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Review

Resistance to ceftazidime/avibactam in infections and colonisations by KPC-producing Enterobacterales: a systematic review of observational clinical studies

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ABSTRACT

Objectives: Ceftazidime/avibactam (CAZ-AVI), approved in 2015, is an important first-line option for *Klebsiella pneumoniae* carbapenemase-producing Enterobacterales (KPC-E). Although still uncommon, resistance to CAZ-AVI has emerged and may represent a serious cause of concern.

Methods: We performed a systematic literature review of clinical and microbiological features of infections and colonisations by CAZ-AVI-resistant KPC-E, focused on the in vivo emergence of CAZ-AVI resistance in different clinical scenarios.

Results: Twenty-three papers were retrieved accounting for 42 patients and 57 isolates, mostly belonging to *K. pneumoniae* ST258 harbouring D179Y substitution in the KPC enzyme. The USA, Greece and Italy accounted for 80% of cases. In one-third of isolates resistance was not associated with previous CAZ-AVI exposure. Moreover, 20% of the strains were colistin-resistant and 80% were extended-spectrum β-lactamase (ESBL)-producers. The majority of infected patients had severe underlying diseases (39% cancer, 22% solid-organ transplantation) and 37% died. The abdomen, lung and blood were the most involved infection sites. Infections by CAZ-AVI-resistant strains were mainly treated with combination therapy (85% of cases), with meropenem being the most common (65%) followed by tigecycline (30%), gentamicin (25%), colistin (25%) and fosfomycin (10%). Despite the emergence of resistance, 35% of patients received CAZ-AVI.

Conclusion: Taken together, these data highlight the need for prompt susceptibility testing including CAZ-AVI for Enterobacterales, at least in critical areas. Resistance to CAZ-AVI is an urgent issue to monitor in order to improve both empirical and targeted CAZ-AVI use as well as the management of patients with infections caused by CAZ-AVI-resistant strains.

1. Introduction

Ceftazidime/avibactam (CAZ-AVI) is a novel β-lactam/β-lactamase inhibitor combination available since 2015. Compounding avibactam with ceftazidime overcomes resistance due to Ambler class A, class C and some class D β-lactamases [1]. CAZ-AVI is approved for use in (i) complicated intra-abdominal infections, (ii) complicated urinary tract infections, (iii) hospital-acquired pneumonia including ventilator-associated pneumonia (VAP) and (iv) infections due to aerobic Gram-negative organisms in patients with limited treatment options [2].

Nowadays, CAZ-AVI is mostly used for treating severe *Klebsiella pneumoniae* carbapenemase-producing Enterobacterales (KPC-E) infections, commonly associated with high morbidity and mortality rates, increased medical costs and prolonged hospital stay [3].

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At least 33 000 people died in Europe (approximately one-third in Italy) in 2015 as a result of multidrug-resistant pathogens, especially KPC-E [4]. KPC-E are of particular concern due to the high level of endemicity observed in several areas worldwide and they have been indicated by the US Centers for Disease Control and Prevention (CDC) as an urgent threat and one of the greatest global public-health challenges [5].

Only a few active antibiotics are available for KPC-E infections, and CAZ-AVI has become an important first-line option. Recently, the Infectious Diseases Society of America (IDSA) indicated CAZ-AVI, meropenem/vaborbactam and imipenem/cilastatin/relebactam as the preferred agents for KPC-E infections outside of the urinary tract [6]. This is mainly because their introduction has made it possible to treat severe KPC-E infections with β-lactams, an option that was unfortunately lost in the last decade, with clinicians forced to use last-resort and possibly suboptimal options such as polymyxins [7]. Indeed, although certainly useful as salvage therapy when nothing else works, polymyxins are hampered by possibly nephrotoxicity and potential impaired efficacy, especially in lung infections [8]. Apart from toxicity issues, clinical data have demonstrated the superiority of novel β-lactam/β-lactamase inhibitor combinations over polymyxins [6,9].

In light of this, the opportunity to retain CAZ-AVI activity against KPC-E in the long-term should not be wasted, therefore this agent should be used wisely according to antimicrobial stewardship principles (correct dosage for the correct duration, and for the correct indications) in order to maximise its efficacy and to delay the emergence and spread of resistance [10].

The first CAZ-AVI-resistant strain was reported in the clinic in 2015, from a patient with no history of CAZ-AVI exposure [11]. Since then, other episodes of colonisation or infection due to CAZ-AVI-resistant strains have quickly been reported in the literature, although overall resistance to CAZ-AVI was reported at very low rates in large prevalence and surveillance studies [12]. Resistance to CAZ-AVI is commonly due to the presence of metallo-β-lactamases since their activity is not restored by avibactam [13]. Other mechanisms include increased expression of the bla_{KPC} gene, specific mutations in genes encoding carbapenemases, changes in cell permeability (i.e. loss of porins) and expression of efflux pumps [14,15]. In some cases, restoration of susceptibility to meropenem can occur, mostly due to amino acid substitutions and conformational changes in the active site of carbapenemase enzymes, leading to very low minimum inhibitory concentrations (MICs) [16–18]. In these cases, the use of carbapenems is not indicated because, following their use, MICs could increase and resistance to CAZ-AVI persist [12,16,19]. Despite this, meropenem is commonly used as anti-KPC-E option, in combination with colistin and aminoglycosides, in order to avoid the risk of treatment failure. To treat infections caused by KPC-E resistant to CAZ-AVI, new molecules are now available, including meropenem/vaborbactam, imipenem/relebactam and cefiderocol. Meropenem/vaborbactam is a combination of a known carbapenem and a new non-β-lactam β-lactamase inhibitor derived from boronic acid. Vaborbactam is capable of restoring the activity of meropenem against β-lactamase-producing Enterobacterales, including KPC-E [20]. Similarly, relebactam is a non-β-lactam, bicyclic diazabicyclooctane β-lactamase inhibitor of class A and class C β-lactamases, including KPC-E. Addition of relebactam significantly improves the activity of imipenem [20]. Cefiderocol is a new parenteral catechol-substituted siderophore cephalosporin that enters the periplasmic space of bacterial cells using the iron transport system. Of note, this drug shows high stability against various types of β-lactamases, including serine-based and metallo-type carbapenemases [21]. The emergence of resistance to CAZ-AVI induced the European Centre for Disease Prevention and Control (ECDC) to provide a rapid risk assessment in 2018 [22].

Table 1
Criteria used for literature inclusion and exclusion

Inclusion criteria
• Case reports or case series regarding in vivo emergence of resistance to CAZ-AVI in patients infected or colonised by KPC-producing Enterobacterales
Exclusion criteria
• Reports regarding other micro-organisms (neither KPC-producers nor Enterobacterales) • Only in vitro studies • Reports related to surveillance studies (aggregate data) • Reports in languages other than English • Reports that were multiple publications of a primary study

CAZ-AVI, ceftazidime/avibactam.

Here we performed a systematic review of the available observational literature on the clinical and microbiological features of patients with infection or colonisation due to CAZ-AVI-resistant KPC-E in order to provide an overview and critical appraisal of the available evidence on resistance to CAZ-AVI in several clinical scenarios. In particular, our analysis aimed to evaluate (and summarise): (i) all clinical studies in which resistance to CAZ-AVI in KPC-E was reported; (ii) the characteristics of CAZ-AVI use (e.g. monotherapy versus combination therapy); (iii) patients' outcomes; (iv) the involved resistance mechanisms; and (v) the therapeutic options undertaken against CAZ-AVI-resistant isolates.

2. Methods

This systematic review was performed according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) [23].

2.1. Protocol and registration

The study protocol was registered and made publicly available on https://osf.io/87bjh.

2.2. Literature search strategy

Information sources were represented by two major databases, MEDLINE and Embase [24], screened from inception until to 30 April 2020 using the following combination of keywords: (ceftazidime/avibactam[Text Word] OR ceftazidime-avibactam[Text Word] AND KPC[Text Word] AND resistance[Text Word]). Records were de-duplicated before entering the subsequent phase of the review.

