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Carbapenemases in *Klebsiella pneumoniae* and Other *Enterobacteriaceae*: an Evolving Crisis of Global Dimensions

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INTRODUCTION

Klebsiella pneumoniae is encountered as a saprophyte in humans and other mammals, colonizing the gastrointestinal tract, skin, and nasopharynx; it is also found in various environmental niches (soil, water, etc.) (11). In the past, it was considered an important causative agent of community-acquired (CA) infections, including a severe form of pneumonia. Recently, while CA

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TABLE 1 Types, classification, variants, and species distribution of plasmid-mediated carbapenemases encountered in *Enterobacteriaceae*

Type	Molecular class (subclass)[a]	Functional group[b]	Variants	Species
KPC	A	2f	KPC-2 to -13	*K. pneumoniae*, *E. coli*, *Klebsiella oxytoca*, *S. marcescens*, *Enterobacter* spp., *C. freundii*, *Salmonella enterica*, *Raultella* spp.
VIM	B (B1)	3a	VIM-1, -2, -4, -5, -6 VIM-11, -12, -13, -19, -23 VIM-24, -25, -26, -27, -32	*K. pneumoniae*, *E. coli*, *K. oxytoca*, *S. marcescens* *Serratia liquefaciens*, *Enterobacter* spp., *C. freundii* *Morganella morganii*, *Proteus stuartii*, *P. mirabilis*
IMP	B (B1)	3a	IMP-1, -3, -4, -6, -8 IMP-11, -24, -27	*K. pneumoniae*, *E. coli*, *K. oxytoca*, *S. marcescens* *Enterobacter* spp., *Citrobacter* spp., *P. mirabilis*, *Proteus rettgeri*, *Shigella flexneri*, *M. morganii*
NDM	B (B1)	3a	NDM-1, -4, -5, -6	*K. pneumoniae*, *E. coli*, *Enterobacter* spp., *K. oxytoca*, *C. freundii*, *M. morganii*, *Providencia* spp.
OXA	D	2df	OXA-48, -163, -181	*K. pneumoniae*, *E. coli*, *C. freundii*, *P. mirabilis*

[a] See reference 109.
[b] See reference 37.

pneumonia due to *K. pneumoniae* has become rare, novel manifestations of CA infections, such as liver abscess complicated by endophthalmitis and other metastatic infections, have been described (140).

In the early 1970s, both the epidemiology and spectrum of infections caused by *K. pneumoniae* changed dramatically when this bacterium was established in the hospital environment and became a (still) leading cause of nosocomial infections. Not only is it found in the gastrointestinal tracts of patients, at frequencies as high as 80%, but high carriage rates have also been recorded for patient nasopharynges and hands (212). This considerable efficiency of colonization, enhanced by acquired resistance to antibiotics, enables *K. pneumoniae* to persist and spread rapidly in health care settings (119). Although not inherently resistant to antibiotics, since it produces only moderate amounts of chromosomal penicillinases, *K. pneumoniae* is a notorious "collector" of multidrug resistance plasmids. During the 1970s to 1980s, these were commonly plasmids encoding resistance to aminoglycosides. Later, however, *K. pneumoniae* became the index species for plasmids encoding extended-spectrum β-lactamases (ESBLs)—mostly TEMs and SHVs active against newer cephalosporins—along with a variety of genes conferring resistance to drugs other than β-lactams (212). The successive addition of genetic elements encoding resistance to aminoglycosides and extended-spectrum β-lactams, coupled with the rapid accumulation of chromosomal mutations conferring resistance to fluoroquinolones, left carbapenems as the first-choice drugs for the treatment of health care-associated infections caused by *K. pneumoniae*.

This was true until approximately 2000, when we began witnessing a global crisis of unprecedented dimensions due to the rapid dissemination of multidrug-resistant (MDR) *K. pneumoniae* strains producing "carbapenemases" encoded by transmissible plasmids. Later, other clinically important enterobacterial species, including *Escherichia coli*, acquired carbapenemase genes (202). Thus, it appears probable that as in the ESBL "era," *K. pneumoniae* again functions as a pool of potent β-lactamases. The clinically most important carbapenemases in *Enterobacteriaceae* are the class A enzymes of the KPC type and the zinc-dependent class B metallo-β-lactamases (MβLs), represented mainly by the VIM, IMP, and NDM types. The plasmid-expressed class D carbapenemases of the OXA-48 type complete the picture (Table 1) (107, 166, 202).

Carbapenemase-producing enterobacteria (CPE) cause serious infections in debilitated and immunocompromised patients, in association with prolonged hospital stays and increased mortality rates, ranging from 24% to as high as 70%, depending on the study population (14, 24, 28, 67, 175, 187, 203, 233, 274). Given the critical condition of these patients, treatment should be timely, aggressive, and rapidly efficacious. However, therapeutic options are obviously limited, and unfortunately, the introduction of new antimicrobials such as tigecycline or the "reinvention" of colistin has far from entirely resolved this problem, as discussed in a later section.

In this review, we attempt to (i) describe the microbiological and epidemiological characteristics of carbapenemase-producing *Enterobacteriaceae* and (ii) present in a critical manner the available data regarding the antimicrobial treatment and infection control practices used to combat infections caused by these bacteria.

GENETIC CONTEXT, SUBSTRATE SPECTRA, AND β-LACTAM RESISTANCE PHENOTYPES

KPC Carbapenemases

KPC β-lactamases (KPC-2 to KPC-13; molecular class A) (www.lahey.org/studies) exhibit activity against a wide spectrum of β-lactams, including penicillins, older and newer cephalosporins, aztreonam, and carbapenems (Table 2) (191). Structural studies and comparisons with the TEM-1 and SHV-1 penicillinases indicated that positioning of the catalytic residues in KPCs may allow accommodation of the bulky α-substituents of carbapenems in a manner facilitating the subsequent acylation and deacylation steps (123).

bla_{KPC} genes detected so far in *K. pneumoniae* are all carried on plasmids. Sequences adjacent to bla_{KPC} genes display rather limited diversity, suggesting a single or at least a limited number of original sources. Segments of the Tn*3*-related Tn*4401* transposon, occurring in four isoforms, are invariably present upstream of

TABLE 2 Hydrolytic efficiencies of representative carbapenemase variants against various β-lactam substrates

β-Lactamase	Hydrolytic efficiency (k_{cat}/K_m) (s^{-1} μM^{-1})[a] against:								Reference
	Imipenem	Meropenem	Ceftazidime	Cefotaxime	Aztreonam	Cefoxitin	Cephalothin	Penicillin G	
KPC-2	0.29	0.27	ND	0.10	0.08	0.002	0.84	1.90	271
KPC-3	1.90	1.40	0.03	0.50	ND	0.50	3.50	ND	4
VIM-1	0.13	0.26	0.08	0.68	—	0.20	5.10	0.04	93
VIM-2	3.80	2.50	0.05	5.80	—	1.20	11.8	4.0	74
VIM-4	23.0	0.90	ND	ND	—	ND	36.0	3.10	137
VIM-5	0.29	0.05	0.001	0.09	—	ND	ND	0.26	95
VIM-19	6.0	2.0	0.02	30.0	—	0.50	ND	5.0	227
VIM-27	0.26	ND	ND	0.82	—	0.03	8.30	ND	198
IMP-1	1.20	0.12	0.18	0.35	—	2.0	2.40	0.62	136
IMP-4	0.35	0.18	0.07	0.14	—	ND	0.43	0.08	51
NDM-1	0.21	0.25	0.03	0.58	—	0.02	0.40	0.68	273
NDM-4	0.46	0.31	0.06	1.20	—	—	0.50	ND	189
OXA-48	0.14	<0.001	0.001	0.05	—	ND	0.15	6.10	217

[a] ND, not determined; —, no hydrolysis detected.

bla_{KPC} (63, 180). Tn*4401* is bracketed by 39-bp imperfect inverted repeats and bounded by different 5-bp target site duplications (Fig. 1, structure I) (180). These structures indicate the operation of a replicative transposition mechanism (typical of Tn*3*-like transposons) that allows spread of KPC-encoding sequences among different genetic units and has resulted in the emergence of distinct KPC-encoding plasmids belonging to various Inc groups, such as FII (probably derivatives of the characteristic FII virulence plasmid of *K. pneumoniae*), L/M, and N (63). The same genetic structures have been identified in KPC-positive isolates of other enterobacterial species (Table 1).

In line with their substrate spectra, KPC enzymes confer on enterobacteria decreased susceptibility or resistance to virtually all β-lactam antibiotics. Moreover, there have been studies reporting

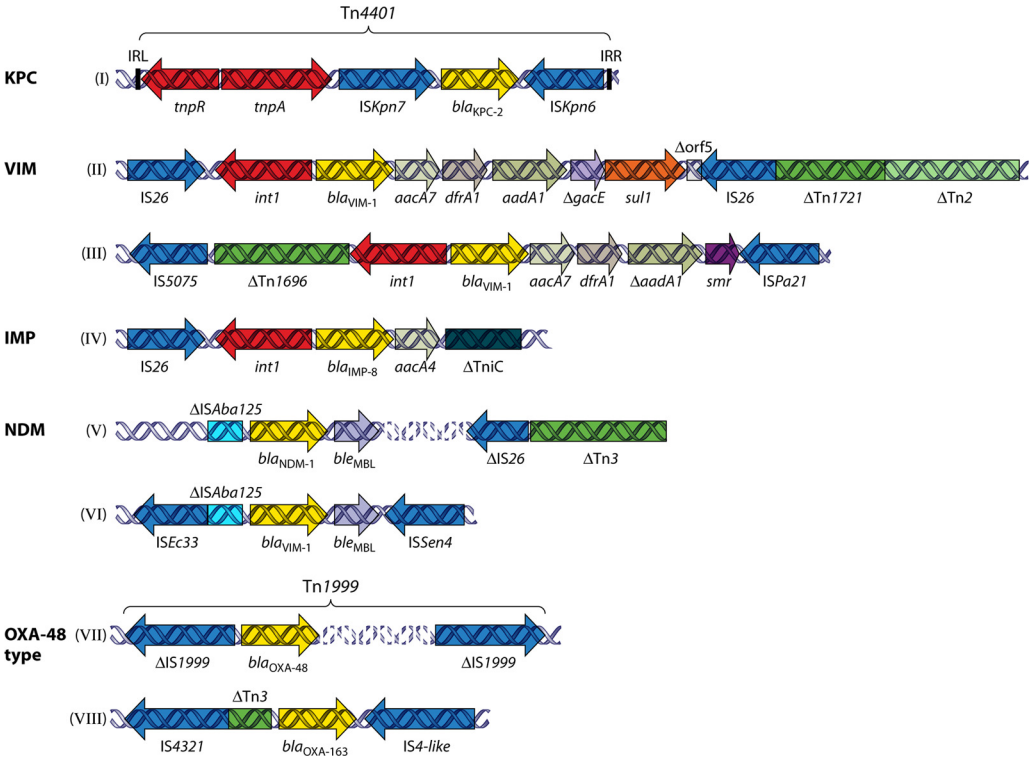

FIG 1 Schematic depiction of representative sequences from enterobacterial plasmids, showing the association of carbapenemase-encoding genes with various mobile elements. (I) The bla_{KPC-2}-containing Tn*4401* transposon from plasmid pNYC (GenBank accession no. EU176011) (180). (II and III) Representative VIM-encoding sequences from plasmids pNL194 (GenBank accession no. GU585907) (167) and pCC416 (GenBank accession no. AJ704863) (59), respectively. (IV) A bla_{IMP}-carrying sequence from plasmid pFP10-2 (GenBank accession no. HQ651093) (146). (V and VI) Sequences containing bla_{NDM-1} carried by a plasmid from *K. pneumoniae* 05-506 (GenBank accession no. FN396876) (273) and by plasmid p271A (GenBank accession no. HQ162469) (218), respectively. (VII and VIII) The OXA-48-encoding transposon Tn*1999* from plasmid pA-1 (GenBank accession no. AY236073) (217) and the $bla_{OXA-163}$-containing segment from plasmid p6299 (GenBank accession no. HQ700343) (216), respectively.

on the emergence of KPC-positive *K. pneumoniae* exhibiting a decreased outer membrane permeability that enhances β-lactam resistance levels (134). Although the MICs of carbapenems vary, the latest Clinical and Laboratory Standards Institute (CLSI) and European Committee for Antimicrobial Susceptibility Testing (EUCAST) breakpoints classify most KPC-producing isolates as resistant to these drugs (55; www.eucast.org). It should also be noted that carbapenem and oxyimino-β-lactam MICs are commonly higher for species with derepressed production of their chromosomally encoded AmpCs, such as enterobacters, than for *K. pneumoniae* (166, 191).

