MICROBIOLOGICAL PROFILE

Dalbavancin has a similar microbiological profile to the other available glycopeptides. Against MRSA, dalbavancin has demonstrated to be 16-fold more potent than daptomycin, and 32-fold more potent than vancomycin and linezolid. Dalbavancin is also the most potent agent against coagulase-negative staphylococci (CoNS) (MIC_{90} 0.06 mg/L).

Overall, dalbavancin is 16-fold more potent against β-hemolytic streptococci ($MICs_{90}$ 0.03-0.047 mg/L) than vancomycin (MIC_{90} of 0.75 mg/L). All vancomycin-susceptible *Enterococcus faecalis* and *Enterococcus faecium* isolates are inhibited by dalbavancin at ≤ 0.25mg/L, but is not active against enterococci with VanA-mediated glycopeptide resistance and only partially active against VanB isolates [2].

PHARMACODYNAMIC AND PHARMACOKINETIC PROFILE

In staphylococcal animal models the clinical efficacy of dalbavancin has been related to AUC/MIC values ≥ 1000. The main pharmacokinetic properties of dalbavancin are as follows: approximately 93% is binding to serum albumin after an intravenous dose; excretion is through non-microsomal metabolism with inactive metabolites and up to 42% of the dose through the kidneys by glomerular filtration; and a terminal elimination half-life can exceed 200 hours. Dose adjustment is required in patients with severe renal impairment (creatinine clearance <30 mL/min) who do not undergo hemodialysis, and caution is recommended in Child-Pugh class B and/or class C hepatic impairment [3].

CLINICAL EFFICACY

Current indications for the use of dalbavancin in the ABSSSIs come from the pivotal studies DISCOVER 1 and DISCOVER 2 trials (dalbavancin vs vancomycin/linezolid 1:1, double-blind, double dummy, non-inferiority trials), that showed noninferiority of dalbavancin in both DISCOVER 1 and DIS-

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Lipophilic side chain:

- **Enhancement binding to bacterial cell membrane**
- **Faster and more potent bactericidal activity**
- **Increase in half-life**

Amidated carboxyl side group:

- **Increase anti-staphylococcal activity**

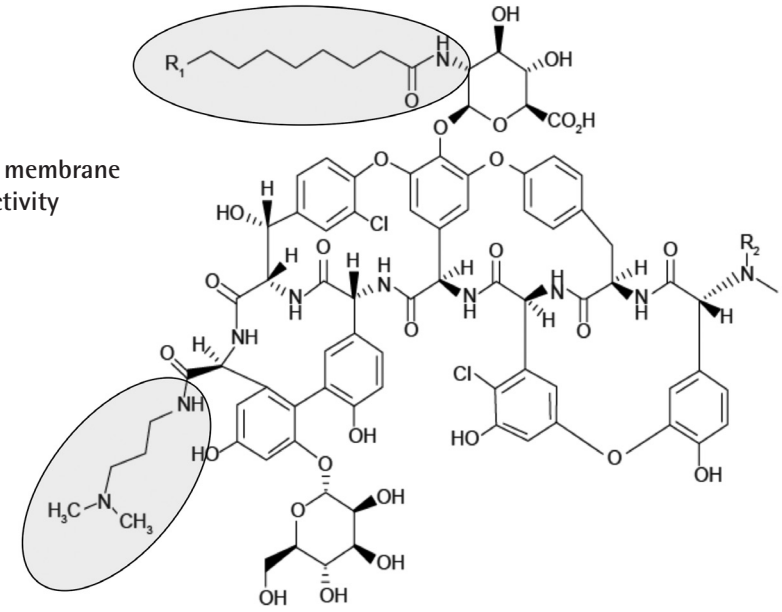

Figure 1 | **Structure of dalbavancin**

Table 1 | **Potential indications of dalbavancin in real-life clinical practice**

Treatment	Prophylaxis
Consolidation therapy in acute infections[a]	Recurrent processed
Catheter-related staphylococcal bacteremia	Recurrent cellulitis
Infective endocarditis	Recurrent enterococcal cholangitis
Osteomyelitis	Recurrent enterococcal urinary tract infection
Spondylodiscitis	Vascular implants at risk of staphylococcal bacteremia
Acute septic arthritis	
Diabetic foot infections	
Prosthetic joint infections	
Suppressive treatment of chronic infections	
Chronic osteomyelitis	
Chronic prosthetic joint infections	

[a]Particularly in infections with prolonged treatment (≥ 14 days)

COVER 2. Dalbavancin was better tolerated than vancomycin/linezolid and significantly fewer patients in the dalbavancin group experienced diarrhea (0.8% vs 2.5%; *P*=0.02) or pruritis (0.6% vs 2.3%, *P*=0.01) compared to the vancomycin/linezolid group. A secondary analysis did identify significantly longer duration of therapy in the vancomycin/linezolid treatment group as compared to the dalbavancin arm (38.0% vs 31.0%; *P*=0.008) [4].

The long terminal half-life of dalbavancin allows its use as consolidation therapy in acute infections that require prolonged treatment, suppressive treatment of chronic infections and prophylaxis of some recurrent processes caused by Gram-positive cocci (Table 1). The use of dalbavancin for bone and joint infections (BJIs), including prosthetic joint infections (PJIs) has also been assessed in several retrospective studies and one randomized clinical trial. In osteomyelitis the cure rates ranged from 65% to 100%. The worst results have

been observed in postoperative, chronic and diabetic foot osteomyelitis and when dalbavancin was mainly used as a first or rescue regimen after failure of a previous treatment. The experience of dalbavancin in PJIs is smaller. The cure rate ranged from 33% to 93%. The lack of information in many cases about surgical management and the heterogeneity of PJIs included make it difficult to draw conclusions on the efficacy of dalbavancin in these processes. The use of dalbavancin in BJIs is supported by the activity of dalbavancin against staphylococcal biofilms and its bone and articular tissue penetration that exceeds the MIC_{90} of *S. aureus* for extended periods of time after a significantly shortened dosing regimen [4].

In catheter-related bloodstream infection (CR-BSIs) caused by gram-positive pathogens (included *S. aureus* and CoNS), a phase 2, open-label, randomized, controlled, multicentre study has shown a superior efficacy of dalbavancin compared with vancomycin [5]. In infective endocarditis treated with dalbavancin, clinical cure rates range from 50% to 100%. The best results have been observed when dalbavancin was used as a consolidation therapy after blood culture clearance rather than as a rescue strategy. As in BJIs, the diversity of the therapeutic regimens used and the fact that most patients have previously received other antibiotics, are two important limitations to knowing the efficacy of dalbavancin. In some of the published studies of dalbavancin in BJIs and IE, a reduction in the length of hospital stay (LOS) and economic cost has been observed [6].

SAFETY PROFILE

Dalbavancin, in all published evidence, has been shown to be safe and less nephrotoxic than other glycopeptides. Drug-drug interactions are uncommon with other comedications.

CONCLUSIONS

Dalbavancin, in addition to its indication in ABSSSIs, represents an effective and safe therapeutic alternative in clinically stable patients with other infections requiring prolonged treatment to shorten the LOS.

CONFLICTS OF INTEREST

The authors declare no conflict of interests.

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Update on antimicrobial pharmacotherapy

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ABSTRACT

Community-acquired pneumonia (CAP) is one of the leading causes of admission to emergency departments. Ceftaroline is a fifth-generation cephalosporin with a potent *In vitro* activity against *Streptococcus pneumoniae, Haemophilus influenzae* and *Staphylococcus aureus,* the three most important pathogens causing CAP. Three randomized and double-blind clinical trials compared the efficacy of ceftaroline versus ceftriaxone in patients with CAP and the results of each trial and a meta-analysis, concluded the superiority of ceftaroline in terms of clinical success. In particular, the major difference was observed among patients with CAP caused by *S. aureus.* Accordingly, ceftaroline has been included as a first-line option in the recent clinical guidelines for the management of CAP.

Keywords: Community-acquired pneumonia, clinical cure, *S. pneumoniae*, *S. aureus*, ceftaroline

INTRODUCTION

Community-acquired pneumonia (CAP) is one of the leading causes of emergency department care and hospital admission. The most recent guidelines for the management of this entity were published in 2019 by two American Societies[1] and among the most notable changes is the incorporation of ceftaroline as a first-line option for the treatment of CAP in patients with a severe infection, but without risk factors for *Pseudomonas aeruginosa* or methicillin-resistant *Staphylococcus aureus* (MRSA). For this reason, we are going to summarize the main characteristics of this fifth-generation cephalosporin.

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MECHANISM OF ACTION

Like all beta-lactams, ceftaroline inhibits the transpeptidase activity of PBPs, including PBP1a, PBP2b and PBP2x of *Streptococcus pneumoniae* responsible of penicillin resistance, as well as PBP2a of MRSA. In the latter case, inhibition is produced by an allosteric effect consisting in the binding of a ceftaroline molecule at a point distant from the active centre inducing a conformational change in PBP2a that now allows the binding of another molecule of ceftaroline at the active centre leading to the inhibition of this enzyme. The in vitro activity exhibited by ceftaroline is bactericidal and time-dependent [2].

SPECTRUM

It is active against Gram-positive microorganisms including viridans group streptococci, ß-haemolytics and *S. pneumoniae* with a MIC_{90} < 0.25 mg/L. It is of note its activity against third-generation cephalosporin-resistant strains of pneumococcus. A study of strains from around the world collected between 2015 and 2016 showed that ceftaroline was the ß-lactam with the highest intrinsic activity (lowest MIC) against pneumococcus [3]. For *S. aureus* and coagulase-negative *Staphylococcus*, ceftaroline has a MIC_{90} < 0.5 mg/L, although for MRSA strains the MIC_{90} is 2 mg/L. Its activity against other Gram-positive cocci such as enterococcus is moderate (*E. faecalis*) or they are resistant (*E. faecium*).

Against Gram-negative bacilli, its activity is superimposable to that of a third-generation cephalosporin. For *Haemophilus influenzae* and *Moraxella* the MIC_{90} is < 0.12 mg/L and for the susceptible Enterobacterales < 0.5 mg/L. Extended-spectrum beta-lactamase (ESBL) and AmpC-producing strains are resistant to ceftaroline. *P. aeruginosa* and other non-fermenting Gram-negative bacilli are resistant. Activity against anaerobic microorganisms is limited to Gram-positive cocci (*Peptococcus* and *Peptostreptococcus*), while Gram-negative bacilli (*Prevotella*, *Bacteroides*) are resistant.

The breakpoint for susceptibility proposed by EUCAST for *S. pneumoniae* is ≤ 0.25 mg/L, for *H. influenzae* ≤ 0.03 mg/L, for *S. aureus* ≤0.5 mg/L (1 mg/L in case of pneumonia) and for Enterobacterales ≤ 0.5 mg/L.

The association with daptomycin is often synergistic against MRSA and the association with ampicillin may be synergistic against *E. faecalis* [4].

PHARMACOKINETICS AND PHARMACODYNAMICS

It is administered intravenously in a 60-minute infusion that allows a maximum serum concentration of 28 mg/L with 600 mg. It has a half-life of 2.5h and the protein binding is 15-20%. About 20% is metabolized in the liver but it does not modify the activity of cytochrome P-450 isoenzymes. Elimination is mostly urinary (90%) and 64% in active form. Although data are scarce, diffusion to cerebrospinal fluid is 5-9% of the serum concentration, corresponding to 1-2 mg/L.

The pharmacodynamic parameter that predicts its clinical efficacy is the time that the antibiotic free fraction remains above the MIC between two consecutive doses (*f*T > MIC). The value required to obtain a 2-log reduction in bacterial load is 35% for *S. aureus* and 51% for *S. pneumoniae*. In both cases the probability of achieving these values with the 600 mg/12h dose infused over 60 minutes is >90% for the cut-off points established by EUCAST [5].

CLINICAL EFFICACY

A meta-analysis of 3 clinical trials in patients with CAP and with similar inclusion criteria summarized the clinical efficacy of ceftaroline. In two studies, the comparator was ceftriaxone at a dose of 1g/24h and in the third 2g/24h. The outcome variable in all 3 studies was clinical cure defined as resolution of symptoms without modification of antibiotic 8-15 days after completion of treatment. The conclusion of the meta-analysis is that ceftaroline was superior to ceftriaxone in both the intention-to-treat and clinically evaluable populations [6]. The results were consistent across the different sub-analyses according to age, co-morbidity and PORT scale. In addition, the percentage difference in clinical cure rate was approximately 10 points higher in the ceftaroline arm in cases with documented *S. pneumoniae* and Gram-negative bacilli (*E. coli, K. pneumoniae*) infections, but reached a difference of more than 20 points in those patients with S. aureus isolation. A subsequent analysis including only the two studies using the 1g of ceftriaxone as a comparator assessed the time to recovery of the two treatment options and showed that a significantly higher percentage of patients in the ceftaroline arm reached clinical stability earlier [7]. These data support the incorporation of ceftaroline in the recent clinical guidelines for the management of CAP. On the other hand, not many data are available in patients with severe pneumonia (criteria for ICU admission), but recently our group reported at the congress of the Spanish Society of Infectious Diseases and Clinical Microbiology our experience in patients with these characteristics and through a case-control study we were able to observe a decrease in in-hospital mortality among patients who received ceftaroline.

ADVERSE EVENTS

The main adverse effects are related to skin hypersensitivity reactions and gastrointestinal disturbances (diarrhea, nausea). In 10% of patients the Coombs' test becomes positive without evidence of hemolysis. Neutropenia has been reported in patients receiving more than 3 weeks of treatment.

CONCLUSION

Empirical treatment of moderate or severe CAP requiring hospital admission or 24h of observation should include a ß-lactam. Ceftaroline is an alternative that has demonstrated greater clinical efficacy than ceftriaxone in several clinical trials. The greatest difference between the two options has been seen in patients with *S. aureus* infection, which is to be expected given the low intrinsic activity of ceftriaxone against this pathogen. This makes ceftaroline the ß-lactam of choice when *S. aureus* is suspected (e.g. co-infection with influenza virus). The greater benefit observed in patients with moderate CAP, a prevalence of *S. pneumoniae* strains with intermediate susceptibility to ceftriaxone of 10% in many areas of the world [3] and a higher incidence of *S. aureus* among severe forms of CAP suggest that treatment of patients with severe CAP should include ceftaroline for at least the first 48-72h until microbiological results are available. Further studies on its efficacy in this population group are needed in the future.

CONFLICTS OF INTEREST

AS has received honoraria for lectures and advisory boards from Pfizer, MSD, Shionogi, Menarini, Angelini and Gilead.

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Update on antimicrobial pharmacotherapy

Ceftobiprole: a clinical view

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ABSTRACT

Ceftobiprole is a broad-spectrum, fifth-generation cephalosporin currently approved for community-acquired and non-ventilator-associated hospital-acquired pneumonia. High bactericidal and anti-biofilm activity has been exhibited in *in vitro* and animal models. This, together with its synergism with other antibiotics against gram-positive bacteria, makes it an ideal candidate for treatment of complex infections, such as those associated with devices or infective endocarditis. More clinical data are needed to achieve drug positioning.

Keywords: ceftobiprole, MRSA, synergy, anti-biofilm.

Ceftobiprole is a broad-spectrum, fifth-generation cephalosporin, currently approved for community-acquired pneumonia (CAP) and hospital-acquired pneumonia (HAP) excluding ventilator-associated one [1,2]. This drug exerts potent bactericidal activity against several gram-positive and gram-negative pathogens, as well as *Streptococcus* spp. (including most *Enterococcus faecalis*) and *Staphylococcus* spp. [including methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase-negative *Staphylococcus* (CNS)], *Haemophilus influenzae*, *Moraxella catarrhalis*, most of the *Enterobacterales* group, and *Pseudomonas aeruginosa* (Table 1). On the other hand, ceftobiprole shows reduced or no activity against *Enterococcus faecium*, *Proteus vulgaris*, most Gram-negative anaerobes, *Acinetobacter baumannii*, *Burkholderia cepacian* complex, and *Stenotrophomonas maltophilia*. With respect to difficult-to-treat bacteria, ceftobiprole has activity against derepressed AmpC producers, but not against extended-spectrum β-lactamases (ESBLs), carbapenemases or metallocarbapenemase-producing *Enterobacterales*. Epidemiological surveillance studies have shown excellent susceptibility rates (close to 100%) in *S. aureus*, MRSA, CNS, *S. pneumoniae* and *E. faecalis*, although with more discreet results in *Enterobacterales* and *P. aeruginosa*. With respect to the latter, ceftobiprole susceptibility rates of about 70% were reported in a recent international cohort that included 1064 isolates [3,4]. To our knowledge, there are no published data concerning the susceptibility of *P. aeruginosa* to ceftobiprole in Spanish isolates, although in a recent conference communication, only 59% of 95 Spanish isolates tested were susceptible. A limitation is that we do not know which hospitals participated in that study and that may have conditioned these results [5]. Ceftobiprole shows several pharmacokinetic and pharmacodynamic properties that make it a very interesting molecule: high bactericidal activity, proven in experimental models (*in vitro* and animal studies), low protein binding (16%), a high volume of distribution, and predominantly renal excretion (70-90%). Consequently, concentrations of ceftobiprole found in feces after 7 days of therapy are very low, as was demonstrated in a study in healthy volunteers [6]. This may be associated with a low rate of *Clostridioides difficile* infection [1,2].

The data sheet recommends 500 mg every 8 hours administered as a 2-hour intravenous infusion, demonstrating linear pharmacokinetics if higher doses than usual are used. It exhibits two very promising features that may help to place it in a wide range of complex infections in the near future. First, it shows *in vitro* synergy with different antibiotics, highlighting combinations with daptomycin against MRSA, and with piperacillin/tazobactam and amikacin against *P. aeruginosa* [7,8]. Second, it has very good activity against biofilm, once again showing synergy with rifampin and vancomycin [9]. These characteristics could make it an excellent option against MRSA or CNS infective endocarditis, endovascular or prosthesis-related infections, osteomyelitis, among others. While the clinical data about the efficacy of ceftobiprole in these scenarios has increased in recent years, it is not easy to draw solid conclusions because it has been used in most cases as combined or

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Table 1 **Spectrum of ceftobiprole compared with other antimicrobials**

	Staphylococcus aureus		Coagulase negative staphylococci	*Streptococcus pneumoniae*		*Pseudomonas aeruginosa*		*Enterobacterales*	
	MSSA	MRSA		Non-MDR	MDR /PRSP	CAZ-S	CAZ-R	Non-ESBL	ESBL
Ceftobiprole									
Ceftaroline									
Cefepime									
Ceftazidime									
Ceftriaxone									
Meropenem									
Pip/tazobactam									
Linezolid									
Daptomycin									
Vancomycin									

MSSA: methicillin-susceptible *Staphylococcus aureus*; MRSA: methicillin-resistant *S. aureus*; MDR: multidrug resistant; PRSP: penicillin-resistant *Streptococcus pneumoniae*; CAZ: ceftazidime; S: susceptible; R: resistant ESBL: extended-spectrum β-lactamases; Pip/tazobactam: Piperacillin/ tazobactam

salvage therapy [10-13]. There are some ongoing clinical trials, such as the one establishing the efficacy and safety of ceftobiprole versus daptomycin in the treatment of *S. aureus* bacteremia, including infective endocarditis [14].

Ceftobiprole is generally well tolerated with a low rate of adverse effects. The most common ones are dysgeusia, nausea, vomiting, and diarrhea, although hyponatremia and myoclonus have also been reported on rare occasions [1,2,11].

In our opinion, the use of ceftobiprole as empirical treatment in nosocomial infections is limited because the number of *P. aeruginosa*-susceptible strains is not well established in our media and because it has no activity against ESBLs strains. Own susceptibility data are needed for the adequate positioning of the drug in this regard. Nevertheless, ceftobiprole may have a role as targeted therapy to carry out antimicrobial diversification in nosocomial infections, replacing standard combinations such as ceftazidime plus and vancomycin. It could also be useful as salvage therapy in combination with daptomycin in MRSA infections, although comparisons with other combination options, such as daptomycin plus fosfomycin, or daptomycin plus fosfomycin are needed. Preliminary data shows that ceftobiprole is at stable for up to 24 hours at 25ºC and protected from light, which also allows for potential administration in outpatient parenteral antimicrobial therapy [15].

In the meantime, we look forward to more observational studies and data from clinical trials that will help us to establish definitively new indications for ceftobiprole.

CONFLICTS OF INTEREST

LELC has served as scientific advisor for Angelini, speaker for Angelini, ViiV, Gilead and Correvio, and has served as trainer for ViiV. PMMPC declare no clonflic of interest.

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Ceftolozane-tazobactam: When, how and why using it?

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ABSTRACT

Ceftolozane-tazobactam is currently the most active antipseudomonal agent, including multidrug-resistant extensively drug-resistant strains. Tazobactam provides additional activity against many extended-spectrum beta-lactamases *Enterobacterales*. Ceftolozane-tazobactam is formally approved for complicated urinary tract infection, complicated intra-abdominal infection, and hospital-acquired and ventilator-associated bacterial pneumonia. The clinical and microbiological success is over 70-80% in many series. However, resistant mutants to ceftolozane-tazobactam have been already described. Combination therapies with colistin or meropenem could be among the strategies to avoid the resistance emergence.

Key words: Ceftolozane-tazobactam, *Pseudomonas aeruginosa*, multidrug resistant, extensively drug resistant, extended spectrum β-lactamase.

INTRODUCTION

Ceftolozane-tazobactam (TOL-TAZ) combines a new antipseudomonal cephalosporin (ceftolozane) with enhanced antipseudomonal activity with a classic β-lactamase inhibitor (tazobactam). It exhibits bactericidal properties through inhibition of bacterial cell wall biosynthesis, which is mediated through penicillin-binding proteins (PBPs). Ceftolozane is a potent PBP3 inhibitor and has a higher affinity for PBP1b and PBP1c compared with other β-lactam agents. PBP1b and PBP1c are present in *Pseudomonas aeruginosa*. Moreover, ceftolozane has high stability against amp-C type betalactamases, which are frequently present in *P. aeruginosa*, and it is significantly less affected by the changes in the porin permeability or efflux pumps of the external membrane of gram negatives. Because of this ceftolozane has higher antipseudomonal activity than other antipseudomonals. Further, due to the combination with tazobactam, TOL-TAZ inhibits class A serine-betalactamases and extended-spectrum beta-lactamases (ESBL). TOL-TAZ also acts against non-ESBL class D oxacillinases, but it lacks activity against carbapenemases [1].

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SPECTRUM OF ACTIVITY

TOL-TAZ is an effective combination against several multidrug-resistant (MDR) Gram-negative bacilli, particularly MDR or extensively drug-resistant (XDR) *P. aeruginosa*. It is also active against AmpC and ESBLs producing *Enterobacterales*, but with a limited activity against ESBL-producing *Klebsiella pneumoniae*. Further, it remains activity against *Streptococcus* spp. (excluding *Enterococcus* spp.) and some anaerobes (*Bacteroides fragilis* and non-Bacteroides Gram-negatives) [2,3].

APPROVED INDICATIONS

TOL-TAZ was first approved for the treatment of adults with complicated intra-abdominal infection (cIAI) (in combination with metronidazole 500 mg every 8 hours) and complicated urinary tract infection (cUTI), including pyelonephritis. The dosage approved for these indications was 1.5 g 3 times a day. It was lately approved for adults with hospital-acquired and ventilator-associated bacterial pneumonia (HABP/VABP) at a dosage of 3 g every 8 h [2].

CLINICAL EXPERIENCE

The efficacy of TOL-TAZ in *P. aeruginosa* and ESBL *Enterobacterales* infections has been evaluated in several studies to the date (Table 1).

