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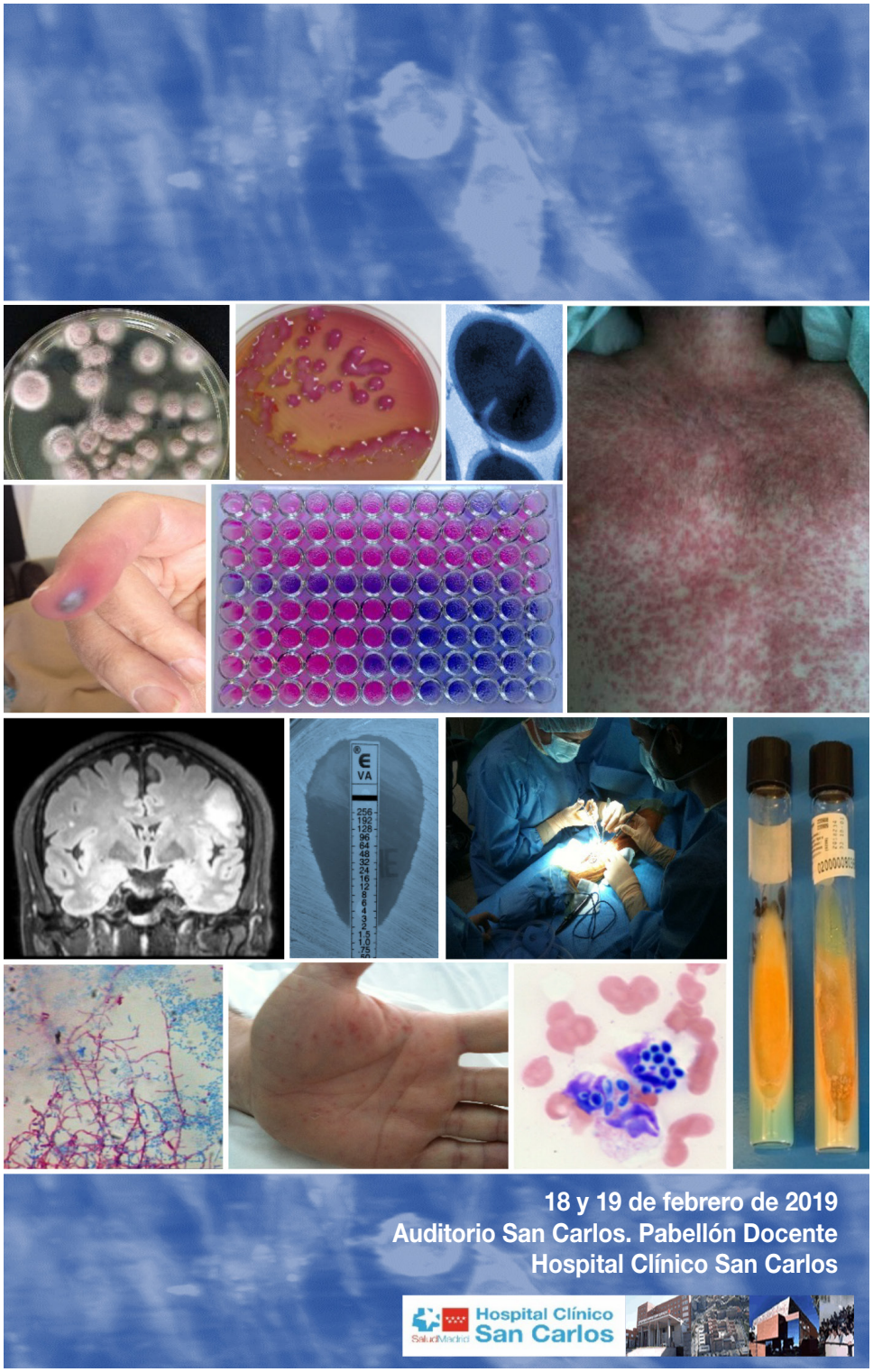
IX Updating Course of Antimicrobials and Infectious Diseases



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Introduction

Update in Infectious Diseases 2019

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ABSTRACT

The IX Updating Course of Antimicrobials and Infectious Diseases included a review of the main issues in clinical microbiology, epidemiology and clinical aspects for a current approach of infectious pathology. The present introduction summarizes about the most important meetings related to infectious diseases during 2018 (ECCMID, IAS, ASM and ID Week). In addition, the course provides a practical information to focus on nosocomial infection models, with immunosuppressed patients or complex multidrug-resistant pathogens. The closing lecture of this year reviewed the infection during donation process.

Key words: Clinical Microbiology and Infectious diseases, current concepts.

INTRODUCTION

Last February, the IX 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 57/094976.9/18, www.infeclinico.es) 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 attracted more than 450 professionals of all specialties related to infection, the teachers made an update of the most relevant aspects on clinical microbiology and infectious diseases.

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Current supplement 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 be able to contrast the results. Revisions have been grouped under 3 headings to guarantee a greater educational character. First of them was an update in infection related meetings during 2018, and we have selected the European Congress of Clinical Microbiology and Infectious Diseases or ECCMID, the American Society of Microbiology Microbe or ASM Microbe 2018, the International AIDS Society meeting or IAS 2018 and the Infectious Diseases Week or ID Week 2018. For the second section, a practice approach of epidemiology and clinical management of nosocomial infections. For the last heading an update in management of immunosuppressed patients. The closing lecture of this year reviewed the infection during donation process.

UPDATE IN INFECTION RELATED MEETINGS DURING 2018

Dr. Emilia Cercenado tried to summarise the ASM Microbe 2018, which took place in Atlanta (GE), focusing on the most important aspects in terms of new techniques of microbiological diagnosis that have improved the diagnosis of infectious diseases, resistance to antimicrobials and new antibiotics. There were 24 plenary sessions, 84 symposia, 25 meet-the-expert sessions, 20 workshops, and more than 2000 abstracts were presented. Among all the new technologies that have been developed for the diagnosis of infections, Dr. Cercenado highlighted the technique ATR-FTIR, a technique to quickly obtain the fingerprint of the whole-organism to allow bacterial identification and discrimination of different subspecies [1]. She also talked about magnetic resonance for detecting microorganisms in clinical samples, as well as laser dispersion for the detection of microorganisms in organic fluids and in the screening of urine samples for the diagnosis of ITUs. Finally, she mentioned Microfluidic [2] and genome sequencing as

very promising techniques. Regarding resistance to antimicrobials, Dr. Cercenado presented the study where the transferable gene *mcr*-1 that confers resistance to polymyxins was first described in China in 2015 [3]. In another study, presented at ASM Microbe, the presence of a chromosomally transferable *mcr*-5 gene was first described in a clinical isolate of *P. aeruginosa* resistant to colistin in the United States. She also dealt with the resistance to carbapenems among *P. aeruginosa* isolates, which, although it is generally chromosomally encoded, several studies presented at the ASM Microbe conference describe an increase in the appearance of plasmid resistance and transferable carbapenem between this species. Finally, new families of antimicrobials are emerging with new mechanisms of action, as well as new drug associations, which are active against multiresistant bacteria. She stressed odilorhabdins; the novel siderophore cephalosporins, such as GT-1; the new tetracycline eravacycline; and other antibiotics or antifungals recently marketed (delafloxacin; plazomycin, rezafungin).

The last European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) which took held in Madrid (Spain), last April 2018, focused in three different aspects: microbiology diagnosis, resistance to antimicrobials, and new antimicrobials. All of this microbiology diagnosis techniques were summarized by Dr. García-Lechuz [4]. The (MALDI-TOF MS) is a primary method [5] for the identification of microorganisms, that only requires little amount of bacteria and allows high-throughput (Rodriguez-Sánchez B, et al; P2236). An interesting experience in identification of non-tuberculous Mycobacteria isolates was presented by Rodriguez-Sánchez B, et al; P2405. Another technique like PCR-MALDI could replace current real-time PCR technology detecting bacterial (Green J, et al; P2376) and fungal species. Lastly, there were some experiences with Sepsis Flow Chip (SFC) assay, based on multiplex PCR and low-density DNA arrays, detecting Gram-positive and Gram-negative bacteria and fungi, and, in the same assay, the most common antibiotic resistance genes [6]. The AMR Direct Flow Chip assay (Galiana A, et al; P2288) detects the main genetic resistance determinants in a single step. This assay was compared to next-generation sequencing (NGS) techniques and showed sensitivity and specificity values close to 100%.

The immunochromatographic tests (ICT) are a good option and has been recently developed for Carbapenemase-producing *Enterobacteriaceae* (CPE) detection from cultures on solid media. This can help to rapidly identify patients with CPE BSI (Bloodstream infections), optimize the treatment of patients and reduce the mortality. The NGS analyze the entire human genome or to sequence thousands of genomes (Vincent AT et al, 6). Sanger sequencing and NGS can be used for detection of HIV drug resistance mutations (García-Arata MI, et al; P1902). With NGS you can have the results in three labor days and for a low price. The findings made NGS an effective new strategy and a useful tool in the detection of HIV resistance.

The antimicrobial resistance was also reviewed by García-Lechuz. The multidrug-resistant Gram-negative bacteria is a worldwide problem. Colistin is one of the last resort antimicrobials for the treatment of infections caused by multidrug-resistant Gram-negative bacteria but in recent years, the resistance is increasing, [7, 8]. In a study presented by Mendes AC, et al (P0417) there were isolates of *Klebsiella pneumoniae* producing KPC-3 and mcr-1, surviving after polymyxin treatment in vitro and in vivo. One study analyzed the impact of the mechanism of resistance to carbapenems in Gram-negative on mortality. The highest crude mortality was observed in *K. pneumoniae* (KPC and OXA-type had higher mortality than metallo-betalactamases (MBL)) followed by *Acinetobacter baumannii* (OXA-type was higher than MBL) and *Pseudomonas aeruginosa*. (Pezzani MD, et al; P1052).

Emergence of ceftolozane-tazobactam resistance is caused by structural mutations in intrinsic (AmpC) or acquired (OXAs) beta-lactamases. Other resistance mutations include specific large chromosomal deletions and PBP3 mutations (Oliver A; S0387). Ceftazidime-avibactam resistance appear after mutation in KPC-2 or KPC-3 (Humphries RM; S0386). Animals like cows, pigs, veals, calves and poultries can act as reservoirs of antimicrobial resistance genes. Colistin-resistant *E. coli* from animals may represent a potential risk to human health (Lei L, et al; O1050). Among new antimicrobial agents was important to mention the FDA approved delafloxacin, meropenem-vaborbactam and other antimicrobial agents are in End-stage clinical development like cefiderocol, eravacycline, imipenem-relebactam, omadacycline or plazomicin.

There are many studies, clinical trials, prospective studies to show us the new antimicrobial agents' effect. For example, the phase III clinical trials IMPACT 1 and 2, analyzed efficacy of oral cadazolid versus vancomycin. Cadazolid showed no inferiority and was safe, well tolerated and could potentially be an alternative therapy for *Clostridium difficile* infection. In the study REVIVE-2 (O0424) iclaprim was non-inferior to vancomycin. In the OASIS-2 phase III clinical trial (O0425), Omadacyclin was non-inferior to twice-daily oral linezolid in the treatment of adults with skin and soft tissue infections. Against multidrug resistant Gram-negatives, the clinical trial (RESTORE) (O0427) compares imipenem-relebactam versus colistin and imipenem for *Pseudomonas* spp and *Klebsiella* spp infections. The patients treated with imipenem-relebactam had a favourable overall response. In the other side, in TANGO II study, meropenem-vaborbactam was associated with increased clinical and microbiologic cure. The new agent cefiderocol, has a great activity against carbapenem-resistant *Enterobacteriaceae* and meropenem-resistant *Pseudomonas* spp, showed no inferiority in the phase III APEKS trial in complicated urinary tract infection cUTI. The antipseudomonic agent, murepavadin, showed great activity against *Pseudomonas* spp in HABP/VABP phase II clinical trial. Eravacyclin showed similar results than meropenem or ertapenem in the IGNITE trials (O0421). Related to community-acquired infections, lefamulin (phase III clinical trial LEAP-1) and omadacyclin (phase III clinical trial OPTIC) were compared with moxifloxacin, with non-inferiority results including the PORT risk class III to V (P0276). The Merino trial, comparing piperacillin-tazobactam and meropenem for treating blood stream infections, showed no differences in microbiological eradication and test of cure between the two groups

but the difference in mortality rate was significantly lower in meropenem branch.

ID Week is an annual scientific meeting of the Infectious Diseases Society of America, the Society for Healthcare Epidemiology of America, the HIV Medicine Association and the Pediatric Infectious Diseases Society. ID Week 2018 was held in October in San Francisco. Dr Emilio Bouza made a selection of symposia, reunions and abstracts that drew his attention. He pointed out the conference was focused on medical education and updating on the attendees and the event on topics like adult infectious disease (ID), pediatric ID, global ID and HIV. Dr Bouza focused attention on some topics from the 74 symposiums: antibiotics policy, new antimicrobials, situation of human microbioma and the outstanding increase of apiaceous use with associated infections. Among the new antimicrobial in research, he mentioned tetracyclines, inhibitors of beta lactamase and a new antifungal, ibrexafungerp, with a new action mechanism.

In the communications section he selected those issues related to *S. aureus*, *C. difficile* community-acquired infections, its overdiagnosis in colonized cases, control of requests trough stewardship, the value of quantifying the PCR tests for *Clostridium difficile* infection (CDI) by evaluating the positivity cycle of the amplification curves, decreasing of relapses with bezlotuxumab, faecal transplant with capsule, Gram-negative bacterial infections and ceftolozano-tazobactam susceptibility *in vitro* of *K. pneumoniae* and *Pseudomonas aeruginosa*, advantages of stewardship, rational antifungal treatment applying T2 Candida testing, asymptomatic influenza, baloxavir marboxil in high risk influenza patients, aspergillosis among patients with influenza, injection opioid drug use as an emerging risk factor for candidemia and *S. aureus* bacteremia. In the conclusions, Dr. Bouza called attention on a more representative presence of infectology over microbiology, the low amount of basic science, and the American opiates abuse concerns.

Last conference was the 22th International AIDS Conference and was summarized by Dra Núñez-Orantos. In this conference Dra Nuñez highlighted the GEMINI and DIAMOND studies and in the second time the PARTNER study. GEMINI-1 and -2, published by Cahn et al [9], showed that the virologic efficacy of 2-drug regimen of Dolutegravir (DTG) plus Lamivudine (3TC) was non-inferior to 3-drug regimen of DTG plus Emtricitabine (FTC)/Tenofovir disoproxil fumarate (TDF) in treatment-naive patients at Week 48. The main objective was to establish the percentage of participants with a viral load below 50 copies/ml at 48 weeks after starting the study. In conclusion, a dual therapy with 3TC + DTG in naive patients could be an alternative to a triple therapy based on TDF + FTC + DTG. DIAMOND Study was a prospective multicenter study evaluating Darunavir/Cobicistat/Emtricitabine/Tenofovir Alafenamide (D/C/F/TAF) in a rapid initiation model of care over 48 weeks. In this trial, a high proportion of patients using D/C/F/TAF achieved HIV-1 RNA <50 copies/ml. No patients discontinued treatment. The results of the mean HIVTSQs score indicated a high level of satisfaction. These findings suggest that D/C/F/TAF should be considered an adequate option of treatment.

The PARTNER2 study shows in serodifferent men having sex with men (MSM) couples reporting condomless sex when HIV-positive partner virologically suppressed, Rodger A et al [10], Bavinton BR et al [11]. The main finding of the study was that no within-couple HIV transmissions were observed among 783 serodifferent MSM couples who reported condomless sex while the HIV-positive partner was receiving suppressive ART. This data shows that the risk of HIV transmission from an HIV-positive partner who has undetectable HIV-1 RNA is effectively zero. The PREVENIR study, Molina et al. [12] showed the real-life data of the PrEP (pre-exposure prophylaxis) application in Paris. At an average follow-up of 7 months, the incidence of HIV in both groups was 0, and it was estimated that 85 HIV infections had been prevented.

UPDATE IN NOSOCOMIAL INFECTION

The acronym ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* spp) [13] was coined by US researcher Louis B Rice to designate a particular group of microorganisms that have been mainly associated with nosocomial antimicrobial resistance. In his conference, Dr. Cantón exposed data from the European Antimicrobial Resistance Surveillance Network [EARS-Net] that show an alarming increase of resistance among the ESKAPE bugs in Europe in the recent years [14], with a huge impact in mortality and economic cost of infections caused by these organisms. He focused his speech in the current situation in Spain, particularly in carbapenem resistance; emphasizing the emergence, persistence and rapid dispersion of this resistance mechanism, with a dominance of OXA-48 producing *K. pneumoniae* isolates [15]. Dr. Cantón pointed out the increased number of colonized patients 3 and the presence of multi drug resistance high-risk clones as the main factors contributing to the emergence and spread of carbapenemase producing *Enterobacteriaceae* throughout the country. To end up, he highlighted the appearance of resistance to new β-lactam–β-lactamase inhibitor combinations, showing interest in a recently described KPC mutation that confers resistance to ceftazidime-avibactam while restoring carbapenem susceptibility [16], stressing the need of developing new antibiotic compounds.

Relevant aspects of antibiotic selection in the treatment of acute invasive infections by *P. aeruginosa* were reviewed. Regarding the MIC of antibiotics, it was stated that for the treatment of severe or high bacterial load infections, produced by microorganisms exhibiting MIC ≥4 mg/L of the β-lactam, only elevated doses administered by continuous or extended infusion reach free antibiotic concentrations exceeding 4-times the MIC [17] and that for the aminoglycosides, the greatest efficacy for a treatment is obtained when Cmax/MIC ≥10 [18]. It was also commented that in infections with high bacterial load, an early and rapid ≥ 2 log10 CFU/mL decrease produced by the antibiotic treatment might decrease bacterial density allowing an optimal contribution for microorganism eradication [19]. And to avoid selection of resistant mutants,

antibiotics [like aminoglycoside, ciprofloxacin or levofloxacin] associated with the β-lactam during the first 48-72 h, should be administered at doses achieving concentrations over the corresponding MPCs. The authors exposed that in certain infection sites, the possibility of directly introducing the antibiotic into the infectious foci using the inhalatory, intrathecal or other routes to increase antibiotic concentration in the foci should be considered. The relevance of early administration of an appropriate antibiotic treatment when the infection presents clinical or biological severity criteria, the patient suffers important immunodepression or comorbidities or has advanced age was also highlighted [20]. Finally, the current clinical experience with monotherapy and combination therapy for the treatment of acute invasive infections by *Pseudomonas aeruginosa* was presented.

Doctor Azanza warned in his presentation about the need to review the posology of most anti-infectives. In the past, dosing guidelines were chosen by selecting those with the ability to exceed MIC and based on tolerance criteria. The discovery of the importance of PK/PD relationships highlights the importance of reviewing these posological guidelines. Dr. Azanza talked about the three types of PK/PD relationships that have been established for antibiotics. The first one is the concentration dependent model, which uses the inhibitory coefficient (Cmax/MIC) as a reference parameter. This coefficient indicates that the effect of a drug fundamentally depends on the coefficient between the highest concentration reached and the minimum effective concentration. The drugs that belong to this group show that the higher administrated doses, the greater activity is presented, without the administration interval being especially relevant. Consequently, it is recommended to administer one daily dose. An example of this group of drugs would be aminoglycosides, to which he referred in a study on nephrotoxicity induced by aminoglycosides [21].

The second PK/PD model uses as a defining parameter the AUC / MIC, which takes into account both the MIC and the time period in which the concentration values remain above it. These antibiotics must be administered in a dose that will generate the highest possible plasmatic concentration, and at intervals that avoid the presence of subinhibitory concentrations. This group would include vancomycin, to which he referred in a study on vancomycin-induced nephrotoxicity [22]. The third and last model is the time-dependent model, based only on the time of effectiveness, the time in which the plasma concentration remains above the MIC. The choice of dosage regimen is simple for drugs with high half-life elimination, such as beta-lactams. During his presentation, Dr. Azanza highlighted a study on pharmacokinetics and pharmacodynamics in beta-lactams [23]. In this PK/PD model, the problem relies on the administration of drugs with short half-lives (less than 2h), which will require many daily endovenous doses.

Catheter-related bloodstream infections (CRBSI) is a common cause of nosocomial infection associated, resulting in substantial morbidity, mortality, increased length of hospital stays and higher health-care costs.

Dr. Garnacho focused her presentation on giving updated recommendations and key aspects concerning to the diagnosis and management of adults with CRBSI, based on a review of the new clinical practice guidelines for the management of this entity, recently published by the Spanish Society of Infectious Diseases an Clinical Microbiology (SEIMC) and the Spanish Society of Intensive and Critical Care Medicine and Coronary Units (SEMICYUC) (24). Some aspects were summarized by Dr. Garnacho, emphasizing the fact that an accurate diagnosis of CRBSI becomes essential because of the serious consequences associated with inaccurate or failed diagnoses.

The guidelines define the clinical characteristics, along with other factors, to establish a clinical suspicion and initiate a microbiological diagnosis, as well as, indicating the conditions needed to consider the CRBSI as complicated. The guidelines also highlight the recommendation that a catheter culture must only be obtained when a CRBSI is suspected, thus avoiding unnecessary cultures [25]. Catheter removal is the most suitable approach for the diagnosis of CRBSI at least in the critical care setting. However, withdraw or replacement of a suspicious central venous catheter may not be feasible in many cases, so then conservative techniques may be employed in the diagnosis. In this regard, Dr. Garnacho made a summary of the main diagnostic methods for CRBSI, such as semiquantitative or quantitative culture of catheter tip, quantitative or differential time to positivity blood cultures, among others. In addition, molecular-based rapid diagnostic testing, which has evolved recently for the early identification of microorganisms involved in bloodstream infections, are contemplated in the cited guidelines due to its usefulness for improving the diagnosis, especially in patients under antibiotic therapy [26, 27]. Regarding treatment, Dr. Garnacho highlighted the importance of choosing the empiric anitimicrobial agents based on an assessment of the risk factors for infection, the severity of the clinical picture and the likely pathogens based on local ecology and catheter site of insertion, as well as the importance of oral sequencing treatment.

Sepsis, that can be defined as a life-threatening organ dysfunction caused by a deregulated host response to infection, is the major cause of mortality from any infectious disease worldwide [28]. Dr. Del Pozo described the importance but also the limitations and challenges, of applying antimicrobial stewardship programs to sepsis. The goals of antimicrobial stewardship are to achieve optimum clinical outcomes, and to ensure cost effectiveness and minimum unintended consequences, including toxic effects, selection of pathogenic organisms, and resistance. The combination of inadequate diagnostic criteria for sepsis, with the extraordinary time pressure to provide broad-spectrum antimicrobial therapy is troubling from a stewardship perspective [29]. There are several challenges to face. Firstly, the diagnosis of severe sepsis may be delayed because physicians or nurses may not identify the progression of sepsis, and/or because some patients may not show obvious systemic manifestations of the process. Secondly, patients may have differences in the timing of their presentation and concurrent conditions confounding the diagnosis.

Thirdly, treatment may be delayed once the diagnosis is made [30]. Another aspect to take into account is the microbiological diagnosis. The first 3-6 hours after the clinical suspicion are critical to establish therapeutic measures that improve prognosis. Therefore, a microbiological diagnosis in less than 6 hours would undoubtedly benefit the optimal management of patients. Unfortunately, rapid molecular-based diagnostic tests usually provide little information on antimicrobial susceptibility. Dr. Del Pozo emphasized that despite all the challenges surrounding antimicrobial stewardship programs when we talk about sepsis, they can lead to significant benefits for clinical outcomes, adverse events and costs. This can be done by adhering to local guidelines for empirical therapy, multidisciplinary bedside consultation, optimized antibiotic dosing, and integration of rapid diagnostic techniques in the decision-making process. Nevertheless, there is still a long way to go on this topic.

Clostridium difficile infection (CDI) is the most common cause of nosocomial antibiotic-associated diarrhea worldwide and additionally due the high risk of recurrence (12%-40%) has led to multiple emergency therapies as fidaxomicin (FDX), faecal microbiota transplantation (FMT) and monoclonal antibodies [31, 32]. Dr. Salavert reviewed new strategies for effective prevention of recurrent CDI (rCDI) and he emphasized that FDX compared to vancomycin treatment, was associated with a lower rate (~50%) of second-occurrence relapses 4 weeks after the infection in patients with no prior episode of CDI. Hence, FDX is recommended from the first episode of infection in patients with recurrence risk factors (elderly people, concomitant antibiotic use and severe underlying disease) [33], but due to its higher cost, this use is reserved for patients with first or later recurrences. Otherwise, FMT has a rate of cure of rCDI about 90% when associated to antibiotic cessation and may be offered to patients with rCDI who have had at least two recurrences, or one recurrence and risk factors for further episodes [34]. However, in Spain it is still not a routine procedure and the potential benefit of FMT in primary CDI remains uncertain. Finally, he explained that a new approach to the prevention of rCDI is the administration of monoclonal antibodies *against C. difficile* toxin B. Bezlotoxumab is the first of this kind and is currently approved for the prevention of rCDI in patients on treatment for CDI and who are at high risk for recurrence [35]. In the near future, some of new molecules (cadazolid, ridinilazole, auranofin and thuricin CD) might be effective alternatives to fight against CDI and prevent more effectively rCDI.

UPDATE ON THE INFECTION OF THE IMMUNOCOMPROMISED PATIENT

Febrile neutropenia (FN) is a common complication in patients with hematologic malignancies receiving chemotherapy and is associated with high morbidity and mortality. Infections caused by multidrug-resistant bacteria represent a therapeutic challenge in this high-risk patient population, Dr. Gudiol reviewed the most relevant issues included in the recently published Consensus Document of the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC) and the Spanish Association of Hematology and Hemotherapy (SEHH) on the management of febrile neutropenia in patients with hematologic malignancies [36]. Stratification of patients should include validated models such as the MASCC index score [37]. Many factors should be considered when choosing empirical antibiotic treatment in patients with FN. These include the risk of infection associated with the severity of neutropenia, possible focus of infection, clinical manifestations (e.g., hypotension, sepsis, septic shock), local epidemiology, previous infection or colonization by multidrug-resistant organisms, previous use of antibiotics, and presence of allergies and potential toxicities. Antibiotic treatment should be selected and modified according to the suspected clinical focus of infection). Furthermore, reducing the exposure to unnecessary antibiotic is a cornerstone in the fight against antimicrobial resistance.

In patients with FN and clinically documented infection, antibiotic treatment can be discontinued when clinical signs and symptoms of infection have resolved and the patient remains afebrile for at least 72 hours [38], avoiding the standard approach of maintenance until neutrophil recovery. Gram-negative bacteria are the leading cause of infection in onco-hematological patients with febrile neutropenia, and emergence of multidrug resistance among these organisms is a matter of concern [39]. The use of a β-lactam with activity against P. aeruginosa is recommended, in monotherapy or in combination with another regimen. Special attention was given to the treatment of extended-spectrum betalactamase-producing *Enterobacteriaceae* (ESBL-E). Beta-lactam and beta-lactam inhibitor combinations (mainly piperacillin-tazobactam) should be considered as carbapenem-sparing alternatives for the treatment of low-risk patients who do not have a high-inoculum infection and present without severe sepsis or septic shock [40]. Extended infusion is strongly recommended [41]. Patients considered to be at low risk for complications can be treated with oral antibiotics and outpatient follow-up after 48-72 hours [42].

In her presentation, Dr. García-Vidal reviewed relevant aspects related to chemoprophylaxis of mould infection. Firstly, she explained that IFI prevention should be made a priority objective in at-risk patient, such as hematopoietic stem cell transplant recipients (HSCT), solid organ transplant recipients (SOT) and patients with hematological malignancies, all those with acute myeloid leukemia. In addition to these classic risk groups, the speaker exposed that the use of novel treatments like immunomodulatory and immunosuppressive agents has increased the risk of IFIs in patients with chronic lymphoproliferative disorders [43]. Secondly, Dr. García-Vidal stated that clinical guidelines for the management of invasive diseases caused by Aspergillus, recently published, recommend posaconazole as a first line antimould prophylactic [44]. In addition, she commented that the pharmacokinetic and pharmacodynamic properties of isavuconazole offer potential for use in fungal prophylaxis, salvage therapy or in combined regimens [45].

Latent infection in patients receiving biological therapies was reviewed by Dr. Fernandez-Ruiz. First, he highlighted the most prevalent latent infection in our area is Tuberculosis, in which the host's adaptive immune system ultimately depends on the dynamic equilibrium between pro-inflammatory and anti-inflammatory cytokines. TNF-α cytokine, exerts a major role in the structural maintenance of tuberculous granulomas, and theoretically the use of agents targeting tumor necrosis factor (TNF-α) increase the risk of reactivation of latent tuberculosis infection (LTBI), and progression to active disease [46]. Nevertheless, it is noteworthy that no cases of tuberculosis were reported in the randomized clinical trials (RCTs), despite the lack of specific risk-minimization measures in these studies [47]. Post-marketing follows up, reported by the FDA, revealed the first cases of adverse events, allowing to delineate the risk of LTBI reactivation in patients receiving TNF-α-targeted therapies [48]. Notice that such risk increase is not uniform across different agents: the use of etanercept is consistently associated with a lower incidence of LTBI reactivation as compared to monoclonal antibodies targeting TNF-α [49]. Moreover, the risk of active tuberculosis also varies according to patient age (with higher incidence in older groups) and the background rate of LTBI in the overall population. Finally, he described different strategies for screening latent tuberculosis infection: the tuberculin skin test (TST) and the interferon (IFN)-γ release assays (IGRAs), the last one has the advantage of better reproducibility and specificity than TST. There is general consensus in performing both tests and, eventually, a chest X-ray examination prior to the initiation of TNF-α-targeted. However, the optimal screening sequence to avoid an unacceptable number of false-positive results is still not well established. Regarding to patients diagnosed with LTBI, tuberculostatic treatment is mandatory and the administration of the anti-TNF-α agent should be delayed for 30-60 days [50]. A 6 to 12-month course of isoniazid monotherapy (300 mg daily) remains as the first-line option, but alternative regimens have been successfully tested in recent trials.

Dr Fernández-Ruiz made a brief mention about reactivation of viral pathogens able to establish chronic or latent infection within the host, like Hepatitis B viral infection. This balance between the host's immune surveillance and the virus can be disrupted by immunosuppressive therapy, leading to viral replication that can evolve into life-threatening hepatitis. Mayor risk is clearly associated with the use of anti-CD20 monoclonal antibodies in HBsAg-positive patients, and lower substantially risk is observed among HBsAg-negative/anti-HBc-positive patients ("hidden infection") [51].