MβLs

MβLs constitute a class of enzymes (molecular class B) that, despite their significant amino acid sequence diversity, share three distinct functional properties: (i) capability of hydrolyzing carbapenems, (ii) resistance to mechanism-based inhibitors, and (iii) susceptibility to chelating agents such as EDTA. The latter property results from their unique mechanism of hydrolysis, in which divalent cations, most commonly Zn^{2+}, are essential for the nucleophilic attack of the β-lactam ring. Phylogenetic analysis suggests the existence of three MβL lineages: B1, B2, and B3 (13). In addition to chromosomally encoded MβLs, subgroup B1 includes the acquired enzymes of the VIM, IMP, GIM, SPM, SIM, AIM, DIM, and NDM types, all of which, surprisingly, are still of unknown origin (61). Of these, several variants of the VIM, IMP, and NDM types have been encountered in *K. pneumoniae* and other *Enterobacteriaceae* (Table 1). These β-lactamases are active against penicillins, older and newer cephalosporins, and carbapenems, although significant variations in hydrolytic efficiency exist even between enzymes of the same type (Table 2). Also, they are incapable of inactivating aztreonam. This is due mainly to the fact that B1 MβLs bind monobactams with a very low affinity. Moreover, docking experiments have indicated that positioning of the drug within the active site does not favor hydrolysis (213).

The bla_{VIM} and bla_{IMP} variants identified in *K. pneumoniae* so far occur as gene cassettes incorporated into the variable regions of class 1 integrons (Fig. 1, structures II, III, and IV) (254), with the exception of a class 2 and a class 3 IMP-encoding integron (174, 235). In contrast, bla_{NDM} genes are not associated with integrons (Fig. 1, structures V and VI) (114, 218, 273). The wide variety of *K. pneumoniae* plasmids encoding MβLs implies the operation of mobilization mechanisms. Two non-mutually exclusive possibilities are likely to account for this dissemination of MβL genes in distinct plasmids: (i) reshuffling of MβL cassettes among plasmid-borne integrons and (ii) *en bloc* mobilization of MβL gene-containing structures through transposition and/or recombination events. Indeed, insertion elements (ISs) such as IS*26*, IS*Ec33*, IS*Sen4*, and IS*Aba125*, either alone or as parts of transposons (e.g., Tn*3* and Tn*1696*), commonly flank MβL-encoding regions (Fig. 1, structures II to VI) (42, 59, 114, 165, 167, 215, 218, 273). At least some of the latter elements apparently participate in the spread of MβL-encoding sequences among different genetic units.

The three MβL types encountered in *K. pneumoniae* have also been identified on numerous occasions in other enterobacterial species, including *E. coli* (VIM, IMP, and NDM), *Enterobacter cloacae* (mainly VIM and IMP), *Serratia marcescens* (mainly IMP), and *Proteus mirabilis* (mainly VIM) (Table 1) (61, 166). In these species, most genetic platforms of MβL genes are similar to those found in *K. pneumoniae*.

The baseline phenotype expected from MβL-producing enterobacteria includes (i) resistance to amino-, carboxy-, and ureidopenicillins, penicillin-clavulanate combinations, and cefoxitin; (ii) decreased susceptibility to piperacillin-tazobactam and oxyimino cephalosporins; and (iii) elevated MICs of carbapenems compared to the epidemiological cutoff values. In actual fact, however, such a minimal resistance phenotype is observed rarely, if at all, among clinical isolates, since MβL producers most often possess additional mechanisms that increase carbapenem resistance levels, such as elevated expression of the MβL itself and, most importantly, impaired outer membrane permeability (40, 152). The latter mechanism apparently plays a significant role in determining carbapenem resistance levels, as also indicated by the wide range of carbapenem MICs among MβL producers belonging to the same species and even to the same lineage (40, 152, 166). Also, ESBLs such as SHVs, often encountered among VIM producers (152, 224), expand the resistance phenotype to include aztreonam resistance.

OXA-48

OXA-type β-lactamases (molecular class D), such as OXA-23, OXA-24/40, and OXA-58, encountered frequently in acinetobacters, exhibit relatively weak carbapenemase activity (256). In 2001, OXA-48, a distinct OXA enzyme (<50% amino acid sequence identity with the other OXA enzymes) with significant carbapenemase activity, was identified in *K. pneumoniae* (217). Its hydrolytic efficiency against imipenem is approximately 10-fold higher than those of the acinetobacter OXAs (256). Data from the crystal structure of OXA-48 and molecular dynamics studies suggest that the process of carbapenem hydrolysis is different from that for the other OXA carbapenemases. For OXA-48, hydrolysis relies on the rotation of the carbapenem's α-hydroxyethyl group within the active site in a manner that allows movement of the deacylating water toward the acylated serine residue (73). Consequently, OXA-48-producing *K. pneumoniae* isolates exhibit elevated MICs of carbapenems that are still frequently lower than the respective breakpoints. OXA-48 also hydrolyzes penicillins and early cephalosporins, but its activity against oxyimino cephalosporins is weak (217). Other carbapenem-hydrolyzing variants of OXA-48 (OXA-163 and -181) have also emerged in *K. pneumoniae* (216, 220, 221). bla_{OXA-48} is invariably carried by transmissible plasmids responsible for its spread among *K. pneumoniae* and other enterobacterial species, such as *E. coli* and *Citrobacter freundii* (Table 1) (46, 217). Moreover, the plasmid-borne bla_{OXA-48}-containing sequences are associated with IS*1999*, an IS*4* family element involved in mobilization and expression of β-lactam resistance genes (Fig. 1, structures VII and VIII) (8). This association provides further possibilities for bla_{OXA-48} to be transferred to other genetic units.

GLOBAL SPREAD OF CPE

Producers of KPC Types

A rapid and extensive dissemination of KPC-producing *K. pneumoniae* was first noticed in the northeastern parts of the United States during the first decade of the 21st century. Surveillance studies suggested that the epicenter of this epidemic was the state of New York (25, 27, 29). Later, isolates producing KPC-2 (270) and KPC-3 (a point mutant of KPC-2) (4) became established in hospitals in neighboring states, apparently due to transfer of col-

onized patients (83, 84, 126). During the same period, KPC-producing *K. pneumoniae* also emerged in Latin America (171, 201, 252) and Israel (139). Other countries, such as China (257) and Greece (102), soon followed. The Chinese data originate from a limited number of hospitals; thus, the actual extent of spread of KPC producers in China remains unknown. In Greece, KPC-positive *K. pneumoniae* became dominant in tertiary care hospitals, reaching epidemic proportions in a matter of approximately 2 years (99). In Northern and Western European countries, KPC prevalence remains low. In these countries (e.g., Switzerland, Ireland, United Kingdom, France, Sweden, Norway, the Netherlands, and Denmark), most reports concern sporadic isolates introduced by patients from high-prevalence areas (181, 230, 266). Nevertheless, a multihospital outbreak has already occurred in France (43). Higher prevalences have been reported from Poland and Italy, where KPC producers appear to be established in various regions (12, 103). The rapid global dissemination of KPC-producing *K. pneumoniae* implies multiple transmission routes. According to a widely held scenario, an important event was the introduction of KPC-positive *K. pneumoniae* from the United States to Israel, followed by spread to neighboring countries and via Greece to other European countries (260). However, index cases to confirm this scenario were not identified with certainty (154). KPC enzymes have been detected in a large number of *K. pneumoniae* sequence types (STs) (99). Nevertheless, the vast majority of isolates with these enzymes worldwide belong to ST258. This ST is strongly associated with KPC production and with isolates exhibiting multidrug resistance, but one could also speculate on additional—though yet unknown—inherent traits responsible for its high rate of transmissibility. Whatever these eventually turn out to be, KPC-producing ST258 *K. pneumoniae* can undeniably be regarded as one of the most successful multidrug-resistant nosocomial pathogens known to date.

Not unexpectedly, KPC-producing isolates of various other enterobacterial species, including *E. coli* and *E. cloacae*, have been reported in settings where the prevalence of KPC-positive *K. pneumoniae* is high. Outbreaks of KPC-producing *E. coli* have occurred in health care facilities in various countries, including the United States, Israel, and Greece (29, 105, 160, 249). Also, sporadic KPC-positive isolates of a wide variety of other enterobacterial species have been described worldwide (Table 1) (166, 191).

Producers of MβLs

K. pneumoniae strains producing enzymes belonging to any of the three MβL families (VIM, IMP, and NDM) have already achieved international spread, though significant local differences do exist. VIM-positive *K. pneumoniae* was first observed around 2001 to 2003 in Southern Europe and was introduced later to Northern Europe (e.g., Germany, France, and the Scandinavian countries) and the United States, mostly through colonized patients transferred from high-prevalence areas (61). Isolation rates of VIM-positive *K. pneumoniae* in Northern Europe and the United States remain low, though some infection clusters limited to single hospitals have been reported (107). In addition, sporadic cases have been recorded in Tunisia (129), South Korea (272), and Venezuela (155). Until recently, VIM-producing *K. pneumoniae* and other enterobacteria were frequently isolated in Mediterranean countries, reaching epidemic proportions only in Greece (107, 251). However, up-to-date surveillance data from this country indicate that these organisms have been in decline since 2009 (G. L. Daikos, unpublished data).

Acquisition of IMP MβLs by *K. pneumoniae* was described during the 1990s, primarily in Japan, as well as in Taiwan and Singapore (225). IMP-positive *K. pneumoniae* clinical isolates remain frequent in Japan (94). IMP-4-producing *K. pneumoniae* strains have also caused hospital outbreaks in China (162) and Australia (206). In addition, IMP-positive clinical enterobacteria, such as *S. marcescens* and *E. cloacae*, have been reported in the same area, i.e., Japan, South Korea, and Taiwan (225). Dissemination of IMP-producing *Enterobacteriaceae* in the rest of the world appears to be limited, with single cases identified in Turkey, Lebanon, Brazil, and the United States (3, 72, 148, 174). As usual, limitations and differences in surveillance systems in different countries inevitably affect the reliability and comparability of international epidemiological data on IMP (or indeed VIM)-positive *K. pneumoniae*.

