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Pseudomonas aeruginosa Positivity and Sensitivity in Invasive Bloodstream Infections Using Automated Bactec in Tertiary Care Teaching Hospital of North India

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Background: *Pseudomonas aeruginosa* is one of the most commonly encountered gram-negative aerobic bacilli in the differential diagnosis of several probable hospital-acquired infections. Hence, the present study is designed to determine the Pseudomonas positivity and sensitivity in Invasive bloodstream infections using automated Bactec systems as the Antibiotic Sensitivity Profiles differ from one clinical setting to another.
Material and Methods: All the blood culture samples received in the Department of Microbiology for culture by Bactec Bd fx from July 2015 to June 2016 were included in the study. The blood culture was observed in the Bactec bd fx system for at least 5 days before they are reported as sterile.
Results: Among the total 1275 cultures which were positive for bacteria, 931(73.02%) were positive for gram-negative bacteria. Among the total of 931culture which were positive for gram-negative bacteria, *Pseudomonas aeruginosa* was isolated in 120(12.89%) cultures. Maximum was found in the age group of 0-1 years 33(27.50%) followed by 19-45 years 26(21.67%).

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Pseudomonas spp isolates were 100.00% sensitive to Colistin followed by Levofloxacin 84.44%, Piperacillin Tazobactum 82.50%, PB 77.50%, Amikacin 75.00%, Cefepime 75.00% while Pseudomonas spp isolates were 90% resistant to Ampicillin followed by Ceftazidime clavulanic acid 82.64% and Aztreonam 70.31%.

Conclusion: *Pseudomonas aeruginosa* is one of the most common organisms among Gram-Negative isolates and the most commonly isolated in the neonate and infant age group. All the Pseudomonas isolates showed maximum sensitivity to Colistin followed by Levofloxacin, Piperacillin Tazobactum, Amikacin while they were most resistant to Ampicillin followed by Ceftazidime clavulanic acid and Aztreonam. Some alternative novel techniques need to be developed to counter the increasing menace of antibiotic resistance in this particular pathogenic bacteria

Keywords: Blood stream infections; culture, bactec; antimicrobial resistance; pseudomonas positivity and sensitivity.

1. INTRODUCTION

Pseudomonas aeruginosa is one of the most commonly encountered gram-negative aerobic bacilli in the differential diagnosis of many probable hospital-acquired infections. We have to consider this pathogen due to the fact because that it can cause severe life-threatening hospital-acquired infections, cystic fibrosis patients, in immunocompromised patients, in invasive prosthetic device-related infections, keratitis in contact lens users, burn patients, and antibiotic-resistant in most of cases, and is associated with a high mortality rate [1].

In bloodstream infections (BSIs) mortality due to *P*seudomonas *aeruginosa* remains an issue of serious concern and data for survival benefits of various antimicrobial treatment therapies are contradicting in nature. Combination therapy for the treatment of *P*seudomonas *aeruginosa* BSI is initiated due to different parameters: first, to provide coverage for the suspected pathogen while results of identification and susceptibility testing are awaited, second, to avoid resistance to emerge during treatment and third, due to possible synergistic activity in the initiated regimen resulting in better clinical results and outcome [2].

Pseudomonas aeruginosa bloodstream infection is associated with increased duration of hospital stay, patient mortality, and increased healthcare costs having a significant impact on patients' or their families' financial condition and a burden on the economy as a whole [3]. Blood cultures are a diagnostic tool and play an important role and valuable role whenever required to determine the causative pathogen in cases where there is clinical evidence of sepsis or FUO or unknown systemic infection [4].

Indira Gandhi Medical College (IGMC) Shimla is a Tertiary care hospital and patients with above mentioned clinical conditions are treated both in the outpatient department(OPD)and inpatient department (IPD) and usually require blood culture to establish the etiological diagnosis.

Previously no study has been done in IGMC Shimla using an automated Bactec BD FX machine for blood culture. Hence, the present study is designed to determine the Pseudomonas positivity and sensitivity in Invasive bloodstream infections using automated Bactec systems.

1.1 Aims and Objectives

To determine Pseudomonas positivity and sensitivity in Invasive bloodstream infections using automated Bactec systems.

