Original article:

Prevalence and Antimicrobial susceptibity pattern of *Pseudomonas aeruginosa* isolated from various clinical samples in a tertiary care hospital, Bathinda

Amandeep Kaur[1] Satnam Singh[2] Amarjit Kaur Gill[3] Narinder Kaur[4]

[1]Assistant Professor & PhD Student,Department of Microbiology, Adesh Institute of Medical Sciences and Research,Bathinda

[2]Assistant Professor, Department of Pharmacology, Adesh Institute of Medical Sciences and Research,Bathinda

[3]Professor and Head, Department of Microbiology, Adesh Institute of Medical Sciences and Research,Bathinda

[4]Associate Professor, Department of Microbiology, Adesh Institute of Medical Sciences and Research,Bathinda

Corresponding Author-Ms.Amandeep Kaur

Abstract

Introduction: *Pseudomonas aeruginosa (P.aeruginosa)* is one of the important bacterial pathogens isolated from various samples.It almost exclusively infects hospitalized patients with lowered host resistance.

Aims and objectives: Success of antimicrobial therapy depends on the appropriateness of the choice of antibiotics that should be used on the basis of prior knowledge of the susceptibility pattern of the agent.Therefore, the current study was undertaken to determine the antimicrobial susceptibility pattern of *P.aeruginosa* in a tertiary care hospital of Adesh Institute of Medical sciences and Research(AIMSR), Bathinda.

Material and methods: The study was conducted in Department of Microbiology, AIMSR over a period of one year and six months from July 2014 to December 2015. A total of 1443 samples were collected from patients admitted in ICU(545 samples) and different wards (898 samples)of the hospital.All samples were processed according to standard microbiological procedures. All confirmed *P.aeruginosa* isolates were subjected to antimicrobial susceptibility testing by Kirby Bauer disc diffusion method. .**Results:** In the present study, a total of 1443 samples were studied. A total of 118 *P.aeruginosa* were obtained from 1443 samples accounting for isolation rate of *P.aeruginosa* to be 8.2%(118/1443).Sensitivity to Polymixin B and Colistin was 100% followed by Imipenem(82.2%), Meropenem(66.9%), Piperacillin-Tazobactum(65.2%), Netimicin(58.4%). Similar sensitivity was shown towards Gentamicin, Amikacin and Ciprofloxacin(56.7%)and very low level of sensitivity was recorded for ceftazidime (37.2%) and cefepime(38.9%).

Conclusion: .This study would help and guide the physicians in prescribing the right combinations of antimicrobials to prevent the emergence of multidrug resistant strains of *P.aeruginosa* in the hospital environment.

Keywords: *P.aeruginosa*, antimicrobial susceptibility, pus, urine

Introduction

Pseudomonas aeruginosa (*P.aerugonosa*) is a ubiquitous organism present in many diverse environmental settings,and it can be isolated from living sources including plants, animals and humans.The ability of *P.aeruginosa* to survive in minimal nutritional requirements and to tolerate a variety of physical conditions has allowed the organism to persist in both community and hospital settings.[1] *P.aeruginosa* is seldom a member of