In stark contrast, the results of the internationally concerted effort and resources allocated for the elucidation of the transmission routes and public health impact of enterobacteria, mainly *E. coli* and *K. pneumoniae* strains producing NDM, the most recently identified MβL type, were spectacular. These efforts produced a wealth of data regarding the epidemiology of NDM producers. The epicenter of their epidemic is the Indian subcontinent, where the high isolation frequency of these microorganisms in health care facilities, as well as their extensive spread in various environmental niches, has been documented repeatedly (130, 192). Furthermore, the bla_{NDM} genes have spread to various enterobacterial species other than *K. pneumoniae* and *E. coli* (Table 1) (255). Also, a second reservoir of NDM-producing *K. pneumoniae* strains seems to exist in the central Balkans, but its link with the Indian epidemic remains uncertain (108, 150). In contrast, the recent spread of NDM producers in Western Europe, North America, Australia, and the Far East has clearly been attributed to patients who originated mainly from India, Pakistan, and Bangladesh (192). A characteristic of NDM-producing *K. pneumoniae* isolates has so far been their rapid dissemination; indeed, infected or colonized humans without obvious connection to the Indian epidemic are increasingly being reported in several countries (125, 190, 214).

Producers of OXA-48

OXA-48-producing *K. pneumoniae* was first detected sporadically in Turkey, in 2001 (217). Hospital outbreaks in the main cities of this country soon followed (45). About the same time, OXA-48-positive *K. pneumoniae* isolates were also identified in other Middle Eastern and North African countries (46, 62) as well as in Western European countries, including the United Kingdom, Belgium, France, Germany, and the Netherlands. Emergence of OXA-48 producers in the latter countries has been attributed mainly to colonized patients transferred from North Africa (107). Recently, an important outbreak due to an OXA-48-producing *K. pneumoniae* strain was reported in a Dutch hospital (220). However, there are no indications of an overall significant spread of these microorganisms across Europe. Although the Middle East and North Africa remain the main foci of infection, the recent isolation of *K. pneumoniae* isolates producing OXA-48-type enzymes in India (47), Senegal (173), and Argentina (216) suggests an expansion that can safely be considered global. Additionally, the recent isolation of OXA-48 producers belonging to species

other than *K. pneumoniae* underlines the spreading potential of bla_{OXA-48} (Table 1) (46).

DETECTION OF CPE

Counterintuitive as it may sound, carbapenemase production by enteric bacilli does not necessarily confer significant resistance to carbapenems. Before the introduction of the new carbapenem breakpoints by CLSI and EUCAST in 2010 (55; www.eucast.org), carbapenemase-positive isolates (as determined by phenotypic tests) with relatively low carbapenem MICs were reported without interpretation of their susceptibility status. This directly implied the possibility of therapeutic failure for carbapenem regimens, passing to clinicians a mixed message of dubious value. Today, after the introduction of the new, lower breakpoints, the situation has been simplified: laboratories report the MICs of carbapenems irrespective of carbapenemase production. On the other hand, various enterobacterial isolates lacking enzymes with appreciable carbapenemase activity may exhibit elevated MICs of carbapenems. Consequently, this may exclude from use a viable group of antibiotics. Application of simple and reliable carbapenemase-detecting tests nevertheless remains useful for monitoring of carbapenemase-producing microorganisms in order to inform appropriate infection control policies in health care settings.

A large-scale and cost-effective approach for deciding which isolates are carbapenemase producers based solely on phenotypic tests should rely on the epidemiological cutoff (ECOFF) values for nonsusceptibility. These values depend on carbapenem MIC distributions of carbapenemase producers as opposed to wild-type strains. Considering the respective distributions for *K. pneumoniae* and *E. coli* compiled by EUCAST, MICs of $\geq$1 μg/ml for imipenem and $\geq$0.5 μg/ml for meropenem and ertapenem have been proposed (57). According to the CLSI, which has not defined ECOFFs, selection of isolates for testing can rely on clinical breakpoints: isolates that test intermediate or resistant to at least one carbapenem as well as resistant to a "subclass III cephalosporin" (cefotaxime, ceftazidime, ceftriaxone, cefoperazone, or ceftizoxime) should be tested further. Ertapenem is considered the most sensitive indicator (29). It should be noted, however, that use of this drug may cause specificity problems: decreased permeability, combined with either production of CTX-M or overproduction of AmpC β-lactamases, can significantly affect the MIC of ertapenem and therefore lower the detection specificity (57, 264).

Screening criteria, however, may and should be adapted depending on the epidemiological situation in a given ecological setting. Application of the CLSI criteria is expected to be adequate in settings where carbapenemase producers have already been established. On the other hand, occasional adoption of less stringent criteria (i.e., the use of lower cutoffs or reducing the concentrations of the selective agents used for screening) in low-prevalence settings may facilitate the timely detection of CPE emergence or early dissemination and therefore allow the swift implementation of measures preventing their further spread. The potential prevention benefits of such an approach are likely to counterbalance the burden of increased false-positive results.

There have been numerous studies that deal with technical issues of carbapenemase detection methods, comparing their performances mainly for *K. pneumoniae* and *E. coli*. We therefore briefly review these methods and their principles.

MHT

The cloverleaf or modified Hodge test (MHT) is based on the inactivation of meropenem or ertapenem by whole cells of carbapenemase-producing organisms. MHT has been used extensively as a phenotypic method for the detection of carbapenemase activity (55), and it is the only carbapenemase detection method recommended by the CLSI for screening purposes. There are, however, various shortcomings with MHT. The assay cannot distinguish the type of carbapenemase involved. Most importantly, false-positive results have been observed with isolates producing CTX-M-type ESBLs or increased amounts of AmpC β-lactamases (cephalosporinases) (166, 200). Moreover, sensitivity problems (false-negative results) may occur, mainly with MβL-producing enterobacterial isolates exhibiting weak carbapenemase activity (166). Also, MHT is probably unreliable in detecting NDM-1-producing *K. pneumoniae*, though the relevant observations regard a limited number of isolates (49, 169). Replacement of Mueller-Hinton agar by MacConkey agar has been proposed as a means to increase the sensitivity of MHT for detection of isolates producing MβLs or OXA carbapenemases. Improved performance was attributed to the enhanced release of periplasmic enzymes caused by the bile salts included in MacConkey medium (141). This modification, however, has not been evaluated systematically. Overall, MHT, although remaining a convenient assay, cannot be used as the sole method for the detection of carbapenemase-positive *Enterobacteriaceae* in the clinical laboratory.

Detection of MβLs Based on Chelating Agents

Phenotypic detection of MβL producers in the clinical laboratory is based mainly on the specific inhibition of MβLs by EDTA (193). Additionally, various techniques utilizing other chelating agents, such as dipicolinic acid and 1,10-phenanthroline, as well as thiol compounds such as 2-mercaptopropionic and mercaptoacetic acid, have been developed (166). Use of a combination of chelators, e.g., EDTA plus 2-mercaptopropionic acid, has also been proposed (124). These compounds, by depriving the MβL of hydrolytically essential Zn divalent cations, render it inactive against β-lactams. The most common MβL detection tests employ a disk of a hydrolyzable β-lactam (typically a carbapenem, though ceftazidime has also been used extensively) placed close to a disk with a given amount of an MβL inhibitor (most commonly EDTA), hence the term "double-disk synergy test" (DDST). Formation of a synergy pattern is indicative of MβL production. A drawback of this approach is that interpretation is subjective and cannot be quantified. Alternatively, the β-lactam disk is potentiated with an inhibitor, and the diameter of its inhibition zone is then compared with that of the β-lactam disk alone, hence the term "combined disk test" (CDT). An increase of the inhibition zone diameter above a predefined cutoff value denotes MβL activity. Various gradient diffusion methods (e.g., Etest [bioMérieux, Solna, Sweden]), utilizing strips containing imipenem and EDTA, are based on the same principle. In general, MβL detection methods based on β-lactam–chelator combinations perform well for *K. pneumoniae* and *E. coli*, while they have not been tested systematically for other enterobacterial species. Also, the user should always consider the potentially detrimental effects of chelating agents on bacterial growth. Additionally, interpretation difficulties are to be expected with MβL producers exhibiting low carbapenem MICs.

Detection of KPCs Based on Boronates

Phenotypic detection of KPC production is based on the susceptibility of KPCs to boronic acid and its derivatives, i.e., phenylboronic and 3-aminophenylboronic acid. Boronate derivatives, which structurally resemble β-lactams, have long been used in probing the function of β-lactamases, especially class C enzymes. In 2008, Pasteran et al. (201) observed that boronates preferentially inhibit KPC-type β-lactamases. This report was soon followed by studies proposing detection techniques using boronic acids combined with a carbapenem, mostly in the CDT format (75, 104, 248). As with the above-described MβL detection tests, experience with the boronate-based detection of KPC producers is limited mainly to *K. pneumoniae*. Specificity problems may arise with isolates producing AmpC-type β-lactamases (cephalosporinases), since boronic acid derivatives are potent inhibitors of these enzymes. The problem can be alleviated partly by the simultaneous use of cloxacillin, which preferentially inhibits cephalosporinases (104). It should also be noted that boronate-based assays are ineffective in detecting KPC-positive *K. pneumoniae* in the case of coproduction of VIM β-lactamase (100).

Detection by Use of Chromogenic Media

At least two selective agar media allowing different carbapenemase-producing microorganisms to be recognized are commercially available: CHROMagar-KPC (CHROMagar; BBL) and Brilliance CRE agar (Thermo Fisher Scientific). Species are distinguished by colony color. The reliability of these media has not yet been evaluated rigorously. Nevertheless, they have been used successfully for surveillance cultures on various occasions (196, 208, 229).

Molecular Detection of Carbapenemase Genes

Many clinical laboratories employ "in-house" PCR-based methods for the detection of carbapenemase genes to get around the problems of phenotypic detection methods and to reduce reporting times. In addition, PCR-based methods allow detection of OXA-type carbapenemases for which specific phenotypic tests have not been developed. Simplex PCR assays targeting a single carbapenemase type have been used successfully in numerous studies, although there is no consensus regarding the oligonucleotide primers that should be used for each *bla* gene group. Multiplex and real-time PCR methods that allow the identification of multiple carbapenemase gene types and that further shorten the detection time, in the case of real-time PCR, have also been utilized (20, 70, 172, 219, 253). Also, real-time PCR assays can be followed by a melting curve step to allow the accurate identification of carbapenemase gene variants (163). PCR- and hybridization-based kits for detection of the main carbapenemase gene types, for example, Hyplex MBL ID and Hyplex CarbOxa ID kits (BAG Health Care, Lich, Germany), have also been developed by the industry. Although manufacturers claim that these methods have the potential to be used directly on clinical samples (9), their diagnostic usefulness remains to be evaluated systematically in different settings. Microarray technology was recently added to the list of molecular methods aiming at the rapid and reliable identification of multiple resistance determinants. The Check-KPC ESBL microarray and its expanded version, Check-MDR CT102 (Check-Points Health BV, Wageningen, Netherlands), have been used successfully for detection within a single reaction tube of a wide variety of *bla* genes, including most clinically relevant carbapenemase genes (58, 179). Nevertheless, the term "macroarray" may be more suitable given the relatively small number of genes tested.

