Original Article

Emergence Of Polymyxin B As A Viable Treatment Option In Comparision To Newer Antimicrobials In Intensive Care Units: A Study In North India

Meher Rizvi, Naushaba Siddiqui *, Fatima Khan, Asfia Sultan, Parvez A. Khan Indu Shukla, Haris M.Khan

Department of Microbiology, Jawahar Lal Nehru Medical College, Aligarh, India

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ABSTRACT

Background: Multidrug resistance (MDR) among gram-negative bacilli has increased substantially limiting the choice of antimicrobials. This study was conducted with the objective to determine the efficacy of tigecycline , polymyxin B, newer floroquinolones and newer carbapenems against MDR gram negative isolates.

Methods: 90 clinical samples were obtained from ICU patients. On the basis of antibiotic susceptibility to first line antibiotics isolates were divided into 3 groups- a) sensitive to all the first line drugs, b) sensitive only to injectable and c) resistant to all antibiotics except imipenem. These groups were then tested against enoxacin (10 µg), gemifloxacin (5µg), moxifloxacin (5µg), prulifloxacin (5µg), ertapenem (10µg), faropenem (5µg), tigecycline (15µg) and polymyxin B (300 units). Isolates were screened for ESBL, AmpC, CRE and MBL.

Results: All the isolates in group 1 were uniformly sensitive to all the new antimicrobials tested. In group 2 susceptibility profile was as follows- 100% sensitive to polymyxin B, 16.6% to tigecycline, 10% to enoxacin, 3.3% to gemifloxacin, moxifloxacin, prulifloxacin, ertapenem and faropenem. In group 3, 81.5% of the isolates were sensitive to polymyxin B, 13.2% to tigecycline, 3.3% each to gemifloxacin and ertapenem.. Isolates of the three groups were uniformly sensitive to imipenem(100%). 2(6.67%) of the isolates were ESBL producers and 30 (33.3%) were AmpC producers. No CRE and MBL were detected.

Conclusion: Polymyxin B emerged as most effective antimicrobial in group 2 and group 3 with 100% and 81.5% sensitivity respectively. Use of polymyxin B will prevent injudicious use of imipenem and will decrease escalation of MBLs in our facility.

***Corresponding author:**
Dr. Naushaba Siddiqui, Department of Microbiology, J.N Medical College, AMU, Aligarh (UP) INDIA
Phone: + 919897520952
E-mail: naushsid@gmail.com

Introduction

The prevalence of multidrug resistance (MDR) among gram-negative bacilli has increased substantially over the years.[1]Infections by these MDR gram-negative bacilli often lead to prolonged hospitalization, increased mortality, and greater costs of treatment.[2] The emergence and spread of these pathogens in health care settings, has lead to an acute shortage of effective antibiotics which can be effectively used in initial empiric therapy.[3]The emergence of extended spectrum β lactamases (ESBL) and AmpC production by Gram negative bacteria further limits the choice of antimicrobials.[4]Ultimately carbapenems are used as the drugs of last resort in the treatment of life threatening infections.

Unfortunately in recent years carbapenem resistance is increasingly being reported in Gram negative bacteria.[5] Given the alarming state of drug resistance, clearly there is an urgent need for newer antimicrobial agents with novel mechanisms of action to reduce the burden on carbapenems and thus in the process decrease the emergence of carbapenemases.

This study was conducted with the objective to determine the in-vitro activity of tigecycline , polymyxin B, newer floroquinolones and newer carbapenems against MDR gram negative isolates.

Materials and Methods

Study group: The study was conducted in the Department of Microbiology on patients admitted to the Intensive Care Unit, Jawaharlal Nehru Medical College and Hospital. The study group comprised of 90 clinical samples from ICU patients obtained from the following sources: surgical site infections [SSI] (70) ,drains (10), urine (5), tracheal aspirate (3), sputum (1) and Foley's catheter tip (1). Rigorous precautions were taken during sample collection. Clinical significance of the bacteria from tracheal aspirate was assessed as per Shin et al.[6] Briefly, sample was rejected if >10 epithelial cells were seen under low power in a direct smear of agram stained slide. Uncentrifuged urine samples were screened for significant pyuria by direct wet mount. Semiquantitative cultures were put up by using filter paper method.[7] Collection and transport was done as per standard protocol.[7]

Processing of sample*:* Culture was performed on 5% sheep blood agar, Mac Conkey agar and BHI broth. Identification was done as per standard guidelines.[8]

Anti-microbial susceptibility testing*:* Antibiotic susceptibility testing was performed on Mueller Hinton agar by Kirby Bauer disc diffusion technique as per the CLSI guidelines.[8] Bacterial isolates were tested first against routinely used antibiotics: gentamicin (10µg), amikacin(30µg), amoxicillin (20µg), ceftriaxone (30µg), cefotaxime (30µg), cefoperazone+ sulbactum (75/75µg), cefixime (15µg), cefoperazone (75µg), cefepime (30µg), ofloxacin (5µg) piperacillin (100µg), piperacillin tazobactum (100/10µg), tobramycin (10µg) and imipenem (10µg).Antimicrobial susceptibility controls used was Escherichia coli ATCC 25922 and Pseudomonas aeruginosa 27853.

