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In vitro activity of ceftazidime/avibactam against isolates of carbapenem-non-susceptible Enterobacteriaceae collected during the INFORM global surveillance programme (2015–17)

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Objectives: To report data for ceftazidime/avibactam and comparators against meropenem-non-susceptible Enterobacteriaceae collected globally (excluding centres in the USA) from 2015 to 2017 as part of the International Network For Optimal Resistance Monitoring (INFORM) surveillance programme.

Methods: MICs and susceptibility were determined using EUCAST broth microdilution methodology and EUCAST breakpoints. Isolates were screened to detect genes encoding β-lactamases using multiplex PCR assays. MBL-positive isolates were those in which one or more of the IMP, VIM and/or NDM genes were detected.

Results: A total of 1460 meropenem-non-susceptible isolates were collected and, of the agents on the panel, susceptibility was highest to ceftazidime/avibactam, colistin and tigecycline [73.0%, 77.0% (1081/1403) and 78.1%, respectively]. Ceftazidime/avibactam was not active against MBL-positive isolates (n=367); these isolates showed the highest rates of susceptibility to colistin (92.1%, 303/329), tigecycline (71.9%) and amikacin (46.6%). A total of 394 isolates were resistant to ceftazidime/avibactam and, of the 369 isolates that were screened, 98.4% were found to carry a gene encoding an MBL enzyme. Among isolates that were identified as carbapenemase positive and MBL negative (n=910), susceptibility was highest to ceftazidime/avibactam (99.8%). Susceptibility was also highest to ceftazidime/avibactam among isolates that were carbapenemase negative and MBL negative (94/98, 95.9%).

Conclusions: These data highlight the need for continued surveillance of antimicrobial activity as well as the need for new antimicrobials to treat infections caused by meropenem-non-susceptible Enterobacteriaceae, for which the options are extremely limited.

Introduction

Infections caused by carbapenem-resistant Enterobacteriaceae pose a significant treatment challenge, due to the limited number of antimicrobials available to treat them, and are associated with high rates of mortality.[1–4] Indeed, carbapenem-resistant Enterobacteriaceae have been categorized in the critical and highest priority group on a global list generated by the WHO to guide the research and development of new antimicrobial treatments.[5]

Carbapenem resistance among Enterobacteriaceae can be due to one of two important mechanisms.[6,7] One such mechanism is β-lactam hydrolysis via expression of carbapenemase enzymes, such as serine carbapenemases (KPC, OXA-48-like and GES) and MBLs (VIM, IMP, NDM and SPM) and the second is via changes in membrane permeability due to mutations in efflux pumps or porins coupled with ESBL or Ambler class C β-lactamase expression.[6,7]

Ceftazidime/avibactam is a combination of ceftazidime, a broad-spectrum, third-generation cephalosporin, and the β-lactamase inhibitor avibactam.[8–10] Avibactam is a diazabicyclooctane non-β-lactam β-lactamase inhibitor that has *in vitro* activity against Ambler class A β-lactamases, class C β-lactamases and some class D β-lactamases, but does not inhibit MBLs.[8–11]

Ceftazidime/avibactam is approved by the EMA and the FDA for the treatment of adult patients with complicated intra-abdominal infections, complicated urinary tract infections (including pyelonephritis) and hospital-acquired pneumonia (including ventilator-associated pneumonia).[12,13] The EMA has also approved

ceftazidime/avibactam for the treatment of infections due to aerobic Gram-negative organisms with limited treatment options.[13]

The *in vitro* activity of ceftazidime/avibactam and comparator agents against clinical isolates has been monitored through the International Network For Optimal Resistance Monitoring (INFORM) global surveillance programme since 2012 and the activity of ceftazidime/avibactam against carbapenem-non-susceptible Enterobacteriaceae has previously been reported.[14] This study reports the activity of ceftazidime/avibactam against isolates of carbapenem-non-susceptible Enterobacteriaceae collected between 2015 and 2017. Centres in the USA are not included in this study and are reported separately.[15,16]

Materials and methods

Non-duplicated clinical isolates of Enterobacteriaceae were collected between 2015 and 2017 as part of the INFORM surveillance programme. All isolates were collected from hospitalized patients with intra-abdominal, urinary tract, skin and soft tissue, lower respiratory tract or bloodstream infections. One isolate per species per patient was collected. Isolates were collected from five regions (excluding the USA): Africa/Middle East, Asia, Europe, Oceania and Latin America. Demographic data recorded included: culture source; patient location, including hospital ward; and sex and age of the patient.