2.3. Study selection

Two investigators (LP and VV) carried out the first selection of the retrieved records by title and abstract in order to establish eligibility for full-text review. The second step consisted of further screening of full-text articles to define final inclusion in the systematic review according to the following criteria: (i) observational studies (cohorts, case series or case reports); and (ii) description of in vivo resistance to CAZ-AVI among Enterobacterales strains (MIC $>$ 8 mg/L) [25] producing a KPC carbapenemase, whatever infection they were responsible for. Surveillance studies, namely those aimed at assessing the prevalence of given resistant strains and/or resistance mechanisms among large collections of laboratory isolates (aggregate data), were excluded. The inclusion and exclusion criteria are summarised in Table 1. A third reviewer (AEM) was called upon to resolve disagreements with regard to the two-step selection process.

2.4. Data extraction

A pre-conceived data extraction sheet was used to abstract data from the included studies. The task was performed by two investigators (LP and VV). Any disagreement was reconciled through consensus of the entire study group, made up both of clinical microbiology and infectious diseases specialists. The extracted information included authors, publication year, country, number of patients, baseline features of described cases (sex, age, prior exposure to CAZ-AVI, type of infection/colonisation, main co-morbidities, exposure to other antibiotics), microbiological data regarding resistance to CAZ-AVI (mobile element harbouring bla_{KPC} gene, associated resistance genes, replicon/plasmid), antibiotic regimens implemented to counter resistance and clinical outcomes.

2.5. Analysis plan

A descriptive analysis was planned, not testing any a priori hypothesis. Anticipating high heterogeneity of the included studies and limited sample sizes, a narrative summary of findings was favoured over a non-feasible meta-analytic approach.

2.6. Quality assessment

For case series and case reports, an adapted version of the tool proposed by Murad et al. was adopted [26] (Supplementary Table S1). The Newcastle–Ottawa scale was used in the case of observational cohort studies [27].

2.7. Ethics

This kind of study did not require approval by an institutional review board since it relied on already available data from existing medical literature.

3. Results

3.1. Bibliography selection and general features

The initial search identified 361 records. After proper screening of the titles and abstracts, de-duplication and full-text review, 23 articles (all case reports or case series, no cohort studies) were deemed eligible for inclusion. The entire selection process is illustrated in Fig. 1.

The publication year ranged from 2015 to 2020. Nine articles were from the USA, six were from Italy, seven were from other European countries (Greece 3, Finland 1, Germany 1, Spain 1 and Switzerland 1) and one was from Argentina.

A total of 42 patients were described, of which 33 contributed a unique isolate and 9 contributed multiple isolates.

3.2. Microbiological findings

3.2.1. Characteristics of CAZ-AVI-resistant isolates

A total of 57 isolates with resistance to CAZ-AVI were reported (Table 2), of which 19 (33.3%) showed baseline resistance not associated with previous CAZ-AVI-based treatment, while 38 (66.7%) acquired resistance after a treatment with CAZ-AVI. Regarding bacterial species, 55 were *Klebsiella pneumoniae*, 1 was *Citrobacter freundii* and 1 was *Enterobacter hormaechei*. The isolates mainly belonged to sequence type 258 (ST258) ($n = 20$; 35.1%). Other strains belonged to ST147 ($n = 8$), ST307 ($n = 5$), ST512 ($n = 4$), ST1519 ($n = 4$), ST11 ($n = 3$), ST39 ($n = 2$), ST101 ($n = 1$), ST395 ($n = 1$) and ST407 (*E. hormaechei*). The ST was not reported for eight isolates. When reported, the presence of a Tn*4401*-like transposon harbouring the bla_{KPC} gene was described for 19 *K. pneumoniae* isolates (33.3%), while Tn*5403* was reported for the *E. hormaechei* isolate. The plasmidic nature of the bla_{KPC} gene was also described for 26 isolates (45.6%) (Table 3).

Other reported resistance determinants for β-lactams were SHV-type (-11, -12, -128 and -182; $n = 27$ isolates; 47.4%), TEM-1 ($n = 24$ isolates; 42.1%), OXA-type (-1, -9 and -10; $n = 25$ isolates; 43.9%), CTX-M-type (-1 and -15; $n = 8$ isolates; 14.0%), VEB-type (-14 and -25; $n = 10$ isolates; 17.5%) and CMY-type ($n = 1$ isolate; 1.8%). Regarding last-resort antibiotics, resistance genes were reported for aminoglycosides ($n = 21$ isolates; 36.8%) and fosfomycin ($n = 6$ isolates; 10.5%). Notably, although colistin resistance mediated by MgrB alteration was reported in only 1 isolate, phenotypic resistance to colistin was reported for 10 isolates (17.5%) (Table 3).

3.2.2. Isolates with acquired resistance during treatment

Acquired resistance for strains previously exposed to CAZ-AVI was reported in 38 isolates harbouring either KPC-3 ($n = 26$; 68.4%) or KPC-2 ($n = 11$; 28.9%) determinants. The single isolate of *E. hormaechei* harboured a KPC-40 enzyme (Table 2). After CAZ-AVI-based treatment, acquisition of resistance was mostly associated with isolates harbouring the substitution D179Y ($n = 23$; 60.5%), in KPC-3 ($n = 18$; 47.4%) or in KPC-2 ($n = 5$; 13.2%), alone or in combination with other substitutions or resistance determinants (i.e. non-functional porins). When reported, non-functional porins (OmpK35, OmpK36 and OmpK37) were detected in 10 isolates (26.3%). In seven of them, this resistance trait has been reported in combination with the substitution D179Y in KPC determinants. MICs for CAZ-AVI in resistant isolates exposed to antibiotic ranged from 12 mg/L to 256 mg/L. Overall, isolates harbouring the D179Y substitution showed the highest MICs for CAZ-AVI (mostly 128–256 mg/L), either alone or in combination with other resistance determinants, conferring from a 5- to 7-fold increase of the initial MICs (ranging from 0.5–8 mg/L).

Initial MICs for meropenem ranged from 8 mg/L to 128 mg/L. Restoration of susceptibility to meropenem was reported for 20 isolates (52.6%), showing from a 2- to 9-fold reduction of initial meropenem MICs. Notably, 12 (60.0%) of 20 isolates harboured the D179Y substitution in the KPC determinant. Information regarding restoration of susceptibility was not reported for five isolates (13.2%) showing the D179Y substitution in KPC. Moreover, the lowest post-treatment MICs for meropenem (0.25–0.5 mg/L) were observed for isolates harbouring D179Y substitution in KPC determinants.

3.2.3. Isolates with baseline resistance (with no previous CAZ-AVI-based treatment)

Baseline resistance was reported for 19 isolates not previously exposed to CAZ-AVI (Table 2). Resistance was mostly due to the presence of VEB-25 ($n = 9$ isolates; 47.4%) in combination with KPC determinants and non-functional porins. Other resistant isolates showed KPC variants, such as KPC-8 (V240G+H274Y substitutions in KPC-2; $n = 3$; 15.8%), KPC-23 (V240A substitution in KPC-3; $n = 1$; 5.3%), KPC-31 (D179Y substitution in KPC-3; $n = 1$; 5.3%) and KPC-2 with D179Y substitution ($n = 1$; 5.3%). Overall, baseline resistance due to non-functional porins (OmpK35, OmpK36 and OmpK37) was reported in combination with other determinants (KPC-2, KPC-3 or VEB-25) in 11 isolates (57.9%). MICs for CAZ-AVI ranged from 16 mg/L to 256 mg/L, with the highest values mostly associated with the presence of non-functional porins and the VEB-25 determinant, rather than other KPC variants. Baseline MICs for meropenem ranged from 4 mg/L to 2048 mg/L, with the highest value (1024–2048 mg/L) observed in two isolates harbouring multiple copies of the bla_{KPC-3} gene in combination with non-functional porins (OmpK35 and OmpK36). Importantly, the last two isolates also showed resistance to meropenem/vaborbactam.

