Correlation of β -Lactamase Production and Colistin Resistance among *Enterobacteriaceae* Isolates from a Global Surveillance Program

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The increasing use of carbapenems for treating multidrug-resistant (MDR) Gram-negative bacterial infections has contributed to the global dissemination of carbapenem-resistant *Enterobacteriaceae* (CRE). Serine and metallo- β -lactamases (MBLs) that hydrolyze carbapenems have become prevalent and endemic in some countries, necessitating the use of older classes of agents, such as colistin. A total of 19,719 isolates of *Enterobacteriaceae* (excluding *Proteae* and *Serratia* spp., which have innate resistance to colistin) were collected from infected patients during 2012 and 2013 in a global surveillance program and tested for antimicrobial susceptibility using CLSI methods. Isolates of CRE were characterized for carbapenemases and extended-spectrum β -lactamases (ESBLs) by PCR and sequencing. Using EUCAST breakpoints, the rate of colistin susceptibility was 98.4% overall, but it was reduced to 88.0% among 482 carbapenemase-positive isolates. Colistin susceptibility was higher among MBL-positive isolates (92.6%) than those positive for a KPC (87.9%) or OXA-48 (84.2%). Of the agents tested, only tigecycline (MIC₉₀, 2 to 4 μ g/ml) and aztreonam-avibactam (MIC₉₀, 0.5 to 1 μ g/ml) consistently tested with low MIC values against colistin-resistant, ESBL-positive, and carbapenemase-positive isolates. Among the 309 (1.6%) colistin-resistant isolates from 10 species collected in 38 countries, 58 carried a carbapenemase that included KPCs (38 isolates), MBLs (6 isolates), and OXA-48 (12 isolates). These isolates were distributed globally (16 countries), and 95% were *Klebsiella pneumoniae*. Thirty-nine (67.2%) isolates carried additional ESBL variants of CTX-M, SHV, and VEB. This sample of *Enterobacteriaceae* demonstrated a low prevalence of colistin resistance overall. However, the wide geographic dispersion of colistin resistance within diverse genus and species groups and the higher incidence observed among carbapenemase-producing MDR pathogens are concerning.

Multidrug resistance (MDR) in Gram-negative bacilli has limited the options for treating serious infections and is responsible for increased morbidity and mortality rates worldwide (1). Over the last decade, carbapenem-resistant *Enterobacteriaceae* (CRE) have spread globally and now affect patient care in nearly all geographic regions (2). Resistance to carbapenems can be caused by several mechanisms; however, the resistance can most often be attributed to the carbapenemases, which include serine enzymes, such as the KPC and OXA-type enzymes, and the metallo- β -lactamases (MBLs), including IMP-, NDM-, and VIM-type enzymes. Because standard therapies usually include a β -lactam for treating Gram-negative bacterial infections, the limitations of treatment choices imposed by CRE require that alternative therapeutic approaches be considered, including polymyxins, fosfomycin, some aminoglycosides, and tigecycline, which are not affected by mechanisms of carbapenem resistance (3–5). These antimicrobial agents, including colistin, are now being used as last-in-line treatment options for MDR bacterial infections (6). Colistin (also known as polymyxin E) is a cationic antimicrobial peptide that disrupts both the outer and inner membranes of Gram-negative bacteria, and in recent years, it has emerged as a therapeutic option against CRE and MDR pathogens. Unfortunately, there is the potential for adverse outcomes when colistin therapy is relied on for the treatment of infections caused by CRE. These outcomes are a result of a lack of comprehensive coverage against all enteric species, the potential for epidemiological outbreaks, and safety concerns due to adverse toxicology. All these issues must be taken into consideration when selecting colistin as a therapeutic choice for CRE infections (5–8).