Regarding infections caused by *P. aeruginosa*, all these studies included patients treated with a dose of either 1.5 g every 8 h or 3 g every 8 h, with the high dose usually adminis-

Table 1 **Clinical studies evaluating ceftolozane-tazobactam for *P. aeruginosa* and Enterobacterales infections. Adapted from [2]**

Study reference	Design	No. and source of infection	Microorganism	Outcomes
Pseudomonas aeruginosa				
Miller 2016, Antimicrob Agents Chemother	Post hoc analysis of RCT: C-T vs. Meropenem	IAI (C/T: 26 vs. Meropenem 29)	MDR	Clinical cure: C-T 100% vs. meropenem 93.1%
Caston 2017, Antimicrob Agents Chemother	Case series with C-T	6 LRTI, 5 BSI, 3 IAI, 3 others	MDR	Mortality 25%, Clinical cure 75%, Microbiological cure 58.3%
Dinh 2017, Int J Antimicrob Agents	Case series with C-T	7 LRTI, 3 UTI, 2 IAI, 3 others	XDR	Mortality 27%, Clinical cure 67%, Microbiological cure 75%
Haidar 2017, Clin Infect Dis	Retrospective study	18 LRTI, 1 BSI, 1 ITU, 1 IAI	MDR/XDR	Mortality 10%, clinical cure 71,4%
Munita 2017, Clin Infect Dis	Retrospective study	18 LRTI, 6 BSI	CR	Mortality 22.3%, clinical cure 74%, Microbiological cure 100%
Diaz-Cañestro 2018, Clin Infect Dis	Prospective observational study	35 LRTI, 10 UTI, 4 IAI,3 BSI, 6 others	MDR/XDR	Mortality 27.6%, Clinical cure 63.8%, Microbiological cure 70%
Escola Verge 2018, Infection	Retrospective study	14 LRTI, 11 BSI, 6 UTI, 6 SSTI, 4 IAI, 8 others	XDR	Mortality 13.2%, Clinical cure 68.4%-86.6%, Microbiological cure 68.4%
Gallagher 2018, Open Forum Infect Dis	Retrospective study	121 LRTI, 28 UTI, 25 BSI, 20 IAI, 42 others	MDR	Mortality 19%, Clinical cure 73.7%, Microbiological cure 70.7%
Xipell 2018, J Glob Antimicrob Resist	Case series with C-T	8 LRTI, 7 UTI, 6 SSTI, 3 IAI	MDR/XDR/PDR	Mortality 22%, Clinical cure 88%, Microbiological cure 75%
Bassetti 2019, Int J Antimicrob Agents	Retrospective study	32 LRTI, 22 BSI, 21 SSTI, 14 UTI, 13 IAI, 6 others	Non-MDR/MDR/XDR/PDR	Mortality 5%, Clinical cure 83.2%
Pogue 2019, Clin Infect Dis	Retrospective study: C-T vs polymyxin or aminoglycoside	C-T: 64 LRTI, 16 UTI, 13 SSTI, 6 BSI, 7 others Comparator: 75 LRTI, 11 UTI, 6 SSTI, 6 BSI, 6 others	MDR/XDR	Mortality: C-T 20% vs. comparator 25% Clinical cure: C-T 81% vs. comparator 61%
Vena 2019, Clin Infect Dis	Case control study C-T vs polymyxin or aminoglycoside	C-T 16 vs comparator 32: 27 LRTI, 21 BSI	MDR/XDR	Mortality: C-T 18.8% vs. comparator 28.1% Clinical cure: C-T 81.3% vs. comparator 56.3%
Bosaeed 2020, Infect Dis	Retrospective study	LRTI 6, BSI 4, SSTI 3, UTI 2, IAI 3, bone 1	CR	Mortality 21%, Clinical cure 94.7%, Microbiological cure 73.7%
Coppola 2020, Microorganisms	Case series with C-T	SSTI 2, BSI 2, 1 other	MDR	Mortality 0%
Hart 2021, Open Forum Infect Dis	Retrospective study	UTI 45, SSTI 8, IAI 6, BSI 6, bone/joiont 4, brain 3.	MDR	Mortality 19%, clinical cure 68%
Enterobacterales				
Huntington 2016, J Antimicrob Chemother	Post hoc analysis of RCT: C-T vs. Levofloxacin	212 UTI, 7 BSI	186 Enterobacterales 85 ESBL	Clinical cure: C-T 90% vs. comparator 76.8% Microbiological cure: C-T 63% vs. comparator 43.8%
Popejoy 2017, J Antimicrob Chemother	Post hoc analysis of 2 RCT: C-T vs. Levofloxacin C-T vs. Meropenem	UTI: 54 C-T, 46 Levofloxacin IAI: 24 C-T, 26 Meropenem	ESBL	Clinical cure: C-T 97.4% vs. Levofloxacin 82.6% and vs Meropenem 88.5%. Microbiological cure: C-T 79.5% vs. Levofloxacin/Meropenem 62.5%
Arakawa 2019, J Infect Chemother	Nonrandomized open-label trial	90 UIT, 24 BSI	93 Enterobacterales 13 ESBL	For ESBL: Mortality 0%, Microbiological cure 38.5%
Mikamo 2019, J Infect Chemother	Nonrandomized open-label trial	130 IAI	58 Enterobacterales 5 ESBL	For ESBL: Mortality 0%, Clinical cure 100%, Microbiological cure 100%

Abbreviations: RCT, randomized controlled trial; C-T, ceftolozane-tazobactam; IAI, intra-abdominal infection; LRTI, lower respiratory tract infection; BSI, bloodstream infection; ITU, urinary tract infection; SSTI, skin and soft tissue infection; MDR, multidrug resistant; XDR, extensively drug resistant; CR, carbapenem resistant; PDR, pandrug resistant; ESBL, extended spectrum β-lactamase.

tered for high inoculum sources such as pneumonia, osteomyelitis, and abscesses. However, not only the source of infection should be considered to make the decision about the dosage but also the TOL-TAZ minimum inhibitory concentration (MIC). In a study aimed to evaluate the efficacy of different TOL-TAZ doses in patients with lower respiratory infection due to MDR- or XDR-*P. aeruginosa*, *Rodríguez Núñez* et al. found that mortality was significantly lower in patients with *P. aeruginosa* strains with MIC ≤2 mg/L and receiving high dose of TOL-TAZ compared with the group with higher MIC and standard dosage (16.2% vs 35.8%; P = .041). However, in the multivariate analysis only TOL-TAZ MIC >2 mg/L was identified as an independent predictor of mortality [4].

In case of third generation cephalosporin resistant *Enterobacterales*, the results of MERINO-3 (multicentre, parallel group open-label non-inferiority trial design comparing TOL-TAZ vs. meropenem in adult patients with bloodstream infection caused by ESBL or AmpC-producing *Enterobacterales*) will provide a better comprehension about the efficacy of TOL-TAZ in such infections [5].

RESISTANCE MECHANISMS

In vitro and *in vivo* data indicate that *P. aeruginosa* resistance to TOL-TAZ is due to several mechanisms. The most important seems to be a combination of mutations leading to hyperproduction and structural modified AmpC enzymes. It has been also suggested that specific PBP3 mutations may reduce its susceptibility. Finally, although to a minor extent, the overexpression of different efflux pumps could also affect to TOL-TAZ. With respect to acquired β-lactamases, TOL-TAZ shows no activity against metallo-beta-lactamases (MBL) -producing strains. Finally, extended-spectrum mutations in horizontally acquired OXA-type β-lactamases may lead to the emergence of resistance to TOL-TAZ [3].

Regarding *Enterobacterales*, tazobactam has no activity against serine carbapenemases or MBL, and has limited activity against AmpC and some ESBL [6].

COMBINATION THERAPY AGAINST MDR/XDR P. AERUGINOSA STRAINS

In order to avoid the selection of resistance, some studies have addressed the efficacy of combination antibiotic therapy with TOL-TAZ for treating MDR/XDR *P. aeruginosa* strains**.**

In an *in vitro* study aimed to evaluate the antibacterial activity of TOL-TAZ and colistin alone and in combination against a collection of 24 clinical XDR *P. aeruginosa*, *Montero* et al. demonstrated synergistic or additive effect for TOL-TAZ plus colistin (21/24), including TOL-TAZ-resistant strains [7]. The same group also evaluated the efficacy of TOL-TAZ in combination with meropenem against XDR strains in a hollow-fiber model. This approach showed that when TOL-TAZ was administered in combination with meropenem, there was a >4 log10 CFU/ml bacterial density reduction, without resistance emergence. This result suggests that a double beta-lactam strategy based on TOL-TAZ plus meropenem may be a useful combination for treating XDR *P. aeruginosa* [8].

CONFLICTS OF INTEREST

JPH has received honoraria as speaker or for advisory activities from Pfizer, MSD, Menarini, Angelini, Zambon. All other authors declare no conflicts of interest.

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Update on antimicrobial pharmacotherapy

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Ceftazidime-avibactam

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ABSTRACT

Ceftazidime is a 3rd generation cephalosporin active against *Pseudomonas aeruginosa*. Avibactam is an inhibitor of class A, C and some class D β-lactamases. The antibacterial spectrum of ceftazidime-avibactam covers 95% of *P. aeruginosa* isolates and >99% of enterobacteria, including strains carrying extended-spectrum β-lactamases (ESBLs). Selection of resistant mutants in *Klebsiella pneumoniae* and *Enterobacter cloacae* strains producing KPC-3 or KPC-2 after exposure to ceftazidime-avibactam has been described by the appearance of one or more amino acid changes in the Ω-loop of the β-lactamase. These strains usually regain susceptibility to meropenem. There is evidence of a shorter multidrug-resistant organisms colonization period in patients treated with this antimicrobial, which could be beneficial in the treatment of infections caused by bacteria carrying ESBLs or carbapenemases.

Keywords: Ceftazidime-avibactam, *Pseudomonas aeruginosa*, *Enterobatericeae*, KPC-2, KPC-3, decolonization.

Ceftazidime is a 3rd generation cephalosporin active against *Pseudomonas aeruginosa*, which in the 1990s was widely used in monotherapy or associated with an aminoglycoside, in empirical treatment regimens for fever in neutropenic patients [1-3]. With the appearance of extended-spectrum β-lactamases (ESBLs) around the year 2000, its indications were progressively reduced to the targeted treatment of infections caused by *P. aeruginosa*. Avibactam is an inhibitor of class A β-lactamases, including TEM, SHV, CTX-M, KPC, GES, PER, SME; chromosomal class C (AmpC) and plasmid class C such as FOX, MOX, CMY, LAT, ACC, DHA; and some class D such as OXA-48 from *Klebsiella pneumoniae*, and OXA-24, OXA-40 and OXA-69 from *Acinetobacter baumannii*. Activity against OXA-2, OXA-5/10 and OXA-50 is limited; it is not active against class B β-lactamases (metallo-β-lactamases). Against most β-lactamases it behaves as a reversible (non-suicidal) inhibitor. Avibactam forms a covalent bond with the serine of the active center of the β-lactamase but, unlike what occurs with clavulanic acid and tazobactam, the molecule is not hydrolyzed, but is slowly separated and recovers its original structure. This mechanism of action, together with the broad spectrum of activity against the different β-lactamases (including carbapenemases KPC, OXA-48) and an elimination half-life of 2.5 hours (longer than that of clavulanic acid, tazobactam and relebactam), justify the greater effectiveness observed in a hollow fiber infection model, in which the effectiveness of the piperacillin association was compared with each of the three β-lactamase inhibitors, tazobactam, avibactam and relebactam. The % fT > MIC of the combination of piperacillin with avibactam (61.4%-73.6%) was significantly higher than that of tazobactam (13.5%-44.5%) in suppressing bacterial growth of 3 clinical isolates, 2 CTX-M-15-producing *K. pneumoniae* and 1 SHV-12-producing *Escherichia coli* isolate [4].

The antibacterial spectrum of ceftazidime-avibactam (CAZ-AVI) covers 95% of *P. aeruginosa* isolates and >99% of enterobacteria [5]. A 2017 study in 51 Spanish hospitals included up to 30 consecutive healthcare-associated P. aeruginosa isolates collected from each of the participating hospitals and determined the MICs of 13 potentially active antibiotics. Colistin and ceftolozane-tazobactam were active against 94.6% of isolates (MIC50/90 = 1/2 mg/L), followed by CAZ-AVI with 94.2% of sensitive isolates (MIC50/90 = 2/8 mg/L). Four isolates showed mutations in AmpC determinants of resistance to ceftolozane-tazobactam and CAZ-AVI (6). Against enterobacteria, the spectrum of ceftazidime-avibactam is the broadest of the antimicrobials available to date. In a study conducted during 2017-2018 in 70 medical centers in the United States, 3269 enterobacteria were consecutively collected from patients with pneumonia, community or nosocomial origin,

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and tested for sensitivity by broth microdilution methods. The most active agents were CAZ-AVI with susceptibility percentages of 99.9%, amikacin 98.7%, meropenem 97.4% and tigecycline 94.6%, but only CAZ-AVI and tigecycline retained good activity (≥ 90% susceptible) against carbapenem-resistant isolates (97.5% and 92.4% susceptible, respectively). The most active agents against multidrug-resistant *Enterobacteriaceae* were CAZ-AVI with 99.2% of susceptible isolates and amikacin 90.9%, whereas ceftolozane-tazobactam and meropenem were only active against 53.8% and 78.1% of these organisms, respectively. Among ESBL-producing *Enterobacteriaceae* (excluding carbapenemase-producing *Enterobacteriaceae*), the susceptibility rates to CAZ-AVI, ceftolozane-tazobactam, and meropenem were 100.0%, 84.1%, and 98.9%, respectively [7].

In *K. pneumoniae* and *Enterobacter cloacae* strains producing KPC-3 or KPC-2, exposure to CAZ-AVI can select resistant mutants by the appearance of changes in one or more amino acids of the Ω-loop of the β-lactamase. These strains usually regain susceptibility to meropenem [8-10]. The development of resistance in the course of treatment has been observed in patients with pneumonia and renal failure requiring continuous renal replacement techniques [9]. Resistance is probably the consequence of insufficient antibiotic dosage in the presence of a high bacterial load. In vitro, the association of CAZ-AVI with a carbapenem can prevent the selection of these mutants [11]. In *K. pneumoniae*, PBP3 is the main target of ceftazidime, cefepime, and aztreonam, whereas PBP2 is the main target of carbapenems. Complete blockade of both PBPs, obtained with the association of CAZ-AVI with a carbapenem, may have a synergistic effect [12].

Among the non-fermenting gram-negative bacilli, *Burkholderia cepacia* complex, B. gladioli and about 50% of *Achromobacter* strains are susceptible to CAZ-AVI. The susceptibility of *Acinetobacter* spp to ceftazidime is not modified by the presence of avibactam, probably due to its low diffusion through the bacterial wall. *Stenotrophomonas maltophilia, Elizabethkingia meningoseptica* and Aeromonas spp. produce a chromosomal metallo-β-lactamase not inhibitable with avibactam. However, up to 30% of *S. maltophilia* isolates are susceptible to CAZ-AVI. Avibactam restores aztreonam activity against *S. maltophilia* and other GNBs when they, in addition to a metallo-β-lactamase produce an ESBL.

Intestinal colonization by KPC-producing *K. pneumoniae* (Kp-KPC) is an important risk factor for developing systemic infection by the same strain. Different orally administered non-absorbable antibiotics have been used to decolonize or reduce the bacterial load of the intestinal microbiota. On average, these regimens succeed in decolonizing 60% of patients, but after discontinuation of treatment, within a few days/weeks, patients recolonize. In a retrospective, observational, multicenter, retrospective study, we compared the rate of intestinal decolonization of Kp-KPC under treatment with CAZ-AVI alone or associated with other antibiotics (Group A) versus treatment regimens based on other antimicrobial regimens (Group B) in patients with Kp-KPC infection. Eleven of the 12 patients in Group A (91.7%), achieved intestinal decolonization, compared to none of the 24 patients in Group B. Group A patients remained decolonized for a mean follow-up of 39.5 days [13,14]. If these results are confirmed, the possibility of decolonization is a serious argument for considering CAZ-AVI as first-choice treatment in infection by ESBL or carbapenemase-producing enterobacteria.

Clinical experience with the use of CAZ-AVI has been reported in several studies. The results of interest from some of the most relevant studies are briefly discussed below. In an observational study conducted in two ICUs, the clinical course of 102 patients with Kp-KPC bacteremia of intra-abdominal (23.5%), urinary tract (20.6%) and skin and soft tissue (17.6%) origin was analyzed. Patients treated with CAZ-AVI-containing regimens had a lower risk of 30-day mortality or nephrotoxicity (HR 0.231 [95% CI 0.071-0.745], p = 0.014) compared with those receiving colistin-containing regimens.

Another retrospective, observational study analyzed a cohort of 577 adults with KPC-Kp infection, of whom 391 cases developed bacteremia. All were treated with CAZ-AVI, either as monotherapy (n=165) or in association with other active antibiotics (n=412). All-cause mortality 30 days after the onset of infection was 25% (146/577). There was no statistically significant difference in mortality between patients treated with CAZ-AVI monotherapy and those treated with combination regimens (26.1% vs. 25.0%, p=0.79). In multivariate analysis, 30-day mortality was positively associated with the presence of septic shock (P=0.002), neutropenia (P<0.001), with an INCREMENT score >8 (P=0.01), with pneumonia (P=0.04), and with dose adjustment of CAZ-AVI for renal function (P=0.01). Mortality was negatively associated with CAZ-AVI administration by prolonged infusion (P=0.006) [15].

In two intensive care units in Greece, the clinical course of critically ill and mechanically ventilated patients with carbapenem-resistant *Enterobacteriaceae* infection was studied. Forty-one patients were treated with CAZ-AVI and 36 with the best available appropriate antibiotic therapy (other than CAZ-AVI). Significant improvement in SOFA scale score was observed at days 4 and 10 in the CAZ-AVI group compared to the control group (P 0.006 and P 0.003, respectively). Microbiological eradication was achieved in 33/35 (94.3%) patients in the CAZ-AVI group and in 21/31 (67.7%) patients in the control group (P 0.021), and clinical cure was observed in 33/41 (80.5%) vs. 19/36 (52.8%) patients (P 0.010), respectively. The results were similar in patients with bacteremia. Survival at 28 days was 85.4% in the CAZ-AVI group and 61.1% in the control group (log-rank test 0.035). There were 2 and 12 relapses in the CAZ-AVI and control groups, respectively (P 0.042). The CAZ-AVI-containing regimen was an independent predictor of clinical survival and cure (odds ratio [OR] 5.575 and P 0.012 and OR 5.125 and P 0.004, respectively), as was disease severity. No significant side effects were recorded [16].

The association of avibactam with aztreonam is active in vitro against class B β-lactamase-producing enterobacteria. Several studies have been published analyzing the potential clinical efficacy of this association. A prospective observational

study conducted in 3 hospitals in Italy and Greece included 102 patients with bacteremia due to metallo-β-lactamase-producing enterobacteria treated with ceftazidime-avibactam and aztreonam (CAZ-AVI + ATM) or with associations of other in vitro active antibiotics; in 82 cases the infection was caused by NDM-producing strains (79 K. pneumoniae and 3 *Escherichia coli*) and in 20 cases by VIM-producing strains (14 *K. pneumoniae*, 5 Enterobacter species, 1 *Morganella morganii*). Mortality at 30 days was 19.2% in the CAZ-AVI + ATM group vs. 44% in the other antibiotics group (p = 0.007). In a logistic regression analysis, treatment with CAZ-AVI + ATM was associated with lower 30-day mortality (P = 0.01), lower clinical failure at day 14 (P = 0.002), and shorter length of hospital stay (P = 0.007) [17].

In conclusion, the extensive and favorable experience gained with the use of ceftazidime, the antibacterial spectrum of the association of ceftazidime with avibactam (> 99% of *Enterobacteriaceae* and ≈ 95% of *P. aeruginosa* susceptible) and the potential decolonizing effect on the fecal microbiota, make CAZ-AVI one of the first options for the empirical treatment of nosocomial infection with possible involvement of gram-negative bacilli, especially if it presents with severity criteria or occurs in the "fragile" patient. The use of CAZ-AVI also reduces the consumption of carbapenems.

CONFLICTS OF INTEREST

The authors declare no conflict of interests.

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Update on antimicrobial pharmacotherapy

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Cefiderocol, a new antibiotic against multidrug-resistant Gram-negative bacteria

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ABSTRACT

Cefiderocol is a novel catechol-substituted siderophore cephalosporin that binds to the extracellular free iron, and uses the bacterial active iron transport channels to penetrate in the periplasmic space of Gram-negative bacteria (GNB). Cefiderocol overcomes many resistance mechanisms of these bacteria. Cefiderocol is approved for the treatment of complicated urinary tract infections, hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia in the case of adults with limited treatment options, based on the clinical data from the APEKS-cUTI, APEKS-NP and CREDIBLE-CR trials. In the CREDIBLE-CR trial, a higher all-cause mortality was observed in the group of patients who received cefiderocol, especially those with severe infections due to *Acinetobacter* spp. Further phase III clinical studies are necessary in order to evaluate cefiderocol´s efficacy in the treatment of serious infections.

Keywords: Cefiderocol; multidrug-resistant Gram-negative bacteria; carbapenemases; extended-spectrum beta-lactamases

INTRODUCTION

Cefiderocol is a novel catechol-substituted siderophore cephalosporin, structurally similar to cefepime and ceftazidime [1,2]. Cefiderocol binds to the extracellular free iron, and uses the bacterial active iron transport channels to penetrate the outer cell membrane and enter the periplasmic space, overcoming many of the resistance mechanisms of Gram-negative bacteria (GNB), including efflux pump up-regulation and porin channel mutations [1]. Moreover, the side-chain properties allow cefiderocol to remain highly stable against hydrolysis by various β-lactamases, including serine β-lactamases and metallo-β-lactamases [1]. In the periplasmic space, cefiderocol primarily binds to the penicillin-binding protein 3 (PBP3) and disrupts the cell wall synthesis, which results in the lysis and death of the bacteria. Cefiderocol also has affinity for PBP1a of *Pseudomonas aeruginosa* and PBP2 of *Klebsiella pneumoniae* [2]. Cefiderocol is highly active against a broad range of aerobic GNB, including carbapenem- and colistin-resistant bacteria, but has no activity against most Gram-positive bacteria and anaerobic bacteria [1]. It was approved by the Food and Drug Administration (FDA) in 2019 for the treatment of complicated urinary tract infections (cUTI), hospital-acquired bacterial pneumonia (HAP) and ventilator-associated bacterial pneumonia (VAP), and in 2020 by the European Medicines Agency (EMA) for the treatment of infections produced by aerobic GNB in adults with limited treatment options, after consultation with an infectious disease specialist.

CLINICAL EFFICACY TRIALS

Most experience with cefiderocol derives from clinical trials. The APEKS-cUTI trial (ClinicalTrials.gov, number NCT02321800) was a phase 2, multicentre, double-blind, parallel-group non-inferiority study performed at 67 hospitals in 15 countries, which enrolled patients with a clinical diagnosis of cUTI with or without pyelonephritis or those with acute uncomplicated pyelonephritis [3]. Patients were randomly assigned to receive 1 h intravenous infusions of cefiderocol (2 g) or imipenem-cilastatin (1 g each) every 8 h, for 7-14 days. Patients with an infection cause by a carbapenem-resistant bacterium were excluded. The primary endpoint was the composite of clinical and microbiological outcomes 7 days after the end of treatment. Between 2015 and 2016, 452 patients were randomly assigned to cefiderocol (303) or imipenem-cilastatin (149), of whom 448 patients (300 in the cefiderocol group and 148 in the imipenem-cilastatin group) received treatment.

