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Original article

Prevalence and antimicrobial susceptibility profile of *Pseudomonas* species isolated from blood cultures from a tertiary care hospital of Haryana, India

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ABSTRACT

Objectives: Bacteremia by *Pseudomonas* species is a global health challenge as it is a major pathogen associated with significant morbidity and mortality. Increasing prevalence of MDR *Pseudomonas* in health care settings has worsened the situation globally.

Materials and methods: The study was carried out in department of Microbiology, Pt. Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences (Pt. B.D.S P.G.I.M.S), Rohtak, Haryana, India.

Results: Most sensitive antibiotic for the treatment of *Pseudomonas* was found to be PIT (92.48%), followed by HLG (85.45%), CIP (84.70%), MRP (80.30%), IMP (76.28%). The maximum resistance was shown by antibiotic CAZ (46.41%) and AT (44.33%). There was no resistance observed against Polymyxin-B by *Pseudomonas* species.

Conclusion: MDR *Pseudomonas* species is the third most prevalent blood stream pathogen causing mortality and morbidity in hospital settings. As it shows resistance to multiple antibiotics hence it should be treated as per antimicrobial susceptibility testing. Proper hospital infection control practices can prevent the spread of MDR *Pseudomonas* saving the life of many patients.

Keywords: Bacteremia; Blood culture; *Pseudomonas aeruginosa*; Multidrug resistance

Introduction

Bacteremia is the presence of viable bacteria in blood and culturing that considered as a gold standard for the diagnosis of bacteremia[1,2]. Blood stream infections (BSI) are highly dangerous and sometime cause death of pediatric as well as adult patients. Also it cause severe economic burden on global economy[3]. Sepsis is described as serious organ dysfunction which may cause death, is induced by a deregulated host response to infection which triggered by the pathogenic bacteremia[4]. Bacteria can enter the body through skin fissures or blood vessels on the surface, as well as by the direct entry of infected material into the blood circulation or inhalation droplet through air. BSIs are becoming more common, resulting in

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significant mortality and morbidity. Bacteria, fungi, protozoa, and viruses are among the microorganisms that can cause BSIs and more than 90% of BSI caused by bacteria alone[5].The bacteremia which cause infections in the patients are grouped in two major categories that is Gram negative and Gram-positive species. However cases of infection caused by the gram negative bacteremia is more prevalent than gram positive bacteremia, also gram negative bacteremia acquire more resistance to the antibiotics[6]. The resistance of the gram negative bacteremia and prevalence in the patients has sparingly studied. Detailed study of the bacterial infection and antibiotic resistance of the bacteria is crucial and necessary for strong public health concern. The most common microorganisms isolated from the blood cultures are gram negative, gram positive bacteria and yeasts. Around 60 to 70% of BSIs caused by Gram negative bacteria which includes *Klebsiella* sps., *Acinetobacter* sps., *E. coli*, *Salmonella* sps., and *Pseudomonas* sps. on the other hand, Gram positive bacteria particularly *Staphylococcus aureus*, *Staphylococcus epidermis*, *Streptococcus pyogenes*, *Streptococcus pneumonie* etc. are responsible for 20-40% BSIs[7-16.]

This study has been designed because *Pseudomonas* species are emerging as one of most common multidrug resistant pathogen in hospital settings and a major pathogen linked with substantial morbidity and mortality. Additionally it may cause severe infections due to mutation, production of type III Exotoxins and (T3SS) and four effecter proteins namely, exoS, exoT, exoU, and exoY. All these factors are responsible for poor clinical results in MDR *P. aeruginosa* infections[17,18]. In addition to all these factors its ability to form biofilms under different conditions also make it a dreaded pathogen[11]. So continuous evaluation of its prevalence, morphological characteristics and mechanisms of drug resistance is necessary to minimize it adverse effects on society.