With the increased usage of colistin to treat serious infections, there are beginning to be reports of resistance to colistin

in isolates of CRE (9–11). Resistance can be caused by several mechanisms. It can be due to modifications of lipid A that alter the net charge of the cellular lipopolysaccharide (LPS), thereby reducing its affinity for polymyxins (12). In addition, mutations that activate the PhoPQ and PmrAB two-component signal transduction systems that regulate LPS modification, including alterations of the negative regulator encoded by *mgrB*, have been reported (12–15). Several species of *Enterobacteriaceae* are naturally resistant to polymyxin derivatives, including *Proteae* and *Serratia* spp., and studies have documented a higher prevalence of these pathogens following the increasing usage of colistin, which was associated with high mortality rates (16, 17). A recent study documented the *in vivo* evolution of a KPC-producing *Klebsiella pneumoniae* isolate to a stable colistin-resistant phenotype following low-dosage colistin use (13), and colistin heteroresistance among *Enterobacteriaceae* isolates following colistin therapy has also been reported (18). In addition to these resistance issues, related toxicity issues are also a concern (19). Furthermore, several focused studies have documented case reports and the possible hospital transmission of carbapenemase-producing, colistin-resistant *Enterobacteriaceae* (7, 8, 11). These reports provide grow-

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ing evidence of an increasing association of CRE and resistance to colistin. However, to date, the reports of colistin resistance have mainly been anecdotal.

This study evaluated the species associated with and the regional distribution of colistin-resistant *Enterobacteriaceae* collected globally over a 2-year period. The activities of colistin and comparator compounds were analyzed with subsets of β -lactamase-producing isolates. These molecularly characterized isolates with genes encoding carbapenemases and extended-spectrum β -lactamases (ESBLs) provided a better understanding of the activity of colistin against MDR Gram-negative pathogens.

MATERIALS AND METHODS

Excluding *Proteae* and *Serratia* spp., which are intrinsically resistant to colistin, a total of 19,719 isolates of *Enterobacteriaceae* were collected during 2012 and 2013 from 180 sites in 39 countries. Unique isolates were collected from intra-abdominal, genitourinary, skin and soft tissue, and lower respiratory tract infections. The study protocol permitted only one isolate per species per patient. Basic patient demographic data (age and gender) were collected for each sample, but no identifiable patient-specific information regarding clinical presentation and antimicrobial therapy was recorded.

The MICs of colistin and the comparator agents listed in Table 2 were determined by the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method using reagent-grade antimicrobial powders obtained commercially or from the licensed manufacturing company (20). Aztreonam-avibactam was tested with avibactam at a constant concentration of 4 μ g/ml. After preparation of the colistin stock solution, polysorbate 80 (P-80) was included in the wells at a final concentration of 0.002% throughout dilution preparation and panel production. At the time that this study was initiated and conducted, the CLSI had approved the addition of 0.002% P-80 to the testing media as a surfactant supplement to prevent the binding of the positively charged molecules colistin and polymyxin B to the plastic of the 96-well microtiter plates (January 2012 meeting minutes; <http://clsi.org/standards/micro/microbiology-files/>) (21, 22). Test results for all agents were validated using concurrent testing of quality control (QC) strains recommended by the CLSI (23). In the absence of published QC ranges for colistin tested with P-80, the QC ranges for colistin tested alone were used (21, 23, 24). Because breakpoints were also not available for colistin tested with P-80, susceptibility was determined using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints for colistin tested alone (MIC for susceptible, ≤ 2 μ g/ml; MIC for resistant, ≥ 4 μ g/ml) (25). Susceptibility for tigecycline was determined according to FDA breakpoints (MIC for susceptible, ≤ 2 μ g/ml; MIC for intermediate, 4 μ g/ml; MIC for resistant, ≥ 8 μ g/ml), and susceptibilities to the other comparator agents were interpreted using the CLSI breakpoints (23, 26). No interpretive criteria have yet been defined for aztreonam-avibactam.

