PSEUDOMONAS AERUGINOSA IN BURN INFECTIONS AND ITS ANTIMICROBIAL RESISTANCE

Chinmaya Dash[1], Satyajit Sahu[2], Sulekha Sinha[3], Pallavi Nayak[4]

[1]Associate Professor, Department of Microbiology, IQ City Medical College and Hospital, Durgapur, Kolkata, West Bengal.
[2]Consultant, Department of Plastic Surgery, IQ City Medical College and Hospital, Durgapur, Kolkata, West Bengal.
[3]Associate Professor, Department of Biochemistry, IQ City Medical College and Hospital, Durgapur, Kolkata, West Bengal.
[4]Assistant Professor, Department of Otorhinology and Head and Neck Surgery, IQ City Medical College and Hospital, Durgapur, Kolkata, West Bengal.

ABSTRACT

BACKGROUND

Pseudomonas aeruginosa is the main culprit of hospital acquired infection especially in wards like critical care area and burn units. Treatment for burns has been improved greatly but infections with multi-drug resistant (MDR) strains P.aeruginosa remain the main concern for death especially in critical care areas. We wanted to update the antibiotic sensitivity profile of Pseudomonas aeruginosa isolated in burn wounds among the burn patients at a tertiary care hospital.

METHODS

This is a retrospective study conducted in a tertiary health care center for one year. Data regarding the organism Pseudomonas aeruginosa was collected from the medical records. In this, samples 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 modified Kirby-Bauer's disc diffusion method and the results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

RESULTS

Of a total of 116 patients, P. aeruginosa was isolated from 62 (41.3%) samples. Among all the isolates 42 (67.7%) were MDR and 18 (29%) were XDR. And the isolates show maximum resistance to Ticarcillin-Clavulanic Acid (96.77%), followed by Cefepime (93.54%), Levofloxacin (93.54%), Piperacillin (91.94%), Netilmicin (91.94%), Ceftazidime (90.32%), Doripenem (90.32%), Ciprofloxacin (87.1%), Imipenem (87.1%), Meropenem (85.49%), Piperacillin-Tazobactam (83.87%), Gentamicin (58.06%), Aztreonam (51.61%), Tobramycin (50%) and Amikacin (48.4%).

CONCLUSIONS

The result confirmed the prevalence of MDR strains. Prevention of, dissemination and indiscriminate use of antibiotics is important. Novel infection control practices, hospital antibiotic policy and regular surveillance programmes should be implemented.

KEYWORDS

Burns, Pseudomonas aeruginosa, Multidrug Resistance, Hospital Acquired Infections, Antibiotic Susceptibility Testing.

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BACKGROUND

Burn is described as a traumatic injury to the skin or other organic tissues, mainly caused by thermal or other acute exposures. Decreased immunity, hospital overstaying and breach in protective skin barrier are mostly responsible for the increase in hospital-acquired infections (HAIs) in these patients.[1] Injudicious use of systemic antibiotics and other interventions like surgical debridement and skin grafting facilitated the growth of multidrug resistant bacteria.[2] Even with aggressive antibiotic therapy Pseudomonas aeruginosa can carry out high morbidity and mortality in burn units. Multi drug resistant (MDR) strains are responsible for rising in wound infections, sepsis, and associated deaths worldwide.[3]

Being ubiquitous P.aeruginosa has been found in the contaminated floor, bed rails, and sinks and also from the hands of healthcare workers, which can act as a source of infection in the burn units. Besides transmission through fomites and vectors, these MDR bacteria can be carried into the hospital from the community by the patients as normal flora and can be an important source of infection for the same individual.[4]

Various studies from the USA and Iran indicate that in more than 40% cases there are irrational uses of antimicrobial agents. The widespread application of antimicrobials results in a vicious cycle of increasing level

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Corresponding Author:
Dr. Satyajit Sahu,
Consultant, Department of Plastic Surgery,
IQ City Medical College, Sovapur, Bijra Road,
Jemua, Durgapur- 713206, West Bengal.
E-mail: drsatyajitsahu1981@gmail.com
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and severity of bacterial resistance, which in turn increase in the demands for antimicrobial agents and further aggravating bacterial resistance. To curtail inpatient mortality, morbidity, and the economic burden, it is essential to select appropriate antimicrobial agents. Increase in the frequency of multi-drug resistant (MDR) strains of P.aeruginosa has severely limited the availability of therapeutic options.[2] These strains can be carried by some patients into the critical care areas as asymptomatic carriers and being treated throughout several courses of antibiotic treatment which were administered to treat Pseudomonas and non- Pseudomonas infections. It will further worsen the scenario when it will spread from one patient to another and the persistence of these strains even after several antibiotic treatments.[4] Even drug susceptible strains of Pseudomonas aeruginosa have considerable defences. Like some Enterobacteriaceae species, P.aeruginosa has an inducible AmpC b-lactamase, efflux and the impermeability to common antimicrobials used.