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The most prevalent GNB isolated were *Escherichia coli* and *K. pneumoniae*. The primary efficacy endpoint was achieved by 73% of patients in the cefiderocol group and 55% of patients in the imipenem-cilastatin group, with an adjusted treatment difference of 18.58% (95% CI 8.23-28.92; p=0.0004), in favor of cefiderocol. No unexpected safety concerns were identified. The authors concluded that cefiderocol 2 g three times daily was non-inferior when compared to imipenem-cilastatin 1 g three times daily for the treatment of cUTI.

The randomized, double-blind, parallel-group, phase 3, non-inferiority trial APEKS-NP (ClinicalTrials.gov, NCT03032380), performed between 2017 and 2019 in 76 centers of 17 different countries, enrolled patients diagnosed with HAP, VAP, or health-care-associated Gram-negative pneumonia. The study randomly assigned 148 patients to receive a 3-h intravenous infusion of cefiderocol 2 g every 8 h and 152 participants to receive a 3-h intravenous infusion of meropenem 2 g every 8 h [4]. Treatment was usually prescribed for 7 to 14 days, but could be extended to 21 days based on the investigator's decision. Patients also received intravenous linezolid (600 mg every 12 h) for at least 5 days to ensure coverage of Gram-positive bacteria and of methicillin-resistant *Staphylococcus aureus*. The primary endpoint was all-cause mortality at day 14. *Klebsiella pneumoniae* (32%), *Pseudomonas aeruginosa* (16%), *Acinetobacter baumannii* (16%), and *Escherichia coli* (14%) were the most common GNB isolated, with extended-spectrum beta-lactamases (ESBL) frequently found in Enterobacteriaceae, and carbapenemases most common in *A. baumannii*. Out of 175 of patients, 60% were on mechanical ventilation, and 199 (68%) were admitted to the intensive care unit at randomization. All-cause mortality at day 14 was 12,4% with cefiderocol (18 out of 145 patients) and 11,6% with meropenem (17 out of 146 patients; adjusted treatment difference 0,8%, 95% CI 6,6-8,2; p=0,002 for non-inferiority hypothesis). The overall occurrences of treatment-emergent adverse events (TEAEs), drug-related TEAEs, serious adverse events, and TEAEs leading to study drug discontinuation were similar between treatment groups. The authors concluded that cefiderocol was non-inferior to high-dose, extended-infusion meropenem in patients with GNB nosocomial pneumonia (NP), and considered cefiderocol as a potential option for the treatment of patients with NP, including those caused by multidrug-resistant (MDR) GNB [4].

The CREDIBLE-CR study (ClinicalTrials.gov, NCT02714595) was a randomized, open-label, multicenter, parallel-group, pathogen-focused, descriptive, phase 3 study performed in 95 hospitals in 16 countries between 2016 and 2019 [5]. Patients diagnosed with NP, bloodstream infections (BSI) or sepsis, and cUTI, due to a carbapenem-resistant GNB were included. A total number of 101 patients were randomly assigned to receive a 3-h intravenous infusion of cefiderocol 2 g every 8 h (15% received one adjunctive antibiotic) while 49 were randomly assigned to receive the best available therapy, which was pre-specified by the investigator before randomization (61% received a combination therapy). Overall, 66% of patients who received cefiderocol reached clinical cure, compared to 58% of patients treated with a combination of other antibiotics, while 48% treated with cefiderocol reached microbiological eradication compared to 26% in the comparator group [5]. Notwithstanding, the study raised some concerns as a higher proportion of patients treated with cefiderocol died by the end of the study (34% vs 18%, respectively). Most patients had received cefiderocol for a carbapenem-resistant *A. baumannii* infection (as a single bacteria or in combination with *P. aeruginosa* or *Stenotrophomonas maltophilia*). These results leaded the FDA to point out a potential reduction of cefiderocol´s efficacy in patients with HAP, VAP and BSI, especially due a carbapenem-resistant *A. baumannii* [6].

The clinical trial GAME CHANGER (ClinicalTrials.gov, NCT03869437) is currently in progress. The study´s primary outcome is to compare the 14-day mortality of a 2 g regimen of cefiderocol administered intravenously over 3 hours every 8 hours for a period of 7 to 14 days versus an antibiotic standard therapy for healthcare-associated and hospital acquired GNB BSI. The study is estimated to be completed in February 2022.

CONCLUSION

Cefiderocol is a novel cephalosporin with a promising activity against MDR GNB, including carbapenem-resistant GNB. It would be especially useful for the treatment of GNB with limited therapeutic options as those producing metallo-β-lactamases. Further evaluation in phase III clinical studies are necessary in order to evaluated its efficacy in the treatment of serious infections, especially those produced by carbapenem-resistant *P. aeruginosa*, *A. baumannii*, and *S. maltophilia*.

CONFLICTS OF INTEREST

FLM has received fees from Shionogi for his participation as a medical advisor.

JTS presents no conflict of interest.

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Update on the management of SARS-CoV-2 infection

Rafael Delgado

Comprehensive serological strategy for the diagnosis and monitoring of SARS-CoV-2. From infection to vaccine control

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ABSTRACT

SARS-CoV-2 serology is useful to identify past COVID-19 cases, and it is not useful for acute infection. Levels of specific SARS-CoV-2 anti-N and especially anti-S are expected to be maintained for long periods. At this moment there is not a clear correlate of protection after COVID-19 or vaccination, therefore serological follow up is not indicated in most cases.

Keywords: SARS-CoV-2, COVID-19, Serology, Vaccines

After SARS-CoV-2 infection, most individuals develop a specific antibody response that it is detectable from the first week post-symptoms, being 13 days the median time for IgM or IgG seroconversion [1]. Titres of IgG against SARS-CoV-2 spike (S) protein, correlate with neutralizing activity indicating a potential use of S protein as a vaccine immunogen [2]. Initially some reports found a declining trend of antibodies and neutralizing response during early convalescent period [3-5] although subsequent studies, and our own experience, have shown sustained humoral response, long-term B-cell memory and evidence of affinity maturation beyond the viral replicative phase in the respiratory tract [6-8].

Nevertheless, there is a great heterogenicity in the serological response of different individuals after natural infection by SARS-CoV-2. Early on in the pandemic numerous reports disclosed a rapid decay of the antibody levels [4,5]. Several factors could have been contributed to the initial impression that the immune response to SARS-CoV-2 was a very transient one, including short periods of follow up and technical limitations of diagnostic tests specially those based in detecting anti-nucleoprotein (N) antibodies [3]. More prolonged follow up shows a sustained response in most of the individuals. Anti-S IgM is in many cases transiently expressed during the first months but still can be detectable in over 32% of infected individual by month 7 after recovery, further questioning the diagnostic value of IgM as an acute infection marker of COVID-19 [9]. It has been recently shown that affinity maturation occurs far beyond the replicative phase of SARS-CoV-2 in the airway epithelium, a process that increases the affinity of antibodies and remarkably the breath against variants [8]. In this respect it has been shown the presence of SARS-CoV-2 particles in the gut mucosa, a highly enriched ACE2 cellular milieu. Whether this gut mucosal infection can be the source of antigen presentation and affinity maturation occurs in regional follicular germinal centres and remains to be confirmed [6,8].

Overall, in cohorts of representative COVID-19 cases, a sustained humoral response is present in most of the convalescent individuals up to 12 mpi and data from the analysis of B-memory cells indicate that a considerable number of cells able to activate and produce anti-SARS-CoV-2 antibodies are long term maintained [6].

The immune correlates of protection upon SARS-CoV-2 infection or vaccination are so far unknown, however the levels and the stability of the anti-S specific antibodies and neutralizing response observed, together with the presumptive innate and cellular response capabilities developed, indicate that probably convalescent individuals are protected from systemic disease for long periods. In most of the studies it has been analysed the presence of antibodies in serum and the correspondence with those in respiratory mucosa, that can be more related to susceptibility for infection and transmission, is not clear. This is an issue of the highest relevance that warrants further research. Finally, this sustained immune response needs to be tested against the new SARS-CoV-2 variants that have been described precisely in areas with high attack rates and appear to be scape mutants under selective immunological pressure [10-13].

Vaccination is now in rapid deployment mainly in de-

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Table 1 **SARS-CoV-2 Serologic markers after COVID-19, vaccination, or both.**

	Anti-N	Anti-S/RBD
COVID-19 convalescence	+/++	+/++
Vaccination (Spike: Pfizer/BNT, Moderna, AZ, Janssen)	-	++
COVID-19 and vaccination	+/++	+++

N: SARS-CoV-2 Nucleoprotein. S: SARS-CoV-2 Spike protein. RBD: SARS-CoV-2 Receptor Binding Domain of S protein.

veloped countries and this fact introduces a new complexity in serology interpretation. Main marker used in commercial test and results are described in Table 1. Natural infection by SARS-CoV-2 induces heterogeneous but maintained levels of antibodies against all viral components. So at this moment detection of anti-Nucleoprotein (N) is a hallmark of previous COVID-19 whereas after vaccination only anti-Spike (S) is detected. In convalescent individuals after vaccination there is a remarkable boost of production of anti-S antibodies and in this cases anti-N combines typically with very high levels of anti-S.

CONFLICTS OF INTEREST

The author declares no conflict of interests.

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Infection and infectivity: Utility of rapid antigen tests for the diagnosis of COVID-19

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ABSTRACT

Detection of SARS-CoV-2 proteins is commercially available in the form of lateral-flow rapid antigen test for the point-of-care diagnosis of COVID-19. This platform has been validated for symptomatic and asymptomatic individuals, for diagnosis or screening, and as part of single or sequential diagnostic strategies. Although in general less sensitive than amplification techniques, antigen tests may be particularly valid during the first days of symptoms and to detect individuals with greater viral load, thereby with enhanced chances of viral transmission. The simplicity of antigen tests make them very suitable to discard infection in settings with low pretest probability, and to detect infection in case of higher chances of having COVID-19.

Keywords: SARS-CoV-2; COVID-19; rapid antigen tests; PCR; variants; infectivity

The transmission of SARS-CoV2 occurs mainly in the presymptomatic period and for just around 72 hours after the onset of symptoms (Figure 1). Asymptomatic infected also transmit the infection, although to a lesser extent. Other factors that have been related to greater transmission are contact with infected persons in closed environments or risks from professional exposure.

Tests that detect SARS-CoV-2 antigen (AgT) can be performed quickly and at the same point of care, and therefore can be more accessible with a faster time to result than techniques based on amplification (PCR, TMA). In contrast, AgT are less sensitive than amplification techniques (AmT). From a clinical point of view, the positivity of the AgT indicates infection with high specificity, and additionally it is related to high amounts of virus in respiratory secretions, thus indicating a greater risk of contagion. It is known that AmT remain positive for a long time, so a positive result in these techniques is not always associated with a risk of contagion (Figure 1).

It is accepted that AgT are particularly useful in the following scenarios:

- **Early stages of infection**: Although they cannot detect viruses at levels as low as AmT, AgT can be useful for people who are in the early stages of infection, when virus replication is at its highest. The WHO notes that, in settings where AmT are not available or where AmT result times are too long, AgT

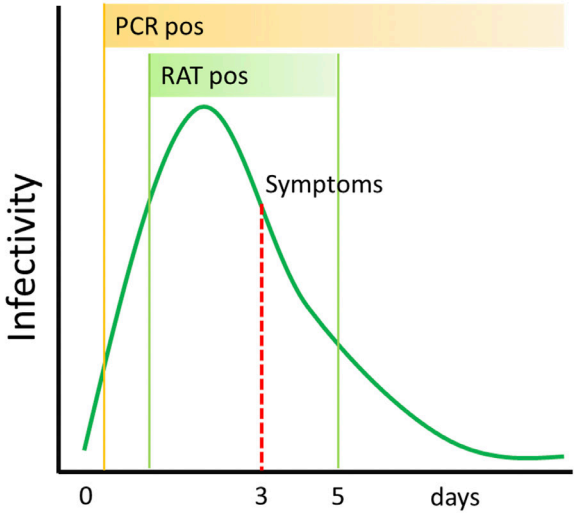

Figure 1 | **Dynamics of SARS-CoV-2 transmission.**

PCR, polymerase chain reaction; RAT, rapid antigen test

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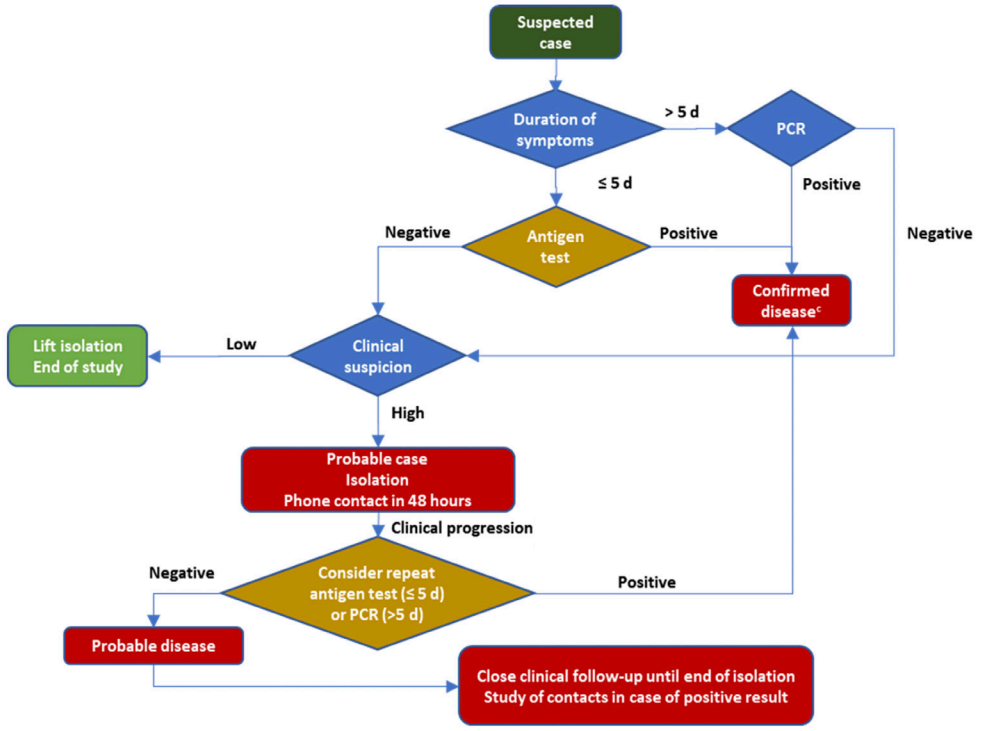

Figure 2 | **Indication of antigen test in symptomatic individuals.**

with minimal sensitivity and specificity of ≥80% and ≥97%, respectively, can be used for diagnosis SARS-CoV-2; in such cases, the test should be done within the first 5 to 7 days of the onset of symptoms [1]. Used for clinical diagnosis in symptomatic patients, positive AgT indicate SARS-CoV-2 infection with high reliability (Figure 2) [2]. However, the negative result may be false as a consequence of the lower sensitivity of the AgT, so if the clinical suspicion is high, an AmT should be performed within the following 48 hours and the patient should be isolated during that time.

According to a study [3], between days 0 to 10 of the acute phase of infection, only a third of patients present elevated levels of RNA in respiratory secretions, which would result in a positive AgT; the other two-thirds have a low viral load so that the AgT most likely will be negative. After 10 days of infection, 90% of patients have such a low RNA level that the AgT will also be negative; finally, there are 10% of patients who, after 10 days of infection may have high levels of RNA and still positive AgT.

-Closed environments: AgT can also be useful in the study of outbreaks, particularly in high-risk closed environments (nursing homes, schools, prisons, etc.); if the frequency of testing is high enough, even despite the lower sensitivity of AgT, it has been shown that AgT can be used successfully to reduce cumulative infection rates [4]. If used for serial testing in this setting, negative AgT does not need to be confirmed by AmT and positive results are diagnostic.

If AgT are used in situations in which the probability of infection is lower (screening of asymptomatic patients or low cumulative incidence in the community) the negative result is reliable because in these settings the negative predictive value of the test increases. However, any circumstance that increases the likelihood of pre-test infection, such as close family contact or high infection rates in the community, should lead to confirmation with AmT any negative AgT.

According to a recent systematic review, the sensitivity of AgT is higher in cases with symptoms compared to asymptomatic contacts (72% versus 58%) and is higher during the first week compared to the second week after the onset of symptoms (78% versus 51%). The sensitivity is also higher in samples with a higher estimated level of viral RNA (95% with Ct <25 versus 41% with Ct> 25). The specificity in general is high (greater than 99%) [5]. In another study, the sensitivity and specificity of AgT, taking AmT as a reference, was 41% and 98%, respectively, in asymptomatic individuals, and 80% and 99% among symptomatic individuals [6]. The mean Ct value for samples that had a negative AgT but a positive AmT result was higher than that of the samples that were consistently positive (Ct of 32 versus 24), confirming that the sensitivity of AgT is higher for high levels of virus in respiratory secretions. There are no studies in this regard, but it is foreseeable that AgT will be more cost-effective for the detection of variants

that are associated with higher levels of RNA [7,8]; in contrast, AgT will probably be less useful for the diagnosis of reinfections or infection in vaccinated patients, situations in which viral RNA levels are expected to be lower.

In another study it has been shown that a positive AgT indicates the presence of viable virus in the sample more reliably than a positive AmT; among 38 samples positive for AmT, AgT was positive in 27/28 samples with positive culture, but only in 2/10 samples with negative culture [9]). Viable virus can also be isolated from a AgT negative samples; in another study, 9% of clinical samples positive for AmT were negative for AgT [10]. Negative AgT are not entirely reliable in indicating that a person with a proven infection is not infectious.

Most AgT target the nucleocapsid protein for the detection of SARS-CoV-2. Mutations found in viral variants are mostly located in the spike protein, but there are several changes in the nucleocapsid protein that could affect the performance of AgT. In several studies, the variant B.1.1.7 (British) was equally well detected by all AgT tested (namely Abbott Panbio, Fortress, Innova, Roche, and Surescreen). No such studies are available for the variant B.1.351 (South African) or B.1.617.2 (Indian) [11].

In summary, AgT are especially useful in these situations:

- To detect infection in patients with active symptoms.
- In patients with high viral load, such as variant-infected patients; probably sensitivity will be lower in the case of reinfections or in the infection of the vaccinated patient.
- In the study of outbreaks, particularly if the test is repeated every 3-5 days.
- To rule out infection if there is a low pretest probability.
- The performance of AgT needs to be monitored as new viral variants emerge.

CONFLICTS OF INTEREST

The authors declare no conflict of interests.

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Role of molecular diagnostics in the clinical management of SARS-CoV-2 infection: advantages and drawbacks

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ABSTRACT

The diagnosis of SARS-CoV-2 is based on the use of nucleic acid amplification tests (NAAT), especially rRT-PCR. The latter also allows us to quickly identify variants of concern. However, its use in follow-up of patients and the correlation between Ct value and the viability of the virus is controversial.

Keywords: COVID-19, SARS-CoV-2, NAAT, cell culture

As with other respiratory viruses, the main test for diagnosing COVID-19 is detection of SARS-CoV-2 in respiratory samples using nucleic acid amplification tests (NAATs) [1], mostly real-time reverse transcription polymerase chain reaction (rRT-PCR) [1,2]. Molecular detection is a highly sensitive diagnostic method; however, the results can remain positive for long periods, even when the patient has clinically recovered and the virus has lost its infectivity. The sustained rRT-PCR positivity of COVID-19 has complicated the discharge of patients, the transfer of patients between various hospital areas, and the reincorporation of health care workers (HCWs) to their jobs. The use of rRT-PCR as a follow-up tool for SARS-CoV-2 infection has led to hypotheses regarding infectivity duration, and even the possibility of reactivation [3].

The performance of rRT-PCR depends on several factors, such as the specimen type, the timing of collection, nucleic acid extraction method, the design of primers and probes and the selection of their viral RNA target, the reagents, and instrument and software used for the rRT-PCR and for the result interpretation.

Regarding specimen type, nasopharyngeal flocked swabs are considered the gold standard for respiratory virus sampling of the upper respiratory tract, while BAL fluid, endotracheal secretions or sputum should be considered when lower tract infection is suspected [4].

In the absence of diagnostic methods with reliable quantification, the cycle threshold (Ct) value obtained in the amplification has been employed as a semiquantitative measure and has been proposed as a parameter for elaborating approaches for removing patients from isolation [5]. Establishing a reliable cut-off Ct value is difficult, given the large number of available rRT-PCR-based diagnostic tests (which amplify different viral regions generally in a multiplex format), the need to use more than one molecular test in most clinical laboratories to meet growing demand, the inclusion of an automated system based on real-time transcription-mediated amplification (which does not provide Ct values), and the use of different types of samples during patient follow-up.

INTER-ASSAY AND INTRA-ASSAY VARIABILITY

We have analyzed the qualitative results obtained in four NAAT assays, three of them based on rRT-PCR, testing 200 respiratory samples obtained during follow-up of patients. We consider a result as true positive when this result was obtained in at least two assays (n = 198 samples).

Table 1 shows the NAATs compared, the regions that each one amplifies, the number of samples with a positive result for each assay, and the agreement obtained with the reference value.

When we compare Ct values obtained on assays that amplify E gene (Panther Fusion LDT, COBAS 6800 and Allplex™) in a subset of 100 samples with Ct values between 30 and 35, according to the results obtained on Panther-Fusion LDT assay, we observe statistically significant differences between median Ct values obtained (Table 2).

Since most commercial rRT-PCRs are multiplex assays, we have analyzed the intra-assay variability of COBAS 6800, without finding statistically significant differences in the median of

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Table 1 Comparison of qualitative results obtained in four NAAT assays for detection of SARS-CoV-2 RNA

	Assay						
	Panther-Fusion LDT	Panther TMA	COBAS 6800		AllplexTM		
Target gene(s)	E	Orf1ab	E	orf1ab	E	rdRP/S	N
Number of positive samples	200	178	180	173	179	165	172
Agreement (%)	99	89	90	87	90	83	86

Table 2 Median (IQR) Ct values and statistical significance obtained amplifying SARS-CoV-2

	Assay			
	Panther Fusion LDT	COBAS 6800	AllplexTM	P value
Median (IQR)	32.0 (31.0-33.0)	30.3 (29.1-32.3)	29.2 (26.8-30.8)	P < 0.001

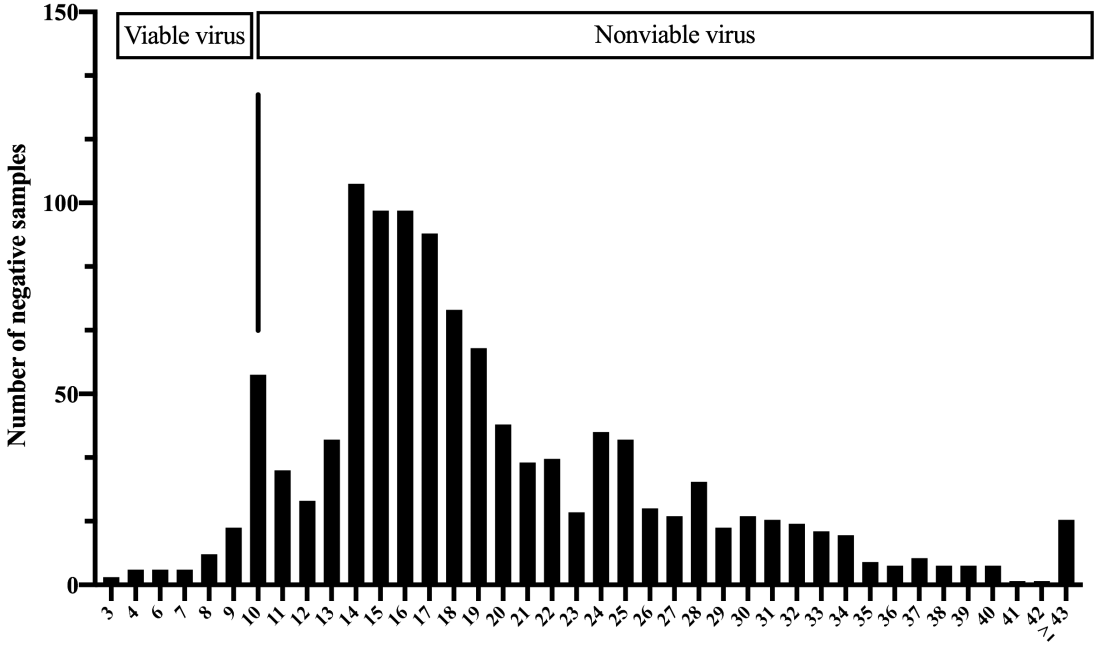

Figure 1 Time (days) for rRT-PCR to becomes negative after the onset of symptoms in a HCW cohort during the first pandemic wave.