2. MATERIALS AND METHODS

2.1 Study Design

Prospective observational study.

2.2 Study Setting

Department of Microbiology, Indira Gandhi Medical College and Hospital, Shimla.

2.3 Study Period

One year from July 2015 to June 2016.

2.4 Inclusion Criteria

1. All the blood culture samples received in the department of microbiology for blood culture by Bactec bd fx system.
2. Patients willing for study.

3. Blood cultures from all age groups.

2.5 Exclusion Criteria

1. Patient not willing to study.
2. Blood cultures showing mixed growth

2.6 METHODOLOGY

All the blood culture samples received in the Department of Microbiology for culture by Bactec Bd fx were included in the study. The blood culture was observed in the Bactec bd fx system for at least 5 days before they are reported as sterile.

The sample to be tested is inoculated into the Bactec ™ plus aerobic/f culture bottle for adults and Bactec ™ Peds plus/f for children which is then inserted into the Bd Bactec fluorescent series instrument for incubation. Each bottle has a sensor that can detect an increase in co2 produced by the growth of microorganisms. The sensor monitors every 10 minutes for fluorescence to increase, which is proportional to the amount of co2 present. A positive reading indicates the presumptive presence of viable microorganisms in the bottle. The positive bottle will be subcultured on Blood agar and MacConkey^{s} agar plates. Following the subculture on solid media from each positive bottle a smear will be prepared for gram staining from that blood culture bottle. The Gram-stained smear will be examined for the presence of microorganisms and presumptive report conveyed to respective departments. The Blood agar and Mac Conkey agar plates will be incubated aerobically at 37ºc for 24 to 48 hrs and then observed for the growth of bacteria. All bacterial isolates will be identified using standard biochemical identification methods. All the positive isolates were stocked.

2.7 Statistical Analysis

The data was analyzed using statistical analysis-epi info7 Tthe data collected was entered into a spreadsheet. The data was checked for any missing values and completed. analysis in terms of demographic variables, positivity in the processed samples, type of species prevalent, was done using statistical software epi-info version 7(7.1.1.0).

3. RESULTS

In the current study, among the total of 5473 samples suspected of BSI's received in the Department of Microbiology, IGMC, Shimla, 1441 were positive. Among the total positive culture, 1275(88.48%) were positive for Bacteria while 166(11.52%) were positive for Fungi. Among the total 1275 cultures which were positive for bacteria, 931(73.02%) were positive for gram-negative bacteria while the rest 344(26.98%) were of gram-positive bacteria.

Among the total of 931culture which were positive for gram-negative bacteria, *Pseudomonas aeruginosa* was isolated in 120(12.89%) cultures. Maximum Pseudomonas spp isolates were found in age group of 0-1 years (27.50%) followed by 19-45 year (21.67%), 46-65 years (18.33%), >66 years (13.33%) , 12.50% in 6-18 years and 6.67% in 2-5 years.(Table 1) (Fig. 1).

Table 1. Distribution of pseudomonas infection (n=120)

Serial No.	AGE-GROUP	Frequency	Percent
1.	0-1	33	27.50%
2.	2-5	8	6.67%
3.	6-18	15	12.50%
4.	19-45	26	21.67%
5.	46-65	22	18.33%
6.	≥66	16	13.33%
7.	Total	120	100.00%

Pseudomonas aeruginosa isolates were 100.00% sensitive to Colistin followed by Levofloxacin 84.44%, Piperacillin Tazobactum 82.50%, PB 77.50%, Amikacin 75.00%, Cefepime 75.00%, Amoxiclav 68.57%, Imipenem 62.50%, Ceftriaxone 60.00%, Ceftazidime 57.14%, Ciprofloxcin 54.17%, Meropenem 54.10%, Gentamycin 41.67%, Piperacillin 33.33%, Aztreonam 23.44%, Ceftazidime Clavulanic acid 16.53%, and Ampicillin 10.00% (Table 2).