This study was conducted with an aim to update the antibiotic sensitivity profile of isolated Pseudomonas aeruginosa isolated in burn wounds among the burn patients at a tertiary care hospital.

METHODS

This is a retrospective study conducted in a tertiary health care center for one year. All adult patients with burn injuries were included in this study.

Data regarding the organism Pseudomonas aeruginosa were collected from the medical records. The pus samples were processed manually in the Central Laboratories. For isolation of the organism MacConkey Agar (Hi-media®) and Blood Agar was used. The colony morphology, motility tests, sugar fermentation tests and biochemical tests such as oxidase test, urease test and IMViC (indole, methyl red, Voges-Proskauer, and citrate) tests used to confirm the isolates as Pseudomonas aeruginosa. Isolation, identification and antibiotic susceptibility testing was carried out as per the Clinical and Laboratory Standard Institute (CLSI) guidelines. Antibiogram was performed using modified Kirby-Bauer's disc diffusion method, and the following antipseudomonal were assessed- Piperacillin 100 µg, Piperacillin-tazobactam 100/10 µg, Ticarcillin-Clavulanate 75/10 µg, Aminoglycosides (Ciprofloxacin 5 µg and levofloxacin 5 µg), Cephalosporins(Ceftazidime 30 µg, Cefepime 30 µg, Cefoperazone-sulbactam 75/30 µg), Fluoroquinolones (Amikacin 30 µg, Gentamicin 10 µg, Netilmicin 30 µg, Tobramycin 10 µg) Monobactams (Aztreonam 30 µg), Carbapenems (Imipenem 10 µg, Meropenem 10 µg, Doripenem 10 µg), and Lipopeptides (Polymyxin B and Colistin). The standard strain Pseudomonas aeruginosa (ATCC 27853®) were used as the control. Epidemiological data was also collected by using the case report form.

The P.aeruginosa was proposed to be multidrug resistant (MDR) when the isolate is resistant to at least three antimicrobial categories within its susceptibility spectrum (including resistant and intermediate). Resistance to one antimicrobial category is defined when the isolate is non-susceptible to at least one agent in the recommended list for susceptibility testing of the corresponding category. The isolates susceptible only to polymyxin B and colistin were categorized under extensively drug resistant (XDR).[5]

Statistical Analysis

Categorical data are expressed in proportions; Continuous data based upon their distribution are tested either by parametric or non-parametric tests.

RESULTS

A total 116 numbers of patients admitted in the burn ward with various degrees of burn during one year of study. Of which 58 (50%) were male and 58 (50% were females. The organisms isolated from the burn patients were Pseudomonas aeruginosa (n=62,41.3%), followed by Klebsiella pneumonia (n=26,17.3%), Acinetobacter sp (n=23, 15.3%), Coagulase negative staphylococcus (n=7, 4.7%), Enterococcus sp (n=6, 4%), Candida spp. (n=4, 2.7%), Proteus mirabilis (n=2, 1.3%) and Nonfermenting gram negative bacilli (n=2, 1.3%). Almost all P.aeruginosa (60, 96.77%) were isolated from pus sample.

The antimicrobial susceptibility pattern among the isolated P.aeruginosa is shown in Table-1, which indicates maximum resistance to Ticarcillin-clavulanic acid (96.77%) followed by Cefepime and Levofloxacin 93.54% and least to amikacin (48.4%). Among penicillin and ß-lactam penicillin group of antibiotics, maximum resistance was for Ticarcillin-clavulanic acid (96.77%) and least to Piperacillin – Tazobactam (83.87%) as shown in Figure-1. As shown in the Figure-2 the organism is showing maximum resistance to cefoperazone sulbactam (96.77%) followed by cefepime (93.54%) and ceftazidime (90.32%). Among fluoroquinolones, the drugs showing maximum resistance was levofloxacin (93.54%) followed by ciprofloxacin (87.1%) which is mentioned in Figure-3. Most commonly used aminoglycosides are amikacin, gentamycin, netilmicin, and tobramycin. Among these P.aeruginosa shown maximum resistant to netilmicin (91.94%) followed by gentamycin (58.06%), tobramycin (50%) and amikacin (48.4%). Resistance pattern among Aminoglycosides is shown in the Figure-4. The antimicrobial susceptibility test was done in the monobactam (Aztreonam) shows resistance in 51.61% of strains. The figure-5 shows the resistance pattern among the carbapenems. Among the carbapenems, maximum resistance is shown with doripenem (90.32%) followed by imipenem (87.1%) and meropenem (85.49%).The drugs used to detect the antimicrobial susceptibility pattern among lipopeptides were polymyxin B and colistin. All the isolates were sensitive to both the lipopeptide antibiotics. Among all the isolates 42(67.7%) were MDR and 18(29%) were XDR P.aeruginosa.