Ct values obtained for the different amplified regions in this case (E gene median Ct value : 29.3 [IQR: 26.6-33.4], orf1ab gene median Ct value: 27.0 [IQR: 25.5-30.5], *p*: 0.15).

ASSESSMENT OF VIRAL VIABILITY

Previous studies [6,7] have shown prolonged viral shedding in patients with severe COVID-19 and its relation to high viral loads. Although we observed a significant positive correlation between low Ct values and the presence of viable virus, this viral load estimate appears insufficient for discriminating samples harboring infective virus. We showed that, in immunocompetent patients with severe forms of COVID-19, viral replication can be detected even with moderate or low viral loads during prolonged periods [8].

Figure 1 shows the discordance between timing for rRT-PCR to become negative and timing for viable virus clearance in nasopharyngeal samples. This is a cohort of health work-

ers (HCW) with mild COVID-19, infected in the first pandemic wave, who were prospectively followed until the rRT-PCR was negative. The virus remains viable for up to 10 days after the onset of symptoms.

SARS-COV-2 VARIANTS SURVEILLANCE

A fast and extensive strategy for detecting SARS-CoV-2 variants of concern (VOC) and variants of interest (VOI) is achieved by testing of all RT-PCR SARS-CoV-2 positive samples with subsequent variant RT-PCR [9]. This approach can have a positive impact on adequate and timely contact tracing, and could facilitate targeted public health measures. In comparison to whole genome sequencing (WGS) this PCR-based screening method is easy to implement in molecular diagnostic laboratories.

CONFLICTS OF INTEREST

The authors declare no conflict of interests.

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Update on the management of SARS-CoV-2 infection

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Ventilatory support and corticosteroid therapy in SARS-CoV-2

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ABSTRACT

It has been almost two years since COVID-19, a disease caused by a new coronavirus called SARS-CoV-2, was declared a pandemic by the World Health Organization. The entire scientific and medical community was put to the test during the following months to find the best therapeutic strategy to save lives. Although some antivirals and anti-inflammatory drugs are being tested in different clinical trials with some controversial results, this short review will focus on corticosteroids usefulness and ventilatory support principles, as they have become two essential therapeutic pillars for those patients who need hospital admission due to respiratory failure.

Key words: SARS-CoV-2, COVID-19, ARDS, corticosteroids, steroids, ventilatory support, high flow nasal cannulas, non-invasive mechanical ventilation, mechanical ventilation

INTRODUCTION

The respiratory system is the most frequently organ affected by SARS-CoV-2. A mean time from the onset of respiratory symptoms to the onset of pneumonia is estimated to be about 5 days and 7 to 12 days from the development of hypoxemia to admission to the Intensive Care Unit (ICU) because of severe respiratory failure.

Despite COVID-19 is not an autoimmune disease, the lack of effective antivirals and the potential lung damage caused by the inflammatory response to infection has justified investigating the usefulness of steroids in several randomised controlled trials (RCTs) with good results [1], becoming the standard of care for this disease when patients need supplementary oxygen. Nevertheless, not all patients respond to steroids and some concerns regarding a potential viral load clearance delay produced by steroids have emerged. On the other hand, correcting hypoxemia and protecting the lungs as much as it is possible, is mandatory even when the patient might not refer dyspnoea. However, it is still not clear whether or not an early intubation might modify the disease clinical course, hence we will review these two complementary therapeutic strategies.

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CORTICOSTEROID THERAPY

Steroids are agonist compounds that bind to the glucocorticoid receptor (GCR), producing a pharmacological response. Its clinical efficacy depends on dosage, timing of initiation, mode of administration, duration, and dose de-escalation. These extensively used drugs have complex actions not always well understood.

The named "genomic" effects of steroids depend on how much the GCR is saturated, while the "nongenomic" effects, normally achieved with higher doses, are independent of its specific receptor, producing interaction with cellular membranes or other cytosolic proteins. The most desirable anti-inflammatory and immunosuppressive effects of steroids are achieved with genomic doses and are induced by the mechanism named transrepression, by which, synthesis of proinflammatory mediators, such as cytokines and prostaglandins is suppressed through downregulation of nuclear factor Kappa-B (NF-kB).

"Low-dose" (prednisone-equivalent doses lower than 7.5 mg/day) produces GCR saturation less than 40-50% with mild adverse effects. Prednisone-equivalent doses of 7.5–30 mg/day ("medium doses") lead to more than 50% receptor saturation, while "high-dose" refers to prednisone-equivalent doses of 30–100 mg/day (dexamethasone -DXM- 20 mg/day) and result in almost complete saturation of cytosolic GCRs. These doses are not recommended for long-term therapy because of the potential serious adverse effects.

Finally, "very high-dose" of steroids (prednisone-equiva-

lent of > 100 mg/day) and "pulse" therapy (prednisone-equivalent of ≥ 250 mg/day, usually given for 1–5 days) saturate all GCRs. These doses induce the full range of genomic effects and have additional effects on pharmacodynamics (receptor off- loading and re-occupancy) and receptor synthesis and expression. Even more, receptor saturation is thought to increase the therapeutic benefit via nongenomic effects. Such very high doses are used clinically in the initial treatment of acute or life-threatening exacerbations of rheumatic diseases, but they have not been proved to be useful on the acute respiratory distress syndrome (ARDS). Very high doses of steroids cannot be used as long-term therapy due to their serious adverse effects [2].

The strong involvement of the inflammatory response to infection on the physiopathology of ARDS [3] has led, for many years, to explore the usefulness of steroids, mainly "high doses", in different RCTs with some controversial results in this setting, but with a globally favourable balance towards steroids in most of the studies performed before the COVID-19 pandemic [4].

The last of these studies, published during the first months of the pandemic, was a Spanish study [5]. Two hundred seventy-seven critically ill patients in 17 ICU with established moderate-to-severe ARDS were randomly assigned to receive treatment with DXM (139 patients) or placebo (139 patients as control group). Treatment group received an intravenous (IV) dose of 20 mg once daily of DXM from day 1 to day 5, which was reduced to 10 mg once daily from day 6 to day 10. Although the study was stopped by the data safety monitoring board due to low enrolment rate after including more than 88% of the planned sample size, the primary outcome, defined as the number of days alive and free from mechanical ventilation (MV) from day of randomisation to day 28, was higher in the DXM group than in the control group (between-group difference 4,8 days [95% CI: 2,57-7,03]; p<0,0001). Secondary outcome, defined as all-cause mortality 60 days after randomisation, was also better in the treatment group while the proportion of adverse events did not differ significantly between both groups.

These good results provoked that, throughout the first wave of the pandemic, many critically ill patients were treated following this DXM regimen, although with some concerns about whether or not steroids might cause a possible delay in virus clearance as it had been published regarding MERS, few years earlier [6].

In fact, the role of viral load in plasma or viral RNAemia has proved to be related with the dysregulated immune response to SARS-CoV-2 in a study of 250 COVID-19 patients with different disease severity (50 outpatients, 100 hospitalized ward patients and 100 critically ill) [7]. The rate of viral RNAemia was higher in the critically ill group (78%) compared to ward patients (27%) and outpatients (2%) ($p < 0.001$). Most severe patients had higher viral RNA loads in plasma than non-critically ill patients, with non-survivors showing the highest values. Viral RNAemia did not show significant associations, in the multivariate analysis, between outpatients and ward patients. On the contrary, both viral RNAemia and plasma viral RNA load were associated with critical illness when in-ward patients were compared to ICU patients. Plasma viral RNA load was also correlated with higher levels of chemokines, biomarkers indicative of systemic inflammatory response (IL-6, CRP, ferritin), activation of NK cells (IL-15), endothelial dysfunction, coagulation activation (D-Dimer and INR), tissue damage (LDH, GPT), neutrophil response and immunodepression (PD-L1, IL-10, lymphopenia and monocytopenia), suggesting a major role of uncontrolled viral replication in the pathogenesis of COVID-19.

Besides these findings, as the pandemic was going on, many observational, retrospective and some small RCT showed globally better results in the group of patients treated with steroids.

In this regard, a meta-analysis [8] of 44 studies (37 retrospective observational studies, 5 RCTs, and 2 studies with historical controls) from the first wave of the pandemic, with a varied population of 20,197 hospitalized patients (28/44 studies) to patients admitted to the ICU (15/44 studies), and one study including discharged patients for viral clearance assessment, showed a significant reduced mortality in the steroid group (OR 0,72 [95%CI: 0,57–0,87]) besides they found a signal of delayed viral clearance, but data in the studies were too sparse to draw any definitive conclusions. Fourteen studies reported a positive effect of steroids on need for and duration of MV. It is worth noting that a trend toward more infections and antibiotic use was present amongst patients who received steroids.

More recently, the results of the controlled open-label Recovery trial [9] comparing a range of possible treatments in patients with COVID-19, regarding those who were randomly assigned to receive a dose (oral or IV) of 6 mg once daily of DXM for up to 10 days or to receive usual care alone have been published [9]. The primary outcome was 28-day mortality. A total of 2,104 patients were allocated to DXM arm and 4,321 to usual care group. Overall, 482 patients (22,9%) in the DXM group and 1,110 patients (25,7%) in the usual care group died within 28 days after randomization (age-adjusted RR 0,83; 95% CI: 0,75-0,93; P<0,001). Very importantly, in the DXM group, the incidence of death was lower than that in the usual care group among patients receiving MV (29,3% vs. 41,4%; RR 0,64; 95% CI: 0,51-0,81) and among those who received oxygen without invasive MV (23,3% vs. 26,2%; RR 0,82; 95% CI: 0,72-0,94) but not in the group of patients that was not receiving any respiratory support at randomization (17,8% vs. 14%; RR 1,19; 95% CI: 0,92-1,55) warning about the importance of customizing the treatment to the clinical status. Even more, although these results seem to be highly favorable to steroids, important limitations have been highlighted [10], for instance, there was no stratification between centers, body mass index and ethnicity were not reported, location of patient at randomization (ward/ICU) is unknown, there was age imbalance in the study population, the distribution of the various factors associated with outcome were not specified for the

different subgroups and for patients receiving MV important details as positive end expiratory pressure (PEEP), FiO_2, PaO_2/FiO_2 were not collected. Another limitation is that good results at the short day-28 mortality endpoint might not translate into longer-term benefit, particularly in COVID-19 patients where those who need MV often require prolonged ICU and hospital stays.

After publishing the beneficial results of DXM in the Recovery trial, particularly for most severe patients, a prospective meta-analysis of 1,703 critically ill patients who had been randomized to receive systemic DXM, hydrocortisone, or methylprednisolone (678 patients) or to be given usual care/placebo (1,025 patients) was performed [1]. Although this meta-analysis includes 7 RCT, only the Recovery trial(9) had been completed when it was published. The rest of the studies had only randomized between 2.9% to 73.14% of the planned sample. Considering this limitation, 222 subjects died among the 678 (32,74%) patients allocated to steroids and there were 425 deaths among 1,025 (41,46%) patients randomized to usual care/placebo (summary OR, 0,66 [95% CI: 0,53-0,82]; p<0,001 based on a fixed-effect meta-analysis). There was little inconsistency between the trial results, so the authors conclude that the administration of systemic steroids to critically ill patients, compared with control group, was associated with lower 28-day all-cause mortality.

Finally, a recent meta-analysis that compared corticosteroids to placebo or usual care in adult patients with COVID-19 ARDS or not COVID-19 ARDS, deserves mention because it only included those RCT of patients on MV [11]. It contains 6 RCT (833 patients) from previously published meta-analyses and 12 additional RCT for a total of 18 RCT (2826 patients) that met eligibility criteria. The authors concluded that the use of steroids probably reduces mortality in patients with ARDS of any cause (2,740 patients in 16 trials, RR 0,82; 95%CI: 0,72–0,95, ARR 8%; 95%CI: 2,2-12,5%), with moderate certainty. Patients treated with steroids for more than 7 days had higher rates of survival compared to those who received a shorter course. This effect was consistent between corticosteroid types and dosage. It is important to highlight that almost all of the included RCT in this meta-analysis started steroids within the first week of ARDS diagnosis, when the exudative-inflammatory phase of ARDS is still active.

Although we have seen that several meta-analyses show a globally beneficial signal for steroids in this setting, clinicians should make an effort to customize the treatment. Remembering that ARDS is not a disease but a "syndrome" helps us understand that steroids will improve the outcome only when the predominantly underlying pathological changes are cortico-sensitive, as might happen in the early exudative phase of the ARDS or when the histologic pattern is characterized by an extensive intra-alveolar fibrin deposit called fibrin "balls" which is recognised as acute fibrinous and organizing pneumonia (AFOP).

At the beginning of the pandemic, some authors questioned whether or not the lung injury caused by coronavirus was a conventional ARDS. They described, based on pulmonary mechanics and function, two patterns: a phenotype L characterized by low elastance, low ventilation to perfusion ratio, low lung weight and low lung recruitability and a phenotype H defined by high elastance, high right-to-left shunt, high lung weight and high lung recruitability [12].

A recent review of the literature [13] does not support the existence of such a clear clinical dichotomy and there seems to be a continuum between both phenotypes, so worsening patients are supposed to progress from type L to type H. A post-mortem study of pulmonary biopsies [14] found, in a patient who died five days after the beginning of fever, a lymphocytic viral pneumonia pattern that could be considered as phenotype L. Nevertheless, for five other patients, who died around 20 days after complaining of symptoms with a phenotype H, the histologic pattern was AFOP, rather than hyaline membranes.

In fact, several studies of autopsies of patients who died due to COVID-19 reveal a wide range of histological lung features. While some of these findings are the landmark of the ARDS such as diffuse alveolar damage (DAD) in up to 87% of cases, there are also different types of vascular injury like large vessel thrombi in 42% of them and platelet-fibrin microthrombi, at least focally, in 84% of cases. It is worth noting that AFOP, commonly responsive to steroids, was seen up to 34% of cases, particularly in those autopsies with a longer disease duration (5–34 days) [15].

For all these reasons, it is difficult to determine the best dose and time to start steroids and for how long they should be given. In the early exudative phase, most benefited patients would be those with low viral load to prevent disease progression without facilitating viral replication, which has been proved to maintain lung injury, but those patients who develop late AFOP would also benefit from steroids. We have seen [16] significantly higher plasma levels of LDH, D-Dimers, CRP and PCT within 5 days of ending a standard regimen of steroid therapy in mechanically ventilated non-survivors of COVID-19-associated ARDS compared to survivors, suggesting a reactivation of inflammation after stopping steroid treatment in those patients with a worse prognosis, so tailoring the duration of therapy to the degree of inflammation and viral status of each patient might be of paramount importance and warrants further investigation.

Although it is very likely that the best effect of steroids will be achieved with a dose sufficient to reach close to maximal GCR saturation (methylprednisolone 80–100 mg or DXM 20 mg/day), to clarify this issue, there will be to wait to the results of the currently open-label, randomized controlled MEDEAS trial (Methylprednisolone vs. DXM in COVID-19 Pneumonia trial, ClinicalTrials.gov Identifier: NCT04636671). This study has planned to enrol 680 patients. The study drug is methylprednisolone given as an initial IV bolus of 80 mg to achieve close-to-maximal GCR saturation, followed by a continuous 8-day infusion to maintain high response levels throughout treatment, with the option of adjusting treatment duration

based on parameters of clinical severity (intubated vs not intubated) and followed by dosage gradual de-escalation to avoid inflammation rebound. The comparator is DXM as given in Recovery trial.

In summary, there seems to be a globally beneficial effect of steroids in COVID-19 respiratory failure when the patient needs at least supplementary oxygen. Although questions regarding the timing for steroids treatment, the optimal dose, duration and type of steroids remain to be clarified, a "high genomic dose" (prednisone-equivalent doses of 30–100 mg/day or DXM 20 mg/day) customized to the inflammatory status of the patient and very likely given when the viral load is decreasing, might be the best approach.

VENTILATORY SUPPORT PRINCIPLES

Although hypoxemia produced by COVID-19 can be well tolerated giving a false sense of safety, severe respiratory failure treatment due to SARS-CoV-2 must follow the general principles of the ARDS and correcting hypoxemia is mandatory.

Nevertheless, besides the high number of patients attended to the ICU, it is still not clear the best time to intubate the patients because some of them can be maintained with non-invasive oxygenation devices, particularly with high flow nasal cannulas (HFNC), avoiding the potential damage caused by invasive MV, known as ventilator-induced lung injury (VILI). The mechanisms of VILI are due to barotrauma (high pressure inside the airway), volutrauma (high tidal volume -TV- that produces high transpulmonary pressure and alveolar overdistention), atelectrauma (injury caused by cyclical opening and closing of unstable alveoli) and biotrauma (lung injury due to inflammatory mediators). The pulmonary distention pressure or "driving pressure = ΔP" is the most common and important modifiable determinant of this VILI, so it is recommended to keep it below 15 cmH_2O. Driving pressure is determined by the difference between the inspiratory (plateau) pressure in the airway (Pp) and the positive end expiratory pressure (PEEP): $\Delta P = Pp - PEEP$. Considering this concept, the main objective of MV, whatever mode chosen, will be protecting the lung while ensuring oxygenation. It is important to remember that transpulmonary pressure (P_L) is the result of subtracting the value of the pleural (esophageal) pressure (Ppl) from the airway inspiratory pressure (Pp): $P_L = Pp - Ppl$, so not only the driving pressure should be kept low but also the P_L should be as low as possible because the greater the stretching forces acting on the lung, the greater the lung injury.

Starting with oxygen through HFNC when oxygenation cannot be assured with the conventional devices (ventimask or ventimask-reservoir) is preferred over non-invasive mechanical ventilation (NIMV) because HFNC will not significantly increase the P_L. On the contrary, when the patient is put on NIMV the most commonly mode is Pressure Support (PS). The clinician programs an inspiratory pressure level that supports spontaneous breathing, while the patient regulates the respiratory rate. The resulting TV will depend on the mechanical characteristics of the respiratory system, the programmed PS level and the effort of the patient. When the respiratory effort is very intense the negative inspiratory pressure will increase P_L causing or aggravating VILI. Sometimes this concept is not well understood, so it is mistakenly assumed that P_L is equivalent to subtracting PEEP from the level of PS applied, but this difference is the driving pressure not the P_L. It is worth noting that as the Ppl is always negative with the spontaneous inspiratory effort, P_L might be much greater when applying positive inspiratory pressure with NIMV in case that inspiratory effort does not decrease.

This is the reason why the increase in P_L generated by the patient, might be higher with low PS levels (trying to decrease driving pressure) that produces an increase in inspiratory effort to maintain an appropriate TV to the mechanical conditions of the respiratory system. Consequently, in PS, transpulmonary pressure will depend on lung compliance rather than on the level of inspiratory pressure set, so this might be detrimental to the lung if the patient receives NIMV or invasive MV.

Taking these considerations into account, a rational approach to manage the respiratory failure might be to begin with HFNC when the ventimask is not able to achieve a minimum safety oxygenation. Monitoring patient´s oxygen saturation measured by pulseoximetry ($SatpO_2$) and his work of breathing is mandatory once he is receiving HFNC because intubation should not be delayed when the $SatpO_2$ does not improve. Although a clear parameter to indicate the intubation has not been stablished, the ROX index (IROX) might be a useful tool to facilitate the decision. It is defined as the ratio of $SatpO_2/FiO_2$ to respiratory rate. The lower the IROX in the following hours after beginning with HFNC, the higher the likelihood of needing MV. An IROX lower than 2.85, lower than 3.47, and lower than 3.85 at 2, 6, and 12 hours of HFNC initiation, respectively, have shown to be good predictors of HFNC failure [17] in respiratory insufficiency due to pneumonia. Another single-centre retrospective study in COVID-19 patients showed that an IROX > 5.37 was significantly associated with a lower risk for intubation after 4 hours of receiving HFNC [18]. Accordingly, once the patient is put on HFNC it seems reasonable observing how $SatpO_2$, work of breathing and respiratory rate change throughout the following hours. When the work of breathing increases or the IROX decreases the intubation should not be delayed because transpulmonary pressure will increase provoking SILI. In case of deciding a trial of NIMV it is important to mention that some patients will move large TV although the clinician programs a low PS. An expired TV greater than 9.5 mL/kg predicted body weight (PBW) has been strongly associated with NIMV failure [19] and delaying intubation might worsen the outcome.

Finally, regarding invasive MV, general guidelines of protective ventilation must be followed [20]. Although discussing in detail these guidelines is out of the scope of this short review, the main principles are the following: a) set TV of 6 mL/kg PBW, b) keep driving pressure below 15 cmH_2O, c) maintain high PEEP levels (>10-15 cmH_2O), d) when PaO_2/FiO_2 ratio <

150 mmHg, neuromuscular blockade for the first 48 hours is recommended and e) in most severe cases, particularly if PaO_2/FiO_2 ratio < 120 mmHg prone positioning for at least 12 hours results beneficial.

CONFLICTS OF INTEREST

The authors declare no conflict of interests.

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Update on the management of SARS-CoV-2 infection

Antiviral therapy and immunotherapy of COVID-19

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ABSTRACT

The pharmacological treatment of COVID-19 has evolved in the months since the description of the disease. Published observational studies and, above all, clinical trials have highlighted drugs that are useful as well as ruled out any benefit from other drugs used at the beginning of the pandemic. The pathogenesis of the disease has suggested that patients may benefit from the administration of both antivirals, mainly in the earliest stages, and anti-inflammatory/immunomodulatory medications in more advanced stages. We present a short review of the drugs used and under investigation for the treatment of COVID-19.

Keywords: COVID-19, SARS-CoV-2, remdesivir, tocilizumab, antivirals, immunomodulators

COVID-19 is a public health challenge, responsible for enormous morbidity and mortality in the population. The causative agent is a coronavirus (SARS-CoV-2) for which effective antiviral treatments were not known, even based on experience with patients infected with other coronaviruses that cause similar respiratory diseases (SARS-CoV, MERS-CoV). The knowledge that has been acquired about the pathogenesis of the disease has highlighted the need to administer effective antiviral treatment against the coronavirus but also drugs with anti-inflammatory / immunomodulatory activity that will alleviate the complications that appear in the second phase of the disease. Herein we briefly review the pathogenic aspects of the disease on which proposals of therapy are based, as well as the drugs that have been evaluated, including those that have been shown to be effective, those in which effectiveness has been ruled out, and those that are currently under investigation.

