

## REVIEW

# Bench-to-bedside review: The role of $\beta$ -lactamases in antibiotic-resistant Gram-negative infections

Karen Bush\*

### Abstract

Multidrug resistance has been increasing among Gram-negative bacteria and is strongly associated with the production of both chromosomal- and plasmid-encoded  $\beta$ -lactamases, whose number now exceeds 890. Many of the newer enzymes exhibit broad-spectrum hydrolytic activity against most classes of  $\beta$ -lactams. The most important plasmid-encoded  $\beta$ -lactamases include (a) AmpC cephalosporinases produced in high quantities, (b) the expanding families of extended-spectrum  $\beta$ -lactamases such as the CTX-M enzymes that can hydrolyze the advanced-spectrum cephalosporins and monobactams, and (c) carbapenemases from multiple molecular classes that are responsible for resistance to almost all  $\beta$ -lactams, including the carbapenems. Important plasmid-encoded carbapenemases include (a) the KPC  $\beta$ -lactamases originating in *Klebsiella pneumoniae* isolates and now appearing worldwide in pan-resistant Gram-negative pathogens and (b) metallo- $\beta$ -lactamases that are produced in organisms with other deleterious  $\beta$ -lactamases, causing resistance to all  $\beta$ -lactams except aztreonam.  $\beta$ -Lactamase genes encoding these enzymes are often carried on plasmids that bear additional resistance determinants for other antibiotic classes. As a result, some infections caused by Gram-negative pathogens can now be treated with only a limited number, if any, antibiotics. Because multidrug resistance in Gram-negative bacteria is observed in both nosocomial and community isolates, eradication of these resistant strains is becoming more difficult.

### Introduction

Much has been publicized recently about the expansion of hospital- and community-based infections caused by

Gram-positive bacteria, especially those caused by vancomycin-resistant enterococci (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA) [1]. However, a second, but potentially more important, threat to critical care is that caused by the multidrug-resistant Gram-negative bacteria. The response of the pharmaceutical companies over the past decade has been to design new drugs that can treat VRE and both hospital- and community-acquired MRSA infections [2]. However, what is lacking from the current pharmaceutical arsenal are drugs to treat multidrug-resistant Gram-negative infections in the hospital setting [3]. Because  $\beta$ -lactam antibiotics have long been a component in the treatment regimen for serious nosocomial infections, any threat to their efficacy must be examined closely.

Among the  $\beta$ -lactam antibiotics of clinical utility for the treatment of infections caused by susceptible Gram-negative bacteria are penicillins such as amoxicillin, oral cephalosporins such as cefpodoxime and cefuroxime axetil, parenteral cephalosporins such as cefepime and ceftriaxone, and the carbapenems such as doripenem, ertapenem, imipenem, and meropenem. Combinations of penicillins with  $\beta$ -lactamase inhibitors also play prominent therapeutic roles, with amoxicillin-clavulanic acid being a major factor in the treatment of community infections and piperacillin-tazobactam being important for serious hospital-acquired infections. In this review, the role of  $\beta$ -lactamases will be discussed as a major cause of resistance to these safe and widely prescribed drugs.

### Infections associated with Gram-negative bacteria

Infections caused by Gram-positive bacteria represented the majority of serious infections prior to the late 1950s. Thus, it is not surprising that the increased use of penicillins to treat the associated diseases caused  $\beta$ -lactam resistance to arise in the clinical setting, first in the staphylococci and then in Gram-negative bacteria. When penicillins lost their utility as monotherapy for most disease states, penicillins and cephalosporins that were more potent were developed in an effort to retain the favorable clinical properties of the  $\beta$ -lactam antibiotics. In addition to predictable efficacy in a number of clinical indications, these agents have continued to

\*Correspondence: karbush@indiana.edu  
Department of Biology, Indiana University, Jordan Hall A311, Bloomington, IN 47405, USA

**Table 1. Common  $\beta$ -lactam antibiotics that may be used as monotherapy to treat infections caused by Gram-negative bacteria**