The carbapenems to which isolate susceptibility was tested included imipenem, meropenem, ertapenem, and doripenem. Isolates that were nonsusceptible to one or more of the tested carbapenems were analyzed for genes encoding ESBLs (TEM, SHV, VEB, CTX-M type, PER, GES), plasmid-mediated AmpC enzymes (CMY, DHA, ACT, MIR, MOX, FOX, ACC), and carbapenemases (KPC, NDM, IMP, VIM, SPM, OXA-48 type). Genes were detected using a combination of microarray analysis (Check-MDR CT101; Check-Points B.V., Wageningen, Netherlands) and multiplex PCR assays as described previously (27). Detected genes encoding ESBLs, carbapenemases, and plasmid-mediated AmpC enzymes were sequenced, and the sequences were compared to those of the genes in public databases available from the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) and the Lahey Clinic (www.lahey.org/studies/). If more than one carbapenemase gene was detected in a single isolate, a hierarchy of tiered analysis was as follows: MBL with or without an OXA-48-type enzyme or KPC, followed by KPC with or without an OXA-48-type enzyme and then an OXA-48-type enzyme only.

To assess the clonality of a subset of the population, semiautomated repetitive extragenic palindromic PCR (rep-PCR) analysis was employed. Briefly, genomic DNA was extracted using a MoBio UltraClean microbial DNA isolation kit (Carlsbad, CA). The obtained DNA was adjusted to a concentration of approximately 25 ng/ μ l and amplified using a DiversiLab bacterial fingerprinting kit (bioMérieux, Marcy l'Etoile, France) following the manufacturer's recommendations. Detection of the rep-PCR products was performed using a DiversiLab system (bioMérieux), and the products were analyzed with DiversiLab software (v.3.6). The results included a dendrogram generated via a pairwise similarity matrix and virtual gel images of the fingerprint for each DNA sample.

RESULTS

Among the large global collection of *Enterobacteriaceae* isolates evaluated in the present study, 309 (1.6%) isolates among 10 species without intrinsic resistance to the colistin class tested resistant to colistin (Table 1). Compared to the colistin resistance of other genera, isolates of *Enterobacter* spp. were more commonly resistant to colistin, with over two-thirds (39.1%) of *Enterobacter asburiae* isolates being resistant to this agent. In contrast, there was a notable difference in the percentage of colistin-resistant isolates among the various species of *Enterobacter*, with 3.0% resistance being detected among *Enterobacter cloacae* isolates and only 0.4% resistance being detected among *Enterobacter aerogenes* isolates. The next most common species with a colistin-resistant phenotype was *K. pneumoniae* (2.4%), with lower percentages of colistin resistance (0.1 to 0.6%) being observed among the *Citrobacter freundii*, *Citrobacter koseri*, *Escherichia coli*, and *Klebsiella oxytoca* isolates tested (Table 1).

The regional distribution of colistin resistance among the isolates from this sample showed a similar incidence of resistance in Europe (1.8%), Latin America (1.5%), Middle East-Africa (1.4%), North America (1.3%), and the Asia-Pacific (1.3%). The distribution of colistin resistance by country demonstrated colistin resistance in all countries sampled, with the exception of Ireland. However, sampling in that country was done for only 1 year at one site, resulting in the collection of only 61 isolates of *Enterobacteriaceae* from that country. Among the countries with colistin resistance, higher percentages were observed in Greece (5.0%), Italy (4.7%), and Romania (3.2%). The rate of colistin resistance was 2 to 3% in Hungary, Spain, the Netherlands, Turkey, Brazil, Chile, and Thailand. The remaining 28 surveyed countries had an incidence of colistin resistance below 2% for the species of *Enterobacteriaceae* included in this survey (Table 1).

Within the overall collection of isolates, 482 carbapenemase-producing *Enterobacteriaceae* isolates were observed in 30 countries, and the rate of colistin susceptibility was notably reduced to 88.0% among the isolates in this subset of the population compared to the rate among isolates that did not express a carbapenemase enzyme (Fig. 1A). The 58 colistin-resistant, carbapenemase-producing isolates included 55 *K. pneumoniae* isolates and 3 *E. asburiae* isolates that were collected from 16 countries in diverse geographic regions. Thirty-nine (67.2%) of the isolates carried additional ESBL enzymes consisting of CTX-M, SHV, and VEB variants (data not shown). The carbapenemase most frequently identified among both colistin-resistant and -susceptible isolates was KPC. Among the 58 colistin-resistant isolates, 38 (65.5%) contained KPC. The majority of KPC-carrying colistin-resistant isolates were collected from six medical centers in Greece (19 isolates) and Italy (10 isolates) and were analyzed by the DiversiLab rep-PCR to determine their genetic relatedness. A total

