Carbapenemase-producing *Enterobacteriaceae:* A diagnostic, epidemiological and therapeutic challenge

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Abstract

The global spread of carbapenemase-producing *Enterobacteriaceae* (CPE) has become a public health problem. Not all CPE are resistant to carbapenems creating a diagnostic and therapeutic challenge. Furthermore, as resistance to carbapenems can also be mediated by other β-lactamases combined with defects in membrane permeability, their detection can be difficult by microbiology laboratories that lack molecular tools, which may limit and often delay the correct antibiotic selection. There is only limited evidence regarding infection control measures to contain the spread of CPE. However, recomendations have been published from the World Health Organization (WHO) and the European Prevention Center and Disease Control (ECDC). Because of the lack of randomized control trials, treatment regimens are mostly based on observational clinical studies. Several of those studies have reported that combination therapy with two or more *in vitro*-active agents including a carbapenem is superior to monotherapy. On the other hand, a new β-lactamase inhibitor in combination with ceftazidime has shown clinical efficacy Against KPC and some OXA-type producing Enterobacteriaceae.

Key words: Carbapenem-Resistant Enterobacteriaceae, Antibiotic Resistance

Enterobacteriaceae productoras de carbapenemasas: Un reto diagnóstico, epidemiológico y terapéutico

Resumen

La diseminación global de las *Enterobacteriaceae* productoras de carbapenemasas (EPC) se ha convertido en un problema de salud pública. No todas las EPC son resistentes a los carbapenémicos, por lo que representan un reto diagnóstico y terapéutico. Adicionalmente, como la resistencia a los carbapenémicos puede ser mediada por otras β-lactamasas en combinación con cambios de la permeabilidad de la membrana plasmática, su detección puede ser difícil en laboratorios de microbiología que no cuentan con técnicas de diagnóstico molecular, lo que puede restringir y frecuentemente retrasar el inicio de la terapia antimicrobiana adecuada. La evidencia respecto a las medidas para la contención de las EPC es escasa. Sin embargo, existen recomendaciones por parte de la Organización Mundial de la Salud y del European Prevention Center and Disease Control (ECDC). Debido a la ausencia de estudios controlados y aleatorizados, los esquemas terapéuticos se basan en estudios clínicos observacionales. Varios de estos estudios han reportado mejores resultados con la terapia combinada de dos o más agentes activos in vitro, incluyendo a los carbapenémicos, en comparación con la monoterapia. Por otra parte, un nuevo inhibidor de β-lactamasas en combinación con ceftazidime, ha mostrado eficacia clínica contra infecciones por *Enterobacteriaceae* productoras de KPC y algunas carbapenemasas de tipo OXA.

Introduction

Because *Enterobacteriaceae* are a common cause of community- and hospital-acquired infections, antimicrobial resistance in these bacteria have a significant impact on antibiotic use and patient outcomes. The emergence and spread of Carbapenemase-producing *Enterobacteriaceae* (CPE) have become a public health problem in recent decades due to their great ability to spread and became endemic in several countries of the world, including Colombia[1]. Their presence has been associated with a negative impact on health care systems, since they are a frequent cause of nosocomial infections (2–8) with reported mortality rates up to 50% (7–10). The diagnosis of CPE in the clinical laboratory is difficult because they can test susceptible to carbapenems despite the presence of a carbapenemase, according to the minimal inhibitory concentration (MIC) breakpoints defined by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST), causing a "silent dissemination" in many hospitals[11,12]. CPE can be resistant to a broad range of antibiotics, leaving only very few therapeutic options. Some "second-line" drugs such as ti-

gecycline, colistin, fosfomycin, aminoglycosides and occasionally quinolones may remain active against CPE. The combination of a carbapenem in high doses and prolonged infusion with one or two of the previously mentioned drugs has been associated in many reports with better outcomes for high-risk patients[13]. Unfortunately, none of these studies was designed with the purpose of comparing different treatment regimens making it more difficult to choose the right combination[14].

In the last years, new β-lactamase combinations such as ceftazidime–avibactam and meropenem–vaborbactam have become available for the treatment of CPE. Early results suggest they are safer and more efficacious for the treatment of CPE infections compared with some of the older agents, particularly polymyxin regimens. Other new drugs active against selected CPE isolates are in different stages of development and/or approval by the Food and Drug Administration agency of the United States (FDA) or the European Medicines Agency (EMA). Among the antibiotics evaluated are imipenem-relebactam, plazomicin, cefiderocol, eravacycline, and aztreonam-avibactam[15].

The aim of this review is to focus on the current evidence regarding the diagnosis, prevention, control and treatment of CPE infections, with emphasis on the association between different treatment options and mortality. In addition, ceftazidime/avibactam will be reviewed due to its near launch in Colombia.

Diagnostic challenge

Resistance to carbapenems in *Enterobacteriaceae* is caused by two principal mechanisms[16,17]:

• Expression of a cephalosporinase (extended spectrum β-lactamase (ESBL) or plasmid or chromosomal AmpC) combined with defects in membrane permeability.

• Expression of a carbapenemase combined with permeability defects of the outer membrane.

