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Clinico-microbiological study of *Pseudomonas aeruginosa* in wound infections and the detection of metallo- β -lactamase production

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Metallo beta lactamase; Multidrug resistance; Nosocomial; *Pseudomonas aeruginosa*; Wound infection

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Abstract

Pseudomonas aeruginosa is a common opportunistic pathogen of humans among the Gram-negative bacilli. Clinically, it is associated with nosocomial infections like burns and surgical-site wound infections and remains a major health concern, especially among critically ill and immunocompromised patients. This is a prospective laboratory-based 2 year study conducted to isolate *P. aeruginosa* from wound specimens and the antimicrobial susceptibility pattern with reference to metallo- β -lactamase (MBL) production. Two hundred and twenty-four samples of *P. aeruginosa* isolated from wound specimens were included in the study. Antimicrobial susceptibility was done as per Clinical Laboratory Standard Institute (CLSI) guidelines. MBL-producing *P. aeruginosa* was detected using the EDTA disk diffusion synergy test. Statistical analysis was done using the SPSS 11 package (SPSS Inc., Chicago, IL). Out of the 224 *P. aeruginosa* isolates, 100% were susceptible to polymyxin B and colistin, 92.8% were sensitive to imipenem, 38% showed resistance to gentamicin followed by ceftazidime (31.69%) and meropenem (33.03). Sixteen (7.14%) isolates showed MBL production. Infection caused by drug-resistant *P. aeruginosa* is important to identify as it poses a therapeutic problem and is also a serious concern for infection control management. The acquired resistance genes can be horizontally transferred to other pathogens or commensals if aseptic procedures are not followed.

Introduction

Over the years, there has been a notable increase in antibiotic resistance among Gram-negative bacteria recovered from hospitalised patients, especially from critically ill patients (1). Infections caused by *P. aeruginosa* are often severe and life-threatening and are difficult to treat because of its limited susceptibility to antimicrobial agents and the high frequency of the emergence of antibiotic resistance during therapy. Infections caused by multidrug resistant (MDR) Gram-negative bacteria, especially MDR *P. aeruginosa* (MDRPa), have been associated with increased morbidity, mortality and costs (2,3). In Latin America, *P. aeruginosa* is a major cause of nosocomial infections, ranking second in wound infections (4,5).

Metallo- β -lactamases (MBLs) are class-B enzymes that hydrolyse carbapenems and are encoded by genes such

as imipenemase (IMP) and verona integron-encoded metallo- β -lactamase (VIM) (6). They are further described as the enzymes that require divalent cations, usually zinc, as metal co-factors for their enzymatic activity. In recent years, the

Key Messages

- It is a challenge to treat wound infections caused by *P. aeruginosa* because of its intrinsic resistance to many antibiotic classes and its ability to acquire resistance
- Therefore, to avoid the risk of poor outcomes, there is a need for antibiotic treatment guided by antimicrobial susceptibility and for following strict aseptic procedures during wound care

MBL genes have spread from *P. aeruginosa* to *Enterobacteriaceae*. Their continued spread is going to be a major therapeutic challenge (7).

Knowledge of the responsible bacterial flora in wound infections and its prevalence and resistance is crucial in making fast and reliable therapeutic decisions. Hence, the present study was aimed at finding the antibiogram of *P. aeruginosa* from wound specimens and detecting the production of MBL by the resistant isolates in order to help formulate an effective antibiotic strategy.

Materials and methods

This is a descriptive study done from October 2012 to March 2014 in the Department of Microbiology, Kasturba Medical College, Mangalore. The samples collected were pus, serous discharge, tissue and slough from traumatic ulcers, burn and surgical wounds and diabetic ulcers. Exclusion criteria were the absence of polymorpho nuclear (PMNs) during gram stain evaluation.

Antimicrobial susceptibility was done using the Kirby Bauer disk diffusion method and interpreted based on the Clinical Laboratory Standard Institute (CLSI) guidelines (8). Piperacillin (100 mcg), piperacillin/tazobactam (100/10 mcg), ceftazadime (30 mcg), imipenem (10 mcg), meropenem (10 mcg), gentamicin (10 mcg), amikacin (30 mcg), ciprofloxacin (5 mcg), colistin (10 mcg), polymixin B (300 mcg), cefoperazone (75 mcg) and cefoperazone/salbutam (75/30 mcg) EDTA disk synergy tests were conducted to screen MBL-producing strains among carbapenem disk-resistant isolates of *P. aeruginosa* (9).