An issue with all molecular methods is that the range of resistance genes to be detected is predefined, so these methods may miss novel gene types.

Detection of Carbapenemase Activity by Spectrophotometry

Assessment of carbapenemase activity by spectrophotometry is carried out using crude or partially purified enzyme extracts and a carbapenem, commonly imipenem. It is considered the reference method for the verification of carbapenemase activity. This laborious and technically demanding approach, however, is limited to reference laboratories.

Detection of Carbapenemase Activity by Mass Spectrometry

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is the latest advancement in the recognition of carbapenemase activity. The method is based on the ionization in high vacuum of the material under examination and its subsequent acceleration in an electrical field. The sizes of fragments can be inferred from the time of flight within the electrical field. MALDI-TOF MS has been introduced in the clinical laboratory mainly as a means for species identification. However, the method is highly versatile and can be used for the recognition of various compounds, including antibiotic degradation products. Recently, MALDI-TOF MS was used successfully to identify carbapenem hydrolysis products, thus confirming carbapenemase activity in Gram-negative isolates (35, 115). However, experience with this methodology is still limited.

ANTIMICROBIAL AGENTS AGAINST CPE

In Vitro Activity

Susceptibility of the infecting isolate is one of the key factors in deciding on a suitable antimicrobial chemotherapy. *In vitro* susceptibility data from numerous studies throughout the world indicate that colistin, tigecycline, and fosfomycin are the most effective antibacterials against clinical enterobacteria producing either KPCs or MβLs. Drug effectiveness, however, differs depending on the extent of spread of resistant isolates in each setting. Indeed, a growing number of studies indicate that the activity of these drugs is decreasing rapidly (7, 21, 80, 85, 88, 96, 128, 142, 151, 156, 185, 186, 226, 243, 247, 275, 277). In addition, rates of susceptibility to fluorinated quinolones are generally low, reflecting the multidrug-resistant nature of CPE. Low susceptibility rates are also found for other, clinically less important antimicrobials, such as nitrofurantoin (breakpoints are available only for *E. coli*) and chloramphenicol, both of which are drugs that are tested less frequently. Among the currently used aminoglycosides, only gentamicin has so far retained good activity against producers of KPCs and acquired MβLs of the VIM type. The majority of NDM producers, however, are resistant to all clinically available aminoglycosides due to coproduction of 16S rRNA methylases (18). Of the β-lactams, the most effective compounds seem to be the carbapenems, which at first sight appears to be paradoxical. However, applications of different carbapenem breakpoints by the CLSI and EUCAST must be taken into account. It has been estimated that in

Greek hospitals, some 10 to 15% of CPE (a bacterial population consisting mainly of KPC-2-producing *K. pneumoniae* strains) appear resistant by the CLSI interpretive criteria but susceptible according to EUCAST (Daikos, unpublished data). Aztreonam, though withstanding hydrolysis by acquired MβLs, exhibits limited activity against the respective CPE due to the frequent coproduction of ESBLs, mainly of the SHV and CTX-M types (60). Finally, temocillin, a 6-α-methoxy derivative of ticarcillin, seems to exhibit moderate activity against KPC-producing *K. pneumoniae* and *E. coli*, but the relevant data are limited (1).

The data summarized above, obtained from a wide variety of settings, should nevertheless be treated with caution given the different methodologies used. Additionally, there have been reports that automated systems have "inherent" problems in reliably determining carbapenem MICs for CPE (33, 101, 199, 244, 246). Also, there are difficulties in interpreting carbapenem susceptibility data for KPC producers due to heterogeneous resistance-like phenomena (191).

In Vitro Synergy

Given the limited therapeutic options for the management of infections caused by carbapenemase-producing *K. pneumoniae* strains, several investigators have evaluated combinations of different antimicrobial agents for potential synergistic effects against these organisms, relying mostly on time-kill methods. It should be noted, however, that in most synergy studies the most frequent compounds used were the polymyxins (polymyxin B and polymyxin E).

In 2005, Bratu and colleagues found that polymyxin B, at 0.5 times its MIC, exhibited synergistic activity with rifampin against 15 of 16 KPC-positive *K. pneumoniae* isolates. A synergistic effect was also seen in 10 of these isolates when polymyxin B was combined with imipenem (30). In a later study, it was found that a triple-drug combination of polymyxin B with rifampin and doripenem at 1/4 their MICs exhibited high bactericidal activity, defined as a decrease of ≥3 log CFU/ml in 24 h, for five *E. coli* and two *K. pneumoniae* clinical isolates, all producing KPC-type enzymes (250). Similarly, the combination of colistin (polymyxin E) and tigecycline has been reported as synergistic against KPC-positive *K. pneumoniae* isolates in time-kill experiments (222). Interactions of colistin and imipenem were also examined in time-kill experiments with 42 VIM-producing *K. pneumoniae* isolates from a Greek hospital (241). In general, the combination of imipenem with colistin exhibited improved bactericidal activity against isolates that were susceptible either to both agents or to colistin alone. More specifically, the combination was synergistic against 50% of the colistin-susceptible isolates and indifferent against the remaining 50%, irrespective of the imipenem MIC. In contrast, for isolates that were nonsusceptible to colistin, the combination was antagonistic for 55.6% of the isolates and synergistic for only 11% of the isolates. These data are partly reminiscent of those reported by Elemam et al. (79), who examined the interactions of polymyxin B with several antimicrobials against 12 KPC-producing *K. pneumoniae* isolates that had elevated polymyxin B MIC values, using a broth microdilution assay in a checkerboard pattern. Synergy was observed with the combinations of polymyxin B plus rifampin and polymyxin B plus doxycycline, at achievable serum drug concentrations for the antimicrobial agents tested. Less pronounced synergy was noted with polymyxin B and tigecycline, whereas no synergy was evident between polymyxin B and the other antimicrobial agents tested, including imipenem and gentamicin.

Given that fosfomycin retains its activity against the majority of CPE, it is reasonable to consider administering this compound against CPE infections, but always in combination with another antimicrobial agent, as the rate of mutation to fosfomycin resistance is worringly high (188). Recently, the interactions of fosfomycin with meropenem, colistin, and gentamicin against KPC-positive *K. pneumoniae* isolates were studied using time-kill experiments (238). Combinations of fosfomycin with meropenem and colistin were synergistic in 64.7 and 11.8% of KPC-producing *K. pneumoniae* isolates, respectively, whereas the combination with gentamicin was indifferent. In addition, combinations of fosfomycin with meropenem, colistin, and gentamicin prevented the development of resistance to fosfomycin in 69.2, 53.8, and 81.8% of examined isolates, respectively. Similar results were obtained by another study that demonstrated synergy of fosfomycin with imipenem, meropenem, doripenem, colistin, netilmicin, and tigecycline for 74, 70, 74, 36, 42, and 30% of 50 KPC-producing *K. pneumoniae* isolates, respectively (228).

Time-kill assays have also been used to comparatively assess the activities of aztreonam and carbapenems. As mentioned previously, aztreonam is not hydrolyzed by MβLs and therefore is a potentially useful agent against MβL producers. A time-kill study assessed the *in vitro* activity of aztreonam in comparison to carbapenems against VIM-1-producing ESBL-negative *K. pneumoniae* isolates (197). Aztreonam exhibited slow bactericidal activity that was sustained for 24 h, whereas carbapenems resulted in more rapid bacterial killing for the first 6 h but regrowth to the level of antibiotic-free controls at 24 h.

In Vitro Pharmacodynamic Models

In a chemostat model simulating human pharmacokinetics, it was shown that optimized doses of meropenem (simulation of 2 g every 8 h, infused over 3 h, in humans) can achieve bactericidal activity against KPC-producing *K. pneumoniae* isolates with low meropenem MICs, despite the presence of an active carbapenemase. In this model, actual meropenem concentrations were significantly lower than intended, presumably due to rapid *in vitro* hydrolysis of meropenem by the released KPC enzyme. Despite this situation, meropenem achieved a rapid, ≥3-log CFU reduction of all KPC isolates within 6 h, but this effect was maintained for only two of the three KPC-producing isolates (with meropenem MICs of 2 and 8 μg/ml) for which adequate drug exposure had been attained (32).

The effect of tigecycline alone or in combination with meropenem was assessed in an *in vitro* pharmacodynamic model simulating human epithelial lining fluid drug concentrations against five KPC-producing *K. pneumoniae* isolates displaying meropenem MICs between 8 and 64 μg/ml and tigecycline MICs between 1 and 2 μg/ml. Tigecycline alone did not produce a reduction in bacterial density in any of the isolates studied, except for one with a tigecycline MIC of 1 μg/ml, in which an initial reduction was nevertheless followed by rapid regrowth. Meropenem alone, on the other hand, produced a rapid bactericidal effect for isolates with meropenem MICs of 8 and 16 μg/ml, but this effect was not maintained and was also followed by regrowth. Unlike monotherapy with tigecycline or meropenem, their combination caused a significant reduction in CFU/ml at 24 and 48 h for isolates with tigecycline and meropenem MICs of ≤2 and ≤16 μg/

ml, respectively, compared to the case with either agent alone. None of the studied regimens, however, was able to maintain a significant bactericidal effect for periods over 48 h (262).

Experimental Infection Models

Several investigators have examined the efficacy of different agents, alone or in combination, against CPE isolates by using different experimental infection models. Daikos et al. (66) assessed the activity of two dosing regimens of imipenem (30 and 60 mg/kg of body weight every 2 h [q2h]) against VIM-1-producing *K. pneumoniae* isolates in the neutropenic murine thigh infection model. Animals were infected with three VIM-1-positive isolates (with imipenem MICs of 2, 4, and 32 μg/ml) and a susceptible clinical isolate (with an imipenem MIC of 0.125 μg/ml) not producing any β-lactamase with broad-spectrum activity. The bactericidal effect was greatest against the susceptible non-VIM-1-producing isolate, intermediate against the "susceptible" VIM-1 producers (imipenem MICs of 2 and 4 μg/ml), and minimal against the resistant VIM-1 isolate (imipenem MIC of 32 μg/ml). However, with administration of a higher dose of imipenem (60 mg/kg q2h) and attainment of a drug exposure (cumulative percentage of a 24-h period that the drug concentration exceeds the MIC under steady-state pharmacokinetic conditions [$\%T_{MIC}$]) of approximately 40%, a more pronounced antibacterial effect against all VIM-1-producing isolates, including the highly resistant one, was achieved.