Isolates were shipped to a central reference laboratory [International Health Management Associates (IHMA), Inc., Schaumburg, IL, USA] where their identities were confirmed using MALDI-TOF MS (Bruker Biotyper MALDI-TOF, Bruker Daltonics, Billerica, MA, USA). Isolates collected in China were tested in a central laboratory in China. The panel of antimicrobials used was: amikacin, aztreonam, cefepime, ceftazidime, ceftazidime/avibactam, colistin, doripenem, imipenem, levofloxacin, meropenem, piperacillin/tazobactam and tigecycline. Susceptibility testing was performed to determine MICs using broth microdilution panels according to EUCAST guidelines;[17] MICs were then interpreted according to EUCAST breakpoints version 8.0.[18] Isolates with intrinsic resistance to colistin were not included in the analysis of antimicrobial activity against colistin. In this study, carbapenem non-susceptibility among Enterobacteriaceae was defined as an isolate with a meropenem MIC ≥4 mg/L. Avibactam was tested at a fixed concentration of 4 mg/L in combination with doubling dilutions of ceftazidime.

All isolates included in this study, excluding isolates collected in China, were screened to detect and identify genes encoding MBL carbapenemases (IMP, VIM, NDM, GIM and SPM), serine carbapenemases (KPC, OXA-48-like and GES), ESBLs (TEM, SHV, CTX-M, VEB, PER and GES), original-spectrum β-lactamases [OSBLs; TEM and SHV that did not contain substitutions at amino acid positions 104, 164 or 238 (TEM) or 146, 238 or 240 (SHV)] and plasmid-mediated AmpC β-lactamases (ACT, CMY, DHA and FOX) using published multiplex PCR assays.[19] Detected β-lactamase genes were amplified using flanking primers and sequenced. Sequences were compared against databases provided by the NCBI (www.ncbi.nlm.nih.gov) and the Lahey Clinic (www.lahey.org/studies). In this study, MBL-positive isolates were those in which one or more of the IMP, VIM, NDM, GIM and/or SPM genes was detected; conversely, MBL-negative isolates were those in which none of the IMP, VIM, NDM, GIM or SPM genes was detected.

Results

Between 2015 and 2017, a total of 1460 meropenem-non-susceptible isolates were collected in five regions (excluding the USA) as part of the INFORM study (Table 1). The majority of isolates were collected in Europe (54.7%) followed by Latin America (24.2%) and Asia (15.8%); few isolates were collected in Africa/Middle East (4.2%; *n*=61) and Oceania (1.2%; *n*=18). Lists of the participating countries in each region are shown in Table S1 (available as Supplementary data at *JAC* Online). Approximately one-third (32.9%) of isolates were collected from patients in ICUs (Table 1). Most isolates were from patients aged ≥18 years (94.0%) and 58.5% of patients were male. Isolates were most commonly from respiratory (28.6%), genital/urinary (22.7%) or integumentary (20.3%) culture sources. Of all the isolates included in this analysis, 1137 (77.9%) were *Klebsiella pneumoniae*.

Among all isolates collected during the study, susceptibility to ceftazidime/avibactam varied between regions (Table 2); the highest rates were detected in Latin America (87.5%) and Europe (76.8%); approximately half of isolates collected in Africa/Middle East and Asia were susceptible (50.8% and 48.3%, respectively). Two out of the 18 isolates collected in Oceania (11.1%) were susceptible to ceftazidime/avibactam. In each region, susceptibility to ceftazidime/avibactam was higher than to the other β-lactam antimicrobials on the panel. Susceptibility was much lower to ceftazidime alone (1.6%; all regions combined) when compared with the combination of ceftazidime and avibactam (73.0%; all regions combined).

Table 1. Patient demographics and culture sources for isolates of meropenem-non-susceptible Enterobacteriaceae (*N*=1460) collected as part of the INFORM programme (2015–17)

Demographic parameter	*n* (%) of isolates (*N*=1460)
Region	
Africa/Middle East	61 (4.2)
Asia	230 (15.8)
Europe	798 (54.7)
Oceania	18 (1.2)
Latin America	353 (24.2)
Patient location	
inpatient	1310 (89.7)
outpatient	68 (4.7)
unknown	82 (5.6)
ICU	481 (32.9)
non-ICU	890 (61.0)
unknown	89 (6.1)
Age (years)	
<18	73 (5.0)
18–64	757 (51.8)
≥65	615 (42.1)
unknown	15 (1.0)
Sex	
female	603 (41.3)
male	854 (58.5)
unknown	3 (0.2)
Culture source	
body fluids	97 (6.6)
cardiovascular	199 (13.6)
gastrointestinal	117 (8.0)
genital/urinary	332 (22.7)
integumentary	297 (20.3)
respiratory	418 (28.6)