	Antimicrobial	Sensitive (S)	Intermediate (I)	Resistant (R)		Antimicrobial	Sensitive (S)	Intermediate (I)	Resistant (R)
Penicillin & ß-Lactam	Piperacillin	03 (4.84%)	02 (3.23%)	57 (91.94%)	Aminoglycosides	Amikacin	23 (37.1%)	09 (14.5%)	30 (48.4%)
	Piperacillin/ Tazobactam	05 (8.06%)	05 (8.06%)	52 (83.87%)		Gentamicin	26 (41.94%)	00	36 (58.06%)
	Ticarcillin/ Clavulanate	02 (3.23%)	00	60 (96.77%)		Netilmicin	02 (3.23%)	03 (4.84%)	57 (91.94%)
						Tobramycin	26 (41.94%)	05 (8.06%)	31 (50%0
Cephems	Ceftazidime	06 (9.68%)	00	56 (90.32%)	Monobactams	Aztreonam	28 (45.16%)	02 (3.23%)	32 (51.61%)
	Cefepime	04 (6.45%)	00	58 (93.54%)	Carbapenems	Imipenem	05 (8.06%)	03 (4.84%)	54 (87.1%)
FQs	Ciprofloxacin	02 (3.23%)	06 (9.68%)	54 (87.1%)		Meropenem	05 (8.06%)	04 (6.45%)	53 (85.49%)
	Levofloxacin	00	04 (6.45%)	58 (93.54%)		Doripenem	02 (3.23%)	04 (6.45%)	56 (90.32%)

Table 1. Antimicrobial Susceptibility Patterns of The Isolated Pseudomonas Aeruginosa

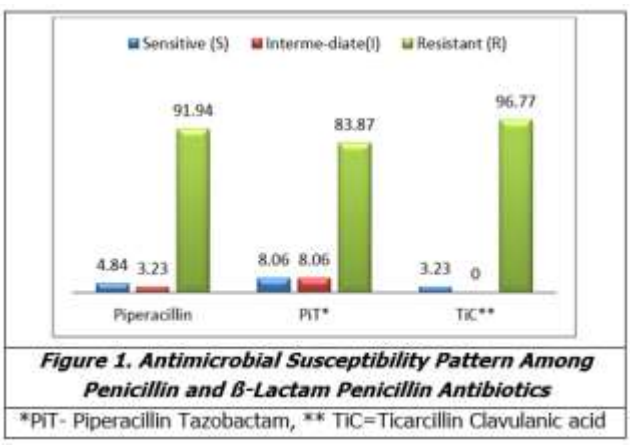

Figure 1. Antimicrobial Susceptibility Pattern Among Penicillin and ß-Lactam Penicillin Antibiotics