Infection type	Phenotype	Possible $\beta$ -lactam antibiotics <sup>a,b</sup>
Bacterial meningitis	Wild-type	Cefotaxime or ceftriaxone, cefepime, meropenem
Intra-abdominal	Wild-type	Amoxicillin-clavulanic acid <sup>c</sup> , piperacillin-tazobactam, ticarcillin-clavulanic acid, cefoxitin, cefotetan
	ESBL-producing	Carbapenems <sup>d</sup>
Osteomyelitis	Wild-type	Ceftazidime, cefepime
Otitis media	Wild-type	Amoxicillin $\pm$ clavulanic acid <sup>c</sup> , cefdinir <sup>c</sup> , cefpodoxime <sup>c</sup> , cefprozil <sup>c</sup> , cefuroxime axetil <sup>c</sup> , ceftriaxone
Lower respiratory infections and pneumonia	Wild-type	Amoxicillin-clavulanic acid <sup>c</sup> , piperacillin-tazobactam, ticarcillin-clavulanic acid, aztreonam, cefdinir <sup>c</sup> , cefpodoxime <sup>c</sup> , cefprozil <sup>c</sup> , cefuroxime axetil <sup>c</sup> , cefepime, cefotaxime, ceftriaxone, ceftazidime
	ESBL-producing	Carbapenems
Gonorrhoea	Non- $\beta$ -lactamase-producing	Penicillin G
	$\beta$ -lactamase-producing	Cefixime <sup>c</sup> , cefpodoxime <sup>c</sup> , ceftriaxone, cefotaxime, cefuroxime
Skin and skin structure	Wild-type	Carbapenems Ampicillin-sulbactam, piperacillin-tazobactam, ticarcillin-clavulanic acid
	ESBL-producing	Carbapenems

<sup>a</sup>Antibiotics listed are based on those recommended in the 2009 Sanford Guide [4], assuming that the causative Gram-negative bacteria are susceptible to these agents. <sup>b</sup>Agents are dosed intravenously unless otherwise noted. <sup>c</sup>Oral dosing. <sup>d</sup>Carbapenems for infections caused by Enterobacteriaceae include doripenem, ertapenem, imipenem, and meropenem. For infections caused by *Pseudomonas aeruginosa*, ertapenem should not be used. ESBL, extended-spectrum  $\beta$ -lactamase.

demonstrate a pharmacodynamic and safety profile that makes them attractive therapeutic agents.

As shown in Table 1, the most common families of  $\beta$ -lactams that are used to treat infections caused by Gram-negative pathogens include extended-spectrum cephalosporins such as ceftriaxone and cefepime, penicillin- $\beta$ -lactamase inhibitor combinations such as amoxicillin-clavulanic acid and piperacillin-tazobactam, and the carbapenems [4]. Infection types range from uncomplicated community-acquired infections such as otitis media to serious nosocomial infections, including ventilator-associated pneumonia. Orally administered  $\beta$ -lactams such as amoxicillin-clavulanic acid and the oral cephalosporins cefixime, cefpodoxime, and cefuroxime axetil are recommended for community infections. For nosocomial infections that are not resistant to cephalosporins, parenteral drugs that may be effective include the injectable penicillin- $\beta$ -lactamase inhibitor combinations and cephalosporins. Carbapenems are often reserved to treat the most serious infections caused by many multidrug-resistant pathogens as they are most able to escape at least some of the common  $\beta$ -lactam resistance mechanisms that affect the other  $\beta$ -lactam families. However, the continued use of these antibiotics for important disease states has maintained the pressure on many pathogenic and commensal bacteria to retain enzymatic inactivation mechanisms that render penicillins ineffective in many disease states. This pressure has resulted in a surge in  $\beta$ -lactam resistance

due to inactivating enzymes, particularly in Gram-negative pathogens.

### Background of $\beta$ -lactamase resistance

In 1940, an enzyme produced by a strain of *Bacillus coli*, now known as *Escherichia coli*, was shown to destroy the ability of penicillin to kill bacterial cells [5]. This first report of  $\beta$ -lactamase activity occurred before widespread use of penicillin, demonstrating the presence of  $\beta$ -lactam-inactivating enzymes in the natural environment. These enzymes have the ability to hydrolyze the  $\beta$ -lactam chemical bond that distinguishes  $\beta$ -lactam antibiotics from other antibacterial agents, thereby rendering the molecules incapable of killing bacteria.