Clinical and epidemiological importance of carbapenemase detection:
The resistance to carbapenems mediated by the production of carbapenemases is of high clinical, therapeutic and epidemiological relevance because

• They cause hospital outbreaks associated with clones and plasmid dissemination
• Depending on the molecular class (table 1), they can have a high hydrolytic activity against carbapenems, reducing their efficacy and therefore the combination of antibiotics such as polymyxins, aminoglycosides, fosfomycin and tigecycline might be necessary[18].
• The simultaneous production of other β-lactamases and activation of other resistance mechanisms leads to multi-resistance and pan-resistance, leaving none or very limited therapeutic options.
• Infections caused by carbapenemase-producing bacteria are associated with higher mortality rates[19].

Although the CLSI and the EUCAST do not recommend the routine screening for carbapenemases for therapeutic purpose (only to control its dissemination) (20,21), the antimicrobial resistance committee of the Colombian Association of Infectious Diseases (ACIN), recommends the routine testing for the presence of this enzymes based on the following key aspects:

1. Carbapenemases have special pharmacodynamical implications. For example, KPC enzymes have the highest hydrolytic activity against cephalosporins and carbapenems while OXA-48 enzymes have only weak activity against these antimicrobials[13].

Table 1. Classification of the most relevant carbapenemases in the clinic

Ambler-Bush Class	Carbapenemase	Most frequently identified in	Key characteristics
A	KPC	*K. pneumoniae* and other *Enterobacteriaceae*	• Variable level of carbapenem hydrolysis. • Some are chromosomal (SME, NMC) others are mainly plasmidic (KPC). • Inhibition by boronic acid, avibactam and partial inhibition by clavulanic acid
	SME	*S. marcescens*	
	IMI, NMC, GES	*Enterobacteriaceae*	
B	**Metallo-β-lactamases (VIM, IMP, GIM, SPM and NDM)**	*Enterobacteriaceae (E. coli, Klebsiella, Enterobacter, Providencia, Citrobacter, Proteus mirabilis)*	• Mainly plasmidic • High hydrolytic activity against carbapenems and cephalosporins • No activity against aztreonam by definition • Inhibition with EDTA and dipicolinic acid • No inhibition with boronic acid, clavulanic acid or avibactam
D	OXA-48-family	*Klebsiella pneumoniae, E.coli* and other *Enterobacteriaceae*	• Low hydrolysis of oximino-cepaholsporins and moderate hydrolysis of carbapenems • No inhibition with EDTA, dipicolinic and boronic acid. Partial inhibition with avibactam

Table adapted from Bush[52]

2. KPC might be associated with higher mortality rates in comparison with other carbapenemase families such as metallo-β-lactamases (MBLs)[22].
3. Aztreonam is not a substrate of class B carbapenemases, thus classifying these enzymes and testing the susceptibility for aztreonam, allows the use of this antibiotic in combination with a second drug for MBLs.
4. The use of new inhibitor combinations such as ceftazidime/avibactam and meropenem/vaborbactam requires the identification of the enzyme class since these treatment regimens are inactive against class B carbapenemases[23].
5. Some therapeutic strategies, such as the combination of two carbapenems, have been used successfully only in class A CPE (KPC)[24].
6. KPC is endemic in Colombia and due to its high capacity of dissemination in the nosocomial environment and between patients, it is key to identify them from the infection control perspective.

In the following section, the most frequently used methods for the detection of these enzymes in clinical isolates and clinical samples will be reviewed. The screening of colonized patients will not be addressed.

Phenotypic tests for the detection of carbapenemases
Production of carbapenemases in *Enterobacteriaceae* should be suspected and confirmed when intermediate susceptibility or resistance to any carbapenem is observed applying the current CLSI breakpoints[25].

Phenotypic laboratory tests used for the detection of these enzymes can be divided into "capture" tests and "differentiation" tests. It is recommended to apply them together based on the arguments presented above[26].

- Capture Tests (table 2): These tests seek to determine the presence or absence of carbapenemases in a given isolate. According to the technique used, the turnaround time can range from 15 minutes to 24 hours. These methods do not allow differentiating between individual enzymes, or classifying carbapenemases into class A, B or D.
- Differentiation and classification tests (table 3): These methods allow to identify the class to which an enzyme belongs to (class A, B or D). It is important to emphasize that due to the diversity and versatility of these enzymes, to date there is not a single phenotypic test capable of detecting and differentiating all classes of enzymes in a single step. The microbiology laboratory in collaboration with the institutional antimicrobial stewardship committee needs to decide which of these tests have higher applicability and represent a better cost-effectiveness for the institution. Colombia, as well as many other countries is frequently reporting isolates that produce simultaneously more than one type of carbapenemase (for example VIM plus KPC or NDM plus KPC)[27]. The co-expression of various enzymes decreases the efficiency of phenotypic tests to differentiate these enzymes correctly.

Molecular tests for the detection of carbapenemases
Currently, there are several molecular platforms based on polymerase chain reaction (PCR) for the detection of carbapenemases in *Enterobacteriaceae* and other gram- negative glucose non-fermenting bacteria. Among the advantages of these techniques are[28,29]:

- Higher sensitivity and specificity compared with conventional phenotypic tests as PCR based techniques are not affected by the co-expression of other enzymes and/or defects of outer membrane permeability.
- Less turnaround time: These tests take approximately one hour compared to other methods that require up to 24 hours.
- Possibility of detecting enzyme co-expression: These platforms can detect the co-production of class A, B or D carbapenemase enzymes simultaneously.

