RAPID RISK ASSESSMENT

Emergence of resistance to ceftazidime-avibactam in carbapenem-resistant Enterobacteriaceae

12 June 2018

Main conclusions and options for response

Multidrug-resistant gram-negative bacteria (MDR GNB), and especially carbapenem-resistant Enterobacteriaceae (CRE), are spreading in healthcare settings and the community, in Europe and globally.

Ceftazidime-avibactam (CAZ-AVI) is a novel antibiotic combination with activity against certain CRE - i.e. those producing *Klebsiella pneumoniae* carbapenemase (KPC) and some class D β-lactamases (e.g. OXA-48).

Reports of CAZ-AVI-resistant (CAZ-AVI-R) CRE strains that have developed resistance during treatment with CAZ-AVI, alone or in combination with other antibiotics, or in treatment with antibiotics other than CAZ-AVI, were published soon after the launch of CAZ-AVI and these reports are of concern.

Resistance genes encoding the production of carbapenemases are found on plasmids and are part of epidemic clones, heightening the likelihood of spread and exchange of resistance determinants between species.

Reports on the emergence of CAZ-AVI-R CRE soon after its launch represent a public health threat in the EU/EEA and beyond, with the potential for adverse patient outcomes in various settings. In the EU/EEA, only sporadic cases (two patients in two different countries) have so far been reported, but CAZ-AVI-R CRE will most probably have the propensity to spread within healthcare settings and across borders, as has been seen for other CRE.

Timely detection of even a single CAZ-AVI-R CRE isolate and immediate implementation of appropriate infection prevention and control (IPC) measures are warranted. Measures to prevent the spread of CAZ-AVI-R CRE should be embedded as part of a multimodal strategy and adhered to by all in healthcare settings, with support from leadership.

Options for actions to reduce identified risks

1. Actions to prevent the emergence of CAZ-AVI-R CRE in hospitals and other healthcare settings

Improved microbiology laboratory methods and capacity for detection of CAZ-AVI resistance

Antimicrobial susceptibility testing (AST) is necessary so that clinicians can make informed decisions regarding continuation of empiric CAZ-AVI therapy, such as CAZ-AVI de-escalation or escalation.

Hospitals and other healthcare settings therefore need to have access to microbiology laboratory services with capacity to perform AST for CAZ-AVI using appropriate genetic methods, or by sending isolates to reference laboratories. Appropriate microbiological samples should be obtained prior to commencing treatment. The identity of the species and the resistance to CAZ-AVI should be confirmed in both clinical and screening samples.

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It is essential that microbiology laboratories communicate results in a timely manner to clinical staff and the IPC team to ensure proper patient follow-up and application of infection prevention and control precautions to minimise the risk of onward transmission.

In Europe, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) has recommended standards for AST. Elsewhere other international standards may be used - i.e. those of the Clinical and Laboratory Standards Institute (CLSI).

Optimising appropriate therapeutic decision-making

Initial empiric antimicrobial treatment, taking into consideration the patient's epidemiological risk factors, local epidemiological data, antibiotic exposure history and the clinical situation, can be followed by re-evaluating and tailoring treatment once relevant microbiological culture results become available. It is strongly suggested that early input should be sought from infection specialists (e.g. infectious disease physicians or clinical microbiologists) to optimise selection of the antibiotic regimen.

Optimising prudent use of antibiotics and implementation of antibiotic stewardship programmes

As part of good antimicrobial stewardship and, as stated by the European Medicines Agency (EMA) in the approved EU product information, when CAZ-AVI is 'indicated for the treatment of infections in adult patients caused by aerobic multidrug-resistant gram-negative organisms for which there are limited treatment options' it should be administered 'only after consultation with a physician with appropriate experience in the management of infectious diseases'.

Enhanced epidemiological surveillance

To facilitate early identification, the spread and prevalence of CAZ-AVI-R CRE, microbiology laboratories should consider implementing prospective, or at least sentinel, CAZ-AVI susceptibility testing on Enterobacteriaceae. This can be performed by using the disk diffusion method currently described by EUCAST which does not require any extra time or resources other than the acquisition of CAZ-AVI disks.

Screening 'at-risk' patients on admission to healthcare settings, which also includes performing AST on isolates, requires appropriate resources at the ward level, within the IPC, surveillance and antimicrobial stewardship teams and the microbiology laboratory.

In the European Survey on Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) project, all EU/EEA countries reported having a national surveillance system for CRE in place supported by national reference identification of carbapenemase genes. Performing additional phenotypic testing for CAZ-AVI resistance on isolates collected within those sentinel, survey-based systems would be valuable to further evaluate the emergence of CAZ-AVI R CRE in the EU/EEA.

2. Actions to prevent transmission of CAZ-AVI-R CRE in hospitals and other healthcare settings

Identifying patients at high risk for carriage of CAZ-AVI-R CRE

Patients who are carriers of CAZ-AVI-R CRE can act as reservoirs for the transmission of these bacteria to other patients within the same healthcare setting, to other healthcare settings locally or across borders.