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COVID-19 STAGES

Soon in the course of the pandemic, Siddiqi and Mehra made a proposal for staging the course of the disease for clinical and therapeutic purposes (Figure 1) [1]. According to this proposal, COVID-19 would have an initial phase dominated by viral replication and a late phase in which the appearance of inflammatory phenomena marks the course of the disease. These two phases overlap in an intermediate phase. In the early-middle stage of COVID-19, when active replication of the virus is eventually present in all patients, the use of an antiviral to halt the propagation is justified. Moreover, the early use of these types of compounds may prevent progression to the inflammatory phase and subsequent complications, and can even reduce the risk of mortality. In the middle-late-stage, anti-inflammatory/immunomodulatory therapy has demonstrated efficacy in diminishing mortality and its use is justified. The use of both antiviral and immunomodulators seems to be warranted for a successful management of the patients

Antivirals. A significant number of antiviral drugs have been evaluated (Table 1). Currently only remdesivir has been approved as an antiviral drug for the treatment of COVID-19. Its usefulness has been confirmed in clinical trials and in observational studies [2-4] although there have been discrepancies in some studies [5]. Currently, most clinical guidelines from health organizations recommend its use for the treatment of patients with COVID-19 who require oxygen therapy to achieve oxygen saturations greater than 94% and who do not require invasive ventilation [6,7]. A 5-day course appears to be adequate with no differences when a longer, 10-day course is used.

No other drug has been shown to be effective in the treatment of COVID-19 in humans, although various drugs with *in*

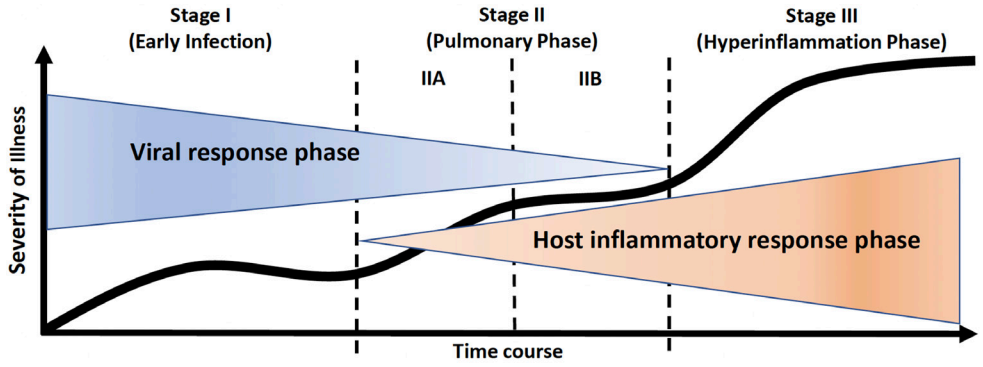

Figure 1 Evolution of COVID-19 and proposal of staging [adapted from Siddiqi et al. (1)]

vitro activity have been used (lopinavir / ritonavir, chloroquine / hydroxychloroquine, azithromycin, betaferon). Clinical studies have shown the absence of benefit from these drugs with an increased risk of toxicity in some cases [8-10]. Other drugs are under investigation. Of special interest, molnupiravir and some monoclonal antibodies (sotrovimab) have shown efficacy in the treatment of mild disease, avoiding clinical progression and the need for hospitalization [11,12].

Anti-inflammatory/immunomodulatory drugs. Anti-inflammatory/immunomodulatory treatment has also been widely explored. Low-dose glucocorticoids for a few days have been shown to reduce mortality in randomized clinical trials [13]. There is a broad consensus regarding the recommendation to use corticosteroids in patients requiring oxygen therapy, including the most serious patients who require admission to intensive care units and mechanical ventilation, given the benefits observed in mortality. Only patients who are in multi-organ failure seem not to benefit from steroid administration.

In addition to steroid treatment, various immunomodulatory drugs have been evaluated, mainly IL-6 and IL-1 inhibitors and JACK inhibitors. Results with tocilizumab, an IL-6 inhibitor, have been variable. The most recent data and meta-analysis results support its use, especially in patients showing data on inflammatory activity [14,15]. There is less data with anakinra, an IL-1 inhibitor. A recent meta-analysis of randomized clinical trials and observational studies concluded that anakinra could decrease mortality in patients with moderate-severe pneumonia and, as in the case of tocilizumab, especially in the presence of signs of hyperinflammation [16]. Less conclusive are the data with the JAK inhibitors (baricitinib, imatinib), although the studies carried out do not rule out a beneficial effect associated or not with antivirals [17,18].

Tabla 1 Key therapeutic classes for the treatment of COVID-19. Drugs under investigation

Antivirals	Immunomodulators
Remdesivir	Corticosteroids, eg, dexamethasone
(Hydroxy)chloroquine	IL-6 inhibitors (eg. tocilizumab)
Lopinavir/ritonavir	IL-1 inhibitors (eg, anakinra)
Interferon	JAK inhibitors (eg, baricitinib)
Azythromicin	Intravenous immunoglobulin
Ribavirin	
Oseltamivir	
Baloxivir	
Favipiravir	
Molnupiravir	
Umifenovir	
Nitazoxanide	
Ivermectin	
Monoclonal antibodies	
Convalescent plasma	

RECOMMENDATIONS

Despite the coincidence of clinical trials and observational studies in showing the benefits of drug treatment for COVID-19, there are wide discrepancies in all cases, including randomized clinical trials conducted with methodological rigor. These discrepancies have been transferred to the guidelines of scientific societies and health organizations, which have interpreted the results differently and have issued recommendations contradictory on occasions.

With this note of caution, we can dare to affirm that most guidelines are in favor of the administration of antiviral and anti-inflammatory/immunomodulatory drugs for the treatment of COVID-19. Recommendations include administration of remdesivir, steroids, and tocilizumab in population groups in which they have shown benefit in clinical trials.

In a rapidly changing scenario, the interest aroused by the disease has launched clinical trials with other drugs and strategies that, presumably, should improve the management of affected patients in the coming months.

CONFLICTS OF INTEREST

The authors declare no conflict of interests.

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Update on the management of SARS-CoV-2 infection

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Vaccination strategies against SARS-CoV-2: General impact on the development of the pandemic

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ABSTRACT

In this article, we will review the main vaccination strategies currently being implemented by the health authorities and analyze the main vaccines authorized by the EMA. As practical aspects of vaccination, we must make it clear that until collective immunity is reached, the preventive measures being implemented will have to be kept in place. In the words of the WHO Accelerator Project, "*There is no time to waste in the fight against COVID-19. No one is safe until everyone is safe.*"

Keywords: COVID-19 pandemic, vaccine, vaccination strategies, herd immunity, mRNA technology, adenoviral vectors.

Vaccination strategies against COVID-19 are part of the scope of responsibilities of the COVID-19 Vaccination Working Group of the Inter-Territorial Council Vaccination Communications and Registry, and up to the time of writing this article, five previous updates have been published. This is clearly a topic of great current importance but also subjected to continuous change. In fact, from February to the present date, there have been important changes, and it is possible that when this monographic issue is published, additional changes will have been made, including a possible 6th update of the vaccination strategy.

In this article and before going into the specific details of the vaccination strategy, we will briefly review concepts we have heard in the media and that have become colloquial, although they are possibly not always sufficiently clear. The first of these is collective, group or herd immunity. Underlying these three terms – collective, group or herd – is a concept and, at the present time, a need to achieve the greatest possible proportion of immunity in a given population, either by natural immunity – in which the majority of the population contracts the disease – or by artificial immunity, that is, by vaccination of the population. As is logical in the face of COVID-19, all people were initially vulnerable, and after a year of the pandemic, we have barely reached 25% collective immunity in areas with a higher cumulative incidence of the disease. Thus, it is necessary to reinforce that immunity through vaccination to reach the highest possible vaccination coverage is the only way to ensure adequate control of the pandemic. Therefore, the other relevant concept is vaccination coverage; considering that the majority of the population is susceptible to acquiring the disease, it is logical that this coverage must be close to 100%. That is, the main objective, as indicated by the Ministry of Health, is "to reduce the morbidity and mortality caused by this disease through vaccination against COVID-19, in a context of increasing availability of doses and protecting the most vulnerable groups". In Spain, from December 27 to April 1, nearly 8 million vaccine doses were administered, with already noticeable results in reducing hospitalizations and deaths [1,2].

Therefore, it is necessary to have safe and effective vaccines produced on a large scale because the majority of the population must be vaccinated, and any strategy must protect the most vulnerable. In the first case, fortunately, great effort has been made by numerous multinational organizations and public and private entities, especially pharmaceutical laboratories, that have understood the needs of the world population and the urgency of the situation. Thus, the WHO launched the Solidarity Vaccine Trial initiative [3] to contribute to obtaining safe and effective vaccines in record time and ensure that when these vaccines are available, they reach the world population. Approximately 200 vaccines have been tested, and several of them have reached phase III clinical trials and been approved by regulatory agencies for subsequent administration. Although vaccines have been developed in record time, at no time has research rigour been lost. In fact, most of these

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vaccines have been produced at the manufacturers' risk while clinical trials were still underway, and the vaccines were authorized knowing that data from completed phase III trials will only be available by the end of 2022. The need to have vaccines available and reduce morbidity and mortality due to COVID-19 led to faster approval, but all vaccines are subject to review and changes in technical specifications according to the what the results of vaccinating the population show.

As mentioned, several vaccines have been authorized, but we will comment only on some of the data from those that were approved by the European Medicines Agency (EMA).i.e., four vaccines, two that use mRNA technology and two that use adenovirus viral vectors.

The first of these is the Pfizer/BioNTech BNT162b1 and BNT162b2 or Comirnaty vaccine. Clinical trial results were published in The New England Journal of Medicine (NEJM) at the end of 2020 [4]. It is an mRNA vaccine that, once administered, encodes the S protein fragment of SARS-CoV-2; given that mRNA is very sensitive to temperature, this vaccine has to be kept between -70-80 °C, which requires special logistics, which initially was assumed to be a complication but that, at the present time, has been solved. The vaccination schedule includes two doses, 3 weeks apart. These aspects have been changed in the fact sheet, as it has been shown that the thermostability and conservation of the vaccine have improved. Other aspects to highlight are its efficacy, approximately 95% with a 95% confidence interval (CI) between 90-97%; 14 days after the first dose, individuals are up to 90% protected, but there are still no data to allow single-dose vaccination schedules. Regarding efficacy, it should also be noted that in addition to good immunogenicity, the vaccine triggers a robust cellular response mediated by CD4+ and CD8+ cells with a Th1-polarized profile, which has great value because it indicates that the vaccine generates good memory immunity. Regarding reactogenicity, the vaccine has been well-tolerated in all age groups, although greater reactogenicity is observed after the second dose, and after some allergic reactions were reported, the decision was made to contraindicate it for people with a history of severe allergic reactions to any of the vaccine components.

The second vaccine is the Moderna mRNA-1273 vaccine, whose phase III results were also published in NEJM [5]. Similar to the previous vaccine, this vaccine also uses mRNA encoding the S-2P glycoprotein, in this case consisting of the S protein stabilized in its prefusion conformation by two consecutive proline substitutions at positions 986-987. Additionally, the mRNA is encapsulated in lipid nanoparticles. The vaccination schedule is two separate doses, in this case 4 weeks apart, and it also shows thermostability at -70-80 °C. Regarding efficacy, it also exceeds 94% (95% CI 89-97%) and has good immunogenicity with a robust cellular response, producing CD4+ neutralizing antibodies with a Th1 cytokine profile. This vaccine has a good safety profile, and similar to the previous one, adverse effects are stronger after the second dose.

The third authorized vaccine, Oxford/AstraZeneca ChAdOx1/AZD1222, is a viral vector vaccine based on the complete S glycoprotein of SARS-CoV-2 that is vehicularized in an adenovirus, the chimpanzee adenovirus ChAdOx1. Clinical trial data were published in NEJM, and there was a subsequent publication with updated efficacy data in the Lancet [6,7], indicating 76% efficacy after the first dose and 82% after the second dose (95% CI 63-92%). Additionally, after an exhaustive review of the published data, it has been considered that the best vaccination schedule is to administer a second dose 12 weeks after the first dose. The vaccine has good thermal stability that allows storage between 2-8°C. Regarding safety, similar to the other vaccines, there is local and general reactogenicity that increases after the second dose. In the phase III trial, some participants used paracetamol to prevent some symptoms; therefore, the possibility of using paracetamol as a preventive measure for some symptoms, such as local pain, is included in the fact sheet. Notably, some adverse vascular effects have been recently reported, such as thromboembolism, at a frequency of 1 case/1 million doses administered, and these have been analysed by the EMA, but a causal relationship has not been established. The vaccine shows good efficacy after the second dose, and good immunogenicity and a cellular response with memory immunity have been quantified.

Last is the Janssen vaccine, based on human adenovirus 26 as a non-replicating viral vector containing the complete S glycoprotein, with an acceptable safety profile and lower reactogenicity in those over 65 years of age. The regimens tested were 1 and 2 doses, and it was found that a single dose is effective for all participating population groups; thus, it was decided to indicate only one dose. Its efficacy is 67% (95% CI 59-73%) for all study participants, maintaining this level in all groups studied by age and comorbidities. As a critical fact, the impact of vaccination with a single dose on hospitalizations and deaths has been very beneficial. Therefore, its data sheet highlights the protective effect against moderate, severe (up to 72%) and critical (up to 86%) forms of COVID-19. Regarding thermostability, the vaccine can be stored between 2-8°C. Finally, regarding safety, its profile is comparable to those for the other currently authorized vaccines [8].

There are still many questions about vaccines that will be answered with time, but the research has not stopped nor will it stop because there are many unknowns to be resolved. New trials are underway to determine the efficacy of the current vaccines against new variants of the virus, and the results obtained are encouraging, especially against the British strain for most vaccines and against the South Africa and Brazil strains for some of them; much remains to be investigated and advanced regarding this aspect, and in the coming months, we will surely have more information [9].

Recently, in the last document updating the vaccination strategy, the Ministry of Health provided data on vaccine effectiveness that show that in the cohort study being carried out, 52% of vaccinated participants were protected after the first dose administered; this rate was 71% for the Comirnaty vaccine. In turn, in the study that used the screening method,

64% of vaccinated participants were protected with the first dose, with an effectiveness in reducing hospitalizations of 26% and reducing deaths of 35%; at 7 days after the second dose, 88% of the vaccinated participants were protected, with an effectiveness in reducing hospitalizations of 77% and reducing deaths of 87% [1,2].

In conclusion, as practical aspects of vaccination, we must make it clear that until collective immunity is reached, the preventive measures being implemented will have to be kept in place. In the words of the WHO Accelerator Project, *"There is no time to waste in the fight against COVID-19. No one is safe until everyone is safe*."

CONFLICTS OF INTEREST

The authors declare no conflict of interests.

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Update on the management of SARS-CoV-2 infection

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COVID-19: Impact on prescribing and antimicrobial resistance

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ABSTRACT

The onset of the COVID-19 pandemic challenged healthcare systems focusing their activity on patients infected with SARS-CoV-2. Previous experience with co-infections and superinfections in patients infected with other coronaviruses (SARS-CoV and MERS), the influenza patients admitted to hospitals and prevention of the unknown led to the increased empirical use of broad-spectrum antibiotics in hospitals. The breakdown of antimicrobial stewardship and infection control programs determine an increase in infections due to multidrug-resistant bacteria, particularly in intensive care units. Most of these infections are related to high-risk carbapenemase-producing clones and occasionally with resistance to new β-lactam-β-lactamase inhibitor combinations. On the contrary, in the primary care, there has been a decrease in the use of antimicrobials during the first wave, although it would not have had a significant impact on pathogens associated with community-acquired infections. The accumulated experience reaffirms the need to maintain antimicrobial stewardship and infection control programs in future health crises.

Key words: COVID-19; antimicrobial resistance; antimicrobial prescription; co-infections; superinfections.

INTRODUCTION

The impact that the COVID-19 pandemic has had on antimicrobial prescription and antimicrobial resistance has been a widely debated topic. From a general perspective, arguments against the rise of antimicrobial resistance and antimicrobial use during the pandemics are based mainly on the reinforcement of hygiene and infection control measures, as well as deep changes in social behavior [1]. In healthcare facilities, improved adherence to hand hygiene, infection control precautions and surfaces cleaning, should decrease the spread of multidrug-resistant (MDR) bacteria circulating in these settings. In parallel, healthcare-associated infections (HAIs) or superinfections, especially those caused by MDR bacteria, will decrease and therefore there should not be an increase in antibiotic consumption for the treatment of these infections. On the other hand, social distancing in the community prevents contact between people, hindering the transmission of SARS-CoV-2 but potentially also of community bacterial pathogens such us *Streptococcus pneumoniae* or extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli*. For this reason, it could also reduce the antimicrobial consumption in the community setting.

On the contrary, there are also arguments in favor of a possible increase in antimicrobial resistance and antimicrobial use, especially in healthcare facilities [2]. The pandemic has disrupted healthcare services in many countries due to overload of health care centers caused by COVID-19 patients. This situation may have interrupted infection control and antimicrobial stewardship activities, facilitating the spread of MDR bacteria and the inadequate use of antibiotics. Moreover, the lack of prevalence data on bacterial co-infection at the early stages of the pandemic and the potential development of superinfections by the presence of multiple risk factors and long hospital stays, especially in critical ill patients, could significantly increase antibiotic consumption in COVID-19 patients. Finally, the Microbiology laboratories have also directed their efforts to the diagnosis of SARS-CoV-2 infection, which may have had a negative impact on the development of other activities related to diagnosis of bacterial infections, screening and surveillance for MDR bacteria. So that, the combination of all of these factors may contribute to exacerbate the problem of antimicrobial resistance [2].

Based on the current evidence, in this review we will discuss the impact of the COVID-19 pandemic on antimicrobial

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prescription and antimicrobial resistance, emphasizing the role that co-infections and superinfections might have played in this impact.

CO-INFECTIONS AND SUPERINFECTIONS

It is important to distinguish between co-infections and secondary bacterial infections or superinfection. Co-infections are those that are present on admission while secondary infections are HAIs resulting from patient care during hospitalization.

It is well known that bacterial co-infections are a frequent complication of viral respiratory tract infection such as influenza. It has been reported that during the 2009 H1N1 influenza pandemic between 18-30% of patients requiring intensive care unit (ICU) admission had bacterial co-infection, resulting in a worse prognosis and greater use of healthcare resources [3]. With other coronavirus-originated infections such as SARS-CoV-1 and MERS-CoV, the prevalence of bacterial co-infections is not well defined, especially due to the lower number of recorded cases. The largest SARS-CoV-1 series estimated that 11% of patients have co-infections, predominantly secondary infections [4] and a multicenter study conducted in Saudi Arabia found that 19% of patients with MERS-CoV infection admitted to the ICU had bacterial co-infections. Thus, taking into account this experience, bacterial co-infection in COVID-19 patients was estimated to play an important role during the first wave of the pandemic.

Different studies have analyzed the occurrence, risk factors and aetiology of bacterial co-infections in COVID-19 patients. A recent meta-analysis described that the percentage of patients with bacterial co-infections at the time of admission was as low as 3.5% (95% CI 0.4-6.7%), so it can be considered an infrequent complication in these patients [4]. However, the percentage of co-infections is higher among patients requiring admission to the ICU and can reach values of 30% [5]. Some of the risk factors that have been associated with co-infections are advanced age and comorbidities, such as chronic kidney disease, diabetes, and chronic heart disease [5]. Regarding the aetiology of community-onset bacterial co-infection, the microorganisms most frequently isolated from respiratory and blood samples are *Staphylococcus aureus* followed by *Streptococcus pneumoniae* and *Haemophilus influenzae* [5]. On the other hand, pathogens such as *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* or *Legionella pneumophila* causing the so-called atypical pneumonias are rarely detected in these patients [3,5].

Most importantly, COVID-19 patients are at risk of acquiring secondary infections during hospitalization and this risk increases especially with the severity of COVID-19 disease and length of stay. It has been described that the prevalence of secondary infections varies between 4-22% and this prevalence can be higher than 45% among patients admitted to the ICU [5]. The average time to develop a secondary infection is between 1 and 2 weeks, with pneumonia and bloodstream infections (BSIs) being the most frequent [5]. The predominant pathogens have been gram-negative bacteria, especially *Klebsiella pneumoniae*, following by *Pseudomonas aeruginosa*. These pathogens have been associated mainly with hospital and ventilator-acquired pneumonia, particularly in the ICU cohorts. Among the gram-positive bacteria, coagulase-negative staphylococci as well as *Enterococcus faecalis* and *Enterococcus faecium,* following by *S. aureus* are the most common and mainly caused of BSIs [5-7].

ANTIBIOTIC PRESCRIPCION DURING THE COVID-19 PANDEMIC

Hospital setting. Despite the low prevalence of co-infections and secondary infections in patients with COVID-19, a high percentage of them have received antimicrobial treatment. In a recently published meta-analysis performed by Langford et al. of 3.338 hospitalized and critical COVID-19 patients across 24 studies reported an antibiotic prescription prevalence of 74% (95% CI 68.3-80.0%), with fluoroquinolones, macrolides, cephalosporins and β-lactam-β-lactamase inhibitor combinations being the most commonly used antimicrobial families [4]. Prescriptions were higher among elderly patients and those admitted to ICU. Moreover, in this study the estimated co-infection rate was 8%, so that antibiotic prescription was much higher than the rate of co-infections, suggesting that a high number of prescriptions were unnecessary [4]. Along with these data, a recently published multicenter study in Spain on the use of antimicrobials in a cohort of 13.932 COVID-19 patients showed that in 34% of these patients, antibiotic prescription was inadequate [8].

Different studies that have analyzed antibiotic consumption prospectively during the first wave of COVID-19 pandemic describe different trends over time. In the first months of the pandemic, they describe an increase in the consumption of amoxicillin-clavulanate, ceftriaxone or azithromycin, while over the months the consumption of broad-spectrum antibiotics such as carbapenems, daptomycin, linezolid, ceftaroline and even novel cephalosporin-β-lactamase inhibitor combinations increases, especially in ICU [9,10]. This biphasic trend in antibiotic prescribing is associated with the evolution of COVID-19 cases in hospitals. The early stages corresponded to high hospital admission rate and the antibiotic empirical coverage of all cases of COVID-19 pneumonia in the absence of real data on bacterial co-infection in these patients. In addition, it was recommended that azithromycin be included in the treatment of patients with severe or mild-moderate COVID-19 disease because of its immunomodulatory properties. In Spain, data on antibiotic use in hospitals reported on the website of the National Plan to Combat Antimicrobial Resistance (PRAN) (https://resistenciaantibioticos.es/es/profesionales/vigilancia/mapas-de-consumo/consumos-antibioticos-en-hospitales) showed a significant increase in the use of azithromycin during the first 6 months of 2020 (Figure 1). However, randomized trials have not demonstrated a clinical benefit from the use of azithromycin, while it may increase the risk of side effects such

as prolongation of QT interval [11]. The second phase, characterized by an intensive consumption of broad-spectrum antibiotics corresponds to a phase of accumulation of patients in the ICU with severe disease and suspected or confirmed superinfections caused by nosocomial pathogens, including MDR.

In order to avoid overuse of antibiotics, the current WHO guidelines for the clinical management of COVID-19, do not recommend the use of empirical antibiotic therapy in patients with suspected or confirmed mild-moderate COVID-19 unless there is clinical suspicion of a bacterial infection [12]. In this scenario, it is important the role of antimicrobial stewardship programs on supporting the optimal selection of empirical therapies and the rapid de-escalation of treatment once SARS-CoV-2 infection is confirmed.