TABLE 1 Distribution of 309 colistin-resistant *Enterobacteriaceae* isolates from global surveillance^a

Organism (no. of isolates)	No. (%) of colistin-resistant isolates ^b	Region	Country(ies) (no. of isolates)
<i>Citrobacter freundii</i> (769)	4 (0.5)	Europe	Portugal (1), Spain (1)
		Asia-Pacific	Thailand (1)
		North America	United States (1)
<i>Citrobacter koseri</i> (476)	3 (0.6)	Europe	Germany (1), Italy (1), Turkey (1)
<i>Enterobacter aerogenes</i> (989)	4 (0.4)	Asia-Pacific	Thailand (1)
		North America	United States (3)
<i>Enterobacter asburiae</i> (215)	84 (39.1)	Europe	Austria (2), Belgium (2), Czech Republic (1), France (2), Germany (2), Greece (1), Hungary (6), Italy (2), Netherlands (4), Poland (1), Romania (2), Spain (6), Sweden (2), Turkey (5)
		Asia-Pacific	Australia (2), China (10), Japan (1), Malaysia (1), Philippines (2), South Korea (1), Taiwan (5), Thailand (1)
		North America	United States (10)
		Latin America	Argentina (1), Brazil (4), Colombia (2), Mexico (2)
		Middle East-Africa	Israel (1), Kenya (1), Nigeria (1), South Africa (1)
<i>Enterobacter cloacae</i> (1,543)	46 (3.0)	Europe	Austria (1), Denmark (1), France (1), Germany (1), Greece (1), Italy (1), Netherlands (3), Spain (2), Sweden (1), Turkey (2), United Kingdom (1)
		Asia-Pacific	Australia (2), China (5), Malaysia (1), Philippines (3), Taiwan (2), Thailand (4)
		North America	United States (4)
		Latin America	Argentina (2), Mexico (1), Venezuela (1)
		Middle East-Africa	Israel (2), Kuwait (4)
<i>Enterobacter kobei</i> (61)	6 (9.8)	Europe	Hungary (1), Italy (1), Russia (1)
		Asia-Pacific	Thailand (1)
		North America	United States (1)
		Middle East-Africa	South Africa (1)
<i>Enterobacter ludwigii</i> (22)	3 (13.6)	Europe	Greece (1), Sweden (1)
		Latin America	Colombia (1)
<i>Escherichia coli</i> (8,452)	24 (0.3)	Europe	Austria (1), France (2), Germany (1), Italy (3), Russia (1), Spain (4), United Kingdom (2)
		Asia-Pacific	China (1), Japan (1), Malaysia (1), South Korea (1), Taiwan (1), Thailand (1)
		North America	United States (3)
		Middle East-Africa	South Africa (1)
<i>Klebsiella oxytoca</i> (1,377)	2 (0.1)	Europe	Italy (1), Netherlands (1)
<i>Klebsiella pneumoniae</i> (5,613)	133 (2.4)	Europe	Belgium (1), Czech Republic (3), France (3), Germany (1), Greece (25), Hungary (1), Italy (21), Netherlands (1), Poland (2), Romania (12), Russia (3), Spain (3), Sweden (1), Turkey (5), United Kingdom (1)
		Asia-Pacific	Australia (3), China (1), Malaysia (1), Taiwan (1), Thailand (1)
		North America	United States (8)
		Latin America	Argentina (6), Brazil (3), Chile (13), Colombia (2), Mexico (1), Venezuela (1)
		Middle East-Africa	Israel (4), Kuwait (2), South Africa (3)

^a Excludes species naturally resistant to colistin.^b EUCAST breakpoints (MIC for susceptible, ≤ 2 $\mu\text{g/ml}$; MIC for resistant, ≥ 4 $\mu\text{g/ml}$) were applied.

