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Susceptibility profile and β-lactamase content of global *Pseudomonas aeruginosa* isolates resistant to ceftolozane/tazobactam and/or imipenem/relebactam—SMART 2016–21

James A. Karlowsky[1,2]*, Sibylle H. Lob[1], Mark A. Estabrook[1], Fakhar Siddiqui[3], C. Andrew DeRyke[3], Katherine Young[3], Mary R. Motyl[3] and Daniel F. Sahm[1]

[1]IHMA, Inc., Schaumburg, IL, USA; [2]Department of Medical Microbiology and Infectious Diseases, Max Rady College of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada; [3]Merck & Co., Inc., Rahway, NJ, USA

*Corresponding author. E-mail: jkarlowsky@sharedhealthmb.ca

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Objectives: To determine susceptibility profiles and β-lactamase content for ceftolozane/tazobactam-resistant and imipenem/relebactam-resistant *Pseudomonas aeruginosa* isolates collected in eight global regions during 2016–21.

Methods: Broth microdilution MICs were interpreted using CLSI breakpoints. PCR to identify β-lactamase genes or WGS was performed on selected isolate subsets.

Results: Ceftolozane/tazobactam-resistant [from 0.6% (Australia/New Zealand) to 16.7% (Eastern Europe)] and imipenem/relebactam-resistant [from 1.3% (Australia/New Zealand) to 13.6% (Latin America)] *P. aeruginosa* varied by geographical region. Globally, 5.9% of isolates were both ceftolozane/tazobactam resistant and imipenem/relebactam resistant; 76% of these isolates carried MBLs. Most ceftolozane/tazobactam-resistant/imipenem/relebactam-susceptible isolates carried ESBLs (44%) or did not carry non-intrinsic (acquired) β-lactamases (49%); 95% of imipenem/relebactam-resistant/ceftolozane/tazobactam-susceptible isolates did not carry non-intrinsic β-lactamases. Isolates that carried indicators of strong PDC (*Pseudomonas*-derived cephalosporinase) up-regulation without a mutation known to expand the spectrum of PDC, or non-intrinsic β-lactamases, showed an 8-fold increase in ceftolozane/tazobactam modal MIC; however, this rarely (3%) resulted in ceftolozane/tazobactam resistance. Isolates with a PDC mutation and an indicator for PDC upregulation were ceftolozane/tazobactam non-susceptible (MIC, ≥8 mg/L). MICs ranged widely (1 to >32 mg/L) for isolates with a PDC mutation and no positively identified indicator for PDC up-regulation. Imipenem/relebactam-resistant/ceftolozane/tazobactam-susceptible isolates without non-intrinsic β-lactamases frequently (91%) harboured genetic lesions implying OprD loss of function; however, this finding alone did not account for this phenotype. Among imipenem-non-susceptible isolates without non-intrinsic β-lactamases, implied OprD loss only shifted the distribution of imipenem/relebactam MICs up by 1–2 doubling dilutions, resulting in ∼10% imipenem/relebactam-resistant isolates.

Conclusions: *P. aeruginosa* with ceftolozane/tazobactam-resistant/imipenem/relebactam-susceptible and imipenem/relebactam-resistant/ceftolozane/tazobactam-susceptible phenotypes were uncommon and harboured diverse resistance determinants.

Introduction

Pseudomonas aeruginosa is an important pathogen that primarily causes respiratory tract infections in hospitalized, debilitated and immunosuppressed patients.[1] Ceftolozane/tazobactam and imipenem/relebactam are bactericidal anti-pseudomonal agents that retain activity against many resistant phenotypes[2,3] and are agents of choice for the treatment of patients with MDR and difficult-to-treat *P. aeruginosa* infections.[4,5] Ceftolozane/tazobactam and imipenem/relebactam are both indicated for the treatment of patients with hospital-acquired and ventilator-associated bacterial pneumonia, complicated urinary tract infections, including pyelonephritis, and complicated intra-abdominal infections.[6,7]

Ceftolozane/tazobactam is an advanced-generation, anti-pseudomonal cephalosporin/β-lactamase inhibitor combination. Ceftolozane has greater stability than other cephalosporins against chromosomal class C (AmpC) β-lactamases, including *Pseudomonas*-derived cephalosporinase (PDC), and is a poor inducer of AmpC enzymes.[5,8,9] Mutations in bla_{PDC} can cause increased hydrolysis of cephalosporins, including slow hydrolysis of ceftolozane.[10–14] Ceftolozane is a weak substrate for pseudomonal efflux systems and is unaffected by OprD loss.[5,8,9] PDC subtypes with mutations that increase hydrolysis of ceftolozane and ceftazidime, or isolates producing PDC at very high levels have also been reported to contribute to ceftolozane/tazobactam resistance in limited numbers of isolates.[8,10,12,13,15–18] MBLs, serine carbapenemases (e.g. KPC), VEB, PER and GES ESBLs, and some OXA β-lactamases (e.g. OXA-14) hydrolyse ceftolozane.[8,13,14,19] Tazobactam contributes very minimally to the antipseudomonal activity of ceftolozane.

Imipenem/relebactam partners relebactam, a diazabicyclooctane inhibitor of class A (e.g. ESBLs, KPC) and class C β-lactamases, with the carbapenem imipenem.[20] Relebactam does not induce AmpC and is a potent inactivator of PDC-1, the WT AmpC of *P. aeruginosa*, and its variants.[8,15,21] Imipenem, however, is a potent inducer of PDC.[22] Imipenem/relebactam activity against *P. aeruginosa* is unaffected by efflux pump-mediated resistance and less affected by OprD loss than imipenem alone.[15] It also retains *in vitro* activity against isolates with KPC-3 or PDC mutations that generate resistance to ceftazidime/avibactam or ceftolozane/tazobactam.[23] Imipenem/relebactam is inactive against isolates of *P. aeruginosa* carrying MBLs, some GES subtypes (e.g. GES-5), VEB-type ESBLs, carbapenemase variants of OXA-type β-lactamases, and isolates with porin defects that also hyperproduce AmpC at very high levels.[15–17,24]

P. aeruginosa resistant to ceftolozane/tazobactam and imipenem/relebactam remain uncommon in North America and Western Europe; both ceftolozane/tazobactam and imipenem/relebactam retain activity against the vast majority of *P. aeruginosa* isolates resistant to carbapenems and other antipseudomonal β-lactams.[2,3,5,25] Surveillance data from outside of North America and Europe that systematically compare the anti-pseudomonal activities of ceftolozane/tazobactam- and imipenem/relebactam and describe molecular mechanisms of resistance in both ceftolozane/tazobactam- and imipenem/relebactam-resistant clinical isolates have not been published. Therefore, in the current study, we evaluated the susceptibility and β-lactamase content of ceftolozane/tazobactam-resistant and imipenem/relebactam-resistant *P. aeruginosa* collected in eight global regions from 2016 to 2021 as part of the Study for Monitoring Antimicrobial Resistance Trends (SMART) global surveillance programme. In addition, for ceftolozane/tazobactam-resistant and imipenem/relebactam-resistant *P. aeruginosa* collected during 2020–21 we identified loss-of-function mutations in non-β-lactamase genes of interest (*oprD*, *ampD*, *ampDh2*, *ampDh3*, *dacB* and *mpl*) as well as other mutations in *ampD* and *ampR* known to impact PDC regulation.