Table 2
Microbiological data of ceftazidime/avibactam (CAZ-AVI)-resistant isolates

Reference	No. of isolates	Bacterial species	Clinical sample	KPC variant[a]	CAZ-AVI exposure at time of culture (days, sample)	Mechanism of resistance to CAZ-AVI	Initial CAZ-AVI MIC (mg/L)	CAZ-AVI MIC (mg/L) after treatment	Restored susceptibility to MEM	Initial MEM MIC (mg/L)	MEM MIC (mg/L) after CAZ-AVI treatment
Humphries et al. [11,15]	1	KP	Blood	KPC-3	No previous exposure	Truncated OmpK35; substitutions T333N and R191L in OmpK36	32	32, no previous CAZ-AVI exposure	No	512	512
Shields et al. [28]	6	KP	#1,#2, sputum #3,#4, urine #5,#6, BAL	KPC-3	#1, 10, sputum #2, 24, sputum #3,#4, 19, urine #5,#6, 15, BAL	Substitutions in KPC-3: #1,#2, D179Y+T243M #3, V240G #4–#6, D179Y	#1,#2, 2 #3,#4, 4 #5,#6, 2	#1,#2, 256 #3, 32 #4, >256 #5, 128 #6, 64	Yes	#1,#2, 128 #3–#6, 32	#1, 0.5 #2, 0.25 #3, 8 #4, 4 #5, 0.25 #6, 0.125
Shields et al. [16]	1	KP	Respiratory secretions, blood	KPC-3	10, blood	Substitutions in KPC-3: A177E+D179Y	1	128	Yes	16	0.25
Giddins et al. [29]	5	KP	#1,#2, BAL #3, tracheal aspirate #4,#5, blood	KPC-2	#1, 12, BAL #2, 21, BAL #3, 22, tracheal aspirate #4,#5, 23, blood	#1–#3, substitution D179Y in KPC-2 #4,#5, truncated OmpK35 (AA349); non-functional OmpK36 (insertion of IS*1*); bla_{KPC-2} multiple copies	3	#1–#3, >256 #4,#5, 12	#1–#3, yes #4,#5, no	128	#1,#2, 2 #3, 1.5 #4,#5, >128
Raisanen et al. [30]	1	KP	Blood	KPC-2	44, blood	15 AA insertion (AVYTRAPNKDDKHSE) in KPC-2 at position 259	1	>16	Partially	>32	16
Gaibani et al. [31]	2	KP	#1, BAL #2, blood	KPC-3	#1, 17, BAL #2, 17, blood	Substitution D179Y in KPC-3; truncated OmpK35 (AA88)	8	≥256	#1, partially #2, no	≥32	#1, 8 #2, ≥32
Gottig et al. [32]	1	KP	Rectal swab, bronchial secretion, wound swab, intraoperative biopsies	KPC-3	14, rectal swab	Substitution D179Y in KPC-3 (formerly KPC-31)	4	>256	No	NS	NS
Athans et al. [33]	2	KP	#1, blood #2, abscess fluid	KPC-2	#1, 33, blood #2, abscess fluid	Substitution D179Y in KPC-2; disrupted OmpK35, OmpK36 and OmpK37	4	#1, 128 #2, >256	#1, yes #2, partially	≥16	#1, 2 #2, 4
Galani et al. [34]	8	KP	#1,#4,#5,#7,#8, rectal swab #2,#6, blood #3, bronchial secretion	KPC-2	#1, NS, rectal swab #2–#8, no previous exposure	#1, deletion T216 in VEB-1 (formerly VEB-14), duplication of Gly134-Asp135 in OmpK36, truncated OmpK37 (AA251) #2–#8, substitution K234R in VEB-1 (formerly VEB-25), truncated OmpK35 (AA173)	#1, NS #2–#8, 64	#1, 64 #2–#8, no previous CAZ-AVI exposure	#1, no #2–#8, no previous CAZ-AVI exposure	#1, NS #2–#8, 64	#1, >64 #2–#8, no previous CAZ-AVI exposure
Voulgari et al. [35]	2	KP	#1, blood #2, BAL	#1, KPC-2 #2, KPC-3	No previous CAZ-AVI exposure	Substitution K234R in VEB-1 (formerly VEB-25)	#1, 64 #2, >256	No previous CAZ-AVI exposure	No previous CAZ-AVI exposure	>32	No previous CAZ-AVI exposure
Cano et al. [36]	5	KP	#1, respiratory secretion #2,#3,abdominal drainage #4, abdominal aspirate #5, rectal swab	KPC-3	#1, 12, respiratory secretion #2, 16, abdominal drainage #3, 20, abdominal drainage #4, abdominal aspirate #5, rectal swab	#1,#2, substitution A172T in KPC-3 (formerly KPC-39) #3, substitutions L169P+A172T in KPC-3 #4, substitution D179Y in KPC-3 (formerly KPC-31) #5, substitutions A172T+T243A in KPC-3	2	>16	#1,#2,#5, no #3,#4, yes	>16	#1,#2,#5, >16 #3, 1 #4, 2

(*continued on next page*)

Table 2 (*continued*)

Reference	No. of isolates	Bacterial species	Clinical sample	KPC variant[a]	CAZ-AVI exposure at time of culture (days, sample)	Mechanism of resistance to CAZ-AVI	Initial CAZ-AVI MIC (mg/L)	CAZ-AVI MIC (mg/L) after treatment	Restored susceptibility to MEM	Initial MEM MIC (mg/L)	MEM MIC (mg/L) after CAZ-AVI treatment
Hemarajata et al. [37]	1	KP	Blood	KPC-2	22, blood	Substitution L169P in KPC-2 (formerly KPC-35), insertion in OmpK36 (GD at position 132) and in OmpK35 (insertion of G at position 122 causing frameshift at AA42)	0.5	16	Yes	>16	1
Coppi et al. [38]	2	KP	#1, urine #2, blood	KPC-3	No previous CAZ-AVI exposure	Double copy of bla_{KPC-3}, alteration of OmpK35 (AA89-truncated) and OmpK36 (Asp135-Thr136 duplication)	#1, 32 #2, 64	No previous CAZ-AVI exposure	No previous CAZ-AVI exposure	#1, 1024 #2, 2048	No previous CAZ-AVI exposure
Antonelli et al. [39]	1	KP	Rectal swab	KPC-3	14, rectal swab	Substitution D179Y in KPC-3 (formerly KPC-31), non-functional Ompk35 (AA89 truncated) and OmpK36 (Gly134-Asp135 duplication)	Susceptible, MIC value NS	>64	Yes	Resistant, MIC value NS	2
Mueller et al. [40]	1	KP	Rectal swab	KPC-3	24, rectal swab	Insertion (269-ProAsnLys-270) in KPC-3 (formerly KPC-41)	4	>128	Yes	8	1
Gaibani et al [41]	3	KP	Blood	#1,#3, KPC-3 #2, mutated KPC-2	No previous CAZ-AVI exposure	#2, substitution D179Y in KPC-2 #1,#3, truncated OmpK35 (AA38), insertion in OmpK36 (GD at position 134–135) truncated OmpK37	#1, 32 #2, 16 #3, >256	No previous CAZ-AVI exposure	No previous CAZ-AVI exposure	>32	No previous CAZ-AVI exposure
Gaibani et al. [42]	1	KP	Rectal swab	KPC-3	18, rectal swab	Substitution D163E in KPC-3 (formerly KPC-36), truncated OmpK35 (AA42), insertion in OmpK36 (GD at position 134–135), truncated OmpK37	8	16	No	Susceptible, MIC value NS	>256
Venditti et al. [43]	2	KP	Respiratory secretions	KPC-3	#1, 30, BA #2, 25, BA	Substitution D179Y in KPC-3 (formerly KPC-31), defective OmpK35	#1, 4 #2, 1.5	#1, 256 #2, 96	Yes	>32	#1, 3 #2, 1
Galani et al. [44]	1	KP	Urine	KPC-23	No previous CAZ-AVI exposure	KPC-23 (substitution V240A in KPC-3), truncated OmpK35 (AA89)	16	No previous CAZ-AVI exposure	No previous CAZ-AVI exposure	512	No previous CAZ-AVI exposure
Shields et al. [45]	5	KP	#1, BAL #2,#3, BAL #4, BAL #5, respiratory secretions	KPC-3	#1, 11, BAL #2,#3, 11, BAL #4, 7, BAL #5, 12, respiratory secretions	Substitution D179Y in KPC-3 (formerly KPC-31)	#1, 2 #2,#3, 2 #4, 4 #5, 2	#1, 64 #2, 64 #3, 32 #4, 64 #5, 64	NS	NS	NS
García et al. [46]	3	KP	Urine	KPC-8	No previous CAZ-AVI exposure	KPC-8 (substitutions V240G+H274Y in KPC-2), loss of OmpK35	16	No previous CAZ-AVI exposure	No previous CAZ-AVI exposure	4	No previous CAZ-AVI exposure
Castanheira et al. [47]	1	CF	Abdominal fluid drain	KPC-2	11, abdominal drain fluid	Substitutions D176Y/R164S+P174L in KPC-2	4	64	No	64	32
Munoz-Price et al. [48]	2	#1, KP #2, EH	Rectal swab	#1, KPC-3 #2, KPC-40	#1,#2, 47, rectal swab	#1, substitution D179Y in KPC-3 (formerly KPC-31) #2, KPC-40 (substitution T237S in KPC-3)	#1, NS #2, NS	#1, 64 #2, 16	#1, no #2, yes	#1, NS #2, NS	#1, >8 #2, ≤1