The use of carbapenems in the treatment of CPE infections was pursued further by Bulik and Nicolau (34), who evaluated the efficacy of doripenem against KPC-producing *K. pneumoniae* isolates with MICs ranging from 4 to 32 μg/ml in both immunocompetent and neutropenic mice. In these experiments, the authors used doripenem doses simulating human pharmacokinetics observed after administration of 1 or 2 g every 8 h as a 4-h infusion. The 1-g dose simulation was able to produce only a bacteriostatic response for the isolates with MICs of 4 and 8 μg/ml, whereas the 2-g dose simulation achieved a similar effect for isolates with MICs of up to 16 μg/ml. Relative to neutropenic mice, a reduction in bacterial density was observed in the immunocompetent animals, with overall decreases of up to 1 log, with either the 1- or 2-g doripenem dose simulation. A critical interpretation of the animal infection model data just summarized suggests that optimized regimens of carbapenems are able to achieve at least a static effect in severely compromised hosts and a modest bactericidal effect in immunocompetent animals infected with KPC-positive isolates with MICs of up to 8 μg/ml.

The efficacy of carbapenems and aztreonam was also evaluated in a rabbit model of peritoneal abscess caused by an ESBL-negative VIM-producing *E. coli* isolate. MICs of imipenem, meropenem, ertapenem, and aztreonam were 1, ≤0.25, 1.5, and ≤0.25 μg/ml, respectively. Carbapenems and aztreonam were shown to be effective in the treatment of this infection with regard to reductions in bacterial densities and mortality of the animals compared with those of untreated controls. Aztreonam, however, resulted in a more favorable outcome overall than that seen with carbapenems (239).

Comments on Experimental Studies

The number of studies assessing the interaction of antimicrobials with CPE in the laboratory, whether using time-kill assays or experimental animal infections, is remarkably small. In addition, clinically important CPE, such as those producing NDM-type MβLs or OXA-48, have not yet been studied in this manner. It is therefore obvious that additional studies of this kind are required, given the extent and severity of the problem posed by CPE. The synergy data from time-kill studies present some discrepancies. These data should therefore be interpreted with caution, since slight differences in experimental conditions (e.g., a relatively small change in the MIC fraction for one or more drugs) could result in significant changes in the apparent effect of a given combination. Despite these limitations, however, the data from time-kill studies indicate a variety of antibiotic combinations with potential synergistic effects against CPE.

In pharmacodynamic models and, most importantly, experimental infections in animals, the antibiotics preferably evaluated so far have been the carbapenems. This might appear unexpected, since therapy with carbapenems in the majority of human infections caused by CPE would be considered "inappropriate" based on MICs. Yet the relevant data, though limited, may be taken as indicating that approaches such as modification of dosing schemes warrant further attention.

ANTIMICROBIAL THERAPY

In studies examining the outcomes of CPE infections, older age, severity of underlying illness, comorbid conditions of the host, intensive care unit (ICU) stay, resistance to carbapenems, and administration of inappropriate antimicrobial treatment (partly due to CPE multidrug resistance compromising empirical therapy) are the most important independent predictors of treatment failure (14, 67, 175, 205, 233, 274). In the absence of controlled comparative trials, however, an overall critical appraisal of antibiotic treatment schemes inevitably has to be based on a variety of case reports, case series, retrospective studies, and observational studies. Moreover, these studies are focused on *K. pneumoniae*, as clinical experience with other CPE is quite limited. Therefore, the assessment we attempt here lacks many of the characteristics of a rigorous meta-analysis but may provide some guidance on the treatment of CPE-infected patients.

Review of Clinical Studies

We performed a systematic search of MEDLINE and compiled 34 studies containing the necessary information to estimate the efficacies of different antimicrobials in relation to their MICs for the infecting organisms (Tables 3 and 4). A total of 301 patients were identified, including 161 infected with KPC-producing *K. pneumoniae* and 140 infected with MβL-producing *K. pneumoniae*. The vast majority of these patients had serious infections: 244 had bloodstream infections (BSIs), 32 had pneumonia, 8 had urinary tract infections, 4 had tracheobronchitis, 3 had wound infections, and 7 had other infections. Three patients reported as having urinary colonization were excluded. Of the remaining 298 patients, 242 (81.1%) received appropriate therapy (with at least one drug to which the infecting organism was classified as susceptible *in vitro*), while 56 (18.9%) received inappropriate therapy (no drug to which the infecting organism was classified as susceptible *in vitro*). To facilitate comparisons, patients were classified into seven groups according to treatment regimen, as follows: regimen A, combination therapy with ≥2 active drugs, one of which was a carbapenem; regimen B, combination therapy with ≥2 active drugs, not including a carbapenem; regimen C, monotherapy with an aminoglycoside; regimen D, monotherapy with a carbapenem;

TABLE 3 Clinical studies, antimicrobial therapies, and outcomes for patients infected with MβL-producing *K. pneumoniae*

Reference	Country (yr of publication)	Study design	No. of patients with indicated type of infection	Type of MBL (no. of isolates)	Treatment (no. of patients)	Outcome (no. of successes/ no. of failures)
121	Greece (2004)	Case reports	4 (2 BSIs, 1 case of mediastinitis, 1 bone infection)	VIM-1 (4)	Colistin (1)	1/0
86	Greece (2008)				Tigecycline (1)	1/0
56	Spain (2008)				Tigecycline-colistin (2)	1/1
223	Ireland (2010)					
269	Taiwan (2001)	Case series	3 BSIs	IMP-8 (3)	Carbapenem (3)	1/2
143	Taiwan (2004)	Case series	3 (2 pneumonias, 1 BSI)	IMP-type enzyme (3)	Carbapenem (1)	1/0
					Carbapenem-aminoglycoside (2)	2/0
240	Greece (2008)	Case series	17 (14 BSIs, 3 pneumonias)	VIM-1 (17)	Colistin (6)	6/0
					Tigecycline (1)	0/1
					Colistin-aminoglycoside (2)	2/0
					Colistin doxycycline (1)	0/1
					Carbapenem-colistin (6)	5/1
					Carbapenem-aminoglycoside-doxycycline (1)	1/0
175	Greece (2010)	Case-control study	18 BSIs	VIM-1 (17)	Colistin (10)	6/4
				VIM-type enzyme (1)	Colistin-aminoglycoside (8)	4/4
64	Greece (2007)	Retrospective study	28 BSIs	VIM-1 (28)	Carbapenem (8)	7/1
					Colistin (4)	0/4
					Aminoglycoside (3)	2/1
					Carbapenem-aminoglycoside (6)	6/0
					Carbapenem-colistin (1)	1/0
					Aztreonam-aminoglycoside (2)	1/1
					No active drug (4)	2/2
67	Greece (2009)	Prospective observational study	67 BSIs	VIM-1 (67)	Carbapenem (14)	11/3
					Carbapenem-colistin (8)	8/0
					Carbapenem-aminoglycoside (4)	3/1
					Colistin (15)	11/4
					Aminoglycoside (8)	5/3
					No active drug (18)	13/5

regimen E, monotherapy with tigecycline; regimen F, monotherapy with colistin; and regimen G, inappropriate therapy (Fig. 2). It should be noted that the carbapenem susceptibility status was taken as reported in relevant studies in which the previous CLSI interpretive criteria were applied (54).

The lowest failure rate (8.3%) was observed for patients who received combination therapies including a carbapenem (regimen A). In addition, the therapeutic efficacy of this regimen was superior to those of regimens B, E, F, and G (for A versus B, the *P* value is 0.02, the odds ratio [OR] is 4.4, and the 95% confidence interval [95% CI] is 1.19 to 16.19; for A versus E, the *P* value is 0.03, the OR is 6.11, and the 95% CI is 1.22 to 30.58; for A versus F, the *P* value is <0.0001, the OR is 9.84, and the 95% CI is 2.76 to 35.03; and for A versus G, the *P* value is <0.0001, the OR is 11.81, and the 95% CI is 3.24 to 43.06). Combination therapy not including a carbapenem (regimen B), as well as monotherapy with either an aminoglycoside (regimen C) or a carbapenem (regimen D), was nevertheless effective compared to inappropriate therapy (for B versus G, the *P* value is 0.014, the OR is 2.68, and the 95% CI is 1.26 to 5.73; for C versus G, the *P* value is 0.04, the OR is 3.44, and the 95% CI is 1.11 to 10.67; and for D versus G, the *P* value is 0.03, the OR is 2.79, and the 95% CI is 1.14 to 6.86). On the other hand, treatment with tigecycline and colistin as single active agents resulted in failure rates comparable to that observed for patients who received inappropriate therapy (Fig. 2). These observations raise concerns about the use of tigecycline or colistin as a single agent in the treatment of serious carbapenemase-producing *K. pneumoniae* infections and support the notion of administering drug combinations preferentially including a carbapenem when susceptibility data allow.

The limited efficacy of tigecycline revealed by the present analysis is in line with the recent warning issued by the U.S. Food and Drug Administration (FDA) against the use of this agent for serious infections (91a). The FDA, in a pooled analysis of 13 clinical trials, found an increased mortality risk associated with the use of tigecycline compared to other drugs to treat a variety of serious infections. A higher mortality rate was seen most clearly for patients treated for ventilator-associated pneumonia and bacteremia (9/18 [50.0%] tigecycline-treated patients versus 1/13 [7.7%] comparator drug-treated patients). The cause of excess death in these trials most likely was related to progression of the infection. Similarly, in a recent meta-analysis including 15 ran-

TABLE 4 Clinical studies, antimicrobial therapies, and outcomes for patients infected with KPC-producing *K. pneumoniae*

Reference	Country (yr of publication)	Study design	No. of patients with indicated infection	Type of β-lactamase (no. of isolates)	Treatment with active drug (no. of patients)	Outcome (no. of successes/ no. of failures)
252	Colombia (2006)	Case reports	23 (10 BSIs, 10 pneumonias, 1 endocarditis, 1 liver abscess, 1 empyema)	KPC-2 (19)	Carbapenem (4)	3/1
153	USA (2006)			KPC-3 (2)	Colistin (3)	2/1
257	China (2007)			KPC-type enzyme (2)	Tigecycline (1)	0/1
71	USA (2007)				Aminoglycoside (2)	1/1
6	USA (2008)				Tigecycline-colistin (2)	1/1
162	China (2008)				Tigecycline-aminoglycoside (1)	1/0
81	USA (2008)				Colistin-aminoglycoside (1)	1/0
159	USA (2009)				Aminoglycoside-fluoroquinolone (1)	1/0
17	Israel (2009)				Carbapenem-aminoglycoside (1)	1/0
158	USA (2009)				No active drug (7)	1/6
80	USA (2009)					
116	USA (2010)					
138	Brazil (2011)					
52	Taiwan (2011)					
10	Switzerland (2011)					
113	USA (2011)					
25	USA (2004)	Case series	4 (1 BSI, 2 urinary tract infections [UTIs], 1 pneumonia)	KPC-2 (4)	Carbapenem (1)	1/0
					Carbapenem-colistin (1)	1/0
					Carbapenem-aminoglycoside (1)	1/0
					Colistin (1)	0/1
258	USA (2009)	Case series	21 (5 pneumonias, 5 BSIs, 4 cases of tracheobronchitis, 5 UTIs, 1 case of meningitis, 1 surgical site infection [SSI])	KPC-3 (21)	Carbapenem (4)	2/2
					Tigecycline (5)	4/1
					Aminoglycoside (3)	3/0
					Carbapenem-tigecycline (1)	0/1
					Tigecycline-aminoglycoside (1)	1/0
					No active drug (7)	3/4
154	Greece (2009)	Case series	13 (9 pneumonias, 4 BSIs)	KPC-2 (13)	Aminoglycoside (2)	2/0
					Tigecycline-colistin (8)	6/2
					Colistin-aminoglycoside (3)	3/0
83	USA (2009)	Case series	7 (3 BSIs, 1 UTI, 3 urinary colonizations)	KPC-2 (1)	Colistin (1)	0/1
				KPC-3 (6)	Colistin-aminoglycoside (2)	0/2
					No active drug (4)	0/4
182	USA (2009)	Case series	3 BSIs	KPC-2 (3)	Tetracycline-aminoglycoside (1)	1/0
					Colistin (3)	1/2
237	Greece (2010)	Case series	17 (11 BSIs, 2 SSIs, 1 UTI, 2 pneumonias, 1 case of cholangitis)	KPC-2 (17)	Colistin (11)	6/5
					Tigecycline (1)	1/0
					Aminoglycoside (1)	1/0
					Colistin-aminoglycoside (2)	1/1
					Tigecycline-colistin-aminoglycoside (1)	1/0
					No active drug (1)	1/0