Methods

Bacterial isolates

From 2016 to 2021, 266 clinical laboratories in 61 countries (Table S1 available as Supplementary data at JAC-AMR Online) collected 254 901 isolates of Gram-negative bacilli as per the isolate collection protocol for the SMART global surveillance programme described previously.[2] A total of 41 645 (16.3%) were *P. aeruginosa*. Isolates from China and India were not included in this report because molecular data were not available for the entire study period. The prevalence of *P. aeruginosa* was approximately three times higher among lower respiratory tract infection isolates (27.7%; 26 888/96 947) than among intra-abdominal (10.5%; 6257/59 399), urinary tract (8.3%; 5130/61 860) and bloodstream infection isolates (9.1%; 3262/35 958); specimen source was not specified for 108 *P. aeruginosa* isolates. All isolates were sent to one of two central laboratories (IHMA, Monthey, Switzerland or IHMA, Schaumburg, IL, USA), where organism identity was confirmed using MALDI-TOF MS (Bruker Daltonics, Billerica, MA, USA), and antimicrobial susceptibility and molecular testing were performed.

Antimicrobial susceptibility testing

MICs were determined by the CLSI broth microdilution method.[26] Isolates were tested on custom-made dehydrated broth microdilution panels manufactured by TREK Diagnostic Systems (Thermo Fisher Scientific, Oakwood Village, OH, USA) in 2016 and 2017 and on frozen broth microdilution panels prepared at IHMA in 2018, 2019, 2020 and 2021. MICs were interpreted using 2022 CLSI MIC breakpoints.[27]

Screening for β-lactamase genes by PCR and WGS

P. aeruginosa isolates with ceftolozane/tazobactam MIC values of ≥8 mg/L (intermediate and resistant)[27] or imipenem or imipenem/relebactam MIC values of ≥4 mg/L (intermediate and resistant)[27] were screened for β-lactamase genes by PCR (2016–19)[28,29] or underwent short-read WGS (2020–21),[30,31] as previously described. Non-β-lactamase genes of interest (*oprD*, *ampD*, *ampDh2*, *ampDh3*, *dacB* and *mpl*) were queried by pairwise alignment against a reference sequence from PAO1 (NC_002516) for loss-of-function mutations. Permeability (*oprD*, porin) mutations can cause loss of function resulting in decreased periplasmic concentrations of some antimicrobial agents (e.g. imipenem).[32] AmpD, and its homologues AmpDh2 and AmpDh3, are enzymes involved in recycling peptidoglycan. AmpD saturation induces AmpC expression via AmpR. Loss, truncation or mutation in AmpD increases AmpC expression by up to approximately 50-fold.[33,34] *dacB* (*pbp4*) is likewise involved in recycling peptidoglycan; its loss or truncation leads to saturation of *ampD*, inducing *ampC* expression via AmpR; its expression is vastly increased if *ampD* is correspondingly lost.[33] Mpl is also involved in recycling peptidoglycan; its loss or truncation leads to increased expression (non-induced) of AmpC.[35] Loss-of-function mutations were defined as a frameshift leading to a premature stop codon, non-sense mutation, insertion or deletion of greater than 20 codons, or ablation of the start or stop codon in the reference sequence without an immediately adjacent replacement. Other mutations known to impact PDC regulation in *ampD* and *ampR*, as well as mutations that expand the substrate profile of PDC (e.g. Ω-loop mutations) were also examined (Table S2, Figure S1).

β-Lactamase genes were not identified in the vast majority of ceftolozane/tazobactam-intermediate (MIC, 4 mg/L) or imipenem- and imipenem/relebactam-intermediate (MIC, 8 mg/L) isolates and, therefore, these isolates were excluded from the molecular analysis for this report, which focused solely on ceftolozane/tazobactam- and imipenem/relebactam-resistant isolates. Table S3 lists all variants of non-intrinsic β-lactamases detected, excluding MBL and KPC variants, in ceftolozane/tazobactam-resistant/imipenem/relebactam-susceptible, imipenem/relebactam-resistant/ceftolozane/tazobactam-susceptible and ceftolozane/tazobactam-resistant/imipenem/relebactam-resistant subsets of *P. aeruginosa* isolates collected globally during 2016–21.

A total of 669 *P. aeruginosa* isolates (1.6% of 41 645 *P. aeruginosa* isolates) were not available for molecular characterization and were not included in the denominators used for carbapenemase rate calculations.

In addition, 1323 randomly selected *P. aeruginosa* isolates collected in 2020 and 2021 that met the testing criteria were also not molecularly characterized (25.1% of 5266 *P. aeruginosa* isolates collected in 2020 and 2021 that qualified for molecular characterization). For each clinical laboratory, the percentage of qualified isolates collected during 2020–21 that were not characterized was considered when calculating carbapenemase rates.

Results

The proportions of *P. aeruginosa* isolates resistant to ceftolozane/tazobactam or imipenem/relebactam collected in eight global regions during 2016–21 is summarized in Figure 1. Ceftolozane/tazobactam- or imipenem/relebactam-resistant *P. aeruginosa* were uncommon (<5%) in Australia/New Zealand (0.6% ceftolozane/tazobactam-resistant and 1.3% imipenem/relebactam-resistant), the USA (2.4% and 3.2%), Canada (2.0% and 4.3%) and Western Europe (4.5% and 4.5%). Resistance to ceftolozane/tazobactam and imipenem/relebactam was highest in Eastern Europe (16.7% and 12.7%) and Latin America (12.2% and 13.6%), and 8%–9% in the Asia/Pacific region (8.2% and 8.3%) and Middle East/Africa (8.6% and 8.5%). Globally, 7.9% of isolates were ceftolozane/tazobactam resistant and 7.9% imipenem/relebactam resistant. Figure 2 depicts the estimated proportions of *P. aeruginosa* isolates carrying carbapenemase and ESBL genes in the same eight global regions during 2016–21. Regional patterns of resistance to ceftolozane/tazobactam and imipenem/relebactam correlated with estimated proportions of isolates carrying carbapenemases [primarily MBLs supplemented by lower numbers of serine carbapenemases (GES, KPC)] and ESBLs. MBLs were present in >5% of *P. aeruginosa* isolates from Eastern Europe (9.5%), Latin America (7.9%), Middle East/Africa (5.9%) and Asia/Pacific (5.9%), and in 1.8% of isolates from Western Europe, 0.3% of isolates from the USA, 0.1% of isolates from Canada and 0.04% of isolates from Australia/New Zealand.

Table 1 summarizes the *in vitro* susceptibility of ceftolozane/tazobactam-resistant and imipenem/relebactam-resistant *P. aeruginosa* isolates collected during 2016–21 to 10 antimicrobial agents. For this analysis, isolates from some regions/countries were combined because of low numbers (Australia/New Zealand region isolates were combined with Asia/Pacific isolates and isolates from the USA and Canada were combined). Notable observations included that in the USA/Canada region, susceptibility to imipenem/relebactam among ceftolozane/tazobactam-resistant *P. aeruginosa* was 54.0% and susceptibility to ceftolozane/tazobactam among imipenem/relebactam-resistant *P. aeruginosa* was 67.1%; these percentages were much higher than in other regions of the world (<23%). These findings appear to be associated with a higher prevalence of isolates carrying MBLs and/or GES-type β-lactamases in regions outside of the USA and Canada (Figure 2). Non-intrinsic β-lactamases (i.e. any β-lactamase other than PDC or OXA-50-like) were not identified in the majority of ceftolozane/tazobactam-resistant and imipenem/relebactam-resistant isolates from the USA/Canada region, while in other regions these phenotypes were frequently associated with MBL carriage (Figure S2). Ceftolozane/tazobactam-resistant and imipenem/relebactam-resistant isolates were most susceptible to amikacin in all global regions with three exceptions: percent susceptible values were higher to aztreonam than amikacin for ceftolozane/tazobactam-resistant isolates in Latin America and Middle East/Africa and for imipenem/relebactam-resistant isolates in Middle East/Africa.