Using this set of antibiotics, ESBLs , Amp Cs and CRE's were detected as follows:

Detection of extended spectrum beta lactamases : Screening of possible ESBL production was done by using ceftriaxone (30µg) and cefoperazone (75µg). Those isolates with zone diameters less than 25mm for ceftriaxone and less than 22mm for cefoperazone were subsequently confirmed for ESBL production. Confirmation was done by noting the potentiation of the activity of cefoperazone in the presence of cefoperazone sulbactum.[8]

Detection of inducible and derepressed *AmpC* beta lactamase: Detection of AmpC betalactamase was done on isolates resistant to ceftriaxone (30µg), cefixime (15µg), cefoperazone (75µg) and cefoperazone sulbactum (75/75µg).Induction of AmpC synthesis was based on the disc approximation assay using imipenem as inducer.[8]

Detection of CRE (Carbapenem resistant Enterobacteriaceae): Isolates demonstrating zone sizes of less than16 mm around imipenem were identified as CRE.

Detection of Metallo-beta-lactamases: MBL were detected by modified Hodge test and Double Disc synergy test using EDTA.[9]

Study groups: On the basis of susceptibility profile to first line drugs and drug resistance markers, the isolates were divided into 3 groups:

Group 1- 30 bacterial isolates susceptible to all the routinely used/ tested antibiotics.

Group 2- 30 bacterial isolates resistant to all the routinely tested antibiotics except to injectable drugs (amikacin, gentamycin, cefoperazone+sulbactum, piperacillin+tazobactum, tobramycin). This group contained ESBL producers.

Group 3- 30 bacterial isolates resistant to all drugs except imipenem. This group consisted of AmpC producers.

Susceptibility to newer antimicrobials: These group were were further tested against enoxacin(10 µg), gemifloxacin(5µg), moxifloxacin(5µg), prulifloxacin(5µg), ertapenem(10 µg), faropenem (5µg) tigecycline (15µg)

and polymyxin B (300 units). All discs were obtained from HiMedia, India.

Result

The organisms isolated were *Escherichia coli* (n=45), *Klebsiella pneumoniae*(n=20), *Citrobacter species* (n=15), *Serratia species* (n=5), *Acinetobacter species* (n=4) and *Proteus mirabilis* (n=1).. Amongst them 2 (6.67%) of the isolates were ESBL producers and 30(33.3%) were AmpC producers. Table 1 shows the susceptibility pattern to injectable antibiotics in group 2. All the isolates were susceptible to amikacin (100%), 3(10%) to gentamicin, 2(6.67%) each to cefoperazone/sulbactum, tobramycin and piperacillin/tazobactum. No MBL and CRE were detected. Table 2 shows comapres susceptibility of the three groups to newer antimicrobials. All the isolates in group 1 were uniformly sensitive to all the routine and the newer antimicrobials tested. In group 2 which also contained ESBLs, susceptibility profile was as follows- 100% sensitivity was observed to polymyxin B, 16.6% to tigecycline, 10% to enoxacin, 3.3% to gemifloxacin, moxifloxacin, prulifloxacin , ertapenem and faropenem. In group 3, 81.5% of the isolates were sensitive to polymyxin B, 13.2% to tigecycline, 3.3% each to gemifloxacin and ertapenem. All the isolates were resistant to moxifloxacin, prulifloxacin, enoxacin and faropenem. Group 3 isolates showed high level of resistance to both aminoglycosides and floroquinolones. Isolates of the three groups were uniformly sensitive to imipenem (100%). Figure 1 shows trend of antimicrobial sensitivity in different groups. Barring imipenem, only polymyxin B followed by tigecycline demonstrated encouraging results. Both polymyxin B and tigecycline worked better in group 2 than in group 3.

