Original Article:
Pan-resistance among gram-negative clinical isolates at a tertiary care hospital in south India

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ABSTRACT

Background: The emergence of multi and pan resistance among Gram negative bacteria in the last decade has forced the medical community in using infrequently used antimicrobials in treating these infections.

Methods: The present study was designed to look into the activity of certain older antimicrobial agents against Gram-negative clinical isolates resistant to all common antibiotics including carbapenams. Members of enterobacteriaceae family, *Acinetobacter* species and *Pseudomonas aeruginosa* isolated and identified in our laboratory during 2011 were included in the study. The antimicrobial susceptibility testing was done as per Clincial and Laboratory Standards Institute (CLSI) guidelines by disc diffusion technique.

Results: From January-December 2011, out of a total of 11,658 samples processed, 157 (1.3%) isolates of Gram-negative bacilli were resistant to all beta-lactams, carbapenem, fluroquinolones and aminoglycosides. E.coli was the predominant isolate (n=50; 31.8%) followed by *Klebsiella* (n=37, 23.6%); 28 (17.8%) isolates were acinetobacter species. *P. aeruginosa* constituted 17 separate isolates other than the above 157 isolates. Of the unconventional agents tested, polymyxin B was the most effective agent with 33.1% strains sensitive to it and another 5/17 (29.4%) of *P. aeruginosa* isolates. Other agents in the decreasing order of sensitivity were chloramphenicol (25.5%), tetracycline and nitrofurantoin (14%) each, and cotrimaxazole (5.7%).

Conclusions: Our study has highlighted the importance of including certain not-so-common antimicrobials in the sensitivity panel, particularly while testing multidrug-resistant isolates since they still possess some degree of activity against such isolates and may prove useful in clinical setting.

Key Words: *Pan - antibiotic resistance, Gram negative bacteria, India*

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INTRODUCTION

Gram-negative bacteria possess resistance mechanisms affecting various classes of antibiotics which has emerged as a global problem. Emergence of such broad spectrum resistance affecting β-lactams and carbapenems, flouroquinolones and aminoglycosides which are the major classes of antimicrobials for treating serious infections and the recent surfacing of New Delhi metallo-beta-lactamase-1 (NDM-1) strains[1] has prompted the apprehension of a possible post-antibiotic era in a not too distant future. The isolation of these Gram-negative bacteria in the laboratory, which are resistant to almost all the antimicrobials in the armamentarium has renewed the interest in the usage of some infrequently, rarely used agents in the treatment of infections caused by these organisms. The aim of the present study was to document the prevalence of pan- resistant Gram-negative bacilli in a tertiary care hospital setting and their sensitivity profile.

MATERIAL AND METHODS

This study included all the Gram-negative isolates obtained from various clinical samples processed at the Department of Microbiology during the period January to December, 2011.The strains were identified by conventional methods.[2] Antimicrobial susceptibility testing was performed by Kirby Bauer's disc diffusion technique on Muller Hinton agar as per Clinical and Laboratory Standards Institute (CLSI) guidelines.[3] The following antimicrobial agents were used for drug-sensitivity testing, as per departmental policy.

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Enterobacteriaceae and Acinetobacter group

First Line

Ampicillin (10 µg), amoxycillin-clavulunate (20/10 µg), cefotaxime (30 µg), cefoperazone-sulbactum (75/10 µg), imipenem (10 µg), ciprofloxacin (5 µg), amikacin (30 µg), gentamicin (10 µg), piperacillin-tazobactum (100/10 µg) and cotrimoxazole (25 µg).

Second line

Cefepime (30 µg), cefoxitin (30 µg), ceftazidime (30 µg), chloramphenicol (30 µg), tetracycline (30 µg), netilmicin (30 µg), meropenem (10 µg), and polymyxin B (300 U).

Pseudomonas species

First line

Ampicillin, amoxycillin-clavulunate and cotrimoxazole were replaced with ceftazidime (30 µg), netilmicin (30 µg), and polymyxin B (300 U).