Transmission can occur when known CAZ-AVI-R CRE carriers are transferred between, or admitted to a healthcare facility without informing the receiving facility that they are carriers of CAZ-AVI-R CRE. To prevent this type of transmission, it is essential that when a patient is transferred, hospital staff in charge of patient admissions are informed of a patient's carrier status from previous records or via communication with other facilities.

Transmission can also occur when patients who are unrecognised CAZ-AVI-R CRE carriers are transferred between, or admitted to a healthcare facility. Active and passive surveillance are necessary for the detection of CAZ-AVI-R CRE carriers. Early identification of CAZ-AVI-R CRE carriers using screening cultures is an important tool for outbreak prevention in high-risk areas, and for outbreak identification and control. Sites that could be considered for sampling are rectal swabs or faeces as a minimum, and additional body sites as deemed appropriate.

Active screening of such patients can identify CAZ-AVI resistance easily when performing AST, by using the CAZ-AVI disk-diffusion offered by EUCAST. If existing screening programmes do not routinely incorporate testing for CAZ-AVI resistance for suspected isolates, patients who are carrying CAZ-AVI R CRE may go undetected.

Patients should be 'screened' by history for certain key epidemiological risk factors placing them 'at-risk' of carriage. These risk profiles are outlined in a recent ECDC guidance document for CRE and could be extrapolated.

Implementation of infection control measures in at-risk patients suspected as being CAZ-AVI-R carriers

Application of standard IPC precautions for all patients in all healthcare settings is essential for the reduction of the risks of microorganism transmission, including unrecognised carriers of CAZ-AVI-R CRE, in healthcare. IPC processes

and measures should be part of a multimodal strategy, with awareness and commitment of all healthcare workers as the main goal.

In addition, rapid implementation of preliminary supplemental IPC measures for interactions with 'at-risk' patients who are suspected carriers of CAZ-AVI-R CRE and are awaiting confirmation from screening is the cornerstone in preventing cross-transmission in healthcare settings. These measures, which are similar to measures recommended for 'at-risk' patients who are suspected CRE carriers in a recent ECDC guidance document, include:

- pre-emptive isolation of 'at-risk' patients, on admission, in a single *en suite* room or with toilets designated for use by known carriers, or with a commode;
- active screening for CAZ-AVI-R CRE carriage on admission;
- contact precautions.

Preventing in-healthcare transmission from patients who are known carriers of CAZ-AVI-R CRE

When a patient is identified as CAZ-AVI-R CRE carrier or infected, additional precautions, similar to those for CRE carrier or infected patients, should be implemented in addition to standard IPC precautions. These include:

- contact precautions;
- isolation of CAZ-AVI-R CRE carrier of infected patients in a single *en suite* room or with toilets designated for use by known carriers, or with a commode, or patient cohorting in designated cohort areas with allocated toilets;
- nurse cohorting - i.e. assigning designated staff for the duration of each shift, to care for patients known to be CAZ-AVI-R CRE carriers or infected;
- active screening of patients who have been in contact with a known CAZ-AVI-R CRE carrier or infected patient;
- enhanced environmental cleaning, including terminal cleaning and disinfection of rooms after discharge of CAZ-AVI-R CRE carrier or infected patients.

3. Specific actions to rapidly contain outbreaks of CAZ-AVI-R CRE

Measures to implement in an outbreak setting include all the above measures targeting CAZ-AVI-R CRE carriers or infected patients, plus the following:

- prompt initiation of an epidemiological investigation and environmental sampling to establish the source(s) of CAZ-AVI-R CRE;
- cross-sectional patient screening for CAZ-AVI-R CRE carriage;
- regular active surveillance cultures to detect CAZ-AVI-R CRE carriage for patients admitted to and remaining in high-risk areas within a hospital or other healthcare facility;
- education and practice audits to improve compliance of healthcare workers with hand hygiene and contact precautions.

4. Preparedness in EU/EEA countries

Member States should consider alerting clinicians and microbiologists in healthcare facilities and local and reference laboratories, to raise awareness of CAZ-AVI resistance in CRE and adapt laboratory testing practice at primary and reference levels.

To ensure timely notification and implementation of IPC actions, appropriate infrastructure is necessary so that local, regional and national health authorities can be immediately informed of any new CAZ-AVI-R isolate.

5. Actions to prevent cross-border spread

Documentation and interfacility notification of known carriage or infection by CAZ-AVI-R CRE during cross-border patient transfer would optimise the early and effective implementation of measures to prevent the spread of CAZ-AVI-R CRE.

Moreover, gathering reliable epidemiological data by notifying cases to public health authorities and exchanging information are important activities to enable informed and coordinated action by public health authorities across the EU/EEA. Public health authorities should issue notifications on the Early Warning and Response System (EWRS) where relevant, as per Article 9 of Decision 1082/2013/EU on serious cross-border threats to health. Use of the Epidemic Intelligence System (EPIS) is encouraged to ensure transparent and timely information sharing among the participating public health authorities in order to detect public health threats at an early stage.