Today, over 890 unique  $\beta$ -lactamases have been identified in naturally occurring bacterial isolates [6,7]. These enzymes have been separated into groups, either according to the amino acid sequences of the enzymes or according to their inactivating properties for different classes of  $\beta$ -lactams. The molecular classification scheme divides  $\beta$ -lactamases into four classes based on the amino acid sequences of the proteins [8-10], whereas numerically more functional groups have been assigned based on the hydrolysis and inhibition profiles of the enzymes [7,10,11]. The major functional groups of current clinical importance are shown in Table 2, where enzyme groups are commonly named according to the most important class of  $\beta$ -lactam that is inactivated. Correlations between the

**Table 2. Major groups of  $\beta$ -lactamases in Gram-negative bacteria that threaten the role of  $\beta$ -lactam antibiotics**

Functional group <sup>a</sup>	Molecular class <sup>b</sup>	Common name	$\beta$ -Lactams to which resistance is conferred	
			Primary <sup>c</sup>	Secondary <sup>d</sup>
1	C	Cephalosporinase	Penicillins, cephalosporins	Carbapenems, monobactams
2b	A	Penicillinase	Penicillins, early cephalosporins	$\beta$ -lactamase inhibitor combinations
2be	A	Extended-spectrum $\beta$ -lactamase	Penicillins, cephalosporins, monobactams, $\beta$ -lactamase inhibitor combinations	None
2d	D	Cloxacillinase	Penicillins, including oxacillin and cloxacillin	None
2df	D	Carbapenemase	Carbapenems and other $\beta$ -lactams	None
2f	A	Carbapenemase	All current $\beta$ -lactams	None
3	B	Metallo- $\beta$ -lactamase	All $\beta$ -lactams except monobactams	None

<sup>a</sup>Classification based on Bush, Jacoby, and Medeiros [11] and Bush and Jacoby [7]. <sup>b</sup>Classified according to primary amino acid sequence [8-10]. <sup>c</sup> $\beta$ -lactams that are resistant solely as a function of  $\beta$ -lactamase production. <sup>d</sup> $\beta$ -lactams that are resistant as a function of  $\beta$ -lactamase production, usually at high levels, in combination with efflux or porin modifications.

molecular and functional categories are also provided in this compilation.

### Descriptions of $\beta$ -lactamase groups

#### Cephalosporinases

Group 1/Class C cephalosporinases are among the most abundant  $\beta$ -lactamases on the basis of the number of organisms that produce these enzymes. These cephalosporinases, frequently named as species-specific AmpC enzymes, are often found in most Enterobacteriaceae as chromosomal enzymes. At low production levels, they can abolish the antibacterial activity of cephalosporins and can also demonstrate inactivating capabilities toward other  $\beta$ -lactams, especially when produced at high levels. These enzymes are generally present at a low (basal) level but may be induced to high levels in the presence of selected inducing agents such as amoxicillin or clavulanic acid. Cephalosporinases may also be produced at very high levels in the absence of an inducer, in a 'derepressed' state. This has been reported to occur after selection of stable mutants during therapy with broad-spectrum cephalosporins, but not cefepime [12,13]. These mutants often arise as a result of a multistep process, with elevated cephalosporin MICs (in the high susceptible or intermediate range) observed in those organisms with impaired permeability characteristics prior to selection of high-level AmpC mutants that are fully resistant [14].

Treatment of organisms producing an inducible AmpC cephalosporinase has created some controversy. Although a group of investigators has recommended that any AmpC-inducible Enterobacteriaceae be regarded as resistant to all third-generation cephalosporins [13], the clinical data supporting this recommendation are mixed [14]. Studies have indicated that the selection of resistant *Enterobacter* spp. has ranged as high as 19% in a 1991 study (6/31), with higher rates observed in patients with bacteremia [15], but more recent studies have not corroborated those observations [12]. For example, in an

18-month study (2005-2006) of 732 patients infected with Enterobacteriaceae capable of producing AmpC  $\beta$ -lactamases, treatment with broad-spectrum cephalosporins resulted in resistance in 5% (11/218) of the patients compared with 0% (0/20) of the patients treated with cefepime, with the emergence of resistance being more frequent in *Enterobacter* spp. [12].

The suggestion has been made that therapeutic decisions be made on the basis of specific cephalosporin MICs rather than on the presence of an inducible AmpC enzyme [14]. This year, the Clinical and Laboratory Standards Institute (Wayne, PA, USA) lowered susceptibility breakpoints for cefotaxime, ceftriaxone, and ceftazidime against the Enterobacteriaceae [16]. Lower breakpoints should help to decrease the number of 'susceptible' isolates that may be likely to emerge as resistant strains following therapy with broad-spectrum cephalosporins but will allow cephalosporins to be used against highly susceptible organisms. The new cephalosporin breakpoints will also classify as resistant those ESBL-producing Enterobacteriaceae that will not respond pharmacodynamically to approved therapeutic doses, thereby reducing the need for specific ESBL testing other than for epidemiological purposes [16].

