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REVIEW

AmpC β-lactamase-producing *Enterobacterales*: what a clinician should know

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Abstract

Background *Enterobacterales* are among the most common causes of bacterial infections in the community and among hospitalized patients, and multidrug-resistant (MDR) strains have emerged as a major threat to human health. Resistance to third-generation cephalosporins is typical of MDRs, being mainly due to the production of extended spectrum β-lactamases or AmpC-type β-lactamases.

Objective The objective of this paper is to review the epidemiological impact, diagnostic issues and treatment options with AmpC producers.

Findings AmpC enzymes encoded by resident chromosomal genes (cAmpCs) are produced by some species (e.g., *Enterobacter* spp., *Citrobacter freundii, Serratia marcescens*), while plasmid-encoded AmpCs (pAmpCs) can be encountered also in species that normally do not produce cAmpCs (e.g., *Salmonella enterica, Proteus mirabilis, Klebsiella pneumoniae* and *Klebsiella oxytoca*) or produce them at negligible levels (e.g., *Escherichia coli*). Production of AmpCs can be either inducible or constitutive, resulting in different resistance phenotypes. Strains producing cAmpCs in an inducible manner (e.g., *Enterobacter* spp.) usually appear susceptible to third-generation cephalosporins, which are poor inducers, but can easily yield mutants constitutively producing the enzyme which are resistant to these drugs (which are good substrates), resulting in treatment failures. pAmpCs are usually constitutively expressed. Production of pAmpCs is common in community-acquired infections, while cAmpC producers are mainly involved in healthcare-associated infections.

Conclusions To date, there is no conclusive evidence about the most appropriate treatment for AmpC-producing *Enterobacterales*. Carbapenems are often the preferred option, especially for severe infections in which adequate source control is not achieved, but cefepime is also supported by substantial clinical evidences as an effective carbapenem-sparing option.

Keywords Beta-lactamases · AmpC · *Enterobacterales* · *Enterobacter* · Gram negative · Antibiotic resistance

Introduction

Increasing resistance of Gram-negative bacteria to β-lactam antibiotics currently represents one of the main concerns worldwide. The primary mechanism of resistance is the production of β-lactamase enzymes, which have the ability to hydrolyze β-lactams. In the last three decades, members of the order *Enterobacterales* producing enzymes capable of hydrolyzing also the expanded-spectrum cephalosporins have emerged as one of the main threats for human health, becoming endemic in many countries [1]. Extended spectrum β-lactamases (ESBLs) and AmpC-type β-lactamases (from now on abbreviated as AmpCs) represent the two groups of β-lactamases mainly involved in expanded-spectrum cephalosporins resistance, but display several peculiarities between each other [2].

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The mechanisms that underlie the AmpC-mediated resistance are not easy to understand for clinicians not familiar with clinical microbiology, since many peculiarities are related to the effective and variable expression of the enzyme by the different bacterial strains carrying the gene on the chromosome or having acquired it by plasmids. Moreover, the literature on infections due to AmpC-producing *Enterobacterales* is sparse and very heterogeneous, pending to date the results of adequate randomized controlled clinical trials.

In this review, mostly aimed at clinicians such as Infectious Diseases specialists, Internists and General Practitioners, we summarize the most important points about the epidemiological impact, dynamics, recognition and treatment of AmpC-producing *Enterobacterales*.

Methods

A literature search was performed using PubMed, through October 2018. The following terms were searched in combination: AmpC, β-lactamases, *Enterobacteriaceae, Enterobacterales, Enterobacter*, Gram-negative, resistance, cephalosporins, and treatment. References of retrieved articles, guidelines, and review articles were manually searched to ensure identification of studies not found in the initial literature search. The selection was limited to publications written in English. After de-duplication, all authors independently screened titles and abstracts, and finally full texts, to identify all potentially relevant studies, resolving discrepancies through discussion and consultation between them.

AmpC β-lactamases: an overview

AmpCs are enzymes encoded by the chromosomes of several bacterial species. Their evolutionary history indicates that they are very ancient enzymes, originated over two billion years ago, so preceding antibiotic introduction for clinical use and reflecting the evolution of resistance mechanisms to natural β-lactams produced by microorganisms for biological competition [3]. Despite having been differently called in the 1940, the first enzyme reported inactivating penicillin was indeed an AmpC, in *Escherichia coli*, before penicillin had been introduced in clinical use [4, 5].

The term AmpC defines a class of enzymes that belong to the molecular class C according to the Ambler's structural classification of β-lactamases (whereas the ESBLs found in *Enterobacterales* typically belong to Class A). A serine residue is contained within the active site of both AmpCs and ESBLs, but the protein sequences of each class are remarkably different, leading to structural and mechanistic differences in β-lactam hydrolysis [2, 5]. In the functional classification scheme of β-lactamases (Bush–Jacoby [6]), based on the hydrolysis and inhibition profiles of the enzyme, AmpCs are assigned to group 1, which is characterized by an overall greater hydrolysis of cephalosporins (including cephamycins) vs. penicillin G (hence the name "cephalosporinases" that has also been used for these enzymes), and resistance to inhibition by β-lactam-based β-lactamase inhibitors, such as clavulanate, sulbactam and tazobactam.

AmpCs are either found as resident enzymes, encoded by chromosomal genes (cAmpCs), in some species of *Enterobacterales*, but can also be found as acquired plasmid-mediated enzymes (pAmpCs); these represent two distinct situations, by both a microbiological and clinical points of view. pAmpCs have spread widely among *Enterobacterales*, although their overall prevalence has remained far lower than that of ESBLs. In Italy, a country endemic for third-generation cephalosporin (3GC)-resistant *Enterobacterales*, the ESBL/pAmpC ratio was found to be approximately 12:1 [1].

