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Original Research Article

Prevalence and antibiotic sensitivity pattern of pseudomonas aeruginosa isolates from respiratory samples, pus samples and body fluids in a tertiary care hospital, Kashmir

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ABSTRACT

Pseudomonas aeruginosa has established itself as an important pathogen because of its ability to survive the hostile environments and causing life threatening infections. This gram negative bacillus has acquired resistance to different classes of antimicrobials thereby increasing the morbidity and mortality. In the present study we aim to analyze the rate of isolation of this opportunistic pathogen from various body fluids as well as respiratory and pus samples and the antimicrobial resistance profile of all these isolates. Standard microbiological techniques were used for its isolation, culture and identification followed by the antimicrobial sensitivity testing using disk diffusion method. Of the 3530 samples received over a two year period, the presence of *Pseudomonas aeruginosa* was detected in 71 samples (9.16%). A higher resistance was observed for ceftriaxone, ceftazidime and ciprofloxacin but a considerable number of isolates were sensitive to carbapenems. Most of the isolates were sensitive to polymyxin B.

1. Introduction

Pseudomonas aeruginosa accounts for nearly 10% of all hospital acquired infections and is considered the fifth most common pathogen among microbes prevailing in hospital environments.[1] This bacterium is frequently isolated as an opportunistic pathogen in recurrent infections of hospitalized and immune-compromised patients.[2,3] The capability of surviving a variety of environmental conditions makes it a ubiquitous pathogen allowing it to persist on numerous living and non-living surfaces due to minimal nutritional requirements.[4]

The pyogenic infections are either mono-microbial or poly -microbial with an average of 5-6 organisms often involved in the infections that are caused by a mixture of aerobic and anaerobic organisms. Among the mono-microbial species, *Staphylococcus aureus* and *Staphylococcus epidermidis* are the most common organisms likely to be encountered followed by Gram-negative bacilli, such as *Klebsiella* spp., *Pseudomonas* spp., *Escherichia coli*, *Proteus* spp., *Citrobacter* spp., *Acinetobacter* spp., *Enterobacter* spp. and others.[5]

Pseudomonas aeruginosa is a well established pathogen in victims of severe burns causing bacteremia, chronic lung infection in cystic fibrosis patients, and otitis media or malignant otitis externa.

Recently this bacteria has acquired resistance to various antimicrobial agents.[6,7] With the widespread use of antibiotics such as quinolones both in the hospital and community settings, multidrug resistant *Pseudomonas aeruginosa* isolates continue to escalate rapidly.[8] This analysis correlated well with an increase in number of *Pseudomonas aeruginosa* among our laboratory isolates over a period of time. Hence the present retrospective study was carried out to observe the pattern of infections caused by *Pseudomonas aeruginosa* and susceptibility pattern of these isolates from respiratory samples, pus samples and body fluids collected in the department of microbiology, Sher-i-kashmir institute of medical sciences

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Continuous monitoring of the prevalence of *Pseudomonas aeruginosa* isolates in a particular region and their antibiotic sensitivity profile is becoming a necessity due to potentially rapid spread of resistance and the implications of infections on patient morbidity, mortality and health care costs.

2. Objectives

The aims of this study were:

1. To determine the prevalence of *Pseudomonas aeruginosa* in our setup.
2. T o understand the current statistics of the antimic robial resistance pattern of this gram negative opportunistic pathogen.

3. Materials and Methods

3.1. Study setting

The study was conducted in the department of Microbiology, Sher-i-kashmir institute of medical sciences Medical College Hospital, Bemina, Kashmir.

3.2. Study design and period

This is a retrospective observational study conducted over a period of two years from January 2017 to December 2018.

3.3. Inclusion criteria

All respiratory samples, pus samples and body fluids collected from patients of all ages and both genders visiting the in-patient and out-patient departments.

The various clinical specimen included pus samples or swabs from wounds, bed sores, burn wounds and aural swab; respiratory samples like sputum, broncheo-alveolar lavage (BAL), endotracheal aspirates (ETA) as well as body fluids like ascitic and pleural fluid.

3.4. Methods

All samples were processed according to standard laboratory methods.[9] All isolates were observed for colony morphology, microscopic examination and relevant biochemical tests before the final identification of that bacterial species.[10]

An array of identification tests including colony morphology for size, shape and pigmentation on different culture media, color of colonies on MacConkey agar (non-lactose fermenting pale colonies), gram staining (gram negative bacilli), motility, oxidase test (positive) and failure to ferment glucose were used to identify isolates as *Pseudomonas* species. *Pseudomonas aeruginos*a was identified by a battery of tests which included indole test, methyl red test, citrate test, urease test, triple sugar iron agar, arginine dihydrolase activity, reduction of nitrate to nitrite and production of a classical bluish green pigmentation.[10]