175	Greece (2010)	Case-control study	19 BSIs	KPC-2 (19)	Colistin (10)	2/8
					Colistin-aminoglycoside (9)	4/5
274	Greece (2011)	Case-control study	53 BSIs	KPC-2 (53)	Carbapenem (1)	0/1
					Colistin (7)	3/4
					Tigecycline (5)	3/2
					Aminoglycoside (2)	2/0
					Colistin-aminoglycoside (2)	2/0
					Carbapenem-aminoglycoside (1)	1/0
					Tigecycline-colistin (9)	9/0
					Tigecycline-aminoglycoside (4)	4/0
					Carbapenem-tigecycline (1)	1/0
					Carbapenem-tigecycline-colistin (2)	2/0
					Tigecycline-colistin-aminoglycoside (1)	1/0
					No active drug (18)	7/11

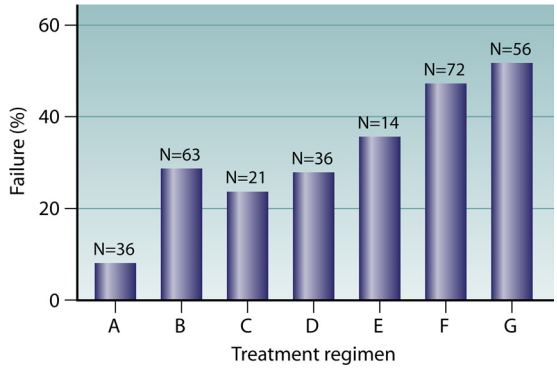

FIG 2 Outcomes of infections caused by carbapenemase-producing *Klebsiella pneumoniae*, according to treatment regimen. Regimen A, combination therapy with ≥2 active drugs, one of which was a carbapenem; regimen B, combination therapy with ≥2 active drugs, not including a carbapenem; regimen C, monotherapy with an aminoglycoside; regimen D, monotherapy with a carbapenem; regimen E, monotherapy with tigecycline; regimen F, monotherapy with colistin; regimen G, inappropriate therapy. Regimen A was superior to regimens B, E, F, and G (for A versus B, E, F, and G, the *P* value was 0.02, 0.03, <0.0001, and <0.0001, respectively). Regimens B, C, and D were superior to regimen G (for B versus G, $P = 0.014$; for C versus G, $P = 0.04$; and for D versus G, $P = 0.03$).

domized clinical trials, the overall mortality was higher for patients treated with tigecycline than for those treated with other antibacterial agents, including levofloxacin, carbapenems, ceftriaxone, and ampicillin-sulbactam (267).

The decreased clinical effectiveness of tigecycline in severe infections could be attributed partly to the pharmacokinetic/pharmacodynamic (PK/PD) profile of the drug. Tigecycline demonstrates mainly bacteriostatic activity against Gram-negative organisms, and the attainable drug concentrations at several anatomic sites are suboptimal. The peak serum concentrations achieved with the standard dosing regimen of the drug (50 mg twice daily) range from 0.6 to 0.9 μg/ml, while those attained in the urine and in the epithelial lining fluid are severalfold lower (2, 36, 88, 210). The drug concentrations attainable by this standard dosing regimen, combined with this drug's MIC profile for current CPE isolates, render it unlikely for tigecycline to cure CPE infections at anatomic sites where drug concentrations are suboptimal. Therefore, this drug should be used with caution against CPE, preferentially in combination with another active agent and after due consideration of the attainable drug concentration at the anatomic site of infection and of the MIC for the infecting organism.

Rather disappointing results were also observed with colistin monotherapy, since 34 of 72 (47.2%) colistin-treated patients had adverse outcomes. The poor performance of colistin monotherapy against CPE infections has also been noticed previously (112). Nevertheless, when colistin was combined with tigecycline or an aminoglycoside, the failure rate decreased to 32% (17 of 53 patients failed treatment). More impressively, however, when it was combined with a carbapenem, the failure rate decreased dramatically, to 5% (1 of 17 patients failed treatment). The inferior clinical efficacy of colistin monotherapy may be associated, among other factors, with a suboptimal dosing regimen of the drug. In a retrospective study that evaluated patients with multidrug-resistant Gram-negative infections who received several daily dosages of colistin, multivariate analysis of survival data

TABLE 5 Results of carbapenem monotherapy in 50 CPE-infected patients from 15 studies[a]

MIC of carbapenem (μg/ml)	No. of patients	No. of successes	No. of failures	% Failure
≤1	17	12	5	29.4
2	12	9	3	25.0
4	7	5	2	28.6
8	6	4	2	33.3
Subtotal	42	30	12	28.6[b]
>8	8	2	6	75.0[b]
Total	50	32	18	36

[a] See references 25, 64, 67, 81, 113, 143, 153, 159, 162, 240, 252, 257, 258, 269, and 275.
[b] $P = 0.02$, odds ratio = 7.5, and 95% confidence interval = 1.32 to 42.52.

showed that a lower total daily dosage of intravenous colistin was associated with increased mortality (90). It is therefore critical to administer an adequate total daily dosage of colistin to critically ill patients, particularly to those who are on renal replacement therapy, in order to accomplish efficacious levels according to current recommendations (97). An additional factor that could be detrimental to a patient's outcome is the delay in attaining an efficacious drug concentration with the standard treatment regimen of colistin. This could be overcome by administering a loading dose of the drug (211).

Although colistin has been used extensively in critically ill patients infected with multidrug-resistant Gram-negative organisms, its optimum dosing regimen remains to be defined. Animal infection models have shown that the ratio of the area under the concentration-time curve for the free, unbound fraction of the drug (*f*AUC) to the MIC is the PK/PD index that is linked most strongly to an antibacterial effect, indicating the importance of achieving adequate time exposure to colistin across the day by administering the drug twice or three times a day (77, 78). In contrast, however, several features of this drug, such as its prolonged half-life, its concentration-dependent killing, and a phenomenon known as "adaptive resistance" that has not been appreciated adequately (50, 68, 92, 236), favor a once-daily dosing regimen, provided that such a scheme is not proven to be more nephrotoxic. Thus, a better understanding of the complex PK/PD features of colistin will be essential in devising dosing regimens with improved efficacy against CPE infections.

Among the publications available in MEDLINE, we were able to identify 15 studies reporting on 50 patients infected with carbapenemase-positive *K. pneumoniae*, all of whom had received carbapenem monotherapy (meropenem or imipenem). Twenty-nine of the respective isolates exhibited carbapenem MICs of ≤2 μg/ml. In seven and six isolates, the MICs were equal to 4 and 8 μg/ml, respectively. The remaining eight isolates were inhibited *in vitro* by carbapenem concentrations of >8 μg/ml. Note that, as indicated by the reported outcomes of these patients, the therapeutic efficacy of carbapenems increased from 25% for a MIC of >8 μg/ml to 66.7% for a MIC of 8 μg/ml, 71.4% for a MIC of 4 μg/ml, and 72.4% for a MIC of 2 μg/ml or less (Table 5). Clinical experience with carbapenem monotherapy is indeed limited. Yet we may consider the above data to indicate that carbapenems could provide some therapeutic benefit in infections caused by carbapenemase-producing *K. pneumoniae*, even for strains with intermediate susceptibility to carbapenems. It should be pointed out here that these observations do not contradict the findings of the experimental infection models discussed herein or those of human PK/PD studies (34, 118, 131, 145). Carbapenems display time-dependent bactericidal killing when free drug concentrations remain above the MIC for 40 to 50% of the time between dosing intervals. The probability of attaining a 50% T_{MIC} target for an isolate with a MIC of 4 μg/ml is 69% for the traditional dosing regimen (e.g., 30-min infusion of 1 g every 8 h for meropenem) and increases to 100% for the high-dose/prolonged-infusion regimen (e.g., 3-h infusion of 2 g every 8 h for meropenem). For a MIC of 8 μg/ml, only the high-dose/prolonged-infusion regimen displays a relatively high probability (85%) of bactericidal target attainment (131).

Whether we can use carbapenems in the presence of a carbapenemase is an issue that remains to be answered (65, 245). However, faced with the daily challenge of managing critically ill patients and the dearth of alternative therapeutic options, some of which have not been investigated satisfactorily and/or whose efficacy in certain situations remains questionable, use of a carbapenem against an organism with a MIC of ≤4 or even ≤8 μg/ml, using a high-dose/prolonged-infusion regimen and in combination with another active agent, preferentially gentamicin or colistin, seems reasonable.

The number of CPE isolates exhibiting resistance to almost all available agents is worryingly high in various settings (85). Given that fosfomycin displays good *in vitro* activity against most CPE, this agent could be selected as salvage therapy in situations where therapeutic options are very limited (89). Although the main indication of fosfomycin remains the treatment of lower urinary tract infection, some investigators have included this drug in various combination schemes to treat systemic infections caused by CPE (87, 164). Available data, however, are too limited to allow a sound hypothesis as to its efficacy. Also, the potential of fosfomycin to rapidly select resistant mutants during therapy is a matter of consideration (188).

The clinical data reviewed here allow for some reasonable notions but not for solid conclusions, since it was not possible to measure and adjust for certain important variables (e.g., host-related factors, severity of infections, and dosing and timing of initiation of treatment). Thus, we cannot exclude the possibility that our analysis, in some cases, might have resulted in biased associations between antimicrobial treatment and outcome. Nevertheless, given that the majority of patients infected with CPE are debilitated, with various underlying diseases, and that more than 90% of them have severe infections (BSIs or pneumonias), it is unlikely that residual confounding could account to an appreciable extent for the significantly different failure rates between treatment groups.

CPE IN HEALTH CARE SETTINGS

Epidemiology

The prevalence of CPE, primarily *K. pneumoniae*, in several institutions in areas of endemicity may vary between 20 and 40% (28, 99, 133, 233). Initially, CPE appeared to cause hospital-acquired infections, mainly in ICU patients (28, 29, 67, 233, 265). More recently, however, they have spread in different health care settings, including long-term care facilities (LTCF) (16, 53, 83, 161, 249).