As ESBL-producing strains, also pAmpC producers may exhibit multidrug-resistant phenotypes, due to co-expression of multiple plasmid-mediated resistance determinants to non-β-lactams (including quinolones, cotrimoxazole and/or aminoglycosides), limiting the number of treatment options. Otherwise, the majority of *Enterobacterales* producing cAmpCs often retain high levels of susceptibility to fluoroquinolones and aminoglycosides [7, 8].

Enzymatic activity and inhibitors of AmpCs

In general, AmpCs exhibit a broad substrate specificity including penicillins (e.g., penicillin G; aminopenicillins such as amoxicillin and ampicillin; carboxypenicillins such as carbenicillin and ticarcillin; ureidopenicillin such as piperacillin), narrow-spectrum cephalosporins (e.g., cefazolin, cephalothin, cefamandole and cefuroxime), oxyimino-cephalosporins (e.g., cefotaxime, cefpodoxime, ceftazidime and ceftriaxone), cephamycins (e.g., cefoxitin and cefotetan) and aztreonam (variable), and their expression can confer resistance to all these compounds. The hydrolysis rate for fourth-generation cephalosporins (e.g., cefepime and cefpirome) is usually low, and that for carbapenems is very low, so that susceptibility to these drugs is usually maintained. Temocillin, a semi-synthetic 6-α-methoxy derivative of ticarcillin, is highly stable against most β-lactamases, and retains *in vitro* activity against ESBL- and AmpC-producing *Enterobacterales* [9].

Amino acid insertions, deletions, and substitutions have been described for both plasmidic and chromosomal AmpCs enhancing catalytic efficiency toward ceftazidime and other oxyimino-β-lactams: these variants have been termed extended-spectrum AmpC cephalosporinases (ESAC) [6]. As with other β-lactamases, the resistance mediated by AmpCs is enhanced by the presence of porin alterations which impair antibiotic entry across the outer

membrane. In this case, the very weak carbapenemase activity exhibited by some AmpCs can contribute a phenotype of reduced susceptibility or resistance to carbapenems, with ertapenem being usually more affected [6, 10].

Concerning inhibitors, AmpCs are usually resistant to β-lactam-based inhibitors (e.g., clavulanate, sulbactam and tazobactam), while being inhibited by the new non-β-lactam-based inhibitors (e.g., diazabicyclo-octanes, such as avibactam and relebactam, and boronates, such as vaborbactam). However, these general functional features may exhibit some variability in different AmpCs, in terms of substrate specificity and susceptibility to inhibitors (e.g., tazobactam inhibits cAmpC in *Morganella morganii*) [2, 5].

The lack of inhibition by β-lactam-based inhibitors represents a characteristic that sharply differentiates AmpCs from ESBLs. Other main differences are represented by the high susceptibility to cefepime and the resistance to cephamycins that AmpC producers usually show. AmpCs are located in the periplasmic space, where they can intercept and destroy β-lactams before interaction with the PBP (penicillin-binding protein) targets. In addition to the relative stability of the fourth-generation cephalosporins to AmpC hydrolysis, also the rapid penetration of these molecules across the outer membrane due to their zwitterionic structure account for their preserved activity against most AmpC-producers.

Chromosomal AmpCs (cAmpCs)

Genes encoding AmpCs are located on the chromosome of some clinically relevant Gram-negative pathogens, including several members of the order *Enterobacterales*. In particular, we must remember those belonging to the so-named ESCPM group, acronym indicating the following species: *Enterobacter* (*Enterobacter cloacae* complex, *Enterobacter aerogenes*), *Serratia marcescens, Citrobacter freundii, Providencia stuartii*, and *Morganella morganii. Enterobacter* spp. represent the prototype of this group.

The main feature of cAmpCs is represented by the variable level of expression of the *ampC* gene by the different species: expression can be constitutive or inducible. When expression is inducible, several β-lactams can act as inducers making clinically relevant the mechanism of resistance. By definition, inducible expression is reversible, but strains producing inducible cAmpCs can easily segregate mutants in which expression is stably de-repressed eventually resulting in constitutive expression (also named "de-repressed mutants").

Based on the propensity of the different antimicrobials of inducing cAmpC expression and of being hydrolyzable by the induced enzyme, we can distinguish the following situations:

1. *Inducer/labile* β-lactam compounds: aminopenicillins, first-generation cephalosporins, cefoxitin, cefotetan. These drugs induce *ampC* expression and are inactivated by the enzyme. Strains producing an AmpC enzyme either inducibly or constitutively are typically resistant to these drugs. Also clavulanate strongly induces cAmpC production (and does not have an inhibitory activity on it).
2. *Inducer/stable* β-lactam compounds: the typical example are carbapenems, which are strong inducers of *ampC* expression, but are overall stable. Strains producing inducibly or constitutively an AmpC enzyme usually remain susceptible to carbapenems, unless in the presence of porin alterations that reduce outer membrane permeability.
3. *Weak inducer/labile* β-lactam compounds: ureidopenicillins (e.g., piperacillin), third-generation cephalosporins and aztreonam. In this case, strains with an inducible AmpC usually appear susceptible to these compounds, while those constitutively producing the enzyme are resistant. Since mutants constitutively producing the enzyme are easily selected from strains with inducible production, the use of these antibiotics should be considered with caution with isolates of cAmpC-producing species, despite apparent in vitro susceptibility. A similar consideration could apply to piperacillin–tazobactam, for which in vitro susceptibility is often retained because piperacillin and tazobactam are only weak inducers, since AmpCs hydrolyze piperacillin and are usually not inhibited by tazobactam. Therefore, if the clinician suspects that the pathogen could produce a cAmpC, in our opinion the use of this antibiotic for severe infections should be considered with caution, regardless of susceptibility reports.
4. *Weak inducer/stable* β-lactam compounds: cefpirome and cefepime (fourth-generation cephalosporins). They usually retain activity against AmpC producers, unless in the presence of AmpC variants that exhibit increased activity against fourth-generation cephalosporins [5, 6].

The regulation mechanism of cAmpC expression is very complex (see Fig. 1).

