ORIGINAL ARTICLE

Pseudomonas bacteremia; in vitro susceptibility pattern in a tertiary care hospital

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INTRODUCTION

Pseudomonas sp. is ubiquitously present worldwide of which *Psedomonas aeruginosa* is a major nosocomial pathogen, which survives in moist environments and colonizes the respiratory tract of mechanically ventilated patients (1). *P. aeruginosa* bacteremia occurs most frequently in critically ill patients, particularly those who are immunocompromised, such as cystic fibrosis patients, burn victims and ICU patients (2).

P. aeruginosa has been implicated as the eighth most common pathogen causing nosocomial blood stream infections; alone it contributes to 10-20 % of nosocomial infections (2). *P. aeruginosa* has emerged as quite a challenging pathogen for clinicians. MDR, XDR, and PDR phenotypes elaborate inactivating enzymes, such as extended-spectrum beta-lactamases (ESBL) and metallo-β-lactamases (MBL), that make beta-lactams and carbapenems ineffective (3). ESBL-producing *P. aeruginosa* was initially detected in Europe in the mid-1980s, and MBL-producing P. aeruginosa was first reported from Japan in 1991. Resistant strains of *P. aeruginosa* have become a growing concern worldwide (4). *P. aeruginosa* resistance to carbapenems has been reported to be emerging at a rate of 20% (5).

Multidrug-resistant *P. aeruginosa* (MDR) infection has been reported to be associated with increased morbidity which includes increased length of stay, invasive procedures (i.e., bronchoscopy, tracheostomy, catheter implantation), higher incidence of surgery and increased mortality rates, as compared to MDS *Pseudomonas* sp. Infections (6).

Antibiotic resistance has been shown to vary by location. The resistance profile of multidrug-resistant strains, therefore, requires enhanced monitoring, especially for empiric treatment. Obtaining regional resistance data is important for establishing guidelines for appropriate antibiotic use, and may help control the rate of antibiotic resistance. Aim of the present study was to determine the sensitivity pattern of *pseudomonas sp.* isolated from bloodstream infections, and the prevalence of multidrug resistance, extensive drug resistance and pan drug resistance.

METHODS

This is a retrospective cross-sectional study of all the *Pseudomonas* isolates isolated from blood samples of patients with fever/sepsis, received at the department of microbiology of Govind Ballabh Pant Institute of Postgraduate Medical Education and Research, New Delhi over a period of 19 months.

Blood was collected 10 ml for adults and 5ml for paediatric patients and diluted in a ratio of 1:10 added to blood culture bottles with BHI broth. These blood culture bottles were then incubated aerobically at 37°C. The samples were subcultured after overnight incubation, day 3 and day 5. The samples were subcultured on blood agar and MacConkey agar and incubated at 37°C for a duration of 18 hours. *Pseudomonas* sp. was identified as per standard bacteriological methods (7).

The antibiotic sensitivity patterns of these isolates were studied by using the Kirby Bauer Disc Diffusion method on Mueller-Hinton agar, in accordance with CLSI Guidelines, and using Hi-media antibiotic discs (8). The antibiotics discs which were tested included piperacillin-tazobactam (100/10ug), amikacin (30 ug), gentamicin (10 ug), tobramycin (10 ug), netimicin (30 ug), cefepime (30 ug), ceftazidime (30 ug), ciprofloxacin (5 ug), levofloxacin (5 mcg) and meropenem (10 ug), imipenem (10 ug) and colistin (10 ug). For this study, multi-drug resistance was defined as resistance of a *Pseudomonas* isolate to at least three of the following four drugs: amikacin, imipenem, ceftazidime and ciprofloxacin. These antibiotics were chosen because they are representative of their antibiotic classes (9). Antibiotics with intermediate susceptibility CLSI were considered resistant in the study analysis.

Although definitions of MDR *Pseudomonas* in other studies were noted (10), it is worth mentioning that currently, no international consensus for the definition of multidrug resistance exists, making direct comparison of literature

very difficult. Extensively drug resistant (XDR) from the MDR *Pseudomonas* isolated were those isolates resistant to all the antibiotics except colistin (9). Pan drug resistance (PDR) is resistance to all antibiotics.