NOTE: Numbers preceded by '#' indicate sequential number of isolates.

MIC, minimum inhibitory concentration; MEM, meropenem; KP, *Klebsiella pneumoniae*; BAL, bronchoalveolar lavage; AA, amino acid; NS, not specified; BA, bronchoaspirate; CF, *Citrobacter freundii*; EH, *Enterobacter hormaechei*.

[a] KPC variant before CAZ-AVI-based treatment (when occurred).

Table 3
Molecular data of ceftazidime/avibactam-resistant isolates

Reference	No. of isolates	ST	Associated resistance genes	Mobile element harbouring KPC gene	Replicon/plasmid [a]
Humphries et al. [11,15]	1	258	*bla*$_{SHV-11}$, *bla*$_{SHV-12}$	Tn*4401d*	IncX3-pUCLAKPC
Shields et al. [28]	6	258	*bla*$_{TEM-1}$, *bla*$_{SHV-11}$, *bla*$_{OXA-9}$, *aadA1, aac(6′)-Ib, strAB, sul2, dfrA14*	ΔTn*1331*–Tn*4401d*	IncFIA-pBK30683
Shields et al. [16]	1	258	NS	NS	NS
Giddins et al. [29]	5	307	*bla*$_{CTX-M-1}$, *bla*$_{OXA-1}$	Tn*4401e*	IncA/C, IncFIB$_K$
Raisanen et al. [30]	1	39	*bla*$_{SHV-11}$	NS	NS
Gaibani et al. [31]	2	1519	#1,#2, *bla*$_{TEM-1A}$, *bla*$_{OXA-9}$, *bla*$_{SHV-11}$, *aac(6′)-Ib, aadA2, aph(3′)-Ia, aac(6′)-Ib*-cr, *oqxA, oqxB* #1, *mgrB*	NS	#1,#2, IncFIIK, IncFIB(pQIL), IncFIBK(Kpn3), IncFIB(pKPHS1), IncX3, ColRNAI #2, Col(BS512)
Gottig et al. [32]	1	101	NS	NS	NS
Athans et al. [33]	2	NS	*bla*$_{TEM-1}$, *bla*$_{SHV-11}$, *bla*$_{SHV-12}$	NS	NS
Galani et al. [34]	8	#1, 39 #2–#8, 147	#1,#2, *bla*$_{SHV-11}$, *bla*$_{OXA-10}$, *bla*$_{TEM-1B}$, *rmtB1* #1, *bla*$_{VEB-14}$ #2–#8, *bla*$_{VEB-25}$	NS	IncA/C2
Voulgari et al. [35]	2	#1, 147 #2, 258	#1,#2, *aadA1, aadA2, aph(2′′)-Ia, aph(3′′)-Ia, aph(3′′)-Ib, aph(6)-Id, rmtB1,* *bla*$_{VEB-25}$, *bla*$_{OXA-10}$, *bla*$_{TEM-1}$, *oqxA, oqxB, fosA, mdfA, cmlA1, floR2, arr-2, sul1, sul2, tetA, tetG, dfrA12* #1, *bla*$_{SHV-11}$ #2, *aac(6′)-Ib*-cr, *bla*$_{SHV-182}$, *mphA, catA1, dfrA14, dfrA23*	NS	#1, IncA/C2, IncR, IncFIB(pKPHS1), IncFIB(pQil), IncFII(K), IncA/C2, IncFIB(K) #2, ColRNAI, IncX3
Cano et al. [36]	5	NS	NS	NS	NS
Hemarajata et al. [37]	1	258	*bla*$_{OXA-9}$, *bla*$_{SHV-11}$, *bla*$_{TEM-1A}$	NS	NS
Coppi et al. [38]	2	258	#1, *aph(3′)-Ia, aadA2, aac(3)-IIa, aac(6′)-Ib3, bla*$_{OXA-1}$, *catA1, catB3, dfrA12, dfrA14, qnrB1, sul1, aac(6′)-Ib* #2, *bla*$_{TEM-1}$	Tn*4401a-1*, Tn*4401a-2*	#1, IncFII$_{K7}$-IncFIB$_k$, IncFIB$_k$-ColE #2, IncFII$_{K7}$-IncFIB$_k$, IncFIB$_k$–ColE
Antonelli et al. [39]	1	512	*bla*$_{SHV-11}$, *bla*$_{TEM-1}$, *aph(3′)-Ia, aac(6′)-Ib, ant(3′′)-Ia, catA1, sul1, dfrA12, mphA*	NS	NS
Mueller et al. [40]	1	395	*bla*$_{TEM-1}$, *bla*$_{SHV}$, *bla*$_{CMY}$	NS	IncFII-type
Gaibani et al. [41]	3	#1, 512 #2, 258 #3, 1519	#1, *bla*$_{SHV-11}$, *aac(6′)-Ib, oqxA, oqxB, aac(6′)-Ib*-cr, *sul1* #2, *bla*$_{SHV-12}$, *aadA2, aph(3′)-Ia, aac(6′)-Ib*-cr, *oqxA, oqxB, sul1* #3, *bla*$_{TEM-1A}$, *bla*$_{OXA-9}$, *bla*$_{SHV-11}$, *aadA2, aph(3′)-Ia, aac(6′)-Ib*-cr, *oqxA, oqxB, sul1*	Tn*4401*	#1, IncFIB (K), IncFIB(pKPHS1), IncX3, ColRNAI #2, IncFIIK, IncFIB(K), IncX3, ColRNAI #3, IncFIB (pQIL), IncFIB(pKPSH1), IncFIB(K), IncFII(K), IncX3, ColRNAI, Col(BS512)
Gaibani et al. [42]	1	1519	*bla*$_{TEM-1A}$, *bla*$_{SHV-11}$, *bla*$_{OXA-9}$, *aac(6′)-Ib, oqxA, oqxB, aac(6′)-Ib*-cr, *fosA*	Tn*4401a*	IncFIB(pQIL), IncFIB(K), ColRNAI, Col(BS512), IncX3
Venditti et al. [43]	2	512	*bla*$_{TEM-1A}$, *bla*$_{SHV-128}$, *bla*$_{OXA-9}$, *aac(6′)-Ib, aadA2, aph(3′)-Ia, fosA, mphA, catA1, oqxA, oqxB, sul1, dfrA12*	NS	IncFII(K), IncFIB(pQil)
Galani et al. [44]	1	258	*bla*$_{TEM-1A}$, *bla*$_{SHV-11}$, *bla*$_{OXA-9}$, *aac(6′)-Ib, aph(3′)-Ia, aadA2, fosA, catA1, sul1, tetA, dfrA12*	Tn*4401a*	IncFIIk-FIB
Shields et al. [45]	5	258	NS	NS	NS
García et al. [46]	3	11	*bla*$_{CTX-M-15}$	NS	NS
Castanheira et al. [47]	1	NS	NS	NS	NS
Munoz-Price et al. [48]	2	#1, 258 #2, 407	*#2, bla*$_{OXA-9}$, *bla*$_{TEM-}$1, *aac(6′)-Ib, aadA1, strB, strA, sul2, dfrA14*	#2, Tn*5403*	NS

NOTE: Numbers preceded by '#' indicate sequential number of isolates.
NS, not specified.
[a] Replicon/plasmid content of study isolates as indicated in original reports.