Expert rules for reporting antimicrobials when CPE are detected:
Class A carbapenemases (detection of KPC or inhibitory enzymes by boronic acid)

- Report as resistant aminopenicillins, combinations of β-lactams plus β-lactamase inhibitors and all cephalosporins including ceftolozane/tazobactam.
- Report as resistant ertapenem (regardless of the MIC result)
- Report meropenem and doripenem with the MIC result and interpretation according to the breakpoints. Include a footnote warning the carbapenemase detection.
- Report aminoglycosides, quinolones, tigecycline, colistin and fosfomycin according to the breakpoints.
- Test and report ceftazidime/avibactam susceptibility using a MIC method (E-test or broth microdilution). Interpret results using the current EUCAST or CLSI breakpoints.

Class D carbapenemases (detection of OXA-48 or enzymes not inhibited by boronic acid and EDTA/dipicolinic acid)

- Report as resistant: aminopenicillins, combinations of β-lactams plus β-lactamase inhibitors as well as first and second-generation cephalosporins and aztreonam.
- Report the results of third and fourth generation cephalosporins with the MIC result and interpretation according to the breakpoints. Include also a footnote warning the detection of a CPE.
- Report ertapenem as resistant (regardless of the MIC result)
- Report meropenem and doripenem with the MIC result and interpretation according to the breakpoints. Include a footnote warning the carbapenemase detection
- Report aminoglycosides, quinolones, tigecycline, colistin and fosfomycin according to the breakpoints.
- Test and report ceftazidime/avibactam susceptibility using a MIC method (E-test or broth microdilution). Interpret results using the current CLSI breakpoints.

Table 2. Phenotypic tests for the detection and differentiation of carbapenemases

Test	Diagnostic performance	Turnaround time	Scope	Limitations
Modified Hodge Test	Sensitivity: 72-100% Specificity: 100%	18-24 h	Detection of KPC and OXA-48 in acceptable range for *K. pneumoniae* and *E. coli* isolates	False positives with chromosomal AmpC-producers and ESBL-producers with permeability defects False negatives for metalocarbapenemasas
Synergy test with boronic acid	Sensitivity: 92% Specificity: 94%	18-24 h	Differentiation of class A carbapenemases (KPC)	False positives with chromosomal AmpC-producers of *Enterobacter* and *Serratia* Sensitivity changes according to the carbapenemase variant Does not detect metalocarbapenemasas Changes in distance of the disks and / or their potency diminishes the diagnostic performance of the test
Synergy test with EDTA	Sensitivity: 92% Specificity: 94%	18-24 h	Differentiation of class B carbapenemases (NDM-VIM)	False positives due to porin-alterations Sensitivity changes according to the carbapenemase variant Does not detect serine-carbapenemases Changes in distance of the disks and / or their potency diminishes the diagnostic performance of the test
Modified carbapenem inactivation test (mCIM and eCIM)	Sensitivity: 98-100% Specificity: 99-100%	18-24h	Detection of class A, B and D carbapenemases. Addition of EDTA to eCIM, allows differentiation of class B carbapenemases (NDM, VIM, etc)	Does not differentiate between carbapenemases of classes A and D Some false positives with AmpC-producing *E. cloacae complex*
Carba NP/Blue Carba	Sensitivity: 84% Specificity: 100%	15 min-2h	Detection of class A, B and D carbapenemases	Does no differentiate individual enzymes Some false positive with OXA-48 variants and with mucoid strains of *Klebsiella spp*.
Lateral flow immunoassay	Sensitivity: 100% Specificity: 95-100%	15 min	Detection of class A, B and D carbapenemases	Low number of validation studies and low local experience False positives with some OXA-type enzymes without carbapenemase activity

Adapted from Tamma et al. and Simner et al[26,53].

Class B carbapenemases (detection of NDM/VIM/IMP or enzymes inhibited by zinc chelators such as EDTA or dipicolinic acid)

- Report as resistant aminopenicillins, combinations of β-lactams plus β-lactamase inhibitors
- Report as resistant all cephalosporins, including ceftolozane/tazobactam.
- Report ertapenem as resistant (regardless of the MIC result)
- Report meropenem and doripenem with the MIC result and interpretation according to the breakpoints. Include a footnote warning the carbapenemase detection
- Report aminoglycosides, quinolones, tigecycline, colistin and fosfomycin according to the breakpoints.
- Test and report aztreonam susceptibility according to the current CLSI breakpoints.

Quality control: To guarantee reliable results that translate into appropriate therapeutic decisions, internal and external quality control is definitive to ensure clinically relevant reports. Quality control must be carried out in accordance with the volume of work of the microbiology section, the endemicity of carbapenemases in the institution, country and/or region, and the type of methods performed by the laboratory. Table 4 suggests some of the quality control strains that can be used to evaluate phenotypic and molecular tests. Discrepancies should be confirmed using molecular test.

Note: The modified Hodge test was removed from the CLSI recommendations for the detection of carbapenemases. However, the ACIN Bacterial Resistance Committee considers that this test is still useful in regions of high endemicity of KPC in *K. pneumoniae* and *E. coli*. Due to its limitations, a negative result does not exclude the presence of carbapenemases, which must be screened with an alternative method.

