Original article:

Antibiotic susceptibility pattern of gram negative bacilli isolated in a super-specialty hospital-Are we gradually losing the battle against superbugs?

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Abstract:

Introduction: Multi-drug resistant Gram negative bacilli are increasingly being isolated from hospitals throughout the world. The aim of this study was to determine the antimicrobial susceptibility profile of Gram negative bacterial isolates obtained from various clinical samples of patients admitted in different ICUs and wards of a super-specialty hospital during 2014.

Materials and Methods: A cross-sectional study was conducted in a super-specialty hospital from January to December 2014. Various clinical specimens obtained from patients admitted in different wards and Intensive Care Units (ICUs) of this hospital were subjected to culture and sensitivity as per the requisition received from clinicians. Bacterial isolates (both Gram negative and positive respectively) obtained from these samples were identified as per standard guidelines. The antibiotic susceptibility profile of Gram negative bacterial isolates was recorded as per standard guidelines.

Results and Discussion: 81% of the bacterial isolates were Gram negative bacilli and only 19% were Gram positive cocci. Members of the family Enterobacteriaceae (*Escherichia coli, Klebsiella spp.* and *Proteus spp.* taken together) were the major Gram negative bacteria isolated during the study period followed by *Acinetobacter spp.* and *Pseudomonas aeruginosa*. The in-vitro susceptibility of isolates belonging to the family Enterobacteriaceae to β-lactam/β-lactamase inhibitor combinations, cephalosporins, carbapenems, fluoroquinolones, aminoglycosides, trimethoprim-sulfamethoxazole, tigecycline and nitrofurantoin was 18-47%, 32-48%, 23-51%, 35-56%, 33-46%, 31-40%, 61-71% and 56-71% respectively. While 70% of *Escherichia coli* and 75% of *Klebsiells spp*. isolates were susceptible to colistin, only 2% of *Proteus spp*. The susceptibility of *Acinetobacter baumannii* isolates to aminoglycosides, β-lactam/β-lactamase inhibitor combinations, carbapenems, cephalosporins, fluoroquinolones, trimethoprim-sulfamethoxazole, tigecycline and colistin was in the range of 27-30%, 12-15%, 31-39%, 15-21%, 23-38%, 10%, 68% and 39% respectively. The susceptibility of *P. aeruginosa* isolates to aminoglycosides, β-lactam/β-lactamase inhibitor combinations, carbapenems, cefepime, fluoroquinolones, colistin was in the range of 31-35%, 15-59%, 31-42%, 56%, 39-49% and 89% respectively.

Conclusion: Since the discovery of new antimicrobial agents has slowed down substantially over the last decade, we are left with limited therapeutic options. Antibiotic susceptibility pattern like the one presented in this study further adds to our woes and forces the entire medical community to think seriously about rational usage of these drugs.

Key words: Enterobacteriaceae, Acinetobacter baumannii, Pseudomonas aeruginosa, antibiotic susceptibility profile

Introduction:

The emergence of antimicrobial resistance has posed a major challenge for health care professionals worldwide. Continuous monitoring of antimicrobial resistance pattern in health care set-ups is the key to determine appropriate therapeutic options especially among critically ill patients. A considerable number of critically ill patients, in particular those staying in Intensive Care Units (ICUs), acquire different infections following hospitalization.[1-4] Several factors such as severity of underlying illness resulting in impaired defense mechanisms, length of hospital stay, usage of invasive devices and monitoring procedures and exposure to broad-spectrum antibiotics are associated with an increased risk of acquiring nosocomial infections. The frequent usage of broad-spectrum antibiotics results in selection of so-called 'super-bugs' which are mostly multi-drug resistant Gram negative bacilli. Colonization and subsequent serious infections with these microorganisms results in increased morbidity and mortality among hospitalized patients.[5-8] The aim of this study was to determine the antimicrobial susceptibility profile of Gram negative bacterial isolates obtained from various clinical samples of patients admitted in different ICUs and wards of a super-specialty hospital during 2014.