Primary care. In contrast to the hospital setting, the prescription of antimicrobials clearly decreased in the early stages of the pandemic. This fact has been pointed out in different publications, although the data collected are less when compared to those obtained in the hospital setting. The reasons for this decrease would have been the discontinuation of non-essential care in primary patients during the wave of the pandemics, the shift from in-person office care visits to telemedicine consultation, and the possible decrease in respiratory infections due to lock down and distancing [13]. A systematic review concludes that there was not yet evidence to conclude that remote consulting had a significant impact on antibiotic prescribing in primary care [14], although several publications and data from web sites alert of this situation

At least two studies have been published with data from the United States showing evidence of a dramatic reduction in antimicrobial consumption during the first wave of the pandemic. One of these studies estimated that between January and May 2020 there was a 33% decline in antimicrobial dispensing, with the months of greatest impact being April and May 2020 [13]. This reduction affected all groups of antimicrobials, although in the case of azithromycin, there was a 5% increase from February to March 2020 period, with a subsequent decrease in the next months (71%). Another study in this country confirmed these data without a subsequent increase during the months of May to July for azithromycin and the other antimicrobials [15].

In Australia, with a follow-up period until September 2020, an abrupt reduction in the dispensing of antimicrobials was also observed from March to May 2020, affecting to a greater extent those used in the treatment of respiratory infections. Subsequently, the dispensing of antimicrobials increased, although it did not reach pre-pandemic values with many of them [16].

In the United Kingdom, publications include data from specific health care geographic areas. One of these, conducted in northwest London, similarly showed an overall reduction in antimicrobial prescribing during the lock down (17). However, in some age groups there was an increase for some of the antimicrobials. In this regard, amoxicillin-clavulanic acid increased between February to April 2020 among patients above 50 years old, while declining in younger age groups during April. This study also showed that 31.5% of patients diagnosed with COVID-19 and not admitted in a hospital received antimicrobial treatment within the time window of 14 days before or after the diagnosis. The most prescribed antibiotics were amoxicillin (34.9%), doxycycline (27.4%), clarithromycin (9.3%), phenoxymethylpenicillin (5.7%) and amoxicillin-clavulanate (4.5%).

A Dutch study comparing follow-up data of prescriptions for outpatients also observed a decrease in pneumonia, mastoiditis, pyelonephritis and gastrointestinal infections, as well as in antimicrobial treatments [18].

In Spain, using data from the PIRASOA program in Andalusia a before and after cross-sectional study comparing antibiotic use in the community patients the first and second quarters of 2019 and the same quarters in 2020 also showed a significant decrease in antimicrobial prescribing, being 7.6% in defined daily doses per 1000 inhabitants per day (DID) [19]. This decrease occurred for all antimicrobials except azithromycin, which remained stable over the studied period. The Spanish Agency of Medicines and Health Products (AEMPS), in the section of its website dedicated to the National Plan to Combat Antimicrobial Resistance (PRAN), provides monthly data on antimicrobial consumption (https://resistenciaantibioticos.es/es/profesionales/vigilancia/mapas-de-consumo/consumo-antibioticos-humana). Unlike in the hospital setting, in the first wave of the pandemic in Spain (March to April 2020) there was a general decrease in the consumption of antimicrobials compared to the same period of the previous year, maintaining this decrease for the rest of the year (Figure 1).

IMPACT OF ANTIMICROBIAL CONSUMPTION ON ANTIMICROBIAL RESISTANCE DURING THE COVID-19 PANDEMIC

The impact of changing antimicrobial prescribing on antimicrobial resistance has been highlighted by several authors [7, 20-23]. The evidence so far has been clearest in the hospital setting where the relevant increase in prescribing of antimicrobials with wide-spectrum, the higher selection density, the difficulty to continue the adherence to epidemiological barrier measures and the discontinuation of antimicrobial stewardship programs may have been the cause of the increase of antimicrobial resistance.

There is a growing number of reports of superinfection caused by ESKAPE pathogens, especially carbapenemase-producing *Enterobacterales*, in patients with severe COVID-19 during hospitalization [7]. The local ecology, represented by the pool of resistance genes and circulating high-risk clones (HiRCs) plays an important role in the epidemiology of MDR bacteria in the hospital setting during the COVID-19 pandemic. Moreover, the selective pressure exerted by the heavy use of antimicrobials favors the selection and co-selection processes of these HiRCs and the breakdown of infection control measures facilitates their dissemination and transmission among patients [20].

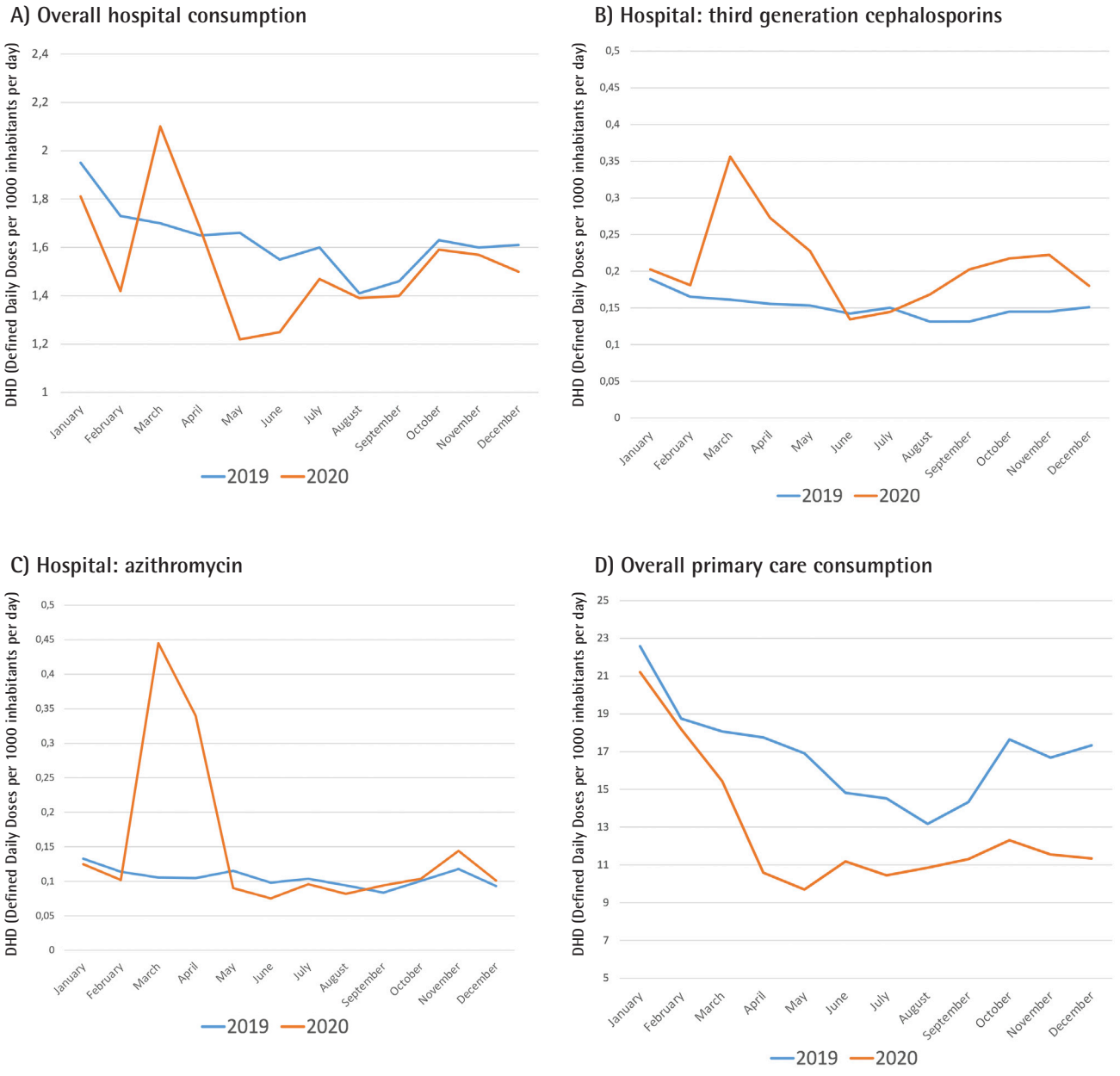

Figure 1 | **Comparative antimicrobial consumption in Spain in 2019 and 2020. Data obtained from the national consumption surveillance system of the Spanish National Plan against antimicrobial resistance (https://resistenciaantibioticos.es/es/profesionales/vigilancia/mapas-de-consumo): Overall antimicrobial consumption in the hospital setting (A), the corresponding to third generation cephalosporins (B) and azithromycin (C), and in the primary care (D)**

A study carried out in an Italian ICU during the first COVID-19 wave reported a significant increase in the incidence of carbapenemase resistant *K. pneumoniae* (CR-Kpn) acquisition (from 6.7% in 2019 to 50% in March-April 2020), despite infection control measures and performing surveillance cultures to identify CR-Kpn carriers. Among the factors proposed by the authors that may be related to the transmission of CR-Kpn in the ICU are the high intensity of care, the prolonged contact of healthcare workers (HCWs) with the patient or the presence of a large number new HCWs from other departments and without work experience in the ICU setting [24]. A high colonization pressure by MDR bacteria, as reported in this article, significantly increases the risk of transmission and at the patient level is also an important risk factor for the subsequent

development of infections. One hospital in New York City reported 13 patients with severe COVID-19 who subsequently developed superinfections with carbapenemase-producing *Enterobacterales*, including KPC-producing *K. pneumoniae* and NDM-producing *Enterobacter cloacae* [25]. All but one of these patients were under mechanical ventilation at the time of infection and 5 of the 13 patients died. Genomic sequencing identified that the majority of *K. pneumoniae* isolates belonged to multiple lineages of the HiRCs-ST258 harbouring bla_{KPC-2} and that these lineages were linked to isolates recovered in the hospital between 2011 and 2016, highlighting the importance of local clonal pool. Another noteworthy fact of this outbreak is the emergence of resistance to ceftazidime-avibactam in the one ST258-bla_{KPC-2} isolate of *K. pneumoniae* in patient with ventilator associated pneumonia [25]. The circulation of HiCRs, especially of KPC-producing *K. pneumoniae* in the ICU, has resulted in the use of intense broad-spectrum antimicrobials including new antibiotics such as ceftazidime-avibactam or meropenem-varbobactam increasing the risk of selection of strains resistant to the new antimicrobials as the case described above [25,26].

The situation in primary care would have been different than that depicted in the nosocomial setting. Antimicrobial consumption was drastically reduced in the former and the ecological impact on resistance could have been the reverse of that observed in the latter. However, the behaviour in the extra-hospitalary setting may not be so evident. It is well known that a high increase in antimicrobial consumption leads to a rapid increase in resistance, although a decrease in consumption does not translate into a rapid decrease in resistance.

So far, no studies have been published showing that the decrease in antimicrobial consumption during the COVID-19 pandemic in primary care, at least in the first wave, has had a positive effect on resistance in pathogens from the respiratory tract such as *S. pneumoniae* or *H. influenzae* or from the urinary tract such as *E. coli*. This could be due to a lower selection density in the extra-hospitalary environment, to the adaptation of previously selected resistant bacteria and their non-clearance over time and to the possible co-selection effect exerted by different antimicrobials, even if consumption had been low. It would also due to the fact that the decline in the antimicrobial use was not too long to have an ecological impact. However, the interconnection of different health care compartments might have also impact on the antimicrobial situation. In fact, the tremendous increment of the azithromycin use might have had a role on resistance, at least in *S. pneumoniae* and *H. influenzae*, and wide-spectrum antimicrobials on *E coli*. Further studies are needed to demonstrate these hypotheses, including the impact on the microbiome and resistome.

CONCLUSIONS

Although co-infection plays an important role in other viral infections such as influenza, studies in patients with COVID-19 have described that the prevalence of bacterial co-infection at admission is low. In the early stages of the pandemic, experience from the management of influenza and the lack of real data about the prevalence of bacterial co-infection in COVID-19 patients led to an increase in the prescription of antimicrobials traditionally used in the treatment of community-acquired pneumonia such as cefotaxime, ceftriaxone or amoxicillin-clavulanic acid. Throughout the evolution of the pandemic, an increase in superinfections has been observed, particularly in patients with severe COVID-19 disease who required prolonged ICU admissions. These infections have often been caused by MDR microorganisms and have required broad-spectrum antimicrobial treatment, including new antibiotics such as ceftazidime-avibactam. The overcrowding of hospitals and especially ICUs has led to a breakdown in infection control measures and antimicrobial stewardship activities. This may have led to outbreaks caused mainly by pre-exiting HiRCs circulating in hospitals, which are subject to intense selection and co-selection processes due to the use of broad-spectrum antimicrobials.

The data published so far in the extra-hospitalary setting are scarce, although the decrease in antimicrobial consumption may have been limited to the first wave, so the effect on resistance may have been of little relevance.

In any case, the COVID-19 pandemic and its consequences on antimicrobial resistance has demonstrated the necessity to maintain the antimicrobial stewardship and infection control programs. Moreover, to learn about the gaps during this period to avoid breakdown of these activities in futures similar situations.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Update on the management of SARS-CoV-2 infection

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Respiratory co-and superinfections in COVID-19

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ABSTRACT

There are few publications on the impact of coinfection and superinfection in patients with COVID-19. Patients with higher severity are much more prone to secondary bacterial, fungal or viral infections. The overuse of antimicrobials in many viral infections (including SARS-CoV-2 infections) undoubtedly contributes to the current antimicrobial resistance crisis. In the context of COVID-19, we are witnessing an increase in multidrug-resistant bacterial infections in our hospitals. The heterogeneity of published studies makes it critical to perform more large-scale studies to better understand the pathogenesis of coinfections or superinfections in the COVID19 patient.

Keywords: COVID-19; SARS-CoV-2; antimicrobial resistance; coinfection, superinfection; Antimicrobial overuse

INTRODUCTION

Respiratory tract viral infections generate extraordinary morbidities and mortality rates worldwide, often in a seasonal way. In the last 20 years, we have witnessed four outbreaks of respiratory infections (i.e., SARS-CoV: 2002-2004; H1N1 Influenza: 2009–2010; MERS-CoV: 2012–2020; SARS-CoV-2: 2019-present). During the management of these outbreaks, the attention is firstly focused on the treatment of the viral infection itself and its complications, but it is mandatory to take into account the risk of existing coinfections and/or secondary infections that might develop in these patients. A relevant complication of viral respiratory infections is the potential colonization by other viral, bacteria or fungi, which might be associated with superinfection resulting in high morbidity, and mortality rates. The treatment for secondary bacterial infections is based on broad-spectrum antimicrobials, but this can result in undesirable side effects that undoubtedly have a negative impact on the host normal microbiota, or in other superinfections (e.g., *Clostridium difficile* or fungal infections).

According to the CDC, a superinfection is an infection following a previous infection while a coinfection is an infection concurrent with the initial infection. The difference is temporal: coinfections occur simultaneously, whereas superinfections develop following the initial infection. While the two terms are used interchangeably in medical literature and clinical practice, they are different clinical entities, being this particularly relevant when talking about COVID-19 patients. Superinfections and coinfections can enhance microbial pathogenesis, increasing the morbidity and mortality of viral infections.

THE VIRAL INTERFERENCE PHENOMENON

Epidemiological studies suggest that, following infection with influenza virus, there is a subsequent period of time during which the patient has a lower susceptibility to infection with other influenza viruses. This phenomenon (i.e., viral interference) appears to be independent of antigenic similarities between the viruses [1]. Viral coinfections may have different consequences. The most common is the above mentioned viral interference, where one virus competitively suppresses replication of the other. Interference between closely related viruses eventually results in elimination of the secondary coinfecting virus and is denoted as superinfection exclusion. The occasions where persistently infected cells withstand the challenge of a heterologous virus are termed superinfection suppression. Besides diminished viral replication (i.e., interference), coinfections with certain viruses may also trigger enhancement of the replication of one or both of the confecting viruses. In other cases, coinfection has no effect on the virus replication, and thus all the coinfecting viruses can coexist (i.e., accommoda-

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tion). Coinfection may modulate viral virulence and cell death, thereby altering disease severity and epidemiology. However, genetic recombination between coinfecting viruses depends on the similarity between the coinfecting viruses.

RESPIRATORY TRACT VIRAL-BACTERIAL SUPERINFECTION

The respiratory tract is susceptible to be colonized by environmental microorganism that circulate in the air. Barriers of the respiratory tract mucosal surface utilize a diversity of strategies to hinder microbe invasion. Physical barrier defenses include immunoglobulins, mucus, and beating cilia and separates the external environment from the internal host tissues [2]. However, pathogens such as *Streptococcus pneumoniae* target the respiratory tract causing severe damage to the host during their invasion. A classic example of viral-bacteria superinfection is the increased susceptibility of a patient with influenza infection to the acquisition of *Streptococcus pneumoniae*, resulting in a pneumonia that causes greater morbidity and mortality than infection with either pathogen alone. Different studies have shown that up to 65% of laboratory-confirmed cases of influenza infection are complicated by bacterial co/superinfections with the majority ranging between 11% and 35% in a meta-analysis [3]. The influenza A (H1N1) outbreak in 2009 had even developed into a global pandemic while causing seasonal flu epidemics each year. Secondary bacterial infections are one of the leading causes for influenza-associated deaths. The lethal synergism between influenza virus and *Streptococcus pneumoniae* strains accounts for the majority of diseases as well as mortality during influenza epidemics [3].

SARS-COV-1 AND COINFECTIONS/SUPERINFECTIONS

Severe Acute Respiratory Syndrome-related Coronavirus (SARS-CoV-1) was first report in Guangdong Province, China in November 2002. The diagnosis of bacterial co-infections was very high in these patients [4]. These included infections by methicillin resistant *Staphylococcus aureus*, *Klebsiella* spp., *Pseudomonas aeruginosa* or *S. pneumoniae*. Most SARS patients were treated with prophylactic broad-spectrum antimicrobials. Previous studies have shown that human metapneumovirus and other viruses can be also detected from SARS-CoV-1 patients.

MERS AND COINFECTIONS/SUPERINFECTIONS

The first cases of the Middle East respiratory syndrome (MERS) occurred in June 2012 in Saudi Arabia with later outbreaks observed in 2015 and 2018. Due to the high mortality rate of MERS infections, the impact of secondary bacterial infections remains unclear. Nosocomial bacterial pneumonia is however common among MERS patients with ventilator support.

SARS-COV-2 AND COINFECTIONS/SUPERINFECTIONS

Preliminary studies and some evidence from high-burden COVID-19 areas suggest that superinfections are common, particularly in severe cases. Almost all SARS-CoV-2 severe cases results in pneumonia with the inflamed alveolar space resulting an ideal environment for microbial growth [5]. The superinfecting pathogen may be bacteria, other virus or fungi. The presence of secondary bacterial infections in patients infected with SARS-CoV-2 complicates treatment and prognosis. Besides, the risk of superinfection with multidrug resistant bacteria challenges the treatment of severely sick COVID-19 patients in intensive care units.

A study described the incidence and predictive factors of secondary infections in a cohort study of patients hospitalized with COVID-19 at San Raffaele Hospital in Milan [6]. Among 731 patients, a secondary infection was diagnosed in 68 patients (9.3%); 22/731 patients (3%) had at least one respiratory tract infection. The overall 28-day cumulative incidence of secondary infections was 16.4%. Lower tract respiratory infections were caused mainly by Gram-negative pathogens (14/26, 53.8%). Eleven patients were diagnosed with putative invasive aspergillosis. At multivariable analysis, early need for ICU, respiratory failure, and severe lymphopenia were identified as risk factors for the development of secondary infections. In a multicenter study [7]. in China that included 476 COVID-19 patients, secondary bacterial infections were significantly associated with outcome severity. Patients were divided into 3 groups (i.e., moderately ill, severely ill, and critically ill). The critically ill patients had the highest percentage of secondary bacterial infections (34.5%) compared to patients in the moderately ill and severely ill groups (3.9% and 8.3%, respectively). Severe COVID-19 is associated with intensive care unit admission, increased secondary infection rate, and significant worsened prognosis.

Risk factors for secondary infections in severe COVID-19 have not been fully described. A study including critical COVID-19 patients from Shanghai found 57% patients who developed secondary infections [8]. The most common infection site was the respiratory tract. The most frequent pathogens were gram-negative bacteria (50%), followed by gram-positive bacteria (26%), virus (11%), and fungi (7%). Patients receiving invasive mechanical ventilation had a higher risk of secondary infections. Respiratory Infection rate post high flow, tracheal intubation, and tracheotomy were 12%, 30%, and 92%, respectively. Secondary infections led to lower discharge rate and higher mortality rate. Diagnosis of secondary bacterial infections typically requires testing of samples obtained by sputum expectoration/induction, nasopharyngeal/oropharyngeal swabs of respiratory passages, bronchoscopy, thoracentesis, and/or lung tissue biopsy. Conventional diagnostic tests have poor sensitivity in identifying the etiologic organisms responsible for respiratory infections. A study used real-time PCR to detect specific pathogens causing COVID-19 coinfections [9]. They found that 243 (94.2%) patients were coinfected with

at least one of 39 different pathogens. Culture accompanied with metagenomics sequencing increased pathogen diagnostic rate. Bacterial coinfections were predominant (91.8%) over viral (31.5%) and fungal (23.3%) infections. Although this study found no significant association between coinfection rates and outcome severity or mortality, they described interesting coinfection patterns in different clinical groups

COVID-19 AND INCIDENCE OF COMMUNITY-ACQUIRED PNEUMONIA

The impact of the COVID-19 pandemic on the incidence of community-acquired pneumonia is not well defined. One study compared the number of elderly patients admitted to a hospital for community-acquired pneumonia from January to June 2020 with the numbers for the same period in each of the last three years [10]. The number of patients diagnosed with community-acquired pneumonia began to decline in February 2020, and by April 2020 the number was significantly lower than in the same period of the previous three years. There is no evidence on the impact of general infection control measures, such as the use of facemasks or hand washing, on the development of community-acquired pneumonia. However, these measures might have indirectly contributed to reducing the number of cases by preventing common viral infections that could be a trigger for community-acquired pneumonia.

CONCLUSIONS

Data regarding superinfections/coinfections in COVID-19 patients are limited and still emerging. The relatively high incidence of severe infection and mortality in COVID-19 is thought to be in part due to secondary infections, alongside with lack of natural immunity and viral replication in the lower respiratory tract leading to severe lung injury and acute respiratory distress syndrome. We have few detailed clinical studies on co-or superinfections occurring in COVID-19 patients. Since mortality rates from antibiotic-resistant bacterial infections are increasing worldwide, and the numbers of COVID-19 patients are steadily increasing it is critical to analyze this point in detail. The use of broad-spectrum antibiotics is often a routine preventive measure in these patients. Until programs to optimize antibiotic use in these patients are implemented in our hospitals, antibiotic overuse will continue to be unavoidable, impacting the genesis of multidrug-resistance phenomena.

Coronavirus infections are and will likely be a clinical challenge for many years to come. Pandemics due to coronaviruses and other emerging pathogens are inevitable in a globalized world with interconnected societies, travel and commerce. We should invest in being better prepared for the next pandemic by exploring and establishing new pathways to treat pathogens implicated in coinfections and superinfections to avoid deepening the health crisis due to antibiotic resistance.

CONFLICTS OF INTEREST

The author declares no conflicts of interest.