Figure 3 depicts the prevalence of concurrent susceptible and resistant phenotypes for ceftolozane/tazobactam and imipenem/relebactam (i.e. ceftolozane/tazobactam-resistant/imipenem/relebactam-susceptible, imipenem/relebactam-resistant/ceftolozane/tazobactam-susceptible and ceftolozane/tazobactam-resistant/imipenem/relebactam-resistant phenotypes) among

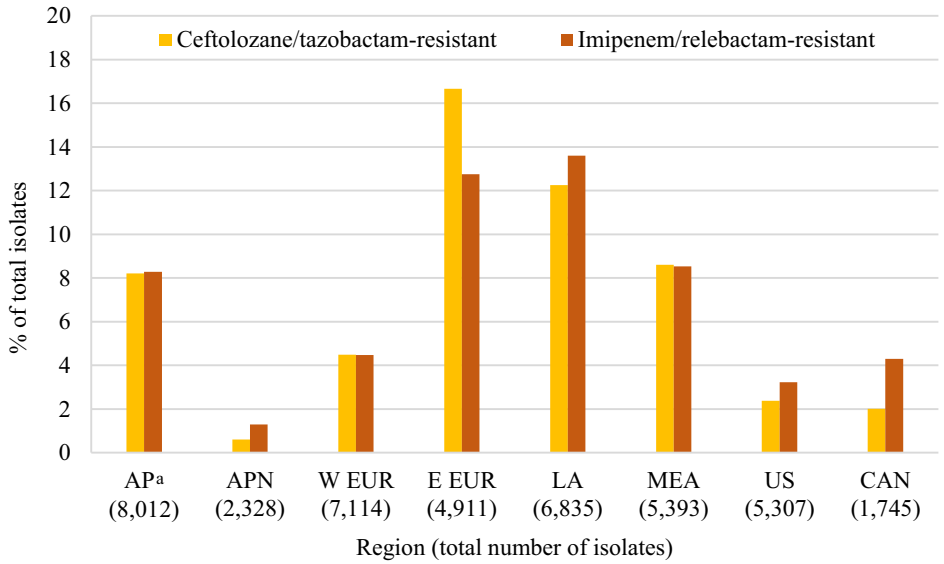

Figure 1. Global region proportions of *P. aeruginosa* isolates that were ceftolozane/tazobactam resistant and imipenem/relebactam resistant, cumulative 2016–21 data. [a]Excludes isolates from Australia and New Zealand. AP, Asia/Pacific; ANZ, Australia/New Zealand; W EUR, Western Europe; E EUR, Eastern Europe; LA, Latin America; MEA, Middle East/Africa; US, United States of America; CAN, Canada.

Table 1. Antimicrobial susceptibility testing results for ceftolozane/tazobactam-resistant and imipenem/relebactam-resistant *P. aeruginosa* isolates, cumulative 2016–21 data

	Percentage of isolates testing susceptible											
	Ceftolozane/tazobactam resistant						Imipenem/relebactam resistant					
Antimicrobial agent	Asia/Pacific (n=672)[a]	Western Europe (n=319)	Eastern Europe (n=818)	Latin America (n=837)	Middle East/ Africa (n=464)	USA/ Canada (n=161)[b]	Asia/Pacific (n=693)[a]	Western Europe (n=318)	Eastern Europe (n=626)	Latin America (n=930)	Middle East/ Africa (n=460)	USA/ Canada (n=246)[b]
Ceftolozane/ tazobactam	0	0	0	0	0	0	15.4	14.8	5.0	16.7	9.6	67.1
Imipenem/ relebactam	13.5	22.6	15.6	8.8	17.0	54.0	0	0	0	0	0	0
Imipenem	7.4	5.6	2.8	3.1	8.8	24.2	0	0	0	0	0	0.4
Meropenem	6.5	6.9	3.1	4.7	8.8	24.8	0.4	0.9	0.8	1.3	0.9	1.2
Cefepime	1.5	4.4	1.5	3.6	3.0	8.7	5.1	11.0	6.2	8.0	13.0	15.0
Ceftazidime	1.0	0.6	0.5	1.4	2.2	3.1	7.4	7.2	4.2	9.9	11.3	23.3
Aztreonam	11.5	17.6	13.6	19.8	27.2	7.5	13.1	23.9	20.9	18.6	31.3	12.6
Piperacillin/ tazobactam	7.1	6.9	7.7	5.7	6.3	10.6	7.5	6.0	3.0	7.5	4.8	15.4
Levofloxacin	2.4	10.3	2.9	4.5	4.7	13.7	3.2	6.3	3.7	4.8	5.0	6.9
Amikacin	29.6	39.5	20.0	18.9	18.1	65.8	35.4	39.9	23.2	27.2	21.3	71.1

[a]Australia/New Zealand region isolates were combined with the Asia/Pacific region isolates because of the low number of isolates with ceftolozane/tazobactam-resistant and imipenem/relebactam-resistant phenotypes in the Australia/New Zealand region.

[b]Ceftolozane/tazobactam-resistant and imipenem/relebactam-resistant phenotype isolates from the USA and Canada were combined into a single region for analysis because of the low number of isolates with ceftolozane/tazobactam-resistant and imipenem/relebactam-resistant phenotypes in both regions.

P. aeruginosa isolates collected during 2016–21. Overall, when resistance to either ceftolozane/tazobactam or imipenem/relebactam was present, cross-resistance to both agents was much more common than resistance to either agent alone, suggesting the presence of common, non-intrinsic resistance mechanisms (e.g. MBLs) in many isolates, with unique agent-specific resistance mechanisms being less common. Globally, 5.9% (2460/41 645) of isolates were resistant to both ceftolozane/tazobactam and imipenem/relebactam. The majority of ceftolozane/tazobactam-resistant and/or imipenem/relebactam-resistant isolates were resistant to both agents in all global regions (Eastern Europe, 11.3% of isolates were ceftolozane/tazobactam resistant and imipenem/relebactam resistant; Latin America, 10.6%; Middle East/Africa, 6.7%; Asia/Pacific, 5.2%; Western Europe, 3.1%) except the USA/Canada (0.8%), where the greatest percentage of isolates (2.3%) were imipenem/relebactam resistant and ceftolozane/tazobactam susceptible.

Figure 4 shows non-intrinsic β-lactamases detected in molecularly characterized ceftolozane/tazobactam- and/or imipenem/relebactam-resistant subsets of *P. aeruginosa* isolates during 2016–21. To be consistent across all study years, only β-lactamases that were included in the screening algorithm for PCR are shown in Figure 4 (i.e. the few non-intrinsic β-lactamases detected by only WGS during 2020–21, such as OXA-233 and PME-like, that would not have been detected by PCR from the years 2016 to 2019 because of the absence of specific primer sets were excluded). Intrinsic AmpC β-lactamases common to *P. aeruginosa* (PDC) are also not shown in Figure 4. Most ceftolozane/tazobactam-resistant/imipenem/relebactam-susceptible isolates carried ESBLs (44% of isolates) or did not have non-intrinsic β-lactamase genes identified (49% of isolates) while most imipenem/relebactam-resistant/ceftolozane/tazobactam-susceptible isolates (95%) did not have non-intrinsic β-lactamase genes identified. The majority of all ceftolozane/tazobactam-resistant/imipenem/relebactam-resistant isolates (76%) carried an MBL. Table S3 identifies in detail the non-intrinsic β-lactamases detected in ceftolozane/tazobactam-resistant/imipenem/relebactam-susceptible, imipenem/relebactam-resistant/ceftolozane/tazobactam-susceptible and ceftolozane/tazobactam-resistant/imipenem/relebactam-resistant subsets of *P. aeruginosa* isolates collected globally during 2016–21.