Antimicrobial susceptibility testing was done by disk diffusion method following the recommendations of CLSI guidelines 2017 against a panel of anti-pseudomonal antimi crobials including imipenem (10μg), ceftazidime (30μg), cotrimoxazole (25μg), ciprofloxacin (5μg), imipenem (10μg), meropenem (10μg), gentamicin (10μg), ceftriaxone (30μg), tobramycin (30 μg), cefixime (30μg), ofloxacin (5μg), amikacin (30μg) and piperacillin- tazobactam (100/10μg) based on standard strengths.[11]

According to the sensitivity pattern, the strains were identified as MDR (isolates resistant to at least one antimicrobial agent in three or more antimicrobial groups), XDR (isolates resistant to at least one antimicrobial agent in six or more antimicrobial groups) and PDR (isolates resistant to all anti-Pseudomonal antimicrobial agents).[12]

4. Results

A total of 3530 pus samples were received in the department of microbiology during these two years out of which 775(22%) showed positive growth on bacteriological culture. Among the positive cultures, 71 (9.16%) isolates were identified as *Pseudomonas aeruginosa* (Figure 1) out of which 29(40.84%) were cultured from pus samples received from out-patient department and 42(59.16%) from pus samples of in-patient department. About 47 patients (66.19%) were males and 24 patients (33.80%) were females. Most of the patients (50.70%) were aged between 25-45 years, while about 42.25% of the patients were below 25 years and 7.05% were above 45 years.

Thirty seven isolates of *Pseudomonas aeruginos*a (52.11%) were isolated from wound swabs only, among which 16.21% samples were received from out-patient department including surgical OPD and the rest 35.90% isolates were cultured from wound swabs of in-patient department. The IPD samples included around 20% samples from casualty wards, 8% from medicine wards and the rest (9%) from other units (burn unit etc). Other samples included were 18 aural swabs (25.35%), 8 tracheal aspirates (11.26%), 5 sputum samples (7.04%) and 3 others (4.22%) like bed sores, pleural fluid etc. All the ear swabs were collected from out-patient departments (Figure 2).

The isolates of *Pseudomona*s spp. from different samples showed variable sensitivities to different antimicrobial agents. Isolates from wound swabs showed more resistance to ceftazidime (70%), ceftriaxone (70%) and ciprofloxacin (65%). Least resistance was observed for polymyxin-B (5.5%), meropenem (24.4%), imipenem (24.4%) and piperacillin-tazobactam (30%). Among the isolates from aural swabs, highest sensitivity was seen to amikacin (83.3%) followed by ciprofloxacin (72.2%). The isolates from tracheal aspirates showed total resistance to ceftriax-

one, ciprofloxacin, ofloxacin, piperacillin-tazobactam and carbapenems (100% resistance). (Table 1)

A total of 18 isolates (25.35%) were found to be multidrug resistant (MDR). Only 5 isolates (7.04%) were extensively drug resistant (XDR). All XDR isolates were cultured from tracheal aspirates. None of the isolates were found to be pan- drug resistant

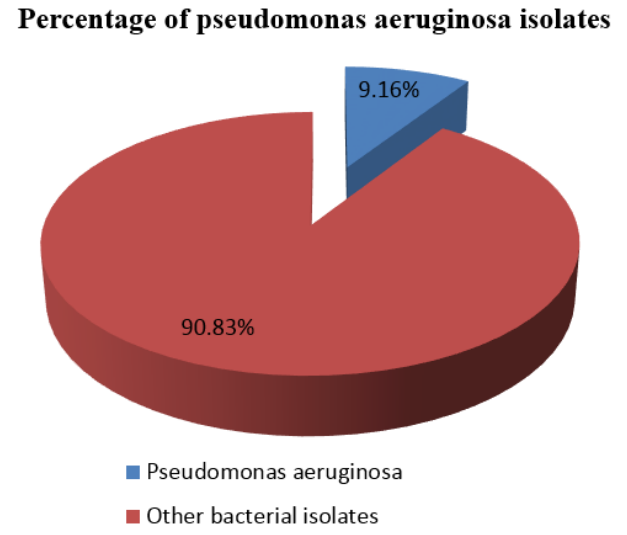

Fig. 1: Rate of isolation of Pseudomonas aeruginosa among the pathogenic bacterial isolates

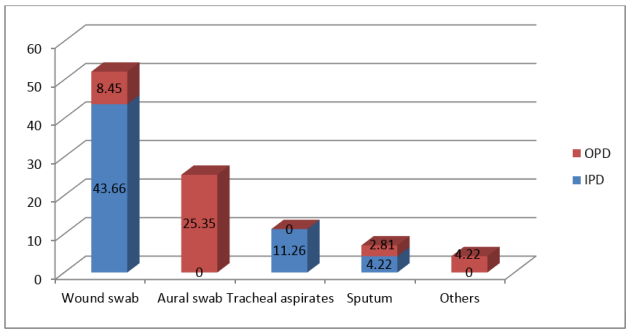

Fig. 2: Percentage isolation of Pseudomonas aeruginosa from various clinical samples

5. Discussion

Pseudomonas aeruginosa has established itself as a super bug because of the intrinsically evolved antibiotic resistance mechanisms. It has become one of the leading causes of hospital-acquired as well as community-acquired infections due to significant changes in the microbial genetics. In addition to this, the indiscriminate use of antibiotics has resulted in the rapid spread of acquired multidrug resistance (MDR) that has become a global problem.

