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Original Research Article

Conventional Methods of Identification of *Pseudomonas aeruginosa* and its Antibiotic Resistance in Clinical Isolates of a Tertiary Care Hospital in South India

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Abstract

Introduction: *Pseudomonas aeruginosa* is an emerging pathogen that can survive in a wide range of environmental conditions. It is a leading cause of hospital-acquired infections. It is inherently resistant to many drugs. There is a need for appropriate identification of the organism in conventional setup among non-fermenters. In the present era where there is a highlight about antibiotic stewardship, it is necessary to have an idea about the emerging antibiotic resistance trends for better infection control practices.

Aims and objectives: The main objective of this study was to know the characteristic features and antibiotic resistance of *pseudomonas aeruginosa* isolated from all clinical samples in a tertiary care hospital.

Materials and Methods: A prospective study was conducted for 18 months. All the isolates presumptively identified as *pseudomonas aeruginosa* from various clinical samples received at the department of microbiology were included in the study. Identification of the isolates was done using conventional methods of identification, Further characterization was done using pseudomonas isolation agar, utilization of amines. Antibiotic sensitivity pattern was studied for the anti-pseudomonal group of antibiotics. The blue-green pigment was demonstrated in nutrient agar and pseudomonas isolation agar.

Results: In our study all P. aeruginosa isolates showed growth at 42^0C, attack carbohydrates like glucose, xylose oxidatively, produce blue-green pigment, utilizes citrate, decarboxylases arginine. Among 105 isolates the antibiotic-sensitive pattern was imipenem (95%), meropenem (94%) and ceftazidime (65%).

Conclusion: The ability of *pseudomonas aeruginosa* to acquire various resistance mechanisms and the emergence of carbapenem resistance emphasizes the need for antimicrobial studies in the future.

Keywords: *Pseudomonas aeruginosa*, antibiotic resistance,

Introduction

Pseudomonas aeruginosa is a ubiquitous gram-negative microorganism whose genetic makeup, virulence factors, and metabolic versatility have helped it to survive and thrive in a variety of environmental conditions. This ability has enabled it to emerge as one of the most successful opportunistic pathogens. [1,2] Pseudomonas is a non-fermenter gram-negative bacilli with the inability to ferment sugars. It can grow easily on general media and survive easily in a temperature range of 4^0c to 40^0c. Phenotypic characteristics of P. aeruginosa which assist in preliminary identification are its typical odor (described as flower-like, "grape juice", or "fresh tortilla"), pigmented colonies, and β-hemolysis on blood agar P. aeruginosa produces pigments like pyocyanin, pyorubin, and pyoverdin, Selective media like cetrimide agar, pseudomonas isolation agar are used to enhance pigment production. [3,4] Based on the known drug resistance mechanisms and emerging resistance mechanisms, we can divide them into three categories as intrinsic resistance, acquired resistance, and adaptive resistance. The genetic makeup of the organism contributes to intrinsic drug resistance. Acquired resistance refers to resistance acquired through drug-resistant genes from other organisms or as a result of mutation mechanisms. Adaptive resistance is due to various stimuli in the environment. Antimicrobial resistance has become one of the most important problems that face clinicians. According to the World Health Organization (WHO), antimicrobial resistance has been declared a public health threat. Therefore, there is a need to study microorganisms' antibiotic resistance patterns, which can guide clinicians to provide empirical therapy during life-threatening infections. Moreover, resistance patterns differ from one region to another and change continuously over time even in the same place Hence, there is a need for local annual resistance surveillances. [4,5,6,7] P. aeruginosa has developed resistance to many antipseudomonal drugs usually used in the treatment, which in turn has limited the availability of effective therapies for this infection. The purpose of this study is the identification and characterization of P. aeruginosa in a conventional setup with special reference to antibiotic resistance in all the isolates.

Materials and Methods

The present study was done for 18 months at Sri Siddhartha Medical College and Hospital, Tumkur, India. It is a descriptive and cross-sectional study. Institutional ethical committee approval was taken for this study.

