Review

Detection, treatment, and prevention of carbapenemase-producing *Enterobacteriaceae*: Recommendations from and International Working Group

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The prevalence of carbapenemase-producing *Enterobacteriaceae* (CPE) has increased during the past 10 years. Its detection is frequently difficult, because they do not always show a minimum inhibitory concentration (MIC) value for carbapenems in the resistance range. Both broth microdilution and agar dilution methods are more sensitive than disk diffusion method, Etest and automated systems. Studies on antimicrobial treatment are based on a limited number of patients; therefore, the optimal treatment is not well established. Combination therapy with two active drugs appears to be more effective than monotherapy. Combination of a carbapenem with another active agent — preferentially an aminoglycoside or colistin — could lower mortality provided that the MIC is ≤4 mg/l and probably ≤8 mg/l, and is administered in a higher-dose/prolonged-infusion regimen. An aggressive infection control and prevention strategy is recommended, including reinforcement of hand hygiene, using contact precautions and early detection of CPE through use of targeted surveillance.

Keywords: Carbapenemase producing *Enterobacteriaceae*, Carbapenemases, Detection, *Klebsiella pneumoniae*, Multiple drug resistance, Colistin, Infection control, Treatment

Introduction

Carbapenems (e.g. ertapenem, imipenem, meropenem, and doripenem) are often the antimicrobials of last resort to treat infections due to extended-spectrum beta-lactamase (ESBL) or plasmid-mediated AmpC (pAmpC)-producing organisms of the *Enterobacteriaceae* family. These pathogens are frequently also resistant to other antibiotic classes including quinolones, aminoglycosides, trimethoprim–sulfamethoxazole, and other classes.[1–3] Carbapenems are crucial for the management of life-threatening healthcare-associated infections.

Unfortunately, the prevalence of carbapenemase-producing *Enterobacteriaceae* (CPE) has increased during the past 10 years, seriously compromising the therapeutic armamentarium.[4–6] This increasing prevalence of CPE poses a challenge in the treatment of healthcare-associated infections. To ensure their containment, wide dissemination of information and

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robust multifaceted strategies involving microbiologists, clinicians, and decision makers are essential.

The aim of these International Working Group recommendations is to briefly summarize the main current issues and provide practical recommendations on detection, treatment and prevention of CPE in different resources settings. It is not the aim of this paper to replace previous published guidance, but instead to complement it.

Methodology

These recommendations were developed by an International Working Group of clinical microbiologists, infectious disease, infection control, and public-health specialists from seven organizations and scientific societies worldwide, based on their experience in epidemiological, microbiological, and/or therapeutic aspects of infections caused by multidrug-resistant (MDR) *Enterobacteriaceae*. The experts of the International Working Group belong to Argentinean Society of Infectious Diseases (SADI), International Society of Chemotherapy (ISC) Antimicrobial Resistance Working Group, Pan American Association of Infectious Diseases (API), Pan American Health Organization/World Health Organization (PAHO/WHO), Infection Control African Network (ICAN), Mediterranean Society of Chemotherapy (MSC), and Federation of European Societies for Chemotherapy and for Infections (FESCI). The methodology used consisted of reviewing the papers identified through MEDLINE, EMBASE, LILACS, Cochrane Library, and different websites (e.g. Google and Medscape). Furthermore, a review of the references of the most relevant publications that would identify other valuable studies was performed. Important studies included prospective cohort studies, case–control studies, and other descriptive studies. In addition, recommendations made by the US Centers for Diseases Control (CDC, USA) and the European Center for Disease Prevention and Control (ECDC) on this overall topic were carefully reviewed.

Owing to the lack of randomized controlled trials for the treatment of CPE infections, many of the therapeutic recommendations are based on discussion and analysis of the evidence from each of the articles, and the experience of the authors included in the present recommendations.

The work was initially developed electronically between March and May 2012. On 19 May, a face-to-face meeting of some authors was held in Córdoba (Argentina), during the XII Argentine Congress on Infectious Diseases SADI 2012. The final suggestions, review, and full acceptance were completed in November 2012.

Classification of Carbapenemases

Carbapenemase enzymes are encoded by *bla* genes carried on mobile elements (e.g. plasmids and/or integrons) that facilitate their horizontal spread among different Gram-negative species.[7–10] Beta-lactamase enzymes with hydrolytic activity against carbapenems have been identified in each of the four Ambler molecular classes, though those of class A, B, and D have major epidemiological impact.