Table 1-Susceptibility profile of pathogens to Injectable Antibiotics in Group 2

Antibiotics	NO. of isolates (n=30)
Amikacin,	30(100%)
Gentamicin	3(10%)
Cefoperazone+sulbactum	2(6.67%)
Piperacillin+tazobactum	2(6.67%)
Tobramycin	2(6.67%)

*Maximum isolates were sensitive to Amikacin (100%)

Table 2- Comparision of Susceptibility of the Three Groups to Newer Antimicrobials

Antibiotics	Group 1(n=30)	Group 2(n=30)	Group 3(n=30)
Enoxacin	30(100%)	3(10%)	0(0%)
Gemifloxacin	30(100%)	1(3.3%)	1(3.3%)
Moxifloxacin	30(100%)	1(3.3%)	0(0%)
Prulifloxacin	30(100%)	1(3.3%)	0(0%)
Ertapenem	30(100%)	1(3.3%)	1(3.3%)
Faropenem	30(100%)	1(3.3%)	0(0%)
Tigecycline	30(100%)	5(16.6%)	4(13.2%)
Polymyxin B	30(100%)	30(100%)	24(81.5%)

* Tigecycline and Polymyxin B showed better results in group 2 as compared to group 3

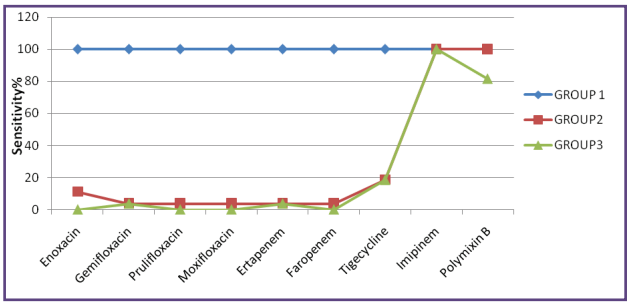

Fig. 1: Trend of antimicrobial sensitivity in different groups

Discussion

Increasing bacterial resistance to the commonly used antimicrobial agents is increasing and is a matter of grave public health concern, particularly in patients with serious and complicated nosocomial infections. The emergence of ESBL and AmpCs, not to mention the MBLs has led to severely limited therapeutic options, resulting in increased morbidity and mortality.

In this study, prevalence of ESBL was 6.67% while AmpC was much higher at 33.3% . In other studies, AmpC levels were usually lower than ESBLs.[10,11,12] The elevated levels of AmpC is alarming as the usage of imipenem increases accordingly.

Polymyxin B emerged as the most effective antimicrobial in group 2 and group 3 with 100% and 81.5% sensitivity respectively. The result was similar to the study done by Castanheira who reported 88.1% of CRE isolates were susceptible to Polymyxin B.[13]There has been resurgence in the use of polymyxins as the drugs of last resort for the treatment of infections caused by MDR gram negative pathogens which are resistant to all other currently available antibiotics. Polymyxin B,a polypeptide cationic antibiotic is active against a variety of gram negative bacilli, including most clinically relavent enterobacteriaceae. It is rapidly acting bacteriocidal agent with dose adjustments required for patients with renal impairment, including decreasing daily dose and extending administration intervals.[14]

In our study, only 16.6% of the bacterial isolates in group 2 and 13.2% of the isolates in group 3 showed susceptibility

to tigecycline but it had a better susceptibility profile than other newer antimicrobials tested including ertapenem and faropenem. Other studies however have reported good activity.[15,16,17]Tigecycline, a newer semi-synthetic glycylcycline derived from minocycline is a promising molecule in the treatment of infections caused by MDR organisms.It is a bacteriostatic agent and has potent invitro activity against several bacteria including ESBL producing Enterobacteriaceae and carbapenem resistant *Acinetobacter* spp. Furthermore, it is unaffected by the known mechanisms of resistance to tetracycline and minocycline such as efflux pumps and ribosomal protective mechanisms.

Although ertapenem is approved for complicated intra-abdominal infections, complicated skin and skin structure infections, community acquired pneumonia, complicated urinary tract infections including pyelnephritis due to susceptible pathogens, and acute pelvic infections,we observed an unexpectedly low sensitivity of 3.7% for ertapenem in our study . This is in sharp contrast to other studies which reported that ertapenem was strongly active against ESBL and AmpC producing gram negative bacteria.[18,19] As there is a need for new oral options for treatment of multidrug resistant gram negative bacteria, we also evaluated the in vitro activity of faropenem, an oral penem. But again resistance ranging from 96.3% to 100% was observed. Other studies have shown better activity of faropenem against MDR bacteria.[20,21]

The newer fluoroquinolones like enoxacin, prulifloxacin, gemifloxacin and moxifloxacin have broad-spectrum bactericidal activity, excellent oral bioavailability, good tissue penetration and favorable safety and tolerability profiles. This is the first study which evaluated the role of newer fluoroquinolones moxifloxacin, prulifloxacin, gemifloxacin and enoxacin in MDR gram negative bacteria from India. However poor results were elicited with low sensitivity (0%-11.1%). Enoxacin was active against 3.7% isolates in group 2 patients. In group 3, the picture was even more dismal.

Conclusion

After assessing 8 drugs of four antimicrobial groups, we recommend Polymyxin B as empiric treatment in seriously ill patients.

Funding

None

Competing Interests

None declared

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