DIFFICULT TO TREAT GRAM NEGATIVE INFECTIONS

C T Deshmukh
Professor Pediatrics, Incharge PICU, Seth G.S. Medical College & K.E.M.Hospital, Mumbai.

Antimicrobial resistance impedes effective treatment of patients with serious infections and is of particular concern for ICU patients. Enterobacteriaceae are among the most prevalent human pathogens and compose 80% of Gram-negative bacteria and 50% of all isolates identified in most hospital laboratories. Pseudomonas aeruginosa and Acinetobacter baumannii are the most prevalent non-fermentative bacterial species isolated from clinical specimens of hospitalized patients. Furthermore, P. aeruginosa is the leading cause of nosocomial respiratory tract infections and is of particular concern for patients who require mechanical ventilation.

The increase in the prevalence of drug-resistant pathogens is occurring at a time when the discovery and development of new anti-infective agents is slowing down dramatically. And hence there is concern that in the near future, we may be faced with a growing number of untreatable infections. In the past decade, several antibiotic-resistant pathogens have been identified as causes of serious infections among patients in hospital. These organisms are typically resistant to multiple classes of antimicrobial agents and are therefore called multidrug-resistant organisms.

Mechanisms of antibiotic resistance – they include:
Enzymatic drug inactivation - β lactamase produced by S. aureus, H.influenzae and resistant to penicillins. Extended spectrum β lactamase produced by Enterobactertaceae and resistant to cephalosporins. Also there are Aminoglycoside inactivating – Enzymes produced by Enterobactertaceae and resistant to gentamicin and other aminoglycosides.

Alteration of the antibiotic target site - Altered penicillin binding proteins – seen in Streptococcus pneumoniae ( resistant to penicillins), Methicillin resistant S. aureus( resistant to methicillin, Cloxacillin). Also altered DNA gyrase or topoisomerase – seen in S. pneumonia, Enterobactertaceae, Pseudomonas aeruginosa (resistant to quinolones)

Prevention of antibiotic access to the target site - Change in outer membrane proteins or porins seen in, Enterobactertaceae and P. aeruginosa ( resistant to aminoglycosides). Also Efflux pump seen in streptococci and S. aureus( resistant to clindamycin, erythromycin, tetracycline)

The common reasons for spread of resistant organisms in hospitals include: New strain introduction (ESBL, MRSA), emergence of new resistant strains due to excessive antibiotic use and selection pressure (ESBL) and clonal dissemination by poor infection control practices (MRSA, VRE).

Multidrug-resistant gram-negative bacilli
We are better aware of resistance to gram positive organisms like Staphylococcus, S. pneumonia etc. However multi drug resistant gram negative bacilli are an even bigger problem facing hospitals particularly the intensive care units.

Multidrug-resistance in gram-negative bacilli is usually defined as resistance to more than two classes of antimicrobial agents. Usually multidrug-resistant gram-negative bacteria are resistant to penicillins, cephalosporins, fluoroquinolones, trimethoprim-sulfamethoxazole and aminoglycosides. Some strains may also be resistant to the carbapenems, and then there are hardly any further antibiotic options.

Three pathogens are particularly problematic: (1) Gram-negative bacilli with extended-spectrum β-lactamases, Carbapenem-resistant Klebsiella species and other Enterobacteriaceae, (2) Multidrug-resistant Pseudomonas aeruginosa including quinolone and carbapenem-resistant strains and (3) Multidrug-resistant Acinetobacter baumannii.

Infections caused by antimicrobial-resistant organisms are associated with increased mortality prolonged hospital stays and excess costs.

Rates of antibiotic-resistant organisms have increased in all hospitals over the past 10 years and it is a global problem. These rates will continue increase unless aggressive control measures are not implemented. The measures include increased surveillance of antibiotic resistance, strict attention to hand hygiene and other infection prevention and control measures and appropriate use of antibiotics.

**Extended-Spectrum β Lactamases**

The introduction of the third-generation cephalosporins into clinical practice in the early 1980s was heralded as a major breakthrough in the fight against β-lactamase-mediated bacterial resistance to antibiotics. These cephalosporins had been developed in response to the increased prevalence of β-lactamases in certain organisms (for example, ampicillin hydrolyzing TEM-1 and SHV-1 β-lactamases in Escherichia coli and Klebsiella pneumoniae).

The first report of plasmid-encoded β lactamases capable of hydrolyzing the extended-spectrum cephalosporins was published in 1983. Other β lactamases were soon discovered which were closely related to TEM-1 and TEM-2, but which had the ability to confer resistance to the extended-spectrum cephalosporins. Hence these new β lactamases were coined extended-spectrum β lactamases (ESBLs).