A variety of Class A carbapenemases have been described; some are chromosome-encoded (e.g. NmcA, SME, IMI-1) and others are plasmid-encoded (e.g. KPC-types, IMI-2, GES-types).[11] KPC-types are the most clinically common enzymes in this group. These carbapenemases are most often carried and expressed by *K. pneumoniae* isolates, but are no longer confined to this organism. In fact, they have been found in *Escherichia coli*, *Klebsiella oxytoca, Salmonella enterica*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Proteus mirabilis*, *Serratia marcescens*, as well as in non-fermenting Gram-negative bacilli like *Pseudomonas aeruginosa*, *Pseudomonas putida*, and *Acinetobacter* spp.[4,12] For KPC producers, the level of resistance to carbapenems may vary markedly, ertapenem being the drug with lowest antimicrobial activity (thus, the highest minimum inhibitory concentrations, MICs). KPC enzymes are generally broadly active against all beta-lactams despite the fact that organisms containing them may test susceptible to some carbapenems other than ertapenem when standard antimicrobial susceptibility tests (ASTs) are implemented (see the section on 'Detection of carbapenemase producers').[13,14] In general, the different MIC levels for imipenem and meropenem among KPC-producing *Enterobacteriaceae* can vary from 1 to >64 mg/l. For instance, for *K. pneumoniae* isolates, several factors should be considered: (1) expression level of the KPC enzyme due to a different asset of the promoter region;[15] (2) co-expression of broad-spectrum and ESBLs (e.g. SHV-11, SHV-12, CTX-M-15);[16] and (3) porin loss (especially OmpK35 and OmpK36 in *K. pneumoniae*).[17]

Class B MBLs are mostly of VIM- and IMP-types, but the recently emerged NDM-type is becoming the most threatening carbapenemase.[18] MBL enzymes are found worldwide and like the KPCs have spread rapidly (especially NDM-1), presenting a serious threat because of their prolific dissemination and their ability to hydrolyze all beta-lactams, with the exception of aztreonam (if no ESBLs and/or AmpCs are co-produced by the isolates). Most MBL producers are hospital-acquired and MDR *K. pneumoniae*, but also include *Pseudomonas* spp. and *Acinetobacter* spp.

Described in 2008 — and retrospectively found in isolates collected in 2006[19–21] — the NDM-producing *Enterobacteriaceae* are now the focus of worldwide attention because of (1) high-level carbapenem resistance (e.g. MICs for imipenem and meropenem $\geq$32 mg/l) is usually observed in the isolates and (2) their rapid global spread, some of which has been

facilitated by extensive international travels. Plasmids carrying the bla_{NDM-1} gene are diverse and can harbour a large number of resistance genes associated with other carbapenemase genes (e.g. OXA-48, VIM-types), plasmid-mediated AmpC cephalosporinase genes (e.g. CMY-types), ESBL genes (e.g. CTX-M-types), aminoglycoside resistance genes (16S RNA methylases), macrolide resistance genes (esterase), rifampin (rifampin-modifying enzymes), and sulfamethoxazole resistance genes. These plasmids are frequently acquired by *K. pneumoniae* isolates, but also by *E. coli* and — surprisingly — by many environmental Gram-negatives.[21–23]

Class D enzymes are mainly represented by OXA-48-like producers (e.g. OXA-48, OXA-162, and OXA-181). Since 2003, these genes have been extensively reported from Turkey as a cause of healthcare-associated outbreaks, and then distributed to Europe, southern and eastern part of the Mediterranean region, and Africa. The rapid spread of *Enterobacteriaceae*-producing the OXA-48 carbapenemase (mainly *E. coli*) linked to the dissemination of a single self-transferable plasmid represents another mode of resistance in healthcare-associated Gram-negative bacilli. Since many of these strains do not exhibit resistance to broad-spectrum cephalosporins, and only decreased susceptibility to carbapenems, their recognition and detection represents a serious challenge.[24] In particular, the clinical microbiologist should be aware that *Enterobacteriaceae* (mainly *E. coli* producing only OXA-48-like enzymes and not co-possessing ESBLs) show: (1) MIC values for imipenem and meropenem of only 0.25–1 mg/l and (2) MIC values for extended-spectrum cephalosporins in the susceptible range.[25]

Detection of Carbapenemase Producers

Detection of CPE is frequently difficult. In fact, these isolates do not always show a MIC value for carbapenems that is in the resistance range and therefore, might go unnoticed for long periods during which, in the absence of good infection prevention and control practices, spread may occur. The detection of carbapenemase producers is based first on AST results obtained by diffusion methods, or by automated systems (e.g. Phoenix, Vitek, Microscan). However, it is important to underline that reference MIC determination methods — such as broth microdilution and agar dilution — are more sensitive than either the disk diffusion, the Etest (bioMerieux) or automated systems.[13,14] In low-income countries, where detection and classification of CPE is difficult to attain, a simplified version for testing and identifying CPE should be considered. Quality-controlled disk diffusion may be used to screen the isolates and those strongly suspicious for carbapenemase production should be sent to national reference laboratories. Then, provisions for confirmation of the presence of CPE should be available in reference laboratories in these countries.

The current (2012) carbapenem breakpoints from Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) are shown in Table 1.