Materials & Methods:

A cross-sectional study was conducted in a super-specialty hospital from January to December 2014. Various clinical specimens which included urine, cerebrospinal, peritoneal, pleural, pericardial and drain fluids, pus, bile, blood, arterial and central venous catheters, sputum and mucus traps respectively obtained from patients admitted in different wards and Intensive Care Units (ICUs) of this hospital were subjected to culture and sensitivity as per the requisition received from clinicians. Bacterial isolates (both Gram negative and positive respectively) obtained from these samples were identified as per standard guidelines.[9] The antibiotic susceptibility profile of Gram negative bacterial isolates in the form of Minimum Inhibitory Concentration (MIC) was recorded using VITEK-2 (Bio Meriux Pvt. Ltd.) automated system as per Clinical & Laboratory Standards Institute (CLSI) guidelines 2014 and European Committee on Antimicrobial Susceptibility testing (EUCAST) guidelines 2014 (as applicable- see footnotes of Table 1). Susceptibility to additional antibiotics namely levofloxacin, norfloxacin, ofloxacin, netimicin, tobramycin & ticarcillin-clavulanate (as applicable for different Gram negative bacterial isolates as per CLSI guidelines 2014) was determined using modified Kirby-Bauer disk diffusion method.[10]

Results and Discussion:

A total of 12,223 clinical samples were received during the study period. Majority of the bacterial isolates (81%) obtained from these samples were Gram negative bacilli and only 19% were Gram positive cocci. Members of the family Enterobacteriaceae (*Escherichia coli, Klebsiella spp.* and *Proteus spp.* taken together) were the major Gram negative bacteria isolated during the study period followed by *Acinetobacter spp.* and *Pseudomonas aeruginosa.* Figure 1 shows the

percentage distribution of various bacterial isolates obtained during 2014. The percentage antibiotic susceptibility profile of Gram negative bacterial isolates obtained from different clinical samples during 2014 has been depicted in Table 1 and Figure 2 respectively.

Resistance rates are steeply rising among several Gram negative pathogens that are often responsible for serious nosocomial infections, including *Acinetobacter spp.*, *Pseudomonas aeruginosa*, and members of the family Enterobacteriaceae.[11] The presence of multi-drug resistant strains of these organisms has been associated with prolonged hospital stays, higher health care costs and increased morbidity and mortality.[11] Recognizing the growing problem of antibiotic resistance, as well as the decreasing investment being made in antimicrobial research and development, the Infectious Diseases Society of America created the Antimicrobial Availability Task Force in March 2003.[12] This task force identified six particularly problematic pathogens, including three Gram negative organisms: *Acinetobacter baumannii*, extended spectrum β-lactamase (ESBL)-producing members of the family Enterobacteriaceae and *Pseudomonas aeruginosa*. The other problematic organisms were the Gram-positive pathogens paricularly, methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Enterococcus faecium* and the filamentuous fungi *Aspergillus spp*.[12]

The first-line antibiotics traditionally used for treating serious infections caused by members of the family Enterobacteriaceae include penicillins, cephalosporins, monobactams, carbapenems, fluorquinolones and aminoglycosides. The frequency of resistance to these first-line agents is increasing and now reach high proportions in many areas of the world.[13-16] In the present study, the in-vitro susceptibility of isolates belonging to the family Enterobacteriaceae (namely *E.coli*, *Klebsiella spp.*, *Proteus spp.*) to β-lactam/β-lactamase inhibitor combinations, cephalosporins, carbapenems, fluoroquinolones, aminoglycosides, trimethoprim-sulfamethoxazole, tigecycline and nitrofurantoin was 18-47%, 32-48%, 23-51%, 35-56%, 33-46%, 31-40%, 61-71% and 56-71% respectively. While 70% of *Escherichia coli* and 75% of *Klebsiells spp*. isolates were susceptible to colistin, only 2% of *Proteus spp*. were susceptible to this antibiotic in-vitro. There are several underlying mechanisms of resistance to different antibiotic groups, which, although not looked into in the present study, may possibly explain our findings.