Closing Conference was presented by Dr. Len, who provided a general up-to-date overview about the revelation of transplantation, the difference between demand and supply and the need of having a look to marginal donors, who could transmit infections to their recipients. Although the number of patients on the waiting list has more than double since 1998, the number of transplants has increased by only about 30% [52]. In any case, the rigorous examination of the donor to detect latent and active infections is essential to prevent the involuntary use of inadequate organs, to optimize the prophylaxis directed against the infection, the preventive therapy or the surveillance measures of infections after transplant. Dr Len analyze two types of transmission of an infection, the expected one, from the donor to the recipient, in which we have prophylaxis or it's controllable, and the unexpected one, where we don't recognize it before the transplant, usually we do not have effective prophylaxis or treatment and, therefore, it has high morbidity and, even, mortality.

Some problems usually block the efforts to prevent unexpected transmission. There are not universal standards for donor evaluation, sometimes it is difficult to differentiate donor-derived infection from the recipient itself, and not all cases of donor-derived infection are published [53]. On the other hand, the causes of unexpected transmission of the infection are, in first place, asymptomatic latent infection not diagnosed in the donor. Considering the current migratory movements, we should not neglect the screening of geographically restricted infections [54] and get a good clinical history of the donor. In second place, absence of diagnosis of active infection as death cause, sometimes because of the lack of early diagnosis and targeted treatment [55]. Without forgetting that the donor may suffer an infectious complication during admission to the intensive care unit, not diagnosed prior to transplantation (e.g. occult bacteremia); and in third place, contamination of preservation fluids [56]. Nowadays, thanks to experience gained, better results are being achieved, and to update information, the Spanish National Transplant Organization, has published a consensus document in collaboration with several scientific societies [57], where in order to advance in prevention of donor derived infection we can act on different directions: improving the screening of infections in donors, with faster, more sensitive and specific tests, involving all the professionals (multidisciplinary team), improving communication between all them (coordination, microbiology, transplant teams) in case of recognizing a risk in a specific donor-recipient procedure, without losing time, in transfer information to the rest of the related transplantations, and finally, with standardized and mandatory notification systems to obtain maximum possible information that allows us to pass from unexpected transmission of the infection to preventable one.

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Highlights at the ASM Microbe 2018, Atlanta (USA)

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INTRODUCTION

The meeting of the American Society for Microbiology, ASM Microbe, is a huge meeting that includes all disciplines of Microbiology, from basic science to clinical aspects [1]. The last ASM Microbe held in Atlanta (USA) last June 2018, included current trends regarding antimicrobial agents and resistance, applied and environmental science, clinical infections and vaccines, clinical and public health microbiology, host-microbe biology, microbial ecology and evolution, molecular biology and physiology, and profession of microbiology. There were 24 plenary sessions, 84 symposia, 25 meet-the-expert sessions, 20 workshops, and more than 2000 abstracts were presented as poster presentations or poster talks. Since it is not possible to summarize in a few pages all the aspects covered in this meeting, this minireview will try to summarize, some of the contributions related to microbiological diagnosis, resistance to antimicrobials, and new antimicrobials.

DIAGNOSTIC MICROBIOLOGICAL TECHNIQUES

Over the past decade, the field of clinical microbiology has experienced a significant development due to new technologies that have improved the diagnosis of infectious diseases. Sensitive diagnostic tests that can be performed at the site of patient care (point-of-care testing) with minimal infrastructure, cost, and training, provide a decrease of the time between sample collection and diagnosis [2]. In this setting, loop-mediated isothermal amplification (LAMP) assays can be used as point of care tests. One study presented at this meeting evaluates the diagnostic performance of a LAMP assay combined with lateral flow dipstick (LFD) as point of care method for the diagnosis of tuberculosis (Nimesh M, et al; abstract 6074). The assay, standardized to detected simultaneously the *Mycobacterium tuberculosis sdaA* gene and the rifampicin resistance gene *rpoβ*, was tested in 18 culture confirmed specimens for pulmonary tuberculosis. All the specimens showed a positive result with LAMP-LFD assay. The diagnostic accuracy of the method was also evaluated in comparison with GeneXpert MTB/RIF assay (3) using 107 clinical specimens from patients with clinical symptoms of pulmonary tuberculosis. Out of 107, 15 specimens were positive with both methods showing high concordance and accuracy in comparison to other methods.

Another study evaluates a LAMP assay (TangenDX™ system) for direct detection of *Candida* species from blood (Zannini A, et al; abstract 5352). The assay detects 5 species of *Candida*. After analyzing 43 spiked blood samples, *Candida* species were detected correctly in 96.1% at a pathogen concentration of 2-3 cfu/ml of whole blood. Sample processing time was 5-7 minutes and time to positive results was 70 minutes. The high sensitivity and the small size of this portable device, make this a reliable diagnostic test.

MALDI-TOF mass spectrometry can be used to perform antimicrobial susceptibility testing by the detection of drug hydrolysis. In addition, this system can also detect resistant strains by peak picking approaches [4]. Two studies aimed to detect specific peaks associated with resistance. In one of them MALDI-TOF MS was able to discriminate between vancomycin-resistant *Enterococcus faecium* (VREfm; 91 strains) and vancomycin-susceptible *E. faecium* (VSEfm; 31 strains). Overall, 30 peaks ranging from 2,212 to 10,225 m/z were detected, and 12 of these were exclusively associated with VREfm: four of them were present in 100% and eight were present in more than 90% of the 91 VREfm strains and absent in all 31 VSEfm isolates, demonstrating the ability to discriminate between VREfm and VSEfm isolates (Ribeiro RL, et al; abstract 6119). Other study (Cordovana M, et al; abstract 6403), based on the detection of the carbapenemase KPC-specific peak at 11,109 m/z in the MALDI-TOF MS spectra of *K. pneumoniae*, developed

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specific algorithms for automated detection of KPC-producing enterobacteria (*E. coli, Enterobacter* spp., *K. oxytoca*, *Citrobacter* spp., and *S. marcescens*), showing a high sensitivity (85%) and excellent specificity (99.9%).

Whole-organism fingerprinting by attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy is a rapid (1 minute), reagent-free technique for bacterial identification and classification with subspecies-level discriminatory capabilities [5]. In one study, by analyzing 93 vancomycin-resistant *E. faecium* (VRE) from two clonal groups, according to PFGE typing, the ATR-FTIR spectroscopy was able to classify the organisms as antibiotic resistant, and showed successful discrimination between two VRE clonal groups, yielding 99% concordance with PFGE results. The authors concluded that this technique, that is performed with a portable instrument, can be easily implemented as a typing method suitable for intrahospital surveillance of VRE outbreaks (Tsutsumi T, et al; abstract 8868). The ATR-FTIR spectroscopy was also applied in other study for the discrimination between fluconazole-resistant and fluconazole-susceptible *Candida auris,* and for the discrimination between *C. auris, C. haemulonii* and *C. duobushaemulonii* with 100% correct classification at the species level in comparison with reference methods and discrimination between fluconazole-susceptible and resistant strains (Lam L, et al; abstract 8961).

Multiplex molecular tests represent an advancement for rapidly and reliably detect and identify causes of infectious diseases. Among these, the syndromic microarray-based nucleic acid assays have shown high positive-predictive values for detection of organisms in blood, CSF, stool, and respiratory samples [6]. Several studies presented at the ASM Microbe have assessed the performance of syndromic PCR panels (FilmArray, Biofire, bioMérieux) for the diagnosis of gastroenteritis, meningitis, and pneumonia, and have evaluated its clinical impact. In a prospective, multi-center study, the authors investigate the impact of a gastrointestinal panel on clinical diagnosis and decision-making, and compared the clinical acuity of patients with positive results obtained exclusively with the panel versus those detected by conventional stool culture. The panel detected a pathogen in 35.3% (669/1,887) of specimens, compared to 6.0% (113/1,887) detected by stool culture. The median time from collection to result was 18 h for the panel compared with 47 h for stool culture. The median time from collection to initiation of appropriate antimicrobial therapy was 22 h for the panel compared with 72 h for stool culture, indicating that the use of the syndromic panel can substantially increase the speed and sensitivity of laboratory diagnosis and inform clinical decisions in patients with acute gastroenteritis (Bateman A, et al; abstract 6215). In a study that performed a total of 216 tests in CSF with the FilmArray meningitis-encephalitis panel and in which they also collected white blood cell counts (WBC), 14 specimens (6.5%) were positive, and all samples with normal WBC were negative. The panel improved the ability to provide rapid PCR results for 14 infectious agents and improved the turnaround to a few hours. The authors suggest that the panel should not be performed in samples that have normal WBC counts (Bolster LaSalle CM, et al; abstract 4565). Another study assessed the clinical impact of a respiratory panel when used as a point-of-care test (results in 1 h) in an urgent care setting (Mortimer L, et al; abstract 6280). A total of 98 patients were enroled with a syndromic panel and 53 tested positive (54%), with 51 testing positive for a viral pathogen (96%) and 2 for a bacterial pathogen (4%). The use of this panel decreased the time to which actionable results were available and improved antimicrobial stewardship. Finally, a multi-center evaluation (8 USA sites) was conducted in order to establish the clinical performance of the Biofire Filmarray® Pneumonia Panel Plus (investigational use only version of the assay). This panel detects 18 bacteria (15 with semi-quantitation), 9 viruses, and 7 antibiotic resistance markers. The overall positive and negative agreement was 93.6% and 99.2%, respectively, compared to the reference methods. For analytes reported with semi-quantitation, the panel demonstrated essential agreement with the correct log bin value +/- 1 bin 97.7% of the time as compared to qNGS, demonstrating that it is a sensitive and specific test for detection of pathogens in sputum and bronchoalveolar lavage (BAL) (Kerr S, et al; abstract 4266).

Magnetic resonance is among the new technologies developed for detecting microorganisms in clinical samples [7]. The T2Bacteria panel, is a novel nanodiagnostic panel that uses T2 magnetic resonance and a dedicated instrument to detect as few as 1-10 cfu/mL of six different organisms (*A. baumannii, E. faecium, E. coli, K. pneumoniae, P. aeruginosa,* and *S. aureus*) directly in whole blood with results in 3-5 hours. In a multicenter prospective study (Nguyen M, et al; abstract 7975), the T2Bacteria panel was compared with blood cultures (BC) for diagnosing bacteremia. A total of 1,427 BC were tested, and the T2Bacteria sensitivity and specificity based on BC results were 90% and 88%, respectively; four samples had T2-/BC+ and 176 samples from 168 patients had T2+/BC- results (24% from patients who had received previous antimicrobial therapy, 23% from patients with positive BC for the same species within the 21 preceding days, 16% from patients with positive non-blood site cultures in the preceding 14 days, 19% from patients with presumed infection but either BC were not drawn or negative, and the remaining were considered false positive results). In summary, the panel demonstrated good performance in detecting bacteremia with lower time to test positivity in comparison with BC, and potential for diagnosing deep-seated infections.

Laser light-scattering is a technology that has been used for the rapid detection of bacterial growth in human specimens and in the screening of urine specimens to facilitate the diagnosis of urinary tract infections. The BacterioScan 216Dx, is a laser light-scattering instrument, that can be used with urine and with BAL specimens. In one study, the authors determined the improvement in performance of this instrument compared to standard methods (Tomaras A, et al; abstract 5577). A total of 55 BAL specimens were analyzed by first diluting them into brain heart infusion broth and subsequently incubating them in the 216Dx for 20 hours, during which optical

measurements were collected every 3-5 minutes. Considering clinically significant bacterial counts of ≥10,000 cfu/mL, 25% of BAL specimens were positive by conventional culture, and the 216Dx demonstrated sensitivity and specificity values of 85.7% and 87.8%, respectively, at 6 h, and 92.9% and 73.2%, respectively, at 8 h, showing that this method can be used as a screening method for BAL specimens with clinically-relevant densities of respiratory pathogens. Laser light-scattering can also be used for rapid antimicrobial susceptibility testing (AST) directly from urine samples. One study presented at this meeting compared the susceptibility results obtained with the 216Dx directly from urine samples, to MIC reports (Vitek 2, bioMerieux; gold standard) from urine culture isolates for 4 antimicrobials (ciprofloxacin, ceftriaxone, cefazolin, and meropenem) against Gram-negative pathogens. Positive urine samples were diluted in Mueller-Hinton broth and inoculated to antibiotic cuvettes of the 216Dx. A total of 112 AST were performed and the concordance with the standard for all 4 drugs was 94.4%, with 3 minor errors, 1 mayor error, and 1 very major error. Results were available within 4 hours, confirming that this technology provides early phenotypic evidence of antimicrobial susceptibility and resistance (Riederer KM, et al; abstract 8903).

Peptide nucleic acid fluorescence *in situ* hybridization (PNA-FISH) is a technology that has been used for the direct detection of bacteria, fungi, and parasites in clinical samples [8]. In one study, the authors evaluated the use of a simple and inexpensive dual-colour fluorescence *in situ* hybridization assay for identifying *Plasmodium knowlesi*. Methanol fixed thin blood smears were hybridized with two fluorescent labeled probes: *Plasmodium* genus and *P. knowlesi* specific probe at 37ºC for 15 minutes. The limit of detection in the *P. knowlesi* FISH assay was 84 parasites per µl in infected monkey blood and 61 parasites per µl for *P. knowlesi* cultured in human blood. The *P. knowlesi*-specific FISH probe detected only *P. knowlesi* and not other species. The assay can be performed in 30 minutes, and could be a potentially useful tool for diagnosing *P. knowlesi* infections in remote laboratories in endemic countries (Shah J, et al; abstract 7321).

Among the future technologies, the development of microfluidic will allow the detection of multiple microorganisms at low cost [9]. One study reports the development and evaluation of a high throughput microfluidic system (BiospectrixTM) that does not require culturing, identifies a broad range of bacteria in less than 1 hour directly from blood, and has a limit of detection of <100 cfu/mL. After blood cells were lysed, bacteria were recovered in a filter and identified by FTIR (Krishnamurthy R, et al; abstract 6885).

Novel technologies, not growth dependent and based on flow cytometry have been developed for the rapid antimicrobial susceptibility testing directly from blood cultures. One of these commercialized methods is the FASTinov®. A study evaluated the performance of FASTinov® for susceptibility testing of *Pseudomonas aeruginosa* directly on positive blood cultures in 2 h. The overall categorical agreement between FASTinov® and broth microdilution was 95%. The highest categorical agreement was observed for gentamicin, ceftolozane-tazobactam and colistin (100%), followed by amikacin and imipenem (97% and 96%, respectively). The highest major error rate was detected for piperacillin-tazobactam, and the very major discrepancies were verified for meropenem (Costa-de-Oliveira S, et al; abstract 7343). Other platforms have also been developed for the rapid antimicrobial susceptibility testing. Among these, SeLux has developed an automated, rapid, low-cost, phenotypic AST platform that enables simultaneous, same-shift susceptibility testing full dilution series of a wide array of antibiotics. The core of this technology is an endpoint assay for bacterial surface area, which enables delineation of truly resistant bacteria from filamented or swelled organisms as can occur prior to lysis. In one study, AST was performed with the SeLux platform and compared against the Clinical and Laboratory Standards Institute (CLSI) broth microdilution reference method on a total of 1,100 bacterial isolates, including d 300 "challenge" isolates, against 20 antibiotics. Susceptibility results with the SeLux platform were obtained within 5 hours from >90% of the isolates tested. Essential and categorical agreement with the reference method were ≥90% for all combinations tested. This platform constitutes an example of next generation phenotyping (Stern E, et al; abstract 5386). Among other new technologies presented at this meeting, the Acuitas® AMR Gene Panel u5.47 include the semi-quantitative molecular detection (real-time multiplex PCR) of 5 bacterial pathogens and 47 antibiotic resistance genes (including genes for aminoglycosides, cephalosporins, sulfonamides, fluoroquinolones, and trimethoprim/sulfamethoxazole) directly in urine specimens or bacterial isolates. The test identified urine pathogens with 96% semi-quantitative consistency compared with urine culture and accurately predicted phenotypic resistance for molecular results from urine with average accuracy from 82% to 94%. The molecular test can be used with statistical algorithms to predict phenotypic resistance to 17 antibiotics, providing results in 2.5 h (Walker G, et al; abstract 8969).

The implementation of the next generation sequencing (NGS) enables a fast microbiological diagnostic method without requiring a predefined range of suspicious pathogens [10]. One study, by using the BGISEQ-100 platform, evaluated the diagnostic efficacy of NGS in clinical samples from 1048 patients with suspected infections (234 blood stream infections, 284 respiratory infections, 312 central nervous system infections, and 218 focal infections). The overall NGS sensitivity was 46.5%, while blood stream, respiratory, focal, and central nervous system infection had a sensitivity of 78.72%, 89.4%, 96.4% and 19.4%, respectively, that was significantly higher than conventional laboratory methods (32.4%). The diagnostic sensitivity of protozoa and virus were the highest (100%), followed by virus (69.5%) and bacteria (74.29%), while fungi had a lowest sensitivity (33.3%). This study highlights the promising potential of NGS in the rapid etiological diagnosis of suspected infectious diseases (Ai JW, et al; abstract 8740). Other applications of NGS presented at

this meeting included the shotgun metagenomics for direct detection and identification of prosthetic joint pathogens in synovial fluid, the identification of organisms and resistance markers directly from urine specimens, and the detection of drug resistant tuberculosis directly from sputum samples, among others.

ANTIMICROBIAL RESISTANCE

Antimicrobial resistance remains a serious threat to public health worldwide, although resistance displays wide variations depending on the bacterial species, antimicrobial group and geographical region [11]. Development of antimicrobial resistance is caused by mutations in bacterial genes, or by acquisition of exogenous resistance genes carried by mobile genetic elements that can spread horizontally between bacteria. A series of plasmid-borne determinants conferring resistance to the last-resort antibiotics, carbapenems, aminoglycosides, and colistin have been described among different bacterial species. Since the first description in China in 2015 [12] of the transferable gene *mcr-1* conferring resistance to polymyxins, several studies have described the spread of *mcr-1* and other variants among *Enterobacteriaceae*. In one study presented at ASM Microbe, the authors describe for the first time the presence of a transferable chromosomally-encoded *mcr-5* gene in a colistin-resistant clinical isolate of *P. aeruginosa* in the USA. The isolate carried a chromosomal copy of *mcr-5* that was flanked by two copies of a novel Tn3-like transposon. The MIC of colistin was 16 mg/L (McGann P, et al; abstract 8054). Other study described the emergence of the colistin resistance gene *mcr-1* in four clinical isolates of *P. aeruginosa* in India, in which the gene was located on the chromosome (Pathak A, et al; abstract 4668).

Although resistance to carbapenems among *P. aeruginosa* isolates is, in general, chromosomally encoded, there is an increased emergence of plasmidic and transferable carbapenem resistance among this species. One study reported a novel carbapenem-hydrolyzing class D β-lactamase in a clinical isolate of *P. aeruginosa* in Korea. The carbapenemase was similar to OXA-10 (by the single substitution Lys103Asn), was located on the chromosome, and harbored by a new class 1 integron, suggesting the further spreading of this carbapenemase among Gram-negative bacteria (Park KS, et al; abstract 4338). Other authors reported the new carbapenemase NDM-11 among *E. coli* isolates in India. This carbapenemase is a variant of NDM-1 with a novel mutation at position 154 (Met to Val) and was located on a plasmid (Rahman M, et al; abstract 4322).

Ceftazidime-avibactam (CAZ-AVI) and ceftolozane-tazobactam (TOL-TAZ) are among the new antibiotics developed for the treatment of multi-drug resistant *P. aeruginosa*, however, isolates conferring resistance to both antimicrobials are described due to a variety of resistance mechanisms [13]. One study described the emergence of a high-risk ST309 *P. aeruginosa* clone harboring the carbapenemases GES-19 and GES-26, and conferring resistance to carbapenems, CAZ-AVI and TOL-TAZ (Khan A, et al; abstract 6989). Two isolates of XDR *P. aeruginosa* isolates were recovered from patients at separate hospitals in Houston (USA), with a class 1 integron carrying multiple resistance determinants including GES-19 and GES-26. One patient cured with the combination of CAZ-AVI plus aztreonam.

Carbapenem-resistant bacteria are increasingly found in hospital settings, but little is known about their prevalence in the environment. One study reported the isolation and characterization of carbapenem-resistant bacteria isolated from samples collected from different public transit stations within the Los Angeles (USA) area. Among 25 samples collected from the validators and from the stations, a total of 34 meropenem-resistant isolates were recovered, and 21 of them were carbapenamase producers, indicating that public transit is an underappreciated reservoir of carbapenem-resistant bacteria (Carlson N, et al; abstract 4905).

NEW ANTIMICROBIAL AGENTS

In recent years, several new antimicrobials have been approved by the FDA [14] and some others are under development. One of the most interesting sessions of the congress was a plenary session dedicated to the description of early new antimicrobial agents [1]. Several new families of compounds with new mechanisms of action and active against multi-drug resistant Gram-negative bacteria were presented as follows: a) the odilorhabdins, that inhibit bacterial translation with a novel mode of action on the ribosome; b) novel siderophore cephalosporins, like GT-1; c) inhibitors of LpxC, a zinc-dependent deacetylase responsible for the biosynthesis of lipid A, an essential component for Gram-negative bacteria; d) Mbx-4191, a pyranopyridine efflux pump Inhibitor in Gram-negative organisms; and e) VNRX-5133, a novel broad-spectrum β-lactamase inhibitor with selective direct inhibitory activity of both serine- and metallo-β-lactamases (MBLs). Moreover, new antimicrobials active against multi-drug resistant *Neisseria gonorrhoeae*, and antifungals active against *Candida auris*, with new mechanisms of action like APX001A, that inhibits Gwt1, an enzyme required for glycosylphosphatidylinositol post-translational modification of surface membrane-anchored proteins in fungi, were also presented. In addition, many poster presentations summarized the activity of other antimicrobials currently in phase 3 of development or recently included in the therapeutic arsenal. Among these, one study presented the activity of TOL-TAZ against *P. aeruginosa* from patients in different risk strata. Overall, the activity was >90% in all risk strata, and also maintained activity against 80% of the meropenem-non-susceptible isolates (Lob S, et al; abstract 4455). Other study presented the activity of CAZ-AVI against *P. aeruginosa* collected in Latin America from 2012 to 2016 (Wise M, et al; abstract 3492), and 92.6% of the isolates were susceptible, although a decrease in susceptibility was observed over the period of study due to the emergence of MBLs. A multicenter study evaluating the activity of

aztreonam-avibactam against *Enterobacteriaceae* and *P. aeruginosa* (Kazmierczak K, et al; abstract 3748) showed that this combination was active against 99.9% of Enterobacterales and against 100% of the MBL-producers. However, the combination showed reduced activity against all *P. aeruginosa* including MBL-positive isolates. Several studies evaluated the activity of imipenem-relebactam (IMI-REL) against multi-drug resistant Gram-negative organisms. In one of these, the overall susceptibility of *Enterobacteriaceae* to IMI-REL was 95.6%, and the activity was particularly high against KPC-positive isolates (98.5%) (Lob S, et al; abstract 4368). Meropenem-vaborbactam is another combination active against multi-drug resistant Gram-negative bacteria. One study that evaluated its activity against KPC-producing *Enterobacteriaceae* (Zhou M, et al; abstract 4966) showed that 97.6% of *K. pneumoniae* and 100% of *E. coli* were susceptible. Zidebactam is a β-lactam enhancer antibiotic (binding to PBP2a) that exhibits β-lactamase-independent, synergistic activity with other beta-lactams. In combination with cefepime shows potent activity against isolates resistant to meropenem, colistin, ceftazidime-avibactam and ceftolozane-tazobactam. In one study that included 1,018 carbapenem-resistant *Enterobacteriaceae* and 262 multi-drug resistant *P. aeruginosa*, >98% of the isolates were susceptible to this combination (Hackel M, et al; abstract 5397). Cefiderocol is a siderophore cephalosporin that has recently been introduced for the treatment of infections due multi-drug resistant organisms. In a worldwide multicenter study, the activity of cefiderocol was analysed against KPC-, NDM-, IMP-, OXA-48, and other OXA-producing isolates, and demonstrated potent *in vitro* activity against meropenem non-susceptible strains of *Enterobacteriaceae*, *A. baumannii* and *P. aeruginosa*, irrespective of the presence of serine- or metallo-type carbapenemases (Tsuji M, et al; abstract 3655). Tebipenem-pivoxil prodrug SPR994, is an oral carbapenem currently under development for the treatment of urinary tract infections. One study assessed the *in vitro* activity of SPR859, against a challenge set of *Enterobacteriaceae* including isolates with plasmid AmpC, ESBLs, KPC, MBLs and OXA-48-like enzymes. As expected, SPR859 showed potent activity only against isolates carrying ESBL and/or AmpC enzymes. These *in vitro* results indicate that SPR994 may be a candidate for stepdown therapy and empirical treatment where the prevalence of ESBL-producing isolates remains elevated (Mendes RE, et al; abstract 4603).

Concerning other non-beta-lactam antibiotics, eravacycline is a novel, fully-synthetic, fluorocycline that has completed phase 3 studies in complicated intra-abdominal infections and is in phase 3 development for complicated urinary tract infections. In a study that evaluated the activity of eravacycline against global isolates of *Enterobacteriaceae, Acinetobacter baumannii* (including carbapenem-resistant), *Stenotrophomonas maltophilia, Staphylococcus aureus* (including methicillin-resistant), and *Enterococcus* spp. (including multidrug-resistant isolates), the MIC50/90 for all *Enterobacteriaceae* (n=3157) was 0.25/1 mg/L, and for multi-drug-resistant-*Enterobacteriaceae* (n=666) was 0.5/2 mg/L. The MIC50/90s for *Enterococcus* spp. and MRSA were 0.06/0.06 and 0.06/0.12 mg/L, respectively, and was also active against *A. baumannii* and *S. maltophilia* (Lawrence K, et al; abstract 5745).

Delafloxacin is a broad-spectrum fluoroquinolone active against Gram-negative (including *P. aeruginosa*) and Gram-positive (including MRSA) organisms that has been approved for the treatment of acute bacterial skin and skin structure infections and that is in clinical development for community-acquired bacterial pneumonia. One study evaluated the *in vitro* activity against a total of 3,629 *Streptococcus pneumoniae* clinical isolates from US and European hospitals including fluoroquinolone-resistant isolates (levofloxacin MIC >4 mg/L, or moxifloxacin MIC >2 mg/L). Delafloxacin demonstrated potent *in vitro* activity against all pneumococci (MIC50/90 0.015/0.03 mg/L) and had excellent activity against all levofloxacin- and moxifloxacin-resistant isolates (Shortridge D, et al; abstract 4784).

Finally, among the new antifungals, rezafungin is a novel echinocandin as active as other echinocandins against common fungal organisms, but with distinctive pharmacokinetics that allow once-weekly dosing and high, front-loaded plasma drug exposure. In a phase 2 study, rezafungin was administered IV and dosed once weekly up to 4 weeks at either 400 mg (group 1) or 400 mg on week 1 and 200 mg thereafter (group 2) for the treatment of candidemia and/or invasive candidiasis. Rezafungin appeared to be safe and well-tolerated at both dosing regimens. The extended half-life and stability of rezafungin will be very useful especially in patients who could be discharged to outpatient therapy (Thompson GR, et al; abstract 7828).

Although antimicrobial resistance poses significant challenges for current clinical care, new classes of antibiotics are being developed, and new drugs hold promise against the challenge of multi-drug resistant bacteria.

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Highlights at the 28th Congress of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), 2018

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INTRODUCTION

The last European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) held in Madrid (Spain), last April 2018, was an undoubtful success for scientific quality and conference affluency [1]. The congress covered the entire field of infectious diseases and clinical microbiology with a significant and important participation of scientist all over the world (more than 12,400 congresists from more than 120 countries). In fact, nowadays ECCMID has become the world congress of reference, for excellence and participation, the main meeting in clinical microbiology and infectious diseases. Designed with more than 250 sessions, the key topics were antimicrobial resistance, novel diagnostic techniques, the role of microbiota, and new antimicrobials. In addition, the congress also covered different aspects of the big four in infectious diseases: bacterial, viral (HIV and hepatitis), fungal and parasitic infections. The complete program included a total number of 3,563 abstracts, more than a hundred of symposiums and oral sessions, 20 educational workshops and 25 experts meetings. This minireview will try to summarize, from an objective point of view, the most important contributions, only focusing in three different aspects: microbiology diagnosis, resistance to antimicrobials, and new antimicrobials.

DIAGNOSTIC MICROBIOLOGICAL TECHNIQUES

Infectious diseases are an important cause of morbidity and mortality but in the past few decades, the diagnostic microbiological techniques have significatly evolved. Thanks to the progress of molecular methods, these not only identify but also detect antibiotic marker genes, fastidious bacteria, and uncultivable microbes [2].

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Nowadays, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has revolutionized clinical microbiology laboratories and they have adopted it as a primary method for the identification of microorganisms [3]. It can be highlighted a study that shows that this technique represents a reliable identification tool for anaerobic bacteria. In spite of the special culture requirements, their low growth-rate and the difficulties to isolate this kind of bacteria, MALDI-TOF MS only requires little amount of bacteria and allows high-throughput (Rodriguez-Sánchez B, et al; P2236). Can also be mentioned the possibility to make direct identification for most of the uropathogens by this method. It can process a large number of urine samples (30 min per sample) with an accuracy of over 90% (Ilki A, et al; P2237). Furthermore, the identification of non-tuberculous Mycobacteria isolates from MGITs® (Mycobacteria Growth Indicator Tubes, Becton Dickinson) can be rapid and reliably performed using MALDI-TOF MS by including a sonication step in the sample processing method (Rodriguez-Sánchez B, et al; P2405).

Following the success of mass spectrometry (MS) for routine identification, several options were considered to extend the clinical applications of MALDI-TOF MS platforms. In one study, an innovative full MALDI-based approach to quickly detect carbapenemase-producing enterobacteria (CPE) in positive blood cultures was evaluated, applying the novel tools of Biotyper system (Bruker Daltonik) directly on the bacterial pellet extracted from the positive bottles (Cordovana M; O0811). KPC-producers were identified by the automated detection of the 11109 KPC-specific peak by the Biotyper software, while an imipenem hydrolysis assay was used to verify the carbapenemase activity. The full MALDI-based approach enabled the rapid detection of different kind of carbapenemases directly from the positive blood culture bottles, with absolute sensitivity and specificity, and allowing a significant shortening of potential reporting time in comparison with the actual routine (30 min-2 h).