Results

A total of 7364 pus, tissue and wound swab samples were obtained in the study period of 18 months. One thousand six hundred and nine pseudomonas isolates from all age groups were isolated from those suffering from wound infections and presenting to KMC Hospital, Ambedkar Circle; KMC Hospital, Attavar, Mangalore and Government Wenlock Hospital. Of the samples collected, 224 were *P. aeruginosa*. Males (63.8%) showed a higher predisposition to the growth of *P. aeruginosa* among the wound specimens. Of the 224 samples, 153 (68.3%) isolates were from the surgical ward, 50 (22.32%) isolates from traumatic wound infections, followed by 16 (7.14%) from orthopaedic patients, 3 (1.33%) were from burn and immunocompromised individuals and the remaining 2 were from others (0.89%). Polymicrobial growth along with *P. aeruginosa* was seen in 33.03% of samples. The main organism involved was *Staphylococcus aureus* followed by the *Klebsiella* and *Acinetobacter* species. Diptheroids may have been colonising flora present on the skin around the wound from where the specimen was collected (Figure 1).

The antibiotic susceptibility pattern of *P. aeruginosa* in this study is as follows (Table 1): the highest sensitivity of 100% was shown by polymixin B and colistin followed by imipenem showing 92.8% sensitivity; the highest resistance was shown by gentamicin showing 38% followed by ceftazidime (31.69%) and meropenem (33.03).

Table 1 Antibiotic sensitivity pattern of *Pseudomonas aeruginosa* from wound infections

Antibiotics	Susceptible (%)	Resistant (%)
Amikacin	170 (75.89)	54 (24.10)
Gentamicin	137 (61.16)	87 (38.83)
Cefaperazone/sulbactam	189 (84.37)	35 (15.65)
Piperacillin/tazobactam	191 (85.26)	33 (14.73)
Ciprofloxacin	159 (70.98)	65 (29.01)
Piperacillin	170 (75.89)	54 (24.10)
Imipenem	208 (92.85)	16 (7.14)
Meropenem	154 (66.96)	70 (33.03)
Ceftazadime	153 (68.30)	71 (31.69)
Cefaperazone	168 (75)	56 (25)
Polymixin B	224 (100)	0
Colistin	224 (100)	0

Out of 224 isolates, 75(33.48%) were MDR, from among which 65 (86.66%) improved with hospitalisation and healed completely before discharge. All the strains that were imipenem-resistant were tested for an EDTA disk diffusion test for MBL detection. Sixteen (7.14%) isolates were MBL producers.

Discussion

During the last few years, there has been growing knowledge and evidence that the persistence of chronic wounds in many cases is because of bacterial infections. One of the most common pathogens in wound infections is *P. aeruginosa*, a very problematic microbe because of its ability to form resistant biofilms and migrate to deeper tissues. *P. aeruginosa* is a leading cause of nosocomial infections such as surgical site infections, burn wound infections, pneumonia, urinary tract infections and bacteraemia (10). In this 18-month study, a total of 21.8% *Pseudomonas* species were isolated from the wound specimens. Of these infections, 4.37% (224) were due to *P. aeruginosa*.

In our study, *Pseudomonas* wound infections were predominant in males, with a male to female ratio of 1.76:1. The highest infection rate was seen in the age group 61–70 years. Similar results were seen in another study also (11). Skin breaches, which can be very debilitating, frequently occur in the elderly because aged skin is more prone to compromise. Adding to this, the older patients had underlying risk factors such as diabetes mellitus, chronic illnesses and malignancies. The patients who were admitted to the intensive care unit (ICU) were given antibiotics. Antibiotic exposure may gradually make a sensitive organism resistant, which makes the treatment option difficult (12). The normal flora, which plays a significant protective role by preventing the colonisation of pathogenic microorganisms, may be removed by antibiotic exposure.

This study shows risk factors such as a hospital stay of 7 days or above and a previous antibiotic history (61%) followed by surgical site infection (54%) and diabetes mellitus (46%) as significant markers. This is similar to the study conducted in a tertiary hospital in Kenya (13) where the main risk factors were previous antibiotic history and a hospital stay of more

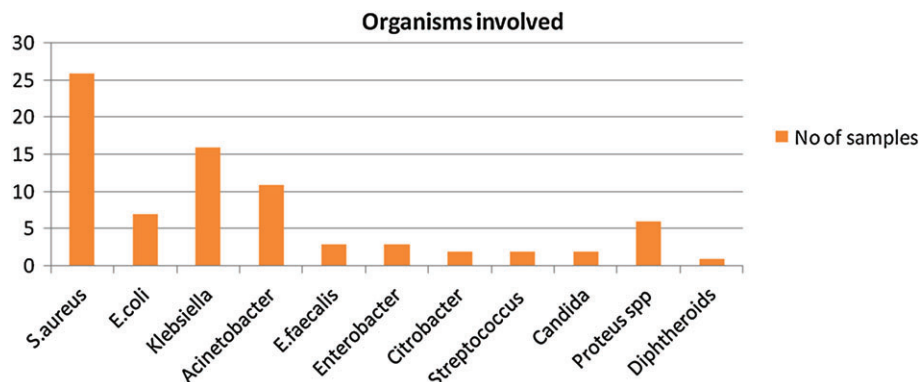


Figure 1 Organisms involved.