*PiT- Piperacillin Tazobactam, ** TiC=Ticarcillin Clavulanic acid

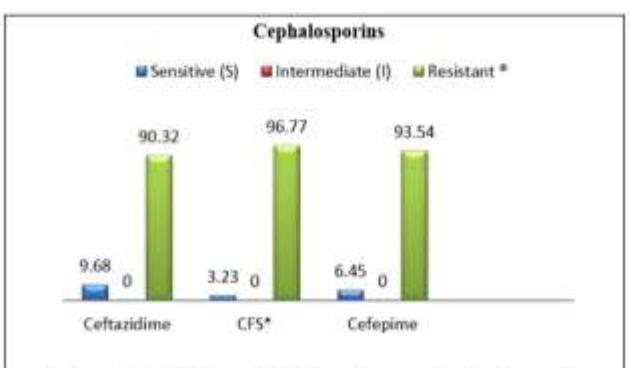

Figure 2. Resistance Pattern Among Cephalosporins

*CFS=Cefoperazone-sulbactam

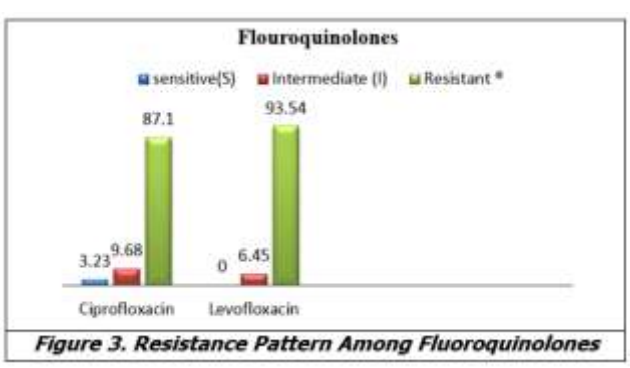

Figure 3. Resistance Pattern Among Fluoroquinolones

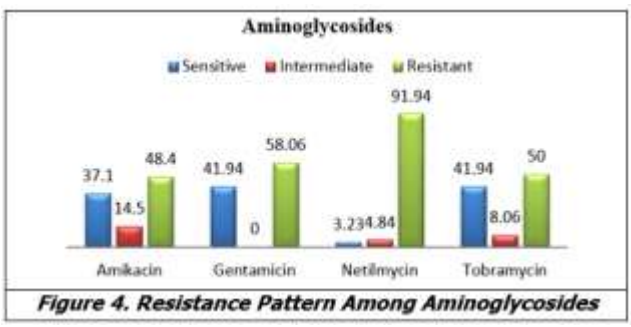

Figure 4. Resistance Pattern Among Aminoglycosides

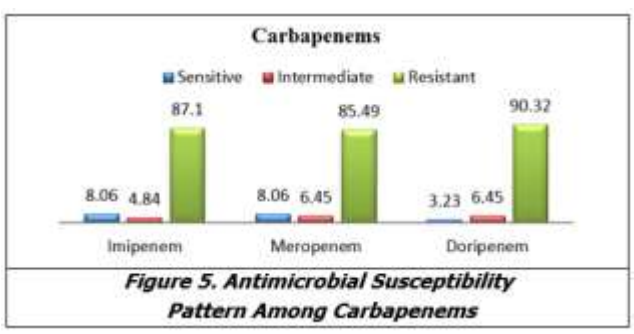

Figure 5. Antimicrobial Susceptibility Pattern Among Carbapenems

DISCUSSION

Being one of the most common causes of Hospital acquired infection and associated with increased drug resistance. Which lead to an increase in mortality and morbidity in critical care areas like burn units.

In our study, we found 41.3% isolates were P.aeruginosa. In studies done by Bhatt et al and Naqvi et al showed 54.9% and 59.6% respectively.[1,6] Which is a little higher than our study. However. Ekrami et al showed a prevalence of 37.5%.[7]

Various penicillins, cephalosporins, carbapenems, monobactams, aminoglycosides, fluoroquinolones, and polymyxins have been used to treat patients infected with P. aeruginosa and are active against most isolates. All, however prone to being compromised by resistance strains. Even drug susceptible strains have considerable defenses.

Derepression of the chromosomal AmpC β-lactamase (PSE-1 and PSE-4. Like classical TEM and OXA enzymes) reduces susceptibility to penicillins and cephalosporins

although the level of resistance depends on the degree of depression, which is more variable than that in Enterobacter mutants. It also may be also efflux mediated. Most commonly extended spectrum ß- lactam like piperacillin and combination of the ß-lactamase inhibitor to ß-lactams are indicated for the treatment of P aeruginosa. In our study, the isolates showed 91.94% resistant to piperacillin. And in the combinations with ß-lactamases, it shows 96.77% resistant to ticarcillin-clavulanic acid and 83.87% resistant to piperacillin-clavulanic acid. Similarly, the study done by Bhatt P et al and Naqvi et al showed 81% and 81.6% resistant to piperacillin respectively.[1,] The study by Radan M et al and Naqvi et al showed 89% and 81.8% resistance to ticarcillin-clavulanic acid which are less than that of our findings.[3,6]

In our study, we found that the isolates showed maximum resistance to cefoperazone sulbactam (96.77%) followed by cefepime (93.54%) and ceftazidime (90.32%). In the study by Bhatt et al 77% showed resistant to ceftazidime and 65% showed resistance to cefepime. Similarly, less resistance was showed to cefoperazone-sulbactam (34.48%) in the study done by Chander et al and 51.67% resistance to ceftazidime in the study done by Shilba et al.[8,9] The higher resistance pattern may be due to irrational use of such antimicrobials.