Inclusion criteria:

P. aeruginosa isolated from various clinical samples were included in the study. Samples included were swabs from ulcers, deep wound infections, sputum, urine, cerebrospinal fluid, vaginal swab, blood, endotracheal aspirates, and ear swab.

Exclusion criteria:

Patients who have received systemic antibiotics for the past one week from their visit to the hospital were excluded.

Sample collection and processing were done according to recommended standard procedures. *Pseudomonas aeruginosa* was identified using conventional methods of identification like colony morphology, pigment production, biochemical reactions. (8,9)

Test for assimilation of amines (9)

A heavy suspension from fresh isolates (18-24 hrs old) from brain heart infusion broth(BHI) (> Mc Farland standard no5 turbidity) grown on 5% sheep blood agar was taken and inoculated into Arginine, lysine, and ornithine decarboxylase broths. 4 drops of the suspension from BHI broth was inoculated into the control with no amino acid with 4 drops of BHI broth suspension. Sterile liquid paraffin of about 4 mm was added over the inoculated broth. The test tubes were incubated at 37^0 c. The results were noted after 7 days. Initially, yellow color due to acid production was observed due to glucose. Purple color in the medium was observed in the tubes where amino acid decarboxylation occurred. Yellow color in the control tube was seen.

Test for pigment production (10,11,12)

The isolated organism was grown on selective media using pseudomonas isolation agar and noted for the development of diffusible blue-green pigment for confirmation of isolates as P. aeruginosa.

Antibiotic sensitivity testing on Mueller-Hinton agar (13)

The antibiotic sensitivity testing was done by Kirby- Beur disc diffusion method using standard CLSI guidelines. The isolated organism was lawn cultured on Mueller – Hinton agar plates antibiotic discs were placed and incubated at 37^0C. The zone size of antibiotics was read using CLSI guidelines. Standard control strain of P. aeruginosa ATCC 27853 was used in the study.

Antibiotic Sensitivity Testing of the organism was done for the following group of antibiotics laid as per CLSI guidelines. Cephalosporin's {ceftazidime (30µg), cefepime (30µg)} Aminoglycosides {gentamicin (10µg), tobramycin (10µg), amikacin (30µg)} Carbapenems {aztreonam (30µg), meropenem (10µg), imipenem (10µg)}, Fluoroquinolones {ciprofloxacin (5µg), levofloxacin (5µg), ofloxacin (5µg), norfloxacin (10µg)} Penicillin {piperacillin (100µg)} Macrolides {azithromycin (15µg)}, Amines {polymyxin B (300 units)}, Combination {piperacillin and tazobactum (100/10µg), ticarcillin & clavulanic acid (75/10 µg)} depending upon clinical sample. All the antibiotic disks were purchased from Hi-Media Laboratories Pvt. Ltd. Mumbai. The observed diameter zone of inhibition was measured using zone measuring scale. Sensitivity was interpreted using CLSI guidelines.

Results:

Characterization of all 103 isolates gave similar reactions. In our study all P. aeruginosa isolates showed growth at 42^0C, attack carbohydrates like glucose, xylose oxidatively, utilize citrate, decarboxylases arginine, and gelatin liquefaction test is positive. (Fig. 1) All the isolates showed diffusible blue-green pigment. (Fig. 2) Out of 103 isolates, 83(80.58%) were males and 20(19.42%) were females. The observed male to female ratio was 4:1. The maximum no. of cases were in the age group of 61-70 (25.2%). 23.3% isolation was in the age group of 51-60 years. The least number isolated were in 0-10 (0.97%) years. Distribution of P. aeruginosa isolated from various samples showed maximum isolates from pus, sputum then urine (Fig. 3). Antibiogram done for anti-pseudomonal antibiotics revealed maximum resistance to Ceftazidime 36 (33%) and Cefepime 21 (18%). (Fig. 4).