of 89.7% of these isolates were clonal, with 26 belonging to one of three clusters of 8 to 10 related strains (Fig. 2). Additional KPC-carrying colistin-resistant isolates were found in seven countries, including countries in Latin America (Argentina, Brazil, Colombia, Venezuela), the United States, Japan, and Israel. We detected six isolates (four *K. pneumoniae* isolates and two *E. asburiae* isolates) that carried an MBL gene, including VIM-1, NDM-1, and IMP-8, and these were isolated from Europe, Africa, and Asia. Fourteen colistin-resistant *K. pneumoniae* isolates with OXA-48-like enzymes (12 isolates carrying OXA-48 and 2 isolates carrying OXA-244) were detected. These were distributed among five countries, but five isolates (35.7%) were collected from Turkey and an additional five isolates were from Romania. Isolates carry-

ing OXA-244, a variant of OXA-48 first described in Spain, were detected from a medical center in Russia (28).

The activities of agents of different drug classes against the set of isolates were assessed. The rate of susceptibility to levofloxacin and β -lactams, except for imipenem, was already below 90% for all the isolates, and as expected for the β -lactams, the activities of those agents against isolates containing a carbapenemase or ESBL enzyme decreased even further (Table 2). The activities of these comparator agents against colistin-resistant isolates were also diminished, and the lowest activity with these agents was seen against carbapenemase-positive colistin-resistant isolates (Table 2). Of the comparator agents tested, only aztreonam-avibactam tested with low MIC₉₀ values against isolates that carried a carbap-

FIG 1 Distribution of colistin (A) and aztreonam-avibactam (B) MIC values against *Enterobacteriaceae* with and without carbapenemase genes collected during 2012 and 2013. Carbapenemase-negative isolates include isolates that were carbapenem resistant and negative for the probed carbapenemase genes and carbapenem-susceptible isolates.

enemase enzyme, including those that possessed MBLs and were colistin resistant ($\text{MIC}_{90\text{s}} = 0.5$ to $1 \mu\text{g/ml}$; Table 2 and Fig. 1B). Tigecycline and amikacin were both highly active against isolates in the overall population (97.9% and 96.6% susceptibility, respectively). However, as was seen with the other agents, the rate of susceptibility to tigecycline and amikacin was reduced among colistin-resistant strains (95.5% and 81.9%, respectively) and was reduced even further among carbapenemase-positive, colistin-resistant isolates (89.7% and 44.8%, respectively).

DISCUSSION

Colistin-resistant, carbapenemase-producing *Enterobacteriaceae* represent a group of pathogens causing infections against which treatment options are currently limited. The testing of agents within the polymyxin class has been debated and scrutinized using various scientific methodologies to standardize MIC values to determine susceptibility and resistance. At the time that this study was initiated, the CLSI *Enterobacteriaceae* Working Group had recommended the addition of 0.002% polysorbate 80 (P-80) to the testing media for colistin and polymyxin B to prevent their binding to the plastic of the microtiter plates used for broth mi-

crodilution (21, 22). Subsequently, the CSLI rescinded this recommendation out of concern that P-80 might act synergistically with polymyxins and has reverted to testing colistin without the addition of a surfactant (22). This results in 2- to 4-fold higher MICs of colistin when it is tested with certain strains, most notably, those with low MIC values ($\leq 2 \mu\text{g/ml}$) (21, 22). Therefore, it is possible that the resistance to colistin that was reported may actually underestimate the percentage of resistance that is detected by the now approved methodology.

The results of this study showed a strong association between the presence of a carbapenemase and increased resistance to colistin among surveillance isolates of *Enterobacteriaceae*. The collection of clinical and treatment histories was beyond the scope of this surveillance study; however, other investigators have reported a correlation between the use of colistin to treat infections caused by CRE and the subsequent emergence of colistin-resistant strains (7–11). In the current study, *K. pneumoniae* was the most common carbapenemase-producing, colistin-resistant species of *Enterobacteriaceae* tested, with a wide geographic dissemination being observed for this MDR pathogen. Although most current