RESULTS

From 3951 sets of blood cultures 676 significant isolates were grown. An isolate was considered significant when a recognized pathogen was isolated from one or more blood samples and the patient had at least one of the following signs and symptoms: fever (>38°C), chills or hypotension and for commensals (e.g., coagulase negative staphylococci) isolation on two or more blood samples drawn on separate occasions. Gram positive cocci were isolated in 178 (26.33%) while Gram negative rods were isolated in 498 (73.66%) cases. *Pseudomonas* sp. constituted 14.05% (95) of the total isolates. ICU, ward and outpatient department (OPD) contributed 73.8% (70), 24.21% (23), 2.10% (2) respectively of the total isolates. Figure 1 shows distribution of *Pseudomonas* sp. among medical and surgical ICUs and wards.

The susceptibility pattern of *Pseudomonas* sp. from ICU and wards was obtained as shown in Table 1. Among aminoglycosides, maximum sensitivity was reported to netilmicin in wards and amikacin in ICU. Most of the isolates were sensitive to levofloxacin in Fluoroquinolone group. Most of the strains were resistant to cephems. *Pseudomonas* sp. isolated from wards and ICU were mostly sensitive to cabapenems (ward-meropenem, ICU-imipenem).

Carbapenem resistance was found to be more in ICU as compared to ward.

MDR, XDR and PDR *Pseudomonas* were analyzed and it was found that six MDR (10.75%), (four from Gastroenterology ICU and two from ward) and four (4.3%) extensively drug resistant (XDR) were isolated (Gastroenterology ICU). No PDR was observed. Five out of six MDR strains (83.3%) were found to be susceptible only to Imipenem, one MDR strain was found to be susceptible to Gentamicin and Amikacin, while all were sensitive to colistin.

DISCUSSION

Pseudomonas sp. bloodstream infection is a serious infection with significant patient mortality and healthcare costs. There is a global emergence of multidrug resistant strains of *Pseudomonas*. In the present study Gram negative rods constituted 73.66%

FIGURE 1: Distribution of *Pseudomonas* sp. among medical and surgical ICUs and wards

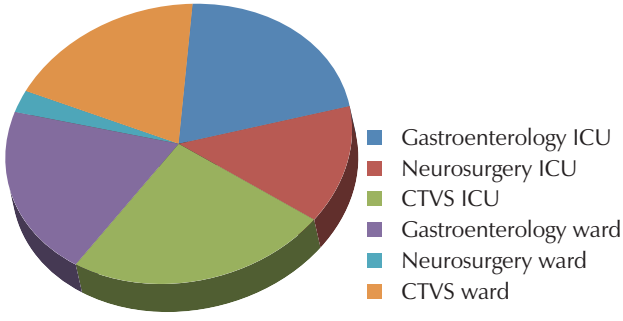

TABLE 1: Susceptibility pattern of *Pseudomonas* sp. in ICU and medicine wards

Antibiotics	Breakpoints (zone diameter)	% Resistance ICU	Medicine wards
Beta lactam/beta lactamase inhibitor			
Piperacillin-tazobactam	21	66.1	65.3
Cephems			
ceftazidime	18	71.2	69.6
Cefepime	18	84.7	78.3
Carbapenems			
Imipenem	19	17.4	13.1
Meropenem	19	44.24	26.1
Aminoglycosides			
Gentamicin	15	61.6	56.6
Amikacin	17	46.2	47.8
Tobramycin	15	88.5	69.6
Netilmicin	15	57.7	43.5
Fluoroquinolones			
Ciprofloxacin	21	94.3	95.7
Levofloxacin	17	38.5	43.5
Lipopeptides			
Colistin	12	0	0

of total isolates from blood and *Pseudomonas* sp. constituted 14% of total. In a study by Lachhab Z et al Gram negative rods constituted 83.6% and *Pseudomonas* sp. was 10% of total blood culture isolates from ICU (5).

Of total of 93 strains of the *Pseudomonas* isolated from the hospital ICU and wards, Piperacillin -tazobactam (Betalactam/ betalactamase inhibitor) 66.1% resistance found in ICU and 65.3% resistance found in wards. Ceftazidime resistance has been found to be 71.2% in ICU and 69.6% in wards. In the research implemented by Van Elder and Ahani Azari *et al*, the high resistance to drugs of the β-Lactam group and sensitivity toward the Amino glycosides and have been observed (11,12).