normal microbial flora in humans. Representative colonization rates for specific sites in humans are 0-2% on skin, 0-3.3% for nasal mucosa,0-6.6% for throat and 2.4-2.6% for faecal samples.[2] However ,colonization ratest may exceed 50% during hospitalization, especially among patients who have experienced trauma to or a breach cutaneous or mucosal barriers by mechanical ventilation, tracheostomy, catheters, surgery or severe burns. Disruption in the normal microbial flora as a result of antimicrobial therapy has also shown to increase colonization with *P.aeruginosa*.[3] *P.aeruginosa* accounts for largest percentage of all non-fermenting Gram negative bacilli isolated isolated from clinical samples.[1] *P.aeruginosa* continues to cause complications in hospital acquired infections. It is increasingly recognized as an emerging opportunistic pathogen of clinical relevance that causes infections in hospitalized patients; particularly in burn patients, orthopaedic related infections, respiratory diseases, infections in immunosuppressed and catheterized patients.[4] Several different epidemiological studies suggest that antibiotic resistance is increasing among clinical isolates. Infections caused by drug resistant *P.aeruginosa* are associated with significant increase in morbidity, mortality, need for surgical intervention, length of stay and overall cost of treatment.[5] The high resistant to cephalosporins may be due to production of extended spectrum beta lactamases [ESBL's).The carbapenems have been drug of choice for treatment of serious infections caused by Gram negative bacteria.However,carbapenem resistance has been observed frequently in *P.aeruginosa*. Resistance to carbapenems is predominantly mediated by MBL's i.e metallobetalactamases that recognize bivalent metal ions, usually zinc for their activity.[6] In the absence of carbapenem hydrolyzing carbapenamases; mainly MBL's, resistance is usually multifactorial. Increased production of *Amp* C encoded cephalosporinase, reduced outer membrane porin *Opr* D expression, increased expression of efflux pump mechanisms are known to contribute to carbapenem resistance.[7,8] Success of antimicrobial therapy depends on the appropriateness of the choice of antibiotics that should be used on the basis of prior knowledge of the susceptibility pattern of the agent.Therefore, the current study was undertaken to determine the antimicrobial susceptibility pattern of *P.aeruginosa* in a tertiary care hospital of Adesh Institute of Medical sciences and Research(AIMSR), Bathinda.The study was approved by the Research and Ethical Committee of our Institute.

Material and methods

The study was conducted in Department of Microbiology, AIMSR over a period of one year and six months from July 2014 to December 2015.

Various samples included in the study were pus exudates from different sites, urine, sputum, Endotracheal secretions, tracheal aspirates, high vaginal swabs, blood and various body fluids. A total of 1443 samples were collected from patients admitted in ICU(545 samples) and different wards (898 samples)of the hospital .All samples were processed according to standard microbiological procedures.[9,10] *Pseudomonas* spp were provisionally identified on the basis of :

- Growth on Mac Conkey's Agar as non-lactose fermenting colonies
- Fruity odor
- Oxidase positive
- Gram negative slender bacilli on Gram staining
- Actively motile in hanging drop preparation

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The confirmation for *P.aeruginosa* was done by various other biochemical tests[9,10]

- K/K reaction in Triple sugar Iron medium
- Oxidative reaction in Hugh Leifson Media(O/F test)
- Indole production test-negative
- Citrate utilization test-positive
- Nitrate reduction test-positive
- Urease test –negative (at 24-48 hrs)
- Lysine and ornithine decarboxylation test-negative
- Arginine dihydrolase test-positive
- Ability to grow at 42^{o}C

All confirmed *P.aeruginosa* isolates were subjected to antimicrobial susceptibity testing by Kirby Bauer disc diffusion method.[11] Antimicrobial discs recommended by Clinical Laboratory Standards Institute(CLSI) were used and interpretation was made according to CLSI guidelines.[12] Following antimicrobial discs were used: ceftazidime (30µg), cefepime (30 µg), piperacillin-tazobactum (100µg/10 µg), aztreonam(30 µg)),Imipenem (10 µg), Meropenem(10 µg), Gentamicin (10 µg), Amikacin (30 µg) , netilmicin (30 µg),Ciprofloxacin(5 µg), Norfloxacin(30 µg for urinary isolates), PolymixinB (300 units) and Colistin(10 µg). All dehydrated media and antibiotic discs were procured from HiMedia Labs,Mumbai,India.

Statistical analysis: Statistical analysis was done using descriptive statistics using simple ratio and percentages method.Microsoft Office 2007 was used to generate tables.