Table 2. Antimicrobial activity against isolates of meropenem-non-susceptible Enterobacteriaceae (*N*=1460) collected as part of the INFORM programme (2015–17)

	Europe (*n*=798)[a]			Latin America (*n*=353)[b]			Asia (*n*=230)[c]			Africa/Middle East (*n*=61)[d]			Oceania (*n*=18)[e]			All regions (*N*=1460)[f]		
Antimicrobial	MIC_{90} (mg/L)	S (*n*)	S (%)	MIC_{90} (mg/L)	S (*n*)	S (%)	MIC_{90} (mg/L)	S (*n*)	S (%)	MIC_{90} (mg/L)	S (*n*)	S (%)	MIC_{90} (mg/L)	S (*n*)	S (%)	MIC_{90} (mg/L)	S (*n*)	S (%)
Ceftazidime/avibactam	≥256	613	76.8	64	309	87.5	≥256	111	48.3	≥256	31	50.8	≥256	2	11.1	≥256	1066	73.0
Ceftazidime	≥256	20	2.5	≥256	1	0.3	≥256	0	0.0	≥256	1	1.6	≥256	1	5.6	≥256	23	1.6
Cefepime	≥32	17	2.1	≥32	7	2.0	≥32	2	0.9	≥32	3	4.9	≥32	1	5.6	≥32	30	2.1
Aztreonam	≥256	53	6.6	≥256	15	4.2	≥256	5	2.2	≥256	7	11.5	128	6	33.3	≥256	86	5.9
Piperacillin/tazobactam	≥256	3	0.4	≥256	0	0.0	≥256	3	1.3	≥256	1	1.6	≥256	3	16.7	≥256	10	0.7
Doripenem	≥16	5	0.6	≥16	7	2.0	≥16	1	0.4	≥16	0	0.0	≥16	1	5.6	≥16	14	1.0
Imipenem	≥16	52	6.5	≥16	20	5.7	≥16	13	5.7	≥16	1	1.6	≥16	7	38.9	≥16	93	6.4
Meropenem	≥16	0	0.0	≥16	0	0.0	≥16	0	0.0	≥16	0	0.0	≥16	0	0.0	≥16	0	0.0
Amikacin	≥64	356	44.6	≥64	200	56.7	≥64	162	70.4	≥64	25	41.0	16	14	77.8	≥64	757	51.8
Colistin[a–f]	≥16	538	70.2	≥16	263	77.6	1	208	92.4	1	55	98.2	1	17	100	≥16	1081	77.0
Tigecycline	2	617	77.3	2	282	79.9	4	178	77.4	2	47	77.0	2	16	88.9	2	1140	78.1
Levofloxacin	≥16	44	5.5	≥16	54	15.3	≥16	15	6.5	≥16	8	13.1	≥16	5	27.8	≥16	126	8.6

MIC_{90}, MIC required to inhibit growth of 90% of isolates (mg/L); S, susceptible.
Isolates were: *K. pneumoniae* (*n*=1137); *E. cloacae* (*n*=95); *E. coli* (*n*=66); *Citrobacter freundii* (*n*=37); *P. rettgeri* (*n*=24); *Klebsiella oxytoca* (*n*=23); *S. marcescens* (*n*=15); *K. aerogenes* (*n*=13); *P. stuartii* (*n*=13); *Enterobacter aerogenes* (*n*=10); *Enterobacter asburiae* (*n*=6); *P. mirabilis* (*n*=5); *Citrobacter farmeri* (*n*=3); *Citrobacter*, non-speciated (*n*=3); *Raoultella planticola* (*n*=3); *Citrobacter koseri* (*n*=2); *Klebsiella variicola* (*n*=2); *Raoultella ornithinolytica* (*n*=2); *Enterobacter kobei* (*n*=1).
[a–f]Isolates with intrinsic resistance to colistin excluded (*P. mirabilis*, *P. rettgeri, P. stuartii* and *S. marcescens*) (number of isolates tested against colistin: [a]*n*=766; [b]*n*=339; [c]*n*=225; [d]*n*=56; [e]*n*=17; [f]*n*=1403).