Susceptibility to ertapenem by disk diffusion has been found to be the most sensitive indicator of carbapenemase production, but when dilution tests are performed, the MICs of imipenem, meropenem, or doripenem are also useful to detect carbapenemase producers. In particular, MICs of ≥ 0.5 mg/l for ertapenem and ≥ 1 mg/l for imipenem and meropenem are an alert to screen suspicious isolates with more adequate phenotypic and molecular tests. With regard to the implementation of ertapenem as indicator of carbapenemase production — as mentioned above — one should be aware that *Enterobacteriaceae* resistant to ertapenem — but susceptible to imipenem and meropenem — could be due to porin loss associated with ESBL or pAmpC production.[26–27]

The modified Hodge test (MHT) is a generic phenotypic test that can be useful to demonstrate the production of carbapenemase enzymes. Multiple isolates (up to eight) can be tested on a single Mueller–Hinton agar plate. However, it is time-consuming and may lack of specificity (e.g. false-positive strains when ESBL or pAmpC are associated to porin loss) and sensitivity (e.g. weak detection of NDM and VIM producers).[4,28–30] Nonetheless, in

Table 1 Current Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria for interpretation of susceptibility testing of carbapenems in *Enterobacteriaceae*

		MIC (mg/l)			Disk diffusion (mm)		
Carbapenem	Criteria[a]	S	I	R	S	I	R
Imipenem	CLSI-2012	≤1	2	≥4	≥23	20–22	≤19
	EUCAST-2012	≤2	4–8	≥16	≥22	16–21	≤15
Meropenem	CLSI-2012	≤1	2	≥4	≥23	20–22	≤19
	EUCAST-2012	≤2	4–8	≥16	≥22	16–21	≤15
Ertapenem	CLSI-2012	≤0.5	1	≥2	≥22	19–21	≤18
	EUCAST-2012	≤0.5	1	≥2	≥25	22–24	≤21
Doripenem	CLSI-2012	≤1	2	≥4	≥23	20–22	≤19
	EUCAST-2012	≤1	2–4	≥8	≥24	18–23	≤17

Note: S, susceptible; I, intermediate; R, resistant.
[a] CLSI document M100, S22 2012; EUCAST document 2.0–2012.

low-income countries, this may be the only available tool for detecting CPE and should be considered as an initial step in the absence of more sophisticated methods.

Boronic acid-based inhibition testing is reported to be sensitive and specific for KPC detection in *K. pneumoniae* when performed with imipenem, meropenem, and cefepime but not with ertapenem, if corresponding isolates co-produce a pAmpC beta-lactamase.[31,32]

Inhibition by EDTA or dipicolinic acid may be used for the detection of MBL activity.[33,34] The Etest MBL strips with meropenem and imipenem plus their specific inhibitors are also useful for detecting MBL producers on the basis of inhibition of MBL activity by EDTA. No validated inhibition tests are available for detection of OXA-48-like carbapenemase producers so far. However, the MHT should retain the ability to detect them.[24]

Currently, there is no screening medium able to detect all types of carbapenemase producers with high sensitivity and high specificity. Agar plates containing imipenem at a concentration of 1 mg/l have been proposed for screening only KPC producers. The chromogenic medium CHROMagar KPC, has been shown to have a sensitivity of 100% and specificity of 98.4% relative to polymerase chain reaction (PCR).[35] However, this selective agar is unable to detect OXA-48-like carbapenemase producers because of the low MICs for imipenem. Recently, a new selective agar plate (i.e. SuperCarba) has shown excellent ability to detect all classes of carbapenemase producers.[36]

The gold standard for identification of carbapenemases is based on the use of molecular techniques — usually PCR-based systems — which may be mainly of epidemiological interest. Several in-house real-time PCRs have been designed and some of them are also commercially available (e.g. Hyplex, CheckPoints).[37,38] However, the main disadvantages of molecular-based technologies for detection of carbapenemases are their cost, the requirement of trained personnel, and inability to detect any novel carbapenemase gene. Often these methods are beyond the scope of less well financed laboratory systems. Thus, there is an urgent need for inexpensive, rapid, sensitive, and specific tests for detection of carbapenemase activity. In this context, microarray technology (e.g. CheckPoints platforms) seems the most versatile method that can be routinely implemented to detect all classes of carbapenemases with high sensitivity and specificity.[39–41]

In Table 2, our recommendations for the identification of CPE are summarized.

Predisposing Factors and Related Infections

As is the case for infections due to other MDR Gram-negatives (e.g. ESBL producers), risk factors for infection include advanced age, severity of the underlying illness, ICU stay, previous antibiotic exposure, invasive devices, organ or stem-cell transplantation, mechanical ventilation, and prolonged hospital stays.[42–45]

Clinical infections are usually healthcare associated and are — in most cases — bacteremia, ventilator-associated pneumonia, urinary tract, and surgical site infections. Infections produced by CPE — mainly *K. pneumoniae* — have been associated with increased cost and length of stay, treatment failures and increased mortality. Overall, the attributable mortality is about 30–50%.[8,46,47]

Antimicrobial Treatment

Experience on antimicrobial treatment of CPE infections and clinical outcomes are based on a limited number of patients, coming from low- to medium-grade evidence studies, and therefore, the optimal treatment is not well established. It is pivotal to stress that for the selection of the antimicrobial agents, the results of the susceptibility tests and location of the infection must be considered for the individual treatment decisions. Also, it is important to remind that patients who are only colonized — but not clinically infected- should not be treated with antimicrobial agents. Professionals taking care of CPE infected patients must be aware that the following recommendations should always be adapted to local epidemiology and patterns of resistance. Considering the dynamic evolution of resistance, in no way these recommendations should be taken as definitive.