There was another study where the development of a PCR-MALDI approach could unlock the potential of a system capable of detecting tens of targets simultaneously, without the need to culture (Green J, et al; P2376). This new technology could provide many clinical advantages and support a syndromic approach to the patient diagnosis. The results showed that combining the large instrument installed base with real-time PCR technologies has the potential to open higher orders of multiplexing which can supersede current real-time PCR technology. PCR-MALDI detection of important fungal species was achieved and this approach could be transferred to any number of applications. With certain pathogens exhibiting poor culture sensitivity, the use of PCR-MALDI has the potential to become a key analytical tool in the microbiology and diagnostics field.

PCR is an important identification method, fast and robust [4]. The Sepsis Flow Chip (SFC) assay is based on multiplex PCR and low-density DNA arrays, which is a novel diagnostic assay for simultaneous rapid-detection of the vast majority of bloodstream pathogens, including Gram-positive and Gram-negative bacteria and fungi, in the same assay, and for the detection of most common antibiotic resistance genes [5]. SFC technique was used to analyse the blood cultures from 96 stratified bacteremic patients (Krishnamoorthi S, et al; P1948). The overall sensitivity and specificity for bacterial identification were 89.16% and 86.96%, respectively and sensitivity and specificity for the identification of antibiotic genetic resistance determinants were 85.89% and 100%, respectively. This new method appears to be a very promising molecular diagnostic tool by combining the high number of distinct pathogens and the genetic resistance determinants identified in a single assay, which will contribute to a rapid and accurate diagnosis of bacteremia. Further investigations should be done to evaluate the usefulness of this assay in combination with clinical multidisciplinary groups.

Another study evaluated the AMR Direct Flow Chip assay (Galiana A, et al; P2288). It detects the main genetic resistance determinants for gram-positive and gram-negative microorganisms in a single step. This assay was compared to GS techniques based on Chrom ID MRSA, VRE and Carba Smart combined with a molecular approach to identify genetic resistant determinants. AMR assay showed sensitivity and specificity values close to 100% demonstrating its ability as a diagnostic test in MDR screening surveillance programs at intensive care units.

Immunochromatographic tests (ICT) are easy and quick alternative diagnostic techniques. The prevalence of CPE is increasing worldwide and the control of their spread is very important, so there were some studies evaluating immuno-chromatography devices for the rapid detection of carbapenemases. For example, K-set OOK® (Resende C, et al; P2332) proved to be a sensitive test (100%) for detecting KPC, OXA-48 and OXA-163 producing Enterobacteriaceae directly from rectal swabs using enrichment broth in different incubation periods. However, since the test is designed to detect only KPC, OXA-48 and OXA-163, false negative results can occur due to the presence of other carbapenemases. For this reason, it should be performed in parallel to culture.

Bloodstream infections (BSI) by CPE are associated with treatment failure and increased mortality, so that it´s so important their rapid detection. Rapid immunochromatographic lateral flow tests (ICT) which detect epitopes specific for a carbapenemase have been recently developed for CPE detection from cultures on solid media. In one study, a protocol was developed and evaluated for the rapid detection of CPE directly from positive BC using a new ICT, which detects OXA-48-like, KPC and NDM carbapenemases (Hamprecht A, et al; O0810). This study demonstrates that with the new protocol OXA-48-like, KPC and NDM carbapenemases can be reliably detected directly from positive BC bottles within 20-30 min (100% sensitivity, 100% specificity). This can help to rapidly identify patients with CPE BSI and optimize the treatment of patients. In addition, the global spread of carbapenem-resistant *Acinetobacter baumannii* (CRAb) has led to an emerging worldwide healthcare problem. There are six identified OXA-subgroups associated with carbapenem-resistance, where OXA-23 is the most prevalent carbapenem-resistance determinant among isolates followed by OXA-72 (OXA-40-like) and OXA-58 worldwide. It has been developed an immunochromatographic lateral flow assay (OXA-23 K-SeT) able to detect OXA-23-like producing strains with 100 % specificity on bacterial colonies (Mertins S, et al; P2330). In this study, new monoclonal antibodies (moabs) to detect OXA-40 and OXA-58 were presented. The resulting triple OXA-23/40/58 detection assay would be able to detect the most prevalent OXA-mediated CRAb. With this rapid detection assay, one can save 12-48 hours in diagnostics, avoiding treatment with inappropriate antibiotics and allowing an earlier intervention to control transmission of CRAb.

The introduction of massive sequencing (next-generation sequencing, NGS) in genomics facilities has meant an exponential growth in data generation. With its ultra-high throughput, scalability, and speed, NGS enables researchers to perform a wide variety of applications. It enables scientists to analyze the entire human genome in a single sequencing experiment, or to sequence thousands to tens of thousands of genomes in one year. Next-generation sequencing-based pathogen detection is already being used by diagnostic laboratories from clinical specimens [6].

A study that compares Sanger sequencing (a commercialized method of DNA sequencing) with NGS for detection of HIV drug resistance mutations in routine was presented (García-Arata MI, et al; P1902). All mutations previously detected by Sanger were accurately detected by NGS. In addition, minority resistance-conferring mutations were also detected by NGS (even in low viral load-samples). This method can be affordable in three labor days and price is comparable or even lower that the conventional sequencing and the platform could be at the same time run for other uses, like genotyping hepatitis C or HIV tropism. All these findings made NGS an effective new strategy and useful tool to use for HIV resistance detection in routine labs.

For prediction of *Mycobacterium tuberculosis* resistance, whole genome sequencing (WGS) has its limitation by the need to isolate mycobacteria by culture. There was a study comparing WGS with Deeplex®- MycTB. This last uses NGS-based targeted deep sequencing for simultaneous prediction of (hetero)resistance to 13 anti-TB drugs/drug classes, MTB genotyping and identification, directly on primary specimens (Gaudin C, et al; P1556). Compared to WGS, Deeplex-MycTB showed high accuracy for rapid identification of MTB drug resistance-associated mutations from clinical specimens. This assay represents an important clinical tool for rapid definition of an optimal patient's treatment.

ANTIMICROBIAL RESISTANCE

Resistance to antibiotics is an important public health threat, which is aggravated by the lack of development of new antimicrobial agents [7]. The emergence of multidrug-resistant Gram-negative bacteria is a growing problem worldwide. Colistin is one of the last resort antimicrobials for the treatment of infections caused by multidrug-resistant Gram-negative bacteria but in recent years, the resistance is increasing [8, 9]. A study presented at ECCMID (Mendes AC, et al; P0417) analysed an outbreak of a KPC-3 and MCR-1 producing *Klebsiella pneumoniae* strain in Europe in 16 patients. All isolates belonged to ST45K24 except one (ST1112). In another study, the authors found that *K. pneumoniae* harbouring a *mcr-1* plasmid is able to survive polymyxin treatment in vitro and in vivo. Interestingly, even without *mcr-1*, polymixin resistance can rapidly emerge after polymixin exposure (Nang SC, et al; P0420). Concerning *MgrB* gene, one study demonstrated that the inactivation of this gene is a common mechanism of colistin resistance among *K. pneumoniae*. The presence of identical mutations/insertions in isolates of the same ST and PFGE profile suggests the occurrence of clonal expansion and cross-transmission (Esposito EP, et al; P0423)

One study analysed the impact of the mechanism of resistance to carbapenems in gram-negative on mortality, 264 outbreaks reported in literature were included. The highest crude mortality was observed in *K. pneumonia* (KPC and OXA-type had higher mortality than metallo-betalactamases (MBL)) followed by *Acinetobacter baumannii* (OXA-type was higher than MBL) and *Pseudomonas aeruginosa*. The highest failure rates in containing the outbreaks were observed in *K. pneumonia* KPC (Pezzani MD, et al; P1052).

The European survey of carbapenemase-producing *Enterobacteriaceae* (EuSCAPE) reveals that carbapenemase-producing *K. pneumonia* isolates focus mainly on five "high-risk" clonal STs (11, 15, 101, 258, 512), founding evidence of geographical clustering of carbapenemase-containing plasmids. Spread of carbapenem resistant isolates is deeply connected to the movement of people who travel more within than between countries (David S, et al; 01149)

Ceftolozane-tazobactam (TOL/Tz) shows high activity against ESBL-producing *Escherichia coli*, but lower against ESBL-producing *K. pneumonia*. Emergence of TOL/Tz resistance may occur during the treatment of MDR/XDR *P. aeruginosa* infections in 10-15% of the cases. It is caused by structural mutations in intrinsic (AmpC) or acquired (OXAs) beta-lactamases and typically shows ceftazidime-avibactam (CAZ-AVI) cross-resistance, but increased susceptibility to carbapenems and penicillins. Other potential low-level resistance mutations include specific large chromosomal deletions and PBP3 mutations (Oliver A; S0387).

A review about CAZ-AVI resistance (Humphries RM; S0386) shows that combination of ceftazidime and avibactam represents a potential alternative to carbapenems for the treatment of multidrug-resistant. Resistance is related to mutation to KPC-2 or KPC-3 omega loop that increases affinity for CAZ but prevents binding of AVI. Mutations to KPC enzymes result in enhanced ceftazidime kinetics rather than reduced avibactam inhibition. In infections with CAZ resistance strains containing certain omega-loop substitutions, the efficacy of the combination may be in question [10]. This mechanism of resistance has been described in patients treated with CAZ-AVI, resulting in microbiological failures. Another mechanism of resistance is due to the combination of increased KPC expression and reduced permeability in patients with no previous exposure to CAZ-AVI. With the increased use of CAZ-AVI, resistance will continue to emerge and plasmids carrying mutant genes may disseminate by horizontal gene transfer [11].

Among Gram-positive bacteria, in one study performed in Barcelona (Càmara J, et al; P0456) 993 enterococci isolates were screened to know the prevalence of transferable linezolid resistance. Through PCR, transferable LZD-resistance was detected in six *Enterococcus faecalis* (*optrA* n=5 and *optrA* plus *cfr* n=1), two of them showed linezolid MIC= 4 mg/L and three linezolid inhibition zone >19mm. The prevalence of the *optrA* gene among enterococci is low and linezolid breakpoints are not sensitive enough to detect transferable linezolid-resistance, being necessary the screening through the antimicrobial susceptibility testing of chloramphenicol. In another study, emergence of an *Enterococcus faecium* strain with variable susceptibility to vancomycin due to the vanX deletion was analysed. *E. faecium* carrying pHVH-V1511 is capable of nosocomial transmission and may develop clinical resistance to vancomycin. These strains may not be detected using standard culture methods for vancomycin resistant enterococci (VRE) (Hansen T, et al; 01147).

Animals can act as reservoirs of antimicrobial resistance genes. Livestock-associated methicillin-resistant *Staphylococcus aureus* has emerged. Pigs, veal calves and poultry play an important role as reservoirs. There is a scatter in the community, which may be partly related to meat consumption and to livestock density. CC398, a new variant of MRSA that has emerged in animals, is at the dawn of its evolution, so close monitoring is necessary. In relation to molecular relatedness of ESBL/ampC *E. coli*, one study of Dorado-García A et al shows that isolates from the general population had higher similarities with those from human clinical settings, surface and sewage water and wild birds, while similarities with livestock or

food reservoirs were lower [12] (Kluytmans AJW; SO392). One study analysed the genetic characteristics of *mcr-1* carrying *E. coli* from companion animals. A total number of 434 *E. coli* isolated were recovered from 1439 nasal/rectal swabs of animals, 56 strains were resistant to colistin and 47 of them were *mcr-1* positive, mainly located on the plasmids which belong to different Inc types. Seven of 35 pets food samples were detected to be *mcr-1* positive. Colistin resistant E. coli from companion animals may represent a potential risk to human health (Lei L, et al; O1050).

NEW ANTIMICROBIAL AGENTS

After the global crisis caused by the threat of antimicrobial resistant bacteria, in the last five years, new agents have been developed on a fast track way and presented for the first time in the last ECCMID congress in Vienna in 2017. Some of them have been recently approved by the regulatory agencies and many of them are under examination of the phase II and III clinical trials. All this information was presented at the 28th ECCMID in Madrid and it is summarized in table 1 and 2.

Among the most important clinical trials, is worth to mention the phase III clinical trials IMPACT 1 and 2, analysed efficacy of oral cadazolid versus vancomycin in the treatment of *Clostridium difficile* associated diarrhoea (O0420). Cadazolid showed no inferiority in clinical cure in IMPACT 1 and similar results than vancomycin in sustained cure (no recurrence in one month). Cadazolid was safe, well tolerated and could potentially be an alternative therapy for *Clostridium difficile* infection.

Another phase III study presented was REVIVE-2 where Iclaprim was non-inferior to vancomycin in the treatment of patients with acute bacterial skin and skin structure infections (O0424). In the same way, monotherapy with once-daily oral omadacyclin was non-inferior to twice-daily oral linezolid in the treatment of adults with skin and soft tissue infections and was safe and generally well tolerated as was shown in the OASIS-2 phase III clinical trial presented (O0425).

Against multidrug resistant gramnegatives, the results of a randomized, controlled, phase 3 trial (RESTORE) comparing imipenem-relebactam versus colistin and imipenem for treating imipenem-nonsusceptible bacterial infections (77% *Pseudomonas*, 55% *Klebsiella*), hospital-acquired/ventilator-associated bacterial pneumonia (HABP/VABP), complicated intra-abdominal infection (cIAI), or complicated urinary tract infection (cUTI) were shown (O0427). Imipenem-relebactam treated patients had a favourable overall response, especially in the pneumonia groups (87.5% vs. 67.5%). By the other hand, excluding patients with prior antibiotic failure for serious gramnegative infections on outcomes in TANGO II, a randomised, open-label comparative trial with best available therapy in patients with cUTI, acute pyelonephritis, HABP/VABP, bacteremia, and cIAI, due to known or suspected carbapenem-resistant *Enterobacteriaceae*, meropenem-vaborbactam was associated with increased clinical cure and microbiologic cure. We have to wait for the results of phase III clinical trial of aztreonam avibactam (vs. meropenem) in patients with intraabdominal infections and ventilator and/or hospital acquired pneumoniae, which began in March last year. Cefider-

Table 1 **Novel betalactam and non-betalactam combinations agents for multidrug-resistant Gram-negatives**

Betalactams	AmpC	ESBL	KPC	OXA	MBL	MDR-PA	MDR-AB	SM
Meropenem/vaborbactam								
Meropenem/nacubactam[a]						Hiper Amp-C		
Imipenem/relebactam[a]								
Aztreonam/avibactam[a]								
Ceftaroline/avibactam[a]								
Cefepime/zidebactam[a]								
VNRX-5133[b]						NA	NA	
Cefepime/AAI101[c]								
Non-betalactams	**AmpC**	**ESBL**	**KPC**	**OXA**	**MBL**	**MDR-PA**	**MDR-AB**	**SM**
Cefiderocol								
Plazomicin								
Murepavadine								
Eravacycline								
Omadacycline								

Class: [a]Diazabicyclooctane; [b]Boronic; [c]Sulbactam like. MBL: metallo-betalactamases; NA: not applicable.
MDR: multidrug-resistant, PA: *Pseudomonas aeruginosa*, AB: *Acinetobacter baumannii*, SM: *Stenotrophomonas maltophilia*

No activity | Some activity | Good to excellent activity

Table 2 The horizon of the new drugs against multidrug-resistant Gram-negatives

Stage	Antimicrobial
FDA approved	Delafloxacin, Meropenem/vaborbactam
End-stage clinical development	Cefiderocol, eravacycline, imipenem/relebactam, omadacycline, plazomicin
Phase II clinical trials	Murepavadine, finafloxacine
Fast-track development	Cefepime-zidebactam, cefepime/tazobactam, cefepime/AAI101, VNRX-5133, meropenem-nacubactam, aztreonam/avibactam ceftaroline/avibactam

Adapted from Avery et al [13].

ocol, a new siderophorus cephalosporin, with a great activity against carbapenemases and *Pseudomonas* resistant to meropenem, showed no inferiority in the phase III APEKS trial in cUTI and we have to wait for the open trial CREDIBLE comparing cefiderocol with the best available therapy in bacteremia, HABP/VABP and cUTI. In addition, a new rapid bactericidal antipseudomonic agent, murepavadin, showed great activity against *Pseudomonas* in HABP/VABP phase II clinical trial. Lastly in c-IAI, eravacyclin, a semisynthetic fluocyclin similar to tigecyclin, showed similar results than meropenem or ertapenem in the IGNITE trials (O0421). Related to community-acquired pneumoniae, two new molecules, lefamulin (phase III clinical trial LEAP-1) and omadacyclin (phase III clinical trial OPTIC) were compared with moxifloxacin, with non-inferiority results including the PORT risk class III to V (P0276).

In the well-known antibiotics field, it is important to mention the results of the Merino trial. This study, from the University of Queensland (Australia), hoped to determine whether piperacillin-tazobactam, a penicillin-based therapy, was as effective for treating blood stream infections as meropenem. The team enrolled 378 adult patients from 32 sites in nine countries from February 2014 to July 2017. The group examined the primary outcome for these patients, which was mortality at 30 days after the randomization; randomization occurred within 72 hours of the initial blood culture. They also noted secondary outcomes including the number of days to resolution, the clinical and microbiological success at day four and the relapse of bloodstream infection or secondary infection. Whilst they discovered no difference between the two groups regarding subsequent infections of drug-resistant bacteria or *C. difficile*, the difference in mortality rate was significant. A total number of 23 patients (12.3%) treated with piperacillin-tazobactam died by day 30 compared with just seven patients in the meropenem group.

And finally, a welcome headline: A 7-day course of antibiotic treatment for Gram-negative bacteremia (GNB) could offer similar patient outcomes compared with a 14-day course, according to new research presented (Yahav D, Turjeman A, Babitch T. Seven versus 14 antibiotic days for the treatment of Gram-negative bacteraemia: non-inferiority randomized controlled trial). In this study, the researchers assessed the 90-day outcomes for 604 patients admitted to three hospitals in Israel and Italy between January 2013 and August 2017, excluding patients with ongoing sepsis or cases where there was an uncontrolled source of infection. The team investigated several primary outcomes, including mortality, and whether a patient was readmitted to hospital or had to remain in hospital longer than 14 days. From the 306 patients who completed a 7-day course of antibiotics, the team discovered 46% experienced these primary outcomes, compared with 50% in the 298 patients who received a 14-day course.

Undoubtedly, these new antimicrobials are the hope for the future added to other strategies of therapy based in new uses of old antibiotics such as fosfomycin (Hutner et al. O1127; or combinations of them as well as the use of bacteriophages (SY040).

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Some highlights of the content of the San Francisco ID-Week, in the area of bacterial infection

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The Infectious Diseases Week (ID Week), is the great annual meeting of the infectious diseases of the United States of America and its international referents. This year, it took place in October 2018 in the city of San Francisco and gathered more than 12,000 people.

The Congress had impressive learning offerings that can be summarised in figures such as: 281 numbered sessions, 74 symposia, some 60 workshops, interactive sessions or meet the professor, 870 poster sessions and 24 oral communication sessions, with some 2500 approved abstracts. The orientation of the congress was clearly educational and aimed at updating the attendees. The organization, understanding the diversity of interests of the attendees, organized a series of tracks that included thematic areas such as: infectious diseases for adults, pediatric infectious diseases, HIV, Transplant Infectious Diseases, etc.

Symposia focused on a wide variety of topics but I was struck by the interest in topics such as antimicrobial stewardship, the new antimicrobials, the situation of the human microbiome and the very striking epidemic of opiate use that affects an enormous number of Americans with devastating consequences in the field of systemic infections. There was no shortage of symposia dedicated to vaccines, the treatment of multi-resistant bacterial infections and other diverse topics.

In the field of antimicrobials, I include a table with those who are at an advanced stage of development and who caught my attention the most (table 1). They include tetracycline derivatives, new β-lactamase inhibitors and a new antifungal, Ibrexafungerp, with action against yeast, filamentous fungi and *Pneumocystis jirovecii*.

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Staphylococcal infection had 202 presentations with very interesting data. One of them was a Spanish study suggesting the superiority potential of the combination of daptomycin associated with fosfomycin versus daptomycin alone in the treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. In this area, I liked a meta-analysis that collected data on the non-inferiority of cefazolin compared with isoxazolic penicillins in the treatment of methicillin-susceptible *S. aureus* bacteremia.

Of the 10 communications related to tedizolid, I was particularly interested in the one that collected safety data on the prolonged use of this drug in osteoarticular infections, showing very good tolerance with long treatment courses.

Following with Gram-positive bacteria, *Clostridioides difficile* was the reason for 238 presentations. Particularly relevant were papers demonstrating the growing importance of *C. difficile* infection (CDI) as a cause of community-acquired infection. A study referred to the convenience of reducing the unnecessary use of laboratory tests for the diagnosis of CDI. One person examined all requests for diagnostic tests in the laboratory and advised on whether or not to perform them, while giving therapeutic advice. This person managed to reduce the use of laboratory tests for CDI by 32%, eliminating unnecessary tests and generating savings of economic resources much greater than their salary.

Again, data were presented on the controversial issue of the meaning of PCR tests in patients with direct detection of *C. difficile* toxin in fecal samples, negative, with very discordant results.

One of the data that motivated several posters was the value of quantifying the PCR tests for CDI by evaluating the positivity cycle of the amplification curves. The data agreed that early amplification cycles are associated with more severe disease, while PCR positivity in late amplification cycles is associated with simple colonization.

With regard to the prevention of recurrences, a study was

Table 1 Some of the new antimicrobials in advanced stages of development

Drug	Company	Family	Target
TP-6076	Tetraphase	Tetracycline	*Acinetobacter*
CF-301	Exebacase	Bacteriophage-derived lysin	Gram-positives
Tebipenem	Spero	Oral carbapenem	*Enterobacteriaceae* ESBL, quinolone resistance
ETX2514+Sulbactam	Entasis	β-lactamase inhibitor	*Acinetobacter*
WCK 5222 Cefepime-Zidebactam	Hartford	β-lactam + β-lactamase inhibitor	Carbapenem-resistant *Acinetobacter*
Ibrexafungerp (SCY-078)	Scynexis	Triterpenoids (glucan synthase inhibitors)	Yeast, filamentous fungi, and *P. jirovecii*

presented evaluating bezlotoxumab as an agent that can be used in ambulatory infusion centres. The administration of bezlotoxumab was associated with a very clear decrease in the risk of CDI recurrences.

Moving to the Gram-negative bacterial infections, there were 252 references to this topic in which we highlighted the 37 relative to ceftolozane-tazobactam. Ceftolozane-tazobactam showed in vitro activity against more than 96% of all *E. coli, K. pneumoniae and P. aeruginosa* isolates in the entire USA. One study offered results from 65 patients with different infections in patients in critical situation, who received ceftolozane-tazobactam. Survival at 30 days was 86%. One study retrospectively compared cohorts of patients treated with ceftolozane-tazobactam and the combination of polymyxin and aminoglycosides.

Two studies compared patients treated with piperacillin-tazobactam with patients treated with other antibiotics demonstrating a higher nephrotoxicity in those treated with piperacillin-tazobactam.

I would like to highlight the great involvement of American Infectious Diseases physicians in antimicrobial stewardship. A total of 504 communications contained the term "Antimicrobial Stewardship" .

Finally, a new concept is making its way into the world of infection counselling, "telemedicine". The term TeleID as a reflection of the work of many electronic consultations on Infectious Diseases and the demonstration that their quality and impact are comparable to direct consultation, open a new way for the way of working in our discipline.

Although they are not the subject of this summary, suffice it to say that the contributions to the world of viral and fungal infection were equally interesting.

CONFLICTS OF INTEREST

The author declares no conflicts of interest.

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Highlights from 22nd International AIDS Conference

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INTRODUCTION

This event is one of the most prestigious conferences worldwide about a single global health issue, and it is a unique forum for the interaction of science, health promotion and human rights. The motto of this edition, "Breaking barriers, building bridges" refers to the need for AIDS programs to be accessible to all people living with HIV in any environment or any country in the world, without distinction and without stigmas. In the literature, studies that investigate the official content of congresses as an indicator of quality are few [1].

Of all the presentations made in this congress, we have selected those topics that can modify or influence during the daily clinical practice regarding novelties in antiretroviral treatment and prevention of HIV infection. In the first issue, we have highlighted the GEMINI and DIAMOND studies. In the second one, we have chosen the second part of the PARTNER study and the presentation of the real-life results of pre-exposure prophylaxis in France.

GEMINI-1 and -2: Dual-Therapy With DTG Plus 3TC Non-inferior to DTG Plus FTC/TDF in Treatment-Naive Patients at Week 48: multicenter, parallel-group, double-blind, randomized phase III non-inferiority trials

This study, published by Cahn et al [2], showed that the virologic efficacy of 2-drug regimen of dolutegravir (DTG) plus lamivudine (3TC) is non-inferior to 3-drug regimen of DTG plus emtricitabine (FTC)/tenofovir disoproxil fumarate (TDF) in treatment-naive patients at Week 48 (91% vs 93% with HIV-1 RNA < 50 copies/mL, respectively). These results are summarized in the figure 1.

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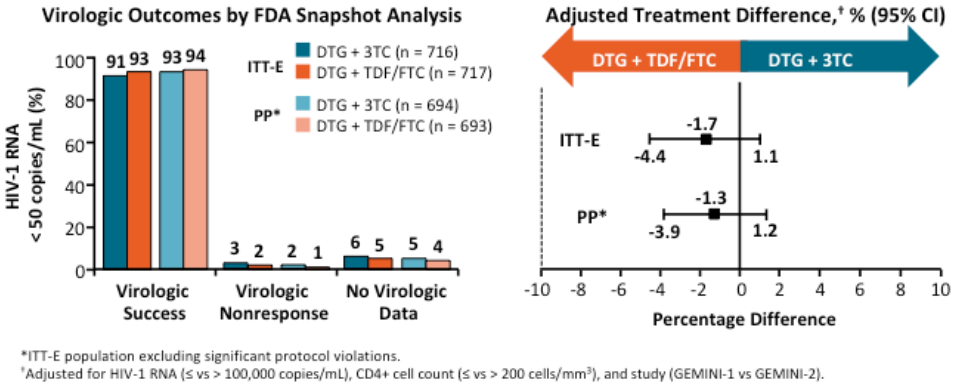
The two studies together had the participation of more than 1,400 patients, who were randomly distributed to receive one or another treatment. They had a viral load of less than 500,000 copies, although there was 2% of participants in each arm with more than 500,000 copies. Stratified results were presented according to viral load and CD4 levels. The main objective of both studies was to establish the percentage of participants with a viral load below 50 copies/ml at 48 weeks after starting the study.

The results showed a virologic efficacy generally consistent across subgroups stratified by baseline HIV-1 RNA. Efficacy of DTG plus 3TC was numerically lower in patients with low baseline CD4+ cell count in Snapshot analysis, but not in treatment-related discontinuation equals failure (TRDF) analysis. The numerical difference is evident in the subset of patients with lower baseline CD4+ cell counts who received dual therapy. Here, 79% of patients receiving dual therapy achieved virologic success compared with 93% of those receiving triple therapy. However, only 1 of these 13 dual therapy recipients without HIV-1 RNA < 50 copies/mL at Week 48 was a true virologic failure. It is also important consider that the percentage of patients with CD4<200 was only 9%.

On the other hand, low rates of confirmed virologic withdrawal through Week 48 (≤ 1% of patients per arm) were observed. No treatment-emergent INSTI mutations or NRTI mutations were seen among participants who met CVW (confirmed virologic failure) criteria.

Finally, the safety findings were comparable between arms and were observed significant differences in impact on renal and bone biomarkers in favour of DTG plus 3TC arm.

In conclusion, a dual therapy with 3TC + DTG in naive patients could be an alternative to a triple therapy based on TDF + FTC + DTG. Since the life expectancies in patients with HIV are extended, it is important to consider use of therapies that reduce cumulative drug exposure and toxicity.



*ITT-E population excluding significant protocol violations.
†Adjusted for HIV-1 RNA (≤ vs > 100,000 copies/mL), CD4+ cell count (≤ vs > 200 cells/mm³), and study (GEMINI-1 vs GEMINI-2).

Figure 1 Results of the GEMINI-1 and 2 studies: Virologic Response at week 48. Adapted from Cahn P et al [2]

Darunavir/Cobicistat/Emtricitabine/Tenofovir Alafenamide (D/C/F/TAF) in a Test-and-Treat Model of Care for HIV-1 Infection: Interim Analysis of the DIAMOND Study

Many studies have shown the benefits of the immediate initiation of antiretroviral treatment (without waiting the results of the resistance study or the viral loud and CD4 data) in patients newly diagnosed with HIV infection, especially in populations with low resources and difficult access to the health system. Benefits in retention of care, morbidity, mortality and time in obtaining virological suppression has been described.

DIAMOND [3] is an ongoing phase 3, single arm, open label, prospective multicenter study evaluating D/C/F/TAF in a rapid initiation model of care over 48 weeks. The main objectives of the study are: a) to asses the efficacy and safety of this regimen in the treatment of newly diagnosed HIV 1 infected naive patients; b) to asses the baseline viral resistance in the study population; and c) to evaluate the results of an HIV Treatment Satisfaction Questionnaire status version (HIVTSQs) at weeks 4 and 24. This is the first phase 3 trial of an STR in a rapid initiation model of care.

In this trial, a high proportion of patients using D/C/F/TAF achieved HIV-1 RNA <50 copies/ml, and 91% (99/109) of patients continued treatment through the interim analysis at week 24. It is a remarkable finding that no patients discontinued treatment due to receipt of baseline resistance reports, and no patients had confirmed virologic failure or discontinued due to lack of efficacy. On the other hand, at weeks 4 and 24, the results of the mean HIVTSQs score approached the maximum of 60, indicating a high level of satisfaction. These findings together with the efficacy, high barrier to resistance, safety profile and convenience of a single table regimen, suggest that D/C/F/TAF should be considered an adequate option of treatment in a quick initiation model of care.

Risk of HIV transmission through condomless sex in gay couples with supressive ART: The PARTNER2 study extended results in gay men

This prospective, observational, multicenter study assessed HIV transmission risk in serodifferent men who have sex with men (MSM) couples reporting condomless sex when HIV-positive partner virologically suppressed [4-6]. PARTNER2 followed MSM from 2014-2018 (included some MSM couples from PARTNER1). Ninety seventy-two couples MSM were recruited, of whom 783 contributed 1.596 eligible couple-years of follow up (CYFU). The main finding of the study was that no within-couple HIV transmissions were observed among 783 serodifferent MSM couples who reported condomless (CL) sex while the HIV-positive partner was receiving suppressive ART. Nevertheless, 15 HIV-negative men acquired HIV infection during follow-up, although none of these infections was phylogenetically linked to the HIV-positive partner. During almost 77,000 within-couple CL sex acts, upper 95% CI for rate of transmission between MSM was 0.23/100 CYFU. This data shows, with more statistical certainty than in the PARTNER1 study, that the risk of HIV transmission from an HIV-positive partner who has undetectable HIV-1 RNA is effectively zero.

