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High Incidence of Multidrug Resistant *Pseudomonas aeruginosa* Isolated from Infected Burn Wounds in a Tertiary Hospital

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A B S T R A C T

Pseudomonas aeruginosa is an important cause of hospital acquired infection especially in patients admitted in critical care units such as intensive care units and burn care units. In recent times, it has emerged as a widespread Multi Drug Resistant (MDR) pathogen which requires antibiotic susceptibility testing on a regular as well as a periodic basis. This study was carried with an aim to determine the antimicrobial resistance pattern and prevalence of MDR *P. aeruginosa* infection among burns patients at a tertiary care center. This retrospective study was carried out from July 2015 to May 2016. In this study 938 non repetitive wound swabs from patients admitted in burn unit were collected and the isolates were identified by conventional phenotypic methods, the antibiotic sensitivity testing of all *P. aeruginosa* isolates was done using Kirby-Bauer disc diffusion method and the results were interpreted according to the Clinical and Laboratory Standards Institute guidelines. Out of these 938 clinical specimens, *Pseudomonas aeruginosa* was isolated in 392(41.79%) samples. Out of these 392 isolates, 335 (85.45%) were found to be MDR *P. aeruginosa*. *P. aeruginosa* showed maximum sensitivity to meropenem and imipenem (30.1%) followed by levofloxacin (11.73%), piperacillin-tazobactum (10.2%). Carbenicillin, ceftazidime and cefoperazone were effective only in 1-5% isolates. Colistin was sensitive in 100% isolates.

Introduction

Pseudomonas aeruginosa is a leading cause of healthcare associated infection especially in patients admitted to critical care units such as intensive care units (ICUs) and burn care centers (Moazami-Goudarzi *et al*., 2012) *Pseudomonas aeruginosa*, a non-fermentative gram negative bacterium, is widely distributed in nature, including hospital environment. *P. aeruginosa* is a ubiquitous micro-organism that can rapidly acquire resistance to different broad-spectrum antibiotics (Indu *et al*., 2014). *P. aeruginosa* is responsible for about 10%–20% of nosocomial infections in the form of septicaemia in intensive-care units (ICUs), cystic fibrosis, burn and wound infections, etc., (Noha *et al*., 2015).

The immune suppression in burn patients in addition to the fact that *P. aeruginosa* has a

predilection for moist and warm wound environments made it a major challenge for burn patients.

MDR *P. aeruginosa* phenotype is defined as a bacterium which is resistant to anti-microbial agents which is included in three or more anti-Pseudomonal anti-microbial classes (carbapenems, fluoroquinolones, penicillins /cephalosporins and aminoglycosides (Magiorakos *et al*., 2011). The mechanism of resistance in MDR *P. aeruginosa* is possibly through the production of several enzymes that inactivate beta-lactams and carbapenems such as extended spectrum beta lactamases (ESBLs) and metallo-β-lactamases (MBLs).

Pseudomonas aeruginosa is naturally resistant to penicillin and most of the b-lactam antibiotics. Carbapenems are the antibiotic of choice for treatment of *P. aeruginosa* infections. However, gradually increasing resistance to carbapenems has become a major concern. Resistance may be mediated by loss of the OprD porin, upregulation of multi-drug efflux pumps and production of certain b-lactamases and carbapenemases (Asghar, 2012; Pier *et al*., 2010).

Mechanisms of aminoglycoside resistance in *P. aeruginosa* are due to reduced uptake of aminoglycosides across the outer and cytoplasmic membranes associated with the production of aminoglycoside-modifying enzymes.

Modification of DNA gyrase is responsible for the development of resistance to quinolones. The use of polymyxins (polymyxin B and colistin) was limited due to their nephrotoxicity. However, emergence of resistance against most effective anti-pseudomonal agents has resulted in the need for use of these agents (Puneet *et al*., 2015). This study was conducted with an aim to determine the antimicrobial resistance pattern and prevalence of MDR *P. aeruginosa* infection among burns patients at a tertiary care center.

Materials and Methods

This retrospective study was conducted in the Dept of Microbiology from July 2015 to May 2016. The wound swabs from 938 patients admitted to the burn unit were collected and *P. aeruginosa* isolates were identified by conventional phenotypic methods for gram staining, colony morphology, motility, oxidase test, Hugh-Leifson oxidation fermentation test, susceptibility to polymyxin B and pyocyanin production. Other bacteria which were isolated were also processed and identified by standard microbiological techniques.

