β-lactam resistance in the *Enterobacteriaceae*

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The development of new β-lactam antibacterial agents within the pharmaceutical ινδυστρψ has shifted into high gear once again due to a rapid development of resistance to older β-lactam compounds within the *Enterobacteriaceae.* Just as antibacterial agents such as cefotaxime, ceftriaxone, and other 3rd-generation cephalosporins (cephs) were created to counteract strains resistant to ampicillin and 1st-generation cephs, 4thgeneration cephs (e.g. cefepime) were created to treat infections refractory to therapy with 3rd-generation cephs and aztreonam. This short review discusses β-lactam antimicrobial resistance mechanisms as well as the experience of the NPHL in the detection of strains producing extended-spectrum β -lactamases.

β-lactam antibiotics act on the bacterial cell by binding to, and thus inactivating, enzymes which are involved in the construction of the cell wall. These enzymes are called penicillin-binding proteins or PBPs. Inhibition of cell wall construction ultimately leads to cell lysis and death. One way bacteria have overcome this challenge is through the production of β-lactamases, which cleave the amide bond in the β-lactam ring, rendering the antibiotic unable to bind PBPs.

The most common β-lactamases responsible for β-lactam resistance within species such as *Escherichia coli* and *Klebsiella pneumoniae* are two closely related enzymes called TEM-1 and SHV-1. These β-lactamases are plasmid mediated and therefore can be transferred from species to species by a process called conjugation. They confer resistance to β-lactam drugs such as ampicillin, piperacillin, ticarcillin, cephalothin, cefazolin and cefamandole. Infections with strains containing a TEM-1 or SHV-1 β-lactamase can be treated effectively with 3rd- and 4thgeneration cephs, cephamycins (cefoxitin and cefotetan) as well as aztreonam, penicillin/β-lactamase inhibitor combinations and the carbapenems.

Unfortunately, through selective pressure in the hospital environment, bacterial strains have been isolated which carry mutant TEM-1 or SHV-1 β-lactamases that are not only able to hydrolyze 1st-generation cephs but are also able to hydrolyze 3rd-generation cephs (see chart) as well as aztreonam. These mutant enzymes are called **extended-spectrum b -lactamases** or **ESBLs**. To date, there have been over 100 ESBLs described throughout the world. Initially, most ESBLs were found in *E. coli* and *K. pneumoniae,* but since the enzymes are encoded on transferable plasmids, they have also been found in other members of the *Enterobacteriaceae* such as *Serratia marcescens* and *K. oxytoca.* Strains harboring ESBLs remain susceptible *in vitro* to the cephamycins, penicillin/β-lactamase inhibitor combinations, carbapenems and cefepime. However, it is not clear at this time whether treatment with any β-lactam besides a carbapenem, and in most cases cefepime, is efficacious in a serious infection.

The TEM -1 and SHV- 1 β -lactamases and their derivatives (ESBLs) are distinct from the **cephalosporinases**, another group of β-lactamases that are encoded on the chromosomes of virtually all gramnegative organisms. These β-lactamases are encoded by a gene called *ampC* and hydrolyze cephalosporins more efficiently than penicillins. Even though an *ampC*-like gene is found in all species of the *Enterobacteriaceae,* certain species such as *E. coli* produce the cephalosporinase in such low amounts that it does not affect an isolate = s susceptibility to β-lactam antibiotics. Other species such as *Enterobacter cloacae* and *Citrobacter freundii* may be induced to generate cephalosporinase at high levels when certain cephs or penicillins (e.g. cefazolin or ampicillin) are present. 3rd-generation cephs do not induce the cephalosporinase and isolates in most

cases can initially be treated effectively with a 3rd -generation ceph. Unfortunately, all species of *Enterobacteriaceae* (including *E. coli*) may potentially develop mutations which are selected during therapy with third-generation cephs (called an ***ampC* mutant**). These mutant bacteria become resistant to all cephs (excluding cefepime), aztreonam, penicillins and penicillin/ β -lactamase inhibitor combinations. Cefepime and the carbapenems retain excellent activity against *ampC* mutants.[1,2]

The laboratory challenge: -detection of ESBLs

Failure to detect resistance to antimicrobial agents may have dire consequences for the patient. Therefore, clinical microbiology laboratories are constantly monitoring and improving their susceptibility testing methods so accurate information will be conveyed to the physician. Fortunately, *in vitro* susceptibility testing accurately reveals an *ampC* mutants**=** resistance to 2nd- and 3rdgeneration cephs and no additional testing is warranted. However, ESBLs are difficult to detect in the laboratory using standard *in vitro* assays and isolates that contain ESBLs may appear susceptible to 3rd-generation cephs or aztreonam. The difficulty arises from the fact that breakpoint panels from commercial automated susceptibility systems currently use an MIC breakpoint for ceftazidime, ceftriaxone, cefotaxime, and aztreonam that is 8 μg/ml. Some strains that express ESBLs have MICs to the 3rd-generation cephs and aztreonam as low as 2 μg/ml. Therefore, a patient may be initially treated with a 3rd-generation ceph based on laboratory results that suggest the organism is susceptible. Two different investigators, using genetically defined and well characterized ESBL isolates, recently found that using cefpodoxime at an MIC breakpoint of 2 μg/ml, accurately detected all strains expressing ESBLs. Commercial automated susceptibility systems already have, or will have in the near future, cefpodoxime on their panels/ cards to help identify strains that contain ESBLs.

Our laboratory is currently defining an ESBL producing isolate as any *Enterobacteriaceae* that is resistant or intermediate to either ceftazidime or ceftriaxone or cefotaxime or aztreonam, yet susceptible to cefoxitin and cefotetan. We confirm each case using a double-disk diffusion test[3], which tests the enzymes susceptibility to β-lactamase inhibitors such as clavulanate. In contrast, *ampC* mutants are resistant to both cefoxitin and cefotetan and are resistant to the inhibitory action of β-lactamase inhibitors. Our protocols for monitoring and testing for ESBLs are available to clinical laboratories. The NPHL is interested in monitoring the frequency of ESBLs throughout Nebraska and isolates may be referred for confirmatory testing or molecular typing. Contact Dr. Paul Fey for more information.

References

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β-lactam Antibiotics

Penicillins

Gram-positive Activity		*Gram-negative Activity*	
-penicillin	-methicillin	-ampicllin	-amoxicillin
-oxacillin	-cloxacillin	-piperacillin	-ticarcillin
-dicloxacillin	-nafcillin	-mezlocillin	-azlocillin
		-carbenicillin	

Penicillin β -lactamase inhibitor combinations

-ampicillin/sulbactam	-piperacillin/tazobactam
-amoxicillin/clavulanate	-ticarcillin/clavulanate

Cephalosporins/Cephamycins*

Generation Classification			
First	***Second***	***Third***	***Fourth***
-cephalothin	-cefamandole	-cefotaxime	-cefepime
-cefazolin	-cefuroxime	-ceftizoxime	
-cephapirin	-cefonicid	-ceftriaxone	
-cephalexin	-cefixime	-cefoperazone	
-cephradine	-cefpodoxime	-ceftazidime	
-cefadroxil	-cefprozil		
-cefaclor	-ceftibuten		
-ceforanide	-cefoxitin		
	-cefotetan		
	-cefmetazole		

Monobactams -aztreonam

Carbacephems -loracarbef

Carbapenems -imipenem
-meropenem

***Separation of first, second, third and fourth generation cephalosporins based on activity alone and not on date of introduction by the company.**