Table 4
Clinical and epidemiological data of patients with infections or colonisations by ceftazidime/avibactam (CAZ-AVI)-resistant isolates

Reference	Country (year)	No. of patients	Sex, age (years)	Prior CAZ-AVI exposure	Main underlying diseases	Infection/colonisation	Antibiotic regimen	Outcome
Humphries et al. [11,15]	USA (2015––2017)	1	F, 62	No	Splenectomy Pancreatic cancer	Liver abscess BSI	SXT + PMB	Improved
Shields et al. [28]	USA (2017)	3	#1, F, 40 #2, F, 50 #3, M, 70	#1, yes (14 days) #2, yes (19 days) #3, yes (15 days)	#1, lung transplant #2, subphrenic abscess #3, oesophageal cancer	#1, pneumonia #2, urinary colonisation #3, pneumonia	#1, MEM + GEN #2, none #3, MEM + COL	#1, died #2, discharged #3, improved
Shields et al. [16]	USA (2017)	1	M, 67	Yes (30 days)	Oesophageal cancer	Intra-abdominal abscess BSI	MEM + drainage MEM	Discharged
Giddins et al. [29]	USA (2018)	1	M, 40	Yes (12 days)	Diabetes Hypertension Acute pancreatitis	Pancreatitis HAP	MEM + PMB	Died
Raisanen et al. [30]	Finland (2019)	1	NS	Yes (34 days)	None	BSI	SXT + COL	Recovered
Gaibani et al. [31]	Italy (2018)	1	M, NS	Yes (17 days)	Liver transplant	BSI HAP	MEM + GEN	Died
Gottig et al. [32]	Germany (2019)	1	F, 60 [a]	Yes (14 days)	Myocardial infarction	Respiratory, intestinal, wound colonisation Sepsis (NS)	CAZ-AVI + TIG	Died
Athans et al. [33]	USA (2019)	1	M, 24	Yes (33 days)	Liver transplant	BSI Subphrenic abscess	GEN + PMB + TIG MEM/vaborbactam + TIG	Recovered
Galani et al. [34]	Greece (2020)	8	#1, NS, 50 #2, NS, 85 #3, NS, 85 #4, NS, 65 #5, NS, 75 #6, NS, 70 #7, NS, 60 #8, NS, 55	#1, yes #2, no #3, no #4, no #5, no #6, no #7, no #8, no	#1, subarachnoid haemorrhage #2, subdural haematoma #3, metastatic cancer #4, subarachnoid haemorrhage #5, subarachnoid haemorrhage #6, acute coronary syndrome #7, metastatic cancer #8, acute coronary syndrome	#1, colonisation #2, CRBSI #3, VAP #4, colonisation #5, colonisation #6, CRBSI #7, colonisation #8, colonisation	#1, NS #2, CAZ/AVI + FOS + MEM #3, ATM + CAZ/AVI + FOS #4, NS #5, NS #6, CAZ/AVI + MEM #7, NS #8, NS	#1, discharged #2, died #3, died #4, discharged #5, discharged #6, died #7, died #8, discharged
Voulgari et al. [35]	Greece (2020)	2	#1, F, 60 [a] #2, M, 30 [a]	#1, no #2, no	#1, cardiopulmonary arrest #2, epidural haematoma	#1, CRBSI #2, respiratory colonisation	#1, NS #2, NS	#1, NS #2, NS
Cano et al. [36]	Spain (2019)	1	M, 47	Yes (12 days)	Pancreatectomy for cancer	Intra-abdominal infection	Imipenem/cilastatin + GEN + TIG	Improved
Hemarajata et al. [37]	USA (2019)	1	M, 40 [a]	Yes (13 days)	Hypertension Alcohol abuse End-stage liver disease End-stage renal disease	BSI	MEM	Discharged
Coppi et al. [38]	Italy (2020)	1	NS	No	Kidney transplant	UTI BSI	Nephrectomy + double carbapenem + TIG	Recovered

(*continued on next page*)

Table 4 (*continued*)

Reference	Country (year)	No. of patients	Sex, age (years)	Prior CAZ-AVI exposure	Main underlying diseases	Infection/colonisation	Antibiotic regimen	Outcome
Antonelli et al. [39]	Italy (2019)	1	NS	Yes (14 days)	Surgical site infection	Intestinal colonisation	None	NS
Mueller et al. [40]	Switzerland (2019)	1	M, 72	Yes (24 days)	Pancreatic cancer	Cholangitis	COL + MEM	Recovered
Gaibani et al. [41]	Italy (2020)	3	NS	No (3/3)	NS	BSI (3/3)	NS	NS
Gaibani et al. [42]	Italy (2020)	1	M, 50	Yes (18 days)	Liver transplant	Intestinal colonisation	None	NS
Venditti el al. [43]	Italy (2019)	2	#1, F, 27 #2, M, 53	#1, yes (30 days) #2, yes (25 days)	#1, liver transplant #2, HIV/AIDS	#1, respiratory colonisation #2, respiratory colonisation	#1, none #2, none	#1, died #2, discharged
Galani et al. [44]	Greece (2019)	1	NS	No	NS	NS	NS	NS
Shields et al. [45]	USA (2018)	4	#1, F, 49 #2, F, 58 #3, M, 73 #4, F, 43	#1, yes (10 days) #2, yes (19 days) #3, yes (15 days) #4, yes (11 days)	#1, lung transplant #2, Intra-abdominal infection #3, oesophageal cancer #4, lung transplant	#1, pneumonia #2, urinary colonisation #3, pneumonia #4, pneumonia	#1, GEN + MEM #2, None #3, COL + MEM #4, CAZ-AVI	#1, died #2, survived #3, survived #4, died
Garcia et al. [46]	Argentina (2020)	3	NS	No (3/3)	NS	Urinary infection/colonisation (3/3)	NS	NS
Castanheira et al. [47]	USA (2018)	1	F, 44	Yes (12 days)	End-stage renal disease Intestinal perforation	Peritonitis	AMK + CAZ-AVI + TIG	Died
Munoz-Price et al. [48]	USA (2019)	2	#1, M, 69 #2, F, 63	#1, yes (47 days) #2, yes (47 days)	#1, liver transplant #2, NS	#1, intestinal colonisation #2, intestinal colonisation	#1, none #2, none	#1, NS #2, NS

NOTE: Numbers preceded by '#' indicate sequential number of patients.
BSI, bloodstream infection; SXT, trimethoprim/sulfamethoxazole; PMB, polymyxin B; MEM, meropenem; GEN, gentamicin; COL, colistin; HAP, hospital-acquired pneumonia; NS, not specified; TIG, tigecycline; CRBSI, catheter-related bloodstream infection; VAP, ventilator-associated pneumonia; FOS, fosfomycin; ATM, aztreonam; UTI, urinary tract infection; HIV/AIDS, human immunodeficiency virus/acquired immune deficiency syndrome; AMK, amikacin.
[a] Approximate age.