Incidence of HIV-Infection in the AVRS Prevenir Study in the Paris Region with daily or On Demand PrEP with TDF/FTC

Molina et al. [7] showed the real-life data of the PrEP (pre-exposure prophylaxis) application in Paris. PREVENIR is a multicenter, open-label, prospective cohort study in Paris that includes HIV-negative adults at high risk of HIV infection with inconsistent condom use. At the beginning of the study, participants choose between daily or on demand prophylaxis, being able to change their arm during the course of the same. A total of 1,594 participants were enrolled, 98% MSM. The primary endpoint was a ≥ 15% reduction in new HIV diagnoses among MSM in Paris vs rate reported by National Surveillance network in 2016. Secondary endpoints were to analyse PrEP adherence, sexual behaviour, and safety. At an average follow-up of 7 months, the incidence of HIV in both groups

was 0, and it was estimated that 85 HIV infections had been prevented. Regarding safety, 11 participants were diagnosed of viral hepatitis, including 7 cases of hepatitis C. Among the groups, the incidence of the first viral hepatitis was 1.1/100 patient-years for daily PrEP and 1.2/100 patients-year for PrEP on demand. In the adherence analysis, it was observed a high rate of correct PrEP use in daily and on-demand groups, being both of 96%. In terms of sexual behaviour, daily PrEP users reported higher numbers of condomless sex acts, and sexual partners at baseline.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Official journal of the Spanish Society of Chemotherapy

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Current status of ESKAPE microorganisms in Spain: Epidemiology and resistance phenotypes

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ABSTRACT

Resistance rates in ESKAPE microorganisms included in the EARS-net surveillance database from Spain have increased in most of the cases. In 2017, multi-drug resistant isolates rose to 5.5% in *Escherichia coli* and 13.0% in *Klebsiella pneumoniae*. Carbapenemase producing *Enterobacterales* (CPE) have also increased in Spain over the last years with a current spread of throughout the country. EuSCAPE project revealed dominance of OXA-48 carbapenemase with lower prevalence of KPC, VIM or NDM enzymes. Increase of faecal carriers and presence of carbapenemases in the so-called high-risk clones have boosted the persistence and dissemination of CPE. One of these clones, the ST307 *K. pneumoniae,* has been associated with the spread of KPC carbapenemases and emergence of KPC variants conferring resistance to ceftazidime-avibactam combination.

Key words: ESKAPE microorganisms; carbapenemase producing *Enterobacterales*, ceftazidime-avibactam

INTRODUCTION

Traditionally in developed countries, the problem of nosocomial antimicrobial resistance has been mainly associated with a particular group of microorganisms, "the ESKAPE bugs"[1]. While many bacteria remain susceptible to antimicrobial agents, this group (composed of *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species) presents a potential series of mechanisms to evade the lethal or inhibitory action of antimicrobial agents. The high antibiotic exposure due to excessive antimicrobial prescription or its inappropriate use, acquisition of resistance mechanisms either by mutational events or gene transfer and clonal spread have been the causes of their increase. Nowadays this group has been extended to other clinically relevant microorganisms and includes the overall *Enterobacterales*, *Clostridioides difficile* and all *Enterococcus* species.

In order to fight against the ESKAPE organisms, strategies such as "10 × '20" proposed by The Infectious Diseases Society of America (IDSA) were developed. The aim of this initiative was the creation of sustainable global antibacterial drug research and development enterprise with the power in the short term to develop 10 new, safe, and efficacious systemically administered antibiotics by 2020 [2] especially the ESKAPE pathogens, continue to increase in frequency and cause significant morbidity and mortality. New antimicrobial agents are greatly needed to treat infections caused by Gram-negative bacilli (GNB). This was necessary due to the decrease in the number of new systemic antibacterial agents approved by the Food and Drug Administration (FDA) in the US and the European Medicines Agency (EMA) in the EU, despite the need for new antibiotic compounds. Moreover, the high rates of resistance among these microorganisms have led the World Health Organization (WHO) to recommend prioritization in the development of new antibiotics against them [3].

In Spain, the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC) made a study with the objective of determining the clinical impact on mortality of multi-drug resistant (MDR) infections in our country. During a one-week period (March 2018), all MDR infections were investigated in 82 hospitals. This involved a total of 903 patients infected by MDR microorganisms of whom 177 died during the first month. If this data were extrapolated to the total of hospitals in the country, this would imply a total of 35,400 annual deaths in patients presenting infections due to MDR microorganisms [4].

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ANTIMICROBIAL RESISTANCE IN ESKAPE ORGANISMS IN SPAIN

Currently, in Spain, resistance among the ESKAPE organisms has mainly increased over the years, a fact documented in the EARS-net surveillance study [5] (figure 1). In *Enterococcus faecalis,* unlike *E. faecium*, a low percentage of resistance (considering the intermediate and resistant categories) to ampicillin (0.8%) and vancomycin (0.1%) whereas a 36.6% of high-level aminoglycoside resistance was observed in 2017. This is very similar to that observed in 2005. On the contrary, in *E. faecium,* ampicillin resistance has increased with respect to 2005 from 49.2% to 83.7%, the high-level aminoglycoside resistance has also experimented an increment (12.5 to 32.3%) but vancomycin resistance rates have remained low (2.4%). In *S. aureus,* methicillin resistance has slightly decreased from 29.4% to 25.3%.

In *Acinetobacter* spp., resistance to carbapenems and aminoglycosides has decreased (68.2% and 56.8%, respectively) and also, the rate of MDR isolates (51.1%) remain minor. In *P. aeruginosa*, we have more resistance than in 2005 to piperacillin-tazobactam (8.2%), ceftazidime (14.6%), carbapenems (20.7%), aminoglycosides (19.3%), fluoroquinolones (23.9%) and overall a higher percentage of MDR microorganisms (10.9%). Focusing on *Enterobacterales*, and specifically in *E. coli* and *K. pneumoniae,* the percentages of resistant isolates have increased in both species: third generation cephalosporin resistance has increased in *E. coli* (1.0% to 13.1%) and *K. pneumoniae* (7.1% to 21.7%). This trend was also observed for aminoglycosides, fluoroquinolones and carbapenems. As a consequence, the percentage of MDR *E. coli* has increased up to 5.5% and the MDR *K. pneumoniae* up to 13.0% in 2017 (figure 1), the latter might also include carbapenemase producers.

CARBAPENEMASES-PRODUCING *ENTEROBACTERALES*

The problem of carbapenems resistance in *Enterobacterales* lies in a series of factors that are promoting their emergence, persistence and rapid dispersion. The increased prevalence of faecal carriage and co-colonization with carbapenemase-producing *Enterobacterales* (CPE), the dispersion of MDR high-risk clones, the presence of co-resistance to other antimicrobials, including colistin resistance and now, the appearance of resistance determinants to new ß-lactam-ß-lactamase inhibitor combinations are the main factor driving this trend. In Europe, the EuSCAPE program performed a survey not only to determine the occurrence of carbapenemase-producing *K. pneumoniae* and *E. coli* in European hospitals but also to depict differences in the spread of different carbapenemases in different countries (figure 2) [6].

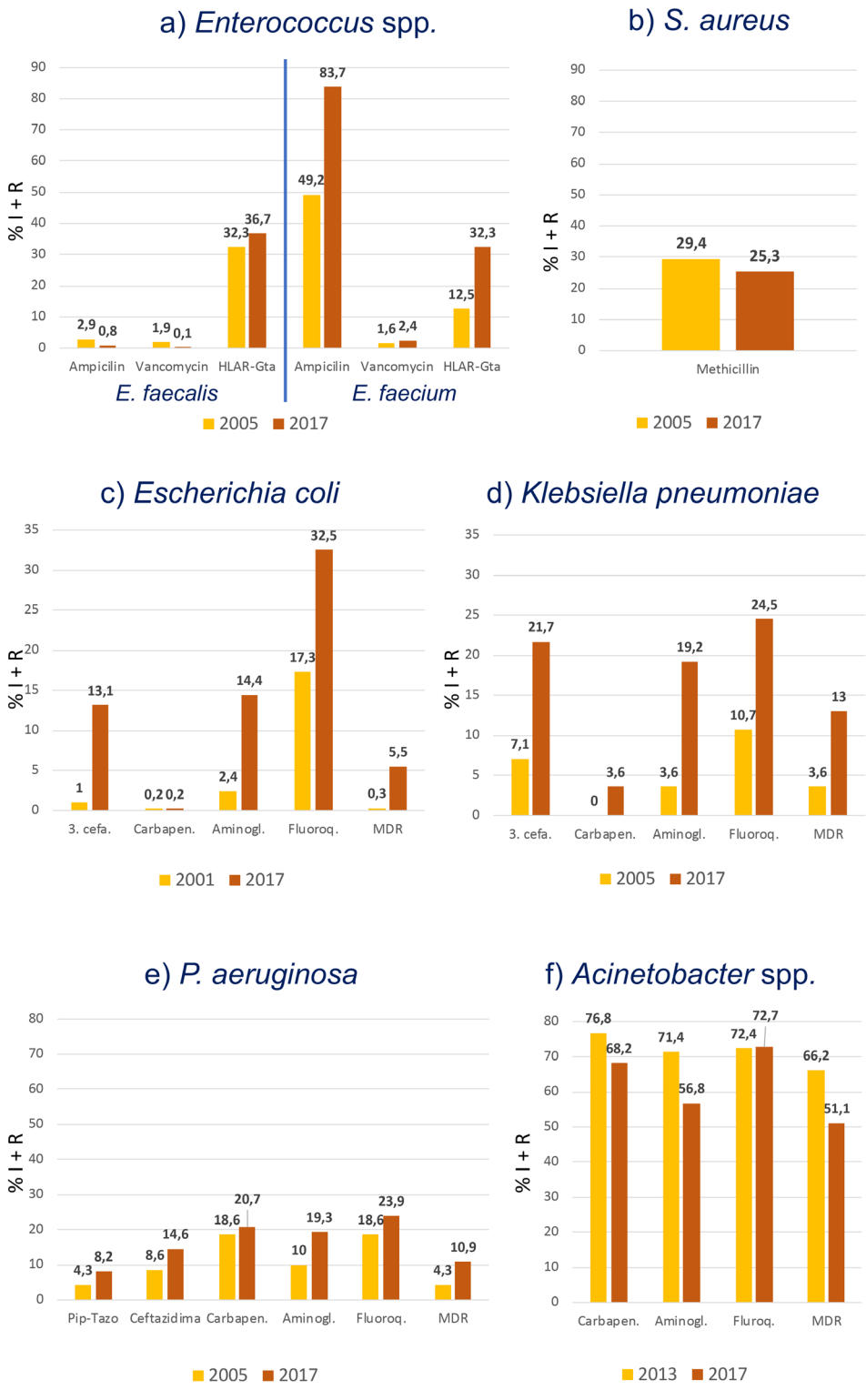
For the same reasons, the complexity in the carbapenemase distribution in *Enterobacterales* in Spain has increased along the last years. The first detection of CPE in our country was associated with sporadic cases of metallo-ß-lactamases (MBLs) in Barcelona in 2003 [7]. Later in 2007, local outbreaks in different hospitals in the Madrid area due to VIM and KPC carbapenemases were described in patients with no history of travel abroad [8, 9]. A national multicenter study performed in 2009 only demonstrated a very low prevalence [10]. The detection of the first imported cases of NDM occurred in 2010 and the appearance of extra-hospitalary cases with no previous sanitary contact [11] has continued until today with local outbreaks and dispersion in different areas of OXA-48-carrying *Enterobacterales* [12]. Higher prevalence of OXA-48 was also highlighted in the EuSCAPE project with lower prevalence of KPC, VIM and NDM carbapenemases.

As mentioned before, one of the reasons of CPE dispersion is the increasing number of patients colonized with CPE. In the article of Hernández-García *et al.* [13], incidence of colonization by CPE in our hospital, during a follow-up period between March 2014 and March 2016, was 2% (161/8,209) of patients, and of these 0.9% were colonized at admission and 1.1% acquired colonization during admission. The principal colonizer was *K. pneumoniae* (54%) followed by *E. coli* (19%) mainly as OXA-48 (64.1%) and VIM-1 (26.8%) producers. Also, 20% of patients were colonized with two or three different CPE (co-colonization).

Other factors fueling emergence and dispersion of CPE is the presence of carbapenemases in the so-called MDR high-risk clones. A recent study performed in Spain analyzing the population structure of CPE revealed that carbapenemases concentrate in a few clones when compared with the susceptible population. These MDR high-risk clones are the cause of multiple outbreaks throughout our country as the one described in Cordoba [14]. This originated in a patient transferred from an Italian hospital, and there was a range of 67 infected and 14 colonized patients and a mortality of 30% due to a *K. pneumoniae*-ST512-KPC-3 resistant to third generation cephalosporins, carbapenems, tobramycin, amikacin, fluoroquinolones and colistin. Also, there is a significant percentage of CPE isolates from clinical samples in ICU admitted patients. This was demonstrated in a recent multicenter study performed in Spain in 8 hospitals, with 23.1% of ESBL-producing *Klebsiella* spp. and 20% of carbapenemase-producing *Klebsiella* spp. [15]. These isolates also showed high co-resistances to non-β-lactam antimicrobials.

EMERGENCE OF CEFTAZIDIME-AVIBACTAM RESISTANCE

Nowadays, we have new compounds available designed to fight against CPE as new ß-lactam-ß-lactamase inhibitors combinations such as ceftazidime-avibactam and meropenem-varbobactamin and in the future imipenem-relebactam. The first one, already available in the US and EU, is a combination of ceftazidime, a classical third generation cephalosporin, and avibactam, a non-ß-lactam (diazabicyclooctane) ß-lactamase inhibitor, that is active against Ambler class A and C ß-lactamases and possesses activity against some Ambler class D enzymes, including OXA-48 producers.



https://ecdc.europa.eu/en/antimicrobial-resistance/surveillance-and-disease-data/data-ecdc / [access February 2019]

Figure 1 Percentage of non-susceptible ESKAPE isolates. Data obtained from the EARS-net data base (https://ecdc.europa.eu/en/antimicrobial-resistance/surveillance-and-disease-data/data-ecdc, access February 2019)

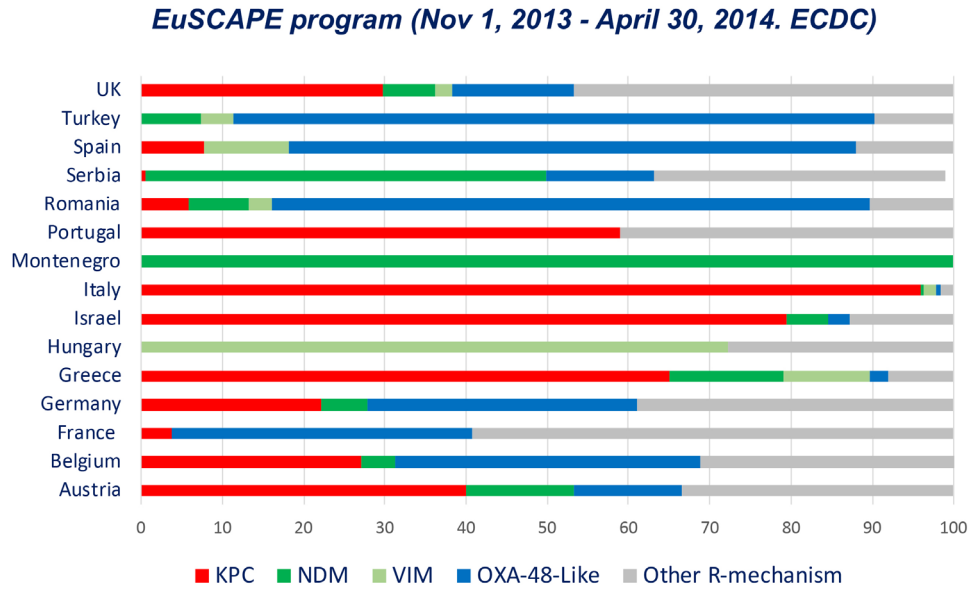


Figure 2 | **Occurrence of different carbapenemases in carbapenemase-producing *Klebsiella pneumoniae* in the European survey of carbapenemase-producing *Enterobacteriaceae* (EuSCAPE) (Data obtained from reference [6])**

Despite its short life in the clinical setting, isolates with acquired resistance to this combination has already been occasionally described. The mechanisms involved in this resistance include: a) Overexpression of extended-spectrum AmpC in *E. cloacae* and mutations in AmpC from *P. aeruginosa*; b) Increased hydrolytic activity of $bla_{\text{CTX-M-14}}$ variants; c) KPC-*K. pneumoniae* with multiple resistance mechanisms such as KPC-3 overexpression plus porin deficiency (*ompK35/ompK36*) and SHV-12 with enhanced efflux activity (*AcrAB*); and d) double or triple $bla_{\text{KPC-3}}$ mutations and $bla_{\text{KPC-2}}$ mutations that confer resistance to ceftazidime-avibactam. Interestingly these last resistance mechanisms might produce a reversion of carbapenem susceptibility, a phenomenon that has been named as "collateral sensitivity".

Many of these resistance mutations appear after ceftazidime-avibactam treatment, such as bla_{KPC} mutations and lead to treatment failure and resistance development [16]. This had been reproduced with in vitro studies subjecting the strains of KPC producing *K. pneumoniae* to various concentrations of antibiotic and counting the number of colonies that grew [17]. Furthermore, in these strains with bla_{KPC} gene mutation, at the same time as the development of ceftazidime-avibactam resistance, there was restoration of meropenem susceptibility occurred during or after ceftazidime-avibactam treatment [18].

In our hospital, we have detected a rapid dissemination of a KPC-3-producing *K. pneumoniae* ST307 clone. In a year period, we detected 353 patients with carbapenemase-producing *K. pneumoniae* isolates of whom 19.2% (68/353) were Kp-ST307-KPC-3 producers. In two patients, ceftazidime-avibactam resistance developed associated with ceftazidime-avibactam treatment due to the emergence of a KPC-3 variant [19].

CONCLUSIONS

In summary, there has been an increase of ESKAPE organisms in Spain during the last years. In addition, CPE have been dispersed throughout all the country with a dominance of OXA-48 producing *K. pneumoniae* isolates but with an increasing prevalence of KPC producers and maintenance of VIM producers. This complexity in the carbapenemase-distribution among *Enterobacterales* is also due to high faecal carriers co-colonized with different CPE, the dispersion of MDR high-risk clones, and the co-resistance with non-ß-lactam antimicrobials, including colistin. Moreover, the emergence of resistance to new β-lactam-β-lactamase inhibitor combinations with restoration of carbapenem susceptibility due to new KPC mutations has also been detected in Spain.

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CONFLICT OF INTEREST

Authors have no conflicts of interest to declare with respect to the contents of this manuscript.

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Antibiotic selection in the treatment of acute invasive infections by *Pseudomonas aeruginosa*

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ABSTRACT

Pseudomonas aeruginosa is characterized by an important intrinsic resistance to antibiotics and it possess an extraordinary ability to develop resistance to nearly all available antimicrobials through selection of mutations. We review some of the pharmacodynamic principles of antibiotics predicting efficacy, clinical experience with monotherapy and combination therapy, and principles for antibiotic treatment for empirical and directed treatment of *P. aeruginosa* invasive infections.

Key words: ***Pseudomonas aeruginosa*, treatment**

PRINCIPLES FOR THE TREATMENT OF INFECTIONS CAUSED BY *P. AERUGINOSA*

Principles guiding election of antibiotic treatment, whether empirical or directed treatment, in case of suspected or confirmed *Pseudomonas aeruginosa* infections, are those also applying to any severe infection, but with some peculiarities as follows:

1) MIC of main antibiotics active against *P. aeruginosa*. The breakpoint used to categorize *P. aeruginosa* as resistant to one β-lactam or aminoglycoside is from 2-times (piperacillin-tazobactam, imipenem, tobramycin, gentamicin) to 8-times (ceftazidime, cefepime) higher than the one used to consider resistant an enterobacteria. Against most clinical isolates of *P. aeruginosa* susceptible to β-lactams, the MIC of an antibiotic is usually at or close to its breakpoint value (2- 8 mg/L). For this reason, high doses of β-lactams are recommended, even if the strain has been categorized as susceptible in *in vitro* susceptibility tests.

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For the treatment of severe or high bacterial load infections, produced by microorganisms exhibiting MIC ≥4 mg/L of the β-lactam, only elevated doses administered by continuous or extended infusion reach free antibiotic concentrations exceeding 4-times the MIC [1].

In most clinical studies [2] but not in all [3]), continuous or extended infusion of piperacillin-tazobactam, cefepime, ceftazidime or meropenem, for the treatment of infections by Gram-negative bacilli (including *P. aeruginosa*) was more efficacious than intermittent administration with respect to the one or more following parameters: clinical cure rate, microbiological eradication, days with fever, length of ICU or hospital stay and decrease in severity (measured by APACHE II) and/or mortality.

The main determinant for clinical response to an aminoglycoside treatment is the Cmax/MIC value [4]. For the reasons exposed below, the greatest efficacy for a treatment is obtained when Cmax/MIC ≥10. For a MIC value for *P. aeruginosa* of 2-4 mg/L of tobramycin and gentamicin, the recommended Cmax is 30-40 mg/L and for amikacin MIC of 8 mg/L, Cmax should be between 60 and 80 mg/L [5]. Usually these values are not achieved with standard doses.

2) Importance of the bacterial load in the infectious foci. In *P. aeruginosa* infectious foci as pneumonia, purulent tracheobronchitis in the intubated patient, secondary peritonitis, neutropenic colitis and skin and soft tissue infections, the bacterial load at antibiotic treatment initiation is usually high (≥10^7-10^8 CFU).

The ability of granulocytes to eradicate microorganisms is saturable [6]. In rat models of pneumonia by *P. aeruginosa*, when the bacterial load was close to or higher than 2.5 x 10^6 CFU/g of tissue, the bacteriolytic ability of granulocytes was surpassed and bacterial growth occurred [7]. The authors of these studies suggested that in infections with high bacterial load, as VAP, an early and rapid ≥2 log10 CFU/mL decrease

produced by the antibiotic treatment might decrease bacterial density below the cut-off level of granulocyte activity saturation, allowing an optimal contribution for microorganism eradication.

Another important consequence of the presence of a high bacterial load is the increased risk of selection of resistant mutants.

3) Mutation ability and development of resistance in *P. aeruginosa*. Frequency of emergence of resistant mutants within *P. aeruginosa* populations ranges from 10^6 to 10^8 depending on the antibiotic [8]. In the presence of agents damaging DNA (fluoroquinolones) and in biofilm-embedded bacterial growth, the basal rate of emergence of resistant mutants can be around 100 times increased [9].

A bacterial density ≥ 10^7 -10^8 CFU at treatment initiation involves high risk of selection and amplification of the resistant subpopulation under the selective antibiotic pressure. Measures to counter this risk include: a) reduction of the bacterial load through the control of the infectious foci (drainage, debridement, de-obstruction or removal of catheter or infected foreign body), b) initiation of treatment with associations of antibiotics not sharing the main resistance mechanism [10], and c) use of doses and/or routes of administration able to generate an antibiotic concentration higher than MIC for potential resistant mutants in the infectious foci.

Antibiotics (aminoglycoside, ciprofloxacin or levofloxacin) associated with the β-lactam during the first 48-72 h, among other purposes to avoid selection of resistant mutants, should be administered at doses achieving concentrations over the corresponding mutant prevention concentrations (MPCs). Although MPCs are unknown and could not be predicted from MIC values, generally for these antibiotics they are from 8 to 12 times higher than the MIC.

At the 2nd-3rd day of treatment, when deescalation to monotherapy is considered, most patients remain colonized by *P. aeruginosa* in mucosa and bronchial secretion (in case of pneumonia, tracheal intubation or previous bronchial pathology), especially if no inhaled antibiotic treatment with tobramycin, colistin or aztreonam had been administered. Persistence of bronchial colonization does not justify by itself prolongation of IV administration of the aminoglycoside more than 3-5 days.

4) Importance of an appropriate empirical treatment. Studies performed in patients with VAP [11] or bacteremia [12] caused by *P. aeruginosa* showed high mortality rates if the initial empirical antibiotic treatment is not appropriate. Early administration of an appropriate antibiotic treatment has special relevance when the infection presents clinical or biological severity criteria, the patient suffers important immunodepression or comorbidities or has advanced age [13]. Treatment initiation with a β-lactam associated with amikacin, ciprofloxacin or colistin (chosen based on local resistance rates) increases the probability of the appropriateness of the initial empirical schedule [11].

5) Value of antibiotic associations. Usually, the association of a β-lactam and an aminoglycoside shows *in vitro* synergistic activity. However, in clinical practice, the potential synergy of the association does not seem to turn into a tangible improvement of prognosis estimated as survival rate. Most studies carried out in patients with bacteremia [12] or VAP [11] by *P. aeruginosa*, as well as several meta-analyses [14], did not found significant differences in mortality rates between patients receiving β-lactam monotherapy and those receiving a β-lactam and aminoglycoside association. Nevertheless, there are several aspects raising doubts with respect to the strength of these results. Most studies were retrospective analyses, treatments were not randomized, the most severe patients tended to be treated with antibiotic associations [15] and analyses were not adjusted by possible confounding factors. In a significant number of patients, the origin of bacteremia was an urinary tract infection or venous catheter removal, thus, non-severe infections and low bacterial load. In addition, in the aminoglycoside arm nephrotoxicity masking the benefits of the association could not be ruled out since renal failure is an important prognostic factor in critically ill patients. On the other hand, in other studies, a favorable effect of the association versus monotherapy has been reported in the treatment of bacteremia caused by *P. aeruginosa* [16], particularly in neutropenic patients [17], in cystic fibrosis exacerbations [18] and in a meta-analysis of studies on bacteremia by Gram-negative bacilli. However, these results are neither conclusive because in the monotherapy arm patients treated with aminoglycosides were often included [19].

In most clinical situations, the treatment of choice for a β-lactam susceptible *P. aeruginosa* infection is β-lactam monotherapy except in the following cases: 1) during the first 72 hours if the infection presents criteria of severe sepsis or septic shock, 2) in the neutropenic patient, and 3) in nervous central system (meningitis, abscess) or endovascular (endocarditis) infections. Use of associations including a β-lactam should be considered even for the treatment of infections caused by β-lactam resistant pathogens, especially if the resistance level is moderate (MIC 2-4 times higher than the breakpoint value). In this situation, the potential synergy with the second antibiotic could revert β-lactam non-susceptibility, if succeed in lowering the MIC below the resistance level.

6) Clinical efficacy of different antibiotics as monotherapy. Clinical experience evidences that monotherapy with β-lactams shows higher efficacy and/or lower toxicity than monotherapy with aminoglycosides [12] or colistin [20] and similar to monotherapy with a fluoroquinolone (ciprofloxacin) [21] in the treatment of Gram-negative infections, including those by *P. aeruginosa*. However, in some infection sites, as in external malignant otitis, prostatitis, or cystic fibrosis bronchial infections, the use of ciprofloxacin may have advantages over a β-lactam, based on the possibility of oral administration, better penetration in the infectious foci and the probable greater activity in biofilms.

7) Measures to increase antibiotic concentrations in the infectious foci. As mentioned in points 1 and 2, to optimize the PK/PD index and to avoid selection/amplification of resistant subpopulations, high (aminoglycosides, fluoroquinolones) and maintained (β-lactams) antibiotic concentrations are required in the infectious foci. Nevertheless, in certain infection sites (as in pneumonia in the intubated patient, ventriculitis, meningitis), even with the maximum tolerated dose, MPCs are not exceeded or the associated toxicity is unacceptably high. In these cases, the possibility of directly introducing the antibiotic into the infectious foci using the inhalatory, intrathecal or other routes (depending on the infection site) should be considered.

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The pharmacodynamic bases of the prescription of antimicrobials

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ABSTRACT

In the past, the dose of an antibiotic was chosen, always from among those that were well tolerated, by considering those with the ability to exceed the MIC of bacteria in plasma. This approach, which has still not widely changed, is contrasted with the pharmacokinetic and pharmacodynamic (PK/PD) relationships, which indicate that the efficacy of antibiotics is directly related to parameters that relate the sequence of concentrations over time with a parameter of the MIC effect *in vitro*. Until now, three types of PK/PD relationships have been established for antibiotics: the inhibitory coefficient (Cmax/MIC), the efficacy time (T>CMI) and the relationship between the exposure of the drug and the MIC (AUC/MIC).

Keywords: Antibiotics, PK/PD relationships

The gradual discovery of the importance of PK/PD relationships means that perhaps we will have to revise the posology of most anti-infectives. In the past, the dose of an antibiotic was chosen, always from among those that were well tolerated, by considering those with the ability to exceed the MIC of bacteria in plasma, in principle the higher the better. An identical approach was established for the choice of the administration interval, which was determined by considering how long the drug maintained concentrations in the plasma that exceeded the active infection. Sometimes, and considering possible problems of access to infected tissues, this meant adding to the above conditions the achievement of plasmatic concentrations that exceeded the MIC of the bacteria, throughout the posological interval by between 4 and 5 times.

Based on these premises, clinical trials are still designed during clinical research using posological guidelines that are often debatable. Therefore, in the field of daily practice, failures occur that are hard to explain and that could potentially have their origin, at least partly, in the lack of similarity between the patients included in clinical trials and the patients treated in health care practice, as the former are selected by following inclusion and exclusion criteria that are unrepresentative of the population where the antibiotics will be used in daily practice.

This approach, which has still not widely changed, is contrasted with the pharmacokinetic and pharmacodynamic (PK/PD) relationships, which indicate that the efficacy of antibiotics is directly related to parameters that relate the sequence of concentrations over time with a parameter of the MIC effect *in vitro*.

Until now, three types of PK/PD relationships have been established for antibiotics: the inhibitory coefficient (Cmax/MIC), the efficacy time (T>CMI) and the relationship between the exposure of the drug and the MIC (AUC/MIC).