For all regions combined, rates of susceptibility to ceftazidime/avibactam, colistin and tigecycline were similar among all meropenem-non-susceptible isolates in the study (73.0%, 77.0% and 78.1%, respectively) (Table 2). Rates of susceptibility to these three antimicrobials varied across the regions, with tigecycline showing the least variability. Susceptibility to colistin was lower in Europe (70.2%) and Latin America (77.6%) when compared with the other three regions (≥92.4%).

Of the 1375 isolates screened for β-lactamases, 1277 (92.9%) possessed at least one carbapenemase gene: 910/1375 isolates (66.2%) were carbapenemase positive and MBL negative, and 367/1375 isolates (26.7%) were MBL positive (Table 3). A total of 98 isolates (7.1%) were carbapenemase negative. By region, the percentages of MBL-positive isolates were: Africa/Middle East 47.5% (29/61), Asia 64.1% (93/145), Europe 23.2% (185/798), Oceania 88.9% (16/18) and Latin America 12.5% (44/353). Eighty-five isolates collected in China were not screened. Ceftazidime/avibactam was not active against MBL-positive isolates; this subset showed the highest rates of susceptibility to colistin (92.1%), tigecycline (71.9%) and amikacin (46.6%) and rates were ≤15.8% for the remaining antimicrobials on the panel.

Among isolates of meropenem-non-susceptible Enterobacteriaceae that were identified as carbapenemase positive and MBL negative, susceptibility was highest to ceftazidime/avibactam (99.8%) (Table 3). Susceptibilities to tigecycline, colistin and amikacin were 79.9%, 69.4% and 52.3%, respectively, and for all other antimicrobials on the panel, susceptibility rates were ≤7.9%.

Table 4 shows the susceptibility of isolates to ceftazidime/avibactam, colistin and tigecycline by year. Among isolates that were carbapenemase positive and MBL negative, rates of susceptibility to colistin were 73.8% (192/260), 70.0% (198/283) and 65.7% (234/356) for 2015, 2016 and 2017, respectively. Among MBL-positive isolates, rates of susceptibility to colistin were 91.5% (86/94), 95.1% (116/122) and 89.4% (101/113) for 2015, 2016 and 2017, respectively.

A total of 394/1460 (27.0%) isolates included in this analysis were resistant to ceftazidime/avibactam. A gene encoding an MBL enzyme was identified in 363 of the 369 isolates of ceftazidime/avibactam-resistant, meropenem-non-susceptible Enterobacteriaceae (the 25 isolates collected in China were not genotyped). The most commonly detected MBLs were NDM-like enzymes (70.7%, 261/369), mostly NDM-1 (59.6%, 220/369) (Table 5). The percentages of isolates carrying an NDM-like gene were 94.7%, 90.0% and 81.8% for isolates from Asia, Africa/Middle East and Latin America, respectively; percentages were lower among isolates from Oceania (6.3%; 1/16) and Europe (58.4%). Genes encoding VIM-like and IMP-like MBLs were also detected. Genes encoding VIM-like enzymes were detected in 83/369 isolates (22.5%), most commonly VIM-1 (16.0%, 59/369). The percentage of isolates carrying a VIM-like gene was higher in Europe (40.0%, 74/185) than in the other four regions (15.9%, 7/44 in Latin America; 6.7%, 2/30 in Africa/Middle East; 0/94 and 0/16 in Asia and Oceania, respectively). Genes encoding IMP-like enzymes were detected in 20/369

Table 3. Antimicrobial activity against the genetically screened isolates of meropenem-non-susceptible Enterobacteriaceae (*N*=1375) collected as part of the INFORM programme (2015–17)