Polymyxins

In vitro susceptibility to polymyxins (i.e. colistin and polymyxin B) among clinical CPE isolates ranges globally from 80 to 100%. However, in some areas,

Table 2 Identification of CPE: summary of recommendations

- The detection of carbapenemase producers can be based on the AST results but with careful attention on the MICs or inhibition diameters for carbapenems. Reference MIC methods are more sensitive than disk diffusion, Etest and automated systems, so they should be used if possible.
- Carbapenem breakpoints are frequently modified, so clinical microbiologists and clinicians should keep them updated.
- Susceptibility to ertapenem can be used for the initial screening of carbapenemase production but then more appropriate phenotypic (e.g. MHT) and molecular methods (e.g. PCR-based or microarray) should be implemented when possible to confirm the presence of carbapenemase genes.
- In low-income countries, and for those laboratories without reference MIC methods, in cases where a CPE is suspected, MHT can be initially used, and then confirmed by a reference laboratory that implement molecular methods.
- This reference laboratory should ideally be always available in low incoming countries.

resistance can be very high due to the clonal spread of resistant strains.[48–50] Colistin is more widely used than polymyxin B. It exhibits a concentration-dependent bactericidal killing, so that the area under the curve (AUC)/MIC ratio is the most predictive pharmacokinetic (PK)/pharmacodynamic (PD) parameter of therapeutic success.[51,52] Colistin is often the only agent active against CPE which achieves adequate serum levels to treat bloodstream infections (BSIs).[42] In the past, polymyxins were used infrequently, largely due to their associated nephro- and neuro-toxicity. However, the emergence of MDR and extreme drug-resistant pathogens led to renewed interest and a significantly increase in its use. Subsequently, various studies have improved the knowledge of PK and PD of colistin demonstrating that it seems to be efficacious and relatively safe.[53] Nephrotoxicity associated with colistin is seen in about 10–15% and — in most cases — is transient and probably related to dosage and duration of treatment.[54–56]

Unfortunately, the most appropriate dosing regimen of colistin to maximize clinical effectiveness has not been well defined, and many studies showed that usual doses [i.e, 3 MU colistin methanesulphonate (CMS) every 8 hours] reaches suboptimal concentrations.[53,57–59] Indeed, current dosing schemes of colistin do not attain serum concentrations that would be sufficient for the treatment of infections caused by pathogens with MICs higher than 0.5 mg/l. In a retrospective study that evaluated patients with infections due to MDR Gram-negatives who received several daily dosages of colistin, multivariate analysis of survival data showed that lower total daily dosage of intravenous colistin was associated with increased mortality.[60,61] Newer PK/PD studies suggest that loading doses might be useful to rapidly achieve active concentrations at the site of infection.[53,55,62,63]

To avoid dosage confusion, clinicians should be aware that 1 mg of colistin base activity is contained in 2.4 mg CMS that is equivalent to 30 000 IU of CMS. Therefore, to better understand the common published regimens, 100 mg of colistin sulphate base is equivalent to 240 mg of CMS and to 3 MU CMS. CMS is a non-active pro-drug of colistin.

Recent data from a PK analysis of critically ill patients showed that to obtain a colistin steady-state plasma concentration of 2.5 mg/l, a 70-kg patient with a creatinine clearance rate of 80 ml/min needs to receive a CMS loading dose of 10 MU, followed by a maintenance CMS daily dose of 10 MU.[63]

Recent studies showed that a loading dose of 6–9 MU, followed by maintenance doses of 4.5–6 MU every 12 hours — always adjusting to renal function — could be more effective than previous prescribed regimens of 3 MU every 8 hours. With this change in dosage, nephrotoxicity was not significantly increased. Titration of dose on the basis of renal function by prolonging dosing interval, instead of by reducing the single dose (according to colistin's concentration-dependent pharmacodynamic behavior), may contribute to the low rate and moderate severity of renal damage.[56]

Proteus spp. and *Serratia* spp. are naturally resistant to colistin. Colistin resistance might develop more frequently in carbapenem-resistant *K. pneumoniae* than in MDR *A. baumannii* or *P. aeruginosa*.[64,65] Increased use of this agent is associated with the emergence of heteroresistant isolates,[66] due to alteration of the membrane lipopolysaccharide structure. The development of resistance during therapy may be related to the presence of heteroresistant subpopulations. This phenomenon was observed in 15 out of 16 MDR *K. pneumoniae* isolates considered susceptible by MIC testing, a result consistent with the very high mutant prevention concentration observed.[67]