The first of these indicates that the effect of a drug fundamentally depends upon the coefficient between the concentration reached and the minimum effective concentration. The drugs that belong to this group (aminoglycosides, colistin, nitroimidazoles and probably rifampicin) present greater activity *in vivo* the higher the administered doses are, without the administration interval being especially important. Consequently, it is recommended to administer the medicinal products in this group in one daily dose. The parameter that defines this relationship is the inhibitory coefficient (Cmax/MIC) and its ideal value appears to be greater than 10. In the case of aminoglycosides and considering the cut-off point of activity *in vitro*, this figure means that a dose of 7 and 20 mg/kg must be administered for gentamicin and amikacin respectively, a high dose that is potentially associated with a risk of renal and cochlear toxicity [1]. With colistin it has been indicated that the optimisation of its efficacy goes from the administration

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of a high loading dose of 9 MU, followed by a dose of at least 4.5 MU every 12 h, intravenously [2].

The second PK/PD model is mixed, as the parameters of interest are the concentrations achieved and how long they are maintained at values greater than the MIC, and consequently the AUC/MIC is the ratio that indicates the efficacy. The antibiotics belonging to this group must be administered in a dose that will generate the highest possible plasmatic concentration, and also at an interval that will avoid the presence of subinhibitory concentrations.

Fluoroquinolones are included in this section, the recognition of which has led to increasing the dose of levofloxacin and of ciprofloxacin [3]. In addition, vancomycin appears to be more effective when administered in regimes that reach AUC/MIC values that are greater than 400 (25,26), a situation that can pose therapeutic problems. Currently it is indicated that when the MIC of the causal strain of the infection is 2 mg/l, it is necessary to administer doses that are associated with nephrotoxicity [4]. In these circumstances it is necessary to verify the seric concentration of vancomycin in the valley immediately before administering the 5th dose (after reaching the state of stationary balance) and adjusting the following doses to obtain the desired values. In the case of linezolid, the optimum value of $AUC_{0\text{-}24h}$ /MIC is 100, which means [5] that it is necessary to administer up to 3 daily doses in the case of strains that are within the cut-off point limit; 4 mg/L.

The best clinical response by daptomycin is achieved with $AUC_{0\text{-}24h}$/MIC >600 [6]. One dose of daptomycin of 6 mg/kg/day generates an AUC_{24h} of around 700 µg h/ml [7] which means an optimum exposure in the case of infection by methicillin-resistant *Staphylococcus aureus* (MRSA) strains with an MIC < 0.5 mg/l [8], but in the case of strains that present higher MIC values it is necessary to administer high doses that are usually 10-12 mg/kg/day.

The $AUC_{0\text{-}24h}$/MIC values of tigecycline that best discriminate between the probability of success or failure, clinical or microbiological, are 12 and 18 µg h/ml respectively [9]. With the usual dose of 50 mg/12 h iv, in a state of stationary balance, an AUC_{24h} in saline is obtained of 4-6 µg h/l, while the MIC_{90} against MRSA strains is 0.25-0.50 mg/l [10]. It is therefore usual to recommend the administration of double the dose.

The third of the models, which includes all β-lactam antibiotics, [11-13] seems to depend especially on maintaining free drugs above the MIC for as long as possible (T > MIC). This parameter, known as the efficacy time, is the reason for discrepancies, because some authors argue that it is not necessary that the estimate of the T>MIC to reach a value of 100%, that is, it might be sufficient for this value to be located at 40-50%. The real-life date provided by health care practice appears to oppose partial or interested readings and it is therefore increasingly evident that β-lactams must be administered at intervals that will cover the MIC of bacteria for as long as possible, that is, they must reach T > MIC = 100%. This result is simple for some drugs that have a very high elimination half-life, but complex in the case of antibiotics, which like the vast majority of β-lactams, present a plasmatic half-life of under 2 h. This is a difficult problem in p.o. administration and also in i.v. administration, which will require the administration of many daily doses or the use of another possible i.v. administration method, which is prolonged or continuous infusion [14, 15].

Logically, taking account of the storage and stability in solution conditions before making prescriptions of these types of infusions becomes a priority.

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Update in nosocomial infection

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Practical approach to the management of catheter-related bloodstream infection

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ABSTRACT

Catheter-related bloodstream infections (CRBSI) is a common cause of nosocomial infection associated resulting in substantial morbidity, mortality, increased length of hospital stays and health-care costs. New clinical practice guidelines for the management of adults with CRBSI have been published in 2018 by the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC) and the Spanish Society of Intensive and Critical Care Medicine and Coronary Units (SEMICYUC). This review focuses on updated recommendations for the diagnosis and management of CRBSI in adults. Prevention of CRBSI is excluded. Our aim is to show some of the key aspects concerning the following topics: diagnosis, empirical and targeted therapy.

Key words: Catheter-related bloodstream infection; guidelines; bacteremia.

INTRODUCTION

Nosocomial bloodstream infections (BSIs) have significant associated morbidity, incur increased hospital costs, and prolonged the length of stay [1]. Attributable mortality ranges between 12% and 25%. Most nosocomial BSIs are associated with intravascular (IV) catheters and central venous catheters (CVCs) in particular.

In the last years, intravascular device insertion has become a very common practice in the hospital and outpatient settings for various purposes, including hemodynamic monitoring, renal replacement therapy, nutritional support, as well as fluid and medication administration. According to national data facilitated by the study of the prevalence of nosocomial infections in Spain (EPINE), it is estimated that approximately 70% of the patients admitted to Spanish hospitals will carry one of these devices at some moment during their stay [2].

Recently published Spanish clinical guidelines provide recommendations about diagnosis and management of catheter-related bloodstream infections (CRBSI) in adults [3]. The experts identified 39 questions being possible to define 103 recommendations with different levels of gradation. Thus, within category A there were 41 recommendations, 29 in category B and 23 C. However, it is worth noting that, regarding the categorization of the recommendation, only 10 could be placed with a quality of the evidence of AI [4]. Nevertheless, other aspects as prevention are therefore excluded. Nonetheless, a manuscript recently published provides a comprehensive review about aseptic measures recommended by scientific societies the insertion and manipulation of vascular catheters [5]. The aim of the present manuscript is to summarize the most relevant recommendations of this Spanish document updating with relevant information recently published.

CATHETER-RELATED BLOODSTREAM INFECTION DIAGNOSIS

It is essential to make an accurate diagnosis of CRBSI because there are serious consequences associated with inaccurate or failed diagnoses, such as unnecessary serious procedural complications and increased morbidity and mortality if the catheter origin of a BSI is not timely removed. The document defines the clinical characteristics and other factors in order to establishing a clinical suspicion and initiate a microbiological diagnostic, as well as, what conditions are needed to consider the CRBSI as complicated.

CRBSI should be clinically suspected in patients with intravenous catheters and onset of fever, chills or other signs of sepsis, even in the absence of local signs of infection, and

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especially if no alternative source is recognized. Several circumstances should increase suspicion such as local sign of infection at the catheter, metastatic infections caused by hematogenous spread of microorganisms or persistent blood cultures for particular microorganisms.

There are a variety of factors associated with poorer outcomes, which make CRBSI as complicated. Factors associated with complicated bacteremia are patients diagnosed with CRBSI and with endocarditis, suppurative thrombophlebitis, septic metastasis, extraluminal infections, septic shock, non-resolving CRBSI, or immunocompromised patients.

DIAGNOSIS CRBSI WITH O WHITHOUT (CONSERVATIVE DIAGNOSIS) CATHETER WITHDRAWAL

The guidelines emphasize the recommendation that a catheter culture must only be obtained when a CRBSI is suspected, thus avoiding unnecessary cultures [6]. Removal of catheter is the most suitable approach for the diagnosis of CRBSI at least in the critical care setting.

Nevertheless, removal of a suspected CVC, may not be feasible or practical for a variety of reasons, such as including limited alternative vascular access or unacceptable complications associated with removal and replacement.

Summary of main diagnostic methods for catheter-related bloodstream infections recommended by new guidelines for the different patient subgroups with suspected CRSBI are the following:

- Semiquantitative culture (roll plate) or quantitative culture performed after sonication or vortex washing the catheter tip are the preferred method for sampling long-term IV catheters. Lamentably, these culture techniques require removal of the catheter.
- In situations where catheter cannot be withdrawal, paired blood cultures obtained simultaneously from a catheter lumen and peripheral blood meets the criteria for CRBSI by quantitative blood cultures (a colony count 3 times greater in a sample drawn through a catheter than from the peripheral vein) or differential time to positivity (DTP) (positivity of blood cultures obtained through the catheter ≥120 min before those obtained from a peripheral vein). The role of DTP for the diagnosis of catheter related candidemia remains controversial.
- Other conservative techniques such as endoluminal brushing, superficial cultures or Gram stain-acridine orange leukocyte cytospin (AOLC) of catheter blood are not widely used in clinical laboratories.

Although molecular-based rapid diagnostic testing has evolved recently for the early identification of microorganisms in BSIs, including infections resulting from vascular catheters [7], these guidelines contemplate the usefulness of these techniques as a potential for the improvement in the diagnosis CRBSI in patients undergoing antibiotic therapy, taken into account these techniques have not been standardized.

CATHETER RELATED BLOODSTREAM INFECTION TREATMENT

One of the most important contribution of this document refers to the no immediate and systematic removal of all catheters in patients with suspected related infection, establishing the criteria that must be fulfilled in order to take this clinical decision. This recommendation is based on two studies that found no differences in outcomes when early CVC removal was compared with a watchful waiting strategy for suspected CRBSI in patients with non-tunneled catheters [8, 9]. These studies excluded patients with neutropenia, solid organ or hematologic malignancy, immunosuppressive drugs or radiation therapy, organ transplants, intravascular foreign bodies, hemodynamic instability, suppuration or frank erythema/induration at the insertion site, as well as bacteremia or fungemia. However, the CVC should be removed early in patients with Gram-negative CRBSI, especially when multi-drug resistant isolates are prevalent [10].

Catheter exchange over a guide is not recommended because it is associated with a higher risk of associated infectious complications [11]. This strategy is contraindicated in patients with documented catheter related infections. Guidewire-assisted exchange to replace a catheter should be limited to patients with very difficult venous access (i.e., extensive burns, morbid obesity, or severe coagulopathy) and without documented catheter infection.

Empirical antimicrobial therapy. Once CRBSI is suspected, empiric antimicrobial therapy should be administered after appropriate cultures are obtained. These guidelines recommend choosing the empiric antimicrobial agent(s) based on an assessment of the risk factors for infection, the severity of the clinical picture and the likely pathogens based on local ecology and catheter site of insertion. Some considerations for appropriate antibiotic therapy are as follows: empiric antibiotics should always cover gram-positive organisms; based on the high frequency of *Staphylococcus* in this type of infections and its potential associated clinical severity. Coverage for other pathogens, gram-negative bacilli or fungi, should be considered especially in episodes presenting as septic shock. It is worth noting that these pathogens are more frequently involved when a femoral catheter is the BSI source [12, 13].

An interesting aspect of the document refers to the conservative management of CRBSI in patients with devices used for hemodialysis. The recommendation indicates that the use of combination antibiotic therapy (local and systemic) offers better results than exclusive systemic antibiotics, with the exception of cases produced by *S. aureus*, in which catheter exchange over a guidewire with systemic antibiotic therapy has been shown to be associated with higher cure rate than a strategy based on local plus systemic antibiotics [14].

Targeted antimicrobial therapy. Appropriate duration of antimicrobial therapy in CRBSI is based on the causative pathogen,

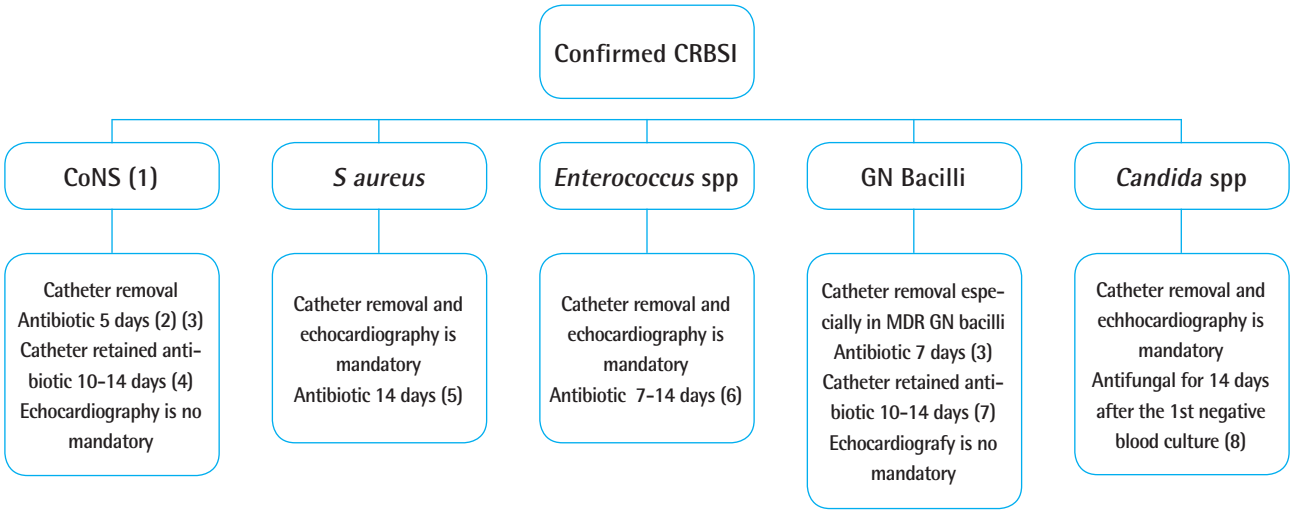

Figure 1 | **Approach to the treatment of a patient with confirmed CRBSI.**

(1) Except *Staphylococcus lugdunensis*, which should be managed as for *Staphylococcus aureus*. (2) Vancomycin is the first option, cloxacillin or cefazolin are the alternatives for methicillin-susceptible strains. (3) In patients with intravascular devices, foreign bodies or in whom markers of inflammation persist after catheter removal therapy, antibiotic therapy for 10-14 days is recommended. (4) Vancomycin in the first option, ALT (Antibiotic Lock Therapy) with vancomycin for 10-14 days. (5) Cloxacillin or cefazolin are the alternatives for MSSA. Vancomycin or daptomycin are the alternatives for MRSA. Complicated episodes require longer courses of treatment (4-6 weeks). (6) Ampicillin is the drug of choice for susceptible strains Vancomycin is the alternative for strains resistant to ampicillin. (7) Only in immunocompetent patients without septic shock and when the isolate is susceptible to antibiotics that are available for ALT. (8) If metastatic complications have been ruled out.

presence of complications, and host factors. Figure 1 shows the approach to the treatment of a patient with confirmed CRBSI.

Guidelines supports the systematic treatment of CRBSI caused by coagulase-negative *Staphylococcus* (CoNS), although this decision does not provide of clear scientific evidence to support it. In fact, recent published studies concluded that inappropriate empirical therapy does not lead to poor outcomes in CoNS-CRBSI bacteremia [15].

One of the most important contributions of the document makes reference to indications for oral sequencing in the treatment of BRCVs. Clinical stability, negativization of blood cultures after catheter withdrawal and the possibility of using oral antibiotics with good bioavailability makes this alternative possible.

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Stewardship in sepsis

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ABSTRACT

Sepsis is the major cause of mortality from any infectious disease worldwide. The goals of antimicrobial stewardship are to achieve optimum clinical outcomes and to ensure cost effectiveness and minimum unintended consequences, including toxic effects, selection of pathogenic organisms, and resistance. The combination of inadequate diagnostic criteria for sepsis with the extraordinary time pressure to provide broad-spectrum antimicrobial therapy is troubling from a stewardship perspective. Use of empirical therapy according to guidelines, de-escalation of therapy, switch from intravenous to oral therapy, therapeutic drug monitoring, use of a list of restricted antibiotics, and bedside consultation can lead to significant benefits for clinical outcomes, adverse events, and costs.

Key words: Sepsis; Stewardship; de-escalation

INTRODUCTION TO SEPSIS

Sepsis, defined as life-threatening organ dysfunction caused by a dysregulated host response to infection, is the major cause of mortality from any infectious disease worldwide [1]. The actual epidemiology of sepsis is currently unknown and extremely variable, since it depends on what we are analyzing, from incidence or prevalence to mortality [2]. Several factors influence, such as poorly classified records of different infectious pathologies and the concept of sepsis in a specific way, poorly or not designed for this purpose, or little information at a global and specific level [3].

A clinical syndrome that is this hard to define, is difficult to diagnose. It is estimated that around 50% of cases of sepsis based on coding are not correctly classified in the USA [4]. There is no one specific test to diagnose sepsis, and a number of different screening tools and biomarkers have been used. Traditional individual markers of sepsis, such as the total white cell count, neutrophil count, and C-reactive protein, lack the specificity to allow them to discriminate between those patients with an inflammatory response to trauma or surgery, for example, and those with an infection. In this sense, procalcitonin has shown to have the best accuracy to identify patients with invasive bacterial infections.

Despite many clinical trials, and the advent of modern intensive care, the mortality of severe sepsis and septic shock continues to be high. Good evidence of a mortality benefit in the early treatment of septic shock exists for two interventions: early goal-directed therapy and appropriate antibiotic therapy.

ANTIMICROBIAL STEWARDSHIP IN SEPSIS

The goals of antimicrobial stewardship (AS) are to achieve optimum clinical outcomes and to ensure cost effectiveness and minimum unintended consequences, including toxic effects, selection of pathogenic organisms, and resistance. However, sepsis represents a unique clinical dilemma with regard to AS. The concept AS is often considered to only include efforts to reduce or restrict use of expensive and broad-spectrum antimicrobials. The real exertion of and AS program should be on getting the right antimicrobial in the right dose to the right patient for the right amount of time [5] (figure 1). So, AS should pursue to achieve optimal clinical outcomes and to diminish drug related toxicity and other adverse events, with the minimum health-care related costs [6].

The combination of inadequate diagnostic criteria for sepsis with the extraordinary time pressure to provide broad-spectrum antimicrobial therapy is troubling from a stewardship

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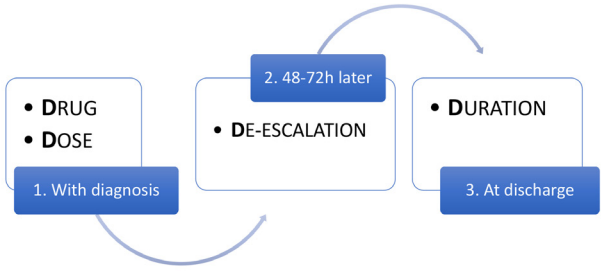

Figure 1 | **When can we do stewardship in sepsis?**

perspective [7]. We have to face several challenges. First, the diagnosis of severe sepsis may be delayed because of physicians or nurses may not identify the progression of sepsis and/or because some patients (e.j., hospitalized, immunosupressed,...) may not show obvious systemic manifestations of the process. Second, patients may have differences in the timing of their presentation and concurrent conditions confounding the diagnosis. Third, treatment may be delayed once the diagnosis is made [3]. An important epidemiological data is to know the origin of sepsis, which it is community in most cases, around 60-70% of whole cases, followed by hospital-acquired outside ICU in 20-30%, while cases of in ICU origin were the least frequent, around 5-9% [3]

MICROBIOLOGICAL DIAGNOSIS OF SEPSIS AND ANTIMICROBIAL STEWARDSHIP

Although approximately 40% of patients with sepsis are culture-negative, identification of a causative organism is essential to de-escalate antibiotics. A rapid response from the microbiology laboratory is a hallmark in hospital settings as in general terms close to 70% of the clinical decisions for the patient's management are based on laboratory results [8]. The first 3-6 hours after the clinical suspicion are critical to establish therapeutic measures that improve prognosis, therefore, a microbial diagnosis in less than 6 hours would undoubtedly benefit the optimal management of patients.

Despite no direct evidence that culture, especially blood culture, is beneficial for patients, indirect evidence supports this approach: de-escalation of antibiotic therapy and switching from intravenous to oral therapy had positive effects on clinical outcomes, adverse events, and costs. Blood cultures, aiming to detect viable microorganisms in blood, are still considered to be the reference standard for the microbiological diagnosis of bloodstream infections during sepsis [9]. However, this culture-based method suffers from important limitations, such as false-negative results because of ongoing antimicrobial therapy, and long time to positivity (usually from 12 hours to 72 hours). In of 50% of cases, bloodstream infections yielded a negative blood culture, and in sepsis even a higher number of blood cultures occur with negative results, which can delay the introduction of an adequate antimicrobial therapy [10]

It is much more interesting to have an etiological diagnosis of sepsis from the patient's direct blood rather than from positive blood cultures after blood incubation. The fastest strategy to identify microorganisms is by direct detection of DNA from blood, as this avoids the enrichment step in blood cultures. The turn-around time of these tests performed directly on blood samples ranges from 3 to 12 hours [1]. A pitfall of rapid molecular-based diagnostic tests for bacterial pathogens is that most of them usually provide little information on antimicrobial susceptibility.

The use of automated electronic sepsis alert system to improve sepsis management represents an area of active research. The widespread introduction of rapid response systems has led to the early identification and the initiation of early intervention to patients within the hospital system [11]. Although it is unlikely that computer programs would be able to tailor therapy in individual patients solely based on software, these programs could be used intelligently to identify key areas that need improvement.

TREATMENT OF SEPSIS AND ANTIMICROBIAL STEWARDSHIP

Timely administration of active antimicrobials has been a keystone of sepsis management even before it was included in the original Surviving Sepsis Campaign (SSC) guidelines [2]. The SSC Guidelines and clinical pathways are now available for several common infections, but the impact of the guidelines on prescribing is difficult to measure accurately. Guidelines recommend that empiric antimicrobial therapy should be based on likely pathogen and local/hospital resistance patterns. However, it is important to note that hospital antibiograms generated from inpatient may not mirror the septic population. Guideline uptake is more likely to be successful if they are tailored to match the local susceptibility patterns, and physicians are more likely to have confidence in guidelines if they are aware of the susceptibility patterns [12]. It is recommended that local susceptibility data should be updated at least annually.

Given the impact of early and broad-spectrum empirical therapy in several studies and the emphasis on this in international guidelines, there is a low threshold for initiating antibiotics in many patients with suspected infection. This has led to the widespread use of antibiotics in critically ill patients, which is often unnecessary or inappropriate. Enforcement of this concept in sepsis would be to cover all potential involved pathogens with the adequate antimicrobials since the first second. De-escalation will take place days later after the patient has been stabilized or when microbiological results (i.e., pathogen identification and definite antibiogram) are available. One area in which AS Programs need to focus on is de-escalation. De-escalation has been generally used in the context of narrowing therapy from broad-spectrum empirical to a nar-

row-spectrum pathogen-directed cover based upon laboratory results (i.e. drug de-escalation). Conceptually, reducing the dose (dose de-escalation), reducing the frequency (frequency de-escalation), switching from parenteral to oral therapy (route de-escalation), or switching from combination therapy to monotherapy are also examples of therapeutic streamlining that help reduce the consumption of antibiotics. [13] This needs systematic education, better diagnostic facilities, clinical microbiologist input, and pharmacy support.

Use of empirical therapy according to guidelines, de-escalation of therapy, switch from intravenous to oral therapy, therapeutic drug monitoring, use of a list of restricted antibiotics, and bedside consultation (especially for *Staphylococcus aureus* bloodstream infection) can lead to signifi cant benefi ts for clinical outcomes, adverse events, and costs, although the quality of evidence is generally low [14].

Antibiotic resistance is a well recognized problem facing modern medicine and it is undeniable that in the last few years, levels of resistance have reached a tipping point. AS is now recognized as a formal strategy for curbing the upward trend in antibiotic resistance. Overuse and/or misuse of antimicrobials may result in selection of multidrug-resistant organisms, high rates of *Clostridium difficile* infections and adverse effects. Restrictive antibiotic policies have been associated with reduced resistance rates in most of the studies we assessed, but inconsistent relations between antibiotic use and resistance rates have been also found [14]. In several studies, increased prescriptions of non-restricted antibiotics were accompanied by concomitant increases in resistance rates.

WHAT ARE THE OBSTACLES THAT PREVENT GOOD STEWARDSHIP PROGRAMS?

The key areas that inform the AS Programs include patterns of prescribing, levels of antimicrobial resistance in a given setting, prescriber education, systematic collection of data in relation to prescribing, and a reliable measure of output. As opposed to structural and operational issues, use of education as an intervention is generally viewed as a medium to long-term strategy that underpins the AS Programs. But educational interventions can also be employed as an immediate tool with defined objectives such as steering the prescribing pattern in order to improve guidelines compliance as discussed above.

It is, thus, imperative to recognize that although the strategic tools can work at a macro level, clinician involvement is the key to successful implementation. The strategists need better clinical support, whereas the clinicians need better facilities in order to change their established practice. Infectious diseases physicians or clinical microbiologists are ideally and traditionally suited for such roles, and for successful implementation of AS Programs it would be vital to formally recognize their effort. This could include time allotment for such activities and also providing opportunity for gaining the Continuing Professional Development points for meetings. Lack of funding and personnel was described recently as a major barrier toward successful implementation of AS Programs [15] The crucial component of payment for physician time would need to be balanced with the cost savings expected as a result

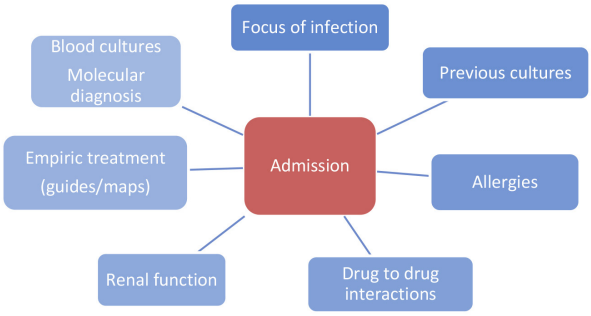

Figure 2 Opportunities of Antimicrobial Stewardship in the Management of Sepsis at admission

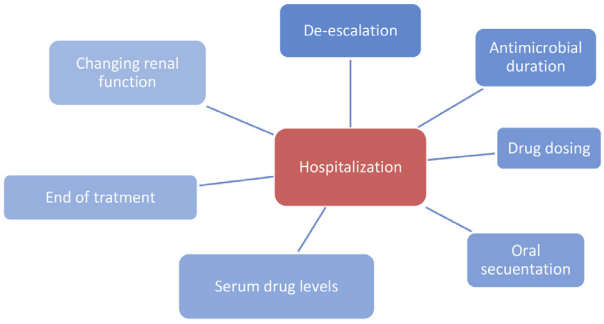

Figure 3 Opportunities of Antimicrobial Stewardship in the Management of Sepsis during hospitalization

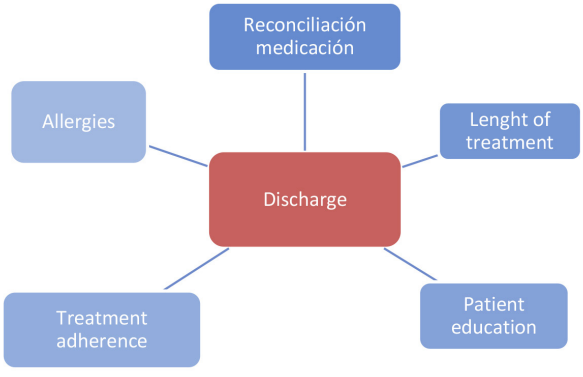

Figure 4 Opportunities of Antimicrobial Stewardship in the Management of Sepsis at discharge

of the AS Programs, and setting clear objectives would help achieve the goal. Rotating membership of AS Programs among colleagues in specialties other than infectious diseases and microbiology would make them feel involved in the strategic process.

Care bundles make it easier to implement the individual components by highlighting them under one goal and make it easier to measure the completeness of a given healthcare strategy. Such strategies have been documented to be successful because when these components are delivered together, their impact is more than when delivered individually (figures 2, 3 and 4).

CONCLUSIONS

The challenge for critical care physicians is thus to correctly diagnose sepsis and improve outcome while reducing antibiotic use. This can be done by adhering to local guidelines for empirical therapy, better risk for multidrug resistance assessment, optimized antibiotic dosing, and integration of rapid diagnostic techniques in the decision-making process.

It is recommended that hospitals implement an AS program to optimize use of antimicrobial agents, decrease antimicrobial resistance, and decrease rates of *Clostridium difficile* infection. there is clearly a need for more randomised multihospital trials to test the effectiveness of interventions on achieving stewardship outcomes and the subsequent effects on meaningful clinical outcomes. Specifically, robust demonstration of direct clinical benefits to individual patients would counteract the view of some health-care providers that stewardship interventions are designed for overall societal benefit, for example by reducing population-level rates of antimicrobial resistance or *Clostridium difficile* infection.

Integrating AS strategies in clinical practice can help upholding the best antibiotic empirical therapy while reducing antibiotic consumption. AS is a multidisciplinary policy and should be embraced by critical care physicians as a solution for balanced antibiotic use. The most effective AS intervention for sepsis will likely include a bundle composed of traditional quality improvement strategies (eg., education, audit, and feedback) combined with rapid diagnostic tests and adequate biomarkers.

QUESTIONS TO REFLECT

1. Are the goals of integrating antibiotic stewardship with the rapid treatment of severe sepsis mutually exclusive?

2. How can we balance rapid antimicrobial choices to select the best antibiotic while protecting members of the community from the further development of antimicrobial resistance

3. What are the practical benefits of a robust antibiotic stewardship program?

4. What are the obstacles that prevent good stewardship programs?

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Doctor, my patient has CDI and should continue to receive antibiotics. The (unresolved) risk of recurrent CDI

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ABSTRACT

Recurrence rate ranges from 12% to 40% of all cases of *Clostridium difficile* infection (CDI) and proposes an exceptional clinical challenge. Conventionally, treatment options of CDI have been limited to regimes of established antibiotics (eg, pulsed/tapered vancomycin) or "improvised" alternative antibiotics (eg. teicoplanin, tigecycline, nitazoxanide or rifaximin) occasionally even in combination, but faecal microbiota transplantation is emerging as a useful and quite safe alternative. In recent years, promising new strategies have emerged for effective prevention of recurrent CDI (rCDI) including new antimicrobials (eg, fidaxomicin) and monoclonal antibodies (eg, bezlotoxumab). Despite promising progress in this area, difficulties remain for making the best use of these resources due to uncertainty over patient selection. This positioning review describes the current epidemiology of rCDI, its clinical impact and risk factors, some of the measures used for treating and preventing rCDI, and some of the emerging treatment options. It then describes some of the barriers that need to be overcome.

Keywords: Clostridium difficile infection, recurrences, fidaxomicin, bezlotoxumab, faecal transplantation.