Several investigators have evaluated the factors associated with

increased risk for acquisition of CPE in the hospital setting (69, 91, 98, 106, 117, 132, 205, 233, 261). Investigators from Israel have shown that poor functional status of the host, prior antibiotic therapy, and stay in the ICU are independent risk factors for colonization or infection with carbapenem-resistant *K. pneumoniae* (233). Other factors that have been associated with CPE include solid organ or stem cell transplantation (122, 204), presence of a biliary catheter (120), multiple invasive devices (157), prior surgery, and the presence of wounds (106). Antibiotic selection pressure may be an additional factor that influences colonization with these organisms. Case-control studies have shown that almost every class of antibiotics can select for CPE (69, 91, 98, 117, 120, 132, 203, 261). What appears to be more important, however, is the cumulative number of prior antibiotic exposures rather than the use of a specific class of antibiotics (69, 205).

Since members of the *Enterobacteriaceae* constitute part of the human enteric flora, once CPE colonize the intestinal tract, carriage may persist for a long time (232). Based on limited experience (90, 116, 207), it appears that colonization with CPE is prolonged and lasts at least several months. A prolonged duration of colonization means a larger reservoir of colonized patients, exerting more colonization pressure, which in turn will result in higher rates of patient-to-patient transmission. CPE cross-transmission occurs more efficiently in health care settings where infection control practices are poor. Indeed, in a surgical unit where hand hygiene compliance was 21%, the probability of a patient becoming colonized with CPE was 7.1% per week of hospitalization, and the incidence of new acquisitions was 9.1/1,000 patient-days (39). It can be supported that once the first case of CPE infection is recognized in a health care facility, these organisms may have already spread widely and colonized a substantial number of patients. Colonization may be extensive and pass largely unnoticed in institutions located in regions of endemicity, as evident in several studies. Calfee et al. (41) reported that 37% of patients with carbapenem-resistant *K. pneumoniae* colonization were first identified by surveillance cultures. During an outbreak of carbapenemase-producing *K. pneumoniae* in Israel, a point prevalence survey demonstrated that 16 (5.4%) of 298 patients screened were colonized with carbapenemase producers, and notably, 11 (69%) of these carriers would have remained undetected without the performance of active surveillance cultures (261).

More importantly, CPE colonization may evolve to infection, with detrimental effects for the host (23, 41, 261). Although data regarding the infection/colonization ratio are very limited, it is estimated that a proportion of colonized patients (10 to 30%) will develop CPE infection. This proportion is probably related to the severity of the underlying disease of the host and appears to be higher for severely immunocompromised patients (e.g., patients in induction chemotherapy for acute myelogenous leukemia or post-allogeneic stem cell transplantation).

Infection Control Strategies

The rapid and worldwide dissemination of a variety of CPE reflects, to various extents, increased antibiotic selection pressure, carriage of the acquired carbapenemase genes by mobile genetic units, and probably the enhanced spreading potential of specific clones, such as *K. pneumoniae* ST258. Notwithstanding these factors, the fact that CPE outbreaks occur principally in settings where infection control practices are inadequate shows that there remains plenty of room for curbing CPE spread.

CPE seem to have a high potential for spread not only from patient to patient within a health care facility but also through "cycling" of patients between institutions in the same region (263) and/or across borders from high- to low-prevalence countries, which, for instance, has happened repeatedly in Europe (107), threatening every health care system. To address this public health threat, it is imperative to formulate a preparedness plan before CPE have the opportunity to become endemic. In areas where this has already happened, on the other hand, control measures must include a multifaceted approach coordinated by the national health authorities, as indicated in a recent study from Israel (234).

In response to this need, both the Centers for Disease Control and Prevention (CDC) and a group of experts from the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) have published guidelines for interventions to control CPE transmission in acute health care facilities (44, 48). These recommendations are based primarily on experience with other MDR organisms (www.cdc.gov/hicpac/mdro/mdro_0.html) and typically include detection, isolation or cohorting, and other enhanced infection control measures. It is expected that the increasing number of studies dealing with CPE epidemiology will soon lead to guidelines specifically targeting CPE.

Tracing of Carriers

Critical to the success of interrupting cross-transmission of CPE in a health care facility is the timely identification of colonized and/or infected patients. Thus, every clinical microbiology laboratory should establish a reliable detection methodology. Additionally, resources and trained personnel should be readily available to carry out point prevalence surveys as well as active surveillance cultures, a demanding yet highly effective approach for detecting carriers (15, 53, 177). The most suitable anatomic sites for surveillance cultures appear to be the perianal area and the rectum (31, 261). In patients with surgical wounds, decubitus ulcers, a urinary catheter, or bronchial secretions, the respective sites could also be screened. Several versions of culture-based techniques have been described. In some of them, differences are limited to the concentration of carbapenem used for the initial screening, with the lowest being that proposed by EUCAST (0.25 μg/ml of meropenem), based on an epidemiologic cutoff. Additionally, several PCR-based techniques for active surveillance have been described (111, 135, 231). PCR assays are rapid and usually more sensitive than culture-based methods. However, their main disadvantages are that they do not provide information on the carbapenemase-producing species and can assess the presence of only already known resistance genes.

In settings with low CPE prevalence, laboratories should monitor clinical culture results to determine whether CPE have been isolated in the facility (48). If a CPE is identified by clinical culture, a point prevalence survey should be performed in selected wards (e.g., ICUs and units where CPE have been identified). Detection of additional CPE carriers should then be followed by active surveillance covering a wider range of patients with potential epidemiological links to persons from whom CPE have been isolated [e.g., patients from the same unit and patients cared for by the same health care worker(s)]. Active surveillance should be continued until no new CPE cases are identified.

In areas where CPE are endemic, an increased likelihood exists for importation of CPE into a previously CPE-free health care facility. Upon admission of patients at increased risk of CPE car-

TABLE 6 Synopsis of 11 successful infection control studies for CPE infections in nonendemic and endemic settings

Reference	Study design	Health care setting and geographic region	Infection control measures		Outcome
			Baseline	Additional	
Studies in regions where CPE are not endemic					
110	Retrospective	36-bed ICU in a tertiary care hospital, Melbourne, Australia	1. Surveillance of culture results 2. Standard precautions 3. Environmental cleaning	1. Universal contact precautions in ICU 2. Single-room isolation of CPE patients (all wards) 3. Restriction of carbapenem use	Decrease of CPE cases from 3 to 1 per month
122	Retrospective	Abdominal surgery center, Paris, France	1. Active surveillance for ESBLs 2. Isolation of CPE patients 3. Contact precautions 4. Environmental disinfection 5. Active surveillance	1. Preemptive isolation of contact patients and newly admitted patients 2. Dedicated nursing staff 3. Limited transfer of CPE patients 4. Antibiotic restriction policy (imipenem) 5. Screening campaign targeting contact patients discharged from the hospital	Rapid control of the outbreak
43	Retrospective	7 hospitals, Paris, France	1. National early warning system for multiresistant isolates 2. Active surveillance for ESBLs 3. Active screening of contact patients 4. Evaluation of duodenoscope disinfection practices	1. Cohorting of CPE cases and contacts 2. Flagging of CPE cases 3. Dedicated health care workers 4. Reinforcing hand hygiene and contact precautions 5. Limited transfer of CPE cases and contacts 6. Revision of duodenoscope disinfection procedure	Rapid control of the outbreak
Studies in regions of endemicity					
127	Retrospective	10-bed ICU in a tertiary care hospital, New York, NY	1. Contact isolation of CPE patients 2. Environmental cleaning 3. Infection control supervising 4. Active surveillance for vancomycin-resistant enterococci and carbapenem-resistant *Acinetobacter*	1. Active surveillance for CPE on admission to ICU and weekly thereafter 2. ICU closure and disinfection 3. Cohorting of CPE patients 4. Dedicated nursing staff 5. Promotion of hand hygiene	Incidence decreased from 9.7 ± 2.2 to 3.7 ± 1.6 CPE cases per 1,000 patient-days
176	Retrospective	20-bed surgical ICU in a tertiary care hospital, Miami, FL	No data	1. Point prevalence surveillance 2. Isolation and contact precautions for CPE patients 3. Dedicated nursing staff 4. Daily chlorhexidine baths on all patients 5. Environmental cleaning after every shift and evaluation with environmental cultures 6. Educational campaigns	Control of CPE spread

177	Retrospective	70-bed long-term acute care hospital, Chicago, IL	1. Active surveillance on admission 2. Baseline point prevalence surveillance	1. Active surveillance cultures for CPE and point prevalence surveys during the intervention 2. Isolation and contact precautions for CPE patients 3. Preemptive isolation of high-risk patients 4. Environmental cultures and enhanced environmental cleaning 5. Daily chlorhexidine baths for all patients 6. Educational campaign	Colonization prevalence of CPE decreased progressively, from 21% to 12, 6, 3, and 0%
83	Retrospective	Long-term acute care hospital, South Florida	No data	1. Active surveillance culture 2. Point prevalence survey 3. Isolation and contact precautions for CPE patients 4. Dedicated nursing staff and equipment	Termination of the outbreak
106	Retrospective	Tertiary care hospital, Puerto Rico	No data	1. Contact precautions for CPE patients 2. Cohorting of CPE patients 3. Dedicated nursing staff 4. Hand hygiene audits 5. ICU closure 6. Restriction of broad-spectrum antibiotics 7. Active surveillance on admission to high-risk units (ICU, diabetes ward) and weekly thereafter	Control of the outbreak
15	Retrospective	Tertiary care hospital, Tel Hashomer, Israel	1. Contact precautions for CPE cases	1. Active surveillance on admission to ICU and in step-down units and weekly thereafter 2. In other departments, active surveillance of patients with epidemiologic links to CPE carriers 3. Daily reporting of CPE cases to hospital manager and the national coordinator	Incidence decreased from 6.93 to 1.8 CPE cases per 10,000 patient-days
53	Prospective intervention study	Tertiary care hospital, Rehovot, Israel	No data	1. Active surveillance on admission to ICU, in roommates of new CPE cases or carriers, and in patients at high risk for carriage 2. Isolation-cohorting and contact precautions for CPE cases and carriers 3. Dedicated nursing staff 4. Environmental cleaning and disinfecting during hospital stay and after discharge 5. Education and training to all medical staff members, patients, and caregivers 6. Automatic warning system	Incidence decreased from 8.2 to 0.5 CPE case per 10,000 patient-days
234	Prospective intervention study	27 acute care hospitals, Israel	No data	1. Isolation-cohorting and contact precautions for CPE patients and carriers 2. Dedicated nursing staff and equipment 3. Mandatory reporting to public health authorities of every CPE case 4. Establishment of Task Force on Antimicrobial Resistance and Infection Control	Monthly incidence decreased from 55.5 to 11.7 CPE cases per 100,000 patient-days

TABLE 7 Experimental antimicrobial agents active against carbapenemase-producing *Enterobacteriaceae*

Drug	Compound type	Relevant target	Reference
BAL30072	Siderophore-containing sulfactam	*Enterobacteriaceae*, including MβL producers	194
Plazomicin (ACHN-490)	Sisomicin derivative	Gram-negative organisms, including carbapenemase producers	149
GSK2251052	Leucyl-tRNA synthetase inhibitor	Gram-negative organisms, including carbapenemase producers	5

riage (such as residency in an LTCF, previous stay in an ICU, prolonged hospitalization in the previous 6 months, or presence of indwelling devices), preemptive isolation while awaiting surveillance culture results can prevent early transmission events (19, 43, 53, 122). Performing a surveillance culture before the discharge of a patient into the community or an LTCF may also be useful to avoid transfer of CPE to additional niches (83, 122). It is also important to communicate the results of screening and to provide alerts for previously identified CPE carriers for every readmitted patient. The surveillance strategy should be defined clearly for each setting and evaluated periodically according to the current situation and available resources (19, 48).