Tigecycline

Tigecycline is a glycylcycline — a bacteriostatic agent — that has a good susceptibility profile *in vitro*. Several studies reported delayed clearance of the organism, recurrence of pathogens, and the need for prolonged administration to achieve favourable outcomes. Tigecycline is a time-dependent active drug; therefore, it is important to prolong the maximum exposure time to maintain serum levels over the MIC; the suitable PK/PD parameter is the AUC/MIC ratio.[68] Recently, a clear PK–PD relationship for $fAUC_{0-24}$/MIC ratio and clinical and microbiological responses has been demonstrated.[69]

Owing to its PK/PD profile, tigecycline is not recommended for treatment of bacteremia, respiratory or other serious infections. The peak serum concentrations achieved with the standard dosing regimen of the drug (50 mg twice daily) range from 0.6 to 0.9 mg/l, while those attained in the urine and in the epithelial lining fluid are substantially lower.[70,71] Considering also the tigecycline's MIC distribution ranging between 1 and 2 mg/l for the majority of contemporary KPC-producing *K. pneumoniae* isolates, the poor therapeutic efficacy of the drug in serious infections can be explained.

Trials with higher dosing schedules are eagerly awaited. *Enterobacteriaceae* with resistance to this drug — caused by point gene mutations — have been reported among clinical isolates.[72,73] An alert by the US Food and Drug Administration[74] advocated for the use of alternative drugs to tigecycline in the case of severe infections. This suggestion stemmed from a pooled analysis of data from comparative trials for different indications, which showed increased overall mortality with tigecycline treatment. However, a recent large prospective non-interventional study of

over 1000 patients — mainly with complicated intra-abdominal infections or complicated skin and skin tissue infections — resulted in no excessive mortality associated with tigecycline. In this study, tigecycline achieved favourable clinical success rates in a population of patients seriously ill and with a high prevalence of MDR pathogens, showing also a good safety and tolerability profile.[75]

Aminoglycosides

Aminoglycoside resistance is increasing among CPE. In susceptible strains, *in vitro* data have shown rapid bactericidal activity of gentamicin against gentamicin-susceptible strains.[76] Other lineages may carry modifying enzymes for gentamicin and other aminoglycosides — namely, amikacin and tobramycin — which have been shown to be less effective against infections due to MDR *K pneumoniae*. When infecting organisms are aminoglycoside susceptible, they are a useful therapeutic option. Published data regarding the use of aminoglycosides as monotherapy against carbapenemase-producing *K. pneumoniae* infections are scarce, and therefore cannot be recommended.

Fosfomycin

Fosfomycin is a naturally occurring phosphonic acid derivative that inhibits cell wall biosynthesis at an earlier stage than beta-lactam antibiotics. This drug displays *in vitro* activity against ESBL-producing *Enterobacteriaceae* (including carbapenem-resistant *K. pneumoniae*).[77] The activity of fosfomycin was evaluated against 68 KPC-producing *K. pneumoniae* isolates, 23 of which were non-susceptible to tigecycline and/or colistin. The susceptibility rates were 93% for the overall group, 87% for the group non-susceptible to tigecycline and/or colistin, and 83% (five out of six isolates) for the extremely drug resistant (i.e. non-susceptible to both tigecycline and colistin) subgroup.[78] Michalopoulos *et al.*, using 2–4 g four times daily fosfomycin in combination with colistin (six cases), gentamicin (three cases), or piperacillin/tazobactam (one case), obtained a promising clinical success rate (100%) in the treatment of serious infections caused by carbapenem-resistant *K. pneumoniae*.[79]

The main consideration regarding the use of fosfomycin as a last resort option for the treatment of CPE infections lies in the potential for emergence of resistance during therapy.[80] Additional data are required to determine the benefit from the administration of fosfomycin as an adjunct to other active agents in the treatment of infections caused by CPE.

Combination therapy for CPE

Polymyxins are commonly used in combination with other antimicrobials, although prospective data to evaluate the efficacy of this approach are not available. Combination therapy may be helpful in preventing bacterial resistance.[42] In terms of outcomes, cumulative experience supports the use of combination therapy in patients with CPE infections.

Qureshi *et al.*,[81] in a retrospective analysis of 41 patients with bacteremia due to KPC-producing *K. pneumoniae*, found that combination therapy was independently associated with survival. The 28-day mortality was 13.3% in the combination therapy group compared with 57.8% in the monotherapy group ($P=0.01$). The most commonly used combinations were colistin, polymyxin B, or tigecycline combined with a carbapenem. Of note, despite *in vitro* susceptibility, patients who received monotherapy with colistin, polymyxin B or tigecycline had a higher mortality of 66.7% (8/12).