There is no consensus of the precise definition of ESBLs. A commonly used working definition is that the ESBLs are β lactamases capable of conferring bacterial resistance to the penicillins, first, second-, and third-generation cephalosporins, and aztreonam (but not the cephamycins or carbapenems) by hydrolysis of these antibiotics, and which are inhibited by β lactamase inhibitors such as clavulanic acid.

They are inhibited by clavulanic acid. This property differentiates the ESBLs from the AmpC-type β lactamases (group 1) produced by organisms such as Enterobacter cloacae which have third-
generation cephalosporins as their substrates but which are not inhibited by clavulanic acid. Selection of stably derepressed mutants which hyperproduce the AmpC-type β-lactamases has been associated with clinical failure when third-generation cephalosporins are used to treat serious infections with Enterobacter spp. In general, the fourth-generation cephalosporin, cefepime, is clinically useful against organisms producing AmpC-type β-lactamases, but may be less useful in treating ESBL producing organisms.

Additionally, the metalloenzymes (group 3) produced by organisms such as Stenotrophomonas maltophilia can hydrolyze third-generation cephalosporins (and carbapenems), but are inhibited by EDTA (a heavy metal chelator) but not clavulanic acid.

DIVERSITY OF ESBL TYPES

SHV
The SHV-type ESBLs may be more frequently found in clinical isolates than any other type of ESBLs. SHV refers to sulfhydryl variable. Within 15 years of the discovery of this enzyme, organisms harboring SHV-2 were found in every continent, suggesting that selection pressure from third-generation cephalosporins in the first decade of their use was responsible. SHV-type ESBLs have been detected in a wide range of Enterobacteriaceae and SHV-producing Pseudomonas aeruginosa and Acinetobacter spp. have also been reported.

TEM
The TEM-type ESBLs are derivatives of TEM-1 and TEM-2. TEM-1 was first reported in 1965 from an Escherichia coli isolate from a patient in Athens, Greece, named Temoneira (hence the designation TEM). TEM-1 is able to hydrolyze ampicillin at a greater rate than carbenicillin, oxacillin, or cephalothin, and has negligible activity against extended-spectrum cephalosporins. It is inhibited by clavulanic acid. Well over 100 TEM-type β-lactamases have been described, of which the majority are ESBLs. One such produced by Klebsiella pneumoniae was found to harbor a novel plasmid-mediated β-lactamase coined CTX-1. The enzyme was originally named CTX-1 because of its enhanced activity against cefotaxime. The enzyme, now termed TEM-3.

CTX-M and Toho-Lactamases
The CTX-M enzymes have been previously reviewed in detail. The name CTX reflects the potent hydrolytic activity of these β-lactamases against cefotaxime. Organisms producing CTX-M-type β-lactamases typically have cefotaxime MICs in the resistant range, while ceftazidime MICs are usually in the apparently susceptible range. Tazobactam exhibits an almost 10-fold greater inhibitory activity than clavulanic acid against CTX-M-type β-lactamases. It should be noted that the same organism may harbor both CTX-M-type and SHV-type ESBLs or CTX-M-type ESBLs and AmpC-type β-lactamases, which may alter the antibiotic resistance phenotype.

Toho-1 and Toho-2 are β-lactamases related structurally to CTX-M-type β-lactamases. (Toho refers to the Toho University School of Medicine Omori Hospital in Tokyo, where a child was hospitalized who was infected with Toho-1 β-lactamase producing Escherichia coli.) Like most CTX-M-type β-lactamases, the hydrolytic activity of the Toho-1 and Toho-2 enzymes is more potent against cefotaxime than ceftazidime.
The number of CTX-M-type ESBLs is rapidly expanding. They have now been detected in every populated continent. Given the widespread findings of CTX-M-type ESBLs in China and India, it could be speculated that CTX-M-type ESBLs are now actually the most frequent ESBL type worldwide.

**OXA**

The OXA-type β-lactamases are so named because of their oxacillin-hydrolyzing abilities. These β-lactamases (group 2d) are characterized by hydrolysis rates for cloxacillin and oxacillin greater than 50% that for benzylpenicillin. They predominantly occur in *Pseudomonas aeruginosa* but have been detected in many other gram-negative bacteria. In fact, the most common OXA-type β-lactamase, OXA-1 has been found in 1 to 10% of *Escherichia coli* isolates. Most OXA-type β-lactamases do not hydrolyze the extended-spectrum cephalosporins to a significant degree and are not regarded as ESBLs. However, OXA-10 hydrolyzes (weakly) cefotaxime, ceftiraxone, and aztreonam, giving most organisms reduced susceptibility to these antibiotics. Some OXA confer frank resistance to cefotaxime and sometimes ceftazidime and aztreonam. The simultaneous production of a carbapenem-hydrolyzing metalloenzyme and an aztreonam-hydrolyzing OXA enzyme can readily lead to resistance to all β-lactam antibiotics.