Pseudomonas aeruginosa isolates were 90% resistant to Ampicillin followed by Ceftazidime Clavulanic acid 82.64%, Aztreonam 70.31%, Piperacillin 66.67%, Gentamycin 58.33%, Ciprofloxcin 45.83%, Mori 44.26% Ceftriaxone 40%, CFZ 38.1%, Imipenem 37.5%, Amoxiclav 31.43%, Amikacin 25%,Cefepime 25%, PB 22.5%, Piperacillin Tazobactum 17.5% and Levofloxacin 13.33% (Table 2).

4. DISCUSSION

In the present study, *Pseudomonas aeruginosa* isolates were 100.00% sensitive to Colistin

followed by Levofloxacin 84.44%, Piperacillin Tazobactum 82.50%, PB 77.50%, Amikacin 75.00%, cefepime 75.00%, while Pseudomonas spp isolates were 90% resistant to Ampicillin, followed by CFZ/CLV 82.64% and Aztreonam 70.31%.

Karlowsky JA et al have shown that P. aeruginosa was highly susceptible to Amikacin (92.3%) and Piperacillin-Tazobactam (91%) similar to the present study [5].

Pseudomonas aeruginosa is an opportunistic and most commonly implicated hospital-acquired pathogen which causes serious infections in patients receiving immune suppressive therapy, burns. iv lines or catheter-related infections and newborns. Bacterial bloodstream infections are considered to be severe infections associated with increased rates of mortality, morbidity, and healthcare costs to the patients. In many Health care providing centers or hospitals, *Pseudomonas aeruginosa* has become the most commonly isolated gram-negative bacterial

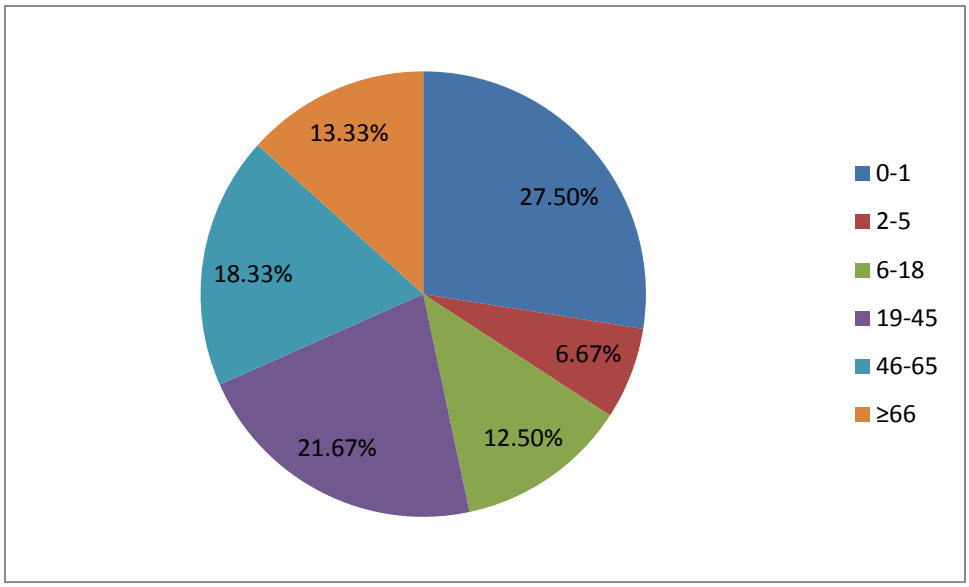

Fig. 1. Distribution of pseudomonas infection (n=120)

Table 2. Sensitivity and resistance of pseudomonas spp

Drugs	Sensitive	ses%	Resistant	res%	Intermediate	int%	Total
Amikacin	30	75.00	10	25.00	0	0.00	40
Amoxiclav	24	68.57	11	31.43	0	0.00	35
Ampicillin	12	10.00	108	90.00	0	0.00	120
Imipenem	25	62.50	15	37.50	0	0.00	40
Aztreonam	30	23.44	90	70.31	8	6.25	128
Ceftazidime Clavulanic	20	16.53	100	82.64	1	0.83	121
Ceftriaxone	24	60.00	16	40.00	0	0.00	40
Ceftazidime	24	57.14	16	38.10	2	4.76	42
Ciprofloxcin	65	54.17	55	45.83	0	0.00	120
cefepime	30	75.00	10	25.00	0	0.00	40
Colistin	40	100.00	0	0.00	0	0.00	40
Gentamycin	50	41.67	70	58.33	0	0.00	120
Levofloxacin	38	84.44	6	13.33	1	2.22	45
Meropenem	66	54.10	54	44.26	2	1.64	122
Polymixin B	31	77.50	9	22.50	0	0.00	40
Piperacill	40	33.33	80	66.67	0	0.00	120
Piperacillin Tazobactum	33	82.50	7	17.50	0	0.00	40