Hirsch *et al.*[12] reviewed 15 studies/reports containing 55 unique patient cases (57 treatment courses). Treatment with aminoglycosides (6/8 patients, 75%), polymyxin combinations (8/11, 73%), and tigecycline (5/7, 71%) appeared to have higher success rates compared to carbapenem (6/15, 40%) and polymyxin (1/7, 14%) monotherapy. The absolute numbers of treated patients were too small for any conclusion to be drawn. Another limitation was that many of the papers were single case reports or small series where precise definitions (e.g. infection versus colonization, success versus failure) were not clear.

Daikos *et al.*[82] performed a prospective observational study to evaluate the importance of VIM production on outcome of patients with *K. pneumoniae* BSIs. The lowest mortality (8.3%) was observed in the group of patients who received combination therapy with two active drugs, one of which was a carbapenem and the other either colistin or an active aminoglycoside, whereas therapy with one active drug resulted in mortality rate of 27% (10/37 patients died) similar to that observed in patients who received inappropriate therapy (28.6%; 4/14 patients died).

Zarkotou *et al.*[83] reviewed outcomes of 53 patients who experienced BSIs caused by KPC-producing *K. pneumoniae.* Appropriate antimicrobial therapy (at least one active drug) was administered in 35 patients. The 20 patients who received combination schemes had favourable infection outcomes, whereas seven of 15 patients given one active drug died ($P=0.001$).

Tzouvelekis *et al.*[84] recently performed a systematic search to evaluate the efficacy of different antimicrobial regimens in the treatment of infections caused by carbapenemase-producing *K. pneumoniae*. A total of 298 patients were identified, 158 infected with KPC- and 140 with MBL-producing *K. pneumoniae*. The vast majority of these patients had serious infections; 244 had BSIs, and 32 pneumonia. One hundred and forty-three patients received monotherapy (only one drug was active *in vitro* against the infecting organism),

99 received combination therapy (at least two drugs were active *in vitro*), and the remaining 56 received 'inappropriate therapy' (no drug was active *in vitro*). Carbapenem susceptibility status was taken as reported in relevant studies in which the previous CLSI interpretive criteria were applied. Overall, combination therapy was superior to monotherapy. By dividing the patients who received combination therapy into two groups on the basis of inclusion of a carbapenem in the treatment scheme, the lowest failure rate (8.3%) was observed in the group who received carbapenem-containing regimens. Monotherapy with an aminoglycoside or a carbapenem was more effective as compared to 'inappropriate therapy', whereas treatment with tigecycline or colistin as single active agents resulted in failure rates (35.7% and 47.2%, respectively) comparable to that observed for patients who received inappropriate therapy (45%). Combinations of carbapenem with colisitin (5.5% of failures) or with an aminoglycoside (6.2%) performed significantly better than when these drugs were used alone or as part of other combinations. On the other hand, combinations of tigecycline (24% of failures), colistin (32%), and aminoglycosides (33.3%) in regimes not including a carbapenem exhibited higher failure rates.

In a recent published multicenter retrospective cohort study, conducted in three Italian hospitals, Tumbarello *et al.*[85] examined 125 patients with BSIs caused by KPC-producing producing *K. pneumoniae*. The overall 30-day mortality rate was 41.6%. A significantly mortality rate was observed among patients treated with monotherapy (54.3% versus 34.1% in those who received combined drug therapy; $P=0.02$). Of note, in multivariate analysis, combination therapy with tigecycline, colistin, and meropenem was independently associated with survival (OR: 0.11; 95% CI: 0.02–0.69; $P=0.01$). In infections caused by *K. pneumoniae* with a MIC value of ≤4 mg/l for meropenem, inclusion of this drug in a combined-drug regimen was associated with a survival rate of 86.6%. Moreover, even in patients with infections caused by isolates with higher meropenem MICs, combined therapy with this drug reached a survival rate of 75%.

Based on the studies analyzed above, it appears that carbapenems retain some therapeutic efficacy against infections caused by CPE, a fact which is supported by human PK/PD studies. Carbapenems display time-dependent bactericidal killing when free drug concentrations remain above the MIC for 40–50% of the time between dosing intervals. The probabilities of attaining 50% T>MIC target for an isolate with a MIC of 4 mg/l is 69% for the traditional dosing regimen (e.g. 30-minute infusion of 1 g every 8 hours for meropenem) and increases to 100% for the high-dose/prolonged infusion regimen (e.g. 3-hour infusion of 2 g every 8 hours for meropenem). Even for a MIC of 8 mg/l, the high-dose/prolonged-infusion regimen displays a relatively high probability (85%) of bactericidal target attainment.[61,86,87]

Although experience with carbapenems in the therapy of infections caused by CPE is still limited, the abovementioned data support the notion that carbapenems may be a reasonable treatment option against these infections provided that: (1) the carbapenem MIC for the infecting organism is ≤4 mg/l and probably up to 8 mg/l; (2) a high-dose prolonged-infusion regimen is administered to drive the PK/PD profile to acceptable exposures; and (3) this class of agents is administered in combination with another active compound, preferably with an aminoglycoside or colistin. The authors of these recommendations stress the fact that probably in many regions or hospitals the MICs for carbapenems are often not available or are usually higher than 8 mg/L. In these situations, carbapenems should not be used as part of a combination regimen to avoid further selection of resistance.