than 7 days. This accounted for 59% of the risk factors followed by surgical site infections and diabetes mellitus accounting for 43%. *Pseudomonas* spp. are often isolated in water and damp areas. Hence, in patients with diabetes, who already have a compromised cell-mediated immune system, *Pseudomonas* may colonise and cause infection. Several reports suggest that host susceptibility and underlying diseases are important predisposing factors (11,14,15).

In the majority of our cases (67%), the growth was monomicrobial. In the case of polymicrobial growth constituting 33% of the cases, *S. aureus* was the most common organism followed by *Klebsiella* and *Acinetobacter*. Polymicrobial involvement was commonly seen in surgical site infection wounds or chronic venous ulcers. Formation of excessive devitalised tissue, increased tension in the wound, haematoma and seromas and foreign bodies in the wound are all factors that predispose patients to a secondary bacterial infection. Most often, the normal flora are responsible for the infection. Patients can get infected with hospital-resistant flora during the hospital stay prior to surgery. The incidence of wound infections, however, varies from surgeon to surgeon, hospital to hospital, one surgical procedure to another and also from one patient to another (16).

P. aeruginosa resistance has been rising to a point where approximately 40% of the isolates are resistant to 'antipseudomonal' drugs, including carbapenems (7,16). However, acquired resistance to antipseudomonal β -lactams such as ticarcillin, piperacillin, ceftazidime, cefepime, aztreonam and carbapenems is considered a major challenge in managing MDR *P. aeruginosa* infections, especially while it is associated with co-resistance to other classes of drugs, namely aminoglycosides and fluoroquinolones (7). Our study shows the highest sensitivity to polymixin B (100%), colistin (100%) and imipenem (92.8%). The highest resistance was shown by gentamicin, showing 38% resistance, followed by ceftazidime and meropenem. Similarly, a study conducted by Peshattiwari (11) shows a resistance pattern against gentamicin (38.09%), amikacin (36.50%), ciprofloxacin (46.82%) and piperacillin (41.26%).

Several mechanisms can contribute to the acquired β -lactam resistance in *P. aeruginosa* that includes the production of β -lactamases, the up-regulation of efflux systems and decreased outer membrane permeability (7). With respect to β -lactamase production, acquired extended-spectrum β -lactamases (ESBL) and MBLs are the predominant emerging resistance

mechanisms in *P. aeruginosa*. *P. aeruginosa* isolates producing MBLs were first reported in Japan in 1991 and since then have been described in various parts of the world, including Asia, Europe, Australia, South America and North America (13). Genes coding for β -lactamase enzymes mutate continuously when exposed to irrational and inappropriate dosages of antibiotic use leading to the development of newer β -lactamases with a broad spectrum of activity (17).

In a study conducted in the S.L.Raheja Hospital, Mumbai on ICU patients, of the 240 *P. aeruginosa* isolates, 60 (25%) were found to be carbapenem-resistant and 50 (20.8%) were MBL producers. Because of this nosocomial pathogen, the average hospital stay was 32 days and was associated with 20% mortality due to septicemia (18). This, however, differs from our study that shows around 33% resistance to carbapenems among which 16 (7.14%) isolates were MBL producers. The prevalence of MBLs in the present study was consistent with the findings of Navaneeth *et al.* and others (19,20). This suggested that the carbapenem resistance in *P. aeruginosa* was mediated predominantly via MBL production.

The occurrence of MBL-positive *P. aeruginosa* isolates in a hospital environment poses not only a therapeutic problem but is also a cause of serious concern for infection control. Such organisms and their ability to participate in horizontal MBL gene transfer with other pathogens in a hospital pose a significant threat of spreading within institutions (7). The emergence of bacterial pathogens resistant to almost all available antimicrobial agents, namely 'superbugs', will severely threaten the therapeutic choices (21). Therefore, there is a greater need for antibiotic treatment guided by antimicrobial susceptibility. Proper hand wash techniques and aseptic precautions taken during wound dressing will help in curbing the spread of the organism from one patient to another.

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The authors declare that they have no conflicts of interest.

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