Plasmid-encoded AmpC cephalosporinases closely related in sequence to chromosomal AmpC enzymes from *Enterobacter cloacae*, *Citrobacter freundii*, or *Aeromonas* spp. also appear in Enterobacteriaceae in organisms that produce at least one other  $\beta$ -lactamase [17]. Although some of the plasmid-encoded AmpC enzymes are inducible, most of the enzymes are produced at much higher levels than seen for basal AmpC cephalosporinases, similar to isolates with derepressed AmpC enzymes [17].

#### Penicillinases

Common penicillinases (functional group 2b, molecular class A) include the SHV-1 enzyme in *Klebsiella pneumoniae* and the TEM-1  $\beta$ -lactamase found in many strains

of *Neisseria gonorrhoeae* [18] and *Haemophilus influenzae* [19]. These two enzymes occurred in high prevalence among the Enterobacteriaceae prior to the introduction of the broad-spectrum cephalosporins such as cefotaxime and ceftazidime [20,21]. The group 2b enzymes are readily inhibited by clavulanic acid, sulbactam, and tazobactam [11]. As a result, infections caused by organisms producing a single penicillinase can be readily treated with a  $\beta$ -lactamase inhibitor combination such as amoxicillin-clavulanic acid, ampicillin-sulbactam, or piperacillin-tazobactam.

### Extended-spectrum $\beta$ -lactamases

Particularly worrisome  $\beta$ -lactamases are found among the extended-spectrum  $\beta$ -lactamases, or ESBLs (functional group 2be or molecular class A). These enzymes were recognized shortly after the introduction of ' $\beta$ -lactamase-stable' cephalosporins and aztreonam. ESBLs were initially identified as variants of the common SHV-1 or TEM-1  $\beta$ -lactamase, often differing from the parent enzymes by only one or two amino acids. After their recognition in the late 1980s concomitantly in Europe [22] and the US [23,24], they became associated with major outbreaks of cephalosporin-resistant infections caused by ESBL-producing *E. coli* and *K. pneumoniae* [22,25]. The genes that coded for these enzymes were generally found on plasmids that conferred resistance to multiple antibiotic classes and that were readily transferable among species [26]. Even during the first reported outbreaks, other Enterobacteriaceae such as *C. freundii*, *Enterobacter aerogenes*, and *Serratia marcescens* were identified as ESBL producers [27].

ESBLs are still associated with major outbreaks of  $\beta$ -lactam resistance. However, the early SHV and TEM variants have been largely replaced by the CTX-M family of ESBLs. The first two CTX-M enzymes were identified at approximately the same time in the early 1990s in western Europe and South America in individual clinical isolates [28,29]. Within a decade, the CTX-M  $\beta$ -lactamases became the predominant ESBL family in many medical centers such that they have largely replaced most of the TEM- and SHV-derived ESBLs throughout the world [30-32].

CTX-M enzymes were particularly slow to emerge in the US [33] but have recently begun to dominate the ESBL populations of some US health centers [34]. The MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) surveillance study of 2007 identified CTX-M genes in 80% of the US medical centers that reported ESBL-producing isolates in their survey [35]. The early CTX-M-producing isolates were frequently resistant to cefotaxime and ceftriaxone but susceptible to ceftazidime because of a strong preference for hydrolysis of the former cephalosporins. However, some members

of the CTX-M family demonstrate high hydrolysis rates for all of the extended-spectrum cephalosporins as a result of single amino acid mutations, resulting in complete cephalosporin resistance in all of the producing pathogens [36,37]. It is possible that regional differences in the emergence of the CTX-M family were due to localized preferences for therapeutic regimens employing cefotaxime or ceftriaxone in Europe and South America compared with more frequent use of ceftazidime in the US and Canada.