pathogen associated with serious hospital-acquired infections, especially within Neonatal or Paediatric intensive care units or General Intensive care units. Pseudomonas infections can easily develop resistance to multiple types of antibiotics seven with synergistic action. The hospital mortality associated with *Pseudomonas aeruginosa* bloodstream infections is reported to be more than 20% in most serious infections and is reported to be highest among patients receiving inappropriate empirical antimicrobial treatment [6].

Bloodstream infection (BSI) due to *Pseudomonas aeruginosa* has a very important clinical impact concerning drug resistance determinants because of the various mechanisms associated with increased drug resistance prevalent in this pathogenic bacteria. It can acquire genes for resistance by any of the mentioned methods like plasmids, integrons, prophages through the methods of horizontal gene transfer and from the same or different bacteria. Horizontal methods include transduction, conjugation. The subsequent production of PER-1 Extended-spectrum Beta-lactamase or Metallo beta-lactamases is due to plasmids and integrons by some serotypes [7]. The increase in Multidrug resistance has made treating these infections quite difficult to handle because of intrinsic or acquired resistance in this serotype. Some recent studies suggest the adaptive factors associated with resistance like the formation of Bio-films and evolution of Multidrug tolerator persistent cells lead to relapsing infections by this serotype. *P*seudomonas aeruginosa strains which were isolated from cystic fibrosis patients were found to have persister cells [8,9], making the *P. aeruginosa* serotypes highly resistant to conventional antibiotics with the ability to become multidrug-tolerant.

Furthermore, the environment has a crucial role in the formation and further proliferation of persister cells. Nutrient deprivation increases the formation of *P. aeruginosa* persister cells via the mechanism called the stringent response that is controlled by the bacterial signaling molecule alarmone (p)ppGpp [10,9] (Nguyen et al., 2011). Due to the above-mentioned factors, It can easily develop resistance even during the ongoing course of treatment. Some new antibiotics are showing good results in *P. aeruginosa* killing and have a lesser rate of resistance development because of their modes of action, efficient drug delivery (e.g. inhaled antibiotics), and their ability to resistance to modification by bacterial enzymes Chatterjee et al., [11] The new antibiotics introduced are Doripenem, Plazomicin, and POL7001. The unnecessary use and misuse of antibiotics can result in side effects and the leading to the development of drug-resistant serotypes [12]. Moreover, the development of new antibiotics is a very costly, limited, and time-taking process. Thus, the dire need for novel therapeutic modalities to treat *Pseudomonas aeruginosa* infections is highly desirable and need of the hour. These novel therapeutic strategies can act either alone or in combination with conventional therapies to combat *Pseudomonas aeruginosa* infections, and they include inhibition of quorum sensing and bacterial lectins, use of iron chelation, phage therapy, vaccine strategy, the use of nanoparticles, antimicrobial peptides, and electrochemical scaffolds Hurley et al., [13].

5. CONCLUSION

Pseudomonas aeruginosa is one of the most common organisms among Gram-Negative isolates and the most commonly isolated in the neonate and infant age group. All the Pseudomonas isolates showed maximum sensitivity to Colistin followed by Levofloxacin, Piperacillin Tazobactum, Amikacin while they were most resistant to Ampicillin followed by Ceftazidime clavulanic acid and Aztreonam Some alternative techniques need to be developed to counter the increasing menace of antibiotic resistance in this particular pathogenic bacteria.

CONSENT AND ETHICAL APPROVAL

As per international standard or university standard guideline Patient's consent and ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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