In vitro synergy data support the use of a colistin/tigecycline combination.[88] Another study[89] suggests that rifampicin, doxycycline, and tigecycline may be useful additions to polymyxin B in the treatment of infections caused by highly-resistant carbapenemase-producing *K. pneumoniae*. Polymyxin B and rifampicin were synergistic *in vitro* against 15 of 16 isolates of carbapenem-resistant *K. pneumoniae*.[76]

In conclusion, although clinical experience for the treatment of CPE infections is quite limited, there is

Table 3 Antimicrobial treatment of CPE: summary of recommendations

- AST results and localization of the infection must be considered for the individual treatment decisions.
- Current dosing regimens of colistin may be suboptimal. A loading colistin dose of 6–9 MU followed by 4.5–6 MU bid could be recommended with no additional nephrotoxicity.
- Tigecycline is not recommended as monotherapy for treatment of bacteremia, respiratory, or other serious infections, unless other options are not available.
- Aminoglycosides should not be used as monotherapy for CPE infections.
- Fosfomycin has not already been widely studied to treat CPE infections, so should be used with caution and always in combination with one active agent — with the possible exception of the urinary tract.
- Combination of a carbapenem with another active agent, preferentially an aminoglycoside or colistin, could lower mortality provided that the MIC of carbapenem for the infecting organism is up to 4 mg/l — and probably up to 8 mg/l — and the drug is administered in a high-dose/prolonged-infusion regimen.
- In cases where the MICs for carbapenems are not available or are higher than 8 mg/l, this class of drugs should not be used as part of a combination regimen to avoid further selection of resistance.

growing evidence that combination schemes containing at least two agents with *in vitro* activity against CPE provide superior therapeutic potential against infections caused by these MDR pathogens.

In Table 3, recommendations for the antimicrobial treatment of CPE are summarized.

Prevention

Patients with unrecognized colonization with CPE have served as reservoirs for transmission during outbreaks.[90] Vigilance on the part of the IPC teams and early detection through laboratory-based targeted surveillance is essential to prevent the spread of CPE. This is particularly important for patients who traveled or were hospitalized in high-risk areas for acquiring CPE (e.g. colonization with NDM or KPC producers in people from endemic areas). Rectal swab culture is the best accepted method for detecting stool carriage.

The US CDC[29] recommends for all acute and long-term care facilities the following core measures: hand hygiene, contact precautions, patient isolation and dedicated staff, minimization of the use of invasive devices — particularly urinary catheter-s, promotion or reinforcement of antibiotic stewardship, and screening for CPE. As supplemental measures for healthcare facilities with CPE transmission, CDC recommends active surveillance and chlorhexidine baths.

The ECDC[91] guidelines are similar to the CDC recommendations and suggest that actions to control CPE in acute healthcare settings should be similar to those targeted to other MDROs, e.g. ESBL-producing *Enterobacteriaceae*. Recommendations are (1) early implementation of active surveillance by rectal screening for CPE carriage; (2) additional precautions for the care of CPE-positive patients, including the wearing of disposable gloves and gown; and (3) cohort nursing by a separate, dedicated team. The ECDC recognize that the use of Standard Precautions, and especially adherence to hand hygiene policies, is the cornerstone for preventing transmission of MDROs, including CPE, in healthcare settings. Additional recommended infection control measures include: active screening cultures on admission or transfer of all high-risk patients; routine use of clinical laboratory screening tests for accurate detection of CPE; pre-emptive isolation of high-risk patients pending the results of the active surveillance and, if positive, continuous active surveillance; contact precautions and isolation or cohorting care for all CPE-colonized patients; dedicated staff and cohort nursing for all isolated patients who are carriers of CPE; prudent use of antimicrobial agents and a system for monitoring compliance with all the aforementioned measures.

As noted, there are no significant differences between US CDC and ECDC.

This International Working Group agrees and endorses the abovementioned recommendations. Many practical and pivotal points should be kept in mind to prevent CPE dissemination in different scenarios and resources' countries and regions, as summarized in the following paragraphs.

Facilities should ensure that healthcare personnel are familiar with proper hand hygiene technique, ensure access to hand hygiene stations, and actively monitor the compliance with this pivotal issue in different areas. Immediate feedback should be provided to staff that miss opportunities for hand hygiene.

Contact precautions ideally should be carried out in a single-patient room preferably with *en-suite* bathroom and toilet facilities. When not available, consultation with infection control is necessary to assess the various risks associated with other patient placement options (e.g. cohorting or keeping the patient with an existing roommate). Contact precautions include wearing a gown, apron, and gloves for all interactions that may involve contact with the patient or potentially contaminated areas in the patient's environment. If placed in a single room, the door must remain closed at all times with a clear notice on the door with instructions for all those entering the room including visitors and healthcare workers. If placed in a cohort facility, contact precautions should be carried out with clearly visible notices around the patients' bed area. Patients, staff, family, and visitors must be aware of, and comply with adopted IPC measures. It is advisable to continue with these precautions until the patient has been discharged from the healthcare facility rather than depend on a negative culture result.