Fluoroquinolones like ciprofloxacin and levofloxacin have been commonly used for the treatment of infections caused by P aeruginosa. The continuous rise and widespread use of quinolones in the hospital as well as in the community leads to the emergence of resistance. Resistance can be developed through mutations on the bacterial target enzymes encoded by the gyrA, gyrB, parC, and parE genes or through the elaborate efflux pumps, such as mexR and nfxB.[10] In our study maximum resistance was shown to levofloxacin i.e. 93.54% followed by ciprofloxacin i.e. 87.1%. Contrary to our findings, in the study done by Bhatt P et al shows 47% of isolates show resistance to levofloxacin but ciprofloxacin shows resistance to71% of isolates.[1] Also in the study done by Shilba et al the resistance rates were 46.67% for ciprofloxacin which is less than to the findings of our study. But the study done by Radan M et al showed 96.7% resistance to ciprofloxacin which has similar high resistance as ours.[9] Like our studies, studies done by Ohmagari N et al showed increased resistance to levofloxacin than ciprofloxacin which is due to overuse.[11]

Upregulation of MexXY-OprM and most importantly increased impermeability confers to aminoglycoside resistance in Pseudomonas aeruginosa. Among the aminoglycosides, maximum resistant to netilmicin (91.94%) followed by gentamycin (58.06%), tobramycin (50%) and amikacin (48.4%). Studies done by Bhatt P et al shows maximum resistant to gentamicin (84%) followed by tobramycin (75%) and amikacin (73%).[1] Similar drug resistant data was reported by Naqvi ZA et al (tobramycin-95.5%) and Nasrabadi BM et al (tobramycin-82%).[6,12] In all the mentioned studies it shows P aeruginosa shows maximum sensitivity to the drug amikacin.

Carbapenems are the preferred antimicrobials to combat with the MDR P.aeruginosa. But it may develop resistance from the organism through combined mechanisms like Metallo-beta lactamase (MBL) production (bla $_{IMP}$, bla $_{VIM}$, bla $_{SPM}$ and bla $_{NDM}$), overproduction of the MexAB-OprM and MexXY efflux pumps, overproduction of chromosome-encoded AmpC β-lactamase, and reduced OprD expression.[13] In our study maximum resistance was shown to doripenem (90.32%) followed by imipenem (87.1%) and meropenem (85.49%). A similar pattern of resistance shown in the Radan M et al all the isolates show 96% resistant to imipenem and meropenem.[3] But less drug resistance is seen in the study done by Rostami S et al,[14] among all isolated P.aeruginosa, 78.5%, 46.7%, and 15% were imipenem, meropenem, and doripenem resistant, respectively.[13] Similarly it was found that 61% isolates were resistant to imipenem and 54% were resistant to meropenem by Bhatt P et al.[1]

Aztreonam a potent monocyclic ß-lactam with good in vitro activity against P.aeruginosa. Our study revealed that the isolates were relatively less resistance (51.61%) to it. Similar studies done by Shilba et al shows 43.33% resistance and in the study of Radan et al shows high resistance i.e 86%.[3,9]

A combination of upregulated efflux, loss of OprD and impermeability to aminoglycosides compromises every drug class except lipopeptides. Lipopeptides (polymyxin B and colistin) are the last alternative available antimicrobial for multidrug resistant P.aeruginosa. All the isolates in our study were sensitive to polymyxin B and colistin. Similar results were seen with the studies done by Bhatt P et al and Radan et al But as per the guidelines from clinical and laboratory standards institute (CLSI), the only approved method is broth dilution.[1,3] Disk diffusion and gradient diffusion methods should not be used for the result analysis. So further studies should be done to assess the MIC level of lipopeptides and interpret the resistance pattern among isolates.

As shown in our study, isolated P.aeruginosa were mostly from pus and it may be a hospital strain or acquired from the community or normal flora of the patient. A similar study done by Varsha et al and Leelavati et al shows potential colonization of P.aeruginosa in patients admitted to the critical care areas of a tertiary care hospital.[15,16] So further studies should correlate between the colonizers and the wound infection in critical care areas of burn wards.

CONCLUSIONS

Treatment for burns has improved greatly but infections with P.aeruginosa leading to death remain the main concern, especially in critical care areas. It is a pathogen with many virulent factors and a matter of concern because it poses a serious therapeutic challenge which leaves the physician or surgeon with very limited options. Materials acting against the MDR P.aeruginosa are limited and toxic. Prevention of, dissemination and indiscriminate use of antibiotics is important. Novel infection control practices, hospital

antibiotic policy and regular surveillance programmes should be implemented.

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