Epidemiological challenge

Due to the fast dissemination of CPE, some countries have implemented national strategies for the containment of these carbapenemases. Israel after the implementation of its national policy in 2007 achieved a decrease in the monthly incidence of nosocomial CPE-infections from 55.5 to 11.7 per 100,000 patient/days. The effective strategy was based

Table 3. Frequently used molecular platforms for Carbapenemase producing Enterobacteriaceae.

Platform	Diagnostic performance	Turnaround time	Scope	Limitations
Cepheid Xpert Carba R®	Sensitivity: 100% Specificity: 98%	1 h	Detection of KPC, NDM, VIM, IMP and OXA-48 enzymes from rectal swabs samples and colonies on agar	Does not detect all the reported variants of some types of enzymes May not detect new carbapenemases
BD MAX CHECK DIRECT CPE®	Sensitivity: 93% Specificity: 97%	1 h	Detection of KPC, NDM, VIM, and OXA-48 enzymes from rectal swabs samples and colonies on agar	Does not detect IMP enzymes Does not detect all the reported variants of some types of enzymes May not detect new carbapenemases
bioMerieux FilmArray® BCID Panel	Sensitivity: 100% Specificity: 100%	1 h	Detection of KPC enzymes from positive bloodcultures	Does not detect carbapenemases other than KPC
bioMerieux FilmArray® Pneumonia panel plus	Sensitivity: 96% Specificity: 97%	1 h	Detection of KPC, NDM, VIM, IMP and OXA-48 enzymes from tracheal aspirates, sputum and Bronchoalveolar lavage	Does not detect all the reported variants of some types of enzymes May not detect new carbapenemases
Sepsis Flow Chip®	Sensitivity: 98% Specificity: 100%	4 h	Detection of GES, VIM, SPM, KPC, NDM, SIM, IMP, SME and OXA enzymes from positive bloodcultures	Laborious Possibility of contamination because it is an open PCR system Does not detect all the reported variants of some types of enzymes May not detect new carbapenemases
Verigene® BC-GN	Sensitivity: 100% Specificity: 100%	< 3 h	Detection of IMP, KPC, NDM, OXA and VIM enzymes from positive bloodcultures	Does not detect all the reported variants of some types of enzymes May not detect new carbapenemases

Adapted from Aziz[54] and Rood[55].

Table 4. Quality control for phenotypic detection of carbapenemases.

Carbapenemase type	Suggested reference strains	Expected results
Serine-carbapenemases (KPC)	*K. pneumoniae* ATCC BAA 1705	MHT (+), BAT (+), EDTA test (-), mCIM (+), eCIM (-), Carba NP (+),
Carbapenem resistance not mediated by carbapenemases	*K. pneumoniae* ATCC BAA 1706	MHT (-), mCIM (-), eCIM (-) BAT (-), EDTA test (-), Carba NP (-)
Metallo-carbapenemases (VIM, NDM)	*E. cloacae* ATCC BAA 2468, *K. pneumoniae* ATCC 2146	mCIM (+), eCIM (+), BAT (-), EDTA test (+), Carba NP (+)
OXA-48 carbapenemases	*K. pneumoniae* ATCC BAA 2524	MHT (+), mCIM (+), eCIM (-), BAT (-), EDTA test (-), Carba NP (+)

eCIM: EDTA-modified carbapenem inactivation method; mCIM: modified carbapenem inactivation method; MHT: Modified Hodge Test, BAT: Boronic Acid Test, Adapted from CLSI 2018[56].

on dedicated task force of appointed professionals. The task force paid site visits at acute-care hospitals, evaluated infection-control policies and laboratory methods, supervised adherence to the guidelines via daily census reports on carriers and their conditions of isolation, provided daily feedback on performance to hospital directors, and intervened additionally when necessary[30].

In the area of infection control, the lack of randomized studies is one of the limitations when it comes to choose the most effective method in a given hospital because recommendations are not based on a high-level evidence.

Different agencies, societies and countries have published guidelines and recommendations for the control of multi-drug resistant gram-negative bacteria. In the last five years, three guidelines have been published which qualify the level of evidence using the methodology described by the Grading of Recommendations Assessment, Development and Evaluation Working Group (GRADE). The first guideline was published in 2014 by the European Society of Microbiology and Infectious Diseases (ESCMID), the second in 2017 by the World Health Organization (WHO) and the third, also published in 2017 by the European Prevention Center and Disease Control (ECDC). The ESCMID Guidelines includes recommendations for extended-spectrum β-lactamases producing *Escherichia coli* and multi-drug resistant *Klebsiella pneumoniae* but not specifically for carbapenemase producers. The WHO guideline is specific for CRE and includes studies published until 2016. Finally, the ECDC guideline reviewed studies until 2013 and focuses not only on prevention measures but also on the identification of patients at risk for CRE infections. Table 5 describes the specific recommendations for CRE control included in each of these guidelines[31–33].

Screening for CPE carriers
It is recommended to perform CPE carrier screening during outbreaks or high endemicity within a hospital, in all patients that meet one or more of the following risk factors:

Table 5. Specific recommendations for the control of CRE by the WHO and ECDC guidelines.