FIG 2 Genetic relatedness of colistin-resistant KPC-positive *K. pneumoniae* isolates collected in Greece (GRE) and Italy (ITL) in 2012 and 2013. OSBL, original-spectrum β-lactamases, which include TEM-1, TEM-2, SHV-1, and SHV-11.

findings document *K. pneumoniae* to be the predominant pathogen with this resistance phenotype, it was shown that this correlation also exists for *Enterobacter* spp. In 2013, an investigator in Ireland reported the first case of infection caused by an IMI-1-producing, colistin-resistant *E. asburiae* strain (29). The present study now also reports cases of infection caused by colistin-resistant *E. asburiae* with NDM-1 in Kenya, IMP-8 in Taiwan, and KPC-2 in Japan.

Although colistin currently maintains a high level of activity against most *Enterobacteriaceae* isolates, the decrease in activity against CRE isolates that carry mobile resistance genes is worri-

some (30). It has also been recognized that increased colistin use is responsible for outbreaks caused by species intrinsically resistant to polymyxins and the increasing isolation of colistin-resistant *Enterobacteriaceae* strains. In Argentina, investigators observed both an increasing frequency of *Serratia marcescens* infections and an outbreak with fatalities caused by an MDR clone that were attributable to increased colistin usage (17). Similarly, a hospital in Spain observed that the previous use of colistin was primarily responsible for the emergence and continuing isolation of colistin-resistant pathogens, including those with KPC enzymes (9). However, it has also been demonstrated that clinical isolates of

TABLE 2 *In vitro* activity of antimicrobial agents tested against *Enterobacteriaceae*^a

Isolate characteristic and antimicrobial agent	MIC (μg/ml)			
	Range	50%	90%	% susceptible ^b
All isolates (19,719)				
Colistin	≤0.12–>4	≤0.12	0.25	98.4
Aztreonam-avibactam	≤0.015–>128	0.06	0.25	NA
Aztreonam	≤0.015–>128	0.12	64	72.6
Tigecycline	≤0.015–>8	0.5	1	97.9
Amikacin	≤0.25–>32	2	8	96.6
Imipenem	≤0.03–>8	0.25	1	94.9
Cefepime	≤0.12–>16	≤0.12	>16	81.8
Levofloxacin	≤0.03–>4	0.06	>4	74.3
Colistin resistant (309)				
Colistin	4–>4	>4	>4	0.0
Aztreonam-avibactam	≤0.015–4	0.12	0.5	NA
Aztreonam	≤0.015–>128	0.5	>128	56.6
Tigecycline	0.06–8	1	2	95.5
Amikacin	0.5–>32	2	>32	81.9
Imipenem	0.06–>8	0.5	>8	71.2
Cefepime	≤0.12–>16	0.25	>16	66.7
Levofloxacin	≤0.03–>4	0.5	>4	58.3
Carbapenemase positive (482)				
Colistin	≤0.12–>4	≤0.12	4	88.0
Aztreonam-avibactam	≤0.015–8	0.25	0.5	NA
Aztreonam	0.03–>128	>128	>128	9.8
Tigecycline	0.06–8	1	2	91.1
Amikacin	≤0.25–>32	16	>32	59.3
Imipenem	0.12–>8	>8	>8	5.8
Cefepime	≤0.12–>16	>16	>16	17.6
Levofloxacin	≤0.03–>4	>4	>4	23.0
Carbapenemase positive, colistin resistant (58)				
Colistin	4–>4	>4	>4	0.0
Aztreonam-avibactam	≤0.015–4	0.25	1	NA
Aztreonam	0.06–>128	>128	>128	8.6
Tigecycline	0.5–4	1	4	89.7
Amikacin	0.5–>32	32	>32	44.8
Imipenem	1–>8	>8	>8	1.7
Cefepime	≤0.12–>16	>16	>16	17.2
Levofloxacin	0.06–>4	>4	>4	6.9
MBL positive, all (81)				
Colistin	≤0.12–>4	≤0.12	1	92.6
Aztreonam-avibactam	≤0.015–4	0.25	1	NA
Aztreonam	0.03–>128	64	>128	30.9
Tigecycline	0.06–8	1	2	92.6
Amikacin	1–>32	16	>32	61.7
Imipenem	0.5–>8	>8	>8	6.2
Cefepime	≤0.12–>16	>16	>16	11.1
Levofloxacin	0.06–>4	>4	>4	38.3
MBL positive, colistin resistant (6) ^c				
Colistin	4–>4			0.0
Aztreonam-avibactam	0.06–2			NA
Aztreonam	128–>128			0.0
Tigecycline	1–2			100
Amikacin	1–>32			50.0
Imipenem	8–>8			0.0
Cefepime	16–>16			0.0
Levofloxacin	4–>4			0.0