Source and date of request

ECDC round table decision based on the recent publications by Giddins et al. [31] and Gaibani et al. [41].

Public health issue

A novel antibiotic (β-lactam/ β-lactamase inhibitor) combination, CAZ-AVI, was developed in response to the need for new antibiotics to combat the rising rates of MDR-GNB worldwide, with a special focus on CRE. This was approved and launched in the USA in 2015 and in Europe in 2016. Even though surveillance studies continue to report very low rates of CAZ-AVI resistance in CRE, recent clinical case reports in the USA and the EU/EEA demonstrate emergence of CAZ-AVI resistance in CRE while receiving treatment with CAZ-AVI or other antibiotics. These reports are of concern in that resistance has emerged in the United States and the EU/EEA countries in under three years since the initial launch of CAZ-AV which would rapidly compromise the effectiveness of the new antibiotic.

This risk assessment will discuss CAZ-AVI resistance in brief, review the recent literature reporting resistance of CRE to CAZ-AVI, and assess the risk for the EU/EEA countries. It will outline options for appropriate detection of resistance and the correct and timely implementation of IPC measures to prevent spread of these highly resistant organisms within healthcare settings and across borders.

Consulted experts

Internal experts consulted (in alphabetical order): Anna-Pelagia Magiorakos, Dominique Monnet.

External experts consulted: Karen Burns (Ireland), Christian Giske (Sweden and EUCAST), Gunnar Kahlmeter (Sweden and EUCAST), and Radu Botgros (EMA).

Disease background information

Multidrug-resistant gram-negative bacteria (MDR GNB), and especially carbapenem-resistant Enterobacteriaceae (CRE) that produce carbapenemases, are spreading in healthcare settings and within the community in Europe and globally [1-5]. Carbapenemases are carbapenem-hydrolysing beta-lactamases that confer resistance to a broad spectrum of beta-lactam substrates including carbapenems [6]. KPC is one of the five major carbapenemase families; the others are VIM, IMP and NDM metallo-beta-lactamases, and the OXA-48-group carbapenemases [7]. KPC is one of the most prevalent carbapenemases globally, with many variants reported to date, of which the most commonly found globally are KPC-2 and KPC-3 [2,5]. The global dissemination of these carbapenemases, especially KPC, is facilitated by the fact that they are frequently part of mobile genetic elements and the successful dissemination of epidemic *K. pneumoniae* clones - e.g. ST258 [8] and ST11 [9].

There are only a few antibiotics available for the treatment of infections caused by CRE; the polymyxins (e.g. colistin), alone, or in combination with other agents, such as tigecycline, aminoglycosides (AG) and fosfomycin have been used [10]. These regimens, however, are frequently suboptimal because of their pharmacokinetics, nephrotoxicity (colistin and AG) and the presence or emergence of antibiotic resistance, prior to or even during therapy [11].

CRE cause difficult-to-treat infections and spread easily within healthcare settings, causing outbreaks [12]. CRE infection is associated with delays in the administration of appropriate empiric antibiotics, poor patient outcomes, increased morbidity and mortality, longer lengths of hospital stay and higher hospital costs, when compared to infections with susceptible organisms [13-15].

Important measures to curtail such spread are the timely detection of these bacteria and resistance mechanisms, good communication between microbiology laboratory and infection control staff for the appropriate implementation of IPC and adherence to an antimicrobial stewardship programme [16-18].

Ceftazidime-avibactam (CAZ-AVI)

CAZ-AVI is a novel antibiotic-enzyme inhibitor combination containing ceftazidime, an extended-spectrum cephalosporin, paired with the novel non-β-lactam, β-lactamase inhibitor, avibactam. It was recently added to the therapeutic armamentarium for the treatment of patients infected with MDR GNB and especially CRE.

Spectrum of activity and indications

CAZ-AVI has activity against Enterobacteriaceae that produce extended-spectrum β-lactamases (ESBLs), Ambler class A β-lactamases —e.g. *Klebsiella pneumoniae* carbapenemase (KPC), class C β-lactamases (AmpC), and some class D β-lactamases - e.g. OXA-48 group. It is not active against class B, the metallo-β-lactamases - e.g. NDM and VIM. The ability of CAZ-AVI to inhibit KPC-producing CRE is of great importance because KPC is one of the most prevalent carbapenemases in Europe and worldwide [2,5].

After showing non-inferiority in phase three clinical trials, CAZ-AVI was approved for the treatment of complicated intra-abdominal infections (cIAIs) [19], complicated urinary tract infections (cUTIs) [19,20], including pyelonephritis, and for hospital-acquired pneumonia (HAP), including ventilator-associated pneumonia (VAP) [21]. The EMA specifically states in the approved EU product information that CAZ-AVI is also 'indicated for the treatment of infections in adult patients caused by aerobic multidrug-resistant gram-negative organisms for which there are limited treatment options' and that, when prescribed for this indication, it should be administered 'only after consultation with a physician with appropriate experience in the management of infectious diseases' [22].