Figure 1: Amino acid decarboxylation, Gelatin hydrolysis test, Oxidation of sugars

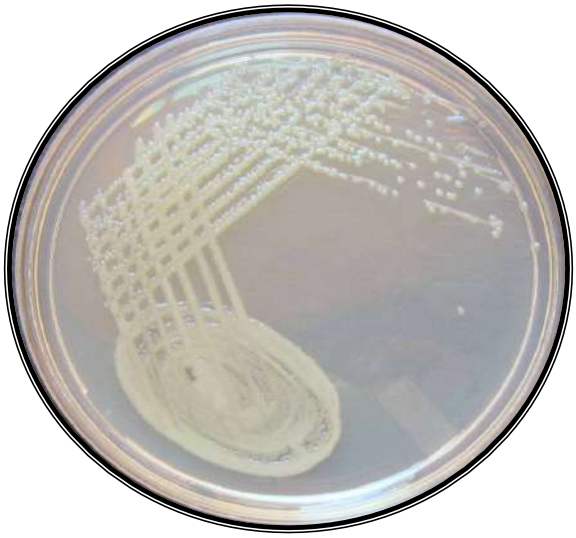

Figure 2: Growth of *P. aeruginosa* on Pseudomonas isolation agar

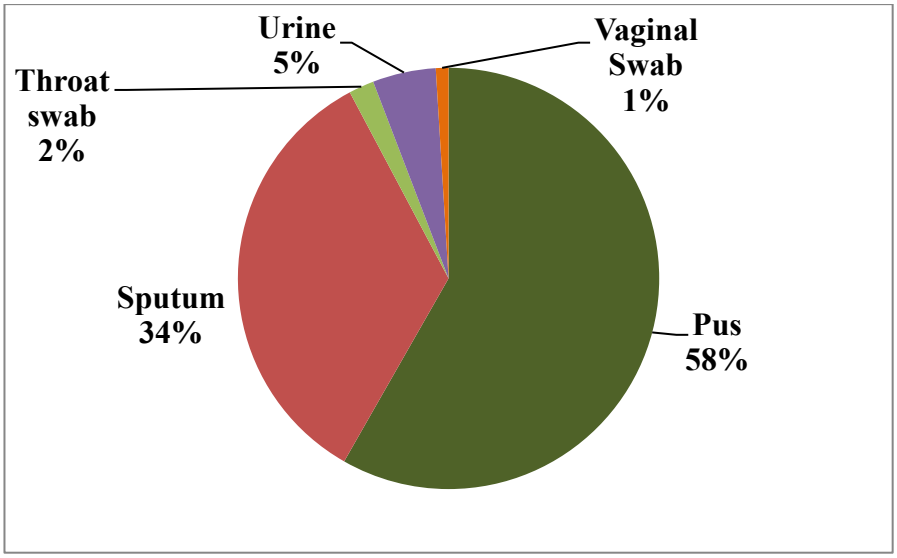

Figure 3: Distribution of Isolates According to Nature of Samples (n=103)

