

PSEUDOMONAS- AN EMERGING NOSOCOMIAL PATHOGENTushar Gautam¹, Anjana Gopi²¹Postgraduate Student, Department of Microbiology, Kempegowda Institute of Medical Sciences, Bangalore, Karnataka, India.²Professor and HOD, Department of Microbiology, Kempegowda Institute of Medical Sciences, Bangalore, Karnataka, India.**ABSTRACT****BACKGROUND**

Pseudomonas aeruginosa is one of the most common pathogens causing nosocomial infections, which is a major threat to patients' safety. It is an opportunistic pathogen with innate resistance to many antibiotic classes. Resistance to carbapenems is often mediated by metallo-beta-lactamase production. It also expresses many extracellular virulence factors. The rising incidence of nosocomial infection stress the need to detect the pathogenic mechanism.

Aims and Objectives-

1. To identify and speciate *Pseudomonas* from various clinical isolates-
2. To detect antimicrobial susceptibility pattern of *Pseudomonas*.
3. To detect metallo-beta-lactamase (MBL) production in *Pseudomonas*.
4. To detect virulence factors in *Pseudomonas* (haemolysin, gelatinase, deoxyribonuclease, bile aesculin, biofilm).

MATERIALS AND METHODS

150 samples were processed. Isolation, identification and speciation of the samples were done by conventional biochemical tests and antimicrobial susceptibility testing using CLSI 2017 guidelines by Kirby Bauer disc diffusion method. Metallo-Beta-lactamase production was detected by combined disc diffusion test and E strip method. Virulence factors were detected by inoculating and incubating them on blood agar, gelatin agar, bile esculin agar, DNase test agar and biofilm by microtitre plate method.

Statistical Analysis- p value was determined by Chi square test using SPSS software

Setting and Design- It was a Descriptive Study conducted between December 2016 and May 2018 in the Department of Microbiology at a tertiary care hospital.

RESULTS

Pseudomonas aeruginosa (50%) was the major isolate. Antimicrobial susceptibility was maximum for meropenem (59%) and amikacin (59%). Metallo-beta-lactamase production was detected in 60 isolates by combined disc diffusion method and it was confirmed by E strip method which detected metallo-beta-lactamase production in 56 isolates. Maximum virulence factor produced was haemolysin (100%).

CONCLUSION

There has been a rising incidence of *Pseudomonas* nosocomial infection with resistance to multiple groups of drugs which is a serious threat. Preventive measures should be taken to prevent the spread of infection.

KEY WORDS*Pseudomonas*, Meropenem, Amikacin, Metallobeta-lactamase, Virulence Factors.

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BACKGROUND

The genus *Pseudomonas* is a complex of many species (spp.) of gram negative, non-fermentative, aerobic bacilli that inhabit soil and water. In their natural habitat, these widely distributed organisms play an important role in the decomposition of organic matter.¹ *Pseudomonads* have been found in many materials used in laboratories and medical practice. In addition to their nutritional versatility, which explains their ubiquity and their importance in the recycling of organic matter, they can be dangerous opportunistic pathogens because of their tolerance to physical, chemical and antibacterial compounds.²

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Corresponding Author:

Dr. Tushar Gautam,

Room No. 105, KIMS Men's Hostel,

24th Main Road, Banashankari 2nd Stage,

Bangaluru-560007, Karnataka, India.

E-mail: drtushargautam@gmail.com

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Pseudomonas spp. are one of the most common gram-negative pathogens associated with infections in ICU patients including bacteraemia, urinary tract infections and surgical site infections, but they predominate as agents of lower respiratory tract infections.³ Nowadays, it is among the most common pathogens involved in nosocomial infections. Hospital reservoirs of the microorganism include respiratory equipment, antiseptics, soaps, sinks, mops and physiotherapy and hydrotherapy pools.⁴ It was also noted that *Pseudomonas aeruginosa* bacteraemia is associated with higher mortality than other gram-negative bacteraemia.⁴ Nosocomial bacterial infections are a major cause of morbidity in hospitalized patients. The patient in the intensive care unit (ICU) has a 5 to 7-fold higher risk of a nosocomial infection compared with the average patient and 20-25% of all nosocomial infections develop in ICUs.⁵