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Update on the management of SARS-CoV-2 infection

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COVID-19 and fungal infections: Etiopathogenesis and therapeutic implications

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ABSTRACT

Invasive fungal infection often complicates patients with severe viral infection, especially those admitted to critical care units. Severe SARS-CoV-2 infection has been no exception and a significant association with *Aspergillus* spp. has been documented, resulting in high patient mortality. In this summary we describe the clinical presentation, the underlying diseases most commonly linked with this association, radiological manifestations and therapeutic management of CAPA.

Keywords: COVID-19; aspergillosis; CAPA; SARS-CoV-2; co-infection

INTRODUCTION

The relationship between invasive aspergillosis (IA) and viral infection, mainly influenza A, in critically ill patients with acute respiratory distress syndrome (ARDS) is widely known nowadays. Since 1972, when this association was first published [1], this association had hardly been described. However, in 2011, and following the remarkable advancements made in diagnostic techniques for influenza infection—that is, a real-time polymerase chain reaction (PCR) performed on nasopharyngeal throat swabs—and invasive aspergillosis, the role of invasive pulmonary aspergillosis (IPA) complicating severe influenza became more evident.

Indeed, several publications demonstrated a strong association between the two diseases [2,3]. In one particular paper, investigators developed the AspICU algorithm [4] to define IPA in critical care patients with viral infections.

One of the most important publications in this field detailed the relationship between viral infections and *Aspergillus* in 40 patients admitted to intensive care units (ICU) in two tertiary hospitals in Belgium. Investigators described up to 23% of patients with severe influenza infections had further complications due to *Aspergillus* [3,5].

That all stated, when patients with IA were compared with those without infection, mortality rates were much higher (51% vs 28%, respectively) [6]. Clinical forms of IA in these patients present some differences with respect to immunosuppressed people, with more atypical findings [7]. There is a high variability in clinical manifestations, ranging from tracheobronchitis to invasive and angioinvasive disease [5].

COVID-19-ASSOCIATED PULMONARY ASPERGILLOSIS

Therefore, when the COVID-19 pandemic arrived, physicians first expected to observe an increase in the incidence of *Aspergillus* spp. cases in relation to SARS-CoV-2 viral infection. In December 2019, autopsy reports described deceased patients with severe SARS-CoV-2 infection who developed co-infection with *Aspergillus* spp. In the following months, different case series were also reported (Figure 1). For example, Marr et al. [8] reported 20 cases of COVID-19-associated pulmonary aspergillosis (CAPA) occurring at Johns Hopkins University (Baltimore, MD, USA) and Hospital Clinic of Barcelona (Barcelona, Spain) before June 2020. Thanks to this international, multicenter CAPA series, we have acquired some key learnings.

First, radiographic manifestations are difficult to interpret. Chest x-rays may not be clear due to diffuse lung damage to the lung parenchyma caused by the viral infection—associated with inflammatory changes—and possible ARDS. Although cavitation and necrosis occur occasionally, no radiographic reports describe manifestations classically seen in angioinvasive cases [3,7].

Second, most of these patients had underlying diseases,

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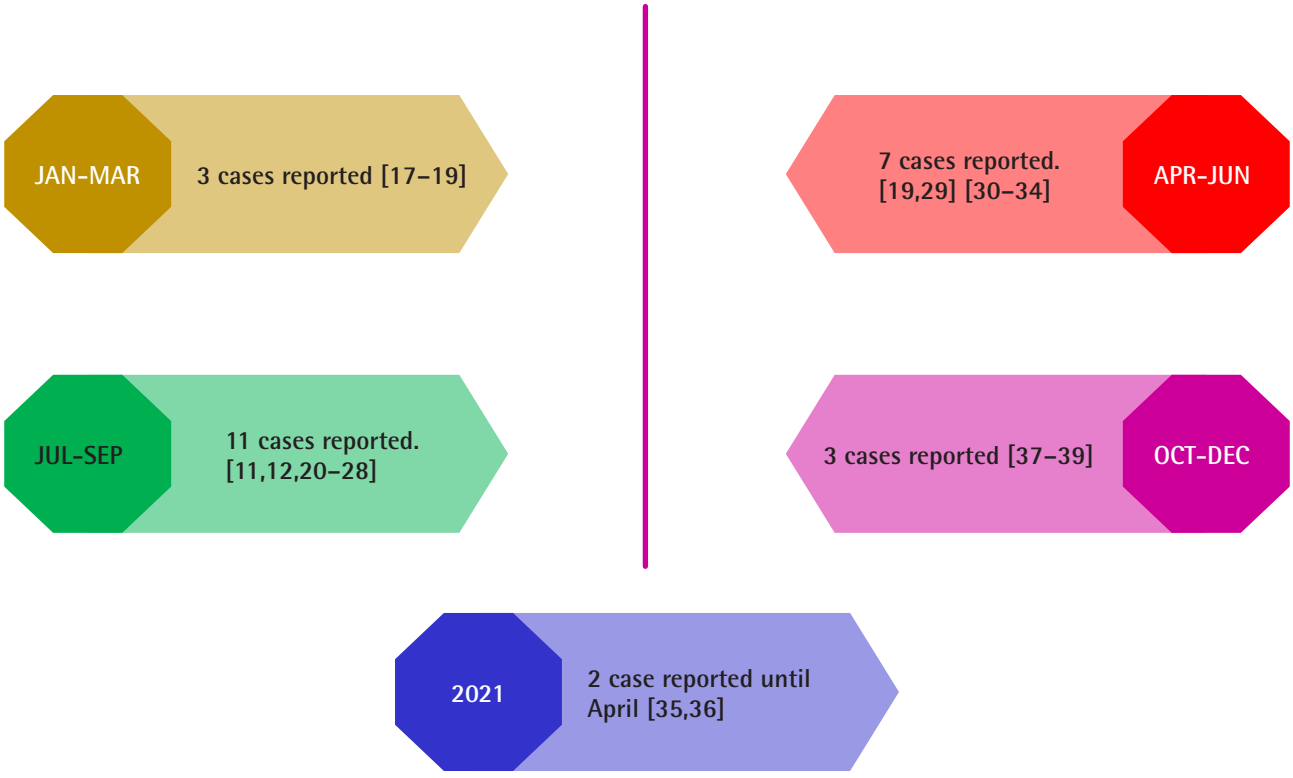

Figure 1 | **Quarterly timeline about cases reported for coronavirus disease–associated pulmonary aspergillosis.**

predominantly prior lung disease that required ICU admission with respiratory support for more than nine days.

Third, performing cultures of respiratory samples, mainly tracheal aspirates, was the most common diagnostic testing approach. In the study by Marr et al. [8], only 25% of serum galactomannan (GM) was positive among patients with CAPA. Conversely, because of invasive diagnostic strategies used in both centers and the lower rate of angioinvasion, 17 of 20 (85%) respiratory cultures tested positive. This observance may be attributable to the fact that these patients were in early stages of the disease. In this series, mortality rates were relatively low.

Furthermore, Salmanton-Garcia et al. [9] reported 186 cases of CAPA across 17 different countries collected from Fungiscope (a global emerging fungal infection registry; https://www.clinicaltrials.gov;National Clinical Trial identifier NCT01731353) and a literature search. In this large series, we found again that 97.8% of patients were admitted to the ICU, and 94.1% required mechanical ventilation. Investigators observed median days until CAPA diagnosis of 10, which was similar to data reported in the multicentric series by Marr et al. [8]. A notable difference between both studies is that up to 60.8% of total CAPA were diagnosed with a positive GM, mainly due to bronchoalveolar lavage (BAL). This data suggests that these patients were in advanced stages of the disease with the presence of angioinvasion. Also, described mortality rates (52.2%) were higher.

Due to the importance of this entity, a group of 22 experts from six continents and 14 countries gathered together to develop guidelines for the management, diagnosis and treatment of CAPA [10]. In this review, they proposed three clinical forms: i) proven aspergillosis when invasive growth of *Aspergillus* was evidenced in histopathological and/or microbiological samples obtained from a sterile tissue; ii) probable aspergillosis when *Aspergillus* spp. evidence was obtained from BAL or blood (culture, GM, or *Aspergillus* PCR); iii) possible aspergillosis when compatible radiological findings were described together with mycological evidence obtained via non-bronchoscopic lavage. In conclusion, should clinical findings elicit suspicion and meet inclusion criteria, it is necessary to initiate diagnostic tests for CAPA, so as to avoid a rapid and undesirable evolution towards more invasive forms.

As high mortality has been reported in cases of CAPA, [11,12] early and adequate therapy is crucial. Traditionally, voriconazole or isavuconazole has AI evidence for the treat-

Table 1	Main advantages of isavuconazole versus voriconazole.
Broad spectrum.	
Linear and predictable pharmacokinetics.	
Not influenced by genetic polymorphisms or by diet.	
Few intervariabilities.	
Does not need therapeutic drug monitoring.	
High volume of distribution; high dose in lung.	
Few interactions with other drugs.	
Few side effects.	
Cyclodextrin-free.	
Can be used in renal failure, dialysis, and hemodialysis.	

ment of IA in main guidelines [13]. However, both drugs are quite different. Table 1 summarizes potential advantages with the use of isavuconazole. Remarkably, isavuconazole has fewer interactions than voriconazole. This fact is of main interest in patients with COVID-19, especially in those who require ICU admission. Baniasadi et al. [14] reported data from a prospective study about drug-drug interactions among patients in ICU, and voriconazole was one of the more frequently involved drugs due to its ability to inhibit CYP3A4 [15]. Secondarily, voriconazole interacts with corticosteroids, some sedative drugs and remdesivir. Isavuconazole is metabolized differently via CYP2C19, CYP2C9, and CYP3A4, which makes the possibility of drug-drug interaction significantly lower. The use of voriconazole must therefore be associated with therapeutic drug monitoring on a weekly basis, given its drug-drug interaction and great interpersonal variability due to genetic polymorphisms of CYP3A4.

Finally, it is important to note that critically ill patients with SARS-CoV-2 infection may suffer from other fungal infections [16]. Like other patients admitted to the ICU, critically ill patients with SARS-CoV-2 can develop candidemia due to prolonged ICU length of hospital stay, invasive medical devices, use of broad-spectrum antibiotics and corticosteroids, etc. More occasionally, though, other fungal infections like *Pneumocystis jirovecci* or mucormycosis have been described.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Approach to COVID-19 pandemic management in Madrid. Chronic of a year

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ABSTRACT

After more than a year of pandemic, the international medical community has changed the perception of fear to one of respect for SARS-COV-2. This has been the consequence of the integral study of all the dimensions of the disease, from viral recombinant capacity to transmissibility, diagnosis, care and prevention. This document summarizes the main strategic lines of study and approach to the pandemic in Madrid.

Keywords: COVID-19, management, Madrid

The region of Madrid is one of the most populated communities in Spain with a density in 2019 of 834 inhabitants/km^2, 12% higher than the next region in terms of population density, which is Barcelona [1]. It represents the largest labor and logistics node in the country. It is also one of the main immigration destinations and connects with the rest of the country through a developed road network and the largest railway and airport connection in the national territory. Spain is home to the oldest population in Europe and Madrid has the largest number of social-healthcare centers with more than 100 beds [2]. All these are influential elements in the spread of SARS-COV-2 infection.

After the first pandemic impact, all of Spain began to work to adapt to the changes that the pandemic itself generated, mainly related to sociodemographic movements (work, academic or vacation). Since then, the graph of the evolution of the pandemic has been marked by two facts, the development and distribution of vaccines (or lack thereof in some areas of the planet) and the knowledge by massive sequencing of the emergence of viral variants, as a result of the ease of viral recombination and the relaxation of preventive measures in the interaction between people. In Madrid, work was carried out along the four strategic lines described below:

1. Diagnostic development (implementation of diagnostic techniques, development of seroprevalence studies, sequencing for early diagnosis of new variants, etc.) to know the burden of the disease and control of outbreaks.
2. Promotion of prevention measures, implementation of perimetral restriction, people capacity control and sectorization of centers with a higher risk of contagion. Once the vaccine is available, development of distribution and administration circuits.
3. Construction of infrastructures and increase in human resources for diagnosis, treatment and prevention of infection.
4. Search for tools to predict changes in the balance of contagion.

DIAGNOSTIC DEVELOPMENT

Despite the shortage of equipment and consumables at the end of the first wave, an effort was made to increase the diagnostic capacity of the Microbiology Departments of all hospitals, and a special effort was made to implement molecular diagnostic platforms. Overall, diagnostic activity increased to 26,300 PCR tests per day in September, 27,400 at the end of October and more than 28,000 in November 2020. Thus, more than 2 million PCR tests for SARS-CoV-2 were performed from September through December 2020. This figure has already been well exceeded in February 2021 [3,4].

Point-of-care (POC) antigen testing is for WHO [5] particularly useful in the diagnosis of infection if PCR results are not available at short notice, or in case the healthcare system is overwhelmed. They are also recommended in the study of contacts, especially in case of outbreaks or in areas of high community transmission, contexts in which the predictive values are high enough to allow effective infection control, which

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is justified because even in the absence of symptoms the viral load levels are similar to those of symptomatic cases.

Madrid pioneered the implementation of these antigenic tests, developing a document of recommendations on their use, published in this journal and in different care settings (primary care, pediatrics, emergency, social and health centers) [6]. From week 41 to the end of 2020, half of the more than 100,000 SARS-CoV-2 diagnostic tests performed each week in suspected cases were rapid antigen tests. This strategy, implemented in hospital emergency and primary care settings, increased the ability to diagnose COVID-19 cases. Rapid antigen tests were also used in the screening of asymptomatic subjects, achieving a ratio of 250 tests per COVID-19 case detected. All these results demonstrate that the acceptability and simplicity of point-of-care diagnosis of SARS-CoV-2 overcomes the limitations of test sensitivity, including those observed in asymptomatic subjects, and makes antigen testing a valuable tool for monitoring and controlling the pandemic [3]. Four months later, antigen tests can be performed in pharmacies and are even available for sale for self-testing. At present, antigenic tests for clinical use have not lost sensitivity against viral mutational variants [7]. According to data provided by the Ministry of Health to the ECDC, the diagnostic capacity of CM has reached 21 tests (PCR or AT) per case detected by the end of 2020, an average of 2,341 diagnostic tests per 100,000 inhabitants [8].

Another diagnostic challenge that points to the burden of disease and is crucial for the development of health strategies are seroprevalence studies. These studies, carried out in vulnerable or exposed persons (residents in social and health care centers, people with disabilities, law enforcement officers, prison officers, etc.) and as a complement to the national study [9], have helped to understand the traceability of infections and their modulating factors (cohabitation, transport, groupings, work, etc.). A clear example is the analysis of residents in health care facilities [10], which has made it possible to sectorize the staff, manage opening and visiting hours, the cadence of screening of workers and the tracing of infection flows in outbreaks among residents, etc [11].

The latest diagnostic challenge to date is the implementation of mass sequencing in disease management. The classical nucleic acid sequencing procedure has for years been the Sanger method, designed to generate single amplicon sequences. However, the development of massive whole genome sequencing techniques, based on next-generation sequencing (NGS) methods, allows millions to billions of DNA copies to be sequenced in a single run [12]. Despite its bioinformatics requirements, this methodology is gradually being incorporated into genomic studies in clinical care and epidemiological surveillance at a cost and scale hitherto inaccessible [13]. For the time being, its main application has been the characterization of the successive phenotypically and genotypically different SARS-CoV-2 variants that have emerged in recent months. This characterization has improved knowledge of the spread and severity of the infection. In addition, its use makes it possible to identify cryptic forms of transmission, generating opportunities for health interventions. The main variants of concern include Alpha (B.1.1.7), which emerged in September 2020 in the United Kingdom, Beta (B.1.351; B.1.351-V1), which emerged in South Africa in October 2020, Gamma (P.1, a descendant of B.1.1.28), initially detected in Brazil in December 2020, and Delta (B.1.617), which appeared in India. Among the variants of interest, the most studied are the Epsilon (B.1.429; CAL.20C/B.1.427) identified in California in May 2021 [14]. In Spain the Beta, Gamma and Epsilon variants have circulated very rarely. Alpha has been until recently the majority [15]. Currently, the Delta B.1.617 variant seems to predominate. This variant has recently been subdivided into two: Delta (B.1.617.2) and Kappa (B.1.617.1) [16]. The new variant of interest Lambda, initially distributed in Peru and Chile, has joined this expanding set of strains [17]. A connotation of epidemiological surveillance lies in its obligatory and agile adaptability to changing and unforeseen situations, such as those of the present pandemic. Thus, the integration of molecular epidemiology with traditional epidemiology is a necessity that cannot be postponed. To this end, the creation of multidisciplinary groups for the implementation of consensual practices between microbiologists, bioinformaticians, clinicians, epidemiologists and health authorities represents the best strategy.

PROMOTION OF PREVENTIVE MEASURES

The successive waves of COVID-19 cases currently occurring in European countries, and until vaccination rates achieve the expected herd immunity, are forcing policy makers to make decisions that will impact not only the spread of the pandemic, but also the socio-economic future of their regions. Spain was one of the first and hardest hit by the COVID-19 pandemic in Europe. The rapid spread of the first wave of COVID-19 overwhelmed the healthcare system, leading the government to declare a State of Emergency on March 14, 2020, and to impose one of the tightest closures in Europe, in line with those imposed in Italy and France [18-20]. These restrictions succeeded in reducing the spread of COVID-19, but led to a 17.8% drop in GDP compared to the first quarter of 2020, which placed Spain among the three OECD countries with the largest GDP decline during the second quarter of 2020 [21]. In a mathematical model of confinement-testing, while policies based on increased testing rates would lead to higher healthcare costs, increased mobility restrictions and confinement would be associated with a larger decline in GDP, with differences of up to 4.4% points [22]. Alternative strategies to control the spread of COVID-19 lead to different economic outcomes. Decision makers can use these tools to identify the most appropriate strategy taking into account epidemiological and economic outcomes.

Instead of maintaining strict containment with complete mobility restrictions, a strategy of perimeter containment by basic health zones was implemented in CM. The CM has 286 Basic Health Zones (ZBS) with a median of 21875 inhabitants (IQR 11083) per area. From September 21, 2020 until spring 2021 when confinements were suspended, half of all of them

were confined. This type of confinement, by districts or small municipalities, only in those areas with the highest density of occurrence, minimized the social and economic impact of the closure of commercial activity. The perimeter restriction not only acts on the mobility of citizens, in fact, it includes many other associated measures such as limiting the capacity and opening hours of commercial activity, capacity control or diagnostic-monitoring campaigns as the main interventions. In any case, the measures applied in the CM to confined areas caused a more pronounced decrease in the incidence of cases in the perimeter areas than in those where these restrictions were not applied.

On December 2, 2020, the Technical Working Group on COVID-19 Vaccination of the Vaccination Program and Registry of the Ministry of Health published the Strategy for vaccination against COVID-19 in Spain [23]. After the arrival of the first doses of the vaccine developed by Pfizer-BioNTech pharmaceuticals, the Community of Madrid began, between December 2020 and February 2021, vaccination in health centers in the region. An enormous effort was deployed to vaccinate both the institutionalized population in their residences and the non-institutionalized elderly dependents in their homes. On February 25, vaccination against COVID-19 began in health centers for people over 80 years of age. The different age and comorbidity risk groups were progressively vaccinated, following national and international bioethical criteria for efficacy and safety. After vaccinating the most at-risk patients, vaccination of the rest of the population began. Three mass vaccination centers were opened (HEEIZ, Wizink Center and the Wanda Metropolitano soccer stadium), where, by means of a prior self-appointment request via internet, vaccination could be carried out, first during daytime hours and from July 2021, 24 hours a day [24]. The vaccination process began with mRNA vaccines (mainly Pfizer and to a lesser extent Moderna), to which the AstraZeneca vaccine was added at the end of February and the Janssen vaccine in April. Due to international reports of thrombotic side effects with the use of these last two vaccines, at present, practically all vaccination is being carried out with mRNA vaccines. By August 23, 2021, of the 8,800,000 doses delivered, more than 7 million will have been with mRNA vaccines. On this date, the vaccination percentage of the population is 67%.

INFRASTRUCTURE AND HUMAN RESOURCES

Already in the first week of March 2020, the hospitals had created Covid-No Covid care circuits to guarantee the safety of healthcare workers and patients. From the third week onwards, the healthcare capacity of the Community of Madrid was exceeded and these care circuits were insufficient. It was necessary to set up medicalized infrastructures to support a growing demand for patients. The most effective model for a temporary hospital was the reconversion of an existing structure with the capacity to accommodate crowds, the IFEMA fairgrounds [25]. Fairgrounds are usually built in large, well-connected areas for patient transport and clinical logistics. They have ample spaces that include administration areas, restrooms, air conditioning, pre-installations to incorporate portable equipment, telephony, and wired and Wi-Fi Internet connection. They usually have high ceilings to better control air recirculation and spaces can be set up for patient, bed and trolley passage areas, as well as for dirty waste disposal circuits. In addition, areas can be set aside for patients to walk around, reducing the risk of thromboembolic disease, and where they can control desaturation themselves. All this deployment is quick and easy to set up. The main need in COVID-19 patient care was the high need for oxygen, to ensure optimal ventilatory therapy for all admitted patients. To ensure ventilatory support, more than 25 km of soldered copper tubing circuit was installed and connected to external oxygen towers. This operation was completed in 72 hours. During the period in which the IFEMA hospital was open (March-May 2020), 3,817 patients were hospitalized with mild to moderate grade, with Charlson Comorbidity Score scores between 0 and 3. 91% had a diagnosis of pneumonia (53% were bilateral) and an oxygen saturation of less than 91% [25].

The major healthcare challenge is the construction of new structures designed specifically for the treatment of infectious diseases. Examples are centers such as Huoshenshan or Leishenshan in Wuhan, built in 10 days and with a capacity for more than 2,000 people, the one in Zhengzhou with a capacity for 800 or other smaller ones such as Fuqing or Weihai with less than 400 [26]. The design of these hospitals contemplates the sectorization by wards and Halls of the care areas, the access circuits and the pre-installation of any clinical equipment, beyond those necessary for patient care. This allows the reuse of the facility for other public health purposes, from vaccination, rehabilitation or radiological screening centers to the installation of hybrid operating rooms to alleviate the surgical waiting list. This versatility has been developed in the Hospital de Emergencias Enfermera Isabel Zendal (HEEIZ) in Madrid, completed at the end of the second wave, with 1,056 beds, 20 of them for critical care and another 30 for convertible semi-critical care. It has a laboratory, radiology area, heliport and easy road access [27]. From December 2020 to April 2021, more than 5,500 patients with bilateral pneumonia and respiratory failure have been treated. A total of 16.4% required admission to semi-critical care and 4.1% to ICU (data not published). At present, it is a COVID monographic center, which offers excellent performance in ventilatory support, however, it is designed for multipurpose functions and represents the paradigm of adaptability in healthcare and an investment in public health.

The Regional Public Health Laboratory (LRSP) is a service dependent on the General Directorate of Public Health (DGSP) and its mission is to provide analytical and technical support to the Public Health Programs in the field of public health protection and surveillance. For more than 20 years it has been carrying out this activity through microbiological and physicochemical analyses of water and food, as well as clinical samples related to epidemiological surveillance. The pandemic situation forced to increase the performance of the Clinical Microbiology section, to carry out seroprevalence studies

in Sociosanitary centers and other fragile populations, for the microbiological diagnosis and control of outbreaks in the out-of-hospital context and for the implementation of novel diagnostic techniques that could modulate the evolution of the pandemic. The number of technicians and the number of work shifts were increased, but soon the capacity of the facilities was exceeded, and a new headquarters had to be built to meet the growing need for microbiological results and the other functions of the LRSP. For this purpose, the project for the construction of a new headquarters was undertaken, on an area of 2,400 m^2 included in the HEEIZ complex, which was also completed within 100 days.