### OXA $\beta$ -lactamases

The 'oxacillinase' family of  $\beta$ -lactamases, the OXA enzymes, was originally named to reflect the ability to hydrolyze oxacillin (or cloxacillin) [19]. Recently, a highly important subgroup of the OXA enzymes was identified as a causative factor for decreasing carbapenem susceptibility in non-fermentative bacteria such as *Acinetobacter* spp. and *Pseudomonas aeruginosa*. The OXA enzymes in *Acinetobacter* spp. are mainly chromosomally located, with little apparent transfer among strains [38]. Although these enzymes are structurally related to the older OXA  $\beta$ -lactamases, these new members of the OXA family have carbapenem-hydrolyzing activity. Because hydrolysis rates may be slow compared with other carbapenemases, full carbapenem resistance may require an additional resistance mechanism such as a porin mutation or upregulated efflux pump [39]. Importantly, carbapenemases within the OXA family have the ability to confer carbapenem resistance to organisms that may already have intrinsic resistance to multiple antibiotic classes [40]. This is especially evident in the *Acinetobacter* spp. that have been identified from soldiers and civilian populations returning from military duty in the Middle East [40].

### Serine carbapenemases

Another emerging family of  $\beta$ -lactamases is the serine carbapenemase group (group 2f/class A). This group of enzymes has the ability to hydrolyze most  $\beta$ -lactam antibiotics, including the carbapenems that are generally stable to hydrolysis by all other  $\beta$ -lactamases that have serine at the active site of the enzyme [11]. Early reports of this group of chromosomal enzymes were from single isolates of Enterobacteriaceae in the western US and London, followed by small outbreaks in Boston and Chicago a decade later [41]. The first plasmid-encoded serine carbapenemases in the KPC enzyme family were reported from the mid-Atlantic region of the US, again in single *Klebsiella* isolates (from the late 1990s) that were not immediately transferred within the reporting hospitals [42]. However, KPC-producing *K. pneumoniae* strains soon began to spread into the New York City metropolitan area, then on to Israel, France, and now to



many other areas of the world, including southern Europe, southeast Asia, and South America, with high clonality among and within geographical locations [43]. In addition, the genes encoding the KPC enzymes have spread into other Enterobacteriaceae as well as into *P. aeruginosa* and *Acinetobacter* spp. [41,44].

### Metallo- $\beta$ -lactamases

Metallo- $\beta$ -lactamases, or MBLs (functional group 3/ molecular class B) belonging to another carbapenemase family, were initially recognized as species-specific  $\beta$ -lactamases with limited contributions to the overall resistance profile in most medical centers [41]. These zinc-requiring chromosomal enzymes appeared in the same strain with other  $\beta$ -lactamases that generally had higher hydrolysis rates for penicillins and cephalosporins. Although these enzymes have relatively weak  $\beta$ -lactamase activity against all  $\beta$ -lactams except monobactams (aztreonam), their distinctive property is the ability to hydrolyze carbapenems [41]. The MBLs were thereby responsible for carbapenem resistance in organisms such as *Stenotrophomonas maltophilia*, *Aeromonas* spp., and a small population of *Bacteroides fragilis*. However, the identification of IMP-1, a plasmid-encoded MBL, in 1990 [45] changed our perspective on the group 3  $\beta$ -lactamases.

Plasmid-mediated carbapenem resistance has now become a serious clinical issue in parts of the world such as southern Europe, Japan, Brazil, and Asia. The initial IMP-1 enzyme now belongs to a family with 26 variants; the VIM family of MBLs has 23 variants [6]. These enzymes were first identified in non-fermentative bacteria such as *P. aeruginosa* and *Acinetobacter baumannii* but have now spread to many of the Enterobacteriaceae, including *Enterobacter* spp., *E. coli*, *C. freundii*, *Klebsiella* spp., and *S. marcescens* [41]. They are frequently associated with integrons (genetic dissemination systems) containing multiple antibiotic resistance determinants that are easily transferred among species. As with the species-specific MBLs, the plasmidic MBLs appear in organisms that almost always produce at least one other  $\beta$ -lactamase with an overlapping hydrolysis profile. Thus, it is possible that the MBL gene may be lost in the absence of specific antibiotic pressure, especially carbapenem therapy.