Results

In the present study, a total of 1443 samples were studied. Out of these,709 samples showed growth on culture and total of 118 *P.aeruginosa* were obtained from 1443 samples processed ;thus accounting for isolation rate of *P.aeruginosa* to be 8.2%(118/1443) **(Table 1)** Thirty samples showed double growth of organisms and among them, eighteen samples showed growth of another organism alongwith *P.aeruginosa.* **(Table 2)**

Table 1:

Total samples processed	1443
Total culture positive samples	709
Samples showing double growth	30
Total isolates obtained	739
Total Gram negative bacteria isolated	477
Total Non-fermenting bacteria isolated	223
Total *P.aeruginosa* isolates	118

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Table 2: Organisms showing growth alongwith P.aeruginosa

Organisms	**No. of samples**
P.aeruginosa + Klebsiella pneumoniae	8
P.aeruginosa + Escherichia coli	5
P.aeruginosa + Staphylococcus aureus	3
P.aeruginosa + Acinetobacter baumannii	1
P.aeruginosa + Candida albicans	1

Isolation rate was more from males(78/118=66.1%%)than females(40/118=33.8%)Isolation rate was slightly higher from ICU samples (46/545=8.44%) than IPD samples(72/898=8.01%) Maximum no. of *P.aeruginosa* isolates were obtained from pus samples(43/118=36.4%) followed by urine(32/118=27.1%) **Table 3** shows sample wise distribution of *P.aeruginosa* isolates.

Table 3: Isolation rate of *P.aeruginosa* (N=118)from various clinical samples

Name of sample	No. of *P.aeruginosa* isolates	Percentage
Pus	43	36.4%
Urine	32	27.1%
Tracheal aspirate	22	18.6%
Endotracheal secretions	9	7.6%
Sputum	5	4.2%
Blood	3	2.5%
Pleural fluid	2	1.7%
High vaginal swabs	2	1.7%

It was observed that isolates of *P.aeruginosa* were resistant to most of the routinely used anti-pseudomonal drugs. Sensitivity to Polymixin B and Colistin was 100% followed by Imipenem(82.2%), Meropenem(66.9%), Piperacillin-Tazobactum(65.2%), Netimicin(58.4%). Similar sensitivity was shown towards Gentamicin, Amikacin and Ciprofloxacin(56.7%).50% isolates of P.aeruginosa were sensitive to Aztreonam and very low level of sensitivity was recorded for ceftazidime(37.2%) and cefepime(38.9%). **Table 4** shows Antimicrobial Susceptibility profile of *P.aeruginosa*.

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Table 4: Antimicrobial Susceptibility profile of *P.aeruginosa*

Name of the antimicrobial agent (Concentration)	Sensitive n(%)	Resistant n(%)
Ceftazidime(30µg)	44(37.2%)	74(62.8%)
Cefepime(30µg)	46(38.9%)	72(61.1%)
Piperacillin-Tazobactum(100 µg/10µg)	77(65.2%)	41(34.8%)
Aztreonam(30µg)	59(50%)	59(50%)
Gentamicin(10µg)	67(56.7%)	51(43.3%)
Amikacin(30µg)	67(56.7%)	51(43.3%)
Netilmicin(30µg)	69(58.4%)	49(41.6%)
Ciprofloxacin(5µg)-In 86 non-urinary isolates	48(55.8%)	38(44.2%)
Norfloxacin(30µg)-In 32 urinary isolates	18(56.2%)	14(43.8%)
Imipenem(10µg)	97(82.2%)	21(17.8%)
Meropenem(10µg)	79(66.9%)	39(33.1%)
Polymixin B(300 Units)	118(100%)	0(0%)
Colistin(10 µg)	118(100%)	0(0%)

Discussion

P.aeruginosa is one of the important causes of morbidity among hospital patients. In the present study, the isolation rate of *P.aeruginosa* from all processed samples was 8.2%(118/1443), which is comparable to other studies by Parmar et al[13], Patel et al[14], More et al[15] and Kalidas et al [16] who have reported it as 8.1%, 8.9% , 9.2% and 6.1% respectively. However, Rajat et al[17,] Javiya et al[18] and Shenoy et al[19] reported it to be higher i.e-15.8%, 20.28% and 31.52% respectively. A study by Juyal et al [20] reported very low isolation rate of *P.aeruginosa* i.e- 3.63%. Prevalence of *P.aeruginosa* among total isolates has been reported to be 15.9%(118/739) in this study which is very close to study by Parmar et al(15.9%) [13]. However, according to Rajat et al [17] prevalence of *P.aeruginosa* among total isolates was almost double than our study (31.3%) In the present study, sex-wise prevalence of clinical isolates shows that infections caused by *P.aeruginosa* are more common in males(64%) as compared to females(36%).This is comparable with studies of Rajat et al[17], Javia et al[18], Jamshaid et al[21] and Rashid et al.[22] In the present study, highest percentage of *P.aeruginosa* was recorded from pus samples(36.4%) followed by urine samples(27.1%).These results are in line with various other studies where prevalence was also found higher in samples of pus and urine. [13,15,17,23,24]