In lieu of screening for specific β-lactamase genes by PCR (as was done for isolates from 2016 to 2019), *P. aeruginosa* isolates from 2020 and 2021 underwent WGS. This permitted identification of chromosomally encoded resistance mechanisms in addition to β-lactamase genes. Table S2 lists the additional resistance mechanisms identified in isolates in which non-intrinsic β-lactamases were not detected.

Putative resistance mechanisms or mutations known to contribute to elevated MIC values for ceftolozane/tazobactam or imipenem/relebactam were identified in 26 (54.2%) of 48 isolates with a ceftolozane/tazobactam-resistant/imipenem/relebactam-resistant phenotype in which non-intrinsic β-lactamases were not detected (Table S2). An Ω-loop mutation in OXA-2 or OXA-10, or PDC mutation previously demonstrated to expand the substrate profile of the enzyme with or without an indicator of AmpC up-regulation was present in 18 (37.5%) of the 48 isolates. Figure S1 shows the frequency distribution of ceftolozane/tazobactam

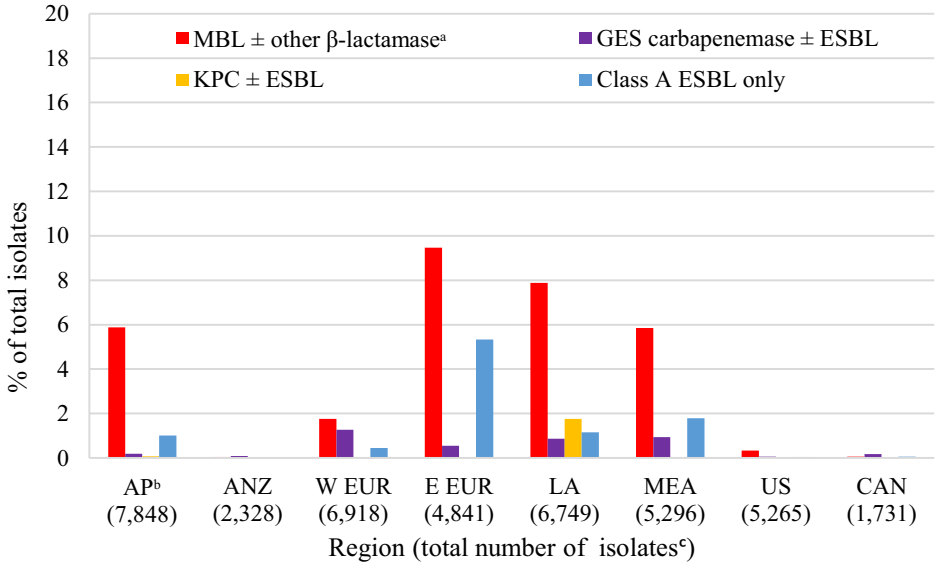

Figure 2. Estimated proportion of isolates carrying carbapenemases or non-intrinsic Class A β-lactamases among *P. aeruginosa* isolates collected by the global SMART surveillance programme from 2016 to 2021. [a]Includes 53 isolates that co-carried a GES carbapenemase (E EUR, $n=47$; MEA, $n=6$) and 49 isolates that co-carried KPC (AP, $n=5$; LA, $n=43$; USA, $n=1$) among a total of 1730 MBL-positive isolates. [b]Excludes Australia and New Zealand. [c]Excludes isolates not available for molecular characterization. AP, Asia/Pacific; ANZ, Australia/New Zealand; W EUR, Western Europe; E EUR, Eastern Europe; LA, Latin America; MEA, Middle East/Africa; US, United States of America; CAN, Canada.

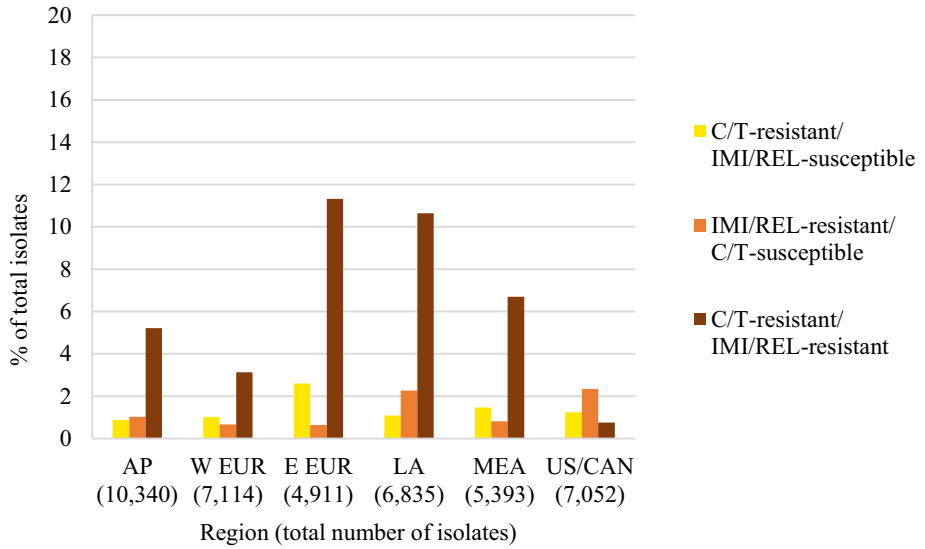

Figure 3. Prevalence of ceftolozane/tazobactam-resistant/imipenem/relebactam-susceptible, imipenem/relebactam-resistant/ceftolozane/tazobactam-susceptible and ceftolozane/tazobactam-resistant/imipenem/relebactam-resistant phenotypes among *P. aeruginosa* isolates collected by the global SMART surveillance programme from 2016 to 2021. AP, Asia/Pacific; W EUR, Western Europe; E EUR, Eastern Europe; LA, Latin America; MEA, Middle East/Africa; US/CAN, United States of America and Canada. AP includes Australia and New Zealand (ANZ) in this figure. C/T, ceftolozane/tazobactam; IMI/REL, imipenem/relebactam.