Organism group and antimicrobial	MIC_{50} (mg/L)	MIC_{90} (mg/L)	MIC range (mg/L)	MIC interpretation		
				S (%)	I (%)	R (%)
Carbapenemase positive (MBL negative)[a] (*n*=910)[d]						
ceftazidime/avibactam	1	2	≤0.015–16	99.8	—	0.2
ceftazidime	128	≥256	0.25 to ≥256	2.1	2.5	95.4
cefepime	≥32	≥32	0.12 to ≥32	2.4	3.1	94.5
aztreonam	≥256	≥256	0.03 to ≥256	2.6	0.1	97.3
piperacillin/tazobactam	≥256	≥256	64 to ≥256	0.0	0.0	100
doripenem	≥16	≥16	0.25 to ≥16	1.0	7.3	91.8
imipenem	≥16	≥16	1 to ≥16	3.5	32.7	63.7
meropenem	≥16	≥16	4 to ≥16	0.0	25.7	74.3
amikacin	8	≥64	≤0.25 to ≥64	52.3	12.1	35.6
colistin[d]	0.5	≥16	≤0.06 to ≥16	69.4	—	30.6
tigecycline	1	2	0.12 to ≥16	79.9	14.3	5.8
levofloxacin	≥16	≥16	0.03 to ≥16	7.9	2.3	89.8
MBL positive[b] (*n*=367)[e]						
ceftazidime/avibactam	≥256	≥256	4 to ≥256	1.1	—	98.9
ceftazidime	≥256	≥256	32 to ≥256	0.0	0.0	100
cefepime	≥32	≥32	1 to ≥32	0.8	2.7	96.5
aztreonam	128	≥256	0.015 to ≥256	15.8	4.1	80.1
piperacillin/tazobactam	≥256	≥256	4 to ≥256	1.1	0.5	98.4
doripenem	≥16	≥16	2 to ≥16	0.0	1.1	98.9
imipenem	≥16	≥16	1 to ≥16	2.5	26.7	70.8
meropenem	≥16	≥16	4 to ≥16	0.0	26.4	73.6
amikacin	16	≥64	≤0.25 to ≥64	46.6	12.3	41.1
colistin[e]	0.5	1	≤0.06 to ≥16	92.1	—	7.9
tigecycline	0.5	4	0.06 to ≥16	71.9	12.3	15.8
levofloxacin	≥16	≥16	0.03 to ≥16	9.3	6.3	84.5
Carbapenemase negative (MBL negative)[c] (*n*=98)[f]						
ceftazidime/avibactam	1	4	0.12 to ≥256	95.9	—	4.1
ceftazidime	≥256	≥256	0.12 to ≥256	4.1	2.0	93.9
cefepime	≥32	≥32	0.25 to ≥32	4.1	4.1	91.8
aztreonam	≥256	≥256	0.06 to ≥256	4.1	2.0	93.9
piperacillin/tazobactam	≥256	≥256	2 to ≥256	4.1	0.0	95.9
doripenem	4	≥16	0.25 to ≥16	5.1	28.6	66.3
imipenem	4	≥16	0.25 to ≥16	49.0	27.6	23.5
meropenem	8	≥16	4 to ≥16	0.0	80.6	19.4
amikacin	8	≥64	0.5 to ≥64	61.2	12.2	26.5
colistin[f]	0.5	≥16	0.12 to ≥16	82.8	—	17.2
tigecycline	0.5	2	0.03–8	81.6	10.2	8.2
levofloxacin	≥16	≥16	0.06 to ≥16	15.3	7.1	77.6

MIC_{50}, MIC required to inhibit growth of 50% of isolates (mg/L); MIC_{90}, MIC required to inhibit growth of 90% of isolates (mg/L); S, susceptible; I, intermediate; R, resistant; —, no intermediate breakpoint.

[a]Isolates tested positive for one or more of the serine carbapenemases tested (KPC, OXA-48 and GES) but negative for the MBL genes tested (IMP, VIM, NDM and SPM).

[b]Isolates tested positive for one or more of the MBL genes tested (IMP, VIM, NDM, GIM and SPM).

[c]Isolates tested negative for all carbapenemase genes tested (IMP, VIM, NDM, GIM, SPM, KPC, OXA-48 and GES).

[d–f]Isolates with intrinsic resistance to colistin excluded (*P. mirabilis*, *P. rettgeri, P. stuartii* and *S. marcescens*)(number of isolates tested against colistin: [d]*n*=899; [e]*n*=329; [f]*n*=93).

isolates (5.4%), most commonly IMP-4 (3.8%, 14/369). The most commonly detected serine β-lactamases were SHV-type (62.9%, 232/369), CTX-M-type (59.6%, 220/369) and TEM-type (55.3%, 204/369) enzymes. No gene encoding an MBL enzyme was identified in six isolates collected in Europe (*n*=4), Asia (*n*=1) and Africa/Middle East (*n*=1); these included two *K. pneumoniae* isolates co-carrying KPC-3, SHV-type and TEM-type enzymes, three *K. pneumoniae* isolates carrying SHV-type or CTX-M-type ESBLs and TEM-

Table 4. Antimicrobial activity against the genetically screened isolates of meropenem-non-susceptible Enterobacteriaceae (*N*=1375) collected as part of the INFORM programme (2015–17)

