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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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