MICs among *P. aeruginosa* isolates from 2020–21 in which non-intrinsic β-lactamases were not detected, stratified by carriage of PDC mutation and the presence of AmpC regulatory mutations. In Figure S1(A), isolates carrying PDC without a known mutation, with or without mutations in accessory genes known to increase PDC expression, are shown. In Figure S1(B), isolates carrying a mutation within PDC, with or without mutations in accessory genes known to increase PDC expression, are shown. Indicators of strong (up to ~50-fold) AmpC up-regulation (loss-of-function mutation in *dacB* or *ampD*, or activating mutation in *ampR*) correlated with an 8-fold increase in the modal ceftolozane/tazobactam MIC value compared with isolates in which no indicative

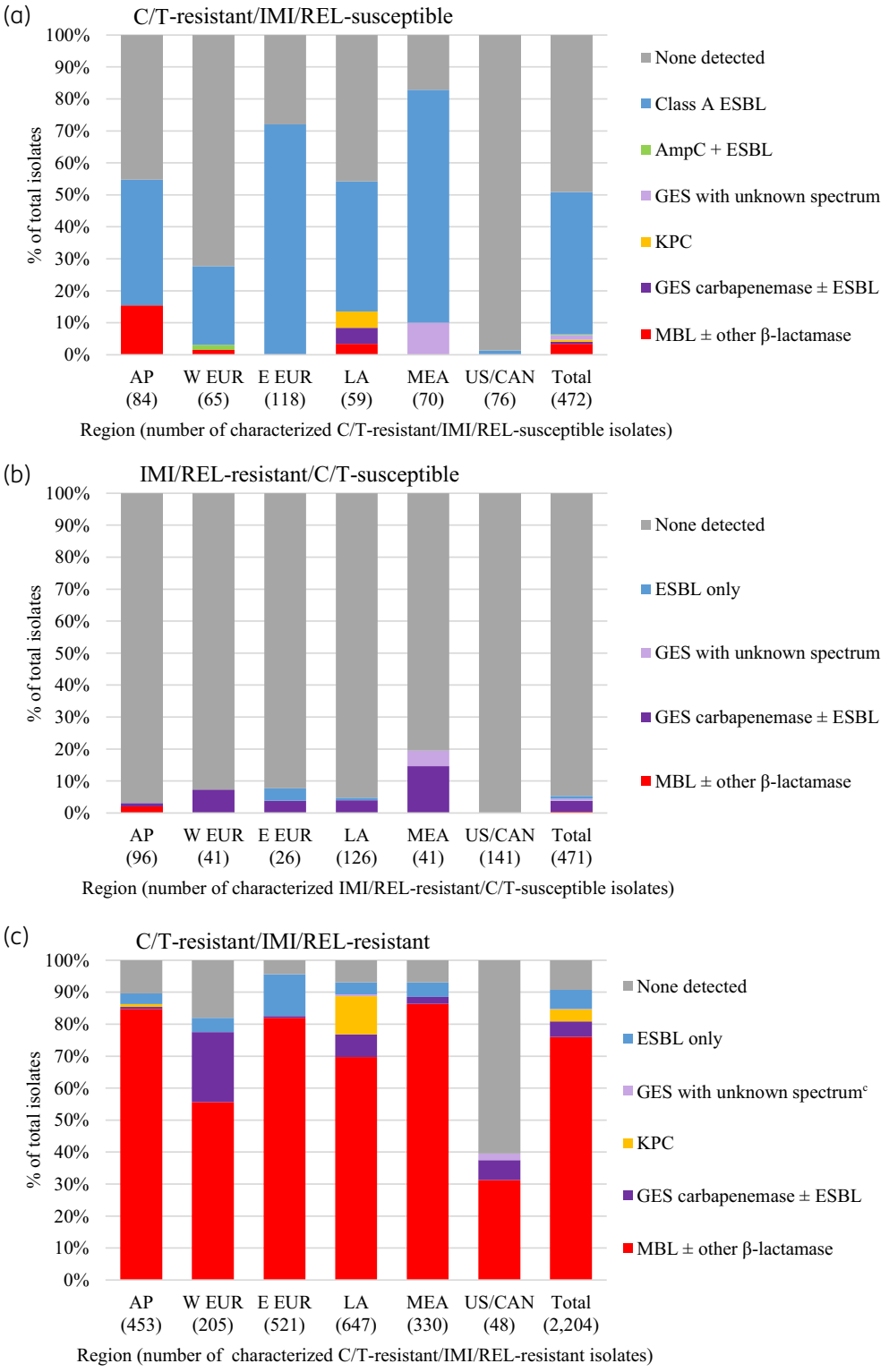

Figure 4. Non-intrinsic β-lactamases detected in molecularly characterized isolates of ceftolozane/tazobactam-resistant/imipenem/relebactam-susceptible, imipenem/relebactam-resistant/ceftolozane/tazobactam-susceptible and ceftolozane/tazobactam-resistant/imipenem/relebactam-resistant subsets of *P. aeruginosa*, cumulative 2016–2021 data. Intrinsic AmpC β-lactamases common to *P. aeruginosa* (PDC) are not shown. A representative sample of 80%–94% of isolates of each phenotype collected in each region were characterized. C/T, ceftolozane/tazobactam; IMI/REL, imipenem/relebactam; AP, Asia/Pacific; W EUR, Western Europe; E EUR, Eastern Europe; LA, Latin America; MEA, Middle East/Africa; US/CAN, United States of America and Canada. AP includes Australia and New Zealand (ANZ) in this figure.

mutation was observed (from 0.5 to 4 mg/L). Indicators such as *mpl* loss of function, and *ampDh2* and *ampDh3* mutation (lacking mutations in *dacB*, *ampD* or *ampR*), which are associated with weaker up-regulation of PDC (~5–10-fold), did not show as strong a correlation with increased ceftolozane/tazobactam MIC values as did mutation in *dacB*, *ampD* or *ampR*. Regardless, ceftolozane/tazobactam resistance was rarely observed, even in isolates with indicators of strong PDC up-regulation alone (3% resistance) unless a PDC mutation known to expand substrate profile was also present. None of the 17 isolates carrying an indicator for PDC up-regulation and a mutated PDC tested susceptible to ceftolozane/tazobactam, whereas a wide range of MIC values (1 to >32 mg/L) were observed among isolates with a mutated PDC and no indicator for PDC up-regulation. This finding could be owed in part to the fact that PDC up-regulation is not directly measurable by WGS and it has poor negative predictive power for this phenotype.

Any OprD loss of function was closely associated with imipenem/relebactam resistance in imipenem/relebactam-resistant/ceftolozane/tazobactam-susceptible isolates (91.0% of 145 isolates with this phenotype) and ceftolozane/tazobactam-resistant/imipenem/relebactam-resistant isolates (38.5% of 65 isolates with this phenotype) (Table S2); however, this finding alone does not account in totality for either of these phenotypes (Figure S3). Figure S3 depicts the impact of OprD loss of function on the frequency distribution of imipenem/relebactam MICs in imipenem-non-susceptible *P. aeruginosa* isolates from 2020 and 2021 in which non-intrinsic β-lactamases were not detected. OprD loss correlates with a shift in the distribution of imipenem/relebactam MIC values up by one or two doubling dilutions. This mechanism alone correlates with a considerable number of isolates (22.7%) testing in the intermediate category for imipenem/relebactam, although the majority of isolates (67.3%) with OprD loss of function were still imipenem/relebactam susceptible and approximately 10% were imipenem/relebactam resistant.