	2015			2016			2017		
Organism group and antimicrobial	MIC_{90} (mg/L)	S (%)	R (%)	MIC_{90} (mg/L)	S (%)	R (%)	MIC_{90} (mg/L)	S (%)	R (%)
Carbapenemase positive (MBL negative)[a] (*n*=910)[d]		(*n*=261)[e]			(*n*=287)[f]			(*n*=362)[g]	
ceftazidime/avibactam	2	100	0.0	4	100	0.0	2	99.4	0.6
colistin[d–g]	≥16	73.8	26.2	≥16	70.0	30.0	≥16	65.7	34.3
tigecycline	2	77.0	6.5	2	87.8	4.9	2	75.7	6.1
MBL positive[b] (*n*=367)[h]		(*n*=96)[i]			(*n*=138)[j]			(*n*=133)[k]	
ceftazidime/avibactam	≥256	4.2	95.8	≥256	0.0	100	≥256	0.0	100
colistin[h–k]	2	91.5	8.5	1	95.1	4.9	8	89.4	10.6
tigecycline	4	67.7	11.5	4	79.0	11.6	4	67.7	23.3
Carbapenemase negative (MBL negative)[c] (*n*=98)[l]		(*n*=38)[m]			(*n*=28)[n]			(*n*=32)[o]	
ceftazidime/avibactam	4	94.7	5.3	2	100	0.0	4	93.8	6.3
colistin[l–o]	≥16	73.0	27.0	≥16	80.0	20.0	0.5	96.8	3.2
tigecycline	4	78.9	15.8	1	96.4	0.0	2	71.9	6.3

MIC_{90}, MIC required to inhibit growth of 90% of isolates (mg/L); S, susceptible; R, resistant.
[a]Isolates tested positive for one or more of the serine carbapenemases tested (KPC, OXA-48 and GES) but negative for the MBL genes tested (IMP, VIM, NDM, GIM and SPM).
[b]Isolates tested positive for one or more of the MBL genes tested (IMP, VIM, NDM, GIM and SPM).
[c]Isolates tested negative for all carbapenemase genes tested (IMP, VIM, NDM, GIM, SPM, KPC, OXA-48 and GES).
[d–o]Isolates with intrinsic resistance to colistin excluded (*P. mirabilis*, *P. rettgeri*, *P. stuartii* and *S. marcescens*) (number of isolates tested against colistin: [d]*n*=899; [e]*n*=260; [f]*n*=283; [g]*n*=356; [h]*n*=329; [i]*n*=94; [j]*n*=122; [k]*n*=113; [l]*n*=93; [m]*n*=37; [n]*n*=25; [o]*n*=31).

Table 5. β-Lactamase genes detected by genotyping in ceftazidime/avibactam-resistant, meropenem-non-susceptible Enterobacteriaceae isolates (*N*=369) collected as part of the INFORM programme (2015–17)

		Number of isolates[a]					
Gene	type	Africa/Middle East (*n*=30)	Asia (*n*=94)	Europe (*n*=185)[b]	Oceania (*n*=16)	Latin America (*n*=44)	all regions (*n*=369)
MBL genes	NDM	27	89	108	1	36	261
	VIM	2	0	74	0	7	83
	IMP	0	4	0	15	1	20
Serine β-lactamase genes	SHV	16	61	126	7	22	232
	CTX-M	22	74	93	4	27	220
	TEM	16	63	87	15	23	204
	OXA	1	16	22	1	0	40
	CMY	1	7	18	0	0	26
	DHA	1	5	12	0	0	18
	VEB	0	1	11	0	0	12
	KPC	0	1	5	0	1	7
	GES	0	0	0	0	2	2
	FOX	0	0	1	0	0	1
	PER	1	0	0	0	0	1
	ACT	0	0	0	0	0	0
No gene detected		1	0	0	0	0	1

[a]Includes co-carriers.
[b]Includes one isolate co-carrying NDM-type and VIM-type MBLs.