TABLE 2 (Continued)

Isolate characteristic and antimicrobial agent	MIC (μg/ml)			
	Range	50%	90%	% susceptible ^b
KPC positive, all (313)				
Colistin	≤0.12–>4	≤0.12	4	87.9
Aztreonam-avibactam	≤0.015–8	0.25	0.5	NA
Aztreonam	0.06–>128	>128	>128	1.3
Tigecycline	0.06–8	1	2	91.1
Amikacin	≤0.25–>32	32	>32	49.5
Imipenem	0.12–>8	>8	>8	1.6
Cefepime	≤0.12–>16	>16	>16	16.9
Levofloxacin	≤0.03–>4	>4	>4	16.0
KPC positive, colistin resistant (38)				
Colistin	4–>4	>4	>4	0.0
Aztreonam-avibactam	≤0.015–4	0.5	1	NA
Aztreonam	0.06–>128	>128	>128	7.9
Tigecycline	0.5–4	1	4	86.8
Amikacin	0.5–>32	32	>32	26.3
Imipenem	2–>8	>8	>8	0.0
Cefepime	≤0.12–>16	>16	>16	18.4
Levofloxacin	0.06–>4	>4	>4	5.3
OXA-48 positive, all (76)				
Colistin	≤0.12–>4	≤0.12	>4	84.2
Aztreonam-avibactam	0.03–1	0.25	0.5	NA
Aztreonam	0.06–>128	128	>128	23.7
Tigecycline	0.06–4	1	2	93.4
Amikacin	0.5–>32	4	16	90.8
Imipenem	0.12–>8	4	>8	19.7
Cefepime	≤0.12–>16	>16	>16	30.3
Levofloxacin	≤0.03–>4	>4	>4	34.2
OXA-48 positive, colistin resistant (12)				
Colistin	4–>4	>4	>4	0.0
Aztreonam-avibactam	0.03–0.5	0.25	0.5	NA
Aztreonam	0.12–>128	128	>128	16.7
Tigecycline	0.5–4	1	2	91.7
Amikacin	1–>32	8	16	91.7
Imipenem	1–>8	8	>8	8.3
Cefepime	0.5–>16	>16	>16	25.0
Levofloxacin	>4	>4	>4	0.0
ESBL positive, all (3,964) ^d				
Colistin	≤0.12–>4	≤0.12	0.25	97.7
Aztreonam-avibactam	≤0.015–32	0.06	0.25	NA
Aztreonam	≤0.015–>128	64	>128	7.3
Tigecycline	≤0.015–8	0.5	2	96.3
Amikacin	≤0.25–>32	4	32	89.7
Imipenem	≤0.03–>8	0.25	1	91.9
Cefepime	≤0.12–>16	>16	>16	21.8
Levofloxacin	≤0.03–>4	>4	>4	30.5
ESBL positive, colistin resistant (92) ^d				
Colistin	4–>4	>4	>4	0.0
Aztreonam-avibactam	≤0.015–4	0.25	0.5	NA
Aztreonam	1–>128	128	>128	4.4
Tigecycline	0.12–4	1	2	94.6
Amikacin	0.5–>32	8	>32	64.1
Imipenem	0.06–>8	1	>8	53.3
Cefepime	0.25–>16	>16	>16	14.1
Levofloxacin	0.06–>4	>4	>4	13.0

(Continued on following page)

TABLE 2 (Continued)