The most common mechanism of resistance to β-lactam antibiotics among members of the family Enterobacteriaceae is the production of the enzymes β-lactamases (both intrinsic or chromosomal mediated and acquired or plasmid mediated).[17] Plasmid-mediated AmpCs are increasingly found as a cause of cephalosporin resistance among members of this family in many areas of the world, although their frequency is heterogeneous according to the geographical area. Other mechanisms of resistance to β-lactams include porin loss, efflux pumps, and modified targets (penicillin-binding proteins [PBPs]). When combined with β-lactamases, some of these mechanisms may also confer resistance to carbapenems.[18] Also, it is worth noting that many non-β-lactam agents such as quinolones, aminoglycosides and trimethoprim sulfamethoxazole are often found to be useless against ESBL producing organisms because the plasmids carrying the ESBL gene also harbour genes encoding resistance to these drugs.[18] Resistance to quinolones most commonly results from the accumulation of chromosomal mutations in DNA gyrase (GyrA) then in

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topoisomerase IV (ParC). Also, decreased membrane permeability or an over expression of efflux pump systems cause lower intracellular concentration of the drug, which is associated with decreased susceptibility. Several plasmid-mediated mechanisms have also been enumerated, which include the Qnr proteins (which act by protecting the antibiotic target), the modified aminoglycoside acetyltransferase AAC(6')-Ib-cr and the efflux pump QepA. The association of different mechanisms of quinolone resistance (both plasmid and chromosomal mediated) at the same isolate is common.[18] Resistance to aminoglycosides may be due to several mechanisms like enzymatic modification (which is the most prevalent mechanism), inactivation, alteration of diffusion through the outer membrane due to porin loss, mutations in the target of the antimicrobial by methylation of ribosomal RNA. There are three main types of aminoglycoside modifying enzymes (AMEs) namely, acetyltransferases (AAC), phosphortransferases (APH) and nucleotidyltransferases (ANT) which modify this class of antibiotics thereby, preventing it's attachment to bacterial ribosomes. Recently, a novel mechanism causing high-level resistance to all aminoglycosides mediated by a 16S rRNA methylase, which causes methylation of the aminoglycoside binding site has been described.[18] Development of resistance to carbapenems during therapy due to porin loss has been repeatedly described in depressed chromosomal and plasmid-mediated AmpC producers.[18] Colistin is a bactericidal antibiotic with concentration-dependent activity. It has been mainly used in the treatment of invasive infections caused by multidrug-resistant (particularly carbapenem-resistant) Gram-negative bacteria. Colistin is active only against Gram-negative aerobic bacilli, including most Enterobacteriaceae, nonfermentative bacilli (e.g. *Acinetobacter spp., P. aeruginosa*, and *Stenotrophomonas maltophilia*) and other Gram negative bacilli like *Haemophilus influenzae*. Among the Enterobacteriaceae, *Proteus spp., Providencia spp., Serratia spp.* and *Edwardsiella spp.* are all resistant to colistin.[18] Tigecycline has also shown good in vitro activity against AmpC-hyperproducing Enterobacteriaceae, but clinical experience is limited.[18] Nitrofurantoin is an agent approved for use in uncomplicated urinary infections, and is active against many ESBL producers.[18]

Most of the *Acinetobacter baumannii* isolates obtained during the study period were resistant to all major groups of antibiotics with in-vitro susceptibility to aminoglycosides, β-lactam/β-lactamase inhibitor combinations, carbapenems, cephalosporins, fluoroquinolones, trimethoprim-sulfamethoxazole, tigecycline and colistin lying in the range of 27-30%, 12-15%, 31-39%, 15-21%, 23-38%, 10%, 68% and 39% respectively. *Acinetobacter calcoaceticus-baumannii complex* is emerging as a multiresistant nosocomial and community-acquired pathogen.[12] Multi-drug resistant strains of *Acinetobacter spp.* are being isolated with increasing frequency in many nosocomial infections. These pathogens have rapidly developed resistance to currently available antimicrobials via a wide range of mechanisms, including production of aminoglycoside-modifying enzymes, ESBLs and carbapenemases, as well as through changes in outer membrane proteins, penicillin binding proteins and topoisomerases.[19,20] Strains of *Acinetobacter spp.* that are resistant to all aminoglycosides, cephalosporins and fluoroquinolones are commonly seen in many areas.[21]

In the present study, while the in-vitro susceptibility of *P. aeruginosa* isolates to aminoglycosides was in