Intervention

When a CPE carrier is identified, the infection control personnel should be notified immediately. Isolation or cohorting of CPE carriers seems to be the main prevention measure (43, 83, 106, 110, 122, 127, 176, 177, 234). Assignment of dedicated health care workers and use of separate equipment for carriers are additional interventions that have been employed successfully in several outbreaks (19, 43, 122, 176, 177, 234). In the Israeli experience, physical separation of carriers from noncarriers and assignment of dedicated nursing staff to care for carriers on all shifts were the most important components of the intervention measures in halting transmission (234).

In addition to isolation, personnel in acute health care facilities should use contact precautions (wearing a gown and gloves) when caring for patients colonized or infected with CPE in order to minimize indirect transmission of the organism (44, 48; www.cdc.gov/hicpac/mdro/mdro_0.html). In LTCFs, it is only practical to apply contact precautions for those patients who are severely ill and have conditions that may facilitate transmission (e.g., diarrhea or decubitus ulcers) (83). The success of the intervention should be monitored constantly, and when failure is observed, a root cause analysis should be performed (19).

Environmental cleaning and decolonization of patients. Cleaning of the inanimate environment and equipment in proximity to a CPE carrier, along with daily antiseptic (chlorhexidine) baths to cleanse patients' skin, were included in the bundles of intervention measures that successfully controlled two recent outbreaks in the United States (176, 177). Available data do not allow for an assessment of the usefulness of the systematic application of these practices. Indeed, persisting environmental contamination with *Enterobacteriaceae* is limited compared to that of other organisms (144). In addition, culturing of surfaces and equipment to investigate their role in the transmission chain is not usually required, unless the inanimate environment or shared equipment is potentially linked to an outbreak (43). Such an event, however, does not seem to occur frequently with CPE. On the other hand, daily antiseptic baths have been proven efficacious in preventing central vascular catheter-associated bloodstream infections (178). This practice therefore appears to warrant further evaluation with respect to CPE-positive settings.

Selective decolonization of the gut by use of oral gentamicin in patients undergoing chemotherapy and allogeneic stem cell transplantation has achieved a 66% eradication rate of the CPE carrier state (278). This approach should be adopted cautiously, however, as gentamicin is commonly used for the treatment of CPE infections, and its excessive use may select CPE that are resistant to this drug as well.

Judicious antimicrobial use. The role of restriction of antibiotics in controlling CPE needs further evaluation. As mentioned previously, almost every antimicrobial class can select for CPE. In this regard, cumulative exposure to antibiotics is likely to be more important than selection exerted by a specific agent. Therefore, formulary interventions should focus on reduction of overall antibiotic usage (19).

Success Stories

There have been various successful attempts to control CPE outbreaks in both endemic and nonendemic settings. The relevant studies (summarized in Table 6) concern single-center outbreaks, except for one describing a countrywide epidemic in Israel (234). Although some differences in approach did exist, the interventions implemented were largely based on the rationale of infection control strategies mentioned previously, with their main components being surveillance cultures, isolation and cohorting, contact precautions, and assignment of dedicated staff.

The design of these studies does not allow us to accurately classify measures according to their effectiveness. Critical interpretation of the published data, however, suggests that application of a bundle of infection control measures may be required for maximum containment of CPE. Controlled studies and mathematical modeling of CPE transmission and prevention are needed to specify the most appropriate procedures for containment or even eradication of CPE.

NOVEL AGENTS AGAINST CPE

Antibiotics

Not unexpectedly, many new antibiotics under development target multidrug-resistant bacteria. The relatively small number of new antibacterials active against MDRs does not necessarily mean a lack of industry interest but rather reflects the countless difficulties posed from the early stages of designing to the introduction into clinical practice. Although not specifically focused on CPE, some of these experimental antibacterials, belonging to diverse antimicrobial classes, exhibit high activity against these microorganisms. We present below a few indicative compounds, preferentially those that are under advanced clinical testing (Table 7).

Sulfactams. The sulfactams comprise a distinct series of monocyclic β-lactams (with tigemonam being the first member) exhib-

TABLE 8 Experimental β-lactamase inhibitors active against carbapenemases from *Enterobacteriaceae*

Inhibitor	Compound type	Inhibition spectrum (β-lactamase classes)	Susceptible carbapenemases[a]	Reference(s)
BLI-489	Penem	A, C, D	KPC type	209
J-110,411 and J-111,225	1-β-Methyl carbapenem	A, C, B	IMP type	183, 184
Mercaptomethyl sulfones	C-6-substituted penicillin sulfone	B	VIM and IMP types	38
2,3-(*S*,*S*)-Disubstituted succinic acids	Succinic acid	B	IMP type	170
Thiomandelic acids	Thiol	B	VIM and IMP types	147, 268
Avibactam (NLX104)	Diazabicyclo-octanone	A, C, D	KPC type	22

[a] Only carbapenemase types with documented susceptibility to the respective inhibitor are included.

iting potent activity against the *Enterobacteriaceae*. Additionally, other structurally related monocyclic compounds, such as aztreonam, are virtually unaffected by the hydrolytic activity of MβLs. Of special interest is a novel group of siderophore-coupled sulfactams, represented by BAL30072 (194); the coupled siderophores enhance activity against MβL producers by facilitating the entrance rate through the outer membrane, even in strains with defective permeability (195). Animal experiments have further supported the therapeutic potential of BAL30072 against MβL-positive enterobacteria (168).

Plazomicin. Formerly known as ACHN-490, plazomicin is a sisomicin derivative with substitutions at positions 1 and 6 (a hydroxyaminobutyric and a hydroxyethyl group, respectively). This antibiotic resists most aminoglycoside-modifying enzymes (but not 16S rRNA methylases) and exhibits potent bactericidal activity against many MDRs, including CPE (149). It is currently undergoing a phase 2 study for use against complicated urinary tract infections (276).

Aminoacyl-tRNA synthetase inhibitors. Until recently, the aminoacyl-tRNA synthetase inhibitors included only mupirocin, an isoleucyl-tRNA synthetase inhibitor of limited clinical use. Development of novel boron-containing aminoacyl-tRNA synthetase inhibitors added compounds with kinetics suitable for systematic use that are also active against Gram-negative organisms. A promising compound, GSK2251052, which inhibits the leucyl-tRNA synthetase and is effective against CPE and other MDRs, is currently under clinical evaluation (5).

Carbapenemase Inhibitors

Despite almost 3 decades of efforts to develop β-lactamase inhibitors, only three, clavulanic acid, tazobactam, and sulbactam, have been made available as therapeutics. Fortunately, a number of potential β-lactamase inhibitors are under evaluation (for a comprehensive review, see reference 76). The majority of currently tested compounds are β-lactam derivatives, though a number of diverse non-β-lactam substances also exhibit significant inhibitory activity. Only compounds that also happen to inhibit acquired carbapenemases (mainly KPCs and MβLs) at clinically relevant concentrations are mentioned briefly here (Table 8).

Penem derivatives. The penem derivatives include the prototype, BRL 42715, and a series of heterocyclic methylidene penems with significant inhibitory activity against serine β-lactamases (molecular classes A, C, and D). BLI-489, a bicyclic derivative, has been shown to be capable of reducing the MICs of piperacillin against enterobacteria producing KPC enzymes (209).

1-β-Methylcarbapenems. 1-β-Methylcarbapenems have been synthesized by various substitutions in the carbapenem nucleus, which contains a methyl group at position C-1 (the same nucleus as that in doripenem). Interesting members of the group are compounds J-110,411 and J-111,225, which inhibit class A and C β-lactamases, as well as IMP-type MβLs, at low concentrations (183, 184).

Sulfones. C-6-substituted penicillin sulfones are primarily inhibitors of class A β-lactamases. However, compounds with a mercaptomethyl substituent at C-6 exhibit strong inhibitory activity against MβLs (38).

Succinic acids (non-β-lactams). Various succinic acid derivatives [2,3-(*S*,*S*)-disubstituted succinic acids] exhibit potent inhibitory activity against the IMP-type MβLs, restoring carbapenem susceptibility in members of the *Enterobacteriaceae* producing these enzymes (170).

Thiols (non-β-lactams). Thiol compounds, such as thiomandelic acids, are considered effective inhibitors of MβLs, especially those of the VIM and IMP types. They act by bridging the zinc ions of the active site, thus displacing the catalytic water molecule (147, 268).

Avibactam. Formerly known as NXL104, avibactam, a non-β-lactam compound, is likely to be the most promising experimental inhibitor and is expected to be introduced soon into antimicrobial chemotherapy. It is a bridged diazabicyclo (EC 3.2.1) octanone with excellent activity against virtually all serine β-lactamases but no activity against molecular class B enzymes (22). In the current era of carbapenemases, the ability of avibactam to inhibit KPC β-lactamases at very low concentrations (50% inhibitory concentration [IC_{50}] of 38 nM) is important (22, 242). MICs of various newer β-lactams tested against KPC producers in the presence of avibactam have clearly shown the *in vitro* efficacy of these combinations (82). Most importantly, the therapeutic efficacy of the ceftazidime-avibactam combination has been documented for murine infection models (259).

CONCLUDING REMARKS AND PERSPECTIVES

The current public health crisis due to the international spread of carbapenemase-producing multidrug-resistant enterobacteria has caught us unprepared, despite clear signs of this problem arising years ago. Most of the recent papers describing yet another emergence of a CPE or a CPE outbreak conclude, almost invariably, with the urgent need for measures to contain these microorganisms. This, however, begs the question: precisely what measures are to be taken? First of all, a clearer and more accurate picture of the situation at the global level, based on data that are valid for comparisons, is necessary. For instance, in developed countries, it appears that many studies have been conducted in single institutions or a small number of tertiary care hospitals and

cover limited times. Therefore, biased sampling is probable, at least in some cases. Moreover, systematic reports from many African countries, the Balkans, the Middle East, and vast areas in Asia are scarce, if they exist at all. The collection of epidemiological data that are as complete as possible is imperative for the implementation of effective yet affordable and sustainable measures against CPE, especially in the most affected countries. In this respect, if the expressed international concern is indeed genuine, resources from international public health organizations should be mobilized and allocated appropriately.

Some new drugs active against CPE are indeed in an advanced stage of development, but very few of them are expected to be clinically available soon. Thus, in the foreseeable future, we shall continue to rely on the available antibiotics. Nevertheless, the studies summarized above show that there is yet room to improve our therapeutic approaches. Colistin and tigecycline are among the most frequently used agents in the treatment of CPE infections. However, as discussed here, laboratory and clinical data supporting this practice are insufficient. Although it has to be admitted that alternative options have not yet been documented solidly, well-designed clinical trials aiming to (i) determine the optimum dosing regimen of colistin, (ii) define CPE infections that could be controlled effectively by tigecycline, (iii) exploit the PK/PD features of carbapenems, and (iv) unravel the most effective drug combinations may prove valuable.

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