INTRODUCTION

Clostridium difficile infection (CDI) is the most common cause of nosocomial antibiotic-associated diarrhea worldwide, one of the most frequent healthcare-associated infections and the source of a growing number of cases of diarrhea in the community [1, 2]. The current picture of CDI is alarming with a mortality rate ranging between 3% and 15% and a CDI recurrence rate ranging from 12% to 40%, especially when it has been treated with metronidazole or vancomycin. The incidence of subsequent recurrent CDI (rCDI) increases with prior episodes of CDI, 15-35% risk after primary CDI to 35-65% risk after the first recurrent episode. Certain host or pathogen factors have been associated with an increased risk of rCDI or CDI-related adverse outcomes: age ≥ 65 years, compromised immunity, severe CDI, prior CDI episode (s), and infection with the BI/NAP1/027 strain [3]. rCDI is one of the most challenging and a very difficult to treat infections. Standard guidelines provide recommendations on treatment of primary CDI. However, treatment choices for rCDI are limited.

The key to preventing recurrent infection is identifying those patients at the greatest risk (table 1). Factors accepted to present a risk of initial CDI include older age and comorbidities. As with initial infection, the risk of recurrence increases with ageing. Poor baseline health status has also been identified as a risk factor. Past exposure to health care has also been found to be a significant risk factor. It has been found that chronic kidney disease with or without dialysis and chemotherapy increased the risk of recurrence at older ages. Usually, proton pump inhibitor and antibiotic use have also been implicated in risk of recurrence.

Antibiotics are the major risk factor for the promotion and development of an episode of CDI, as well as the prolongation or perpetuation of symptoms and a lesser response to specific treatment. In addition, they are one of the main factors favoring the appearance of recurrences. Antibiotic use causes an antibiotic-related loss of gut microbial communities that protect against gut infection, thereby facilitating the germination and vegetative growth of the organism when it enters the gut of vulnerable people [4]. Frequently, this factor is not easily modified and many patients need to continue receiving antibiotics for the mandatory treatment of their severe or complicated infectious syndromes.

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Table 1 **Proposed and potential risk factors for recurrent *Clostridium difficile* infection (rCDI)**

Risk factors group	Factors included in the group
Host risk factors	• Age ≥65 years
	• Prior CDI episode
	• Host genetics
	• Compromised immune system
	• Chronic renal failure
	• Low *C. difficile*-specific antitoxin antibody levels
Severity of CDI episode	• Severe primary CDI
Pathogen-specific factors	• *C. difficile* strain type (ribotype 027, 078, or 244)
Exogenous factors (Exposures)	• Proton pump inhibitors/antacids
	• Previous fluoroquinolone use
	• Ongoing antibiotic use

CDI: *Clostridium difficile* infection. Note: Data collated from multiple references included in the bibliography.

Table 2 **Classification of antibiotics based on the risk of developing CDI or recurrence**

Low risk	Moderate risk	High risk
Aminoglycosides	Ampicillin	Clindamycin
Vancomycin	Amoxycillin	Quinolones
Metronidazole	Macrolides	Cephalosporins
Rifampicin	Tetracyclines	Amoxycillin/clavulanate
Antipseudomonal penicillins	Cotrimoxazole	Piperacillin/tazobactam
		Carbapenems
		Aztreonam

RECURRENT CDI AND ANTIBIOTICS

Antibiotic therapy causes alterations of the intestinal microbial composition, enabling *C. difficile* colonization and consecutive toxin production leading to disruption of the colonic epithelial cells [5]. The risk of CDI is increased up to six-fold during antibiotic therapy and in the subsequent month afterwards. Although nearly all antibiotics have been associated with CDI, clindamycin, third-generation cephalosporins, penicillins, and fluoroquinolones have traditionally been considered to pose the greatest risk. An

association between CDI and antimicrobial treatment > 10 days has also been demonstrated. Antibiotics which have been less commonly associated with CDI include macrolides, sulfonamides, and tetracyclines (table 2). Even very limited exposure, such as single dose surgical antibiotic prophylaxis, can increase patients risk for both *C. difficile* colonization or infection.

Recurrent CDI can be defined as reappearance of symptoms following the completion of a course of therapy resulting in complete resolution of those symptoms [6]. European guidelines define recurrence as symptoms occurring within 8 weeks after the onset of a previous episode, provided the symptoms from the previous episode resolved after completion of initial treatment. However, studies offer different definitions.

Around a quarter of all patients with confirmed CDI will develop a recurrence. Those patients who have had a first recurrence are at increased risk of further recurrence (or multiply rCDI) – up to 60% of patients with a second recurrence will have further infections. Recurrence can occur either as a relapse with the same strain (as the consequence of germinating resident spores remaining in the colon after antibiotic treatment has stopped) or as a reinfection with a different strain (from an environmental source) [7]. Ultimately, distinction between recurrence and reinfection can only be achieved if the strain of *C. difficile* is "typed" using molecular epidemiology.

Studies comparing patients with recurrent infection, those with non-recurrent infection, and those without infection, have demonstrated both greater use of hospital resources and increased mortality. rCDI has been associated with a 2.5-fold higher hospital readmission rate, four-fold longer hospital stay, and 33% higher mortality rate compared to primary CDI [8].

Recurrences are associated with an impaired immune response to *C. difficile* toxins and/or alteration of the colonic microbiota, but nevertheless recurrent episodes are less severe compared to initial episodes and some studies reported a decline in the proportion of severe cases according to the number of recurrent episodes (47% for initial episodes, 31% for first recurrences, 25% for second, and 17% for third in a Canadian study) [9].

Even though consensus regarding factors associated with CDI recurrence is not universal, algorithms have been developed to predict CDI recurrence with good sensitivity. Scoring prediction models could be important tools for prioritizing more individualized, costly, or resource intensive treatment and prevention strategies. Tools to predict the risk of rCDI could be especially useful as advancements in therapies for prevention emerge. Risk stratification allowing identification of patients at risk for recurrence may translate into a more cost-effective approach to decrease rates of rCDI [3]. Unfortunately, existing models have used limited sample sizes, have not been validated externally, or have been found to perform poorly in predicting rCDI. Studies of models to predict rCDI are summarized in table 3.

Recurrence of symptoms after initial therapy for *C. difficile* presents a clinical challenge. As the incidence of rCDI is rising, there is an unmet need for therapies and strategies to prevent rCDI. Despite the great efforts made over the past

Table 3 List of risk prediction scales and scoring systems for recurrent CDI

Study	Variables o parameters included in the score	Comments
Hu et al. [44]	Age > 65 years Severe disease based on Horn index Additional antibiotic use	Small sample size; Score performed poorly in a prospective internal validation cohort (53.8% sensitive, 76.5% specific); Will require external validation to determine clinical utility.
Miller et al. [45]	Age Treatment with systemic antibiotics during CDI therapy Temperature Leukocyte count sCr Albumin	Useful for predicting severity and response to treatment, but found to be a poor predictor of recurrence.
Zilberberg et al. [46]	Age Community onset CDI Prior hospitalization Fluoroquinolone use at onset of CDI Other high risk antibiotic use at onset of CDI Gastric acid suppression	Derived from a large retrospective single center cohort study and cross-validated in the same population. Performed poorly in an external validation study (C statistic 0.59)
D'agostino et al. [19]	Age > 75 years > 10 unformed bowel movements in 24 hours sCr > 1.2 mg/dL Prior episode of CDI CDI treatment received (vancomycin or fidaxomicin)	Derived and validated from a large prospective dataset. Poorly predictive of recurrent CDI (C statistic 0.54)
Escobar et al. [47]	Comparison of 4 models 1) Basic Model: age, prior GI surgery, Immunosuppression status, Locus of CDI onset, Admission from skilled nursing facility 2) Enhanced Model: Based on 14 variables extracted from EMR 3) Automated Model: Based on several variables generated in real time.	Derived from a large multicenter retrospective cohort and internally validated in a separate cohort. None of the models performed well (C statistics 0.59–0.61).
Viswesh et al. [48]	CDI present on admission Temperature > 37.8 °C at admission Leukocyte count > 15,000 cells/mm^3 Nosocomial CDI Abdominal distention	Derived from a large retrospective single center cohort study and cross-validated in the same population. Will require external validation to determine clinical utility.
Cobo et al. (GEIH-CDI Score) [20]	Age Prior CDI in last year Positive direct toxin test Persistence of diarrhea on day 5 of treatment	Derived from a retrospective multicenter cohort and validated in a separate cohort (including several of the same centers from derivation cohort). Moderately predictive for recurrent CDI
Reveles et al. [49]	Prior 3rd or 4th generation cephalosporin use Prior proton pump inhibitor use Prior antidiarrheals Non-severe CDI Community onset CDI	Large retrospective national cohort of veterans. Clinical prediction rule correlated strongly with recurrence (R2 = 0.94) in an internal validation cohort. Will require external validation to determine clinical utility.

CDI: *Clostridium difficile* infection; sCr: serum creatinine; GI: gastrointestinal; EMR: electronic medical records.

C-statistic [equivalent to the area under the Receiver Operating Characteristic (ROC) curve] is a standard measure of the predictive accuracy of a logistic regression model. C-statistic refer to the probability that predicting the outcome is better than chance. It is used to compare the goodness of fit of logistic regression models. Values for this measure range from 0.5 to 1.0. A value of 0.5 indicates that the model is no better than chance at making a prediction of membership in a group and a value of 1.0 indicates that the model perfectly identifies those within a group and those not. Models are typically considered reasonable when the C-statistic is higher than 0.7 and strong when C exceeds 0.8.

10 years to face the rCDI burden, there are still gray areas in our knowledge on rCDI management. Promising treatments include fidaxomicin (FDX), faecal microbiota transplantation (FMT) and monoclonal antibodies [10].

THERAPIES AND STRATEGIES TO PREVENT RCDI

In general, treatment goals of CDI are to resolve the infection, reduce gut dysbiosis, prevent recurrence, prevent transmission among individuals, improve quality of life, and reduce healthcare costs. Along with medical management, some patients may require surgical intervention (which nowadays is required less commonly). In patients diagnosed with CDI, consideration should be given to discontinuation of the offending antibiotic if clinically appropriate. The use of antibiotics along with treatment for CDI is associated with lower cure rates and higher rates of recurrence.

Then we discuss in this section three current strategies for the prevention and treatment of rCDI.

Fidaxomicin use. FDX is a new class of narrow-spectrum macrocyclic antibiotics that inhibits bacterial RNA polymerase. FDX is a bactericidal drug that seems to be more specific over *C. difficile* than metronidazole and vancomycin, with less disruption of the fecal microbiota [11]. Moreover, FDX also decreases both spore production and relapse rates [12], and its low absorption can prevent systemic side effects, reaching high fecal concentrations and remaining in the gastrointestinal tract with reduced impact on the intestinal microbiota [13]. Compared to vancomycin treatment, FDX was associated with a lower rate (~50%) of second-occurrence relapses 4 weeks after the infection in patients with no prior episode of CDI [14]. A post hoc exploratory intent to treat (ITT) time-to-event analysis showed a 40% reduction in persistent diarrhea, recurrence or death at the 40-day follow-up (95% CI, 26-51%; p<0.0001) [13]. This evidence argues in favor that specific treatment developed against *C. difficile* can greatly improve clinical outcomes, although several patient groups were excluded from the trials. Nevertheless, there is still a margin for further improvement since FDX fails in 12% of treatments. In addition, FDX is effective and safe for the treatment of CDI in critical patients, immunosuppressed patients, or patients with chronic renal failure [15].

Vancomycin and FDX are now recommended as a first line treatment options for CDI. Both are considered to have similar therapeutic efficacy (87.7-88.2% with FDX and 85.8-86.8% with vancomycin) though FDX has a significantly lower recurrence rate (15.4% vs. 25.3%, p < 0.005), respectively [14, 16]. Hence, FDX is recommended from the first episode of infection to ensure maximum efficacy in patients with well-contrasted recurrence risk factors (elderly people, concomitant antibiotic use and severe underlying disease) [17, 18]. Due to its higher cost, real-world use of FDX is likely to be reserved for patients with first or later recurrences. Several studies have developed scoring systems that allow the more high-risk patients to be treated earlier [19, 20] and show cost-effectiveness [21].

Furthermore, in 60 years-old patients and older, extended-pulsed FDX (EPFDX) (200 mg oral tablets, twice daily on days 1–5, then once daily on alternate days on days 7–25) was superior to standard-dose vancomycin for sustained clinical cure of CDI and significant reduction in recurrence rates [22]. A recent Spanish economic model showed that EPFDX is cost-effective compared with vancomycin for the first-line treatment of CDI in patients aged 60 years and older [23].

Faecal microbiota transplantation (FMT). Some patients with CDI (primary episodes and recurrences) that do not respond to conventional antibiotic treatments of first choice may be cured by FMT [24, 25], an intervention first described in treating pseudomembranous colitis in 1958.

FMT procedure is based on transplanting stool from healthy donors (people without diseases as malignancy, metabolic or autoimmune disease or infections like HIV or active hepatitis) in order to restore gut microbiome which is disrupted in CDI, suppressing *C. difficile* overgrowth [26]. Donor feces (≥50 g obtained preferably within <6 hours after evacuation) are diluted with water or normal saline, homogenized and filtrated and are administrated through enema, colonoscopy (100–700 mL of stool suspension delivered to the caecum or terminal ileum, as it seems to obtain a better result), nasogastric or nasojejunal tube, or in capsules. For patients with systemic illnesses, capsules may be the best option, followed by nasoenteric tube, but in patients at risk of aspiration, enema or colonoscopy should be a better choice [27]. However, combination of several of those methods is recommended in complex cases. Common adverse events after FMT, that are usually self-limitated, include gastrointestinal discomfort (abdominal pain, bloating, flatulence, diarrhoea, constipation, vomiting or belching) and endoscopy-related complications (like aspiration during sedation). Before FMT, patients are given antimicrobial therapy directed at CDI for at least 4 days and 1 day before FMT, bowel lavage is performed in most of them.

FMT has proven to be safe and effective, showing a rate of cure of recurrent CDI >90% when associated to antibiotic cessation. Nowadays, following the recommendations of British Society of Gastroenterology and Healthcare Infection Society guidelines from 2018 [28], FMT may be offered to patients with recurrent CDI who have had at least two recurrences, or those who have had one recurrence and have risk factors for further episodes, including severe and complicated CDI. However, in Spain it is still not a routine procedure and the potential benefit of FMT in primary CDI remains uncertain.

In a recent systematic review with meta-analysis of donor features, procedures and outcomes in 168 clinical studies of FMT (including ulcerative colitis and Crohn's disease), a final cure rate for CDI of 95.6% was observed [29]. Cure rates in CDI and final remission rates for inflammatory bowel disease were comparable across all routes of FMT administration. Overall adverse event incidence was <1%, mostly gastrointestinal-related. Adverse event rates did not differ significantly between

routes of FMT administration or indication. Reports of its safety in certain immunocompromised populations, such as those with inflammatory bowel disease, those who have received a solid organ transplant [30] or suffering from a oncohaematological disease, appear reassuring and their outcomes are becoming better known.

Monoclonal antibodies (Bezlotoxumab). A new approach to the prevention of recurrent *C. difficile* infection (CDI) is the administration of monoclonal antibodies against *C. difficile* toxins in addition to antibiotic therapy as a form of passive immunity. Bezlotoxumab is the first of its kind, fully humanized monoclonal antibody directed against *C. difficile* toxin B. Binding to toxin B neutralizes the toxin and prevents damage to colonic cells.

Bezlotoxumab is currently approved for the prevention of rCDI in patients on treatment for CDI and who are at high risk for recurrence. Approved dose is a single 10 mg/kg administered intravenously during active *C. difficile* therapy, up to treatment day 14. Bezlotoxumab does not require dosage adjustment in either renal or hepatic impairment and no drug-drug interactions are anticipated or published [31].

Two phase III clinical double-blind trials (MODIFY I and MODIFY II) studied the ability of this antibody to reduce the recurrence of CDI in 2,655 patients. In these trials, it was shown that the addition of bezlotoxumab to the standard of care antibiotics for primary or recurrence *C. difficile* infections resulted in a lower rate of recurrence compared with placebo (17% vs 28% in MODIFY I and 16% vs 26% in MODIFY II; $p<0.001$). These results representing a 40% relative reduction rate ($p < 0.0001$) and a number needed to treat of 10 patients. Bezlotoxumab had no effect on clinical cure (clinical cure of 80% bezlotoxumab vs 80% placebo). Moreover, the absolute difference in rCDI rate was greater in subpopulations at high risk of CDI recurrence than in the overall population [32].

A post hoc analysis evaluated the efficacy of bezlotoxumab in patients with previously identified "high-risk" rCDI (risk factors including age ≥ 65 years, compromised immunity, severe CDI, prior CDI episode, and infection with ribotypes 027/078/244). All of the categories demonstrated a statistically significant reduction in CDI recurrence, with the exception of infection with ribotypes 027/078/244. When further stratified by number of underlying risk factors, there was a greater impact on prevention of recurrence as the number of risk factors increased. While participants with ≥3 risk factors had the greatest reduction of rCDI with bezlotoxumab, those with 1 or 2 risk factors may also benefit [3]. In addition to the rCDI risk factors evaluated in the above study, data presented at the 2016 IDWeek conference evaluated the efficacy of bezlotoxumab in prevention of CDI recurrence in patients receiving concomitant antibiotics. rCDI was observed in 18% of bezlotoxumab treated patients who received concomitant antibiotics compared with 28% of placebo subjects together with concomitant antibiotics. These preliminary data suggest that the efficacy of bezlotoxumab was maintained in patients with concomitant antibiotics [33].

Bezlotoxumab was generally well tolerated and had a safety profile similar to that of placebo. The most commonly reported adverse drug responses are infusion related reactions (10%). Side effects within 4 weeks of administration reported in ≥ 4% of patients in the MODIFY I and II trials included nausea (7%), pyrexia (5%), and headache (4%). These did not differ significantly from placebo. Heart failure was not seen in preclinical trials but reported in 17 (2.2%) bezlotoxumab and 7 (0.9%) placebo treated patients in Phase III trials. Heart failure was more frequently observed in patients with a history of congestive heart failure [31, 32, 34]. Additionaly, recent studies showed that bezlotoxumab added to standard of care antibiotic therapy compared to standard of care alone is a cost-effective treatment to prevent the recurrence of CDI in high-risk patients especially in patients ≥ 65 years old, with severe CDI and immunocompromised [35, 36].

Its novel mechanism of action, apparent lack of impact on the fecal microbiome, and safety profile make it an attractive adjunctive therapy for prevention of rCDI. One of the weaknesses is that much of the published data come from the drug pharmaceutical sponsored MODIFY trials. Real world clinical experience and independent investigations will be helpful to verify the clinical efficacy in high-risk populations [37].

CONCLUSIONS AND FUTURE DIRECTIONS

Treatment and prevention of rCDI remain difficult. Patients who are able to restore their natural gut microbiota or mount an effective immune response to the toxins and/or the bacterium recover from the infection (figure 1), whereas patients who fail to do so are susceptible to recurrent CDI. Although newer strategies are available or in the pipeline, further studies are required to identify those patients in whom these treatments are likely to be both clinically and cost-effective [38].

Treatment of CDI has become complicated due to the emergence of strains with increased toxigenicity and sporulation rate, together with rampant antibiotics use that disrupts colonization resistance of the colonic microbiota. As a result, there is a critical need for non-antibiotic treatments. Therapies based on inhibiting the toxins, bacterial structures responsible for colonization, virulence and restoration of the gut microbiota are the most important non-antibiotic targets to combat CDI. New discovered targets in *C. difficile* could become the focus of future therapeutic agents. Inhibiting colonization and virulence factors during CDI will disrupt pathogen persistence and decrease exposure to the inflammatory toxins, allowing the immune system to clear the infection [39].

The high risk of recurrence has led to multiple emerging therapies that target toxin activity, recovery of the intestinal microbial community, and elimination of latent *C. difficile* in the intestine. The high incidence of rCDI has driven new research on improved prevention such as the emerging use of probiotics, intestinal microbiome manipulation during antibi-

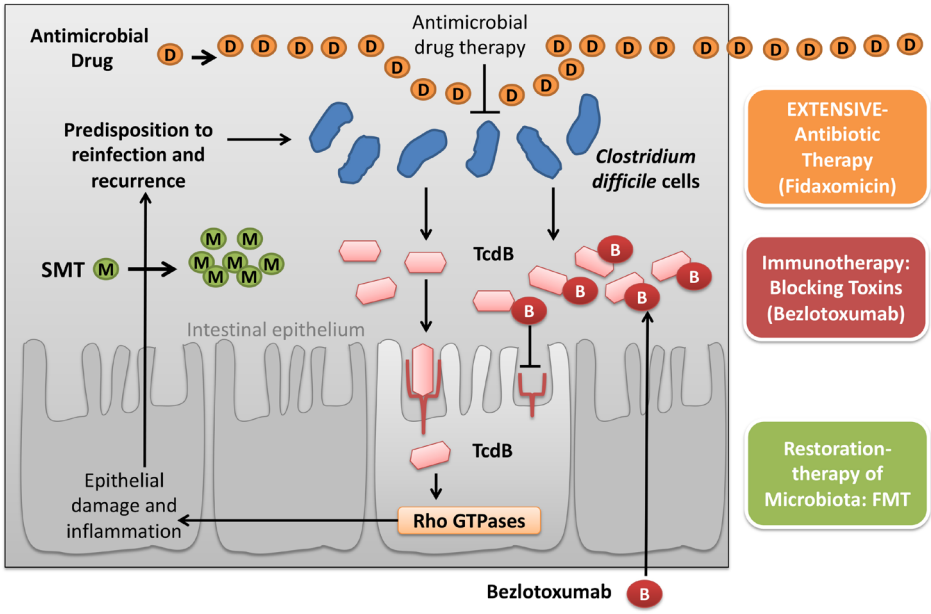

Figure 1 Evaluating and combining strategies against recurrences of CDI

CDI: *Clostridium difficile* infection; TcdB: *C. difficile* toxin B; B: Bezlotoxumab; D: Antimicrobial Drug; M: Microbiota (restored) from selected donors; SMT: Stool microbiota transfer; FMT: fecal microbiota transplant. Arrow: stimulation of the action or effect provocation ; Locked arrow: inhibition effect or blocking action. (modified and adapted from reference number 40)

otic therapies, vaccinations, and newer antibiotics that reduce the disruption of the intestinal microbiome [40]. A novel approach in the manipulation of the microbiome would include the administration of non-toxigenic *C. difficile* strains [4]. In a clinical trial, non-toxigenic *C. difficile* was administered to those with CDI, with the aim of outcompeting toxigenic *C. difficile* from its reservoir within the gut. CDI recurrence rates were 30% in those receiving placebo in comparison with 11% in those receiving non-toxigenic *C. difficile* [41]. While anti-toxin vaccines could be another viable preventative measure, they are currently not as effective and more clinical trials will be needed to identify an efficacious and safe vaccine. Early data suggest reduced seroconversion in older people subjected to this active immunization, those most at risk of CDI, and also very likely in immunosuppressed patients.

At this moment there are more than fifteen antimicrobial molecules under study for CDI treatment in different phases of clinical trials: cadazolid, ridinilazole, surotomycin, rifaximin, rifampin, fusidic acid, tigecycline, LFF571, nitazoxanide, ramoplanin, auranofin, CRS3123, thuricin CD, lacticin 3147, NVB302, and acyldepsipeptide antimicrobials. In comparison with the traditional anti-CDI antimicrobial treatment, some of the novel antimicrobials offer several advantages, such as the favorable pharmacokinetic and pharmacodynamic profile, the narrow-spectrum activity against *C. difficile* that implicates a low impact on the gut microbiota composition, the inhibitory activity on *C. difficile* sporulation and toxins production [42]. Among these novel antimicrobials, the most active compounds in reducing spore production are cadazolid, ridinilazole, ramoplanin, CRS3123 and, potentially, the acyldepsipeptide antimicrobials. These antimicrobials may potentially reduce *C. difficile* environment spread and persistence, thus reducing CDI healthcare-associated acquisition and rCDI. However, some of them, as for example surotomycin, fusidic acid, etc., will not be available due to lack of superiority versus standard of treatment. The most *C. difficile* narrow-spectrum novel antimicrobials that allow preserve microbiota integrity are ridinilazole, cadazolid, auranofin, and thuricin CD.

Another strategy in the prevention of CDI would be a direct action on β-lactam antibiotics. Ribaxamase (SYN-004) is an orally administered β-lactamase that was designed to be given with systemic broad-spectrum antibiotics (intravenous β-lactam antibiotics) to degrade excess antibiotics in the upper gastrointestinal tract before they disrupt the gut microbiome and create a propensity to CDI [4]. In a recent study with patients treated with intravenous ceftriaxone for lower respiratory tract infections, oral ribaxamase reduced the incidence of CDI compared with placebo [43]. The findings of this study support continued clinical development of ribaxamase to prevent CDI.

In conclusion, the novel antimicrobial molecules under development for CDI have promising key features and advancements in comparison to the traditional anti-CDI antimicrobials. In the near future, some of these new molecules might be effective alternatives to fight against CDI and prevent more effectively rCDI.

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Update on the infection of the immunocompromised patient

Update on the management of febrile neutropenia in hematologic patients

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ABSTRACT

Febrile neutropenia is a common complication in patients with hematologic malignancies receiving chemotherapy, and is associated with high morbidity and mortality. Infections caused by multidrug-resistant bacteria represent a therapeutic challenge in this high-risk patient population, since inadequate initial empirical antibiotic treatment can seriously compromise prognosis. Besides, reducing antimicrobial exposure is a cornerstone in the fight against resistance.

Keywords: Febrile neutropenia, hematological disease, empirical antibiotic therapy, targeted antibiotic therapy, antibiotic resistance.

Dr. Gudiol reviewed the most relevant issues included in the recently published Consensus Document of the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC) and the Spanish Association of Hematology and Hemotherapy (SEHH) on the management of febrile neutropenia in patients with hematologic malignancies [1].

Fever is a common sign in patients suffering from chemotherapy-induced neutropenia, but up to 60%-70% of these patients will not have either an identifiable clinical focus of infection or positive cultures. Gram-negative bacteria are the leading cause of infection in onco-hematological patients with febrile neutropenia (FN) in some institutions, and emergence of multidrug resistance among these organisms is a matter of concern [2]. Overall, more than 50% of the main isolated pathogens in neutropenic patients (i.e., *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*) are resistant to cephalosporins, fluoroquinolones, and aminoglycosides. Carbapenem resistance is rapidly increasing and being identified in up to 50% of *Enterobacteriaceae* isolates in some European countries, and its presence has been described as an independent risk factor for mortality in these patients [3].

Many factors should be considered when choosing empirical antibiotic treatment in patients with FN. These include the risk of infection associated with the severity of neutropenia (low versus high risk), possible focus of infection, clinical manifestations (e.g., hypotension, sepsis, septic shock), local epidemiology, previous infection or colonization by multidrug-resistant organisms (MDROs), previous use of antibiotics, and presence of allergies and potential toxicities. The use of a β-lactam with activity against *P. aeruginosa* is recommended, in monotherapy or in combination with another regimen, according to the clinical presentation and the risk of infection due to MDROs. Therefore, an escalation strategy may be used in uncomplicated clinical presentations, in patients without risk factors for MDROs, and in centers with low prevalence of resistance. Conversely, a de-escalation strategy that ensures early initiation of effective treatment is recommended in severely ill patients, in those with risk factors for MDROs, and in settings with high prevalence of resistance. Piperacillin-tazobactam or a cephalosporin with antipseudomonal activity are preferred for escalation strategy. When choosing the de-escalation strategy, imipenem or meropenem may be chosen, but the combination of an antipseudomonal β-lactam plus an aminoglycoside or fluoroquinolone may also be a suitable option. Addition of amikacin or colistin should be considered if there is a risk of infection due to non-fermenting MDROs, and coverage against MDR gram-positives is indicated in cases of hemodynamic instability or risk of methicillin-resistant *Staphylococcus aureus* infection.

Antibiotic treatment should be selected and modified according to the suspected clinical focus of infection, as shown in Table 1.

Classically, antibiotic treatment was maintained until recovery from neutropenia, but evidence supporting this approach is scare. Furthermore, reducing the exposure to un-

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Table 1 **Empirical antibiotic therapy according to clinical focus of infection**

Entity	Antibiotic treatment
Mild oropharyngeal mucositis	- Cefepime
Moderate-severe oropharyngeal mucositis	- Piperacillin-tazobactam - Imipenem or meropenem
Neutropenic enterocolitis	- Piperacillin-tazobactam - Imipenem or meropenem * Consider treating *C. difficile* if high index of suspicion
Skin and soft tissue infection	- Cefepime - Piperacillin-tazobactam - Imipenem or meropenem +/- - Vancomycin, daptomycin, or linezolid (if history of MRSA colonization/infection) * Consider adding clindamycin if severe necrotizing infection
Intravascular catheter infection	- Cefepime - Piperacillin-tazobactam - Imipenem or meropenem +/- - Vancomycin or daptomycin
Pneumonia	- Cefepime - Piperacillin-tazobactam - Imipenem or meropenem +/- - Fluoroquinolones, aminoglycosides, colistin * Consider association with fluoroquinolones or macrolides if pneumonia is community-acquired and an atypical bacterial etiology is suspected. * In patients with MRSA colonization or an epidemiological situation of high endemicity, consider combination with linezolid or vancomycin. * In severely ill patients, those previously colonized/infected with MDR Gram-negative bacilli, or nosocomial cases, according to local epidemiology. * During the flu season, use empirical oseltamivir until PCR results are received. * Consider the possibility of alternative causes (*Pneumocystis jirovecii*, Cytomegalovirus) in risk patients with bilateral infiltrates.
Urinary tract infection	- Cefepime - Piperacillin-tazobactam - Imipenem or meropenem
Acute meningitis	- Cefepime or meropenem + - Ampicillin * In risk patients with suggestive clinical forms, or space-occupying lesions, consider alternative causes (*Cryptococcus*, *Listeria*, *Nocardia*, filamentous fungi, toxoplasmosis, and *Mycobacterium tuberculosis*)
Meningoencephalitis	Use same treatment as acute meningitis, with adding of Acyclovir

MRSA: methicillin-resistant *Staphylococcus aureus*

Table 2 Exclusion criteria for outpatient oral antibiotic treatment

Patients undergoing allogeneic stem cell transplantation or intensive chemotherapy regimens, for example:
– Intensive induction chemotherapy or high-dose cytarabine (Ara-C) or similar as consolidation treatment for acute myeloid leukemia
– DT-PACE chemotherapy for plasma cell leukemia
– BURKIMAB, DA-EPOCH level ≥3 or Hyper-CVAD chemotherapy for lymphoma
Acute organ dysfunction (clinically significant gastrointestinal symptoms, bleeding, oliguria, development of new pulmonary infiltrates, hypoxemia, or the appearance of new neurological symptoms)
Clinically significant comorbidities including pulmonary disease, hepatic or renal dysfunction or any clinically relevant worsening
Clinically significant cellulitis
Central venous catheter infection
Previous colonization/infection with MDR bacteria
Quinolone prophylaxis or previous infection due to fluoroquinolone- or β-lactam-resistant Gram-negative bacteria
Recently admitted to intensive care

necessary antibiotic is a cornerstone in the fight against antimicrobial resistance. In this regard, a multicenter randomized clinical trial (RCT) (the How Long Study) involving high-risk hematologic patients with FN and no etiologic diagnosis to determine the optimal duration of empirical antimicrobial treatment was recently published [4]. In patients in the experimental group, empirical antibiotic treatment was discontinued after 72 hours of apyrexia and all signs and symptoms of clinical infection had disappeared, while those in the control group followed the standard approach of maintenance until neutrophil recovery. The results confirmed that stopping empirical antimicrobials following a clinical criterion regardless of the neutrophil count reduced the number of days of exposure to antimicrobials with no impact on mortality, as well as other secondary outcomes (recurrent fever, secondary infections, etc).