Recommendation	WHO	ECDC	Strength of recommendation; Level of evidence
Implementation of a multimodal strategy that includes different components: hand hygiene, surveillance, contact precautions, isolation measures, environmental cleaning and disinfection	X		Strong; Low – very low
Hand hygiene	X	X	Strong; Very low (WHO) Evidence from observational studies (ECDC)
Surveillance of CPE infections	X	X	Strong; Very low (WHO) Evidence from observational studies (ECDC)
Screening for CPE colonization	X	X	Evidence from observational studies (ECDC)
Contact precaution: use of personal protection elements, limited the transfer of patients, medical elements for exclusive use, prioritized cleaning and disinfection	X	X	Strong; Low – very low (WHO) Evidence from observational studies (ECDC)
Hospitalization in a single room or cohort patients (patients infected or colonized by the same microorganism may share a room)	X	X	Strong; Low – very low (WHO) Evidence from observational studies (ECDC)
Preventive isolation of patients at risk, prior to microbiology results		X	Evidence from observational studies (ECDC)
Compliance with the cleaning and disinfection protocols of the room.	X	X	Strong; Very low (WHO) Evidence from observational studies (ECDC) There is no high level evidence to recommend a type of disinfectant to carry out the disinfection process. Most observational studies employ hypochlorite (WHO)
Taking of environmental isolates during outbreaks	X		Conditional; Very low (WHO)
Monitor the implementation of the multimodal strategy and provide feedback to health workers and administration	X		Strong; Low – very low (WHO)
Implement a antimicrobial stewardship program		X	Evidence from observational studies (ECDC)
Nurses and other health personnel with exclusive dedication to CPE infected or colonized patients		X	Evidence from observational studies (ECDC)
Communication strategies that allow identifying the transfer of patients to the interior or between care centers. Includes electronic alerts.		X	Evidence from observational studies (ECDC)
Antiseptic baths		X	Evidence from observational studies (ECDC)

Adapted from WHO[32] and ECDC[33].

- History of infection or colonization by CPE.
- History of hospitalization in regions or centers whose epidemiology suggests a high incidence of CPE.
- History of hospitalization during the last 12 months.
- History of dialysis or chemotherapy during the last 12 months.
- Epidemiological link with a patient infected with CPE[32,33].

Microbiological samples can be obtained from stool, rectum or perianal area or any other site where an infectious process is evidenced.

Regarding the duration of contact precautions, the ECDC and SHEA (Society for Healthcare Epidemiology of America)

guidelines recommend that contact precautions should be maintained throughout the hospitalization, particularly in cases of XDR and PDR. In contrast, the WHO did not issue any recommendation in its guideline. Banach et al. recently published an article proposing the elimination of contact precautions 6 months after the initial positive CPE culture, when a patient has two negative control rectal or perirectal CPE cultures taken with one-week interval[34].

Therapeutic challenge

Due to the reduced number of randomized clinical trials, the therapeutic approaches for CPE-infections are mainly based on accumulating clinical experience. A wide heterogeneity of

most of the published studies (different types of infections, different groups of patients, a variety of treatment regimens, different methods and outcome definitions) can be a serious obstacle in comparing data, which precludes the possibility of a rigorous meta-analysis. Therefore, it is difficult to recommend a single therapeutic approach[8]. It is important to mention that some of the studies included the term CPE, whereas other studies included carbapenem resistant *Enterobacteriaceae* (CRE). The CDC initially defined CRE as those *Enterobacteriaceae*, which were non-susceptible to ≥ 1 carbapenem and were resistant to 3rd generation cephalosporins. In their November 2015 update, this definition was revised and CRE are now defined as any *Enterobacteriaceae* which are resistant (excluding intermediate resistance) to any carbapenem or are documented to produce a carbapenemase. In addition, for those *Enterobacteriaceae*, which may have intrinsic reduced susceptibility to imipenem such as *Proteus mirabilis*, resistance to a non-imipenem carbapenem is required. The CDC acknowledges that this definition lacks specificity for CPE, especially in low-prevalence areas[35].

In 2017, Trecarichi and Tumbarello performed a literature review of the therapeutic options for CRE and/or CPE infections. The review points out the difficulty of finding comparable studies with a design that allows the identification of the best therapeutic options for patients with these infections[36]. In this review, the authors analyze 11 available studies. However, there are some fundamental limitations when it comes to compare these studies and draw conclusions that is important to take into account when making a therapeutic decision:

All but one of the studies included *K. pneumoniae*-infections. All studies were observational cohorts, which implies certain variability in the clinical conditions of patients, as well as differences in the severity of the infection and the type of CPE/CRE. There is also variation in the site of infection in the different studies, with multiple clinical samples (including blood in variable percentages) and a non-systematic use of the MIC since there are different breakpoints of susceptibility or resistance according to the region of the world (CLSI vs. EUCAST). Furthermore, a detailed definition of "monotherapy" and "combination therapy" is not included in most of the articles. In addition, there is a small percentage of reports of Verona integron-encoded metallo-β-lactamases (VIM) alone or in combination with KPC strains making it impossible to extrapolate the clinical or therapeutic impact of each enzyme. To make the comparison even more difficult, in several studies the isolated strains that expressed carbapenemases were carbapenem-susceptible *in vitro* based on the EUCAST cut-off point. All studies reported different antimicrobial schemes and their association with mortality rates. The different therapeutic approaches were included in the analysis of independent risk factors for mortality in most of the studies; however, there is no adjustment by multivariate analysis for appropriate antibiotic treatment, combined therapy and removal of the source of infection, making the analysis for risk factors and/or mortality associated with CRE/CPE difficult.