In the presence of β-lactams that inhibit the synthesis of the bacterial cell wall, an increased quantity of 1,6-anhydro-N-acetylmuramic acid oligopeptides (muropeptides) are released in the periplasmic space. Muropeptides can enter the cytoplasm via the AmpG transporter (an inner membrane permease) and compete with uridine diphosphate (UDP)-N-acetylmuramic acid peptides for binding with AmpR, a transcriptional regulator that at a baseline state represses

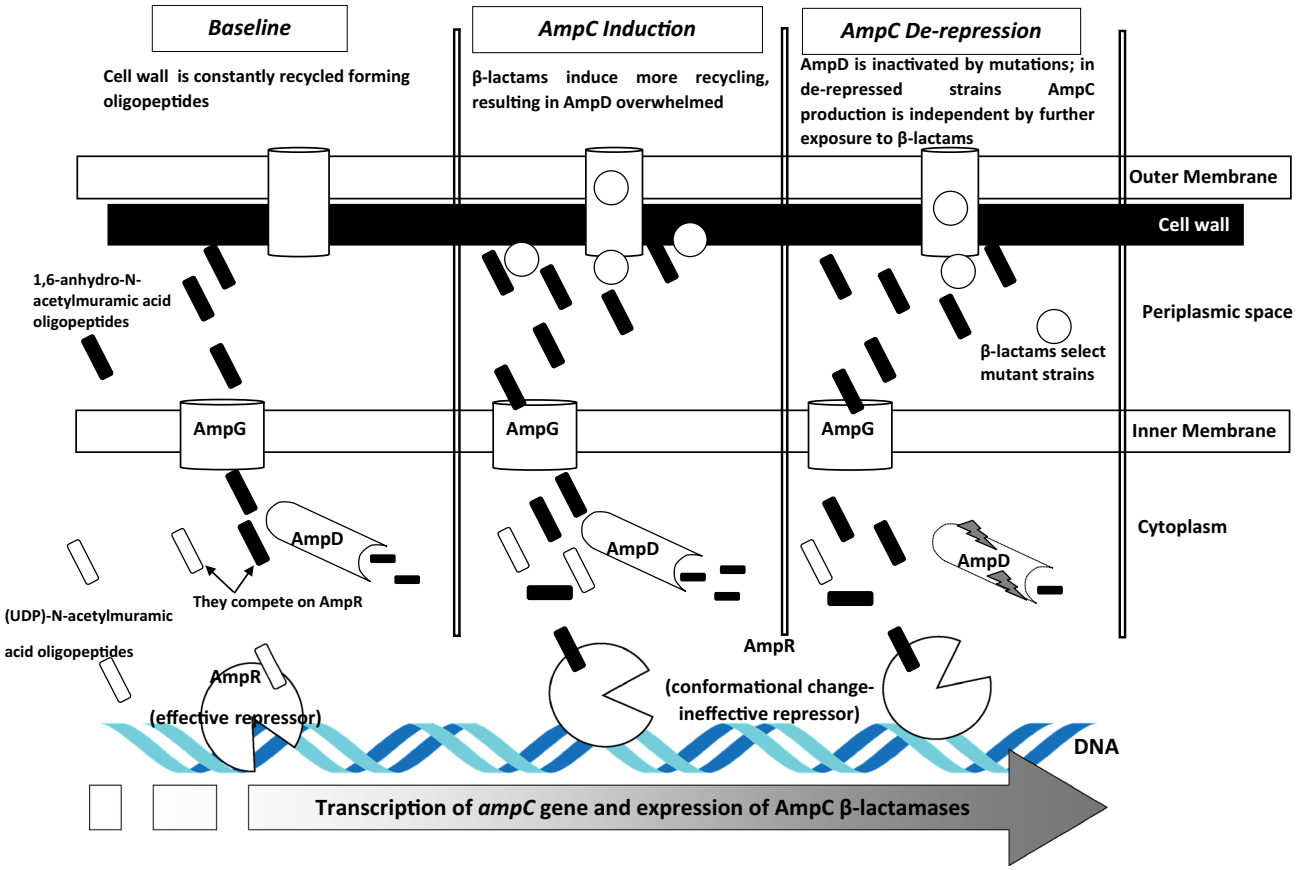

Fig. 1 AmpC regulation: induction and stable de-repression

expression of the *ampC* gene. With increasing 1,6-anhydro-N-acetylmuramic acid peptide binding and decreased UDP-N-acetylmuramic acid peptide binding, AmpR undergoes a conformational change leading to increased transcription of *ampC*. AmpD (*N*-acetyl-muramyl-L-alanine-amidase) is a regulatory protein responsible for cleavage of stem peptides from 1,6-anhydro-N-acetylmuramic acid peptides, which can be recycled for peptidoglycan biosynthesis. So, induction occurs when AmpD enzyme is unable to cleave all of the 1,6-anhydro-N-acetylmuramic acid peptides. Stable de-repression most commonly occurs due to *ampD* mutations reducing its cleaving activity, whereas less frequent causes are mutations in the *ampR* gene [5, 11].

De-repressed mutants may be present at a frequency of 10^{-5}–10^{-7} of the total bacterial population, and may be selected by antibiotic therapy, especially by weak inducer/labile β-lactams. This situation can be detected as early as 24 h after starting therapy or can occasionally be delayed for up to 2–3 weeks. In the landmark study of Chow *et al*. [12], de-repressed mutants of *Enterobacter* spp. constitutively producing cAmpC, resistant to extended spectrum penicillins (i.e., ticarcillin, ticarcillin–clavulanate, piperacillin and mezlocillin) and to 3GCs, were mainly obtained following 3GCs therapy after a mean of 9 days (range 4–18 days). Similarly, Choi *et al*. [13] reported the emergence of mutants of *Enterobacter* spp. and *C. freundii* resistant to 3GCs after treatment with these agents after a median of 7 days (range 3–28 days).