**PER**

The PER-type ESBLs share only around 25 to 27% homology with known TEM- and SHV-type ESBLs. PER-1 β-lactamase efficiently hydrolyzes penicillins and cephalosporins and is susceptible to clavulanic acid inhibition. PER-1 was first detected in *Pseudomonas aeruginosa*, and later in *Salmonella* and *Acinetobacter* isolates as well. Worryingly, a *Pseudomonas aeruginosa* strain producing both PER-1 and the carbapenemase VIM-2 has been detected. The coexistence of these enzymes renders an organism resistant to virtually all β-lactam antibiotics.

**VEB-1, BES-1, and Other ESBLs**

A variety of other β-lactamases which are plasmid-mediated or integron-associated class A enzymes have been recently discovered. They are not simple point mutant derivatives of any known β-lactamases. They are remarkable for their geographic diversity. Novel chromosomally encoded ESBLs have also been described.

VEB-1 confers high-level resistance to ceftazidime, cefotaxime and aztreonam, which is reversed by clavulanic acid. The gene encoding VEB-1 was found to be plasmid mediated; such plasmids also confer resistance to non-β-lactam antibiotics. The patient from whom the β-lactamase was originally described was a Vietnamese infant hospitalized in France.

Bacteria producing *Klebsiella pneumoniae* carbapenemases (KPCs) are rapidly emerging as a cause of multidrug-resistant infections worldwide. Bacterial isolates harbouring these enzymes are capable of hydrolysing a broad spectrum of β-lactams including the penicillins, cephalosporins, carbapenems and monobactam.

**Risk Factors for Colonization and Infection with ESBL Producers**

Patients at high risk for developing colonization or infection with ESBL-producing organisms are often seriously ill patients with prolonged hospital stays and in whom invasive medical devices...
are present (urinary catheters, endotracheal tubes, central venous lines) for a prolonged duration. Several studies have found a relationship between third-generation cephalosporin (particularly ceftazidime) use and acquisition of an ESBL-producing strain. In recent years there has been a wide variety of reports of true community-acquired infections with ESBL-producing organisms mainly in association with diarrhea and urinary tract infections (*Escherichia coli*).

**Recommended Methods for ESBL Detection**

**Screening for ESBL producers**

**Disk diffusion methods**

The disk diffusion methods are proposed for screening ESBL production by *klebsiellae*, *Escherichia coli*, and *Proteus mirabilis*. Laboratories using disk diffusion methods for antibiotic susceptibility testing can screen for ESBL production by noting specific zone diameters which indicate a high level of suspicion for ESBL production. Cefpodoxime, ceftazidime, aztreonam, cefotaxime, or ceftriaxone is used. However, the use of more than one of these agents for screening improves the sensitivity of detection. If any of the zone diameters indicate suspicion for ESBL production, phenotypic confirmatory tests should be used to ascertain the diagnosis.

**Phenotypic Confirmatory Tests for ESBL Production**

**Cephalosporin/clavulanate combination disks.**

The use of cefotaxime or ceftazidime disks with or without clavulanate for phenotypic confirmation of the presence of ESBLs is recommended in *klebsiellae* and *Escherichia coli*. A difference of about 5 mm between the zone diameters of either of the cephalosporin disks and their respective cephalosporin/clavulanate disk is taken to be phenotypic confirmation of ESBL production.

There are many other tests and commercially available kits for diagnosis but all should be as per recommended standard.

**Treatment and outcome of infections with ESBL-producing organisms**

Usually the ESBL producers are resistant to cephalosporins and most of the other drugs like aminoglycosides, quinolones etc. and hence these drugs cannot be used.

β Lactam/ β lactamase inhibitor combinations are usually active against organisms possessing a single ESBL. However many organisms now produce multiple ESBLs which may reduce the effectiveness of these agents. In vitro, the carbapenems (including imipenem, meropenem, and ertapenem) have the most consistent activity against ESBL-producing organisms, and are the drugs of choice particularly in serious infections. The mortality and hospital days stay is more in patients infected with ESBL producing organisms.

The emergence of multidrug-resistant (MDR) Gram-negative bacilli creates a challenge in the treatment of nosocomial infections. While the pharmaceutical pipeline is waning, two revived old antibacterials (colistin and fosfomycin), a newer one (tigecycline) and an 'improved' member of an existing class (doripenem) are the only therapeutic options left.
Pediatric Infectious Diseases Conference –
Clinicomicrobial Fusion 2010 (PIDC 2010), Mumbai, 24th October 2010

Treatment of infection caused by *Klebsiella pneumoniae* carbapenemases bacteria is particularly worrisome as the carbapenems are often agents of the last resort for resistant Gram-negative infections. The optimal treatment of infections caused by KPC bacteria is not well established and clinical outcome data remain sparse. Agents tried include tigecycline and aminoglycosides and polymyxins.