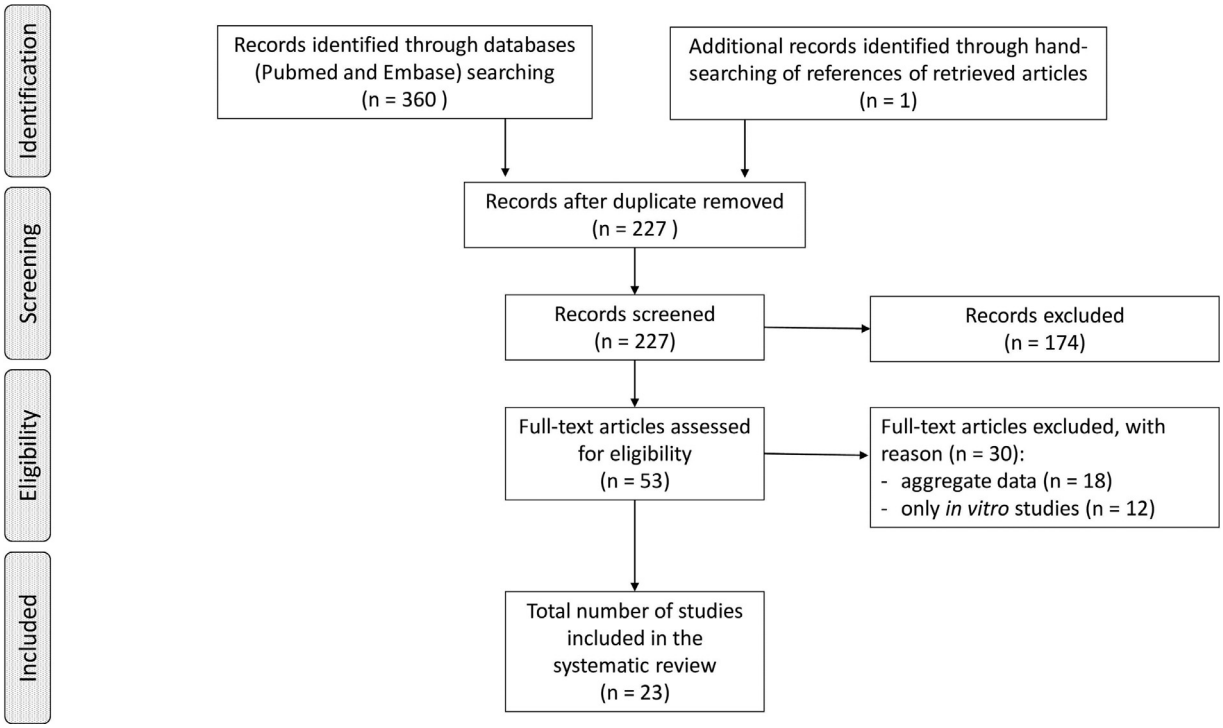

Fig. 1. Literature selection procedure.

3.3. Clinical description of case reports and case series

3.3.1. Clinical and epidemiological data

Our search retrieved 42 patients infected ($n = 27$) or colonised ($n = 15$) by CAZ-AVI-resistant KPC-E (Table 4). Among patients with infections caused by CAZ-AVI-resistant strains, 53% were male and the mean ± standard deviation (S.D.) age was 57 ± 17 years. As predisposing factors, 39% patients had cancer and 22% were solid-organ transplant recipients. CAZ-AVI was administered as intermittent infusion with dosages reflecting the manufacturers' data sheets (i.e. 2.5 g every 8 h) and adjusted for glomerular filtration rate when needed. The mean ± S.D. duration of CAZ-AVI administration was 17 ± 7 days; this long duration is particularly influenced by the presence of patients with intra-abdominal abscess in the case series. The fatality rate of infected patients was 37%. Ten patients (23.8%) developed bloodstream infection (BSI) by CAZ-AVI-resistant strains.

Among colonised patients, the mean age was 54 years and the fatality rate was 15%.

CAZ-AVI in combination with other antibiotics (usually gentamicin or tigecycline; 45% combination regimens for each) was administered to 69% of infected patients before the emergence of CAZ-AVI resistance. Meropenem was part of the regimen in only one case. Conversely, known CAZ-AVI-resistant strains were commonly treated with combination therapy (85% of cases were related to infections), with meropenem being the commonest antibiotic used (65% of cases), followed by tigecycline (30%), gentamicin (25%), colistin (25%) and fosfomycin (10%). One case was treated with a regimen including meropenem/vaborbactam. Despite the emergence of resistance, 35% of patients received CAZ-AVI, in all but one as part of combination therapy.

3.3.2. Detection of CAZ-AVI resistance in the absence of previous CAZ-AVI treatment

The first report of infection due to CAZ-AVI-resistant KPC-3-producing *K. pneumoniae* was that of a 62-year-old woman who underwent a pancreaticoduodenectomy for cancer [11]. She then developed bacteraemic cholangitis abscesses due to carbapenem-resistant *K. pneumoniae*. After failure of combinations regimens including gentamicin, cefepime, colistin, high-dose meropenem and tigecycline, the patient was admitted to the intensive care unit and received CAZ-AVI. Surprisingly, one carbapenem-resistant *K. pneumoniae* isolated from blood turned out to be resistant to CAZ-AVI, likely owing to the combination of porin alterations and increased KPC-3 expression [15]. Apparent synergy between avibactam and meropenem was detected in vitro and a combined regimen of CAZ-AVI, meropenem and polymyxin B was commenced, with possible beneficial effects [11]. Porin alterations and increased KPC-3 expression were also responsible for CAZ-AVI resistance in a pandrug-resistant KPC-producing *K. pneumoniae* causing urinary tract infection and bacteraemia in a kidney transplant recipient in whom bilateral nephrectomy was necessary to resolve the infection [38]. In a laboratory-based surveillance study, three CAZ-AVI-resistant KPC-E were isolated from CAZ-AVI-unexposed patients with BSIs. In this study, CAZ-AVI resistance was conferred by porin alterations plus increased expression of KPC-3 in two of the cases and by mutation of the bla_{KPC-2} gene in the other [41]. No further details regarding clinical history were available, in line with the laboratory-based nature of the study. The same lack of clinical history applies to a few other clinical isolates of CAZ-AVI-resistant KPC-E from some other laboratory-based studies [44–46].

CAZ-AVI resistance in KPC-E isolated from patients without prior CAZ-AVI exposure was also reported by Voulgari et al. who reported two patients harbouring CAZ-AVI-resistant isolates displaying only intermediate susceptibility to tigecycline (one isolated from blood and the other from the lower respiratory tract) and in which resistance to CAZ-AVI was conferred by VEB-25, a variant of VEB-1 that is not inhibited by avibactam [35]. CAZ-AVI resistance due to VEB-25 production was also reported by Galani et al. in seven patients with KPC-E isolates not exposed to CAZ-AVI (two patients with catheter-related BSI, one with VAP and four only colonised) [34]. Clinical improvement was observed in one of

the patients with catheter-related BSI and in the patient with VAP who were treated with combinations of CAZ-AVI plus meropenem plus fosfomycin and CAZ-AVI plus aztreonam plus fosfomycin, respectively (death due to other causes was subsequently registered in both cases). Failure of CAZ-AVI plus meropenem salvage therapy with subsequent infection-related death was conversely registered in the other patient with catheter-related BSI.