CAZ-AVI approval and launch

CAZ-AVI was approved by the US Food and Drug Administration (FDA) in February 2015 and launched for use in the USA in April 2015 under the trade name Avycaz [23]. In the European Union (EU), CAZ-AVI was approved by the European Commission in June 2016 following positive advice from the European Medicines Agency (EMA) and is marketed under the trade name Zavicefta [22].

Laboratory methods and capacity for detection of CAZ-AVI resistance

In order to accurately detect resistance of CRE to CAZ-AVI, healthcare settings should have microbiology laboratories with the ability and capacity to identify CRE isolates and their susceptibilities to CAZ-AVI. This can be performed by using the disk diffusion method which does not require extra time or resources, other than the acquisition of CAZ-AVI disks.

In Europe, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) has recommended standards for AST; elsewhere other international standards may be used - i.e. those of the Clinical Laboratories Standards Institute (CLSI). When resistance to CAZ-AVI is detected, the relevant resistance genes should be characterised. This should be implemented for both clinical and screening samples.

Results from microbiological analyses should be communicated to the clinical staff in a timely manner, and when preliminary and subsequently confirmed findings are clinically or epidemiologically significant, they should prompt immediate and direct contact with clinical staff and the IPC team to ensure proper follow-up.

The reference method for detection of non-susceptibility to CAZ-AVI is broth microdilution. Other methods include disk diffusion and Etes (bioMérieux). Automated susceptibility testing is currently unavailable for CAZ-AVI. The EUCAST [24], the United States Food and Drug Administration (FDA) and the CLSI [25] publish MIC and inhibition zone diameter breakpoints [24-28]. EUCAST and CLSI/FDA breakpoints can be found in Table 1.

Table 1. EUCAST and CLSI/FDA breakpoints for ceftazidime-avibactam (CAZ-AVI)

	EUCAST [24,27]				**CLSI/FDA** [26,28]			
	Broth microdilution		**Disk diffusion** Disk potency CAZ/AVI 10/4 µg		**Broth microdilution**		**Disk diffusion** Disk potency CAZ/AVI 30/20 µg	
	MICs (mg/L)		Zone diameters (mm)		MICs (mg/L)		Zone diameters (mm)	
	S	R	S	R	S	R	S	R
Enterobacteriaceae	≤8	≥8	≤13	≥13	≤ 8/4	≥ 16/4	≥ 21	≤ 20
Pseudomonas aeruginosa	≤8	≥8	≤17	≥17	≤ 8/4	≥ 16/4	≥ 21	≤ 20

MIC, minimum inhibitory concentration; S, susceptible; R, resistant.

Event background information

Resistance of carbapenem-resistant Enterobacteriaceae to CAZ-AVI

Mechanisms of CAZ-AVI resistance in CRE, with a special focus on resistance in KPC-producing *K. pneumoniae*, have been reported and are still being fully elucidated. Such mechanisms include, but are not limited to, mutations in the bla$_{KPC}$ gene [29-31], differences in susceptibilities of KPC subtypes - e.g. KPC-2 and KPC-3 [31-35], and other resistance determinants - e.g. outer membrane proteins (OMPs) such as OmpK36 and ESBLs.

Differences in susceptibilities of KPC subtypes were observed from initial surveillance and *in vitro* susceptibility studies for CAZ-AVI, where it was reported that both KPC-2- and KPC-3-producing *K. pneumoniae* had higher MICs to CAZ-AVI than other KPC subtypes. Papp-Wallace et al. [34] and Livermore et al. [35] reported that certain KPC mutants (e.g. KPC-2 variants and KPC-3) could be selected *in vitro* resulting in avibactam resistance. It also

appears that KPC-3 may exhibit higher MICs than KPC-2, as reported by Shields et al. [36]. Higher KPC-3 MICs to CAZ-AVI have also been reported from other studies [29,30,33,36].

Since the launch of CAZ-AVI in 2015, studies have reported the emergence of resistance to CAZ-AVI in the clinical setting and during therapy with CAZ-AVI and/or other antibiotics. More detailed information and case descriptions are available in the annex but this can be summarised as follows:

In the USA

- In 2015, Humphries et al. [37] from Los Angeles reported the first clinical isolate resistant to CAZ-AVI in a patient who developed a cholangiitic abscess with bacteraemia during an extensive hospitalisation without prior exposure to CAZ-AVI. Among three KPC-3-producing *K. pneumoniae* isolates, one was resistant to CAZ-AVI despite the patient having been treated with various antibiotics, but not CAZ-AVI [37,38]. The authors hypothesised that the patient may have developed resistance to CAZ-AVI through a mutation in the OmpK36 from the selective pressure of cefepime and meropenem.
- In 2016, Shields et al. [39] conducted a retrospective study of thirty-seven patients in Pittsburgh, who had received CAZ-AVI treatment for CAZ-AVI-susceptible CRE infection. Three of these had isolates in which CAZ-AVI resistance had emerged after CAZ-AVI therapy. In 2017, Shields et al. [29] further described these three cases as being KPC-3-producing *K. pneumoniae* isolates with CAZ-AVI resistance and without epidemiological links. The isolates belonged to epidemic clone ST258. All isolates were tested and were found to contain mutant bla_{KPC-3}, which encoded mutant KPC-3 enzymes. In some of the isolates, emergence of CAZ-AVI resistance restored susceptibility to meropenem.
- In 2017, Giddins et al. [31] from New York City reported the stepwise emergence of CAZ-AVI resistance in *K. pneumoniae* isolates harbouring bla_{KPC-2}, belonging to the rapidly emerging ST 307 clone during therapy with CAZ-AVI.