In patients with FN and clinically documented infection, antibiotic treatment can be discontinued when clinical signs and symptoms of infection have resolved and the patient remains afebrile for at least 72 hours. If infection has been microbiologically documented, a minimum of 4 days of apyrexia and 7 days of antibiotic treatment are recommended to stop antibiotic treatment. Neutrophil recovery is not a necessary precondition to determine length of antibiotic treatment.

Special attention was given to the treatment of extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-E). In this regard, the use of β-lactam/β-lactamase inhibitor (BLBLI) combinations as carbapenem-sparing alternatives is a matter of debate. The recently published MERINO trial, failed to demonstrate the non-inferiority of piperacillin-tazobactam compared to carbapenems for the treatment of bacteremia due to cephalosporin-resistant *Enterobacteriaceae*, in terms of overall 30-day mortality [5]. Nevertheless, the study did have some limitations. Of note, a retrospective multicenter international cohort study involving neutropenic high-risk hematologic patients with bacteremia due to ESBL-E (the BICAR study) found no differences in 7, 14 or 30-day mortality rates among patients treated with BLBLI combinations or carbapenems, either as an empirical or a definitive therapy, and after performing a propensity score analysis [6]. Therefore, BLBLI combinations (mainly piperacillin-tazobactam) should be considered as carbapenem-sparing alternatives for the treatment of low-risk patients who do not have a high-inoculum infection and present without severe sepsis or septic shock. Optimized dosing and extended infusion is strongly recommended. In this regard, a recently published RCT involving hematologic patients with FN showed significant better clinical outcomes in patients receiving the empirical β-lactam antibiotic in extended infusion compared with those who received it in bolus [7].

Patients considered to be at low risk for complications can be treated with oral antibiotics and outpatient follow-up after 48-72 hours [8]. Stratification of patients should include validated models such as the MASCC index score [9]. Contraindications for this strategy are signs or symptoms of hemodynamic instability, localized infection, oral intolerance, new clinical signs and symptoms, or isolation of microbiological species non-susceptible to initial empirical therapy. Antibiotic treatment should include a fluoroquinolone with antipseudomonal activity, plus an agent with Gram-positive cocci activity (e.g.: amoxicillin/clavulanate or clindamycin); cefuroxime or cefixime in combination with ciprofloxacin may be an alternative. Fluoroquinolones should be not used empirically if the patient has received these antibiotics as a prophylaxis regimen. Table 2 shows the exclusion criteria for outpatient oral antibiotic treatment.

Finally, antibacterial prophylaxis was also addressed. Due to the high prevalence of quinolone resistance among Gram-negatives, and the risk of resistance development in several Gram-positive and Gram-negative organisms, universal prophylaxis with quinolones is not recommended for low-risk

patients. Individual evaluation for its use should be performed in high-risk patients with profound and prolonged neutropenia. Centers performing fluoroquinolone prophylaxis should implement active monitoring strategies for emergence of resistance.

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Prophylaxis of mould infections

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ABSTRACT

Invasive fungal infection continues to be an important cause of morbidity and mortality in haematological patients. Antifungal prophylaxis in these patients has remarkably increased survival since its introduction. In recent years, new antifungals have been on the rise, being more effective and having less toxicity than previous ones. Nonetheless, the number of patients at risk of fungal infection has also been increasing due to the continuous appearance of new immunosuppressive treatments. As a result of such, we face a changing situation that requires constant updating.

Key words: antifungal prophylaxis, invasive fungal infection, haematological malignancies, haematopoietic stem cell transplant.

INTRODUCTION

Invasive fungal infection (IFI) is currently one of the main causes of infectious mortality in haematopoietic stem cell transplantations (HSCT) and an important cause of morbidity and mortality in onco-haematological patients, mainly those affected by acute leukaemia and myelodysplastic syndromes treated with intensive chemotherapy. The frequency of cases of IFI varies considerably per the underlying disease and treatment administered.

In past decades, there has been a decrease in mortality by invasive aspergillosis (IA) due to earlier diagnosis and new antifungal agents. Nonetheless, the number of patients at risk of fungal infection has experienced an increase due to host defence impairment secondary to intensive chemotherapies and corticosteroid use, longer survival of HSCT, new other immunosuppressive agents and the recognition of new susceptible host such as those with severe influenza infections.

In light of such scenario, IFI prevention should be made priority objective in at-risk patients, especially onco-haematological and HSCT recipients. Although prevention can be approached in several ways, chemoprophylaxis will be our primary focus. The aspects of antifungal chemoprophylaxis that will be presented in depth as follows: patients at risk of IFI caused by moulds, indications for prophylaxis and antifungals agents used.

PATIENTS AT RISK OF INVASIVE FUNGAL INFECTION CAUSED BY MOULDS

Table 1 summarizes the patients with the highest risk of mould infection. One of the highest risk groups of IFI is HSCT recipients. In an American prospective surveillance multicentre study of IFI in HSCT recipients, Kontoyiannis et al [1] describe

Table 1 Patients at high risk of mould infection

Patients at risk of invasive pulmonary aspergillosis
Acute myeloid leukaemia
Allogenic HSCT recipients
Moderate and severe GVHD
Prolonged neutropenia
Other haematological malignancies with biological therapies
SOT recipients (especially heart and lung)
PCNSL receiving ibrutinib
Influenza A (H1N1) infection (especially in immunocompromised patients)

HSCT: haematopoietic stem cell transplantation. GVHD: graft versus host disease. SOT: solid organ transplant. PCNSL: Primary central nervous system lymphoma.

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that the total incidence of IFI did not decrease in spite of common practice with antifungal prophylaxis. They observed that cumulative incidences of aspergillosis increased, whereas invasive candidiasis remained stable. This fact is probably due to current prophylaxis, by which most include fluconazole, without action against moulds.

Another classic group of at-risk patients is solid organ transplantation (SOT) recipients. In a transplant-associated infection surveillance study, Pappas et al [2] observed a slight increase of IFIs during this period, differing according to the type of organ transplantation performed. The increase in IFIs was reflected mainly in the increase in the incidences of *Candida* infections, whilst cumulative incidence of *Aspergillus* infections remained unchanged. Neofytos et al [3] observed that invasive aspergillosis remains a rare complication post-SOT with atypical radiographic presentations and low positive rates of biomarkers. The incidence of invasive aspergillosis was higher in lung (8.3%) and heart (7.1%) transplantation recipients. The median time between transplantation and invasive aspergillosis was 100 days; it was shorter in heart and liver transplantation cases (median 11 and 18 days, respectively). Overall mortality decreased in SOT recipients, but remained high in liver SOT recipients.

In recent years, numerous studies have been conducted to identify risk factors for IFI, but only a few have been made to assess the real incidence in non-transplantation patients with haematological malignancies. Pagano et al [4] reported that amongst non-transplanted patients, those with acute myeloid leukaemia had the highest risk of IFI: about 8% of acute myeloid leukaemia patients would develop mould infections, mainly aspergillosis, and 4%, yeast infections. Almost half of these infections emerged during the first course of induction chemotherapy. IFI-attributable mortality rate was 39%.

Until now, little information was available concerning incidence of IFIs in chronic lymphoproliferative disorders. Novel treatments like immunomodulating and immunosuppressive agents in addition to cytotoxic treatments have increased the risk of IFIs amongst these patients. A recent paper described that IFI in lymphoproliferative disorders has a cumulative incidence of up to 14% in patients with multiple myeloma and 7.8% in patients with chronic lymphocytic leukaemia [5].

Primary central nervous system lymphoma (PCNSL) is an aggressive type of lymphoma with a poor prognosis. The combination of temozolomide, etoposide, doxorubicin, dexamethasone, rituximab, and ibrutinib (DA-TEDDi-R) induced frequent responses, but was associated with aspergillosis infections. Previous studies have reported an incidence of invasive aspergillosis ranging from 5 to 11% using single-agent ibrutinib; incidence increased to 39% when treatment was combined with DA-TEDDi-R [6]. It is tempting to speculate that the concomitant use of ibrutinib and steroids may increase the incidence of aspergillosis. Therefore, one may consider antifungal prophylaxis in patients with PCNSL receiving ibrutinib if this treatment regimen becomes standard.

In another scenario, influenza A infection has been reported to possibly predispose patients with such infection to invasive aspergillosis, especially those patients who are immunocompromised (frequency was 8.8% in patients with acute myeloid leukaemia and transplant recipients) [7]. Schauwvlieghe et al [8] measured the incidence of invasive pulmonary aspergillosis in patients with influenza pneumonia in the intensive care unit. Incidence was 32% and 14% in immunocompromised and non-immunocompromised patients, respectively; and it was associated with high mortality. Influenza was found to be independently associated with invasive pulmonary aspergillosis.

PREVENTION OF INVASIVE FUNGAL INFECTIONS

To date, antifungal prophylaxis is indicated in high-risk haematological patients, those with acute myeloid leukaemia and allogenic stem cell transplants. Most experience in antifungal chemoprophylaxis has been with azole agents. The main studies with the different azole agents are summarised in table 2.

Cornely et al [9] designed a randomised and multicentre study to compare the efficacy and safety of posaconazole as prophylaxis (n=304) in relation to fluconazole or itraconazole (n=240) in patients undergoing chemotherapy for acute myelogenous leukaemia or myelodysplastic syndrome. The group undergoing prophylaxis with posaconazole was superior to those groups undergoing prophylaxis with fluconazole or itraconazole with respect to the prevention of proven or probable invasive fungal infections (2% vs 8%, p<0.001, had significantly fewer invasive aspergillosis (1% vs 7%, P<0.001) and resulted in lower mortality from any cause (16% vs 22%, P=0.048) and longer free survival from proven or probable invasive fungal infection. There were more serious adverse events related to treatment in the posaconazole group (6% vs 2%, P=0.001). The estimated number needed to treat with posaconazole to prevent one IFI, as compared with fluconazole or itraconazole, was 16 patients; and to prevent one death, 14 patients.

In an international, randomised, double-blind trial, Ullman et al [10] compared oral posaconazole (n=301) with oral fluconazole (n=299) for prophylaxis against invasive fungal infections in patients with graft-versus-host disease who were receiving immunosuppressive treatment. Posaconazole was found to be as effective as fluconazole in preventing all IFIs (5.3% vs 9%, P=0.07) and was superior to fluconazole in preventing invasive aspergillosis (2.3% vs 7%, P=0.006). Overall mortality was similar in both groups, but the number of deaths from invasive fungal infections was lower in the posaconazole group (1% vs 4%, P=0.046). The incidence of adverse events was similar in both groups. The estimated number needed to treat with posaconazole to prevent one IFI was 27 patients.

Wingard et al [11] conducted a randomised, double-blind, multicentre study to compare fluconazole (n=295) versus voriconazole (n=305) as IFI prophylaxis in patients undergoing HSCT within the context of a structured fungal screening

Table 2 **Most important antifungal prophylaxis randomized studies in high risk haematological patients.**

Authors	Patients	Antifungal prophylaxis	Results	Others
Cornely et al [9]	Acute myelogenous leukaemia or the myelodysplastic syndrome undergoing chemotherapy	Posaconazole (304) vs fluconazole (240) or itraconazole (58)	Posaconazole was superior in the prevention of IFI (p<0.001) and had lower mortality than any other cause (p=0.048)	More serious adverse events in posaconazole group (p=0.01)
Ullman et al [10]	GVHD who were receiving immunosuppressive treatment	Posaconazole (n=301) vs fluconazole (n=299)	Posaconazole was as effective as fluconazole in preventing all IFI (p=0.07) Posaconazole was superior in preventing invasive aspergillosis (p=0.006). Overall mortality was similar, but lower due to invasive fungal infections in the posaconazole group (p=0.046).	Adverse events were similar.
Wingard et al [11]	Patients undergoing HSCT	Fluconazole (n=295) vs voriconazole (n=305)	Voriconazole trends to be more effective in preventing IFIs (p=0.12) and *Aspergillus* infections (p=0.09). No differences in fungal-free survival at 6 months and overall survival	Severe adverse events were similar.

IFI: invasive fungal infection. GVHD: graft versus host disease. HSCT: haematopoietic stem cell transplantation

programme. Methods used in this study differ from those used in posaconazole trials given that most of the researchers had conducted trials evaluating itraconazole. Those studies showed trends in reduction in frequency of invasive *Aspergillus* infection, but without any clear survival benefits; concerns about tolerability and toxicities were raised. The primary endpoint for Wingard study was therefore freedom from IFI or death at 180 days. Despite the trend of fewer cases of IFIs (7.3% vs 11.2%; P=0.12), *Aspergillus* infections (9 vs 17; P=0.09), and less frequent empiric antifungal therapy (24.1% vs 30.2%; P=0.11) with voriconazole, fungal-free survival rates (75% vs 78%; P=0.49) at 180 days were similar with fluconazole and voriconazole, respectively. Relapse-free and overall survival, as well as the incidence of severe adverse events were also similar. Even though most data on IFI incidence was very much similar to that reported by Ullman, these authors detailed that fungal-free survival at 6 months and overall survival did not differ between fluconazole or voriconazole prophylaxis.

Voriconazole is an important and excellent therapeutic agent; however, due to adverse effects, it necessitates close monitoring, particularly in immunocompromised hosts receiving the drug for a prolonged period. Well-known effects are hepatotoxicity (12-20%), visual disturbances (20-30%) and phototoxicity. Although it is also related with skin cancers (OR 2.6), cardiac arrhythmias, QT Interval prolongation, periostitis (20-25%), central (hallucinations 14%) and peripheral system adverse effects (9%), alopecia and hyponatremia. It will therefore be important to avoid prolonged prophylactic treatments [12]. In addition, in data sheets, voriconazole is contraindicated for patients being administered sirolimus, an immunosuppressive therapy very common used in transplant recipients.

Recently, clinical guidelines for the management of invasive diseases caused by *Aspergillus* have been published by GEMICOMED (Medical Mycology Study Group), REIPI (Spanish Network of Infectious Pathology Investigation), and SEIMC (Spanish Society of Clinical Microbiology and Infectious Diseases). In summary, prophylaxis with an anti-mould agent is recommended for invasive aspergillosis prevention in patients with acute leukaemia; prolonged and profound neutropenia; allogeneic HSCT recipients during the neutropenic phase; and those with moderate to severe graft versus host disease and/or intensified immunosuppression (AI). Antifungal drugs which can be used in high-risk patients include: posaconazole (AI), voriconazole (AI), itraconazole (BII), micafungin (BIII), caspofungin (CIII), aerosolized L-amphotericin B (BI) and intravenous lipidic formulations of amphotericin B (CII) [13]. These authors recommend the use of posaconazole as a first line antimould prophylaxic treatment.

WHY IS POSACONALE SO EFFECTIVE IN PROPHYLAXIS?

Tissue penetration into the site of infection to achieve microbial kill concentrations is a key requirement for efficacy. Posaconazole has much larger volumes of distribution in contrast to voriconazole and high plasma protein binding (>98%). Posaconazole penetrates preferentially into tissue with high lipid content and that which often exhibits tissue/plasma concentration ratios which exceeds 1. This drug exhibits epithelial lining fluid concentration similar to that seen in plasma, but the exposure in alveolar cells is 30 times more than that in plasma [14].

Pharmacokinetic (PK)/pharmacodynamic (PD) parameters

related with efficacy in antifungals are area under the concentration-time curve (AUC)/minimum inhibitory concentration (MIC). Drug peak serum concentration above MIC explains the continued concentrations within the tissue. Therefore, it has been suggested that high intracellular posaconazole concentrations may account for prophylaxis effectiveness.

Isavuconazole is a novel broad-spectrum triazole agent with a safety profile and similar PK/PD parameters to posaconazole, with its indication for treatment of IFI being nowadays restricted due to the lack of clinical experience. Studies on the PK/PD of isavuconazole demonstrated that bioavailability is very high and plasma protein binding is around 98%. It has a large volume of distribution and a long half-life. This, in turn, offers potential for use in fungal prophylaxis, salvage therapy or in combination regimens [15]. However, no current studies demonstrating the efficacy of isavuconazole in preventing IFI in high-risk populations has been conducted.

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Update on the infection of the immunocompromised patient

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Assessment of latent infections in patients receiving biological therapies

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ABSTRACT

The use of biological (or targeted) therapies constitutes a major advance in the management of autoinflammatory and malignant diseases. However, due to the selective effect of these agents on the host's immune response, reactivation of certain pathogens that cause latent infection is to be expected. The most relevant concern is the risk of reactivation of latent tuberculosis infection (LTBI) and progression to active tuberculosis among patients treated with agents targeting tumor necrosis factor (TNF)-α. Systematic screening for LTBI at baseline with appropriate initiation of antituberculous treatment, if needed, is mandatory in this patient population as risk minimization strategy. In addition, reactivation of hepatitis B virus induced by B-cell-depleting (anti-CD20) and anti-TNF-α agents should be also prevented among HBsAg-positive patients and those with isolated anti-HBc IgG positivity (risk of "occult HBV infection"). The present review summarizes available evidence regarding the risk of reactivation of these latent infections induced by newer biological agents, as well as the recommendations included in the most recent guidelines.

Keywords: latent infection; tuberculosis; biological therapy; tumor necrosis factor-α; hepatitis B virus.

INTRODUCTION

The advent of the so-called biological (or targeted) agents has revolutionized the therapeutic approach to many inflammatory, autoimmune and malignant diseases. Indeed, biological therapies —both monoclonal antibodies and small-molecule inhibitors— have the ability of directly targeting the soluble inflammatory mediators, cell surface receptors or intracellular pathways implied in the pathogenesis of the condition, thus sparing normal tissues and minimizing the risk of treatment-related adverse events. In contrast to conventional immunosuppressive and cytostatic drugs, biological agents exert a rather selective effect on immune responses and, presumably, host-pathogen interaction. Although direct attribution of causality is often hampered by other contributing factors (such as the nature and activity of the underlying condition, the presence of comorbidities, or the concurrent use of immunosuppressive therapies), the understanding of the precise mode of action may allow for establishing a mechanistic relationship between a given agent and the expected susceptibility to infection [1]. From a clinical and epidemiological perspective, the most relevant and well-established association links the use of available agents targeting tumor necrosis factor (TNF)-α (summarized in table 1) with the risk of reactivation of latent tuberculosis infection (LTBI) and progression to active disease [2]. Therefore, the present review is mainly focused on this serious and preventable complication.

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REACTIVATION OF LATENT TUBERCULOSIS INFECTION

The role of TNF-α in antituberculous immunity. After the primary infection with *Mycobacterium tuberculosis*, the effective long-term control of LTBI by the host's adaptive immune system ultimately depends on the dynamic equilibrium between pro-inflammatory and anti-inflammatory cytokines. The TNF-α, a pleiotropic cytokine, exerts a major role in the structural maintenance of tuberculous granulomas [3]. Thus, it is to be expected that the therapeutic blockade of TNF-α will result in the progression from LTBI to active tuberculosis. Nevertheless, it is noteworthy that no cases of tuberculosis were reported in the pivotal randomized clinical trials (RCTs) that led to the Food and Drug Administration (FDA) approval of infliximab for rheumatoid arthritis (AR) and Crohn's disease in the late 1990s despite the lack of specific risk-minimiza-

Table 1 **Type, mechanism of action and FDA- and EMA-approved indications of currently available anti-TNF-α agents (modified from ref. [2]).**

Agent	Type	Target	Mechanism of action	Mode of administration[a]	Approved indications
Infliximab (Remicade® and biosimilars)	Human-mouse chimeric IgG1 monoclonal antibody	mTNF-α, sTNF-α	Neutralization, apoptosis, reverse signaling, ADCC, CDC	IV injection every 6-8 weeks	IBD (CD and UC), RA, AS, PsA, plaque psoriasis
Etanercept (Enbrel®)	Fusion protein of the soluble TNFR2/p75 receptor and human IgG1 antibody (hinge, CH2 and CH3 domains of the Fc region)	mTNF-α, sTNF-α, TNF-β	Competitive inhibition, ADCC, CDC (weaker)	SC injection once or twice weekly	RA, AS, JIA, PsA, plaque psoriasis
Adalimumab (Humira®)	Fully human IgG1 monoclonal antibody	mTNF-α, sTNF-α	Neutralization, apoptosis, reverse signaling, ADCC, CDC	SC injection every 2 weeks	IBD (CD and UC), RA, AS, JIA, PsA, plaque psoriasis, hidradenitis, suppurativa, uveitis
Golimumab (Simponi®)	Fully human IgG1 monoclonal antibody	mTNF-α, sTNF-α	Neutralization, apoptosis, reverse signaling, ADCC, CDC	SC injection every 4 weeks	UC, RA, AS, JIA, PsA
Certolizumab pegol (Cimzia®)	PEGylated Fab' fragment of humanized IgG4 monoclonal antibody	mTNF-α, sTNF-α	Neutralization, reverse signaling	SC injection every 2-4 weeks	CD (only FDA), RA, AS, PsA, plaque psoriasis (only EMA)

ADCC: antibody-dependent cell-mediated cytotoxicity; AS: ankylosing spondylitis; CD: Crohn's disease; CDC: complement-dependent cytotoxicity; EMA: European Medicines Agency; FDA: Food and Drug Administration; IBD: inflammatory bowel disease; IV: intravenous; JIA: juvenile idiopathic arthritis; PS: plaque psoriasis; PsA: psoriatic arthritis; RA: rheumatoid arthritis; SC: subcutaneous; sTNF-α: soluble tumor necrosis factor α;mTNF-α: membrane-bound tumor necrosis factor α; UC: ulcerative colitis.

[a]**Maintenance doses once clinical response has been observed; initial doses vary according to the indication.**

tion measures in the study protocols [4, 5]. This circumstance should serve as a reminder of the limited ability of phase 2-3 trials to identify relatively rare but relevant adverse event signals associated with the use of newer biological agents [1]. The relevance of TNF-α in granuloma maintenance is also exemplified by the increased risk of histoplasmosis reported in endemic areas among patients under anti-TNF-α therapy [6].

Clinical evidence and risk factors. Since 2001, when the first cases of infliximab-associated tuberculosis were reported by the FDA Safety Information and Adverse Event Reporting Program [7], a large amount of evidence based on RCTs, open-label extension studies and post-marketing registries allows to delineate the risk of LTBI reactivation in patients receiving TNF-α-targeted therapies [8]. The largest meta-analysis published to date —including 29 RCTs and 11,879 patients— found an overall odds ratio (OR) of 1.94 (95% confidence interval [CI]: 1.10-3.44) for active tuberculosis under anti-TNF-α agents. Subgroup analyses revealed that patients with RA faced an even higher risk (OR: 2.29; 95% CI: 1.09-4.78) [9]. It should be noted that the baseline risk of tuberculosis among RA patients has been found to be higher as compared to the overall population [2]. It is likely that differences in underlying susceptibility to LTBI reactivation according to the condition itself that requires anti-TNF-α therapy may, at least partially, account for the relative lower incidence usually observed in patients with inflammatory bowel disease (IBD).

Such risk increase is not uniform across different agents. The use of etanercept, a dimeric fusion protein consisting of two extracellular ligand-binding portions of the soluble TNF-α receptor linked to the hinge and CH_2 and CH_3 domains of the human IgG1 Fc region, is consistently associated with a lower incidence of LTBI reactivation as compared to monoclonal antibodies targeting TNF-α, such as infliximab or adalimumab (table 1) [10, 11].

Various studies suggest that the previous or concomitant use of conventional synthetic disease-modifying anti-rheumatic drug (csDMARD) and, particularly, corticosteroids has an additive negative impact on the individual susceptibility to tuberculosis [12, 13]. The risk of active tuberculosis also varies according to patient age (with higher incidence in older groups) and the background rate of LTBI in the overall population (with a British registry reporting higher incidence among foreign-born individuals of non-white ethnicity) [14]. The effect of the duration of therapy remains less clear. Some studies have shown that the incidence of associated infection is highest during the first year to gradually decrease thereafter, thus suggesting a progressive reduction over time of the individual risk [2]. However, the observational nature of the data may render such findings prone to misclassification bias due to case mix (i.e. selective treatment switching of patients who are already at an increased risk of infection due to age, comorbidities or prior infectious complications) [15].

Strategies for screening for latent tuberculosis infection. The cornerstone of prevention strategies aimed

at minimizing the risk of tuberculosis in patients receiving TNF-α-targeted agents relies on systematic screening for LTBI at the baseline evaluation, followed by prompt initiation of antituberculous treatment at least one month before the start of biological therapy. In the last few years, various guidelines and position papers have summarized the literature supporting the implementation of such practices, including an official initiative of the ESCMID (European Society of Clinical Microbiology and Infectious Diseases) Study Group for Infections in Compromised Hosts (ESGICH) that performed a comprehensive literature review accompanied by a series of evidence-based recommendations [16].

The diagnosis of LTBI mainly depends on demonstration of a *M. tuberculosis*-specific T-cell-mediated immune response, since by definition the infecting mycobacteria remain in a state of latency and the subject has no attributable symptoms. To this end, two different diagnostic approaches are currently available: the tuberculin skin test (TST) and the more recent interferon (IFN)-γ release assays (IGRAs). In overall terms, the latter approach —that may be based in enzyme-linked immunosorbent assay (ELISA) (QuantiFERON-TB® in different versions, Qiagen, Hilden, Germany) or enzyme-linked immunospot (ELISpot) formats (T-SPOT®.TB, Oxford Immunotec, Oxford, United Kingdom)— has the advantages of better reproducibility and specificity than the TST. This is because a pool of peptides (CFP-10, ESAT-6, TB7.7), which span a specific area (region of difference [RD1]) of *M. tuberculosis* genome, serves as stimulating antigens in IGRAs. Since RD1 is deleted in the Bacillus Calmette-Guérin (BCG) and is not shared by most of non-tuberculous pathogenic and environmental mycobacteria, the use of IGRAs as screening for LTBI would result in a lower false-positive rate. On the other hand, it should be also considered the increased cost of these assays compared to TST and the unexpected rate of reversions and conversions in healthy subjects (i.e. healthcare workers) in which serial IGRA testing was performed over time and no new exposure to *M. tuberculosis* could be apparently identified [17]. Assay variability due to lot manufacturing and pre-analytical and analytical execution defects might explain this finding [8]. Not surprisingly, the concordance rate between IGRA and TST has revealed to be suboptimal.

There is general consensus in performing both tests (TST and IGRA) and, eventually, a chest X-ray examination prior to the initiation of TNF-α-targeted agents to maximize sensitivity [2, 8, 16]. The positivity of any of them should lead to the diagnosis of LTBI and to the administration of antituberculous treatment, regardless of previous history of BCG vaccination. However, the optimal screening sequence to avoid an unacceptable number of false-positive results (and, therefore, unnecessary treatment courses and delays in anti-TNF-α therapy) is still not well established. A 10-year prospective study performed in Spain including 726 patients compared three screening strategies over consecutive periods: two-step TST (either an induration of ≥5 mm in the first test or an increase of ≥5 mm in the second test was considered positive); two-step TST followed by ELISA-based IGRA; and single-step TTS followed by IGRA. The proportion of patients diagnosed with LTBI was lower with the simplified single-step TTS plus IGRA strategy (26.5%) compared with the two-step TST (42.5%) or the two-step TST plus IGRA (38.5%) groups. As expected, BCG-vaccinated subjects had higher positivity rates for TST but not for IGRA. The authors found no significant differences in the incidence of active tuberculosis across the three periods (overall: 2.47 cases per 1,000 patient-years), suggesting that the repeat of TST after a first negative test would be not justified as long as the evaluation is completed with an IGRA [18]. There is also some experience (mostly based on small sized studies performed in low-incidence countries) with the use of a single IGRA as the sole screening, an approach may be particularly useful among patients with psoriasis in which the underlying skin condition often hinders the interpretation of TST [19].

In patients with a baseline negative evaluation for LTBI, the need for periodical retesting during the entire period of anti-TNF-α treatment remains a matter of debate, since the probability of new primary *M. tuberculosis* infection —which is greatly influenced by the background incidence of tuberculosis in the overall population— must be balanced against the risk of false positive results derived from repeated TST and/or IGRA (i.e. single or dual retesting strategy) over time. In the previously mentioned Spanish study, and after a median follow-up of almost 5 years, no cases of active tuberculosis occurred beyond the first year of therapy despite the fact that patients with a negative initial screening were not subsequently retested for LTBI. The authors concluded that retesting should be only considered on the basis of an individual risk assessment for *M. tuberculosis* infection [18]. A prospective study carried out in Greece —with a tuberculosis incidence rate in the overall population lower than that reported in Spain— included 70 RA patients with negative baseline screening (TST, IGRA [QuantiFERON-TB® Gold In-Tube and T-SPOT®.TB assays], and chest X-ray examination) that underwent re-screening following one year of anti-TNF-α treatment. Almost one third of them experienced conversion of at least one of these tests (with conversion rates of 13% for TST and 7% to 10% for IGRAs), despite that no obvious tuberculosis exposure had been recorded within the prior year. Although only 40% of these "converters" received therapy for LTBI, no cases of active tuberculosis were observed during the follow-up [20]. The ESGICH Consensus Document suggests that annual re-screening should be generally considered, acknowledging that clinical significance of test conversions remains unclear [16]. Thus, it seems reasonable that, in low-incidence settings, repeated screening for LTBI would be focused only on those subjects with clinical or epidemiological evidence of new exposure to *M. tuberculosis* since the initial negative evaluation, rather than in a systematic manner [2].