Ceftazidime resistance is mainly mediated by production of β-lactamases such as ESBL (extended spectrum beta lactamases), MBL (metallo-beta lactamases), and occasionally AmpC-β-lactamases (13). Besides production of various β-lactamases, other mechanisms such as the lack of drug penetration due to mutation in porins, loss of certain outer membrane proteins and efflux pumps can also contribute for resistance to β-lactams (14). Horizontal gene spread is considered to be responsible for the high frequency of ESBLs detected in *P. aeruginosa* (15).

In the present study, cefepime resistance has been found to be 84.7% in ICU and 78.3% in wards. This is in stark contrast to findings by Patel *et al* who reported Cefepime to be 15.63% resistant in isolates of *P. aeruginosa* (16), whereas Endimiani *et al* reported that 10-35% of the isolates of the clinical population in North America are resistant to Cefepime (17).

Aminoglycoside resistance has been found to be 63.5% in ICU while 54.3% in ward. In a study by Teixeira B *et al* the frequency of resistant *P. aeruginosa* isolates was found to be higher for the aminoglycosides tobramycin and amikacin (30.7 and 29.9%, respectively). The enzymatic modification of aminoglycosides by aminoglycoside-acetyltransferases (AAC), aminoglycoside-adenyltransferases (AAD), and aminoglycoside-phosphotransferases (APH), is the most common resistance mechanism in *P. aeruginosa* and these enzymes can be coded on mobile genetic elements that contribute to their dispersion (18).

In another retrospective case-control study in Turkey, it was found that the major risk factors for infection or colonization with multi-resistant *P. aeruginosa* were prolonged stay in the ICU, previous and lengthy imipenem usage, and mechanical ventilation (19). Also, in our study, maximum isolates of *Pseudomonas* spp. were from the ICUs irrespective of the type of ICU. High consumption of the antibiotics has led to the increase of vulnerability of the hospitalized patients toward the opportunistic infections.

Prior fluoroquinolone use has been identified as a risk factor for the emergence of imipenem-resistant *P. aeruginosa* (20). Out of 17 imipenem-resistant *P. aeruginosa* isolated during the study period, 15 (90%) showed resistance to ciprofloxacin or levofloxacin, suggesting that cross-resistance may have developed for imipenem due to prior use offluoroquinolones. Similar findings have been reported in a study by Rajkumari N *et al* (9). The widespread use of quinolones inevitably results in increasing cases of resistance.

Carbapenem resistance has been found to be 15.5% for Imipenem and 35% for meropenem. Among the beta-lactam antibiotics, carbapenems with antipseudomonal activity are important agents for the therapy of infections due to *P. aeruginosa*. The development of carbapenem resistance among *P. aeruginosa* strains is multifactorial. Plasmid or integron-mediated carbapenemases, increased expression of efflux systems, reduced porin expression and increased chromosomal cephalosporinase activity have all been defined as contributory factors (21).

Colistin (polymixin E) was one of the first commercially available antibiotics. While toxicity concerns have limited its usage, its potent activity against multidrug-resistant strains of *P. aeruginosa, A. baumannii,* and *K. pneumoniae* in vitro suggests that it may be effective for drug resistant infections. All isolates including multi drug resistant *P. aeruginosa* were found to be susceptible to colistin in a study by Walkty *et al* (22).

Prevalence of MDR is found to 10.75%, XDR 4.43%. No PDR has been found in our study. In a study by Gill JS *et al*, they reported a prevalence of 50% for MDR (resistant to one or more than one antimicrobial agent in three or more antimicrobial categories), XDR 2.3% (resistant to more than one antimicrobial agent in all the antimicrobial categories, except in two or less) and no PDR (3).

CONCLUSION

The study suggests that MDR and XDR strains of *Pseudomonas* sp. are emerging. MDR strains are resistant to commonly used antibiotics and showed maximum sensitivity to carbapenems. For XDR strains, glycopeptides are the only resort. Careful monitoring and surveillance of antibiotic use and bacterial susceptibility, the detection of carbapenem-resistant strains, and the implementation of strict infection control measures become critical for limiting the spread of the underlying resistance mechanisms.

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