Material and Methods:

Study population

The study was carried out at the Department of Microbiology, Post Graduate Institute of Medical Sciences (PGIMS), Rohtak, Haryana, India for a period of two years from 1st January 2021 to 31st December 2022. The study was approved by the institutional human ethical committee of PGMIS, Rohtak. Blood samples were aseptically collected from the patients in sterile culture tube. From the adult patient approximately 10 ml blood and from pediatric patients around 2-3 ml of blood was withdrawn and cultured in brain heart infusion (BHI). The ration of blood and BHI was maintained 1:10. Culture were incubated at 37 °C and sub-cultures were done on blood agar and Mac Conkey agar after 24hours, 72 hours and 7th day (Figure 1). The sample was labeled as sterile or no growth if no bacterial growth was obtained even on 7th day of incubation. Growth of *Pseudomonas* species were identified on the basis of culture characteristics and biochemical reactions by following standard procedures. Morphological assessment using microscope, oxidase, catalase, indole, MR, VP and H_2S gas test were done and recorded[19].

(A) (B) (C)

Figure 1: Growth of *Pseudomonas aeruginosa* on MHA, blood agar and MacConkey agar medium

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Selective culture of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa were selectively grown oncetrimide agar medium, cetrimide inhibits bacterial growth except *P. aeruginosa* and enhances fluorescein and pyocyanin pigment production[20,21].

Antimicrobial susceptibility profiling:

Identification of the bacteria and preliminary antimicrobial susceptibility tests (ASTs) of all isolates were done by means of the Kirby Bauer disk diffusion methods as per the guidelines of Clinical and Laboratory Standards Institute (CLSI). Based on the zones of inhibition (ZOI) ASTs of isolates were categorized into sensitive(S), intermediate sensitive (IS) and resistant(R) for each antibiotic. The results were interpreted using CLSI reference break points[22]. The test was conducted for commonly available antibiotic drugs with following doses Piperacillin-Tazobactam (PIT; 100/10ug), Ceftazidime (CAZ; 30ug), Imipenem (IMP; 10ug), Meropenem (MRP; 10ug), High level Gentamicin (HLG; 120ug), Amikacin (AK; 30ug), Ciprofloxacin (CIP; 5ug), Minocycline (MI; 30ug), Polymyxin-B (PB; 300units), and Aztreonem (AT; 30ug).

A total 185 *Pseudomonas* species were isolated and examined during the period of two years (Table 1).Bacterial isolates which were resistant to three or more classes of antibiotics were defined as multidrug resistant (MDR)[20].

Table1. Occurrence of *Pseudomonas* species including MDR isolates during 2021-2022.

Period	Total samples	*Pseudomonas* isolated	Total MDR Isolated	% MDR *Pseudomonas*
Jan 2021- Dec 2021	13533	95	21	22.1
Jan 2022- Dec 2022	15054	90	18	20.0
Total	28587	185	39	21.1

Results and Discussion:

A total of 28587 blood sample were cultured and analyzed for the prevalence and antimicrobial susceptibility *Pseudomonas* species for the two consecutive year that is 2021-2022. A total of 13544and 15043 blood cultures were examined respectively during the year 2021 and 2022 following standard microbiological procedures. Out of 28587 blood samples, 2201 samples showed positive growth while rests were fails to grow. Among those grown samples total gram negative isolates were 1708and 493 were gram positive. Gram negative cultures included *Acinetobacter* species (588), *Klebsiella* species (n=659), *E. coli* (n=159), *Pseudomonas* species (n=185), *Citrobacter*(n=69), *Proteus* species(n=5) and *Salmonella typhi* and *paratyphi* (n=11) **(Figure 2)**. Gram positive isolates included coagulase negative *Staphylococcus aureus* CONS (n=186), *Staphylococcus aureus* (n=223), *Enterococcus* (n=77), and Group D *Streptococcus*(n=06) **(Figure 2).**The largest number of gram negative bacteria isolated from the blood of patients came to the hospital in the year 2021-2022 were *Klebsiella* species (38.58%) followed by *Acinatobacter* species (34.43%). *Pseudomonas* species, *E.coli*, *Citrobacter*, *Enterobacter* species, *Salmonella* (typhi & paratyphi), and *Proteus* species were 10.83, 9.31, 4.04, 1.87, 0.64 and 0.30 percent respectively.