Resistance in pathogenic bacteria against antibiotics is a challenge for our clinicians for the management of various infections.⁴ A major reason for its prominence as a pathogen is its high intrinsic resistance to antibiotics, such that even for the most recent antibiotics, a modest change in susceptibility

can thwart their effectiveness.⁶ The rapid increase of drug resistance in clinical isolates of this opportunistic human pathogen is of worldwide concern. Unfortunately, *Pseudomonas aeruginosa* demonstrates resistance to multiple antibiotics, thereby jeopardizing the selection of appropriate treatment. Multiple antibiotic resistances in bacterial populations are a pervasive and growing clinical problem, which is recognized as a threat to public health.⁶

MATERIALS AND METHODS

It was a Descriptive study conducted in the Department of Microbiology between December 2016 and May 2018 at KIMS Hospital and Research Centre, Bengaluru. In the present study, 100 isolates of *Pseudomonas* species were obtained from urine, pus, blood, cerebrospinal fluid (CSF) and peritoneal fluid. 50 *Pseudomonas* isolates from sputum and stool samples of healthy individuals were taken as controls. *Pseudomonas* isolated from non-clinical sample (Water analysis) and known case of Malaria/ Typhoid/Dengue positive samples were excluded.

Collection and Processing of Various Samples

All samples were collected under aseptic precautions by standard procedures and processed according to standard guidelines.

Sample collected were subjected to Gram stain and streaked on Nutrient agar, 5% Sheep Blood Agar and MacConkey Agar and incubated at 37°C for 24 hrs. Plate were examined after 24 hrs. And Colonies were identified by the conventional biochemical reactions. Colonies were further speciated by the following tests. Cytochrome Oxidase test, Pigment Production and growth at 42°C, Hugh-Leifson Oxidative fermentation test, Oxidative- fermentative fructose test and Starch Hydrolysis. Antibiogram was done using Kirby Bauer Disc diffusion test according to CLSI 2017 guidelines. Metallo-betaactamase production was detected using combined disc diffusion method using 2µg Imipenem disc and 750 µg Imipenem- EDTA disc. The findings were confirmed by using E Strip method. Virulence factors were detected using Sheep Blood Agar, 3% Gelatin Agar, Bile esculin agar, DNase Agar and Biofilm production was detected using 96 well microtitre plate method.