Also *E. coli* carries a chromosomal *ampC* gene. However, it is almost always expressed at negligible levels and is not inducible; therefore, usually it does not represent a clinically relevant problem in this species. Nevertheless, *E. coli* strains can occasionally exhibit a higher level of production of the cAmpC enzyme by gene duplication or mutations in the *ampC* promoter or attenuator regions [14]. In a recent multicenter Spanish study [15], of 841 bloodstream infections (BSI) due to 3GC-resistant *E. coli*, only 17 cases (2%) were caused by AmpC-producing isolates, according to the relative rarity of the phenomenon in this species, but it is noteworthy to observe that 41.2% of these were cAmpC overproducers, being the remaining carriers of a pAmpC. More recently, a Dutch study [16] on the prevalence among hospitalized patients of rectal carriage of plasmid- and chromosome-encoded AmpC-producing *E. coli* showed a prevalence of 2.4%, contributed by 0.9% of pAmpC-producing

Table 1 Main species of *Enterobacterales* carrying chromosomal AmpCs: a practical classification based on clinical relevance of expression and induction/de-repression phenomena

Species	Comments
Species with cAmpC inducible that can originate de-repressed mutants constitutively expressing high-level of β-lactamases	
Enterobacter cloacae complex *Klebsiella aerogenes* (previously known as *Enterobacter aerogenes*) *Serratia marcescens* *Citrobacter freundii* *Providencia stuartii* *Morganella morganii* *Hafnia alvei*	The acronym ESC indicates the following species: *Enterobacter* (*Enterobacter cloacae* complex, *Enterobacter aerogenes*), *Serratia marcescens, Citrobacter freundii. Enterobacter* spp. represent the prototype of this group: the evidence and data are the most significant, and evidence primarily relates to 3GCs treatment. It is noteworthy to remember that *Citrobacter koseri* lacks a chromosomal *ampC* gene [5] EUCAST rules, for *Enterobacter* spp. (evidence grade A) and *Citrobacter freundii, Serratia* spp., and *Morganella morganii* (evidence grade B), state that if these species are susceptible in vitro to cefotaxime, ceftriaxone or ceftazidime, then the use in monotherapy of these 3GCs should be discouraged, owing to the risk of selecting resistance, or the susceptibility testing results for these agents should be suppressed. Selection of AmpC de-repressed cephalosporin-resistant mutants may occur during therapy. The use of a 3GC in combination with an aminoglycoside may also lead to failure by selection of resistant mutants. Combination with quinolones has, however, been found to be protective. The selection risk is absent or much diminished for cefepime and cefpirome [17] If a 3GC is chosen as monotherapy, it is recommended to repeat susceptibility testing of subsequent isolates PTZ may select for de-repressed mutants, but this effect is weak: the routine suppression of susceptibility testing results may not be justified and there is scarce evidence to support that laboratories should not report susceptibility to this antibiotic [18]. PTZ is an effective option for *Morganella morganii*, as tazobactam inhibits its AmpC [5] For *Providencia stuartii* and *Morganella morganii*, the concepts better verified on the ESC group are mainly extrapolated, and most laboratories infer reporting practices from experience with the more commonly encountered ESC species. So often these species are collectively indicated as ESCPM group
Species with cAmpCs not inducible and expressed at negligible levels	
Escherichia coli	The regulation of cAmpC expression in *Escherichia coli* differs considerably from that in other *Enterobacterales*: this species lacks *ampR*, so AmpC is non-inducible and de-repression does not occur. cAmpC production in *Escherichia coli* normally occurs at levels too low for clinical significance, so this species commonly is susceptible to 2-3GCs (unless it is ESBL- or pAmpC-producing); nevertheless, this species can rarely increase cAmpC production by gene duplication or mutations in the *ampC* promoter or attenuator regions [14]

Note that recent taxonomic studies have narrowed the definition of the family *Enterobacteriaceae*, and some previous members of this family are now included in other families within the order *Enterobacterales*. *Enterobacter aerogenes* changed to *Klebsiella aerogenes*.

cAmpC chromosomal AmpC, *pAmpC* plasmidic AmpC, *2GCs* second-generation cephalosporins, *3GCs* third-generation cephalosporins, *PTZ* piperacillin–tazobactam

strains and 1.4% of strains with cAmpC overproduction due to promoter/attenuator alterations.

Table 1 summarizes a practical classification of the main *Enterobacterales* carrying cAmpC and the importance of

induction/de-repression phenomena on the emergence of resistant strains of clinical interest.

Plasmid-mediated AmpCs (pAmpCs)

More than 20 different AmpCs have been found to be mediated by plasmids: the first was described in 1989 [19], and since then they have been observed globally as a result of horizontal transfer of AmpC-encoding plasmids and clonal expansion. There are several lineages of p*ampC* genes, originating from chromosomal *ampC* genes carried by several Gram-negative species and falling into at least five phylogenetic groups, namely the *Enterobacter* group (MIR, ACT), the *Citrobacter freundii* group (CMY-2-like, LAT, CFE), the *Morganella morganii* group (DHA), the *Hafnia alvei* group (ACC), and the *Aeromonas* group (CMY-1-like, FOX, MOX) [20]. The most prevalent and widely disseminated are the CMY-2-like enzymes, although the inducible DHA-like β-lactamases and some others have also extensively spread [20].

pAmpCs are usually constitutively expressed, conferring resistance patterns similar to that of de-repressed cAmpCs. For this reason, pAmpC-carrying bacteria should always be considered of significant clinical relevance. As an exception to this rule, some p*ampC* genes, such as the bla_{DHA-1}gene, are inducible by β-lactams, with expression regulated similar to that of c*ampC* genes [5].

The most important species of the *Enterobacterales* order that have acquired pAmpCs include *Escherichia coli, Klebsiella pneumoniae* and *Proteus mirabilis*, but other important species are also *Klebsiella oxytoca, Salmonella enterica* and *Shigella* spp. [3, 20].