The strategy for screening for CPE will depend upon the current epidemiological situation of every healthcare facility.

1 Point prevalence cultures

If the review of microbiology records for the preceding 6–12 months shows previously unrecognized CPE, perform a point prevalence culture survey in high-risk units (e.g. intensive care units, units where previous cases have been identified, and units where many patients are exposed to broad-spectrum antimicrobials) to look for other cases of CPE.

2 Surveillance cultures of patients with epidemiological links to persons from whom CPE have been recovered

For example, screening patients of the same unit or who have been cared for by the same healthcare personnel.

3 Active surveillance

This kind of surveillance consists of screening patients who might not be epidemiologically linked

Table 4 Prevention of CPE: summary of recommendations

•For all different types of hospitals, an aggressive infection control strategy is recommended, including managing all patients with CPE using contact precautions and implementing the guidelines for detection of carbapenemase production.
•Infection control teams should be provided of appropriate human and material resources to accomplish their tasks.
•Educational training of all healthcare workers must be maintained continuously; institutions managers must facilitate these and other interventions.
•Hand hygiene should always be reinforced, monitored, and a priority issue of all healthcare institutions.
•Healthcare facilities should always provide resources for and appropriate and sustained compliance with hand hygiene, standard and contact precautions, and heat disinfection of bedpans and urinals.
•Patients under contact precautions should be clearly identified; patients, staff, family, and visitors must be aware of adopted measures, including strict hand hygiene.
•The strategy for screening for CPE — prevalent point cultures, surveillance of related CPE cases, or active surveillance by sending rectal swabs for culture — will depend upon the distinct epidemiological situation of the facility.
•In institutions where CPE are endemic, facilities should consider additional strategies, as educational reinforcement, strengthening of contact precautions, increase frequency of active surveillance cultures, enhance environmental cleaning, improve bedpan and urinal heat disinfection at ward level, chlorhexidine bathing in some situations, and improve communication within and between healthcare facilities.
•Antimicrobial stewardship should be progressively established in facilities where currently it is not being carried out, and reinforced where programs are undergoing.
•Carbapenems, third and fourth generation cephalosporins, and fluoroquinolones should always be carefully used.

to known CPE patients but who meet certain pre-specified criteria. This could include everyone admitted to the hospital, pre-specified high-risk patients (e.g. those admitted from long-term care facilities), and/or patients admitted to high-risk settings (e.g. intensive care units). It is important to underline that the exact impact of active surveillance in preventing CPE spreading is unknown.[29] Screening is carried out by taking a rectal swab and sending it to the laboratory for identification of CPE.

In low- to middle-income countries, active surveillance is often difficult because of the lack of laboratory support and staffing shortages. It is therefore recommended that good infection control — such as mandatory hand washing and contact precautions — be instituted as soon as possible and remain in place until the patient has been discharged. Recent studies have shown the success of implementing at least part of these recommendations.[92–94]

In institutions where CPE are endemic, facilities should consider additional strategies. These include multi-faceted educational reinforcement in different ways to improve hand hygiene, contact precautions (e.g. adopting them preemptively, while results of admission surveillance testing are pending), increase frequency of active surveillance cultures, enhance environmental cleaning, evaluate implementing 2% chlorhexidine bathing in certain areas or high-risk patients, and improve communication about patients with MDR organisms within and between healthcare facilities. The description of all these strategies is beyond the scope of the present statement; in CDC 2012 guidelines, the reader will find wider information and recommendations according to different epidemiological situations.[29]

Other institutions of growing concern are long-term care facilities. Initially, KPC-producing *K. pneumoniae* appeared to be limited to causing hospital-acquired infections, so the abovementioned recommendations were originally made for acute-care hospitals. More recently, outbreaks or high levels of endemicity have been reported both from long-term care facilities[95] and long-term acute care hospitals.[17] Therefore, these recommendations should ideally also be noted by those facilities' managers and staff. For example, contact precautions should be also implemented for CPE colonized or infected residents that are high-risk for transmission.

Finally, antimicrobial stewardship represents a cornerstone of any infection control program and implies a multidisciplinary approach. Resistance is due to a complex interaction of multiple factors, but the selection of resistant pathogens by antimicrobial use is probably the most important variable. A number of epidemiological studies have demonstrated the association between increased antimicrobial use and emergence of resistance. The carbapenems, third and fourth generation cephalosporins, and fluoroquinolones — among others — have been significantly associated with the emergence of CPE.[8,43,91] Thus, these drugs warrant particular attention and should always be carefully used.

In Table 4, our recommendations for prevention of CPE are summarized.

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