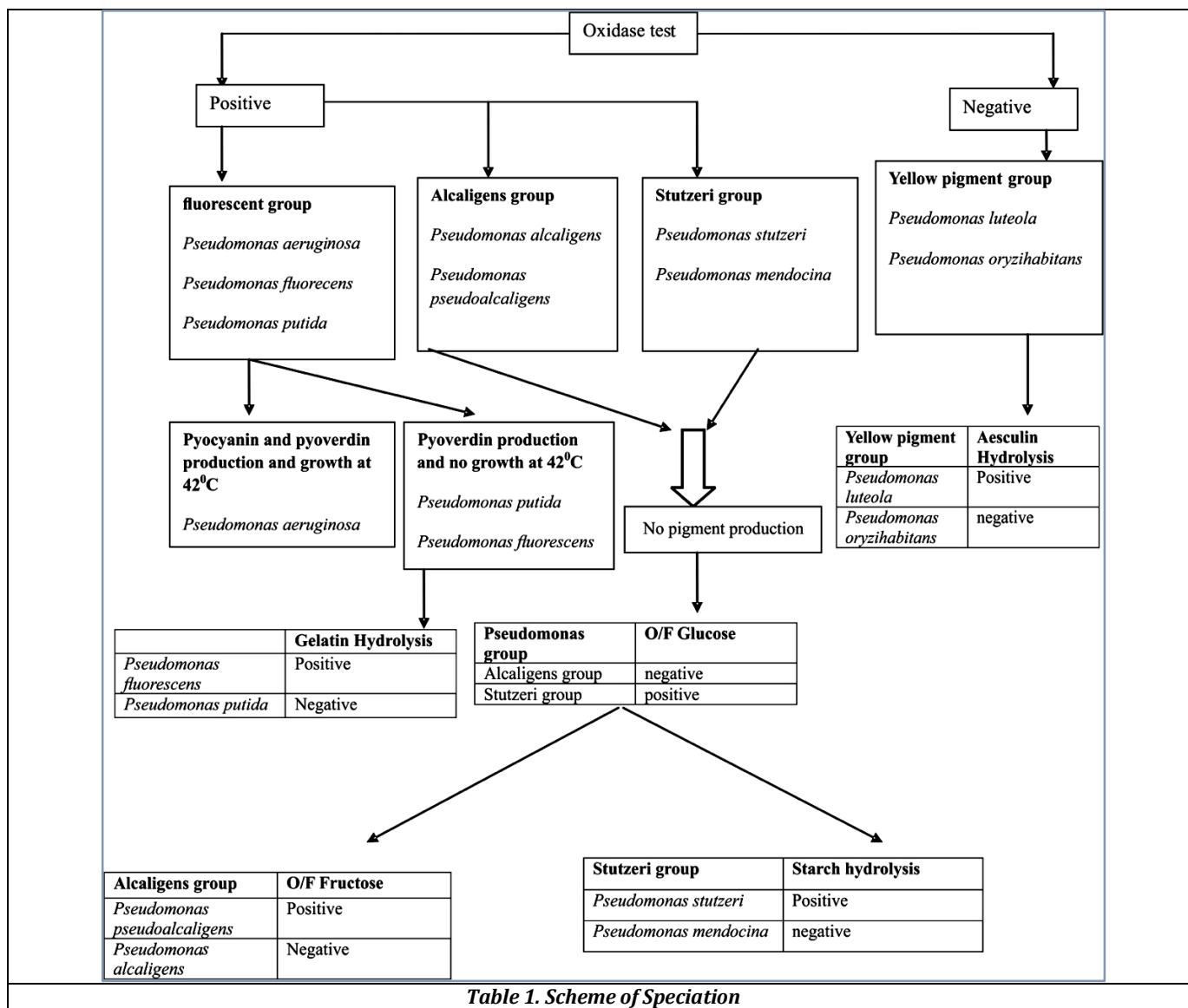


Table 1. Scheme of Speciation

RESULTS

Out of 100 patients, 87 were inpatient and 13 were from outpatient visit. Age of the subjects in this study varied from 1 year to 90 years. Maximum number of isolates (28%) were from the age group of 61-70 years which was statistically insignificant with p value > 0.01.

Male to female ratio was - 3.35:1 and it was statistically significant with p value < 0.001.

Pus sample constituted majority of specimens accounting for 70%. Urine, blood, ET Tube, CVP tip, accounted for 12%, 08%, 07%, 01% and 02% respectively and it was statistically significant with p value <0.001.

Ulcer constituted the majority among all the clinical conditions which is 20%. Out of 100 cases, 32% cases were associated with type 2 diabetes and 1% was HIV positive.

75% of the patient were on antibiotics prior to coming to hospital.

Pseudomonas aeruginosa constituted the majority of species which was 50% and it was statistically significant with p value < 0.01. 65 samples were monomicrobial and 35 samples were polymicrobial.

Pseudomonas was associated with other organism like *Klebsiella*, *Enterococcus spp.*, *Acinetobacter spp.*, *Proteus spp.* Maximum combination (polymicrobial) of *Pseudomonas spp.* was seen with *Klebsiella sp.* Polymicrobial organism with *Pseudomonas* was associated with diabetic foot, cellulitis, and necrotizing fasciitis.

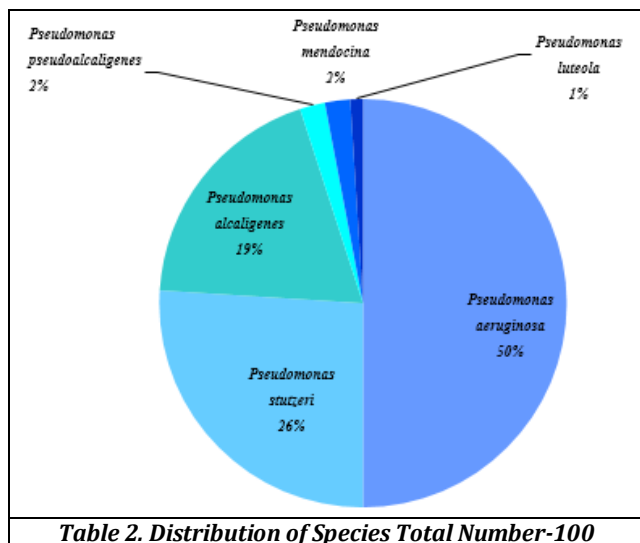


Table 2. Distribution of Species Total Number-100

Among all antipseudomonal antibiotics, amikacin and meropenem constituted the maximum percentage of sensitivity that is 59% for both. Out of 100 isolates, 60 isolates produced MBL by combined disc diffusion method which were confirmed by e strip method which detected MBL in 56 isolates. p value for both methods was statistically significant (p value < 0.01)

Out of 100 isolates, all produced haemolysin, 85 isolates produced gelatinase, 60 isolates produced bile esculin, 06 isolates produced deoxyribonuclease and 17 isolates produced biofilm.