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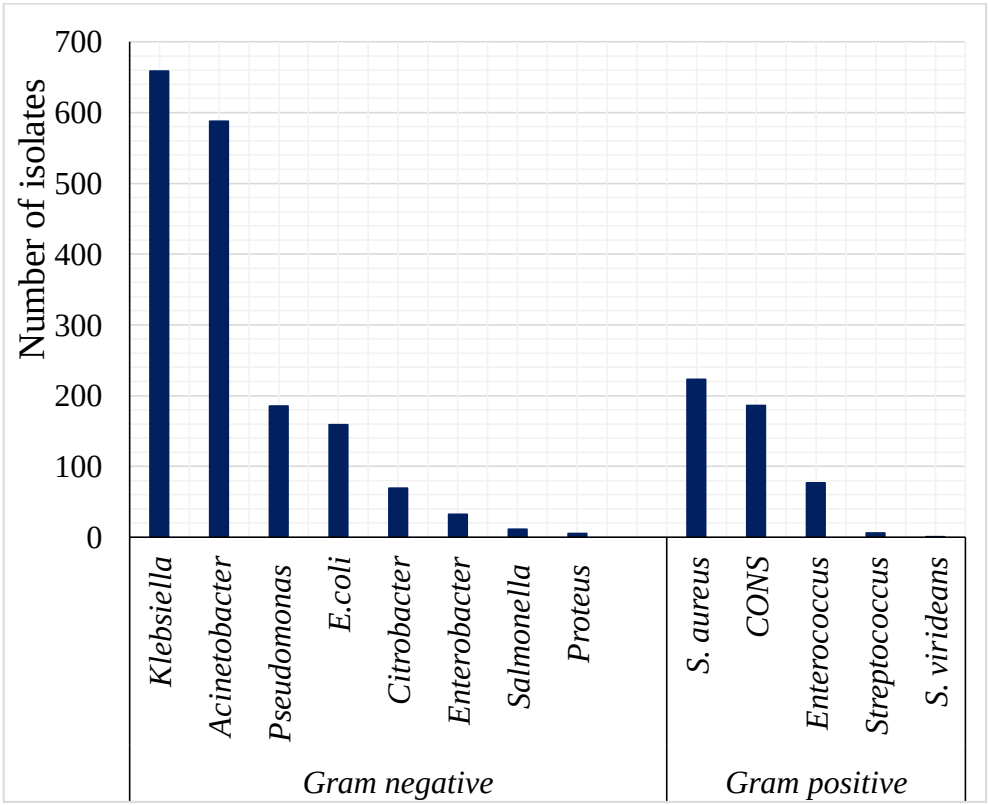

Figure 2. Detection of different types of Gram Negative Bacteria from blood culture during 2021-22.

Prevalence of 10 most prevalent blood stream pathogen were *Klebsiella* species 29.94% (659/2201), followed by *Acinetobacter* species 26.72% (588/2201), *Staphylococcus aureus* 10.13% (223/2201), CONS 8.45% (186/2201), *Pseudomonas* species 8.41% (185/2201), *E.coli* 7.22% (159/2201), *Enterococcus* 3.50% (77/2201), *Citrobacter* 3.13% (69/2201), *Enterobacter* 1.45% (32/2201) and *Salmonella* species 0.50% (11/2201) **(Figure 3)**.