Table 6. Serine β-lactamase genes detected by genotyping in ceftazidime/avibactam-susceptible, meropenem-non-susceptible Enterobacteriaceae isolates (*N*=1006) collected as part of the INFORM programme (2015–17)

	Number of isolates[a]					
Type	Africa/Middle East (*n*=31)	Asia (*n*=51)	Europe (*n*=613)	Oceania (*n*=2)	Latin America (*n*=309)	all regions (*n*=1006)
SHV	28	42	550	2	241	863
TEM	25	38	466	1	200	730
KPC	7	31	352	0	277	667
CTX-M	23	38	281	1	126	469
OXA	21	2	220	1	3	247
CMY	0	3	21	0	0	24
DHA	1	7	6	0	0	14
VEB	0	0	6	0	0	6
PER	0	0	2	0	0	2
FOX	0	0	0	0	1	1
ACT	0	0	0	0	0	0
GES	0	0	0	0	0	0
No gene detected	1	0	12	0	2	15

[a]Includes co-carriers.

type enzymes, and one *Providencia stuartii* isolate in which none of the genes included in the testing algorithm was detected.

A total of 1066/1460 (73.0%) isolates included in this analysis were susceptible to ceftazidime/avibactam (Table 2). Table 6 shows the genotyping analysis for 1006 isolates of ceftazidime/avibactam-susceptible, meropenem-non-susceptible Enterobacteriaceae (the 60 isolates collected in China were not genotyped). The most commonly detected β-lactamases were SHV-like enzymes (85.8%, 863/1006), followed by TEM-like (72.6%, 730/1006) and KPC-like (66.3%, 667/1006) enzymes. In 15 isolates, none of the genes encoding acquired β-lactamases (as tested for in the PCR assay) was detected.

Discussion

In this study, high rates of susceptibility to ceftazidime/avibactam have been demonstrated among MBL-negative isolates of meropenem-non-susceptible Enterobacteriaceae.

Among all isolates of meropenem-non-susceptible (meropenem MIC ≥4 mg/L) Enterobacteriaceae included in this analysis, susceptibility to ceftazidime/avibactam was 73.0% (27.0% were resistant). There was some variability in susceptibility to ceftazidime/avibactam between regions: rates were highest in Latin America and Europe (87.5% and 76.8%, respectively) and in Africa/Middle East and Asia were 50.8% and 48.3%, respectively (11.1% in Oceania; however, only 18 isolates in total were collected in this region). Avibactam does not inhibit MBLs[8] and therefore this variability in susceptibility rates is likely to be greatly influenced by the regional rates of MBLs, which were lowest in Latin America and Europe (12.5% and 23.2%) and highest in Asia and Africa/Middle East (64.1% and 47.5%, respectively). Furthermore, two reports from the US INFORM surveillance programme showed higher rates of susceptibility among carbapenem-resistant Enterobacteriaceae (carbapenem MIC ≥4 mg/L) collected in US medical centres (98.5%, 2012–2014; 97.5%, 2013–16).[15,16] This is likely to be due to low rates of MBL-positive isolates; indeed, Sader *et al.*[16] reported that only 2.1% of carbapenem-resistant Enterobacteriaceae collected during the US INFORM programme (2013–16) were MBL positive.

Genotyping of 1006 isolates of meropenem-non-susceptible Enterobacteriaceae that were susceptible to ceftazidime/avibactam revealed that 98.5% of isolates carried at least one gene encoding a serine β-lactamase; the most commonly detected genes were SHV, TEM, KPC, CTX-M and OXA-48-like. Previous publications have reported that Enterobacteriaceae carrying genes encoding these enzymes are highly susceptible to ceftazidime/avibactam.[14,15,20–22]

In this report, susceptibility to colistin has been presented for isolates that do not possess intrinsic resistance to colistin (*Proteus mirabilis*, *Providencia rettgeri*, *P. stuartii* and *Serratia marcescens* excluded; *n*=57). Among meropenem-non-susceptible Enterobacteriaceae, susceptibility to colistin and tigecycline (77.0% and 78.1%, respectively) was similar to that of ceftazidime/avibactam (73.0%). Unlike ceftazidime/avibactam, colistin and tigecycline were shown to be active against MBL-positive isolates (susceptibility 92.1% and 71.9%, respectively). Changes in susceptibility across a 3 year study cannot be conclusively interpreted; however, we note that susceptibility to colistin appeared to show a trend to decreasing susceptibility among carbapenemase-positive, MBL-negative isolates. Although these isolates were not screened for mechanisms of colistin resistance, chromosomal or plasmid-mediated resistance has been reported among carbapenemase-positive isolates in other studies,[23–27] and continued surveillance of susceptibility among Enterobacteriaceae to colistin is essential. In the case of the MBL-positive isolates, this yearly decrease in susceptibility to colistin was not seen (91.5%, 95.1% and 89.4% for 2015, 2016 and 2017, respectively).