Discussion

From 2016 to 2021, resistance to both ceftolozane/tazobactam and imipenem/relebactam was uncommon (<5%) among clinical isolates of *P. aeruginosa* from Australia/New Zealand, the USA, Canada and Western Europe (Figure 1), confirming results from earlier studies.[2,3,5,25] Resistance to ceftolozane/tazobactam and imipenem/relebactam was highest in Eastern Europe and Latin America followed by the Middle East/Asia and Asia-Pacific regions. Regional percentages of isolates concurrently resistant to both ceftolozane/tazobactam and imipenem/relebactam correlated with estimated proportions of isolates carrying carbapenemases (Figure 2). Estimated MBL rates ranged from 9.5% of isolates from Eastern Europe to <1% of isolates from the USA, Canada and Australia/New Zealand (Figure 2). In total, 76% of *P. aeruginosa* isolates from all global regions that were concurrently ceftolozane/tazobactam resistant and imipenem/relebactam resistant were MBL positive (Figure 4). Most ceftolozane/tazobactam-resistant/imipenem/relebactam-susceptible isolates (95%) carried ESBLs or did not have non-intrinsic β-lactamase genes identified, and nearly all (95%) imipenem/relebactam-resistant/ceftolozane/tazobactam-susceptible isolates did not have non-intrinsic β-lactamase genes identified. In the absence of a PDC mutation known to expand its substrate profile, ceftolozane/tazobactam resistance was only observed in 3% of isolates with indicators of strong PDC up-regulation (mutation in *dacB*, *ampD* or *ampR*) (Figure S1). In contrast, all isolates carrying an indicator for PDC up-regulation and a mutated PDC tested ceftolozane/tazobactam non-susceptible while isolates with a mutated PDC and no indicator for PDC up-regulation demonstrated a wide range of MIC values (1 to >32 mg/L) (Figure S1).

Previously, Fournier and co-workers identified 42 *P. aeruginosa* isolates with ceftolozane/tazobactam MICs ≥8 mg/L from a collection of clinical isolates amassed by 36 hospital laboratories in France in 2015; 50% of the 42 isolates harboured an MBL, OXA-14, OXA-19, OXA-35, GES-9 or PER-1 enzyme; 38% showed extremely high production of PDC as a result of mutations in AmpR and enzymes composing the peptidoglycan recycling pathway, such as AmpD, PBP4 (encoded by *dacB*) and Mpl (producing ceftolozane/tazobactam MICs of 8–16 mg/L), in the absence of mutation in PDC; and the remaining 12% of isolates encoded a PDC variant known to expand its substrate profile to include ceftolozane (as ceftolozane/tazobactam) and ceftazidime.[14] Our results confirm these different mechanisms of ceftolozane/tazobactam resistance using a much larger, global dataset of *P. aeruginosa* isolates.

Previous reports have identified sporadic *P. aeruginosa* isolates that developed cross-resistance to ceftolozane/tazobactam, ceftazidime/avibactam and/or cefiderocol during therapy due to amino acid substitutions, insertions and/or deletions specifically within the AmpC (PDC) Ω-loop or adjacent AmpR regions, sometimes in combination with ESBLs.[13,17,19,36–43] These Ω-loop mutants maintain susceptibility to, or result in lower MICs of, imipenem, imipenem/relebactam and/or piperacillin/tazobactam. Mutations in the Ω-loop widen the AmpC binding pocket to permit cephalosporins with bulkier R2 side chains (e.g. ceftolozane with its 2-methyl-3-aminopyrazolium R2 side chain) to enter, resulting in increased catalysis of both ceftolozane and ceftazidime[12,44] and enable carbapenems to rotate their bulky 6α-hydroxyethyl side chain within the AmpC binding pocket to prevent hydrolysis.[45] Mutations in PBP3, the multidrug efflux transporter MexB and the DNA polymerase subunits gamma and tau have also been identified as infrequent mechanisms of ceftolozane/tazobactam resistance.[40,41]

We observed that in imipenem-non-susceptible *P. aeruginosa* isolates from 2020–21, OprD loss shifted the distribution of imipenem/relebactam MIC values up by one or two doubling dilutions, resulting in a considerable number of isolates (22.7%) testing in the intermediate category for imipenem/relebactam, although the majority of isolates (67.3%) with OprD loss of function were still imipenem/relebactam susceptible and approximately 10% were imipenem/relebactam resistant (Figure S3). Therefore, most imipenem/relebactam-resistant/ceftolozane/tazobactam-susceptible isolates may have been the result of porin defects in combination with another mechanism such as hyperproduction of AmpC,[15–17,24] which we were unable to determine in the current study based on the methods used.

In the current study, 5%–17% of imipenem/relebactam-resistant isolates from the Asia/Pacific region, Western Europe, Eastern Europe, Latin America and Middle East/Africa were ceftolozane/tazobactam susceptible (Table 1). Similarly, 9%–23% of ceftolozane/tazobactam-resistant isolates from the Asia/Pacific region, Western Europe, Eastern Europe, Latin America and

Middle East/Africa were imipenem/relebactam susceptible. In contrast, in the USA/Canada region, susceptibility to imipenem/relebactam among ceftolozane/tazobactam-resistant *P. aeruginosa* was 54.0% and susceptibility to ceftolozane/tazobactam among imipenem/relebactam-resistant *P. aeruginosa* was 67.1%, similar to previous reports of US isolates[3,46] These findings appear to be associated with a higher prevalence of isolates carrying MBLs and/or GES-type β-lactamases in regions outside of the USA and Canada (Figure 2, Figure S2). Less than 1% of isolates from the USA and Canada were resistant to both ceftolozane/tazobactam and imipenem/relebactam. We also noted that in Western Europe the percent imipenem/relebactam susceptible among ceftolozane/tazobactam-resistant isolates (22.6%) was higher than the percent ceftolozane/tazobactam susceptible among imipenem/relebactam-resistant isolates (14.8%) (Table 1). Such a pattern makes sense in Eastern Europe where there are much higher numbers of ESBLs than in Western Europe (Figure 2). However, the ESBL rate may be at least partly responsible, because the proportion of ESBL-positive isolates in the ceftolozane/tazobactam-resistant subset from Western Europe is still almost 10% (Figure S2). The Asia/Pacific region has a similar percentage of isolates with ESBLs, yet a different susceptibility pattern, so an undetected (non-β-lactamase-mediated mechanism) is probably also contributing to the phenotype differences. Previously, Fraile-Ribot *et al.*[17] reported that among carbapenemase-non-producing *P. aeruginosa* isolates from Spain, imipenem/relebactam inhibited 50% of ceftolozane/tazobactam-resistant isolates and Bail *et al.*[47] reported that ceftolozane/tazobactam had greater *in vitro* activity than imipenem/relebactam (80% versus 63%) against 229 carbapenemase-non-producing *P. aeruginosa* isolates from Brazil.

The current study has limitations. First, detailed patient histories are not documented for any isolate collected by the SMART global surveillance programme. Second, increased expression of genes involved in resistance (e.g. porin, efflux pump and PDC expression) could only be inferred from WGS data and was not directly measured. Third, not all isolates that qualified for molecular testing were characterized; sampling was considered when estimating the proportion of carbapenemases for Figure 2, and a portion of ceftolozane/tazobactam-resistant and imipenem/relebactam-resistant isolates could not be included in Figure 4 and Table S1. Fourth, clinical significance was assigned to isolates based on algorithms in place in each contributing clinical laboratory; therefore, non-invasive isolates may have been included in the study. Fifth, the study would have benefited from the inclusion of additional comparative agents such as ceftazidime/avibactam.

We conclude that resistance to ceftolozane/tazobactam and imipenem/relebactam remains uncommon among recent clinical isolates of *P. aeruginosa* collected across eight global regions. Among ceftolozane/tazobactam-resistant and imipenem/relebactam-resistant subsets, varying proportions of isolates tested as susceptible to the other agent across the eight global regions, depending on the non-intrinsic β-lactamases present. While ceftolozane/tazobactam may be considered the preferred anti-pseudomonal agent, imipenem/relebactam may be preferable over other β-lactam combinations against ceftolozane/tazobactam-non-susceptible isolates. Susceptibility testing of both ceftolozane/tazobactam and imipenem/relebactam should be considered in hospital laboratories, as both agents provide an important treatment option. Susceptibility patterns for both ceftolozane/tazobactam and imipenem/relebactam differ by region, making local antibiogram data critical for clinical decision-making. Development of agents with activity against MBL-producing Gram-negative bacilli remains an unmet medical need.