### Clinical response to $\beta$ -lactamases

#### Nosocomial issues

When broad-spectrum cephalosporins were introduced into clinical practice, ESBL-producing Enterobacteriaceae began to arise prolifically, resulting in the loss of these previously effective agents. In the late 20th century, many resistant Gram-negative bacteria were often treated with carbapenems as single therapeutic agents. At that time, it seemed that almost all  $\beta$ -lactamase-mediated resistances

could be overcome by imipenem or meropenem. These antibiotics were not hydrolyzed by the AmpC cephalosporinases or by ESBLs. Carbapenem monotherapy was the approach taken by some hospitals in which ceftazidime-resistant, ESBL-producing, *K. pneumoniae* isolates represented a majority of the Klebsiella isolates in the intensive care unit (ICU) [46]. For example, in a New York hospital [46] with a large outbreak of infections caused by ESBL-producing pathogens in the early 1990s, cephalosporin usage was reduced 80% throughout the hospital, accompanied by a 71% reduction in ceftazidime-resistant Klebsiellae in the ICU. However, the collateral damage was that overuse of carbapenems resulted in a 69% increase in imipenem-resistant *P. aeruginosa* [46] and in infections caused by imipenem-resistant *Acinetobacter* that were treatable only with polymyxin B or ampicillin-sulbactam [47]. Interestingly, a recent study from a New York ICU showed that strict infection control combined with routine rectal surveillance cultures was the most important factor associated with a reduction in carbapenem-resistant *K. pneumoniae* isolates but not with the reduction of carbapenem-resistant *P. aeruginosa* or *Acinetobacter* spp.; antibiotic usage was not correlated with this reduction [48].

Today, we are seeing situations that are even more complex as multiple broad-spectrum  $\beta$ -lactamases are spreading throughout Gram-negative pathogens. Global dissemination of new  $\beta$ -lactamases is expected, with epidemiological characteristics easily traced with currently available molecular techniques. Recent studies have demonstrated the clonal appearance of a highly homogeneous CTX-M-15-producing *E. coli* strain that was present in both hospital and community isolates from eight countries in Europe, North America, and Asia [49]. The first KPC-2-producing *K. pneumoniae* isolate reported in France was directly traced to a patient who had previously been treated in a New York City hospital [50]; subsequent analyses of KPC-producing *K. pneumoniae* isolates from the Centers for Disease Control and Prevention (CDC) identified a major sequence type that accounts for over 70% of the CDC KPC isolates [43]. OXA carbapenemases have been identified in multiple *Acinetobacter* spp. clones worldwide [40,51,52].

Organisms with multiple  $\beta$ -lactamases responsible for high-level resistance to most  $\beta$ -lactams are also appearing more frequently. A *K. pneumoniae* clinical isolate has been reported to produce eight different  $\beta$ -lactamases, including at least one ESBL, an AmpC, and a KPC enzyme [53]. The MBL VIM-1 was identified in a Greek *K. pneumoniae* isolate that also produced the KPC-2 carbapenemase [54]. Carbapenem-resistant *Acinetobacter* isolates have now been reported with both a VIM-2-like MBL as well as an OXA-23 carbapenemase in the same strain [55]. These organisms are resistant not only to all

$\beta$ -lactams but to many other antibiotic classes as well. Multidrug resistance has recently been reported in KPC-producing *K. pneumoniae* in metropolitan New York City [56] and in MBL-producing isolates [57] because of plasmid-encoded resistance determinants that confer resistance to aminoglycosides, fluoroquinolones, trimethoprim, sulphonamides, and chloramphenicol. The Infectious Diseases Society of America (Arlington, VA, USA) made Gram-negative resistance a major focus in their 2009 list of ESKAPE pathogens that include multidrug-resistant *K. pneumoniae*, *Acinetobacter* spp., *P. aeruginosa*, and *Enterobacter* spp. [3].

On a more optimistic note, perhaps plasmid-encoded MBLs and their co-production with non-carbapenemases may not be the disaster some have envisioned. In the absence of a carbapenem-selective pressure in a patient, it is possible that the MBL gene may be lost, although that organism would still maintain its resistance profile to other  $\beta$ -lactams. This speculation is based on the experiences of Japan and Italy, two countries where plasmid-encoded MBLs were first identified. In Japan and Italy, MBL genes that have spread countrywide after their initial reports have been responsible primarily for small sporadic outbreaks in these countries and have not been a continuous cause of carbapenem resistance throughout all Gram-negative species at a single medical center [58,59]. However, co-production of two carbapenemases, such as a VIM and KPC or a VIM and an OXA carbapenemase, may lead to much more serious consequences in which  $\beta$ -lactam antibiotics could never be useful.