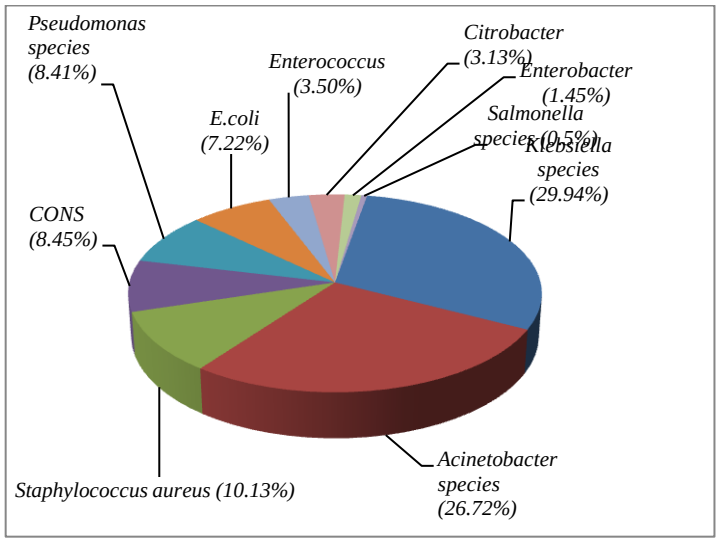

Figure 3. Most prevalent blood stream pathogen isolated

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In a similar study the prevalence of *K. pneumonia* was reported as the third most predominant bloodstream pathogen, whereas, *P. aeruginosa*is fourth most prevalent, proving the main pathogen in these infections. In the present study *Klebsiella* species infection has reached the top and *Pseudomonas* at 3rd position. This proves the significant contribution of this minor subset of Gram-negative species to disease burden.

Earlier studies on contribution blood born bacteria in human infection demonstrated that the contribution of *E. coli* ranged between 9 to 27%, *K. pneumoniae* accounts for 7.5 to 14% of case, *P. aeruginosa* accounts for 1.7 to 9%, and 2.3 to 4.7% for *A.baumannii* [23–25]. In the present study *E.coli* bactremia accounts for 7.22%, *K. pneumonia* 29.94%, *P.aeruginosa* 8.41% and *A. baumannii* 26.72% (table 3) which shows that the infection rate of *K.pneumonae* and *A.baumaniae* has significantly increased and it is almost same for *Pseudomonas*. However these changes in the data may depend on the geographical location, onset status of infection and also on age of the patients.

The order of prevalent blood stream pathogen was *Klebsiella* species > *Acinetobacter* species>*Staphylococcus aureus* > Coagulase Negative *Staphylococcus aureus* CONS > *Pseudomonas* species > *E.coli* > *Enterococcus* > *Citrobacter* > *Enterobacter* > *Salmonella typhi* and *paratyphi*. The similar findings were obtained in the study conducted by the Holmes et al (2021), ten most prevalent species isolated from clinical bacteremia during the year 1997 to 2016 that include *S. aureus, E. coli, K. pneumoniae, P. aeruginosa, E. faecalis, S. epidermidis, E. cloacae, S. pneumoniae, E. faecium,* and *A. baumannii*[26]. Anderson et al (2021) were also reported the six common gram negative bacteria which cause BSI those bacteria were *Escherichia coli, Serratia marcescens, Klebsiella pneumoniae, Enterobacter hormaechei, Citrobacter freundii, and Acinetobacter baumannii,* the current finding is also in accordance with this finding[27]. The frequency of pathogens varied according to geographic location, hospital or community onset status, and age of the patient. In the present study frequency of *Staphylococcus aureus* was 10.1% which was just half of prevalence reported by Diekema et al (2019). In their study, *S. aureus* was the most predominant pathogen (20.7%), and then *E. coli* (20.5%), *K. pneumoniae* (7.7%), *P. aeruginosa* (5.3%), and *E. faecalis* (5.2%). *S. aureus* was the most commonly isolated pathogen from 1997 to 2004, whereas after 2005*E. coli* was the most prevalent pathogen[28].

When the comparison was done in gram negative and gram positive bacteria causing BSIs, it was found that gram negative bacteria was most common blood stream pathogen responsible for 77.6% of bacteremia (1708/2201) and GPC for 22.4%(493/2201) (Table: 5). After *Acinetobacter* and *Klebsiella* species, *Pseudomonas* was the third most prevalent blood stream pathogen among the GNB. Prevalence of bacteremia in patients visiting the hospital. It depicts Gram negative bacteria (GNB): *Klebsiella* species (29.94%) and *Acinetobacter* species (26.72%) as most prevalent followed by gram positive (GPC) *Staphylococcusaureus* (10.13%) and CONS (8.45%). *Pseudomonas* species is the third most prevalent GNB.