In the EU/EEA

- In 2017, Both et al. [40] from Hamburg, Germany, reported the emergence of CAZ-AVI resistance in a clinical *K. pneumoniae* isolate which produced OXA-48 and CTX-M-14. The isolate was from a patient in an intensive care unit on treatment with CAZ-AVI during a hospital outbreak which lasted from October 2015 to March 2016. The patient was extensively treated with CAZ-AVI plus meropenem, and subsequent isolates obtained from blood cultures from a central venous catheter showed resistance to CAZ-AVI. This report demonstrates that resistance can rapidly emerge in non-ST258 clones with various bla_{KPC} variants.
- In 2018, Gaibani et al. [41] from Bologna, Italy, reported the emergence of CAZ-AVI-R *K. pneumoniae* ST1519 isolates harbouring bla_{KPC-3}. The patient was a male with cirrhosis, previously found to be a KPC-producing *K. pneumoniae* carrier, who underwent a liver transplant and after a prolonged course of meropenem, colistin and CAZ-AVI, developed CAZ-AVI-R subpopulations. CAZ-AVI resistance emerged after 17 days of CAZ-AVI treatment.

ECDC threat assessment for the EU

Importance of CAZ-AVI for treatment of CRE and availability of limited treatment alternatives

CAZ-AVI was developed to treat infections with CRE, except those harbouring MBL carbapenemases. The emergence of CAZ-AVI resistance in CRE is extremely alarming because it compromises the activity of a promising new antibiotic, CAZ-AVI, with activity against KPC-producing CRE, as well as other serine β-lactamases, and KPC is among the most prevalent carbapenemases in Europe and worldwide [1-4]. Another element of concern is the fact that resistance to CAZ-AVI emerged very quickly after the approval of CAZ-AVI in the USA in 2015, in the EU/EEA in 2016, and its launch in Germany and the UK in March 2017, and there are plans for CAZ-AVI to be launched in other countries in 2018. In the European market, its availability is of particular importance since CRE are increasingly being isolated from patients with HAI [3,4,17].

Consequences of CAZ-AVI-R CRE for human medicine

The emergence of CAZ-AVI-R CRE is a public health threat to the EU/EEA and can have consequences for patients in different healthcare settings. Even though surveillance studies continue to report very low rates of CAZ-AVI resistance in CRE [32], clinical case reports clearly demonstrate resistance emerging not only during treatment with CAZ-AVI, but also with other antibiotics. In general, CAZ-AVI resistance has most frequently been detected in specific KPC subtypes - i.e. KPC-3 KPC-2.

In the clinical case reports described in the annex to this risk assessment, emergence of resistance to CAZ-AVI is mostly seen in KPC-3-producing, more rarely in KPC-2- or OXA-48-producing, *K. pneumoniae* belonging to highly successful clones, i.e. ST258, ST307, and ST1519 [29,31,37,40,41]. From these reports, it is evident that CAZ-AVI exerts a strong selection pressure that can result in mutations that render the CRE resistant to CAZ-AVI. Moreover, resistance to CAZ-AVI can also emerge from selection pressure during treatment with other antibiotics, e.g. meropenem [37], which underscores the critical need for proactive antimicrobial stewardship programmes targeting the use of all antibiotics.

The spread of CRE, and now the emergence of CAZ-AVI-R CRE, has become an important therapeutic and infection control problem in healthcare settings globally, as they cause difficult-to-treat infections and spread easily within healthcare settings, causing infections and outbreaks [12]. The detection of CAZ-AVI resistance in invasive CRE isolates highlights the risk of co-resistance in pathogens already responsible for infections with limited treatment options and the potential for rapid emergence of resistance.

Infections with highly resistant bacteria are associated with delays in the administration of appropriate antibiotics, poor patient outcomes, increased morbidity and mortality, longer lengths of hospital stay and higher hospital costs when compared to infections with susceptible organisms [13-15]. As a new treatment for CRE infection, emergence of resistance to CAZ-AVI will probably result in similar consequences.