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the range of 31-35%, it was 15-59% for β-lactam/β-lactamase inhibitor combinations, 31-42% for carbapenems, 56% for cefepime, 39-49% for fluoroquinolones and 89% for colistin respectively. *Pseudomonas aeruginosa* is an invasive Gram-negative bacterial pathogen which causes a wide range of severe nosocomial infections, including pneumonia, urinary tract infections and bacteremia.[12] This organism is intrinsically susceptible to only a limited number of antibacterial agents because of the low permeability of its cell wall.[22] In addition to its intrinsic resistance, *P. aeruginosa* has also acquired resistance via multiple mechanisms, including production of β-lactamases and carbapenemases, up regulation of multidrug efflux pumps and finally cell wall mutations leading to a reduction in porin channels. Many small antibiotics, including β-lactams and quinolones, require these aqueous porin channels in order to enter *P. aeruginosa*. In addition, mutation of genes encoding antibacterial targets such as DNA gyrase for fluoroquinolones contributes to resistance in *P. aeruginosa.*[22]

Conclusion:

Considering the results obtained in our study, it appears that multi-drug resistant Gram negative bacterial isolates are ubiquitously distributed in our hospital. This calls for formulation of a policy for rational drug administration. Most nosocomial infections with multi-drug resistant bacteria can be prevented and controlled by following some basic procedures like hand-washing, timely use of appropriate antibiotics, cessation of antibiotic therapy as indicated, timely change or removal of indwelling 'lines' etc. The clinical specimens should be subjected to bacterial culture and antibiotic susceptibility testing prior to initiating antibiotic therapy to determine the appropriate drug. Since the discovery of new antimicrobial agents has slowed down substantially over the last couple of years, we are left with limited therapeutic options. Antibiotic susceptibility pattern like the one presented in this study further adds to our woes and forces the entire medical community to ponder over one raging question time and again: Are we gradually losing the battle against the so called 'superbugs'?

Figure 1: Percentage distribution of bacterial isolates obtained from clinical samples from January to December 2014

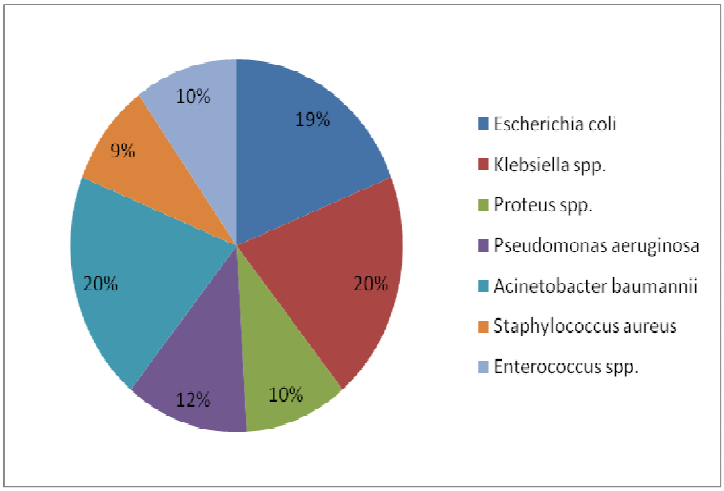
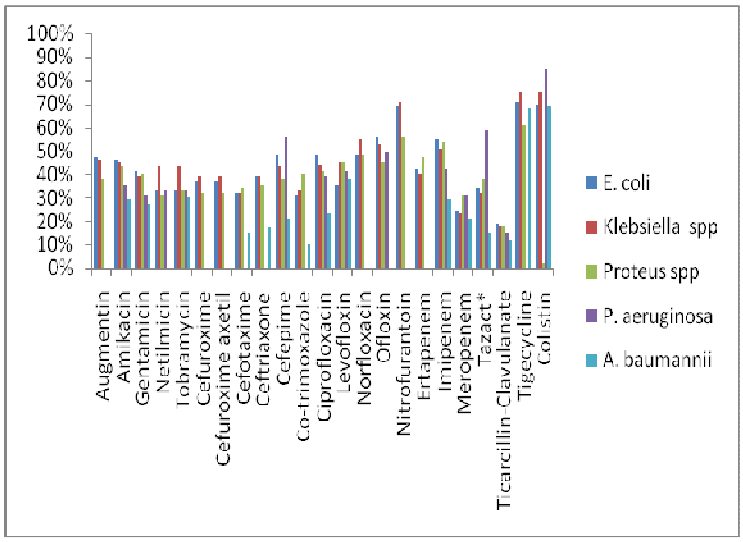