### Community reservoirs

Although the main focus has been on multidrug-resistant infections in the hospital setting, community sources for  $\beta$ -lactamase-mediated resistances are being reported more frequently. Community-acquired ESBLs in locations such as nursing homes or long-term health care residences have been described for several years [31,32,60-62]. As one might expect, the even more deleterious plasmid-encoded carbapenemases are also being described in patients outside the hospital. Nine *E. coli* isolates producing the KPC-2 and KPC-3 carbapenemases, enzymes most frequently found in *K. pneumoniae* strains, were identified in seven nursing homes in the New York City area [63]. In Greece, a set of 45 patients was identified with community-onset urinary tract infections caused by VIM-2 MBL-producing *P. aeruginosa* isolates [64]. It is noteworthy that the first CTX-M-producing *E. coli* isolates in the US were identified from sources that included a number of urinary tract isolates originating from outpatients [34]. Thus, even if infection control procedures are instituted and strictly enforced in hospitals and medical centers,

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resistant Gram-negative pathogens have already been released into the community, where they can reside in relatively healthy people until challenged by changes in disease status, at which time they may become the causative pathogen for a new infection.

### Conclusions

Resistance in Gram-negative pathogens is increasing at an alarming rate. New  $\beta$ -lactamases that are transferred among species on plasmids with multiple resistance factors are also being described as a continuing exercise. The implications for therapeutic use of the workhorse  $\beta$ -lactam antibiotics are sobering. However, it is possible that some of these  $\beta$ -lactamases may exact a high price from the producing organism, especially when multiple  $\beta$ -lactamases are produced by a single strain. Small changes in therapeutic approaches may allow the flora to be altered within a medical center so that bacterial producers of ESBLs or KPCs or MBLs are only a small portion of the nosocomial flora and can be contained in isolated areas. Combination therapy with a  $\beta$ -lactamase inhibitor combination or a carbapenem, and at least one agent from another antibiotic class, may be effective against isolates that have decreased susceptibilities. Use of the maximally approved therapeutic doses should always be considered for serious infections when  $\beta$ -lactam MICs are close to the susceptibility breakpoints. Finally,  $\beta$ -lactam antibiotics should not be used if their MICs are in the highly resistant category, particularly in a hospital known to have high levels of plasmid-encoded  $\beta$ -lactamases that are being transferred throughout the facility; it should be noted that this epidemiological information may need to be determined with the assistance of a reference laboratory. With judicious use of antibiotics and strict infection control procedures, it may be possible to limit the effects of these newer  $\beta$ -lactamases until the time when new antibacterial agents are developed to counteract their effects.

### Abbreviations

CDC, Centers for Disease Control and Prevention; ESBL, extended-spectrum  $\beta$ -lactamase; ICU, intensive care unit; MBL, metallo- $\beta$ -lactamase; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant enterococci.

### Competing interests

KB was employed by Johnson & Johnson (Raritan, NJ, USA) from 1997 to 2009 and now receives retirement compensation from Johnson & Johnson, Bristol-Myers Squibb Company (Princeton, NJ, USA), and Wyeth (Madison, NJ, USA). She received consulting fees from Novexel (Romainville, France), Cubist Pharmaceuticals (Lexington, MA, USA), and Protez Pharmaceuticals (Malvern, PA, USA) in 2009 and is a stockholder of Johnson & Johnson. She is a patent holder on Wyeth patents for  $\beta$ -lactam compounds that were never developed.

She has received compensation from Bristol-Myers Squibb Company and Wyeth, both of which hold patents on  $\beta$ -lactam antibiotics. No organization supported the writing or publishing of this manuscript.

#### Author information

KB was employed from 1973 to 2009 in the pharmaceutical industry, where she studied the mechanism of action and characterization of  $\beta$ -lactamases as they affected the development of new antibiotics. She was responsible for defining the functional classification of  $\beta$ -lactamases which has been in use for over 20 years. Her research supported the development of the  $\beta$ -lactam antibiotics aztreonam (E.R. Squibb & Sons, Princeton, NJ, USA), piperacillin/tazobactam (Lederle Laboratories, Pearl River, NY, USA), doripenem (Johnson & Johnson and Peninsula Pharmaceuticals, Alameda, CA, USA), and ceftobiprole (Johnson & Johnson and Basilea Pharmaceuticals, Basel, Switzerland). She currently serves as an adjunct professor at Indiana University (Bloomington, IN, USA), where she teaches in the biotechnology program.

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