Second line

Aztreonam (30 µg), carbenicillin (100 µg), cefepime (30 µg), meropenem (10 µg), and tobramycin (10 µg).

The media and antibiotic discs were procured from Hi media (India). *E.coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 strains were used as sensitive controls, depending on the nature of isolate. For Polymyxin B, no CLSI guidelines are available for interpretation of disc diffusion technique as regards to enterobacteriaceae and acinetobacter. For these isolates, the recommendation of Galani et al[4] was adopted.

RESULTS

During the year 2011, a total of 11,658 samples such as urine samples (n=9023), sputum samples (n=1835), and catheter tips (n=800) apart from other samples were processed. Of these 11,658 samples, 157(1.34%) isolates of Gram-negative bacilli other than *Pseudomonas* species were obtained which were resistant to all the three major classes of antimicrobials tested, i.e. β-lactams including cephalosporins and carbapenems, aminoglycosides, and fluoroquinolones. The breakup of the isolates is shown in Table 1. *E.coli* was the predominant isolate, primarily from urine. *Klebsiella* species and *Enterobacter* species were isolated in maximum number from the catheter tips. The sensitivity pattern of the 157 isolates to the unconventional/rarely used antimicrobials is shown in Table 2. Of the urinary isolates, about

Table 1: Source of pan-resistant gram negative bacilli

Sample	*Enterobacteriaceae*					*Acineto-*	
	E.coli	*Klebsiella*	*Citrobacter*	*Enterobacter*	*Proteus*	*bacter*	Total
Urine (n=9023)	42	11	14	6	8	16	97
Sputum (n=1835)	1	9	1	3	Nil	5	19
Catheter tips (n=800)	7	17	3	7	Nil	7	41
Total	50	37	18	16	8	28	157

Table 2: Sensitivity pattern observed in 157 pan-resistant isolates

Antibacterial agents	Urinary isolates (n=97) No.(%)	Catheter tip isolates (n=41) No.(%)	Sputum isolates (n=19) No.(%)	Total no. sensitive No.(%)
Chloramphenicol	28 (28.8)	04 (9.7)	08 (42.1)	40 (25.5)
Co-trimoxazole	07 (7.2)	01(2.4)	01(5.3)	09 (5.7)
Nitrofurantoin	22 (22.7)	Not tested	Not tested	22
Polymyxin B	31(31.9)	14 (34.1)	07 (36.8)	52 (33.1)
Tetracycline	16 (16.5)	03 (7.3)	03 (15.8)	22 (14.0)

a quarter of the strains were sensitive to chloramphenicol (28.8%) and nitrofurantoin (22.7%). Overall, 33.1% of the strains were sensitive to polymyxin B, followed by chloramphenicol (25.5%) and tetracycline (14.0%).

During the same year, we isolated 17 *Pseudomonas aeruginosa* strains (14 from urine and 3 from catheter tips) which were pan-resistant. Of these, only 5(29.4%) were sensitive to polymyxin B.

DISCUSSION

The emergence of New Delhi metallo β-lactamase 1 carrying strains of Enterobacteriaceae which are resistant to almost all available antimicrobials[1] has opened up a Pandora's box in the scientific and pharmaceutical community which has already been grappling with the celestial rise in antibiotic resistance among Gram-negative bacteria. In the study we have attempted to find out the magnitude of this multi- and pan drug-resistant Gram-negative bacteria in our hospital and to find out the efficacy of certain out of use/ infrequently used antibacterials against these isolates.

A total of 157 (1.34%) such isolates belonging to *Enterobacteriaceae* group and to the genus *Acinetobacter* could be identified in our study from urine, catheter tips and sputum. In the modern day hospital practice, β-lactams, aminoglycosides and fluoroquinolones remain the most widely used agents for treating all types of Gram-negative infections, and these 157 isolates were resistant to all these agents including carbapenems. With the global spread of extended spectrum beta-lactamase (ESBL) producing strains of Gram-negative bacilli, carbapenems (imipenem, meropenem, ertapenem and doripenem) have typically been the last line antibiotic for these resistant organisms. The emergence of carbapenem resistant Enterobacteria, *Acinetobacter* and *Pseudomonas* has presented fresh challenges. Sporadic reports of carbapenem resistance are available from USA,[5] Greece[6] and other European countries. Similar reports are available from various parts of India.[7-9] To treat such infections clinicians have been forced to use alternative antibiotics such as polymyxins.[10]