3.3.3. Emergence of CAZ-AVI resistance after CAZ-AVI treatment

In 2017, Shields et al. reported three cases of emergence of CAZ-AVI resistance after CAZ-AVI treatment in patients KPC-E infections [28]. The first patient was a lung transplanted woman with urinary tract infection and pneumonia, the second a woman with subphrenic abscess, and the third a man with oesophageal cancer and pneumonia. All of these infections were caused by carbapenem-resistant but CAZ-AVI-susceptible *K. pneumoniae* and were treated with CAZ-AVI. However, an inverse susceptibility phenotype (CAZ-AVI-resistant but meropenem-susceptible) was recorded in subsequent *K. pneumoniae* isolates from the first and third patients who were treated with meropenem plus gentamicin and with meropenem plus colistin, respectively. An unfavourable and a favourable outcome (death in the first patient and survival in the third patient) were ultimately registered. No further therapy was deemed necessary in the second patient in whom the CAZ-AVI-resistant isolates were considered colonisers. In this study, mutations in the bla_{KPC-3} gene were found to be responsible for CAZ-AVI resistance and restored meropenem susceptibility, although the latter was not observed for the second patient [28]. In the same year, the same authors reported another male patient with oesophageal cancer who developed pneumonia from a KPC-3-producing CAZ-AVI-susceptible *K. pneumoniae* treated with a combination of CAZ-AVI and aerosolised gentamicin [16]. Subsequently, the patient developed an intra-abdominal abscess and BSI due to carbapenem-susceptible and CAZ-AVI-resistant *K. pneumoniae*. The abscess was resolved with complete drainage, whereas the bacteraemic event resolved after meropenem monotherapy. Again, mutations in the bla_{KPC-3} gene were deemed responsible for the modified phenotype [16].

Mutations in the bla_{KPC-3} gene leading to KPC variants that conferred resistance to CAZ-AVI in CAZ-AVI-treated patients were also described in other case reports or small case series. Gaibani et al. reported a young liver transplanted man with a BSI due to a CAZ-AVI-susceptible KPC-3-producing *K. pneumoniae*, initially successfully treated with a combination of CAZ-AVI and gentamicin [31]. Two days after treatment discontinuation, the patient developed bacteraemic pneumonia from CAZ-AVI-resistant strains (one with low-level and one with high-level meropenem resistance), treated with a combination of high-dose meropenem and gentamicin with initial improvement, although an unfavourable outcome was eventually registered. A similar scenario was described by Cano et al. who reported a male patient who, following pancreatectomy for cancer, developed a complicated intra-abdominal infection due to a CAZ-AVI-susceptible KPC-3-producing *K. pneumoniae*, with subsequent isolation after CAZ-AVI treatment of a CAZ-AVI-resistant and carbapenem-susceptible *K. pneumoniae* producing a KPC-3 variant [36]. In this patient, clinical improvement was observed after initiation of a combined regimen with gentamicin, tigecycline and imipenem/cilastatin. Two cases of emergent KPC-3 variants conferring CAZ-AVI resistance and reduced meropenem MICs were also reported by Venditti et al. [43]. The first of the two patients had been previously treated with CAZ-AVI plus fosfomycin for VAP and complicated intra-abdominal infection caused by CAZ-AVI-susceptible KPC-3-producing *K. pneumoniae* (developed after liver transplantation). The second patient, who had human immunodeficiency virus (HIV) infection and Kaposi sarcoma, had been previously treated with CAZ-AVI and tigecycline for VAP and bacteraemia due to CAZ-AVI-susceptible KPC-3-producing *K. pneumoniae*.

Of note, development of CAZ-AVI resistance due to mutations in bla_{KPC-3} was also observed in rectal KPC-E colonisers harboured by patients who received CAZ-AVI treatment for other indications (e.g. targeted treatment of a systemic KPC-E infection or empirical treatment) [32,39,40,42,48]. Mutations, this time in the bla_{KPC-2} gene, were registered in the case of emergent CAZ-AVI resistance in six CAZ-AVI-treated patients with KPC-2-producing Enterobacterales colonisation or infection in the USA ($n = 4$), Greece ($n = 1$) and Finland ($n = 1$), not always associated with concomitant restoration of meropenem susceptibility [29,30,33,34,37,47]. Finally, among 19 and 37 patients receiving CAZ-AVI treatment for various types of KPC-2-producing Enterobacterales and KPC-3-producing Enterobacterales infections, CAZ-AVI resistance emerged in 0% (0/19) and 21.6% (8/37) of cases, respectively. Of note, in this latter study development of resistance was independently associated with receipt of renal replacement therapy in patients with microbiological failure ($n = 25$) during CAZ-AVI treatment (odds ratio = 26.7, 95% confidence interval 2.2–317.1; $P = 0.009$) [45]. A summary of the types of infection, antibiotic therapy and outcome of both these latter cases and all other patients with CAZ-AVI-resistant KPC-E described above is available in Table 4. The country-wise distribution of resistant cases and the most important related features are shown in Fig. 2.

4. Discussion

Resistance to CAZ-AVI has become a serious cause of concern [22]. When reported in studies involving >10 isolates, resistance rates mostly ranged between 0% and 4% [41,49–64], with only two studies reporting the higher resistant rate of 8.1% (3/37 isolates) and 12.8% (6/47 isolates), respectively, among KPC-producers [58,60]. However, these data represent the overall rates of resistance to CAZ-AVI reported in the scientific literature, taking into account the diversity of examined populations and the evaluation of different epidemiological or therapeutic contexts. Interestingly, low rates of CAZ-AVI-resistant isolates have also been retrospectively reported in strains isolated before the introduction of CAZ-AVI in clinical practice (2015) [51,55,56]. This is an important point because isolates with baseline resistance to CAZ-AVI, although distributed at very low rates, could represent a reservoir of resistance that could be potentially enhanced under inappropriate CAZ-AVI-based treatment. Notably, an important percentage of isolates with baseline resistance to CAZ-AVI (33.3%; $n = 19$) has also been found in our search.

Our work represents the first review of the literature summarising the emergence of resistance to CAZ-AVI in real-life clinical case reports or case series. Overall, we can speculate that resistance to CAZ-AVI, although uncommon, has rapidly emerged with significant numbers, especially considering the very recent history of this drug. Clinical cases reporting the emergence in vivo of resistance occurred in seven countries, accounting for 42 patients with infections or colonisations sustained by resistant isolates. Notably, 80% of patients were reported in the USA ($n = 14$; 9 reports), Greece ($n = 11$; 3 reports) and Italy ($n = 9$; 6 reports), commonly known as endemic countries for KPC-E (Fig. 2). Intensive use of CAZ-AVI in these countries could be conceivable, hence potentially increasing the local CAZ-AVI resistance rate. To date, more than 50 CAZ-AVI-resistant KPC-E have been reported in clinical cases or case series (i.e. 57 resistant isolates in 23 reports). Almost two-thirds of them were isolated from patients previously exposed to CAZ-AVI and almost all the involved resistant bacteria were *K. pneumoniae* strains. One-fifth of the CAZ-AVI-resistant isolates were also resistant to colistin and ~80% of the isolates were also extended-spectrum β-lactamases (ESBL)-producers.