Finally, the characteristics of the patient population included in the multivariate models of risk factors for mortality could be divided into two categories: (a) those whose cohort included all patients diagnosed with CPE infections, regardless of whether they received active antimicrobial treatment for a small period of time, and (b) those that included only patients with CPE infections who were treated for at least 48 hours with an effective antibiotic treatment (defined as one or more active drugs due to *in vitro* sensitivity against CPE/CRE strains). This important difference of cohorts should be considered, especially in the group of patients who did not receive enough treatment time for a definitive adequate therapy for CPE/CRE-infection, as may represent a bias when analyzing the impact of different treatments on mortality rates.

Evidence for combined therapy

Despite the limitations of design in the studies, some studies have shown lower mortality rates using combinations of two or even three antimicrobial agents. In absence of the ideal study, those with the greatest clinical impact will be reviewed.

In a study by Tumbarello et al., the mortality of patients with bacteremia caused by KPC-producing *K. pneumoniae* who received monotherapy was 54% compared to combined therapy with a mortality rate of 34% at 30 days in 125 patients. Interestingly, patients who received a combination of tigecycline plus colistin and meropenem showed an independent association with higher survival rates at 30 days (Odds Ratio [OR] 0.11)[37]. Similarly, Daikos et al. analyzed the clinical outcomes of 205 patients with bacteremia caused by *K. pneumoniae* producing KPC or VIM, demonstrating an independent association with higher survival rates in patients who received a combined antimicrobial regimen. Combination therapy had the greatest effect on survival in patients with severe sepsis, septic shock, and rapidly fatal underlying diseases[38]. Qureshi et al. also evaluated the results of 41 patients with bacteremia caused by KPC-producing *K. pneumoniae*, finding that combined therapy was independently associated with survival (OR 0.07), and a significantly lower 28-day mortality rate (13.3%) in comparison with monotherapy (57.8%)[39]. In a studied executed by Falcone et al. which involved 141 patients in the ICU with KPC-producing *K. pneumoniae* infections and septic shock, treatment with colistin (Hazard ratio [HR] 0.21) and the use of ≥ 2 active *in vitro* antibiotics as definitive therapy, was associated with favorable results (HR 0.08) in terms of mortality rates[40]. And finally, a study by Trecarichi et al. in 149 patients with hematologic malignancies and *K. pneumoniae* bacteremia who had received ≥48 hours of adequate antibiotic therapy, demonstrated that combined therapy was independently associated with higher survival (HR 0.32)[41].

Nevertheless, there are several publications, although in a lesser amount, which have not found a survival advantage by giving combination therapy. Some of these published studies are:

Gomez-Simmonds et al. evaluated the clinical outcomes of 141 patients with bacteremia caused by carbapenem resistant *K. pneumoniae* according to the number of active *in vitro* agents

received and whether an extended-spectrum β-lactam antibiotic had been administered previously. There was no association between the individual treatment characteristics and clinical outcomes, regardless of the use of monotherapy or combined therapy or a β-lactam. Interestingly, different schemes of meropenem were used: 500 mg every 6 hours, 2 g every 8 hours, 2 g in extended infusion over 3 hours every 8 hours, but no stratification was done for each of the treatment schemes[42]. Likewise, no association was found between combined therapy and survival in their cohort of 118 patients with KPC-producing *Enterobacteriaceae* infections (of which 78 were bacteremia) by De Oliveira et al. The use of polymyxins in this study was an independent predictor of mortality. However, one of the biases of this study was that during the first 10 days of treatment for KPC-producing *Enterobacteriaceae*, 47 patients (40%) had 51 infections due to other etiological agents such as other bacteria (77%), fungi (21%), or virus (2%)[43].

Regarding therapy and the different antibiotic options, there is more evidence for KPC producing *Enterobacteriaceae* than for other bacteria, enzyme types or therapies. The antibiotics with the best evidence will be discussed below.

Evidence for carbapenems in CPE infections
Some studies showed a benefit of meropenem in combined therapy by an association of lower mortality rates; therefore, carbapenems have been one of the most important pillars of combination therapy in CPE. In the following section, we will discuss the literature that has evidence in favor of carbapenems and their association with the minimal inhibitory concentration (MIC) of the antibiotic.

In an article published in 2011, Daikos and Markogiannakis reviewed the clinical data of the treatment and the results of patients infected with carbapenemase producing *K. pneumoniae*, reporting 44 patients who had received carbapenems as monotherapy. The analysis of the clinical result according to the MIC of carbapenem showed a survival rate of 29% with a MIC of > 8 mg/L, 60% for a MIC of 8 mg/L, and 69% for a MIC of 4 mg/L or less. Notably, the mortality rates in the group of a MIC of 4 mg/L or less are similar to those reported for *K. pneumoniae* infections that do not produce carbapenemase, or even CPE infections that have received appropriate combined therapy with a carbapenem. The authors concluded that carbapenems could be a therapeutic option for CPE when the MIC is ≤ 4 mg/L if it is administered at high doses and prolonged infusion and in combination with another active antibiotic[44].