Isolate characteristic and antimicrobial agent	MIC ($\mu\text{g/ml}$)			
	Range	50%	90%	% susceptible ^b
ESBL negative, all (12,839) ^c				
Colistin	≤ 0.12 – >4	≤ 0.12	0.25	98.9
Aztreonam-avibactam	≤ 0.015 –1	0.03	0.12	NA
Aztreonam	≤ 0.015 –64	0.12	0.25	99.9
Tigecycline	≤ 0.015 –8	0.5	1	98.7
Amikacin	≤ 0.25 – >32	2	4	99.5
Imipenem	≤ 0.03 – >8	0.25	0.5	97.4
Cefepime	≤ 0.12 – >16	≤ 0.12	≤ 0.12	99.7
Levofloxacin	≤ 0.03 – >4	0.06	>4	87.8
ESBL negative, colistin resistant (148) ^c				
Colistin	4– >4	>4	>4	0.0
Aztreonam-avibactam	≤ 0.015 –0.5	0.06	0.12	NA
Aztreonam	≤ 0.015 –1	0.12	0.5	100
Tigecycline	0.06–4	1	2	97.3
Amikacin	0.5– >32	2	4	96.0
Imipenem	0.06–4	0.5	2	85.8
Cefepime	≤ 0.12 –16	≤ 0.12	0.25	99.3
Levofloxacin	≤ 0.03 – >4	0.06	>4	86.5

^a Excludes species naturally resistant to colistin.^b Susceptibility percentages are based upon CLSI breakpoint criteria (M100-S23).^c EUCAST breakpoints were applied for colistin, and FDA breakpoints were applied for tigecycline. NA, no breakpoints available.^d MIC₅₀ and MIC₉₀ values were not calculated for subsets containing fewer than 10 isolates.^e ESBL positive, isolates in which a gene encoding an ESBL was detected by PCR.Species included *C. freundii*, *E. aerogenes*, *E. asburiae*, *E. cloacae*, *E. coli*, *K. oxytoca*, and *K. pneumoniae*.^f ESBL negative, isolates with ceftazidime and aztreonam MIC values of ≤ 1 $\mu\text{g/ml}$.Species included *C. freundii*, *E. aerogenes*, *E. asburiae*, *E. cloacae*, *E. coli*, *K. oxytoca*, and *K. pneumoniae*.

colistin-resistant *Enterobacteriaceae* can emerge independently without colistin treatment pressure (31). Regardless, high mortality rates have been observed among patients with infections due to colistin-resistant CRE (30, 32).

On the basis of the higher percentage of colistin resistance observed among *Enterobacteriaceae* isolates producing a carbapenemase, it will be necessary to continually monitor the activity of colistin, although routine testing may not be possible for many clinical microbiology laboratories (22). Two testing methods commercially available in the United States (Etest [bioMérieux, Inc., Durham, NC] and Sensititre Xtra plates for Gram-negative bacteria [Trek Diagnostic Systems, Oakwood Village, OH]) at the time of this writing have not yet been approved for use by the FDA and are therefore to be used only for research purposes. In addition, the accuracy of the results obtained by Etest can be questionable (22). FDA-approved interpretive criteria exist for susceptibility testing by disk diffusion (BBL Sensi-Disc; Becton, Dickinson and Company, Sparks, MD), but confirmation of the results by broth microdilution is recommended because colistin diffuses poorly through solid media (33). Broth microdilution yields consistent results but is usually performed only by reference or academic laboratories (22). With the combination of resistance mechanisms becoming increasingly prevalent in the environments surrounding Gram-negative pathogens, prudent antimicrobial use, infection control, and the rapid development of alter-

native therapies will be required to stave off this growing threat to public health.

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ADDENDUM IN PROOF

Since submission of this manuscript, a transmissible plasmid-mediated colistin resistance gene, *mcr-1*, has been reported [Y.-Y. Liu et al., *Lancet Infect Dis*, 18 November 2015, [http://dx.doi.org/10.1016/S1473-3099\(15\)00424-7](http://dx.doi.org/10.1016/S1473-3099(15)00424-7)].

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