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STUDY OF PREVALENCE OF PSEUDOMONAS AERUGINOSAIN ENVIRONMENTAL OF HOSPITAL

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ABSTRACT
One hundred samples were collected from the beds, earth, and tap water of Al Hussain Teaching Hospital between February 2024 and April 2024. These samples were cultured directly on blood and MacConkey agar, followed by biochemical tests for bacterial identification, and subsequently, molecular tests to detect virulence genes. Eleven isolates were recognized as P. aeruginosa; eight originated from soil, and three from tap water. Biochemical studies, such as the oxidase and catalase tests, as well as culture on MacConkey agar and blood agar were used to identify the isolates. Using the disc diffusion method, the antibiotic susceptibility test to 12 antibiotics was conducted. The findings indicated that all P. aeruginosa isolates are highly resistant (90%) to amoxicillin, cefexime, chloramphenicol, and doxycycline. However, several isolates exhibited resistance to ciprofloxacine (25%), ciprofloxacin (20%), ticarcillin (15%), gentamicin (78%), amikacin (39.58%), and polymyxine B (50%). A few of P. aeruginosa's virulence genes, including pvd A, were found. According to our findings, every strain possesses the pvdA virulence genes, with the exception of two strains that tested negative for the pvdA 1281 bp.

INTRODUCTION
Pseudomonas aeruginosa is a main source of nosocomial contamination, classify second most normal microorganism isolated from patients among the gram negative microorganisms answered to Public Nosocomial Irresistible Observation (NNIS) framework.[1,2] P. aeruginosa is the second most normal reason for nosocomial pneumonia, the third most normal reason for nosocomial urinary tract diseases, and the seventh most normal reason for nosocomial bacteremia.[3,4] P. aeruginosa is a clinically huge astute microorganism which seldom causes infection in sound invulnerable capable people, the rise of multidrug resistant strains in P. aeruginosa confines has expanded overall.[5,6]

Multidrug resistant (MDR) P. aeruginosa is hard to destroy because of raised characteristic obstruction (chromosomally encoded opposition) and its capacity to gain protection from various antimicrobials.[7,8] This nosocomial microbe has different mechanisms of protection against antimicrobials, like as broad spectrum ß-lactamases and metallo-ß-lactamases (MBL), through the change of penicillin-binding proteins (PBP), porin transformations, plasmid enzymatic alteration, DNA-gyrase transformations, and dynamic siphons.[9,10] The clinical issue results from this life form are its capacity to oppose practically all antibacterial specialists, prompting prevail over it when the touchy organic entities are stifled by these specialists.[11] There are restricted quantities of antimicrobial specialists the counter pseudomonal including Penicillins, Cephalosporin, Carbapenems, Aminoglycosides and Fluoroquinolons with dependable action against it.[12,13] Additionally, the restoratively significance of this organic.

MATERIALS AND METHODS
Samples
From February 2024 to April 2024, 100 samples were obtained from beds, soil, and tap water at Al Hussain Teaching Hospital.

Collection of Specimens
A total of 100 swab samples were collected: 25 from tap water, 50 from soil, and 25 from beds. Each swab was carefully transferred into transport medium to maintain moisture and viability until laboratory processing. Specimens were cultured on MacConkey agar and blood agar and incubated aerobically at 37°C for 48 hours.

Identification of *Pseudomonas aeruginosa* Isolates
Colonial Morphology and Microscopic Examination
Colonies grown on Mueller-Hinton agar were identified based on specific characteristics such as diffusible pigments and a sweet grape-like odor. Diagnostic features of *P. aeruginosa* were assessed following

MacFadden's guidelines (2000). Key observations included:

- Colonial morphology: smooth and mucoid with a grape-like odor.
- Pigmentation: yellowish-green or bluish-green.
- Lactose non-fermentation on MacConkey agar.

Microscopic analysis focused on cell shape (rod-shaped), Gram-stain reactions, motility, and capsule presence.

PCR Reaction for Virulence Genes

Preparation of Primers

Lyophilized primers from Alph DNA Company (USA/Canada) were reconstituted in ddH2O to a stock concentration of 100 ρmol/µl and stored at -20°C. A working solution of 10 ρmol/µl was prepared for use.