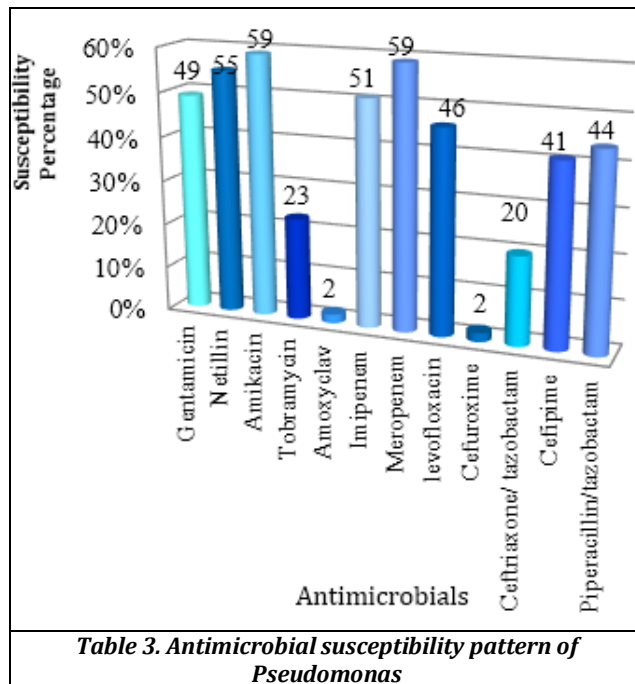


Table 3. Antimicrobial susceptibility pattern of Pseudomonas

DISCUSSION

In this study, a total of 100 *Pseudomonas* were isolated from various clinical sources, both from inpatient and outpatient visits, along with their antimicrobial susceptibility, detection of metallo-beta lactamase production and virulence factors.

In the present study, *Pseudomonas* species was maximum in the age group of 61-70 years. A similar observation was made by B Srinivas et al at, Rajiv Gandhi Institute of Medical Sciences, Srikakulam during February 2010 to January 2012 who showed isolation of *P.aeruginosa* was more common in age above 61 years⁷ and the reason for this age group could be because this age group is highly vulnerable for infection.⁸

Current study reveals that *Pseudomonas spp.* was isolated more commonly in male patients (77%) than in female patients (23%), with male to female ratio 3.35:1. In a study done by Tamil Selvi Sivanmaliappan and Murugan Sevanan at Coimbatore revealed that out of 270 patients, 180 were male patients and 90 were female patients with male to female ratio 2:1.⁹

In our study, total number of Pus samples constituted the majority of the specimen accounting for 70%. A similar study done by Tarana Sarawat et al at Department of Microbiology, Sharda University, Greater Noida revealed that out of 90 isolates of *Pseudomonas aeruginosa*, 30 were isolated from pus constituting the major portion.¹⁰

Most of the *Pseudomonas spp.* were isolated from diabetic patients having Foot ulcers which accounted for 20% (32 patients). In a study done by K Ashwin Alva, P Athiala, Rakesh Rai, and B. Rekha at Father Muller Medical College, Mangalore, Karnataka, out of 100 samples of Diabetic foot ulcers, most common organism isolated was *Pseudomonas spp.*¹¹ In our study one patient was HIV positive and about 75% of the patients were on prior antibiotic. The pathogenicity of these organisms is based on its ability to produce a variety of toxins and proteases and also on its ability to resist phagocytosis.⁹

Of 100 samples, 65 samples were monomicrobial and 35 were polymicrobial.

Maximum combination (Polymicrobial) of *Pseudomonas* sp. was seen with *Klebsiella* sp. Polymicrobial organism with *Pseudomonas* was associated with diabetic foot, cellulitis and necrotizing fasciitis. In the present study, about 50% of the *Pseudomonas* species was *Pseudomonas aeruginosa* followed by *P. stutzeri*, *P. alcaligenes*, *P. pseudoalcaligenes*, *P. mendocina* and *P. luteola* which accounted for 26%, 19%, 02%, 02%, and 01% respectively. In a study done by P Gladstone, P Rajendra, KN Brahmadathan at Christian Medical College, Vellore among patients with respiratory tract infection in Intensive care Units, *Pseudomonas aeruginosa* was the most common isolate which accounted for 42.8% out of total 732 gram-negative bacilli.¹² All the control isolates were pigment producing and were speciated as *Pseudomonas aeruginosa*.