Antibiotic susceptibility testing was done on Mueller Hinton Agar according to CLSI guidelines by Kirby Bauer disk diffusion method. The antibiotic discs used are aztreonam (30ug), piperacillin (100ug) cefoperazone+sulbactam (75/30ug), ceftazidime (30ug), gentamicin (10ug), imipenem (10ug), piperacillin+tazobactum (100/10ug), tobramycin (10ug), meropenem (10ug), polymyxin B (300 units), colistin (10ug) and levofloxacin (5ug). All discs were procured from Hi-Media Pvt Ltd.

MDR *P. aeruginosa* was detected as a bacterium which was resistant to three or more anti –Pseudomonal antimicrobial classes (piperacillin+tazobactum, imipenem, ceftazidime and gentamicin).

Standard strain *Pseudomonas aeruginosa* ATCC27853 was used as a control.

Results and Discussion

Out of 938 wound swab specimens, the most common isolated organism was *P. aeruginosa*

392/938 (41.79%) followed by *Escherichia coli* 115/938 (12.2%), *Enterobacter cloacae* 111/938 (11.8%), *Enterobacter aerogenes* 101/938 (10.7%), *Proteus sp*.80/938(8.5%), *Citrobacter sp*. 49/938(5.2%), Ac*inetobacter* sp 36/938 (3.8%), *Staphylococcus aureus* 26/938 (2.7%) and *CONS* 21/938 (2.2%). (TABLE 1)

Antimicrobial susceptibility testing was carried out for all 392 isolates of *P. aeruginosa* by Kirby -Bauer disk diffusion method. *P. aeruginosa* showed maximum sensitivity to meropenem and imipenem (30.1%) followed by levofloxacin (11.73%), piperacillin-tazobactum (10.2%). Carbenicillin, ceftazidime and cefoperazone were effective only in 1-5% isolates (Fig. 1).

According to the definition of MDR *P. aeruginosa*, 335 isolates out of 392(85.45%) were found to be MDR *P. aeruginosa.*

All the 392 isolates (100%) were found to be susceptible to colistin.

Burn wound represents a susceptible site for opportunistic colonization by microorganisms of exogenous and endogenous origin.

Pseudomonas aeruginosa is a major pathogenic species in the family Pseudomonadaceae. *P. aeruginosa* is associated with colonization of otherwise healthy humans and animals It has the considerable potency to become resistant to many antibiotics and increasingly more antimicrobial resistant strains are being encountered in clinical practice, leaving the treating physician with limited treatment options that lead to a severe adverse outcome.

In the present study, the most prevalent organism isolated from burn patient was *P. aeruginosa* (41.79%). Other studies such as Arslan *et al*., (1999), Naqvi *et al*., (2005), Bhatt *et al*., (2015), Sharma *et al*., (2011) showed a prevalence of *P. aeruginosa* infection among burn patients to be 53.97%, 59.6%, 54.9% and 61.95% respectively.

In the present study 85% isolates were found to be resistant to piperacillin-tazobactum combination. Similarly MozamiGoudarzi and Eftekhar (2012) have shown increased resistance to piperacillin-tazobactum combination (87.2%) and Naqvi *et al*., (2005) showed 81.8% resistance. Similarly S Upadhya *et al.,* (2014) showed 82.4% resistance to piperacillin-tazobactum combination whereas reports by Bhatt *et al*., (2015) and Sharma *et al*., showed 66% resistance and 69.8% resistance respectively.