PCR for *pvdA* Gene

PCR amplification of the *pvdA* gene was conducted following Cotar et al. (2013) using the primers detailed below:

Table 3-14: The specific primer pvdA of gene.

Primer	Sequence	Tm (°C)	GC (%)	Product size
F	5'- - GACTCAGGCAACTGCAAC3'	56	66	1281 bp
R	5'- TTCAGGTGCTGGTACAGG - 3'	56	66	

The reaction mixture and PCR program were consistent with the alg D gene amplification, except for an annealing temperature of 59°C.

Antibiotic Susceptibility Assessment

Disc Diffusion Method

The antibiotic susceptibility of *P. aeruginosa* isolates was evaluated using the disc diffusion method. Results were interpreted based on Clinical Laboratory Standards Institute (CLSI) guidelines.

Statistical Analysis

Chi-square ($\chi 2$) analysis was applied to assess numerical differences among sample groups using SPSS (version 19). A significance level of $P \leq 0.05$ was considered statistically meaningful.

RESULTS AND DISCUSSION

Isolation and Identification of *P. aeruginosa*

A total of 11 isolates of *P. aeruginosa* were identified from 100 collected samples. Primary isolation was performed using selective media. Colonies displayed mucoid, circular morphology, and, on blood agar, some produced β-hemolysis. On MacConkey agar, all isolates grew but did not ferment lactose. Diagnostic pigments ranged from yellowish-green to bluish-green, with a characteristic grape-like odor.

Figure 1: Antibiotics sensitivity test of *P. aeruginosa*isolates.

Biochemical tests confirmed isolates as Gram-negative rods, oxidase-positive, catalase-positive, capable of growth at 42°C, and non-fermenters of sugars.

Molecular Detection of Virulence Genes

Bacterial DNA from the 11 isolates was extracted according to the manufacturer's protocol. DNA purity and concentration were verified using a Nanodrop spectrophotometer and agarose gel electrophoresis. PCR amplification revealed the presence of the *pvdA* gene in most isolates, except for two strains.

Antibiotic Resistance

All isolates showed varying resistance patterns. Resistance rates to key antibiotics were as follows:

- High resistance (90%): amoxicillin, cefixime, chloramphenicol, and doxycycline.
- Moderate resistance: ciprofloxacin (25%), ticarcillin (15%), gentamicin (78%), amikacin (39.58%), and polymyxin B (50%).

Comparison with Other Studies

Results align with findings by Cotar et al. (2013), who reported similar resistance patterns in *P. aeruginosa*. However, discrepancies were noted when compared to other studies regarding resistance rates and virulence gene prevalence. Such variations may reflect regional differences in antimicrobial use and infection control measures.

Molecular Typing and Clinical Implications

Molecular diagnostics proved crucial for identifying virulence factors and antibiotic resistance mechanisms. Advanced molecular methods minimize the need for extensive culture work, saving time in clinical settings. These findings emphasize the importance of continued surveillance and the development of effective treatment protocols to combat multidrug-resistant *P. aeruginosa*.

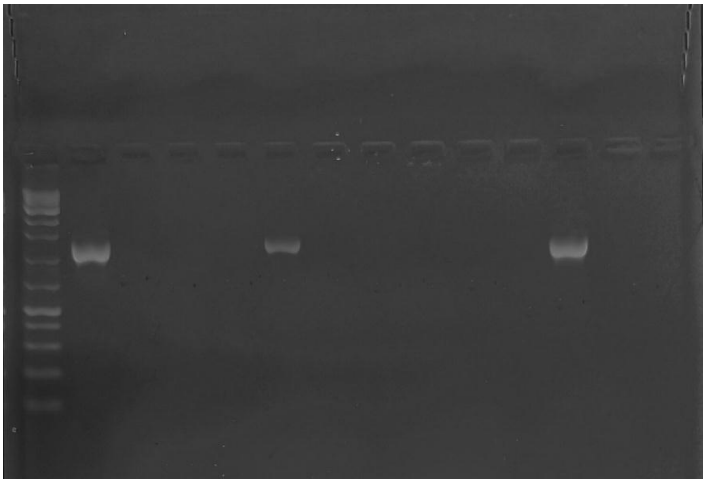

Figure 2: Electrophoresis of *Apvd* PCR product (1310 bp). 1% agarose. N: ladder (1000 plus) bp.

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