In our study, *Pseudomonas* was most susceptible to meropenem and amikacin which accounted for 59 %, followed by netillin, imipenem, gentamicin, levofloxacin, piperacillin/tazobactam, cefepime, tobramycin, ceftriaxone/tazobactam, cefuroxime and amoxiclav, which accounted for 55%, 51%, 49%, 46%, 44%, 41%, 23%, 20%, 02% and 02%. No single antibiotic showed 100% sensitivity against all the isolates of *Pseudomonas*. Sensitivity pattern of this study suggests that most active group of antibiotics against *Pseudomonas* infection is carbapenems and aminoglycosides, this was consistent with the findings of a study done at a Jamnagar, Gujrat, India by Kamria Pooja A, Aring J.B., Sinha Mala, out of 203 clinical isolates of *Pseudomonas aeruginosa* 85% (172) were sensitive to carbapenem group¹³.

In this study, two different methods were used for detection of metalloβ-lactamase (MBL) production which were combined disc diffusion method and E strip method. Out of 100 isolates of *Pseudomonas*, metalloβ-lactamase was detected in 60 isolates by combined disc method which was confirmed by E strip method which detected MBL production in 56 isolates. It was found that combined disc diffusion method was more sensitive than E strip method. In a Study done by Manoharan A et al. at AIIMS, New Delhi, out of 56 imipenem resistant isolate, 48 exhibited metalloβ-lactamase production by combined disc method.¹⁴

Virulence factors play important role in pathogenesis of *Pseudomonas* infection. In the present study, five virulence factors were studied which were haemolysin, gelatinase, bile esculin, DNase, and Biofilm production. Out of 100, all 100 showed haemolysin production, followed by gelatinase, bile esculin, deoxyribonuclease (DNase), and biofilm production which accounted for 85%, 60%, 06% and 17%. In a study done by Georgescu M et al. at Romania haemolysin was produced by 83.33% isolates and DNase produced by 16.67% isolates.¹⁵ Control samples of our study showed 100% haemolysin production followed by gelatinase, bile esculin, deoxyribonuclease, and biofilm which accounted for 98%, 96%, 08%, 16% respectively. Virulence factors produced by control isolates were more than clinical isolates, which is consistent with study done at Sikkim Institute of Medical sciences, Gangtok, on production of slime a virulence marker

in *Pseudomonas aeruginosa* by Prasad S, Ballal M, Shivananda P, where they concluded that slime production of the environmental strains was found to be more compared with clinical isolates which helped the organism to thrive for a longer period in the hospital environment.¹⁶

CONCLUSION

In this study 61-70 yrs. age group was most commonly affected by *Pseudomonas* spp. because in this age there are more immunocompromised patients and hence more vulnerable to *Pseudomonas* infection. Majority of *Pseudomonas* isolates was from male patients as they are more exposed to outside environment in comparison to females.

Pseudomonas was more commonly isolated from pus samples which suggests that *Pseudomonas* is more involved in suppurative infections.

Patients with chronic ulcers with history of diabetes should not be ignored. Such patient should be suspected to be having *Pseudomonas* infection, as there were maximum isolates of *Pseudomonas* from patients with chronic ulcers.

75% of the total patients in our study were on antibiotics in which we got maximum number of isolates of *Pseudomonas* which concludes that *Pseudomonas* is highly resistant to different group of antibiotics.

Pseudomonas aeruginosa remains the most common species among all isolates. In most of the samples it was present as a single organism, but *Pseudomonas* was also isolated along with another organism mostly *Klebsiella* sp. Conditions in which *Pseudomonas* was associated with other organisms were diabetic foot, followed by cellulitis and necrotizing fasciitis.

In our study, *Pseudomonas* was most susceptible to carbapenems and aminoglycosides and was highly resistant to second and third generation cephalosporins which is a serious threat.

For detection of metalloβ-lactamase production, combined disc diffusion method was more sensitive and most of them were confirmed by E strip method, but it may not be practically possible for all laboratories to perform E test due to cost constraints and availability.

Pseudomonas is a nosocomial pathogen. Preventive measures are necessary to prevent the spread of infections.

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