There was a subset of meropenem-non-susceptible Enterobacteriaceae collected in this study (7.1%) that did not carry any of the carbapenemase genes tested (IMP, VIM, NDM, GIM,

SPM, KPC, OXA-48-like and GES) and the susceptibility of these isolates to ceftazidime/avibactam was high (95.9%). It is possible that meropenem non-susceptibility among some of these isolates is mediated by carbapenemases that were not detected with the applied PCR assays. However, the majority of these carbapenemase-negative isolates (90/98) were *K. pneumoniae*, *Escherichia coli*, *Enterobacter cloacae* or *Klebsiella aerogenes*, for which porin defects combined with ESBL and/or AmpC production have been shown in previous studies to reduce susceptibility to carbapenems.[28–30] The majority of isolates in this study were MBL negative and carbapenemase positive, and were susceptible to ceftazidime/avibactam (99.8%). This is consistent with a previous report of European data from the INFORM surveillance programme, which reported that a high percentage (98.5%) of meropenem-non-susceptible (meropenem MIC $\geq$4 mg/L), MBL-negative Enterobacteriaceae isolates collected in Europe between 2012 and 2015 were susceptible to ceftazidime/avibactam.[31]

This analysis, as well as previous reports, has shown that ceftazidime/avibactam is not active against MBL-producing Enterobacteriaceae.[14,21,31] Genotyping of the 369 isolates that were resistant to ceftazidime/avibactam revealed that 98.4% of isolates carried at least one gene encoding an MBL and the majority of MBL-positive isolates (91.2%; 331/363 isolates) co-carried a gene encoding a serine β-lactamase. The most commonly detected serine β-lactamase genes were those encoding SHV, CTX-M, TEM and OXA-48-like enzymes, which have been reported to be highly susceptible to the ceftazidime/avibactam combination.[15,21,22] In a study by Castanheira *et al.*,[15] all of the 1120 isolates of Enterobacteriaceae collected from US hospitals (2012–14) that were positive for CTX-M-type enzymes were susceptible to ceftazidime/avibactam (FDA breakpoints were applied). Ceftazidime/avibactam activity against Enterobacteriaceae isolates carrying SHV- or TEM-type enzymes that were collected in Europe as part of the INFORM programme (2012–15; *n*=165) was high (99.4%).[31] Furthermore, 99.2% of the 242 Enterobacteriaceae isolates collected globally (Europe, Middle East/Africa, Asia-Pacific, Latin America; 2012–15) that were identified as MBL negative and OXA-48 positive were susceptible to ceftazidime/avibactam.[22] Therefore, in our study, ceftazidime/avibactam resistance among meropenem-non-susceptible Enterobacteriaceae that co-carry genes encoding MBLs and serine β-lactamases was most likely to be due to MBL production. There were six ceftazidime/avibactam-resistant isolates in which a gene encoding an MBL could not be detected; resistance of these isolates to ceftazidime/avibactam is most likely to be via a mechanism other than expression of an MBL.

A limitation of this study is that meropenem-intermediate and meropenem-resistant isolates were combined for analysis; this was done to allow comparison with previously published data from ceftazidime/avibactam surveillance studies and other studies of carbapenem-non-susceptible isolates.

In conclusion, we report that meropenem-non-susceptible isolates collected as part of the INFORM global surveillance study (US centres not included) (2015–17) showed the highest rates of susceptibility to ceftazidime/avibactam, colistin and tigecycline among the antimicrobials tested. Susceptibility to ceftazidime/avibactam was high among MBL-negative isolates, and colistin and tigecycline were active against MBL-positive as well as MBL-negative isolates. These data highlight the need for continued surveillance of the activity of these antimicrobials as well as the need for new antimicrobials to treat infections caused by meropenem-non-susceptible Enterobacteriaceae, for which the options are extremely limited. Highly resistant isolates continue to be identified and therefore continued surveillance is required.

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Transparency declarations

K.K. is an employee of IHMA, Inc., which served as the central laboratory for the INFORM programme. G.G.S. is an employee and a shareholder of Pfizer Inc., and a shareholder of AstraZeneca. I.S. received financial support for consumables and staff members for this project.

Author contributions

I.S. participated in data collection and interpretation as well as drafting and reviewing the manuscript. K.K. participated in generation of molecular data, data interpretation, and drafting and reviewing the manuscript. G.G.S. was involved in the study design and participated in data interpretation and drafting and review of the manuscript. All authors read and approved the final manuscript.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online.

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