In a recent Italian survey [1], the overall prevalence of pAmpC producers among *E. coli, K. pneumoniae* and *P. mirabilis* was 1.2%, and the overall prevalence of pAmpC-producing *E. coli* was 0.6%. This finding was similar to the 0.4% prevalence reported by Drinkovic et al. [21] in the Auckland community, where most had a CMY-2-like enzyme and 51% of cefoxitin-resistant *E. coli* were pAmpC producers (37% were instead assumed as hyper-producers of cAmpC) and only a few strains (4%) co-produced pAmpC and an ESBL. In that study, most pAmpC-producing *E. coli* were from community-acquired urinary tract infections, mainly in women, especially if they had previously received β-lactams, but it is noteworthy that a large proportion (43%) of patients were neither hospitalized nor had received any antimicrobial treatment in the previous six months. Furthermore, the isolates exhibited high resistance rates also to non-β-lactam antimicrobials (e.g., norfloxacin, trimethoprim and nitrofurantoin), leaving few treatment options. Harris et al. [22] characterized 70 3GC-resistant *E. coli* isolated from blood in patients enrolled in the MERINO trial from Australia, New Zealand and Singapore: the majority (61.4%) were ST131 isolates, 95% of which carrying bla_{CTX-M} ESBLs; p*ampC* genes (mainly bla_{CMY-2}) were also frequent (17.1%), and more common among non-ST131 isolates. Only two strains carried both bla_{CMY-2} and bla_{CTX-M}. The co-existence of pAmpCs and ESBLs is, therefore, quite rare, even if in a recent Swiss study an overall percentage of 13% of *Enterobacterales* producing both types of enzymes was reported [23].

A large outbreak caused by *K. pneumoniae* producing a FOX-7 AmpC was observed in a neonatal intensive care unit in central Italy from February 2008 to April 2010, with a mortality rate at 14 days in case of sepsis of 28.5%. All isolates were resistant to cefotaxime, ceftazidime and piperacillin–tazobactam, while 76% were susceptible to cefepime and 98–100% to carbapenems [24]. This experience clearly demonstrates that pAmpC-producing *Enterobacterales* can cause large outbreaks with significant morbidity and mortality, underscoring the role of laboratory-based surveillance and infection control measures to contain similar episodes.

The importance of recognizing these situations is underscored by the case of *P. mirabilis* circulating in Italy, where, for over a decade, a multifocal spreading of a clone producing an acquired AmpC β-lactamase (CMY-16) has been detected [25, 26]. In a northern Italian hospital, its prevalence rapidly increased from 0.3% in 2004 to 4.6% in 2006, due to a rapid clonal expansion of the AmpC-positive strain first isolated in 2003 from a geriatric ward. In this case, about 50% of isolates were obtained from hospitalized patients, most frequently in medical wards [26]. The isolates carrying the CMY-16 determinant showed multidrug-resistance and the majority were associated with urinary tract infections; treatment with amikacin or carbapenems was consistently effective, but also piperacillin–tazobactam produced a clinical response in 78% of the cases since tazobactam appeared to be effective at antagonizing the enzyme activity [25, 26]. This pathogen is still today often isolated in Italy, representing the 9% of all 275 isolates of *P. mirabilis* collected in the recent survey mentioned above [1]. For these reasons, every time a *P. mirabilis* is isolated, it is very important a correct phenotypic interpretation of the antibiogram to suspect it (cefoxitin R, cefotaxime and ceftazidime R, cefepime S) and, if possible, to confirm the hypothesis for the most appropriate antibiotic choice.

How to recognize AmpC-producing organisms by antibiogram reading, and when to do further phenotypic and genotypic tests

By reading of the antibiogram it is possible to hypothesize if an isolate is an AmpC-producer only if the gene is significantly expressed leading to relevant β-lactamase activity against target substrates.

Bacterial strains having chromosomal inducible AmpCs are particularly challenging for antibiotic susceptibility reporting since in vitro susceptibility may not correlate with clinical efficacy, as resistance can emerge by selection of mutants overproducing the enzyme during treatment [12, 27]. Indeed, the majority of *Enterobacterales* (such as *Enterobacter* spp. and the other members of ESCPM group) with inducible cAmpCs retain in vitro susceptibility to the oxyimino-cephalosporins (weak inducers). For strains which constitutively produce cAmpC and for most pAmpC-carrying strains (which constitutively produce the enzyme) the situation is quite different, and the classical resistance pattern is represented by the combination of resistance to oxyimino-cephalosporins (such as cefotaxime and ceftazidime), susceptibility to cefepime and resistance to cephamycins (such as cefoxitin; this is why the clinicians should expect and require this molecule to be routinely tested in antibiograms). A cefoxitin MIC > 8 mg/L combined with resistance to ceftazidime and/or cefotaxime may be used as a phenotypic criterion for investigation of acquired pAmpC production in species that are normally lacking these enzymes. This strategy, however, will not detect ACC-1, a pAmpC that does not hydrolyze cefoxitin [20]. For laboratories not testing cefoxitin, susceptibility to cefepime together with resistance to cefotaxime and/or ceftazidime is another phenotypic indicator of potential AmpC production, although less specific [20].

The hypothesis of AmpC production must be confirmed by additional phenotypic and genotypic assays. Phenotypic confirmation tests are generally based on inhibition by either cloxacillin or boronic acid derivatives that are good inhibitors of AmpCs. For *E. coli*, however, these confirmation tests cannot discriminate between acquired pAmpC and constitutive hyper-production of the cAmpC. The presence of acquired pAmpCs can be confirmed using PCR-based methods or with DNA microarray-based methods [20].

The low prevalence of pAmpC-producing *Enterobacterales* precludes routine universal microbiological screening, which is time-consuming and expensive. Unfortunately, it is not possible to identify a clinical profile that would allow targeted screening for pAmpCs, when compared to ESBL producers, because the risk factors and patients' comorbidities are virtually the same [23].