Treatment of latent tuberculosis infection. In patients diagnosed with LTBI, antituberculous treatment is mandatory and the administration of the anti-TNF-α agent should be delayed for 30-60 days [2, 8, 16]. Similar to other high-risk pa-

tient groups with indication for LTBI treatment, active disease must be previously ruled out on the basis of clinical and radiological assessment and, if necessary, sputum smear microscopy. If the patient has active tuberculosis, biological therapy must be postponed for a longer period (at least until sterilization of sputum cultures and clinical improvement have been achieved). In this scenario, it is likely that the subsequent use of etanercept —rather than anti-TNF-α monoclonal antibodies— or other biological agents, such as those targeting interleukin (IL)-6 receptor (certolizumab) or cell adhesion molecules (vedolizumab) should be favored in order to minimize the subsequent risk of tuberculosis relapse.

Isoniazid has potent tuberculocidal activity against intracellular and extracellular micobacteria. A 6- to 12-month course of isoniazid monotherapy (5 mg/Kg [maximum 300 mg] daily) remains as the first-line option for LTBI in patients receiving anti-TNF-α agents [8]. Vitamin B_6 (pyridoxine) supplementation is recommended in adults at risk for isoniazid-induced neuropathy (e.g. diabetes mellitus or alcoholism). The optimal duration of therapy is not well established in the absence of RCTs specifically focused on this population. By analogy with other high-risk groups, such as those with fibrotic pulmonary lesions, it seems that 6- or 9-month isoniazid regimens can be safely compared with 12 months of therapy. In addition, alternative regimens have been successfully tested in recent trials, including 3 months of isoniazid (900 mg) and rifapentine (900 mg) in 12 weekly doses, or 4 months of daily rifampicin (10 mg/Kg [maximum 600 mg] daily) [21, 22]. The available experience with such regimens to prevent LTBI reactivation induced by anti-TNF-α agents is so far limited. A recent single-center non-randomized study compared 41 patients with RA and positive IGRA results that received a 3-month regimen of weekly isoniazid and rifapentine or a 9-month regimen of daily isoniazid. Although not reaching statistical significance, a higher completion rate was found in the former than in the latter group (90.5% versus 78.3%, respectively). Moreover, the occurrence of hepatotoxicity was also lower among patients receiving weekly isoniazid and rifapentine. No cases of active tuberculosis were detected after a two-year follow-up [23]. No experience has been published to date on the use of the 4-month daily rifampicin regimen in patients scheduled to receive anti-TNF-α therapy, although the pivotal trial included about 100 patients per treatment arm with non-HIV-related immunosuppression [22]. Nevertheless, it should be keep in mind that rifampicin acts as a strong liver microsomal enzyme inducer, with the subsequent potential for drug-to-drug interactions [8].

Risk of tuberculosis with other biological therapies. The available evidence points out that the risk of LTBI reactivation and progression to active disease associated to biological therapies appears to be mainly restricted to TNF-α blockade [8]. As noted above, experience derived from pivotal RCTs must be taken with caution since such studies are generally underpowered to detect uncommon adverse events occurring in the mid- and long-term follow-up, as is the case of tuberculosis. Furthermore, most participants in phase 2-3 trials for RA or other rheumatologic conditions, IBD or psoriasis are recruited in low-incidence regions. In addition, and due to the past experience with anti-TNF-α therapy, LTBI screening and treatment is often (but not always) required as per study protocol at patient entry in trials on newer biological agents. Having said this, both the theoretical effect on the antimycobacterial immune response of the targeted pathways and the accumulated clinical experience suggest that anti-IL-17A (secukinumab or ixekizumab) and anti-IL-17 receptor agents (brodalumab) do not meaningfully impact the risk of active tuberculosis [24]. Likewise, the risk of LTBI reactivation under therapeutic blockade of the IL-6/IL-6 receptor pathway (tocilizumab, siltuximab or sarilumab) seems to be lower than that observed with anti-TNF-α agents, although the confounding effect of underlying conditions and prior and concomitant immunosuppressive drugs cannot be ruled out [24]. Finally, it has been shown that the functional abrogation of IL-12 is associated with an increased risk of tuberculosis. This is exemplified by an uncommon condition known as Mendelian susceptibility to mycobacterial disease, which consists of a collection of monogenic disorders. In detail, *IL12RB1* (one of the affected genes) encodes for the common receptor chain whose interaction with IL-12 and IL-23 is inhibited by ustekinumab, a monoclonal antibody targeting the p40 subunit shared by both cytokines. Therefore, the ESGICH Consensus Document recommends LTBI screening before starting treatment with ustekinumab. However, this theoretical risk of active tuberculosis has been not substantiated by clinical experience [24].

REACTIVATION OF HEPATITIS B VIRUS INFECTION

Apart from LTBI, the use of certain biological therapies also poses a risk for reactivation of viral pathogens able to establish chronic or latent infection within the host. Such a concern applies particularly to hepatitis B virus (HBV), not only in patients with chronic HBV surface antigen (HBsAg)-positive infection but also in those who have apparently cleared the virus but remain at risk of "occult" infection (HBsAg-negative, anti-hepatitis B core [HBc] IgG-positive patients, with or without detectable anti-HBs antibodies). In the latter group, HBV DNA may be still detected in the serum and liver tissue in form of episomal covalently closed circular DNA (cccDNA) or integrated into the hepatocyte genome. This balance between the host's immune surveillance and the virus can be disrupted by immunosuppressive therapy, leading to viral replication that can evolve into life-threatening hepatitis, with occasional HBsAg re-seroconversion [25].

The risk of HBV reactivation is clearly associated with the use of B-cell-depleting agents (rituximab and the newer anti-CD20 monoclonal antibodies such as ofatumumab, obinutuzumab or ocaratuzumab), with rates exceeding 30-40% and 10% for HBs-positive and HBsAg-negative/anti-HBc-positive subgroups, respectively [25]. Reactivation of HBsAg-positive infection has been also described with anti-TNF-α agents, although the available evidence is more limited than in the case

of anti-CD20 therapy [2]. A comprehensive literature review that comprised 225 cases published until 2011 revealed that reactivation occurred in 37% of HBsAg-positive patients, substantially lower than the rate observed among HBsAg-negative/anti-HBc-positive patients (5%). Previous immunosuppressive therapy was found to increase the risk of HBV reactivation, and infliximab was associated with a higher rate of liver disease than etanercept. Of note, five patients experienced fatal acute liver failure [26]. However, it is likely that the reactivation rates reported in this study may be overestimated [25]. Anti-TNF-α agents have generally been reported to be associated with a lower incidence of reactivation than that observed with potent immunosuppressive cancer chemotherapy, being most cases restricted to patients with RA rather than IBD or psoriasis.

On the basis of these experiences, systematic screening for HBsAg and anti-HBc IgG is mandatory before initiating anti-CD20 or anti-TNF-α therapy. HBV DNA levels should be determined through PCR-based nucleic acid testing in patients with isolated anti-HBc IgG positivity to exclude the presence of occult infection. There is general consensus to recommend the administration of anti-HBV prophylaxis in HBsAg-positive and HBsAg-negative/anti-HBc-positive patients during the entire duration of anti-CD20 therapy and for at least 12-18 months after the last administration of the monoclonal antibody, since cases of delayed viral reactivation have been reported. Antiviral drugs with high genetic barrier to resistance, such as tenofovir disoproxil fumarate (TDF) or alafenamide (TAF) or entecavir, are usually preferred over lamivudine, particularly for long prophylaxis courses or if baseline HBV DNA level is higher than 2,000 IU/mL [2]. Regarding patients under anti-TNF-α therapy, both the ESGICH [27] and the American Gastroenterological Association [25] support the use of anti-HBV prophylaxis in case of HBsAg or HBV DNA positivity, a recommendation mostly based on non-randomized studies. Due to the lower risk of reactivation among HBsAg-negative/anti-HBc-positive patients (provided that HBV DNA is undetectable at baseline), a preemptive approach with regular monitoring of viral load may be considered with early initiation of antiviral therapy in case of reactivation of an occult HBV infection, although the optimal frequency of monitoring and the threshold for initiating antiviral therapy are not well established.

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Infection in the process of organ donation

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ABSTRACT

The difference between demand and supply has led transplant organizations to look for marginal donors, including those who could transmit infections to their recipients. This potential risk must be thoroughly evaluated to optimize the use of such organs without increasing the incidence of graft dysfunction and the morbidity and mortality of the recipient. This article aims to provide a general and up-to-date overview of this issue.

Keywords: organ transplantation, donor, transmission, infection

INTRODUCTION

Solid organ transplantation (SOT) is the treatment of choice for many patients with terminal diseases. Although the number of patients on the waiting list has more than doubled since 1998, the number of transplants has increased by only about 30% [1]. Therefore, there is a need to increase the number of donors. On the other hand, infectious complications continue to be the main cause of morbidity and mortality after SOT. Some of these complications are caused by pathogens transmitted by the transplanted organ. In fact, transplant physicians have traditionally avoided the use of donor organs with a known transmissible infection or with an increased risk of carrying it despite negative serological tests. However, with the increased availability of tests based on the detection of nucleic acid in real time, the period during which an early viral infection could be overlooked has been greatly reduced and, so, the possibility of transmission. The underutilization of such organs seems to be even more relevant given the fact these donors are frequently younger and with lower comorbidity than other donors. In any case, the rigorous examination of the donor to detect latent and active infections is essential to optimize the results after the transplant and serves to prevent the involuntary use of inadequate organs and the prophylaxis directed against the infection or the preventive therapy or the surveillance measures of infections after transplant.

EPIDEMIOLOGY

There are two types of transmission of an infection from the donor to the recipient: the expected and the unexpected one. The expected one is frequent, it is known before the procedure, we have prophylaxis for it or, in any case, it is controllable. An example would be the transmission of cytomegalovirus from a seropositive donor to a seronegative recipient. On the other hand, we have the unexpected transmission. It is infrequent, we do not recognize it before the transplant, we do not usually have effective treatment or prophylaxis for it and, therefore, it has high morbidity and, even, mortality. An example of this would be the transmission of a West Nile virus infection from a donor who died of encephalitis without diagnosis prior to transplantation. It is on the unexpected transmission that we have to concentrate all our efforts to avoid it. However, and to start with, the information that we have about this concern is limited. First, there are no universal standards for donor evaluation and each society publishes its own recommendations [2–4]. Second, sometimes, it is difficult to differentiate the infection derived from the donor from the recipient's own, especially in the case of latent infections. Third, not all the cases of donor-derived infection (DDI) are published. Since there are no protocols or mandatory reporting systems, there is publication bias. Physicians tend to publish the cases of transmission but not the cases of donors with infection, but without transmission. Finally, most publications are case reports and retrospective literature reviews. The few cohort studies, whether prospective or retrospective, place the

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transmission of the infection from the donor to the recipient around 1% but with a lethality of 40% [5, 6].

CAUSES OF UNEXPECTED TRANSMISSION OF INFECTION

There are several causes that lead to the unexpected transmission of an infection. The first one is the asymptomatic latent infection not diagnosed in the donor. It usually happens when an adequate screening is not performed. As an example, with the current migratory movements, we should not neglect the screening of geographically restricted infections to which the transplant physicians are not familiar [3, 7]. On other occasions, it is the screening tests that fail. The result of a given serology is affected by the haemodilution that potentially donor patients suffer when they require infusion of crystalloids or blood products. In some cases, the haste of the donation decreases the time available to perform the screening tests. In no case should confirmatory diagnostic tests be used as screening because, although they increase specificity, they lack sensitivity enough to rule out infection. Finally, the tests will not be positive immediately after infection. It will take several days from the contact to the detection of the infection. In the case of the serology, that determines the production of antibodies, this time is called the window period. Currently the possibility of identifying the presence of nucleic acids of microorganisms by polymerase chain reaction reduces this time. This is what is called the viral eclipse phase. Thus, the possibility of diagnosing an infection by the human immunodeficiency virus (HIV) decreases from 22 to 9 days and that of the hepatitis C virus (HCV) from 66 to 7 days [8].

The second cause of unexpected infection transmission is the absence of diagnosis of an active infection as the cause of death. This situation is especially worrying and mostly related to the absence of diagnosis of deceased donors with encephalitis. Transmission of rabies virus, lymphocyte choriomeningitis virus or West Nile virus usually leads to the death of the recipient due to the lack of an early diagnosis and the absence of targeted treatment [9]. In parallel, the donor may suffer an infectious complication during admission to the intensive care unit that is not diagnosed prior to transplantation in relation to the invasive procedures to which they are subjected. An example of this situation would be occult bacteraemia [10]. Hence, it is essential to obtain blood cultures at the time of donation. In case of positivity, it is compulsory to prescribe antibiotic treatment in the recipient with the intention of minimizing the possibility of DDI.

The third cause of unexpected transmission of infection from the donor is the contamination of preservation fluids. A recent meta-analysis has shown that the contamination of the preservation fluid can reach 90% [11] but with a low transmission incidence, around 1%. However, such transmission may compromise the functionality of the graft and the life of the patient, especially in the case of the transmission of yeasts or multidrug resistant microorganisms [11, 12].

PREVENTION

It is very important to get a good clinical history of the donor that includes the occurrence of previous infections, vaccination, travel, transfusions of blood products, contact with

Table 1 Recommended screening for latent infections in the donor

Test	Before transplant	Comments
HIV p24 Ag	Always	
HIV Ab	Always	
HBs Ag	Always	
HDV Ab	If HBs Ag +	
HBc Ab	Always	
HBs Ab	Always	
HCV Ab	Always	
Syphilis (CLIA)	Always	If +, perform reagin and treponemal tests
HTLV I/II Ab	Always	If +, confirm by Western-Blott
Trypanosoma cruzii Ab	Selected	In donor of risk zone or descendant in case of heart transplant
CMV Ab	Always	
HIV NAT	Selected	High-risk donor
HCV NAT	Selected	

Ab: antibody; Ag: antigen; CLIA: chemiluminescent immunoassay; CMV: cytomegalovirus; HBs: hepatitis B surface; HBc: hepatitis B core; HCV: hepatitis C virus; HDV: hepatitis delta virus; HIV: human immunodeficiency virus; HTLV: human T-lymphotropic virus; NAT: nucleic acid testing

people with transmissible diseases such as HIV or HCV, sexual habits, and the use of illicit drugs, among others. This information is not always easy to obtain given the circumstances of the act of the donation and that the interview, mostly, will be made to relatives. In any case, this information is required to define a donor as "high risk" [13]. In this type of donor, it is essential to expand the range of diagnostic tests initially recommended for the assessment of the suitability of the procedure (table 1). If, finally, we decide to go ahead, we must inform the recipient of the risks, request an informed consent and closely monitor the recipient in the event of a probable unexpected transmission [14].

Present. As experience is continuously accumulating, better results are observed with donor organs that, in the past, were considered contraindicated. Nowadays, donors with septic shock or multi-organ dysfunction of bacterial origin can be considered, including the heart, provided donors receive adequate antibiotic treatment for a minimum of 24 hours that it is continued in the recipient [15]. Donors with HIV infection can donate their kidneys to recipients with HIV infection as long as the infection is controlled and there are different choices for HIV treatment [16]. Donors with viremic HCV infection can donate kidneys, lungs and heart with similar results to donors without HCV infection because recipients can now be treated with the new direct-acting antivirals agents against HCV infection which are pangenotypic and without interactions with immunosuppressants (calcineurin and mTOR inhibitors) [17, 18]. To update the information in relation to this topic, the Spanish Organización Nacional de Trasplantes (ONT) has published on its website a consensus document in collaboration with different Spanish Scientific Societies [19].

Future. In order to continue advancing in the prevention of DDI we can act on different directions. By improving the screening of infections in donors with the use of faster and more sensitive and, at the same time, more specific tests, such as techniques based on the polymerase chain reaction or mass spectrometry (MALDI-TOF). By shortening the response times of the tests from obtaining the sample to its result with the implementation of point-of-care tests. By involving all the professionals in the decision making related to the transplant in a multidisciplinary team that includes the specialist in Infectious Diseases. By improving communication between all the levels involved (coordination, microbiology, transplant teams) in case of recognizing a risk in a specific donor-recipient procedure so that the information arrives without loss of time to the rest of the related transplantations. And, finally, by creating standardized and mandatory notification systems with the intention of obtaining the maximum possible information that allows us to convert the transmissions of infection not expected into preventable ones. In that sense, initiatives such as the Notify Library (www.notifylibrary.org) promoted by the Centro Nazionale Traplanti of Italy in collaboration with the World Health Organization (WHO) are essential.

CONCLUSION

Although the infection derived from the donor is an infrequent event, its severity is potentially high. The improvement of screening tests is vital to advance in the prevention of transmission. However, once it occurs, the best way to improve its prognosis is to recognize it as soon as possible. And, for this, it is essential to have the mechanisms that ensure the communication within and among transplant teams in a timely manner. However, in spite of everything, the risk of transmission will never be zero.

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Evaluation questionnaire

IX Updating Course of Antimicrobials and Infectious Diseases 2019

1. The infrared spectroscopy technique in Microbiology is used to:

a) Identification of bacteria and fungi
b) Molecular typing
c) Differentiation between antimicrobial/antifungal resistance
d) All of the above are true

2. Regarding the magnetic resonance technique with nanoparticles, it is true that:

a) It is applied in blood samples for the detection of bacteria and fungi
b) It is applied in blood cultures for the detection of bacteria and fungi
c) It is a technique used for the detection of resistance genes
d) All of the above are false

3. Regarding the new antimicrobials, it is FALSE that:

a) Ceftolozane-tazobactam has activity against bacteria producing beta-lactamases type C
b) Ceftazidime-avibactam and ceftolozane-tazobactam have no activity against bacteria producing type B carbapenemases (metallo-beta-lactamases)
c) Imipenem-relebactam has activity against bacteria that produce type B carbapenemases (metallo-beta-lactamases)
d) Ceftazidime-avibactam is active against carbapenemase-producing bacteria of the KPC type

4. MALDI-TOF MS technology has been able to revolutionize the identification of all these microorganisms except:

a) Non-tuberculous mycobacteria
b) Yeast fungi
c) Gram-negative bacteria producing carbapenemases
d) *Chlamydiophytes*

5. One of the following antibiotics has no in vitro activity against multiresistant *Pseudomonas aeruginosa*:

a) Ceftolozane-tazobactam
b) Cefiderocol
c) Eravacycline
d) Plazomycin

6. Betalactamases, vaborbactam and nacubactam inhibitors are capable of inactivating the following Betalactamases except:

a) CTX-M
b) VIM
c) KPC
d) Amp-C

7. All but one of the following antimicrobials are drugs in development at the present time. Please choose the fake one:

a) Tebipenem (Spero)
b) ETX2514 + Sulbactam (Entasis)
c) Ceftazidime-Turbibactam (Sinextro)
d) Ibrexafungerp (SCY-078) (Scynexis)

8. According to a presentation made at the 2018 IDWeek in San Francisco, about Tedizolid, choose the correct statement:

a) It is a drug of the quinolone family
b) Can only be administered via IV
c) It has an oral bioavailability of approximately 50%
d) It has been well tolerated in treatments of up to 4 weeks in patients with osteoarticular infections

9. From the presentations on *Clostridium difficile* infection in the IDWeek of San Francisco 2018, all of the following can be deduced, except one of the following sentences, which is false and should state:

a) CDI has become a growing cause of nosocomial infection in several states of the American Union that surpasses nosocomials
b) All patients whose diagnosis is confirmed only by molecular technique (direct negative toxin) are colonized and do not require treatment
c) Unnecessary *C. difficile* tests can be reduced by hiring a person to act upon the test demand
d) A high Ct of the amplification curve (Ct> 29) allows excluding only colonized patients

10. In the PARTNER2 study, among serodiscordant couples who have sex without a condom, with the virologically suppressed HIV positive member, what was the rate of HIV transmission within the couple?

a) There are no linked transmissions
b) Transmissions unrelated to anal sex insertion, insignificant linked transmissions to anal sex receptive
c) Transmissions not related to receptive anal sex, insignificant linked transmissions with anal insertive sex
d) There are no sex-linked transmissions without a sexually transmitted infection, insignificant sex-linked transmissions with a sexually transmitted infection

11. In the phase III studies, GEMINI 1 and 2, what were the results in terms of efficacy and appearance of resistance with dolutegravir plus lamivudine as a starting therapy?

a) Biterapia inferior to triple therapy, without observing resistance
b) Lower bi-therapy versus triple therapy, the M184V resistance mutation was observed.
c) Non-inferior bi-therapy versus triple therapy, with no resistance observed.
d) Non-inferior biotherapy, but with the appearance of resistance mutation M184V

12. Regarding the results of the use of PrEP in the PREVENT cohort, sign the correct answer:

a) It is estimated that the use of PrEP prevented 85 new HIV infections after 7 months of follow-up
b) Fewer HIV infections were recorded with daily PrEP than with PrEP on demand.
c) No STI was registered in any of the arms of the study
d) The percentage of patients who took PrEP correctly was low, around 55%

13. Indicate the correct answer regarding carbapenemases-producing enterobacteria (EPC) compliance with hand hygiene indications is conditioned by

a) Ceftazidime-avibactam and ceftolozane-tazobactam are equally active against EPCs
b) Only ceftazidime-avibactam is active against EPCs with the exception of those that produce metallo-betalactamases
c) Only ceftolozane-tazobactam is active against all EPCs
d) Neither ceftazidime-avibactam nor ceftolozane-tazobactam have activity against EPCs

14. Indicate the correct answer. Resistance to ceftazidime-avibactam in *Klebsiella pneumoniae* can be produced by

a) Mutations in the sequence of the KPC carbapenemase that determines loss of affinity for avibactam
b) Hyperexpression of carbapenemase KPC together with alterations in the porins
c) Production of a metallobetalactamase
d) All are correct

15. According to the ECDC in its 2017 report, carbapenemic resistance in invasive isolates of *Klebsiella pneumoniae* has increased in recent years in Spain, reaching figures of:

a) 50%
b) 10%
c) 3%
d) There are no resistant isolates

16. *P. aeruginosa* is an important etiologic agent in case of:

a) Ventilation pneumonia
b) Neutropenic patient infection
c) Urinary tract infection in the patient with permanent probing
d) All of the above are true

17. Which of the following antibiotics makes it possible to reach a higher level than CMP (concentration that prevents the selection of mutants) of *P. aeruginosa*?

a) Ertapenem
b) Meropenem
c) Ceftolozane-tazobactam
d) Ceftazidime-avibactam

18. Which of the following statements do you think is right?

a) *P. aeruginosa* isolates resistant to carbapenems tend to be also colistin
b) More than 50% of neutropenic patients with MDR *P. aeruginosa* bacteremia receive inadequate empirical treatment
c) Ertapenem is active against about 50% of *P. aeruginosa* isolates
d) The extensively resistant *P. aeruginosa* isolates do not produce blue-green pigment

19. Point out the correct answer regarding the possible impact of edemas on the antibiotic PK

a) In general it is associated with reduced concentrations of any drug
b) Reduction of concentrations especially affects antibiotics with reduced volume of distribution
c) Only affects drugs that are highly bound to plasma proteins
d) All are incorrect

20. The efficacy time (T> MIC) is the PK / PD parameter related to the efficacy of beta-lactams, which of the following is considered reference values to achieve a bactericidal effect?

a) T> MIC: 40-50%
b) T> MIC: 70-80%
c) T> MIC: 90-100%
d) The efficacy of beta-lactams in AUC / CMI dependent

21. Considering the PK / PD criteria

a) Aminoglycosides can be administered in a single daily dose even in patients with normal renal function.
b) Vancomycin may have to be administered in doses that can cause nephrotoxicity
c) Tigecycline should be administered at twice the dose recommended in its data sheet
d) All are correct

22. Which of the following actions seems correct in a critical non-neutropenic patient with candidmia related to venous catheter by *C. albicans* sensitive to all tested antifungals (echinocandins, amphotericin B, fluconazole and voriconazole) that after catheter removal, 5 days of treatment with an echinocandin and with negative control blood culture removed at 48 h?

a) Continue with the echinocandin
b) De-escalate to voriconazole
c) De-escalate to fluconazole
d) Stop antifungal treatment

23. What is the wrong answer regarding differential blood cultures?

a) It is a diagnostic technique that does not require catheter removal.
b) They are blood cultures extracted simultaneously through catheter and direct venipuncture.
c) It is very suggestive of CRB if the difference is more than 120 minutes between the growth of the samples obtained by the catheter with respect to those obtained from peripheral blood.
d) It is very suggestive of catheter-related candidemia if the difference is more than 240 minutes between the growth of the samples obtained by the catheter with respect to those obtained from peripheral blood.

24. According to CRB by *Staphylococcus* coagulase negative (SCN):

a) The treatment of choice for methicillin-sensitive SCNs is vancomycin and for cefazolin for strains resistant to methicillin.
b) If the catheter was removed, uncomplicated CRBs can be treated with 5-7 days of antibiotic.
c) For patients with intravascular, biomedical devices or persistent signs of inflammation after catheter removal, antibiotic treatment is recommended for 5-7 days.
d) *S. lugdunensis* do not usually cause serious infections.

25. Which definition among the following is best suited to the concept of stewardship in sepsis?

a) Restriction of antibiotic use in the septic patient
b) Use of the antibiotic the most appropriate antimicrobial, at the optimal dose, and for the correct duration in the septic patient
c) Reduction of the economic expense associated with the use of antibiotics in the septic patient
d) Reduction in the number of days of antibiotic treatment in the septic patient

26. The implementation of stewardship programs in the septic patient enables all the following objectives except one ...

a) Decrease in the economic cost associated with the use of antimicrobials
b) Reduction of adverse effects
c) Reduction of the possibility of drug interactions
d) Reduction of the possibility of superinfections

27. Stewardship programs in the septic patient can be implemented ...

a) Ideally at the time of patient admission
b) They can be implemented throughout the patient's hospitalization
c) It is best to implement them upon patient discharge
d) At any of the previous moments

28. Which, or which, among the aforementioned statements are risk factors for recurrence of *Clostridium difficile* infection (CDI) ?; point them out:

a) Lack of adaptive immune response to toxins A and B
b) Use of antibiotics (for other infections) during or after an episode of CDI
c) Hypervirulent strains (such as NAP1/BI/027)
d) All of the above

29. Which of the following antimicrobials with activity against *Clostridium difficile* is characterized by greater protection or preservation of the fecal microbiota ?:

a) Fidaxomycin
b) Cadazolid
c) Ridinylazole
d) Vancomycin

30. One of the pairings described below between anti-*Clostridium difficile* drug and its target or mechanism of action is not correct; Which?:

a) Bezlotoxumab --- *Clostridium difficile* toxin B
b) Rifaximin --- RNA-Polymerase
c) Ridinylazole --- bacterial DNA and toxin production inhibition
d) Actoxumab --- *Clostridium difficile* binary toxin

31. Which of the following statements is true?

a) In the patient with febrile neutropenia, bacterial infection is the most frequent infectious complication
b) Bacteremia of endogenous origin by bacterial translocation is the most frequent bacterial infection
c) The choice of empirical antibiotic treatment in febrile neutropenia depends on the risk factors of multiresistance and local epidemiology
d) All of the above are true

32. In the management of patients with febrile neutropenia:

a) When the fever persists, an antibiotic with activity against gram-positive agents should be added to cover the possibility of vascular catheter infection.
b) If there is a clinical response and the fever remits, the same antibiotic treatment must be started empirically.
c) In stable, asymptomatic patients, and without microbiological documentation, withdrawal of antibiotic treatment is recommended regardless of neutrophil count.
d) Biomarkers are very useful for deciding to discontinue antibiotic treatment.

33. Which of the following statements is true?

a) In low-risk patients, antibiotics can be administered orally and ambulatory management.
b) The MASCC risk index should always guide the indication of hospital admission.
c) The universal use of antibacterial prophylaxis with quinolones is recommended.
d) Due to lack of scientific evidence, the use of new antibiotics recently marketed should not be used in neutropenic patients.

34. In which of these situations would you perform prophylaxis against filamentous fungi?

a) In patients with acute leukemia and induction chemotherapy
b) In patients with graft disease versus the recipient after an allotransplant who require corticosteroids
c) In any situation where the risk of infection by filamentous fungi is greater than 10%
d) All of the above are true

35. Which antifungal has the greatest scientific evidence of efficacy in prophylaxis of filamentous fungi?

a) Isavuconazole
b) Posaconazole
c) Voriconazole
d) Itraconazole

36. Using posaconazole in prophylaxis in patients with acute leukemia and intensive chemotherapy has demonstrated ...

a) Reduce the incidence of fungal infection
b) Reduce the incidence of aspergillosis
c) Decrease overall patient mortality
d) All are true

37. Which of the following anti-TNF-alpha agents has been systematically shown to be associated with a lower risk of reactivation of latent tuberculous infection?

a) Infliximab
b) Adalimumab
c) Etanercept
d) Certolizumab pegol

38. What is the minimum period of treatment with isoniazid from which it is usually considered safe to start treatment with an anti-TNF-alpha agent in a patient with latent tuberculosis infection?

a) One week
b) One month
c) Three months
d) Two weeks

39. What prevention strategy would be necessary to apply to a patient who is going to receive rituximab for a non-Hodgkin lymphoma and who has the following serological markers: positive anti-HBc IgG, positive anti-HBs IgG, negative surface antigen (HBs), Negative HBV DNA?

a) Since it presents both HBs antigen and HBV negative DNA, it would not require any specific prevention strategy
b) HBV DNA monitoring after 1 month and start of treatment with entecavir in case a positive result was obtained
c) Administer prophylaxis with lamivudine during the course of treatment with rituximab
d) He would administer prophylaxis with lamivudine, which he would maintain for at least 6-12 months after the end of treatment with rituximab

40. Which of the following options is not a cause of unexpected transmission of donor infection to the recipient of a solid organ transplant?

a) Preservation liquid contamination
b) Absence of diagnosis of active infection as a complication during donor admission
c) Asymptomatic latent infection diagnosed in the donor
d) False negative of donor infection screening tests

41. Indicate the correct answer:

a) Donor organs with HCV infection cannot be transplanted
b) Donor organs cannot be transplanted with West Nile virus encephalitis
c) Donor cytomegalovirus infection contraindicates the donation of any organ
d) Donor Chagas disease contraindicates liver donation

42. Indicate the wrong answer:

a) Screening for latent infection by PCR reduces the eclipse period
b) In the donation of a solid organ there is no "zero risk" against the transmission of an infection
c) Donor-derived infection is uncommon but potentially lethal
d) Blood cultures should be made to the donor at the time of donation to rule out a hidden bacteraemia

Correct answer sheet

IX Updating Course of Antimicrobials and Infectious Diseases 2019. 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			