**Pseudomonas aeruginosa**

*Pseudomonas aeruginosa* is a leading Gram-negative pathogen that causes nosocomial infections. *P. aeruginosa* causes a wide range of infections, some life threatening, such as bacteremia and pneumonia. In many hospitals, *Pseudomonas aeruginosa* has become the most common gram-negative bacterial species associated with serious hospital-acquired infections, particularly within intensive care units. It may account for nearly 20% of all the nosocomial infections.

The rapid emergence of antibiotic-resistant strains of *P. aeruginosa* poses a significant challenge. In particular, the rate of fluoroquinolone resistance has tripled over the last decade due to widespread prescribing of the fluoroquinolones. Many such strains also show cross-resistance to other structurally unrelated antipseudomonal agents (aminoglycosides, cephalosporins, carbapenems and β-lactamase inhibitor combinations) severely limiting potential treatment options.

The hospital mortality associated with *P. aeruginosa* bloodstream infections is reported to be greater than 20% in most series and is highest among patients receiving inappropriate initial antimicrobial treatment.

The treatment of *Pseudomonas* is difficult. Empiric therapy before cultures is initiated with a combination of anti-pseudomonal agents like aminoglycosides, ceftazidime, Cefoperazone, quinolones and carbapenems. Initial choice would depend on the local sensitivity patterns. Appropriate changes should be made once the culture sensitivity becomes available.

**Acinetobacter baumannii**

*Acinetobacter* is a group of bacteria commonly found in soil and water. It can also be found on the skin of healthy people, especially healthcare personnel. While there are many species of *Acinetobacter* and all can cause human disease, *Acinetobacter baumannii* accounts for about 80% of reported infections.

*Acinetobacter baumannii* has emerged as an important nosocomial pathogen. Hospital outbreaks have been described from various geographic areas, and this organism has become endemic in some of them. *A. baumannii* does not have fastidious growth requirements and is able to grow at various temperatures and pH conditions. These properties explain the ability of *Acinetobacter* species to persist in either moist or dry conditions in the hospital environment, thereby contributing to transmission. This hardiness, combined with its intrinsic resistance to many antimicrobial agents, contributes to the organism’s fitness and enables it to spread in the hospital setting. The role of the environmental contamination in the transmission of nosocomial infections in general and in *A. baumannii* infections in particular is well recognized.
More than 20 years ago, researchers observed acquired resistance of *A. baumannii* to antimicrobial drugs commonly used at that time, among them aminopenicillins, ureidopenicillins, first- and second-generation cephalosporins, cephemycins, most aminoglycosides, chloramphenicol, and tetracyclines. Since then, strains of *A. baumannii* have also gained resistance to newly developed antimicrobial drugs. Although multidrug-resistant (MDR) *A. baumannii* is rarely found in community isolates, it became prevalent in many hospitals.

*Acinetobacter* can colonize and later infect patients particularly in the intensive care unit. They can cause bloodstream infection, Pneumonia (ventilator associated pneumonia) or skin infections.

There are various antibiotics used against *Acinetobacter* infection. As there is increasing resistance, local sensitivity patterns become important. Drugs which are often used are aminoglycosides, quinolones, third generation cephalosporins and carbapenams.

**Conclusion:** Gram-negative bacilli (GNB) are a common cause of sepsis, pneumonia, urinary tract infections, and postsurgical infections in patients in acute care hospitals. Antimicrobial resistance among GNB is increasing worldwide. A direct correlation has been shown between resistance of GNB and patient mortality, cost of patient care, and length of stay in the hospital. The problem of GNB resistance is of particular concern in the intensive care unit (ICU) setting. The most important determinant in the successful management of infections in patients in the ICU is prompt institution of effective empirical antimicrobial therapy; inappropriate empirical therapy affects both patient mortality rates and patient time spent in the ICU. Optimizing empirical therapy requires knowledge of likely antimicrobial resistance patterns.

**References:**

- Journal of Antimicrobial Chemotherapy Lee H. Nguyen1, Donald I. Hsu2, Vaidyanathan Ganapathy3, Kimberly Shriner4 and Annie Wong-Beringer3,4*Reducing empirical use of fluoroquinolones for Pseudomonas aeruginosa infections improves outcome *(2008)* 61, 714–720
- Chest, Marin H. Kollef, Time To Get Serious About Infection Prevention in the ICU, 2006;130:1293-1296