Impact of CAZ-AVI resistance in the clinical setting

Routine testing for CAZ-AVI resistance in Enterobacteriaceae in general, and particularly in CRE, is not currently performed in most microbiology laboratories across the EU/EEA. Thus, resistance to CAZ-AVI will not be detected in the absence of AST, with the associated risk of undetected resistance spreading in the healthcare setting, HAIs, outbreaks and possibly endemicity. This underscores the need for an increase in awareness of the emergence of resistance to CAZ-AVI among infection specialists and the need for both national reference laboratory and local microbiology laboratory capacity building for susceptibility testing to CAZ-AVI. This needs to be coupled with IPC measures applied at the local level, supported by robust with national IPC action plans and guidelines.

Emergence of resistance is alarming and will have an impact on clinical decision-making and antibiotic therapy in a number of ways. Clinicians will need to be careful when selecting empiric antibiotic treatment regimens, since susceptibility of CRE to CAZ-AVI can no longer be assumed. Moreover, other antibiotics aside from CAZ-AVI may see emergence of resistance to CAZ-AVI and resistance can be mediated by a variety of mechanisms, e.g. mutations in porin genes which can emerge from selective pressure even from other antibiotics.

The importance of the prudent use of antibiotics is again highlighted, as is the necessity for clinicians to be vigilant when patients are on treatment with CAZ-AVI since emergence of resistance to CAZ-AVI has been reported during therapy. Follow-up of patients and repeated AST should be considered, particularly if therapeutic failure is clinically suspected or microbiologically confirmed. Adjustments in treatment regimens may also be required depending on the AST results.

Potential for spread of CAZ-AVI-R CRE

Propensity for spread in healthcare settings and the environment

Resistance to CAZ-AVI has mostly been reported in Enterobacteriaceae, and of these, mostly in *K. pneumoniae*, especially those harbouring bla_{KPC} isolates among humans with severe infections.

For Enterobacteriaceae such as *K. pneumoniae* and *E. coli,* the incidence of multidrug-resistant (MDR) isolates, defined as being resistant to three or more antibiotic classes, in both *K. pneumoniae* and *E. coli* isolates is steadily increasing in the EU/EEA, as shown in the latest report by the European Antimicrobial Resistance Surveillance Network (EARS-Net) [42]. In the 2011–12 European point prevalence survey (PPS) of HAIs and antimicrobial use in European acute care hospitals, coordinated by ECDC [43] 44% of all HAIs were caused by *E. coli* and 24% by *K. pneumoniae*.

To date, reports of CAZ-AVI-R CRE have involved sporadic cases, but given the epidemiological behaviour of carbapenemases, it is likely that CAZ-AVI-R CRE has potential to spread within healthcare settings and across borders [4,18]. Certain features of CAZ-AVI-R CRE increase their propensity for spread:

- CAZ-AVI resistance has most frequently been reported in KPC-producing *K. pneumoniae* ST258, a highly successful epidemic clone, which has already spread worldwide, but also in newer clones ST307 and ST1519.
- Additionally, even though resistance during therapy has been reported mostly from the globally prevalent KPC subtype KPC-3, it is also reported in KPC-2 with similar mutations.
- Plasmids harbouring genes that confer resistance to CAZ-AVI can be transferred between bacteria within the same species or between different species via conjugation, passing on the resistance genes.

Risk of spread of CAZ-AVI-R CRE into the ecosystem and community

As a normal component of both the human and animal intestinal microbiomes and a common cause of HAI, *E. coli* and the plasmids it carries are easily transmitted between humans, animals and the environment [44,45], underscoring the importance of One-Health approaches to combatting antimicrobial resistance [44].

Identifying patients at high risk of carriage of CAZ-AVI-R CRE

The principles of control identified in the recently published ECDC guidance document 'Infection prevention and control measures and tools for the prevention of entry of carbapenem-resistant Enterobacteriaceae into healthcare settings: guidance from the European Centre for Disease Prevention and Control' also apply to CAZ-AVI-R CRE [16].

Preventing transmission within healthcare settings from patients who are CAZ-AVI-R CRE carriers

Appropriate mechanisms should be in place in healthcare facilities to ensure that if even one CAZ-AVI-R isolate is detected, IPC measures can immediately be implemented. Active and passive surveillance and good microbiological capacity are necessary for the detection of any such isolate, as well as to establish whether there has been in-healthcare transmission. Standard precautions should be implemented throughout the healthcare setting for all patients at baseline, and if even one CAZ-AVI-R CRE is detected, additional precautions should be implemented immediately to prevent transmission to other patients. Such measures include contact precautions, single room isolation or patient cohorting and dedicated nursing staff for patients who are colonised or infected with CAZ-AVI-R CRE. Emphasis should also be placed on the terminal cleaning of rooms after discharge of patients who carry, or are infected with CAZ-AVI-R CRE. Measures to prevent the spread of CAZ-AVI-R GNB should be quality-driven and embedded as part of a multimodal strategy, adhered to by all in healthcare settings, with support from leadership. Decisions regarding patient placement should be left to the healthcare setting and based on a 'case-by-case' risk assessment.