In the present study, the rate of isolation of *Pseudomonas aeruginosa* from pus samples in our set up was observed to be 9.1 0% of the total culture positivity rates. This was found to be lower as compared to the rate of isolation of 21.85% and 18% for *Pseudomonas aeruginosa* as reported by Shrivastava *et al* (2014)[12] and Gad *et al* (2007)[13] respectively. A lower incidence was reported in a study conducted in Pakistan by Khan *et al* in the year 2008.[14]

In our study, the overall sensitivity pattern of the all isolates of *Pseudomonas aeruginosa* revealed highest resistance to gentamicin (66.5%), ceftazidime (59%), ceftriaxone (58.3%) and ciprofloxacin (42%). Highest sensitivity was observed for polymyxin B (78.5%) and amikacin (68.5%), followed by carbapenems (63.15%). In a study conducted by Jamatia *et al*, *Pseudomonas* spp. were 100% sensitive to imipenem and amikacin.[5] In one of the studies from Iran that was published in the year 2003, the researchers observed that the resistance of antimicrobials to *Pseudomonas aeruginosa* was very high for gentamicin (93.7%), ceftazidime (96%), amikacin (93%) and ciprofloxacin (86%).[15]

In our study, MDR *Pseudomonas aeruginosa* were 25.35% of all isolates of *Pseudomonas aeruginosa* which is in concordance with the study conducted by Shrivastava *et al* (24.70%),[12] Gill MM *et al* (27%)[16] and Ullah F *et al* (29%).[17] In contrast a prevalence of 14% was observed in a study conducted in Houston by Tam *et al.*[18] while a prevalence of 45.20% has been reported in India by Amutha R *et al.*[19]

In our study, prevalence of XDR *Pseudomonas* isolates was 7.04% which is lower from the study conducted by Shrivastava *et al*[12] and Pena C *et al.*[20]

Almost 60% of the XDR isolates were cultured from tracheal aspirates and 40% from wound swabs. None of the isolates from aural swab, all of which were collected from the out-patient department, were found to be XDR. This revealed the specific proliferation of resistant strains in the hospital environment under selection pressure.

In our study, we observed that the isolates from tracheal aspirates collected from ICU and hospital wards were more resistant than other specimen and this pattern is very alarming. Most of these patients were elderly and severely debilitated by chronic illness. This pattern of rising resistance among isolates indicates the improper use of antibiotics in the hospital settings and importance of strict implications of stewardship programmes. Moreover, it also suggests that these commonly used drugs can no more be used as empirical therapy for infections caused by *Pseudomonas aeruginosa*. In fact the irrational and inappropriate use of antibiotics is responsible for the development of resistance of *Pseudomonas* to antibiotic mono therapy.

Hence, there is an urgent need to emphasize the formation of effective antibiotic policy for better management of

Table 1: Antibiotic sensitivity pattern of Pseudomonas isolates from various clinical samples

Antimicrobial agents	Wound swab (n=52.11%)	Aural swab (n=25.35%)	Tracheal aspirate (n=11.26%)	Sputum (n=7.04%)	Others (n=4.22%)
Ceftriaxone (S%)	30%	50%	0%	42%	45%
Ceftazidime (S%)	30%	55.5%	25%	50%	45%
Ciprofloxacin (S%)	35%	72.2%	0%	80%	70%
Ofloxacin (S%)	63%	NT	0%	80%	72%
Amikacin (S%)	31.5%	83.3%	37.5%	70%	92%
Imipenem (S%)	75.6%	NT	0%	80%	97%
Meropenem (S%)	75.6%	NT	0%	80%	95%
Piperacillin-tazobactam (S%)	70%	NT	0%	NT	82%
Polymyxin B (S%)	94.5%	NT	62.5%	NT	NT
Gentamicin (S%)	66.5%	50%	25%	30%	72%

NT= not tested; S%=percentage sensitive; n=percentage of isolates

these patients. This can be done by regular antimicrobial susceptibilty surveillance and monitoring the area-wise resistance pattern of such isolates.[12]

6. Conclusion

Pseudomonas aeruginosa has been commonly reported in nosocomial infections. Increasing resistance of this organism demands constant surveillance of antimicrobial resistance trends, administration of appropriate antibiotics and use of combination therapy along with implementation of infection control practices. This will eventually help in time management and accurate administration of drugs thereby reducing the possibility of development of drug resistance and failure of antimicrobial therapies.

7. Source of funding

None

8. Conflict of interest

The authors declare that they do no t have any conflicts of interest.

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