Human resources are as important as material resources, both for patient treatment and epidemiological control. Between September and December 2020, 11,324 new positions were created in the CM to strengthen the fight against COVID (1,067 medical staff, 5,063 nursing staff, 3,274 auxiliary staff and 1,247 non-health professionals) [28]. In addition, screening activity was boosted in September with 456 new tracers, so that by the end of the year the number of these positions, primary care, Army, and call-centers, amounted to 1,590 (approximately 1 per 4,264 inhabitants).

SEARCH FOR PREDICTIVE TOOLS FOR CHANGES IN THE BALANCE OF CONTAGION

As a complement to the analysis of data from diagnostic tests and confinement, it is necessary to have some predictive tool for changes in incidence, to establish public health strategies and to optimize health care resources in case of a reoccurrence of cases.

Since April 2020, Canal de Isabel II launched an intensive wastewater analysis initiative to monitor the presence and evolution of SARS-CoV-2 in the Community of Madrid, as an early ecological indicator to monitor behavioral changes in the ecosystem. A total of 289 wells of the water network were analyzed to represent the entire population. Each week, samples were taken from all sampling points and analyzed for SARS-CoV-2 concentration (gc/L, genome copies per liter) and physicochemical parameters are also analyzed to validate or rule out what in principle could be an unusual presence of the virus in two different laboratories. The presence and evolution of SARS-CoV-2 in wastewater correlated significantly with 14-day incidence rates and COVID-19 hospitalizations. This information is shared daily with health authorities for advice and decision making [29]. Similarly, other parameters continue to be explored as predictors of changes in disease incidence and its impact on the healthcare system. The threshold cycle of detection of SARS COV 2 on PCR could be one of them. It has been suggested that it could be related on admission to the clinical course and prognosis of the patient with COVID-19 [30]. Its role as a predictor of admission is yet to be confirmed.

Despite all the material and human efforts, the incidence peaks and the onset of new waves continue to be experienced. Demographic movements and the relaxation of prevention measures, especially during holiday periods, in a population already in need of mobility, favor recombination and the development of viral variants. The most direct consequence is the overload of care, especially in primary care. Therefore, it is so important to achieve herd immunity and to carry out epidemiological surveillance of variants by sequencing.

CONFLICTS OF INTEREST

FJC, JSR y PB work part-time as advisors to Council of Health. Madrid. FJMP is Director of Social and Health Coordination, Madrid. JVC is General Director of Health. Madrid. FPR is General Director of Infrastructures. Madrid. JC is Director Director General of Hospitals. Madrid. AZ is Vice-Council of Health. Madrid.

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Evaluation questionnaire

XI Updating Course of Antimicrobials and Infectious Diseases 2021

1. Regarding the genomic surveillance of antibiotic resistance, point out the false statement:

a) It provides a great capacity to improve the study of outbreaks and the surveillance of multidrug-resistant microorganisms.
b) It improves the understanding of bacterial evolution and dissemination.
c) It is already fully incorporated into the surveillance of most pathogenic microorganisms.
d) The ECDC enhances the integration of genomic typing in surveillance through a strategic framework.

2. ¿which of the following is not an objective of the Laboratory Network for Antibiotic Resistance Surveillance (RedlabRA):

a) Obtain a complete and quality microbiological diagnosis in cases of infection and/or colonization by resistant microorganisms under surveillance.
b) Genomic characterization of all antibiotic resistant bacteria.
c) Standardize procedures for detection and characterization of resistance mechanisms.
d) Establish mechanisms for the exchange of information among the laboratories of the network according to the priorities to be established.

3. The coordination of RedlabRA:

a) It is performed by the Ministry of Health.
b) It is exclusively in the hands of clinical microbiologists.
c) It is multidisciplinary and directed by the National Antibiotic Resistance Plan.
d) It is multidisciplinary and directed from the National Center of Microbiology.

4. Mark the correct answer regarding bioinformatics applied to the study of antimicrobial resistance.

a) It is unique to genomic studies
b) Allows a perfect correlation between the genome and the antimicrobial susceptibility profile.
c) Does not allow analysis of resistance mechanisms by MALDI-TOF.
d) None of the above is true

5. Which of the following would not apply to the monitoring and follow-up of carbapenemase-producing *Enterobacteriaceae* (CPE)?:

a) Massive sequencing or ultrasequencing (WGS or NGS)
b) MLST
c) Analysis of outer membrane protein profiles (OMP)
d) Bacterial proteome study

6. Indicate the correct answer regarding bacterial genome databases:

a) It is a tool with little utility in a clinical microbiology laboratory.
b) Allows identification and tracing of the mobility of specific pathogens.
c) There is no national or international initiative in this regard due to the high cost and specialization required.
d) No plasmid information can be stored

7. Of the following statements, indicate the correct one:

a) The stability at room temperature of all antibiotics for parenteral use is known with accuracy.
b) There is no experience with continuous infusion administration of antibiotics in patients cared for at home.
c) There are studies showing that self-administration of parenteral antibiotics on an outpatient basis is a safe practice.
d) Multidrug-resistant microorganisms should not be treated in hospitalization at home due to the risk of infecting cohabitants.

8. According to data from the TADE Registry, which of the following statements is false in relation to the resistance of microorganisms causing infections that are treated in hospitalization at home:

a) The percentage cure rate of improvement of *Escherichia coli* resistant to ceftriaxone is 94%.
b) The percentage of *Staphylococcus aureus* resistant to cloxacillin is less than 10%.
c) Up to 25% *of Pseudomonas aeruginosa* is resistant to imipenem.
d) *Klebsiella pneumoniae* shows resistance rates to amoxicillin-clavulanic acid close to 50%.

9. In relation to the use of antimicrobials for the treatment of infections in hospitalization at home, it is true that:

a) The antibiotic with the broadest spectrum of activity should always be used to ensure that there is no therapeutic failure.
b) Whenever possible, a once-daily antibiotic should be chosen to facilitate hospital discharge, regardless of its spectrum of activity
c) PK/PD ratios should be taken into account.
d) Antibiotics cannot be administered every 8 hours if they are not stable at room temperature.

10. All of the following statements about serology for SARS-CoV-2 in COVID-19 are correct EXCEPT:

a) It is useful in Acute Infection
b) It is useful in past infection
c) It is useful in Seroprevalence Studies.
d) Anti-S (Spike) titters correlate with neutralizing activity.

11. The main target of the neutralizing antibodies in COVID-19 is:

a) N protein (nucleoprotein)
b) E protein (envelope)
c) RBD region of protein S (spike)
d) Viral RNA

12. Vaccination against COVID-19 (mRNA vaccines or Spike expressing Adenoviruses) induces all of the following EXCEPT:

a) Anti-S antibodies
b) Anti-N antibodies
c) Anti-S specific CD4 response
d) Anti-S specific CD8 response

13. Indicate the true answer about SARS-CoV-2 detection:

a) PCR is more sensitive than TMA.
b) Antigen detection tests are useful after 5 days of symptoms.
c) Antigen detection tests are usually positive when the cycling threshold (CT) of PCR is low
d) Antigen detection tests are more useful in asymptomatic patients than in those who are asymptomatic.

14. What has been demonstrated in case of reinfections?

a) Infectivity is lower if antibodies persist.
b) Reinfections are not possible in the presence of antibodies.
c) No mutations causing escape from humoral immunity have been described.
d) The presence of antibodies is associated with more severe reinfections.

15. ¿ What condition allows isolation to be lifted in a patient with a first positive PCR?

a) Determination of low cycling threshold (CT)
b) Negative Ag test
c) Positive IgG
d) Absence of symptom

16. Regarding the significance of Ct in the molecular detection of SARS-CoV-2 indicate the correct answer.

a) An elevated Ct value (> 30) should be used as a criterion for de-isolation as it rules out the patient being infective.
b) Ct is an objective value, easily measurable and interpretable.
c) Ct value depends on sample quality, amplified region and assay used.
d) All molecular techniques report the Ct value.

17. Indicate the correct answer regarding the molecular detection of SARS-CoV-2

a) Rapid rRT-PCR (15 minutes) is the technique of choice for population screening.
b) Transcription-mediated amplification (TMA) is not recommended for the diagnosis of SARS-CoV-2 as it is not considered a PCR technique.
c) Multiple rRT-PCR allows the detection of the different variants of epidemiological interest.
d) Mass sequencing is the technique of choice for epidemiological surveillance.

18. Regarding the use of rRT-PCR as a tool to control SARS-CoV-2 infection, which statement is correct?

a) rRT-PCR is not a good follow-up technique.
b) To de-isolate a patient it is necessary that the rRT-PCR is negative.
c) A post-diagnostic control should always be performed 10 days after diagnosis.
d) It is necessary to perform rRT-PCR screening systematically to social-health personnel to avoid nososcomial outbreaks.

19. In relation to the tools for pandemic management, point out the false answer

a) In low prevalence settings or when there is little suspicion of SC2 infection, rapid antigen tests are associated with a high negative predictive value, thus reliably ruling out infection.
b) Perimetral containment is performed by basic health zone (BHZ). Sometimes not all municipalities or districts of the BHZ are confined.
c) Antigen tests better detect patients with higher Ct.
d) SARS-CoV-2 viral concentration in wastewater is 48-72 hours ahead of changes in incidence density.

20. Which of the following microorganisms is not sensitive to ceftaroline?

a) Methicillin-resistant *Staphylococcus aureus*
b) Penicillin-resistant *Streptococcus pneumoniae*
c) Coagulase-negative *staphylococcus* resistant to oxacillin
d) Extended-spectrum beta-lactamase-producing *Escherichia coli*

21. In patients with community-acquired pneumonia ceftaroline has demonstrated:

a) Similar clinical efficacy to ceftriaxone.
b) A higher rate of adverse effects than ceftriaxone
c) A higher clinical cure rate than ceftriaxone
d) A higher mortality rate than ceftriaxone

22. What is the spectrum of ceftobiprole?

a) Gram + cocci including methicillin-resistant *Staphylococcus aureus* and *Enterococcus faecalis*
b) Non BLEE-producing *Enterobacteriaceae*
c) Pseudomonas aeruginosa, although between 60 and 70% in our environment.
d) All of the above are true

23. For what indications is ceftobiprole approved in Spain?

a) Skin and soft tissue infections
b) Community pneumonia
c) Nosocomial pneumonia excluding that associated with mechanical ventilation
d) b and c are true answers.

24. In relation to the in vitro activity and sensitivity studies of Tedizolid against the following microorganisms, we can treat the clinical infections they cause, except for one of them:

a) Mycobacterium spp.
b) *Staphylococci* resistant to linezolid due to mutations in 23S rRNA.
c) *Corynebacterium* spp.
d) *Listeria monocytogenes*

25. In a recent retrospective multicenter study of real-life uses of tedizolid lasting more than 6 days in 81 patients, the following facts have been observed:

a) Frequent off-label indications (osteoarticular, respiratory infection).
b) Important use of linezolid to avoid toxicities or pharmacological interactions.
c) Lower rates of serious adverse effects (gastrointestinal, myelotoxicity) than with linezolid.
d) All of the above

26. Which of the following answers about dalbavancin is true?

a) It is a glycopeptide
b) It is a lipopeptide
c) It is a lipo-glycopeptide
d) It is eliminated mainly by the hepatic route.

27. Which of the following properties about dalbavancin is false?

a) Its spectrum is limited to gram-positive cocci
b) It is a broad-spectrum antibiotic that includes gram-positive and gram-negative bacteria.
c) It is the antistaphylococcal with the highest intrinsic activity.
d) Methicillin resistance does not affect it.

28. Which of the following answers about dalbavancin is false?

a) It has a very long elimination half-life that allows weekly administration.
b) It is a very appropriate antibiotic for sequential therapy in severe infections that require prolonged treatment once stabilized.
c) It allows shortening hospital stay and reducing costs.
d) It is a nephrotoxic antibiotic.

29. Which of the following statements regarding ceftazidime-avibactam is false?

a) The elimination half-life of avibactam is longer than that of tazobactam.
b) More than 95% of enterobacteria isolated from clinical samples are sensitive to ceftazidime-avibactam.
c) Pseudomonas aeruginosa is often resistant to ceftazidime-avibactam.
d) The association of ceftazidime-avibactam with meropenem can be synergistic.

30. Which of the following statements regarding ceftazidime-avibactam is false?

a) It is a good alternative for the empirical treatment of gram-negative bacilli infections.
b) It is active against anaerobic microorganisms.
c) It can reduce the presence of carbapenemase-producing enterobacteria in the intestinal microbiota.
d) It is not active against methicillin-resistant *Staphylococcus aureus*.

31. What is the mechanism used by cefiderocol to penetrate inside the bacteria to its pharmacological site of action?

a) It binds to the Angiotensin Converting Enzyme type 2 (ACE-2) receptor of the bacterial wall.
b) It acts as a siderophore.
c) It binds to the cell receptor containing sialic acid through a radical of its molecular structure called hemagglutinin.
d) It acts by binding to the teichoic acids of the outermost part of the peptidoglycan layer of the bacterial wall.

32. Point out the incorrect answer about cefiderocol:

a) It is not indicated in Gram-positive bacteria infection.
b) It presents activity against multiresistant Gram-negative bacteria that produce cAMP, BLEE and carbapenemase.
c) It is not indicated for use in upper urinary tract infections.
d) The recommended dose is 2 grams every 8 hours in a 3-hour infusion.

33. Regarding the isothermal amplification techniques (LAMP) for microbiological diagnosis, it is true that:

a) It is not possible to perform them directly on clinical samples.
b) The time required in the laboratory is longer than that of PCRs.
c) They can be used for the detection of *Pneumocystis jirovecii*
d) Inhibition is more frequent with these techniques than with PCR.

34. Immunochromatography tests performed on saliva samples have proven to be useful for the diagnosis of:

a) Hepatitis A
b) Hepatitis B
c) Hepatitis C
d) None of the above is true

35. With regard to the activity of beta-lactams against *Streptococcus pyogenes*:

a) In Spain, the resistance of *S. pyogenes* to beta-lactams is 2-4%.
b) Decreased sensitivity to beta-lactams has been described due to mutations in the pbp2x gene.
c) Resistance is due to beta-lactamase production.
d) Currently, no *Streptococcus pyogenes* strains with decreased sensitivity to beta-lactams have been described in the world.

36. In the ASPEIN study on *Aspergillus* in Spain, in the samples analyzed from 30 hospitals and the Spanish reference center, which antifungal drug had the highest sensitivity (no resistance was detected) against *Aspergillus fumigatus* sensu stricto (excluding cryptic species)?

a) Posaconazole
b) Isavuconazole
c) Amphotericin B
d) Voriconazole

37. In patients undergoing CAR-T (chimeric antigen receptor CD19 T-lymphocyte antigen) therapy, all of the following opportunistic infections are important, but which is the most common to monitor early?

a) Bacteremia due to gram-positive pathogens
b) Herpes simplex type 1 infection
c) *Aspergillus fumigatus* infection
d) Diarrhea by *Clostridium difficile*

38. Indicate which of the following statements regarding the treatment of methicillin-resistant *S. aureus* bacteremia is true:

a) The combination of daptomycin and fosfomycin is more eradicative and with fewer adverse effects than daptomycin alone.
b) Associating cloxacillin with vancomycin or daptomycin prevents relapses or treatment failures.
c) Intermittent therapy with vancomycin is safer and easily reaches the target concentration than continuous therapy.
d) The best PK/PD parameter to maximize clinical efficacy and minimize toxicity of vancomycin is the 24-hour area under the curve concentration.

39. Which of the following conditions a worse prognosis a priori in a patient affected by COVID?

a) Lymphopenia
b) Age > 65 years
c) Fibrinogen elevation
d) C-reactive protein elevation.

40. Which of the following statements is not true?

a) COVID pneumonia has a higher mortality rate than community-acquired pneumonia
b) The decision to discharge from COVID pneumonia cannot be based solely on PSI.
c) COVID pneumonia is an absolute criterion for hospital admission
d) COVID pneumonia usually has a bilateral interstitial pattern.

41. The "Predicovid" prediction model for risk stratifying SARS-CoV-2 infected patients includes all but one of the following parameters. Point it out:

a) Lymphopenia
b) C-reactive protein
c) Renal clearance
d) Dementia

42. Regarding treatment with corticoids in COVID-19, indicate the false answer:

a) Dexamethasone at a dose of 6 mg/day for 10 days vs. placebo decreases mortality in hospitalized patients requiring oxygen therapy.
b) CT findings with ground glass lesions and postmortem anatomopathologic pulmonary studies compatible with diffuse alveolar damage and acute fibrinous organizing pneumonia support that response to corticosteroids improves the prognosis.
c) Methylprednisolone is able, through its binding to glucocorticoid receptor alpha (GC-GR-), to decrease the activity of NF-kappaB in the production of mediators of inflammation, coagulation and fibroproliferation.
d) Corticoids in invasively ventilated patients decrease the days of mechanical ventilation but increase mortality at 6 months.

43. If you have to mechanically ventilate invasively a patient with severe respiratory failure due to SARS-Cov-2 pneumonia/SDRA who arrives at the ED on the tenth day of evolution, indicate the best therapeutic decision in February 2021:

a) I will start 100 mg/day of hydrocortisone
b) I will start dexamethasone at a dose of 6 mg/day
c) Given the high possibility of reactivating the virus, I will not initiate steroids.
d) I will initiate dexamethasone 6 mg/day if the patient is younger than 60 years old

44. In a 50-year-old male patient, with no medical history, with SARS-Cov-2 pneumonia and baseline SatpO2 < 88% on arrival at the emergency department, who maintains a good level of consciousness, indicate the best therapeutic decision:

a) I will initiate oxygen therapy with ventimask and if I do not achieve a SatpO2 >90% I will switch to noninvasive mechanical ventilation.
b) I will start oxygen therapy with ventimask and if I do not achieve a SatpO2 >90% I will switch to high-flow nasal cannulas, placing him in an isolation room and instructing the nurse not to visit him more than once per shift to avoid contagion.
c) He requires early invasive mechanical ventilation to avoid self-induced lung injury.
d) I will start oxygen therapy with ventimask and if I do not achieve a SatpO2 >90% I will switch to high flow nasal cannulas monitoring his clinical situation and IROX.

45. Which of the following is not a criterion for the administration of remdesivir?

a) Need for mechanical ventilation.
b) Administration in the first 10 days of the infection.
c) Need for low-flow oxygen therapy.
d) Respiratory rate of 24 rpm and SpO2 94% in room air.

46. Which of the following immunomodulators is accepted to be effective in reducing mortality in patients with COVID-19?

a) Corticosteroids
b) Intravenous immunoglobulin
c) JAK inhibitors (baricitinib)
d) IL-6 inhibitors (tocilizumab)

47. One of the following antiviral drugs is under evaluation for the treatment of COVID-19:

a) Lopinavir/ritonavir
b) Ribavirin
c) Oseltamivir
d) Favipiravir

48. Herd immunity depends on:

a) Natural immunity
b) Vaccine coverage and efficacy
c) On the transmission force of the corresponding infection
d) All are true

49. Which of the following vaccines uses as vector to induce the immune response the non-replicating adenovirus 26 that carries the S protein of SARS-CoV2?

a) The BioN Tech-Pfizer vaccine
b) Moderna's vaccine
c) J&J/Janssen vaccine
d) The Oxford/Astra Zeneca vaccine

50. Which of the following vaccines uses mRNA encoding protein S encapsulated in lipid nanoparticles to induce the immune response?

a) BioN Tech-Pfizer vaccine
b) The Moderna vaccine
c) The Curevac vaccine
d) All of the above

51. Which of the following vaccines uses as vector to induce the immune response the non-replicating chimpace adenovirus that carries the S protein of SARS-CoV2?

a) The BioN Tech-Pfizer vaccine
b) Moderna's vaccine
c) J&J/Janssen vaccine
d) The Oxford/Astra Zeneca vaccine

52. Indicate the true answer concerning antimicrobial prescribing in the hospital setting during the first peak of the pandemic

a) There was an overall increase
b) There was an overall decrease
c) There was no significant change in prescribing
d) It has not been possible to perform an analysis of prescribing.

53. Indicate the true answer regarding antimicrobial prescribing in the out-of-hospital setting during the first peak of the pandemic

a) There was an overall increase
b) There was an overall decrease
c) There was no significant change in prescribing
d) It has not been possible to perform an analysis of prescribing.

54. The COVID-19 pandemic and the use of antimicrobials in the hospital setting

a) Has not had an impact on nosocomial infection
b) It has been correlated in some centers with outbreaks of multidrug-resistant bacteria.
c) has had an impact on the increase in the spread of resistance mechanisms to new antimicrobials
d) b and c are true

55. The phenomenon of viral interference refers to:

a) The potentiation of the virulence of two viruses when they simultaneously infect a patient
b) The phenomena of suppression and exclusion of the superinfection of two viruses when they simultaneously infect a patient.
c) Interference caused by co-infection of two viruses in diagnostic molecular tests.
d) The modification of the epidemiology of a disease when coinfection by two viruses occurs.

56. What is the most frequent etiology of post-covid pneumonia in our environment?

a) Another virus
b) A filamentous fungus
c) A bacterium
d) A yeast

57. Which of the following factors has NOT been associated with an increased risk of respiratory co-infection in the covid19 patient?

a) A lymphopenia below 700
b) A PaO2FiO2 above 200
c) An ICU admission less than 48h after hospital admission
d) All of them have been associated with an increased risk of respiratory co-infection in the covid19 patient.

58. Aspergillosis in COVID patients....

a) Is diagnosed mainly in elderly patients
b) It is diagnosed in critically ill patients admitted to the ward or ICU
c) It is mainly diagnosed in intubated patients, who have received corticosteroids and have previous pulmonary diseases.
d) It usually affects any patient profile.

59. The diagnosis of CAPA (COVID-19 associated pulmonary aspergilosis) is mainly made by....

a) Cultures
b) Beta-glucan
c) galactomannan
d) PCR

60. The treatment of CAPA (COVID-19 associated pulmonary aspergilosis) ...

a) Should be early
b) Should be initiated upon suspicion of IFI
c) Isavuconazole is a reasonable therapeutic option
d) All are correct

61. One of the following statements is incorrect regarding ceftolozane tazobactam

a) It is active against multidrug-resistant Pseudomonas aeruginosa
b) It is synergistic with colistin.
c) It can be used in complicated intra-abdominal infection.
d) It is not active against BLEE enterobacteria.

62. One of the following statements is not correct regarding ceftolozane tazobactam

a) It is indicated in infections by enterobacteria BLEE
b) The dose in pneumonia is 1 g / 8h.
c) It can be used in complicated urinary tract infection.
d) Mutations in the amp-C gene confer resistance.

Correct answer sheet

XI Updating Course of Antimicrobials and Infectious Diseases 2021. Correct answers

	a	b	c	d
1			X	
2		X		
3				X
4				X
5			X	
6		X		
7			X	
8		X		
9			X	
10	X			
11			X	
12		X		
13			X	
14	X			
15			X	
16			X	
17				X
18	X			
19			X	
20				X
21			X	
22				X
23				X
24		X		
25				X
26			X	
27		X		
28				X
29			X	
30		X		
31		X		
32			X	
33			X	
34				X
35		X		
36			X	
37	X			
38				X
39		X		
40			X	
41	X			
42				X
43		X		
44				X
45	X			
46	X			
47				X
48				X
49			X	
50				X
51				X
52	X			
53		X		
54				X
55		X		
56			X	
57		X		
58			X	
59	X			
60				X
61				X
62		X		