It is important to underline that, in some strains, the coexistence of AmpCs and ESBLs makes the interpretation of the antibiograms even more challenging [20, 23]. In fact, for *Enterobacterales* with inducible cAmpCs, ESBL screening should also be performed with cefepime (stable to AmpC) in phenotypic testing with clavulanic acid [20].

Furthermore, AmpC overproduction in addition to decreased outer membrane permeability due to porin mutations can reduce susceptibility to carbapenems, in particular in plasmid-mediated AmpC producers, conferring resistance patterns similar to that of carbapenemase-producers, and these situations must be distinguished. Anyway, only genotypic methods can clearly discriminate these complex situations.

Clinical evidences

Clinical studies evaluating treatments of infections caused by AmpC-producing *Enterobacterales* are currently scarce, and most evidence is based on retrospective data. Carbapenems are usually considered first-choice options, but alternatives are needed because the rate of carbapenem resistance is rising and alarming [28]. The interpretation of available evidences is complicated by the heterogeneity of the different studies, including infections by different species: either all ESCPM [13] or only ESC organisms actively and stably producing cAmpC [29], or only *Enterobacter* spp. [12, 27, 30], or only members of the *E. cloacae* complex [8, 31]. The isolates were from blood in some studies [8, 12, 30, 31] or from various specimens in others [13, 27, 29, 32], reflecting very different clinical situations.

For *Enterobacter* spp., emergence of resistance to 3GCs during therapy with these agents was 19% in two important studies, being significantly more frequent when the initial site of isolation is blood [12, 27]. Therefore, when an *Enterobacter* spp. is isolated from blood it may be prudent to avoid 3GCs regardless of in vitro susceptibility, but also a biliary tract source seems to be significantly associated with the emergence of resistance [13]. On the other hand, concomitant exposure to quinolones seems to be associated with a decreased risk for emergence of resistance [27].

For infections due to isolates of the ESC group constitutively producing cAmpC, cefepime and meropenem showed no difference in 30-day mortality or length of hospital stay [29], and for BSI due to *E. cloacae*, cefepime represented a reasonable alternative to carbapenems irrespective of the inducible or de-repressed phenotypes, when the prevalence of ESBL producers was low [8]. Cefepime is particularly suggested for isolates with MIC ≤ 2 mg/L, since approximately 97% of patients with such isolates cleared bacteremia within 1 day [30]. Also other authors have shown that cefepime represents an effective option for *E. cloacae* bacteremia caused by strains with MIC ≤ 2 mg/L, resulting inferior to carbapenems on mortality only for higher MIC values [31]. In a systematic review and meta-analysis of studies on BSI due to cAmpC-producing *Enterobacterales*, no strong evidence was found to suggest that β-lactam/β-lactamase inhibitor (BLBLI) agents (specifically piperacillin/tazobactam or ticarcillin/clavulanate), quinolones or cefepime were inferior to carbapenems concerning mortality [33].

The recent report of the British Society for Antimicrobial Chemotherapy/Healthcare Infection Society/British

Table 2 Selection of relevant clinical or review studies dealing with patients with infections due to chromosomal AmpC-carrying *Enterobacterales*

First author, year [references]	Study design and species	Main results of the study and comments
Chow, 1991 [12]	Prospective multicenter observational study of 129 pts with BSI due to *Enterobacter* spp	84% of bacteremias were hospital-acquired. 29% of *Enterobacter* spp. initially isolated were found to be "multiresistant" (defined as resistant to extended spectrum penicillins, i.e., ticarcillin, ticarcillin-clavulanate, piperacillin and mezlocillin, and to 3GCs): almost all these patients had received antibiotics in the previous two weeks. Previous administration of 3GCs was more likely to be associated with an initial multiresistant phenotype than previous administration of other antibiotics (69% vs. 20%; $P < 0.001$) Initial isolation of a multiresistant *Enterobacter* was associated with a higher mortality rate (32% *vs*. 15%; $P = 0.03$) Emergence of resistance during therapy with 3GCs was 19%, while occurred less often with other ß-lactams (0%) Authors suggest that when *Enterobacter* spp. is isolated from blood it may be prudent to avoid 3GCs regardless of in vitro susceptibility
Kaye, 2001 [27]	Retrospective cohort study of 477 pts with isolates of *Enterobacter* spp. (343 *E. cloacae*, 108 *E. aerogenes,* 26 other) initially susceptible to 3GCs	Exposure to 3GCs (specifically ceftriaxone and ceftazidime) was a risk factor for the emergence of resistant strains (RR 2.3; $P = 0.01$). 19% of the patients initially treated with 3GCs subsequently showed emergence of resistance. Exposure to quinolones was associated with a decreased risk for emergence of 3GC-resistant strains (RR = 0.4; $P = 0.03$) Resistance emerged significantly more frequently when the initial site of isolation was the blood rather than urine, tissue (not further specified in the study) or wounds Resistance occurred more frequently among *Enterobacter aerogenes* than *Enterobacter cloacae* (17% *vs*. 9%)
Choi, 2008 [13]	Prospective single-center observational study of 732 pts with infections caused by *Enterobacter* spp. (287 *E. cloacae*, 143 *E. aerogenes*, 11 *E. agglomerans*, and 2 *E. asburiae*), *Serratia marcescens* (113), *Citrobacter freundii* (130), or *Morganella morganii* (46)	73.1% of infections were nosocomial, many in medical wards. Infection sources: pneumonia (26.4%), biliary tract (23.4%), urinary tract (15.3%), primary bacteremia (10.7%), skin and soft tissue (10.5%), intra-abdominal (8.6%); secondary bacteremias were 27.6% The overall emergence of resistance during antimicrobial therapy (main end point) was 1.9%. Resistance to 3GCs (cefotaxime, ceftriaxone and ceftazidime), cefepime, extended-spectrum penicillins, carbapenems, fluoroquinolones, and aminoglycosides emerged during treatment in 5%, 0%, 2%, 0%, 0%, and 1.1% of patients, respectively Resistance to 3GCs during treatment occurred more often in *Enterobacter* spp. (8.3%) than in *Citrobacter freundii* (2.6%), *Serratia marcescens* (0%), or *Morganella morganii* (0%). Note that resistance emerged in 13.3% of patients with *Enterobacter* spp. BSI. Biliary tract infection with malignant bile duct invasion was significantly associated with the emergence of resistance ($P = 0.024$) Authors suggest that for *Enterobacter* spp. it might be prudent to avoid the use of 3GCs in patients with biliary tract infections associated with malignant bile duct invasion and, possibly, skin and soft tissue infections