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Table 5 shows prevalence of *P.aeruginosa* among pus and urine samples reported in other studies. The variation among these studies could be due to the difference in study period, sample size, geographical location and patient population.

Table 5 :Prevalence of *P.aeruginosa* among pus and urine samples reported in other studies and present study

Type of sample	Present study	More et al[15]	Bimla B and Rehha B[23]	Parmar et al[13]	Rajat et al[17]	Pathi et al[24]
Pus	36.4%	14.1%	41.42%	48.6%	71%	23.2%
Urine	27.1%	7.28%	20.08%	19%	16%	29.1%

P.aeruginosa is inherently resistant to many antimicrobial agents, thus posing a great challenge in community acquired and nosocomial infections. In our study also, *P.aeruginosa* was found resistant to most of the commonly used antimicrobial agents.It was extremely resistant to ceftazidime (62.8%) and cefepime(61.1%) which is similar to other studies.Juyal et al[17], Patel et al[14] and Kalidas et al [16] have reported resistance of *P.aeruginosa* for ceftazidime to be 68.8%, 75.4% and 71.3% respectively. Juyal et al[17] reported resistance towards cefepime as 61.4% whereas very low level of resistance was recorded by Bimla B and Rehha B[23](25.3%) and Sadhna et al[25](28%). Higher resistance to cephalosporins may be due to production of extended spectrum beta lactamases(ESBL's) by this bacteria.[14]

In this study, amikacin resistance was recorded as 43.3% which is quite similar to Sarkar et al[26] (40.9%) Bimla B and Rehha B[23](39.3%) and Rajat et al[17](45%). In our study, resistance to netilmicin was 41.6% which is comparable with studies by Bimla B and Rehha B[23](46.6%) and Sadhna et al[25](37%).

In our study, piperacillin-tazobactum was effective antibiotic as only 34.8% resistance was recorded for this antibiotic.This is almost similar to other studies- Patel et al[14](24.1%) and Kalidas et al[16](46.5%). Sensitivity of *P.aeruginosa* to imipenem in our study was 82.2% .Various other studies[14,16,23] have also reported Imipenem as effective antibiotic to treat infections caused by *P.aeruginosa* with sensitivity of 94%, 91% and 89% respectively.In various studies across the world, varying rates of resistance(4-60%) have been reported for imipenem and meropenem.[27] However in our study, resistance towards meropnem(33.1%) was higher as compared to imipenem(17.1%).Overexpression of the *Mex* AB-*Opr* M efflux system is known to affect meropenem efficacy but not that of imipenem.In addition, the *Mex* CD-*Opr* J and *Mex* XY-*Opr* M efflux systems may be involved in reduced susceptibility to meropenem.[28]

Polymixin B and colistin are the most effective antimicrobial agents against P.aeruginosa as 100% sensitivity was recorded for them.However, they are very costly and their nephrotoxicity limits their use and should be therefore used only at the last resort.

Conclusion

This has possibly resulted due to indiscriminate use of antibiotics, lack of awareness, unhygienic conditions and patient non-compliance. The study also indicates that sensitivity pattern changes from

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hospital to hospital and population. Thus, various International authorities emphasize that every hospital should have it's own sensitivity pattern since standard sensitivity pattern may not hold true for every area. This would help and guide the physicians in prescribing the right combinations of antimicrobials to limit and prevent the emergence of multidrug resistant strains of *P.aeruginosa* in the hospital environment.

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