Other studies have also investigated the correlation between treatment with a carbapenem, its MIC and the clinical outcomes. In a study by Tumbarello et al., which included 36 patients with bacteremia caused by carbapenemase-producing *K. pneumoniae*, treated with a combined therapy with meropenem, the patients were stratified according to the MIC: in *K. pneumoniae* with a meropenem MIC of ≤ 8 mg/L, all-cause mortality at 30 days was 15.8%, vs 35.2% for patients with strains with a MIC of ≥ 16 mg/L[37].

A mortality of 19.3% was observed by Daikos et al. in patients with carbapenem-resistant *K. pneumoniae* bacteremia treated with a combination that included a carbapenem, when the MIC for carbapenems was ≤ 8 mg/L, in contrast to an increase in mortality of 35.5% in patients who received a carbapenem in combination therapy when the MIC was > 8 mg/L for carbapenems[38]. A similar effect was observed by Tumbarello et al., describing lower mortality rates at 14 days in patients who received combined therapy that included a carbapenem and its MIC was ≤ 8 mg/L[14]. In all these studies, carbapenems were administered at high doses[14,37,38] and in the studies by Tumbarello et al. prolonged infusion was included[14,37].

Evidence for polymyxins in CPE infections
In January 2019, the first International Consensus Guidelines for the Optimal Use of the Polymyxins was published (45). In this guideline, the question about the treatment of CPE infections using polymyxins in monotherapy or combination therapy is addressed. However, no consensus was obtained due to the heterogeneity in the methodology of the published studies. The authors decided to issue recommendations based on voting, therefore, the level of evidence is low. The recommendations based on the previous methodology are:

- Polymyxins should be used in combination with a second antibiotic with *in vitro* activity against the bacteria
- A second or third agent (with the lowest MIC) should be added to polymyxins if the bacteria is not susceptible to other antibiotics.

Ceftazidime/avibactam as a new therapeutic option
Ceftazidime/avibactam is the combination of the third-generation cephalosporin ceftazidime with a novel β-lactamase inhibitor called avibactam. The initial studies and the clinical conditions for which the drug was approved by the FDA were urinary tract infections, complicated intra-abdominal infections and ventilator-associated pneumonia. However, due to its potent *in vitro* activity against CPE, especially KPC producing *Enterobacteriaceae*, and, partial activity against enzymes of the OXA-48 family, in addition to its high stability against other enzymes such as ESBL and AmpC, this antibiotic presents an excellent profile as a therapeutic option for infections caused by KPC producing *Enterobacteriaceae*. It is important to highlight that this antimicrobial agent has no activity against metallo-enzymes[46].

Shields et al. reported a series of cases, including 37 patients with CRE treated with ceftazidime/avibactam, observing a 76% survival (28 out of 37 of patients) and a clinical cure of 59% (22 out of 37 patients), although this rate did not reach statistical significance in patients who received monotherapy (58% [15/26]) versus combination therapy (64% [7/11]). However, there was a recurrence of CRE-infections in 23% between patients with initial clinical success. Microbiological

failure was diagnosed in ten of 37 patients (27%) and in three of the ten patients (30%) resistance to ceftazidime/avibactam (MIC > 8 mg/L) was detected[47].

In a study conducted by Temkin et al. that involved 38 patients with infections caused by CRE and in some cases *Pseudomonas aeruginosa*, 65.8% of the participants received a second antimicrobial agent with *in vitro* non-resistance of the microorganism. Of these patients, 73.7% had clinical and/or microbiological cure[48]. Carmeli et al. published a study in 16 countries including patients with complicated urinary tract and intra-abdominal infections caused by *Enterobacteriaceae* or *P. aeruginosa* resistant to ceftazidime. Patients randomly received ceftazidime/avibactam or the best available treatment chosen by the physician. The results were comparable, with 91% success in both arms[49].