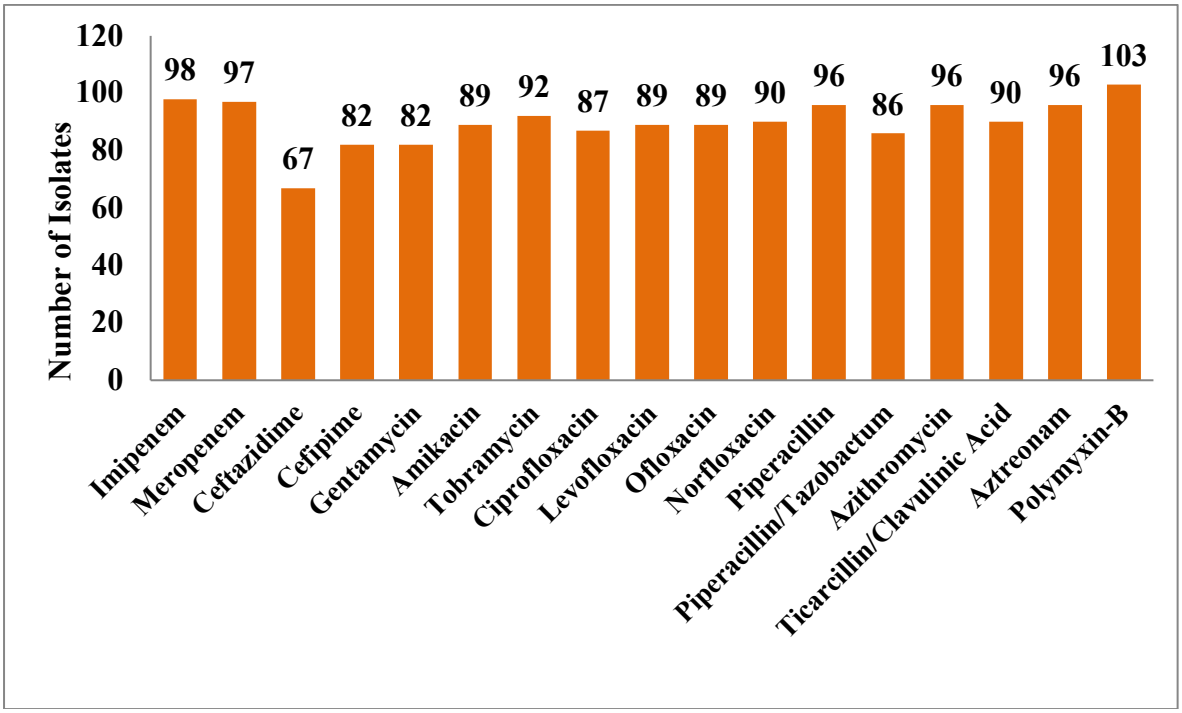

Figure 4: Antibiotics Sensitivity Pattern Observed (n=103 in each group)

Table 1: Characterization of P. aeruginosa in other studies

Study	Oxidase	Growth at 42^0C	Pigment production	Arginine dihydrolase	O/F Glucose	O/F Xylose	O/F Mannitol
Gilardi[10]	+	+	+	+	+	+	V
Hynes WC[110]	+	+	+	NA	NA	NA	NA
Our study	+	+	+	+	+	+	+

Discussion:

Very few studies are available on the characterization of non-fermenters like P. aeruginosa. In our study all P. aeruginosa isolates showed growth at 42 ^{0} c, oxidase-positive, attack carbohydrates like glucose, xylose oxidatively produce blue-green pigment, utilizes citrate, decarboxylases arginine which is similar to studies done by G l Gilardi and W C Hynes. [14,15] (Fig:1). Pigment enhancement was seen by all P. aeruginosa isolates on selective media like Pseudomonas isolation agar due to the presence of irgasan selective agent and glycerol which enhances pigment production and provides a slow release of carbon as a source to a bacterium which was the same as in a study by Fonseca, et al. [11] In our study, we found that among 103 isolates identified as P. aeruginosa 80.58% were male and 19.42% were females with a male to female ratio of 4:1. which is similar to a study conducted by Varaiya, et al. [16] Increased number of males observed may be because they are at risk of infection from the environment. In the present study, most of the patients were in the age group between 61-70 years which is similar to the study of Ullah N et al, [17]. Increased elderly male patients observed can be due to low immunity in them. In our study, we found that maximum isolates were from pus followed by sputum, urine, and others which is similar to studies done by Sunil KB and C. Roopa [18], and Saroj

Golia et al [19]. P. aeruginosa can thrive easily in a moist humid environment. Wound infections exposed to soil and moist environment can be contaminated by P. aeruginosa. Sensitivity was maximum to Polymyxin B. Similar findings were reported by Litwin et al [20] and Suprakash Das et.al [21]. Most P. aeruginosa isolates were sensitive to aminoglycosides and Carbapenems similar to studies conducted by Singh B et al [22], Saha S et al. [23], Hu et al [24], Gautam T, Gopi A [25]. There is a need to improve methodology to identify non-fermenters like P. aeruginosa. Periodic analysis of the antibiogram is helpful for clinicians to provide empiric treatment to patients.

Conclusion:

Key reactions for identification of P. aeruginosa are growth at 42^0C, oxidase-positive, attack carbohydrates like glucose, xylose oxidatively produce blue-green pigment, utilizes citrate, and decarboxylases arginine. Carbapenems and aminoglycosides remain the effective anti-pseudomonal antibiotics in our hospital.

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