Table 2 (continued)

First author, year [references]	Study design and species	Main results of the study and comments
Hilty, 2013 [8]	Retrospective study of 57 *Enterobacter cloacae* responsible for 51 BSI	BSI were frequently hospital-acquired (68%). 59.6% and 31.6% of strains possessed inducible or de-repressed AmpCs, respectively. Patients with BSI due to de-repressed isolates tended to be older and more often immunocompromised than those with inducible isolates. 28-day mortality was higher for BSI due to de-repressed than inducible isolates (29.4% vs. 3.8%; $P=0.028$). Patients showed a favorable outcome (complete or partial response) both with cefepime (88.9%) and carbapenems (92.3%). Authors state that cefepime represents a reasonable alternative to carbapenems for BSI due to *Enterobacter cloacae* irrespective of the inducible or de-repressed phenotypes, when the prevalence of ESBL producers is low (in this study 3.5%)
Tamma, 2013 [29]	Retrospective study of 96 pts with isolates of AmpC-producing ESC (*Enterobacter* spp., *Serratia* spp., *Citrobacter* spp., positive by both phenotypic methods, cefotetan/boronic acid disk tests and cefotetan/cloxacillin Etest strips)	Out of 399 patients with isolation from blood, bronchoalveolar and intra-abdominal fluids of ESC, 96 (24%) had strains actively AmpC-producing. Of all isolates tested, 38%, 15%, and 1% of *Enterobacter* spp., *Serratia* spp., and *Citrobacter* spp., respectively, were positive for expression of AmpC β-lactamase production by both phenotypic methods Between the two groups of patients treated with cefepime or meropenem there was no difference in 30-day mortality (OR 0.63; 95% CI, 0.23–2.11; $P=0.36$) or length of hospital stay (RR 0.96; 95% CI, 0.79–1.26; $P=0.56$) Authors conclude that cefepime may be a reasonable option for the treatment of invasive infections due to AmpC-producing organisms, particularly when adequate source control is achieved
Siedner, 2014 [30]	Retrospective study of 368 pts with BSI due to *Enterobacter* spp.	In multivariable models there was no association between carbapenem use and persistent bacteremia (aOR, 1.52; 95% CI, 0.58–3.98; $P=0.39$) and a non-significant lower OR with cefepime use (aOR, 0.52; 95% CI, 0.19–1.40; $P=0.19$). In-hospital mortality was similar among patients receiving cefepime (aOR, 1.50; 95% CI, 0.73–3.47; $P=0.25$) or carbapenems (aOR, 1.82; 95% CI, 0.82–3.80; $P=0.11$) Authors conclude that cefepime has a similar efficacy as carbapenems for the treatment of *Enterobacter* spp. BSI and suggest this agent particularly for isolates with MIC ≤ 2 mg/L, because approximately 97% of patients with such isolates cleared bacteremia within 1 day of cefepime initiation

Table 2 (continued)

First author, year [references]	Study design and species	Main results of the study and comments
Lee, 2015 [31]	Retrospective study of 144 adults with BSI due to *Enterobacter cloacae* definitively treated with in vitro active cefepime or carbapenems	Over 90% of BSI were hospital-acquired. Critical illness, rapidly fatal underlying disease, concomitant ESBL production and cefepime-SDD category (MIC 4–8 mg/L) were independently associated with 30-day mortality The 30-day mortality rate of patients definitively treated with cefepime infected by cefepime-susceptible isolates was significantly lower than patients infected by cefepime-SDD isolates (16.1% vs. 62.5%; $P < 0.001$) and similar to that of patients definitively treated with carbapenems (16.1% vs. 22.2%; $P = 0.50$). Patients infected by cefepime-SDD isolates definitively treated with cefepime had a higher mortality than those treated with carbapenems (71.4% vs. 18.2%; $P = 0.045$). ESBL production was more common in cefepime-SDD isolates than in those that were cefepime-susceptibles (88.9% vs. 44.2%; $p < 0.001$) Cefepime is an option for bacteremia due to cefepime-susceptible (CLSI MIC ≤ 2 mg/L) *Enterobacter cloacae* but is inefficient for SDD isolates compared with carbapenems
Blanchette, 2014 [32]	Retrospective, matched 1:2, case–control study of 48 pts with bacteremia, pneumonia, skin and soft tissue infection, intra-abdominal and urinary tract infections due to SPICE isolates, treated with ertapenem or cefepime	The primary outcome was clinical response to therapy. No difference in clinical success was found (69% for ertapenem vs. 88% for cefepime, $P = 0.138$) Compared with cefepime, ertapenem has the advantage of a narrower spectrum that does not include activity against *Pseudomonas aeruginosa* and furthermore it is more active against ESBL producers
Harris, 2016 [33]	Systematic review and meta-analysis of eleven observational studies on BSI due to cAmpC-producing *Enterobacterales*	In unadjusted analyses, no significant difference in mortality was found between BLBLIs (specifically piperacillin/tazobactam or ticarcillin/clavulanate) vs. carbapenems for definitive therapy (OR 0.87, 95% CI 0.32–2.36) or empirical therapy (OR 0.48; 95% CI 0.14–1.60) or cefepime vs. carbapenems as definitive therapy (OR 0.61; 95% CI 0.27–1.38) or empirical therapy (0.60; 95% CI 0.17–2.20). Use of a fluoroquinolone as definitive therapy was associated with a lower risk of mortality vs. carbapenems (OR 0.39; 95% CI 0.19–0.78) Conclusions: despite limitations of available data, there is no strong evidence to suggest that BLBLIs, quinolones or cefepime were inferior to carbapenems. The reduced risk of mortality observed with quinolone use may reflect less serious illness in patients, rather than superiority over carbapenems
MERINO II, in progress [36]	RCT, estimated enrollment 100 pts with BSI due to ESCPM. ClinicalTrials.gov Identifier: NCT02437045	Pilot RCT of meropenem *vs*. piperacillin-tazobactam for definitive treatment of BSI caused by AmpC-producing *Enterobacter* spp., *Serratia marcescens, Providencia* spp., *Morganella morganii* or *Citrobacter freundii* Inclusion Criteria: BSI with ESCPM (i.e., likely AmpC-producer), and susceptibility to 3GCs, meropenem and piperacillin–tazobactam from at least one blood culture draw Primary outcome measures: clinical and microbiological outcomes at 30 days (composite end point of: death, clinical failure, microbiological failure; microbiological relapse)