Finally, van Duin et al. compared the efficacy of ceftazidime/avibactam versus colistin against CRE, finding that in the adjusted analysis for greater probability of response, patients treated with ceftazidime/avibactam had a lower mortality rate (9%) and a 64% greater probability of better clinical results than those treated with colistin (with a mortality rate of 32%)[50]. On the other hand, some of the concerns are the likelihood of selection of resistance with KPC-3 due to lower affinity to avibactam[51], which is why, in severe infections, the possibility of combination therapy is raised, which still needs to be studied.
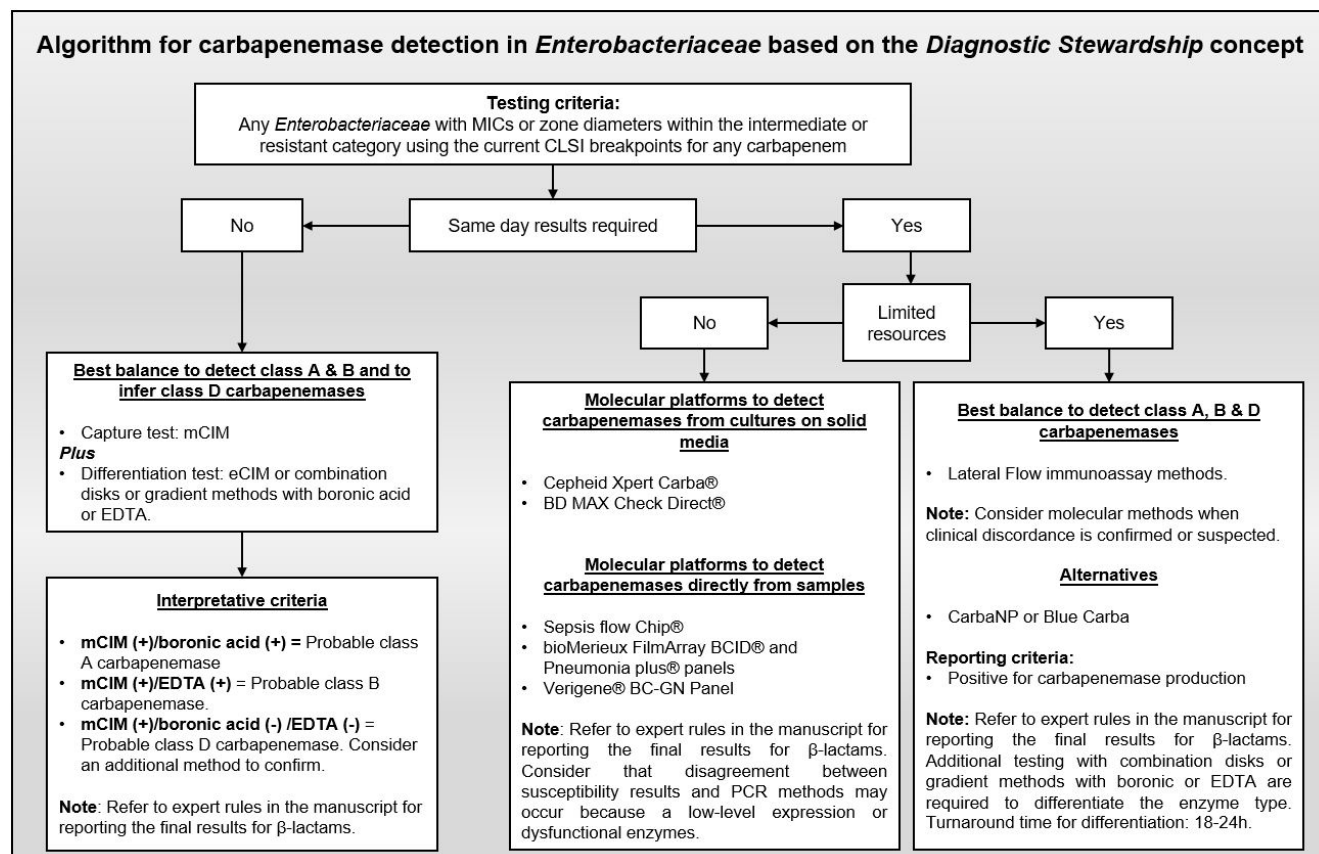
Conclusions

In recent years, carbapenem resistance in *Enterobacteriaceae* has increased dramatically and represents an important threat to global health. The optimal clinical management of CPE infections has not been established because clinical trials have not been performed with this objective. We aimed to summarize in the present review data provided by previous observational clinical studies that have investigated the impact of different treatment strategies and their outcomes. Most of these studies reported that combination therapy with two or more *in vitro* active agents is superior to monotherapy providing a survival benefit. The role of carbapenems in treatment of CPE infections is widely debated; however, the use of carbapenems in association with other active drugs is probably more effective for CPE isolates with carbapenem MICs ≤ 8 mg/L. Ceftazidime/avibactam already launched in other countries has shown clinical efficacy against KPC and some OXA-type carbapenemases. However, its wise use may help limit the selection of resistance.

Disclosure

Algorithm for carbapenemase detection in *Enterobacteriaceae* based on the *Diagnostic Stewardship* concept

Testing criteria:
Any *Enterobacteriaceae* with MICs or zone diameters within the intermediate or resistant category using the current CLSI breakpoints for any carbapenem

Same day results required — No / Yes

No — Limited resources — Yes

Best balance to detect class A & B and to infer class D carbapenemases

• Capture test: mCIM
Plus
• Differentiation test: eCIM or combination disks or gradient methods with boronic acid or EDTA.

Interpretative criteria

• **mCIM (+)/boronic acid (+) =** Probable class A carbapenemase
• **mCIM (+)/EDTA (+)** = Probable class B carbapenemase.
• **mCIM (+)/boronic acid (-) /EDTA (-)** = Probable class D carbapenemase. Consider an additional method to confirm.

Note: Refer to expert rules in the manuscript for reporting the final results for β-lactams.

Molecular platforms to detect carbapenemases from cultures on solid media

• Cepheid Xpert Carba®
• BD MAX Check Direct®

Molecular platforms to detect carbapenemases directly from samples

• Sepsis flow Chip®
• bioMerieux FilmArray BCID® and Pneumonia plus® panels
• Verigene® BC-GN Panel

Note: Refer to expert rules in the manuscript for reporting the final results for β-lactams. Consider that disagreement between susceptibility results and PCR methods may occur because a low-level expression or dysfunctional enzymes.

Best balance to detect class A, B & D carbapenemases

• Lateral Flow immunoassay methods.

Note: Consider molecular methods when clinical discordance is confirmed or suspected.

Alternatives

• CarbaNP or Blue Carba

Reporting criteria:
• Positive for carbapenemase production

Note: Refer to expert rules in the manuscript for reporting the final results for β-lactams. Additional testing with combination disks or gradient methods with boronic or EDTA are required to differentiate the enzyme type. Turnaround time for differentiation: 18-24h.

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