Donkor et al (2023) were reported different GNB isolated from the patients from Ghana and observed their contribution in was 26.4% by *E. coli*, 25.5% by *K. pneumonia*, 12.0% of *Acinetobacter baumannii*, 7.5% by *Pseudomonas aeruginosa* and 23.1% by other Gram-negative bacteria[29]. Nagvekar et al (2020) were reported that among GNB detection, the sequence of prevalence was *E.coli* > *Klebsiella* > *Acinetobacter* >*Pseudomonas*while in the present study the prevalence sequence was *Klebsiella* > *Acinetobacter* > *Pseudomonas* > *E.coli.* This shows that infection rates ofeach different species was varyingamong studies which may be due to patient demographics and geography However, studies constantly proved that this pathogen is highly prevalent in suspected bacteremia cases showing MDR phenotype nearly 21.08%. Hence, detailed discussion about problematic pathogen in the context of bacteremia is important.

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General characteristics and biochemical tests of *Pseudomonas*

It is slender, non-fermenter, gram negative rods. The most common species which causes infections in humans include *Pseudomonas aeruginosa, Pseudomonas maltophilia, Pseudomonas mallei, and Pseudomonas putida* etc. The *Pseudomonas* strains utilizing citrate as carbon source and they were oxidase positive, also showed positive test for catalase and indole, MR, VP. They were showed negative test for H_2S gas (Figure 4).

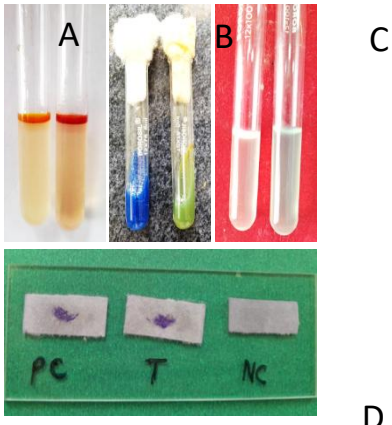

Figure 4. Biochemical tests of *Pseudomonas*: A. Indole negative with positive control; B. Citrate positive with negative control; C. Surface pellicle by *Pseudomonas aeruginosa*; and D. Oxidase test.

In the collected samples of bloods from patients from OPD and IPD, *Pseudomonas* species (n=185) score around 10.83% among gram negative bacteria and further analysis of *Pseudomonas* species revealed that *Pseudomonas aeruginosa* was the most prevalent i.e. 32% (n=60).Upon screening *Pseudomonas* species for the antibiotic sensitivity 39culture were showed multidrug resistant. When comparing the prevalence of *Pseudomonas* between OPD and IPD patients, infection with this species was significantly higher in IPD patients (OPD=13.1%; IPD= 21.8%) (Table 2).

Of 1708 GNB, 185 *Pseudomonas* species were detected which constitutes 10.83% of total GNB detected. In a similar study done by Donkor et al (2023); Bitew et al (2023) and Yamba et al (2023) they reported prevalence of *Pseudomonas aeruginosa* was 7.5%, 8% and 4.5% respectively which was lower than the present findings[24,29,31]. However in a similar study conducted by Ruiz-Ruigómez et al (2020) in BSI patients of Madrid, Spain and reported prevalence of *Pseudomonas* was 22.2% which is at least double of prevalence in present study[32]. In a study conducted by Weiner-Lastinger et al (2020) they reported the prevalence of *Pseudomonas aeruginosa* was 12.8%[33]. This reflects that infection rate of *Pseudomonas* species vary between different studies based on demography and geography.

MDR *Pseudomonas* species

Prevalence of MDR *Pseudomonas* species in the present study was found to be 21.76% in indoor patients (IPD) and 13.13% in outdoor patients (OPD)(Table 2). However the various studies reported the MDR *Pseudomonas* infection in IPD patients up to more than 50%. Almost all studies reported a very higher MDR *Pseudomonas* prevalence in admitted patients. This study also established this fact that majority of *Pseudomonas* infection in in-patients (IPDs). They probably acquired this infection due to lack of awareness of aseptic techniques among the nurses and other supporting staff and spread the infection to newly admitted patients. *Pseudomonas* can survive in extreme environments like disinfectants and antiseptics used in hospital, can colonize on surgical instruments, beds and clothes of doctors and nurses. So proper training, awareness and continuous evaluation is very important to reduce the rate of *Pseudomonas* infection.