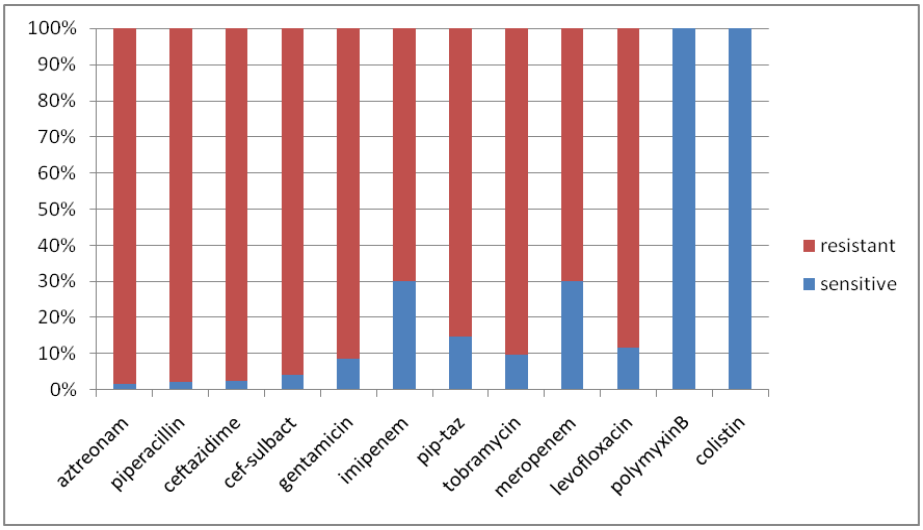
Among aminoglycoside 91% isolates were resistant to gentamicin and 90% isolates were resistant to tobramycin. Similarly Naqvi *et al*., (2005) showed 93.2% resistance to gentamicin and 95.5% resistance to tobramycin. Bhatt *et al*., (2015) showed 84% and 75% resistance to gentamicin and tobramycin respectively. However Upadhaya *et al*., (2014) showed 47.1% and 100% isolates to be resistant to gentamicin and tobramycin respectively.

Carbapenems are the antibiotics of choice for MDR *P. aeruginosa* infection but increasing resistance against carbapenems has now become a serious concern. In this study, it was found that 70% isolates were resistant to meropenem and imipenem. However Bhatt *et al*., (2015) showed resistance to meropenem and imipenem were 54% and 61% respectively. Mozami –Gordarzi and Eftekhar in their study found 94.7% isolates resistant to both imipenem and meropenem. Similarly Upadhaya et al., (2014) showed 94.1% and 88.2% resistance to meropenem and imipenem respectively in their study.

Table.1 Prevalence of organisms isolated from swab culture of burn wound (N=938)

Organism	No of strain isolated	Percentage(%)
Pseudomonas aeruginosa	392	41.7
Escherichia.coli	115	12.2
Enterobacter.cloacae	111	11.8
Enterobacter.aerogenes	101	10.7
Proteus sp	80	8.5
Citrobacter sp	49	5.2
Acinetobacter sp	36	3.8
Staphylococcus aureus	26	2.7
Coagulase negative staphylococci	21	2.2

Fig.1 Antibiogram of *Pseudomonas aeruginosa* isolates



In this study, 85.45% isolates were found to be MDR, which is more than findings of Bhatt *et al*., (2015) who found 76.8% isolates to be MDR. Similarly Saderi *et al*., (2014) showed 69% isolates to be MDR.However S Upadhaya *et al*., (2014) and Moazami-Goudarzi and Eftekhar (2012) showed 100% MDR isolates in burns patients.

The absence of new anti-pseudomonal agents against MDR *P. aeruginosa* has amplified the problem. Therefore, polymyxin B and colistin are becoming the last resort for the treatment of such infections. Colistin should mainly be used as salvage therapy in combination with one or more antimicrobials. In the present study, all strains were found to be sensitive to polymyxin B and colistin

The lack of any new compounds in the near future indicates that national and local surveillance efforts are essential, to provide clinicians with correct information for choosing right antimicrobial therapy

In conclusion, the prevalence of *P. aeruginosa* infections in burn patients was found to be 41.79%, and the prevalence of

multi-drug resistance among these isolates was. Such a high prevalence of MDR *P. aeruginosa* infection in burn patients is a cause for concern because it poses a serious therapeutic challenge due to very limited treatment options. Restriction of 'selected antibiotic usage' and/or infection control policies must be tailored for each institution, to combat the rapid emergence of MDR *P. aeruginosa* in burn patients. Colistin still retains a high sensitivity and could, therefore, be used as a therapeutic alternative in case of multi-drug resistance (Puneet *et al*., 2015). The lack of newer antimicrobial agents with activities against *P. aeruginosa*, makes periodic studies on the antimicrobial resistance patterns very important (Babita *et al*., 2011).

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