3GCs third-generation cephalosporins, *BLBLI* beta-lactam/beta-lactamases inhibitor, *BSI* bloodstream infections, *SDD* susceptibility dose-dependent (CLSI), *SPICE Serratia* spp., *Providencia* spp., indole-positive *Proteus , Citrobacter* spp., and *Enterobacter* spp., *ESCPM Enterobacter* spp., *Citrobacter freundii, Morganella morganii, Providencia* spp., *Serratia marcescens, RCT* randomized controlled trial, *CI* confidence interval, *OR* odds ratio, *aOR* adjusted odds ratio, *RR* relative risk

Infection Association Joint Working Party on treatment of infections caused by multidrug-resistant Gram-negative bacteria [34] recommends that cefepime could be used to treat infections caused by AmpC-producing bacteria if susceptible at the EUCAST breakpoint of MIC ≤ 1 mg/L (conditional recommendation for), but not, even at increased dose, for isolates with MICs > 1 mg/L or those producing both AmpC and ESBL (strong recommendation against). Use of meropenem or imipenem or ertapenem is strongly recommended to treat serious infections with AmpC-producing *Enterobacterales*. This document states that, for infections due to AmpC-producing *Enterobacterales*, temocillin could be used for urinary tract infections and associated bacteraemia, and ceftazidime/avibactam could be used as an alternative to carbapenems but alternatives may be cheaper (grading: conditional recommendations for). Furthermore, since the activity of ceftolozane/tazobactam against *Enterobacterales* with copious AmpC enzyme is variable, and many *Enterobacter* spp. with de-repressed AmpC are resistant [35], this antibiotic should not be used in infections due to AmpC-producing *Enterobacterales* (grading: strong recommendation against use).

Table 2 shows a selection of the most relevant clinical studies enrolling patients with infections due to cAmpC-carrying *Enterobacterales*.

To our knowledge no comparative studies for pAmpC producers are available.

Discussion

Multidrug-resistant *Enterobacterales* are a major concern worldwide. In most cases, the resistance to 3GCs is mediated by ESBL production, but expression of AmpCs may be relevant; nevertheless, little attention to these β-lactamases is currently paid by many clinicians that every day face these complex situations.

We know that pAmpC-producing *Enterobacterales* are often involved in community-acquired infections, while cAmpC-carrying species are mainly involved in nosocomial infections. Therefore, not only the Infectious Diseases specialists, but also Internists and General Practitioners can frequently intercept these pathogens; hence, they must be aware of their epidemiological impact, dynamics, recognition and treatment. Also patients undergoing chronic hemodialysis are at high risk of infections due to cAmpC-producing *Enterobacterales* [37]; therefore, additional specialists, such as Nephrologists, should know this problem.

The clinicians should know that the different species may or may not express the resistance gene, and that some antimicrobials can have different efficacy depending on the possibility of in vivo selection of resistant mutants which constitutively produce the enzyme. In particular, for members of the ESCPM group and mainly for *Enterobacter* spp. and for BSI, it seems prudent to avoid 3GCs regardless of in vitro susceptibility, due to the risk of emergence of resistance during treatment in a significant percentage [12, 27].

To date no RCTs directly comparing different treatment options for AmpC-producing bacteria have yet been completed, and few and very heterogeneous observational studies are the sole source of evidence; thus, there is no conclusive evidence about the best treatment of these problematic situations, and carbapenems are often considered the preferred option. In our opinion, if the infection is severe, if an adequate source control is not achieved, and especially if a monotherapy is chosen, a carbapenem should be the first choice, but there are enough data from the literature to consider cefepime as a reasonable carbapenem-sparing option, especially when the MIC values are in the EUCAST susceptibility range (≤ 1 mg/L), and optimizing the way of administration (extended infusion).

Also the associations of β-lactams with the new β-lactamases inhibitors active on AmpCs (such as avibactam) can represent an interesting option, while we underline that data on ceftolozane mainly refer to *Pseudomonas aeruginosa*.

A phenotype of reduced susceptibility to carbapenems can be co-mediated by AmpC overproduction and outer membrane protein loss in some *Enterobacterales*; to our knowledge, there is no clinical study about the most appropriate treatment of these situations, and in our opinion we should rely on antibiogram results and on the molecules with the most favorable MIC values, taking into account, when appropriate, also non β-lactams antibiotics, such as fluoroquinolones and aminoglycosides.

In summary, we hope that well-conducted trials will be performed to clarify uncertainties about the most appropriate treatment, but pending definitive data, in our opinion, it seems reasonable to rely on the options with the most evidence from the literature (carbapenems and cefepime), taking into account the severity and site of infection, the previous use of antimicrobials known to be inducers, and the level of expression and effective production of AmpC β-lactamase.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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