Figure 2: Percentage antibiotic susceptibility profile of Gram negative bacterial isolates obtained from different clinical samples during 2014



*Tazact: Piperacillin-tazobactam

Table 1: Table showing percentage antibiotic susceptibility profile of different Gram negative bacterial isolates obtained from different clinical samples during 2014

*****Susceptibility of *Acinetobacter baumanni* to netilmicin could not be recorded as only MIC and not zone diameter of netilmicin has been defined for *Acinetobacter baumannii* as per CLSI guidelines 2014. VITEK-2 automated system does not calculate MIC of netilmicin for *Acinetobacter baumannii.* ****** Amoxicillin-clavulanate is not recommended for use against *Pseudomonas aeruginosa* and *Acinetoacter spp.* respectively as per CLSI guidelines 2014. ******* Ertapenem is not recommended for use against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* respectively as per CLSI guidelines 2014. **#** Cefotaxime, ceftriaxone, cefuroxime axetil and cefuroxime are not recommended for use against *Pseudomonas aeruginosa* as per CLSI guidelines 2014. **##** Norfloxacin is not recommended for use against *Acinetobacter baumannii* as per CLSI guidelines 2014; Ofloxacin was used instead of norfloxacin in case of urinary *Pseudomonas aeruginosa* isolates as per CLSI guidelines 2014. **###** Ofloxacin is not recommended for use against *Acinetobacter baumannii* as per CLSI guidelines 2014. **@** Trimethoprim-sulfamethoxazole is not recommended for use against *Pseudomonas aeruginosa* as per CLSI guidelines 2014. **@@** MIC for tigecycline was recorded as per European Committee on Antimicrobial Susceptibility testing (EUCAST) guidelines 2014. As per both CLSI and EUCAST guidelines 2014, tigecycline is not recommended for use against *Pseudomonas aeruginosa.* **@@@** MIC for colistin was recorded for Pseudomonas aeruginosa and *Acinetobacter baumannii* as per CLSI guidelines 2014. However, for members of the family Enterobacteriaceae, MIC for this antibiotic was recorded as per EUCAST guidelines 2014. **$** As per CLSI guidelines 2014, nitrofurantoin is not recommended for use against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* respectively.

Antibiotic Group	*E. coli*	*Klebsiella spp*	*Proteus spp*	*P. aeruginosa*	*A. baumannii*
Aminoglycosides					
Amikacin	46%	45%	43%	35%	29%
Gentamicin	41%	39%	40%	31%	27%
Netilmicin*	33%	43%	31%	33%	Not Applicable
Tobramycin	33%	43%	33%	33%	30%
β-Lactam/β-Lactamase Inhibitor Combinations					
Amoxicillin-cavulanate**	47%	46%	38%	Not Applicable	Not Applicable
Piperacillin-tazobactam	34%	32%	38%	59%	15%
Ticarcillin-clavulanate	19%	18%	18%	15%	12%
Carbapenems					
Ertapenem***	42%	40%	47%	Not Applicable	Not Applicable
Imipenem	55%	51%	54%	42%	29%
Meropenem	24%	23%	31%	31%	21%
Cephalosporins					
Cefepime	48%	43%	38%	56%	21%
Cefotaxime#	32%	32%	34%	Not Applicable	15%
Ceftriaxone#	39%	39%	35%	Not Applicable	17%
Cefuroxime axetil#	37%	39%	32%	Not Applicable	Not Applicable
Cefuroxime#	37%	39%	32%	Not Applicable	Not Applicable
Fluoroquinolones					
Ciprofloxacin	48%	44%	41%	39%	23%
Levofloxin	35%	45%	45%	41%	38%
Norfloxacin##	48%	55%	48%	Not Applicable	Not Applicable
Ofloxin###	56%	53%	45%	49%	Not Applicable
Folate Pathway Inhibitors					
Trimethoprim-sulfamethoxazole@	31%	33%	40%	Not Applicable	10%
Glycylcyclines					
Tigecycline@@	71%	75%	61%	Not Applicable	68%
Lipopeptides					
Colistin@@@	70%	75%	2%	85%	69%
Nitrofurans					
Nitrofurantoin$	69%	71%	56%	Not Applicable	Not Applicable

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