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Table2. Occurrence of MDR *Pseudomonas* among OPD and IPD patients.

Year	Total *Pseudomonas* species detected		MDR *Pseudomonas* detected		Prevalence of MDR *Pseudomonas* (%)	
	OPD	IPD	OPD	IPD	OPD	IPD
2021 (n = 95)	03	92	00	21	0.0%	22.8%
2022 (n = 90)	12	78	02	16	16.7%	20.5%
Total (n = 185)	15	170	02	37	13.1%	21.8%

Antibiotic sensitivity test of *Pseudomonas species*

Antibiotic sensitivity test also reveals that the PIT was most effective drug against these pathogens, which kills around 95% of *Pseudomonas species*. After PIT, HLG and CIP both are equally kills around 85% of pathogens. However, polymyxin B (PB) showed 100% killing of pathogens but it is not preferred drug usually because it cause adverse effects to the host, so, this drug can be used as last option[34]

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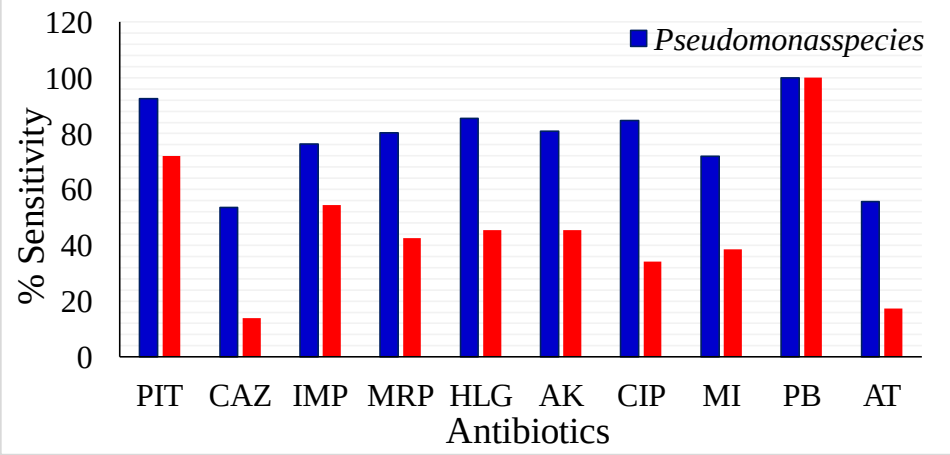

Figure 5. Sensitivity pattern of Pseudomonas species detected during 2021-2022.

Evaluation of the sensitivity of MDR *Pseudomonas* against antibiotics PB, PIT, IMP, HLG, AK, MRP, CAZ, AT, CIP and MI, their sensitivity was 100% against PB. The MDR *Pseudomonas* were most resistance against CAZ that is around 86%, then AT(83%),CIP(66%) and MI(62%) (Figure 5). Comparison of AST pattern among non MDR and MDR *Pseudomonas* showing significant difference. The drug Ciprofloxacin was showed 85% effectiveness against non MDR *Pseudomonas* whereas it was only 34% effective against MDR *Pseudomonas*. Similar pattern of low AST of drugs were also observed in HLG(40.02%),CAZ(39.70%), AT (38.41%), MRP (37.80%),MI(33.42%),IMP (21.87%) and PIT(20.50%) against MDR *Pseudomonas*. Keep aside effectiveness of PB, all other antibiotics were showed varied sensitivity against MDR *Pseudomonas*. Such varied sensitivity signified the construction of antimicrobial susceptibility profile for every isolated strain isolated.