In our study, we have looked into the sensitivity profile for polymyxin B, chloramphenicol tetracycline, co-trimoxazole, and nitrofurantain. Nitrofurantoin is a synthetic antimicrobial agent that has been available in clinical practice for more than 50 years. It still has a role, and continues to be prescribed for uncomplicated urinary tract infections. Our study found 22.7% of ESBL and carbapenemase producing strains of Enterobacteriaceae and *Acinetobacter* species susceptible to this agent, although in certain western countries like Canada the sensitivity is as high as 93%.[11] Nevertheless, in Indian settings, it remains an alternative treatment option for uncomplicated urinary tract infections caused by highly resistant organisms.

The finding in our series of the high frequency of resistance shown by tetracycline and co-trimoxazole and the added problems of their bacteriostatic nature and primarily oral mode of administration, make them unlikely candidates to treat serious, complicated infections. However, in recent years, tigecycline, which is a derivative of minocycline, has shown some promise and has shown excellent activity against ESBL producing *E.coli* isolates.[12] In our study, tigecycline sensitivity pattern could not be included because of the non-availability of the discs on a regular basis. As a result we could use it to test only a quarter of the isolates (38/157), all of which were found to be sensitive. From 2012, tigecycline has been included in the panel of second line agents for all multidrug resistant Gram negative isolates except *Pseudomonads*. However, resistance has already started appearing among Enterobacteriaceae.[13] Furthermore, it cannot be used to treat *Pseudomonas* infections.

The two systemic parenteral agents which we evaluated were chloramphenicol and polymyxin B. Almost a quarter of the strains in our series were sensitive to chloramphenicol. Although this agent is infrequently used but high level resistance exists in nature as has also been observed in a recent study from UK, where, of the 81 carbapenem resistant Enterobacteriaceae, less than 25% of the strains were sensitive to this agent.[13]

There has been a renewed interest in colistin/ polymyxin B as an alternative agent for treating highly resistant serious Gram-negative infections, and its use is on the increase. Although in use from 1959, but the advent of less toxic aminoglycosides in the 1970s resulted in the decline of its use. One-third of the *Enterobacteriaceae* and *Acinetobacter* strains in our study and 29.4% of the *Psuedomonas aeruginosa* were sensitive to polymyxin B and it was the most effective antimicrobial in our series. Colistin resistance has been found to be most frequent among *Acinetobacter baumani,* followed by *Klebsiella* pneumoniae and Psuedomonas aeruginosa.[14] In Asia the rate of Colistin resistant *Acinetobacter* has been found to be less than 28%[15] while it was much less (7.4%) in UK.[13] The problems of nephrotoxicity and neurotoxicity associated with this agent have been addressed to by various workers, and recent reports have shown the incidence of neurotoxicity to be 7% and nephrotoxicity to be 8%-18%.[16] The emergence of tigecycline and colistin resistant isolates in 3.5% strains from India[17] is a danger signal since it leaves the clinician with no other agent to choose from. With the increasing use of polymyxin B and colistin, further selection of resistance to the cationic peptides is expected.[18]

The increasing challenge in treating infection caused by multi- and pan-resistant bacteria has forced the clinicians to resort to unusual or infrequently used antimicrobials or their combinations. As a result we are increasingly encountering reports regarding the efficacy and safety studies of such agents as fosfomycin, nitrofurantion and colistin. The pipeline for new and promising antimicrobials does not look at all promising due to multiple factors as has been discussed in a recent report by Walsh and Tolemen.[19] Dissemination of multidrug-resistant strains or the plasmid mediated transfer of such resistance can only be tackled by a combination of stringent antibiotic policy and stewardship not only at the institutional, but also at a nationwide and global level together with appropriate hospital associated infection control measures.

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