Early identification of carriers using active surveillance cultures in cross-infection epidemic settings is an important tool for outbreak control. Active surveillance cultures should be conducted in accordance with a specified protocol. Sites that could be considered for sampling include nose/throat, axilla, groin, rectum, insertion sites of venous catheters and clinical samples such as urine, faeces, wound drain fluid and respiratory specimens. Further clinical experience will ascertain which sampling sites are the most sensitive for detecting and monitoring patient colonisation with CAZ-AVI-R CRE.

Cross-border spread

Patient mobility and patient transfer have been highlighted as risk factors for the introduction of CRE into healthcare settings within the same region and country and also across international borders [18,46]. CAZ-AVI-R CRE, especially *K. pneumoniae* and *E. coli,* will most probably have similar behaviour and conclusions can therefore be extrapolated from previous voluminous literature on CRE. CAZ-AVI-R CRE will probably be easily introduced into healthcare settings when patients are transferred, because they are highly transmissible if the receiving facility has not been informed or does not screen for them. The introduction of CAZ-AVI-R CRE into the receiving facility can result in carriage and infection of patients and could give rise to outbreaks. Infections with CAZ-AVI-R CRE represent a threat to patient safety due to their resistance to multiple antibiotics, leaving few treatment options and resulting in increased morbidity and mortality.

Preparedness in EU/EEA countries

Risk for the EU/EEA

The emergence of resistance of CAZ-AVI-R CRE is a public health threat to the EU/EEA and can have life-threatening consequences for patients in different epidemiological settings. To date, reports involve sporadic cases, but like all other CRE, CAZ-AVI-R CRE will most probably also have the propensity to spread within healthcare settings and across borders.

Initial reports of resistance in clinical CRE isolates were from the USA, but reports from Europe have now been published (see annex for detailed description). A recent report by Gaibani et al. was from Italy and a previous one by Both et al. from Germany. This is of concern because it shows the ease with which resistance can emerge *de novo* in disconnected geographical areas and underscores the need to raise awareness in Europe and globally of the advent of resistance to CAZ-AVI. This is particularly important because isolates can be easily transferred between healthcare facilities, if they are not detected.

Microbiological laboratories in Europe need to ensure that they have the capacity for CAZ-AVI susceptibility testing, for IPC and also therapeutic purposes. Clinicians need to be vigilant for the possibility of CAZ-AVI resistance in CRE and monitor susceptibilities even during therapy. Furthermore, communication between healthcare systems is paramount so that when carrier patients are transferred the receiving healthcare system is informed of the patient's carrier status.

Improved national and EU/EEA-wide surveillance and communication

Member States should consider alerting clinicians and microbiologists in healthcare facilities and associated reference laboratories, to raise awareness of the emergence of CAZ-AVI resistance in CRE, with the aim of adapting laboratory testing practice at primary and reference levels and reporting the results in a timely manner.

In the European Survey on Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) project, all EU/EEA countries reported having a national surveillance system for CRE in place supported by national reference identification of carbapenemase genes. Performing additional phenotypic testing for resistance to CAZ-AVI on isolates collected within those sentinel, survey-based systems would be valuable and feasible. ECDC plans to initiate a new EU sentinel WGS-based surveillance module for CRE and extensively drug-resistant Enterobacteriaceae (XDRE) infections, by means of periodically repeating structured, pan-EU surveys following the sampling design of the EuSCAPE project. Analysis of WGS data from collected isolates will determine the genetic complement and predict the phenotype by mobilome/resistome/virulome profiling and the identification of genetic markers of high-risk clones and plasmids. This approach would enable identification of CAZ-AVI resistance and help to monitor its geographical distribution across EU/EEA countries.

Documentation of known carriage or infection by CAZ-AVI-R CRE associated with cross-border patient transfer would optimise the implementation of measures to prevent the international spread of CAZ-AVI-R CRE. Moreover, gathering reliable epidemiological data through notification of cases to public health authorities and exchange of information via electronic early warning platforms, such as the Epidemic Intelligence System (EPIS) can be facilitated by ECDC, which will enhance the timely sharing of outbreak experience and control measures. This will enable informed and coordinated risk management action by public health authorities across the EU/EEA.

Disclaimer

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Annex. Reports of emerging resistance to CAZ-AVI in CRE clinical isolates

Since the launch of CAZ-AVI in 2015, studies have reported the emergence of resistance to CAZ-AVI in the clinical setting and during therapy with CAZ-AVI and/or other antibiotics as follows:

- In 2015, Humphries et al. [37] reported the first clinical isolate resistant to CAZ-AVI from Los Angeles (CA), United States in February 2015, prior to the extensive availability of CAZ-AVI. The patient was a 62-year old woman with pancreatic cancer and extensive hospitalisation, with no prior exposure to CAZ-AVI, who developed a cholangiitic abscess with bacteraemia. Two KPC-3-producing *K. pneumoniae* isolates grew, one from a blood culture and the other from a culture of the cholangiitic abscess drain, which were both susceptible to CAZ-AVI. A third KPC-3-producing *K. pneumoniae* isolate grew from a subsequent blood culture which was resistant to CAZ-AVI. This third isolate was collected after the patient had been treated with various antibiotics, but not CAZ-AVI. Both KPC-3 isolates from the blood cultures were found to belong to ST258 [37,38]. Further work showed, among others, the presence of the porin gene OmpK36 which has been shown to cause elevated MICs to CAZ-AVI [36]. Both isolates harboured *bla*$_{SHV-11}$ on the chromosome and *bla*$_{KPC-3}$ and *bla*$_{SHV-12}$ on the sole plasmid [38]. It is hypothesised that the patient may have developed resistance to CAZ-AVI by a mutation in the OmpK36 from the selective pressure of cefepime and meropenem with which she had been treated after the initial blood culture had grown the first KPC-3-producing *K. pneumoniae* isolate.
- In 2016 Shields et al. [39] conducted a retrospective study of patients in Pittsburgh (PA), United States, who had received CAZ-AVI treatment for CAZ-AVI-susceptible CRE infections between April 2015 and February 2016. Thirty-seven patients were treated with CAZ-AVI for three days or more and three of these had isolates in which CAZ-AVI resistance emerged after CAZ-AVI therapy. In 2017, Shields et al. [29] further described these cases as being KPC-3-producing *K. pneumoniae* isolate s with CAZ-AVI resistance, but without epidemiological links. The KPC-3-producing *K. pneumoniae* isolates, which belonged to the successful epidemic clone ST258, as well as all isolates from all three patients were initially CAZ-AVI susceptible and developed resistance during treatment with CAZ-AVI. The first patient had been treated with CAZ-AVI for a KPC-3-producing *K. pneumoniae* pneumonia and urinary tract infection; the CAZ-AVI isolate that was resistant was obtained after therapy. The second patient was treated with CAZ-AVI for a KPC-3-producing *K. pneumoniae* cultured from a subphrenic abscess. The CAZ-AVI-R isolate was obtained after therapy and was thought to be a coloniser. The last patient was treated with CAZ-AVI for a KPC-3-producing *K. pneumoniae* pneumonia. The KPC-3-producing *K. pneumoniae* isolate cultured after treatment was resistant to CAZ-AVI. All isolates were tested and were found to contain mutant *bla*$_{KPC-3}$, which encoded mutant KPC-3 enzymes. The emergence of resistance to CAZ-AVI in some isolates restored susceptibility to meropenem.
- In 2017, Both et al. [40] from Hamburg, Germany, reported the emergence of CAZ-AVI resistance in a clinical *K. pneumoniae* isolate which produced OXA-48 and CTX-M-14. The isolate was from a patient on treatment with CAZ-AVI during a hospital outbreak which lasted from October 2015 to March 2016. The patient had been admitted to the intensive care unit in septic shock after a laparotomy and adhesiolysis with subsequent peritonitis. The original *K. pneumoniae* was cultured from the patient's pharynx for screening purposes and was found to be identical to the outbreak strain. The patient initially received ceftazidime alone for an HAP and subsequent isolates had developed resistance to ceftazidime. The patient was then extensively treated with CAZ-AVI plus meropenem and subsequent isolates obtained from blood cultures from a central venous catheter showed resistance to CAZ-AVI. Resistance in these isolates emerged from mutations in CTX-M-14.
- In 2017, Giddins et al. [31] from New York, NY, USA, reported stepwise emerging resistance in *K. pneumoniae* isolates harbouring *bla*$_{KPC-2}$, belonging to the rapidly emerging ST 307 clone during therapy with CAZ-AVI. The patient was a diabetic male with a history of recent hospitalisation for acute pancreatitis whose initial culture grew *K. pneumoniae* from a pancreatic fluid collection. The patient received meropenem and polymyxin B, and the clinical course was complicated by renal failure. The patient was switched to CAZ-AVI and during the course of therapy three samples were obtained: two from a bronchoalveolar lavage and a third from a tracheal aspirate, the initial one 12 days into therapy. All isolates were *K. pneumoniae* ST307 harbouring *bla*$_{KPC-2}$ and demonstrated a mutation in *bla*$_{KPC-2,}$ as the initial mechanism of resistance, which had previously only been reported in a *bla*$_{KPC-3}$. Interestingly, previous reports mostly involved *K. pneumoniae* isolates harbouring *bla*$_{KPC-3}$, belonging to ST 258. This report demonstrates that resistance can rapidly emerge in non-ST258 clones with various *bla*$_{KPC}$ variants.
- In 2018, Gaibani et al. [41] from Bologna, Italy, reported the emergence of CAZ-AVI-R *K. pneumoniae* ST1519 isolates harbouring *bla*$_{KPC-3}$. The patient was a male with cirrhosis, previously found to be a KPC-producing *K. pneumoniae* carrier, who underwent a liver transplant and after a prolonged course of meropenem, colistin and CAZ-AVI, developed CAZ-AVI-R subpopulations. CAZ-AVI resistance emerged after 17 days of CAZ-AVI treatment.