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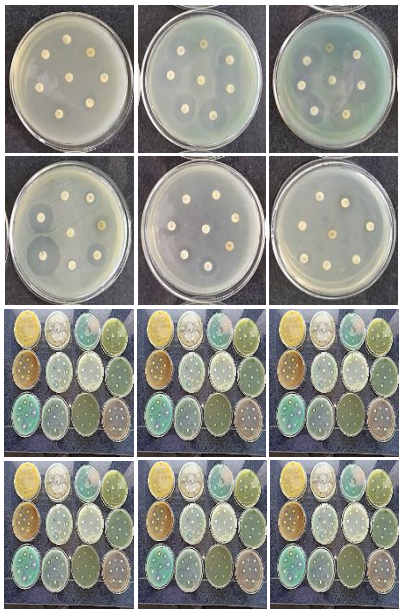

Figure 6. Screening of the antibiotic sensitivity of *Pseudoonas aeruginosa*, showing different antibiotics sensitivity by zones of inhibition.

Antibacterial susceptibility profile: use of proper antibiotics is very vital in treatment of bacterial infections. In this study antimicrobial study showed the varied susceptibility patterns (Figure 6).The most effective antibiotic in this study was found to be Polymyxin-B(100%) however because of its severe adverse effects on the patients this should be used as a last option if all other antibiotics are ineffective[35]. Second most sensitive antibiotic in the present study was found to be PIT (92.48%) followed by HLG (85.45%, CIP (84.70%), AK(80.80%), MRP (80.30%),IMP (76.28%), AT(55.67%)and CAZ(53.59%).

In a similar study done by Ndukwe et al (2021) in Nigeria reported 18.8% sensitivity to gentamycin, and 59.40% sensitivity to ciprofloxacin which are quite lower than our findings[36]. These finding suggest that there is better management for antibiotic prescription in Indian tertiary care hospital as compare to Nigeria.

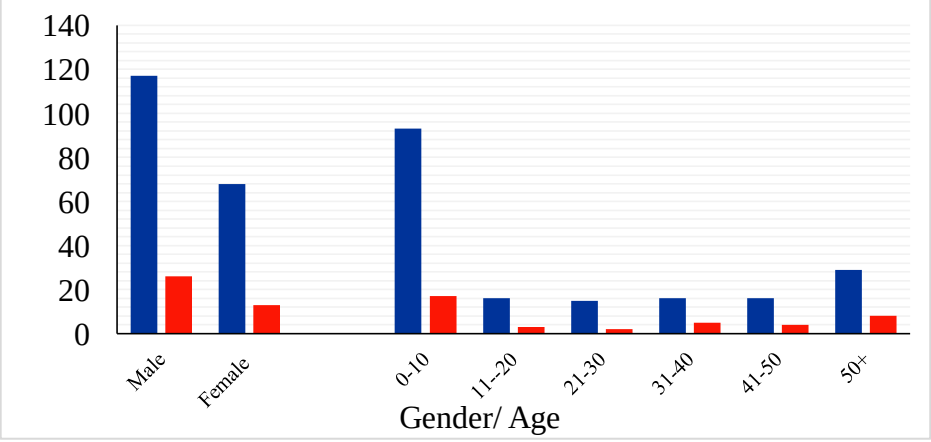

Figure 7. Socio-demographic characteristics of *Pseudomonas* infected patients

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The socio-demographic features of *Pseudomonas* bacteremia demonstrated that the infection caused by *Pseudomonas* was significantly high in males (62%) than females (38%) (Figure 7). Also MDR *Pseudomonas* in males (22.2%) was higher than females (19.12%). Most of the *Pseudomonas* infection was observed in age group of0-10 years which account for more than 50%, while, in aged patients (>50 years) infection by *Pseudomonas* accounts only 15.7%. However, no significant difference was observed between the infection rates in age group of 10 to 50 years.

Conclusion

There should be continuous training of all hospital staff including physicians, nurses, paramedical and supporting staff to reduce infection. The treatment of *Pseudomonas* infection should be done as per recommended protocol and indiscriminate use of antibiotics should completely discouraged at all levels of treatment.

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