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Supplementary data

Figures S1 to S3 and Tables S1 to S3 are available as Supplementary data at *JAC-AMR* Online.

References

1 Reynolds D, Kollef M. The epidemiology and pathogenesis and treatment of *Pseudomonas aeruginosa* infections: an update. *Drugs* 2021; **81**: 2117–31. https://doi.org/10.1007/s40265-021-01635-6

2 Karlowsky JA, Lob SH, Raddatz J *et al*. In vitro activity of imipenem/relebactam and ceftolozane/tazobactam against clinical isolates of gram-negative bacilli with difficult-to-treat resistant phenotypes—study for monitoring antimicrobial resistance trends, United States 2015–2017. *Clin Infect Dis* 2021; **72**: 2112–20. https://doi.org/10.1093/cid/ciaa381

3 Karlowsky JA, Lob SH, DeRyke CA *et al*. *In vitro* activity of ceftolozane-tazobactam, imipenem-relebactam, ceftazidime-avibactam, and comparators against *Pseudomonas aeruginosa* isolates collected in United States hospitals according to results from the SMART surveillance program, 2018 to 2020. *Antimicrob Agents Chemother* 2022; **66**: e00189-22. https://doi.org/10.1128/AAC.00189-22

4 Tamma PD, Aitken SL, Bonomo RA *et al*. Infectious Diseases Society of America guidance on the treatment of extended-spectrum β-lactamase producing Enterobacterales (ESBL-E), carbapenem-resistant Enterobacterales (CRE), and *Pseudomonas aeruginosa* with difficult-to-treat resistance (DTR-*P. aeruginosa*). *Clin Infect Dis* 2021; **72**: 1109–16. https://doi.org/10.1093/cid/ciab295

5 Lizza BD, Betthauser KD, Ritchie DJ *et al*. New perspectives on antimicrobial agents: ceftolozane-tazobactam. *Antimicrob Agents Chemother* 2021; **65**: e02318-20. https://doi.org/10.1128/AAC.02318-20

6 US FDA. Zerbaxa, Highlights of Prescribing Information. https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/206829s008lbl.pdf. 2019.

7 US FDA. Recarbrio, Highlights of Prescribing Information. https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/212819s002lbl.pdf. 2020.

8 Livermore DM, Mushtaq S, Meunier D *et al*. Activity of ceftolozane/tazobactam against surveillance and 'problem' Enterobacteriaceae, *Pseudomonas aeruginosa* and non-fermenters from the British Isles. *J Antimicrob Chemother* 2017; **72**: 2278–89. https://doi.org/10.1093/jac/dkx136

9 Tamma PD, Doi Y, Bonomo RA *et al*. A primer on AmpC β-lactamases: necessary knowledge for an increasingly multidrug-resistant world. *Clin Infect Dis* 2019; **69**: 1446–55. https://doi.org/10.1093/cid/ciz173

10 Cabot G, Bruchmann S, Mulet X *et al*. *Pseudomonas aeruginosa* ceftolozane-tazobactam resistance development requires multiple mutations leading to overexpression and structural modification of AmpC. *Antimicrob Agents Chemother* 2014; **5**: 3091–9. https://doi.org/10.1128/AAC.02462-13

11 MacVane SH, Pandey R, Steed LL *et al*. Emergence of ceftolozane-tazobactam-resistant *Pseudomonas aeruginosa* during treatment is mediated by a single AmpC structural mutation. *Antimicrob Agents Chemother* 2017; **61**: e01183-17. https://doi.org/10.1128/AAC.01183-17

12 Berrazeg M, Jeannot K, Ntsogo Enguéné VY *et al*. Mutations in β-lactamase AmpC increase resistance of *Pseudomonas aeruginosa* isolates to antipseudomonal cephalosporins. *Antimicrob Agents Chemother* 2015; **59**: 6248–55. https://doi.org/10.1128/AAC.00825-15

13 Fraile-Ribot PA, Cabot G, Mulet X *et al*. Mechanisms leading to *in vivo* ceftolozane/tazobactam resistance development during the treatment of infections caused by MDR *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 2018; **73**: 658–63. https://doi.org/10.1093/jac/dkx424

14 Fournier D, Carrière R, Bour M *et al*. Mechanisms of resistance to ceftolozane/tazobactam in *Pseudomonas aeruginosa*: results of the GERPA multicenter study. *Antimicrob Agents Chemother* 2021; **65**: e01117-20. https://doi.org/10.1128/AAC.01117-20

15 Young K, Painter RE, Raghoobar SL *et al*. In vitro studies evaluating the activity of imipenem in combination with relebactam against *Pseudomonas aeruginosa*. *BMC Microbiol* 2019; **19**: 150. https://doi.org/10.1186/s12866-019-1522-7

16 Livermore DM, Warner M, Mushtaq S. Activity of MK-7655 combined with imipenem against Enterobacteriaceae and *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 2013; **68**: 2286–90. https://doi.org/10.1093/jac/dkt178

17 Fraile-Ribot PA, Zamorano L, Orellana R *et al*. Activity of imipenem-relebactam against a large collection of *Pseudomonas aeruginosa* clinical isolates and isogenic β-lactam-resistant mutants. *Antimicrob Agents Chemother* 2020; **64**: e02165-19. https://doi.org/10.1128/AAC.02165-19

18 Ortiz de la Rosa JM, Nordmann P, Poirel L. ESBLs and resistance to ceftazidime/avibactam and ceftolozane/tazobactam combinations in *Escherichia coli* and *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 2019; **74**: 1934–9. https://doi.org/10.1093/jac/dkz149

19 Díaz-Cañestro M, Periañez L, Mulet X *et al*. Ceftolozane/tazobactam for the treatment of multidrug resistant *Pseudomonas aeruginosa*: experience from the Balearic Islands. *Eur J Clin Microbiol Infect Dis* 2018; **37**: 2191–200. https://doi.org/10.1007/s10096-018-3361-0

20 Blizzard TA, Chen H, Kim S *et al*. Discovery of MK-7655, a β-lactamase inhibitor for combination with Primaxin®. *Bioorg Med Chem Lett* 2014; **24**: 780–5. https://doi.org/10.1016/j.bmcl.2013.12.101

21 Barnes MD, Bethel CR, Alsop J *et al*. Inactivation of the *Pseudomonas*-derived cephalosporinase-3 (PDC-3) by relebactam. *Antimicrob Agents Chemother* 2018; **62**: e02406-17. https://doi.org/10.1128/AAC.02406-17

22 Livermore DM. β-Lactamases in laboratory and clinical resistance. *Clin Microbiol Rev* 1995; **8**: 557–84. https://doi.org/10.1128/CMR.8.4.557

23 O'Donnell JN, Lodise TP. New perspectives on antimicrobial agents: imipenem-relebactam. *Antimicrob Agents Chemother* 2022; **66**: e00256-22. https://doi.org/10.1128/aac.00256-22

24 Mushtaq S, Meunier D, Vickers A *et al*. Activity of imipenem/relebactam against *Pseudomonas aeruginosa* producing ESBLs and carbapenemases. *J Antimicrob Chemother* 2021; **76**: 434–42. https://doi.org/10.1093/jac/dkaa456