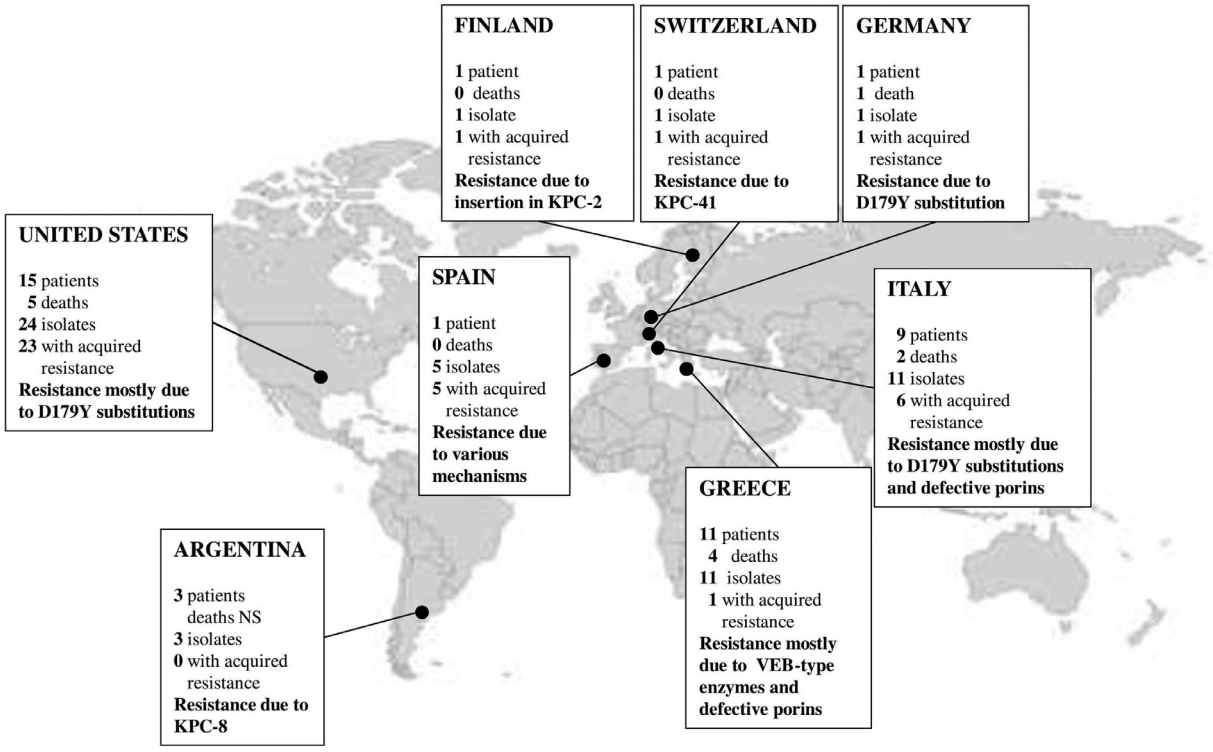

Fig. 2. Country-wise distribution of ceftazidime/avibactam-resistant cases and most relevant features.

An important cause of concern is represented by the high fatality rate related to infected patients (37%). However, this rate is similar to those previously described for infections caused by CAZ-AVI-susceptible KPC-E [65]. The mortality rate of patients with CAZ-AVI-resistant systemic infections (10%) was much lower compared with the overall mortality (37%), hence highlighting the pivotal role of patient co-morbidities (cancer, transplantation, cardiopathy) in increasing the overall mortality rate attributable to CAZ-AVI-resistant isolates. In fact, as previously reported, a higher clinical cure does not necessarily result in a reduction of in-hospital mortality [66]. Moreover, the high percentage of CAZ-AVI-resistant isolates in patients with important co-morbidities mostly reflects the intensive exposure to antimicrobials in this population.

Another major concern is related to the several mechanisms of resistance described so far, determining various levels of resistance to CAZ-AVI. Reported mechanisms of resistance include amino acid substitutions or deletions of the KPC enzyme and permeability defects (i.e. alterations in OmpK35, OmpK36 and OmpK37), sometimes in association with an increased expression of KPC or even ESBL determinants (SHV-, CTX-M- or VEB-type β-lactamases). In particular, most substitutions occurred within the KPC Ω-loop (positions 165–179), thereby enhancing ceftazidime affinity and possibly restricting avibactam binding [17]. Resistant isolates were mostly KPC-3-producing *K. pneumoniae* belonging to ST258. The D179Y variant, both in KPC-2 and KPC-3 determinants, alone or in combination with other substitutions or resistance mechanisms (i.e. non-functional porins), was the most reported resistance mechanism and manifested the strongest phenotypes (CAZ-AVI MICs of 128–256 mg/L), determining a 5- to 7-fold increase of the initial CAZ-AVI MICs. As expected, KPC variants commonly had a plasmidic nature, being mostly harboured by Tn*4401*-like transposons. Despite the mobile genetic nature of mutated enzymes, large outbreaks due to CAZ-AVI-resistant isolates have not been described so far. While KPC mutations were mainly reported following treatment with CAZ-AVI, permeability defects related to non-functional porins have been described even in the absence of previous exposure to the drug (baseline resistance), hence inhibiting the diffusion of AVI across the outer membrane, with higher CAZ-AVI MICs (256 mg/L) mostly associated with the presence of the VEB-25 determinant.

Restoration of susceptibility to meropenem occurred mostly in isolates harbouring the D179Y variant, sometimes reaching very low post-treatment MICs (0.5–0.25 mg/L) and determining a 2- to 9-fold reduction of the initial meropenem MICs. These data could indicate that infections caused by CAZ-AVI-resistant and carbapenem-susceptible *K. pneumoniae* could also, theoretically, be treated with carbapenems. However, in real life, the role of carbapenems in treating patients with infections caused by CAZ-AVI-resistant KPC-E is unclear. In fact, in vitro studies have demonstrated that under selective pressure with carbapenems, the MICs of these compounds can increase, while the organism maintains its resistance to CAZ-AVI [17]. As an important finding, when data were reported, our search revealed 12 patients (28.6%) with infections sustained by CAZ-AVI-resistant KPC-E treated with meropenem-based therapy (alone or in combination). It is of note that six (50%) of them died.

This point represents a serious challenge for the treatment of KPC-E because the application of combination therapy related to the need to protect the activity of CAZ-AVI, but also of carbapenems, should be considered. One of the most recent studies reported the highest percentage of resistant isolates (12.7%) among those treated with CAZ-AVI-based monotherapy, highlighting the possible role of combination therapy in the correct clinical management of CAZ-AVI, although with the limitation of the small sample size and the inherent difficulties in reliably assessing effectiveness of combinations versus monotherapy for the treatment of KPC-E [60,67]. Our search highlights the emergence of resistance to CAZ-AVI when administered either in monotherapy and in a combination regimen. Indeed, considering only infected patients before the emergence of CAZ-AVI resistance, 69% received

CAZ-AVI in combination with other antibiotics (usually gentamicin or tigecycline). Moreover, 85% of CAZ-AVI-resistant strains related to infections were commonly treated with combination therapy, with meropenem as the commonest antibiotic used (65% of cases). Notably, despite the emergence of resistance, 35% of patients received CAZ-AVI, in all but one as part of combination therapy. Given together, these data highlight how an optimal therapeutic regimen for CAZ-AVI, either in monotherapy or combination, remains an unanswered question. Recent IDSA guidance on the treatment of antimicrobial-resistant Gram-negative infections recommended against routine combination therapy for carbapenem-resistant Enterobacterales infections according to previous data reporting no additional benefit of combination therapy [6,68]. However, conflicting evidence exists [69] and we believe this is still an open issue.

Taking into consideration that the main mechanism of resistance to CAZ-AVI is represented by the presence of metallo-β-lactamases, the role of other β-lactam/β-lactamase inhibitor combinations (i.e. meropenem/vaborbactam and imipenem/relebactam) is overall limited. However, the increasing use of CAZ-AVI for treating infections caused by KPC-E could change this situation. Meropenem/vaborbactam has been successfully used in the presence of specific mutations of genes encoding carbapenemases, suggesting a role as salvage therapy [33].

It is likely that the lack of adequate source control alongside an extended antibiotic course (mean duration of 17 days) could have contributed to resistance development. Moreover, the administration of CAZ-AVI as prolonged/continuous infusions (never detected in our search) could be a key strategy to prevent therapeutic failures [70]. However, it is important to underline that the conclusions are limited by the fact that the data at best come from small series, in particular fatality rate data are likely underestimated both for infected and colonised patients owing to some missing data on outcomes, and that further studies are needed to clearly elucidate the most important features involved in CAZ-AVI resistance development.

5. Conclusions

Although CAZ-AVI resistance remains uncommon, it is being increasingly reported and the fatality rate in patients infected with CAZ-AVI-resistant strains appears to be high (almost 40%). Therefore, it is imperative to improve CAZ-AVI use from an antimicrobial stewardship perspective in order both to delay the emergence and spread of further resistance, while at the same time guaranteeing the prompt and correct use of this agent in patients with susceptible KPC-E infections who may benefit from its administration. From this standpoint, the availability of prompt antimicrobial susceptibility testing including CAZ-AVI for Enterobacterales is likely essential. In conclusion, CAZ-AVI resistance is an urgent issue to monitor in order to improve both empirical and targeted CAZ-AVI use as well as the management of patients with infections caused by CAZ-AVI-resistant strains.

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