25 Karlowsky JA, Hackel MA, Takemura M *et al*. *In vitro* susceptibility of Gram-negative pathogens to cefiderocol in five consecutive annual multinational SIDERO-WT surveillance studies, 2014 to 2019. *Antimicrob Agents Chemother* 2022; **66**: e01990-21. https://doi.org/10.1128/AAC.01990-21

26 CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—Eleventh Edition: M07-A11*. 2018.

27 CLSI. *Performance Standards for Antimicrobial Susceptibility Testing—Thirty-Second Edition: M100*. 2022.

28 Nichols WW, de Jonge BLM, Kazmierczak KM *et al*. *In vitro* susceptibility of global surveillance isolates of *Pseudomonas aeruginosa* to ceftazidime-avibactam (INFORM 2012 to 2014). *Antimicrob Agents Chemother* 2016; **60**: 4743–9. https://doi.org/10.1128/AAC.00220-16

29 Lob SH, Biedenbach DJ, Badal RE *et al*. Antimicrobial resistance and resistance mechanisms of *Enterobacteriaceae* in ICU and non-ICU wards in Europe and North America: SMART 2011–2013. *J Glob Antimicrob Resist* 2015; **3**: 190–7. https://doi.org/10.1016/j.jgar.2015.05.005

30 Estabrook M, Kazmierczak KM, Wise M *et al*. Molecular characterization of clinical isolates of Enterobacterales with elevated MIC values for aztreonam-avibactam from the INFORM global surveillance study, 2012–2017. *J Glob Antimicrob Resist* 2021; **24**: 316–20. https://doi.org/10.1016/j.jgar.2021.01.010

31 Bortolaia V, Kaas RF, Ruppe E *et al*. ResFinder 4.0 for predictions of phenotypes from genotypes. *J Antimicrob Chemother* 2020; **75**: 3491–500. https://doi.org/10.1093/jac/dkaa345

32 Quale J, Bratu S, Gupta J *et al*. Interplay of efflux system, *ampC*, and *oprD* expression in carbapenem resistance of *Pseudomonas aeruginosa* clinical isolates. *Antimicrob Agents Chemother* 2006; **50**: 1633–41. https://doi.org/10.1128/AAC.50.5.1633-1641.2006

33 Torrens G, Hernández SB, Ayala JA *et al*. Regulation of AmpC-driven β-lactam resistance in *Pseudomonas aeruginosa*: different pathways, different signaling. *mSystems* 2019; **4**: e00524-19. https://doi.org/10.1128/mSystems.00524-19

34 Kos VN, McLaughlin RE, Gardner HA. Elucidation of mechanisms of ceftazidime resistance among clinical isolates of *Pseudomonas aeruginosa* by using genomic data. *Antimicrob Agents Chemother* 2016; **60**: 3856–61. https://doi.org/10.1128/AAC.03113-15

35 Tsutsumi Y, Tomita H, Tanimoto K. Identification of novel genes responsible for overexpression of *ampC* in *Pseudomonas aeruginosa* PAO1. *Antimicrob Agents Chemother* 2013; **57**: 5987–93. https://doi.org/10.1128/AAC.01291-13

36 Arca-Suárez J, Vázquez-Ucha JC, Fraile-Ribot PA *et al*. Molecular and biochemical insights into the *in vivo* evolution of AmpC-mediated resistance to ceftolozane/tazobactam during treatment of an MDR *Pseudomonas aeruginosa* infection. *J Antimicrob Chemother* 2020; **75**: 3209–17. https://doi.org/10.1093/jac/dkaa291

37 Arca-Suárez J, Lasarte-Monterrubio C, Rodino-Janeiro B-K *et al*. Molecular mechanisms driving *in vivo* development of OXA-10-mediated resistance to ceftolozane/tazobactam and ceftazidime/avibactam during treatment of XDR *Pseudomonas aeruginosa* infections. *J Antimicrob Chemother* 2021; **76**: 91–100. https://doi.org/10.1093/jac/dkaa396

38 Rubio AM, Kline EG, Jones CE *et al*. *In vitro* susceptibility of multidrug-resistant *Pseudomonas aeruginosa* following treatment-emergent

resistance to ceftolozane-tazobactam. *Antimicrob Agents Chemother* 2021; **65**: e00084-21. https://doi.org/10.1128/aac.00084-21

39 Simner PJ, Beisken S, Bergman Y *et al*. Cefiderocol activity against clinical *Pseudomonas aeruginosa* isolates exhibiting ceftolozane-tazobactam resistance. *Open Forum Infect Dis* 2021; **8**: ofab311. https://doi.org/10.1093/ofid/ofab311

40 Tamma PD, Beisken S, Bergman Y *et al*. Modifiable risk factors for the emergence of ceftolozane-tazobactam resistance. *Clin Infect Dis* 2021; **73**: e4599–606. https://doi.org/10.1093/cid/ciaa1306

41 Haidar G, Philips NJ, Shields RK *et al*. Ceftolozane-tazobactam for the treatment of multidrug-resistant *Pseudomonas aeruginosa* infections: clinical effectiveness and evolution of resistance. *Clin Infect Dis* 2017; **65**: 110–20. https://doi.org/10.1093/cid/cix182

42 Skoglund E, Abodakpi H, Rios R *et al*. *In vivo* resistance to ceftolozane/tazobactam in *Pseudomonas aeruginosa* arising by AmpC- and non-AmpC-mediated pathways. *Case Rep Infect Dis* 2018; **2018**: 9095203. https://doi.org/10.1155/2018/9095203

43 Boulant T, Jousset AB, Bonnin RA *et al*. A 2.5-year within-patient evolution of *Pseudomonas aeruginosa* isolates with *in vivo* acquisition of ceftolozane-tazobactam and ceftazidime-avibactam resistance upon treatment. *Antimicrob Agents Chemother* 2019; **63**: e01637-19. https://doi.org/10.1128/AAC.01637-19

44 Slater CL, Winogrodzki J, Fraile-Ribot PA *et al*. Adding insult to injury: mechanistic basis of how AmpC mutations allow *Pseudomonas aeruginosa* to accelerate cephalosporin hydrolysis and evade avibactam. *Antimicrob Agents Chemother* 2020; **64**: e00894-20. https://doi.org/10.1128/AAC.00894-20

45 Lahiri SD, Walkup GK, Whiteaker JD *et al*. Selection and molecular characterization of ceftazidime/avibactam-resistant mutants in *Pseudomonas aeruginosa* strains containing derepressed AmpC. *J Antimicrob Chemother* 2015; **70**: 1650–8. https://doi.org/10.1093/jac/dkv004

46 Lob SH, DePestel DD, DeRyke CA *et al*. Ceftolozane/tazobactam and imipenem/relebactam cross-susceptibility among clinical isolates of *Pseudomonas aeruginosa* from patients with respiratory tract infections in ICU and non-ICU wards—SMART United States 2017–2019. *Open Forum Infect Dis* 2021; **8**: ofab320. https://doi.org/10.1093/ofid/ofab320

47 Bail L, Ito CAS, Arend L *et al*. Activity of imipenem-relebactam and ceftolozane-tazobactam against carbapenem-resistant *Pseudomonas aeruginosa* and KPC-producing Enterobacterales. *Diagn Microbiol Infect Dis* 2022; **102**: 115568. https://doi.org/10.1016/j.diagmicrobio.2021.115568