Evaluation of antibiotic sensitivity pattern in clinical isolates of Pseudomonas aeruginosa in Dhiraj Hospital, Vadodara, Gujarat.

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ABSTRACT:
INTRODUCTION - Pseudomonas aeruginosa is a straight or slightly curved, motile gram-negatiive bacilli, strict aerobe that belongs to the family Pseudomonadaceae. It represents a phenomenon of antibiotic resistance and exhibits all known mutational and enzymatic mechanisms of bacterial resistance thus imperilling the selection of appropriate treatment.

METHOD - Fifty consecutive isolates of Pseudomonas aeruginosa obtained from various clinical specimens were processed at Central Microbiology Laboratory of Dhiraj Hospital between the periods of December 2023 to May 2024. Specimens were cultured on Blood agar, Mac Conkey agar and Nutrient agar and incubated at 37°C for 24 hours. Colonies were identified by its characteristics and biochemical reactions.

RESULTS - A total of 50 isolates of Pseudomonas aeruginosa was found from clinical samples with 56% samples from pus, 24% from sputum, 5% from endotracheal tube secretion, 2% from CSF, and 8% from others. Most of samples were of male 72%. CONCLUSION: Healthcare settings largely contribute as reservoirs of pathogenic strains of Pseudomonas aeruginosa. They are known to utilize their highest levels of intrinsic and acquired resistance mechanisms against most antibiotics.

Key word: Pseudomonas aeruginosa (P. aeruginosa), chronic obstructive pulmonary disease (COPD), ventilator-associated pneumonia (VAP), multi-drug resistance (MDR).

INTRODUCTION:
Pseudomonas aeruginosa is a straight or slightly curved, motilegram-negative bacilli, strict aerobe that belongs to the family Pseudomonadaceae.
It is the pseudomonad most frequently recovered from clinical specimens. It is increasingly recognized as emerging opportunistic pathogen of clinical relevance which causes infection in patients with burn wounds, cystic fibrosis, organ transplants, urinary tract and lower respiratory tract infections which can be severe and even lifethreatening in immunocompromised hosts.
It represents a phenomenon of antibiotic resistance and exhibits all known mutational and enzymatic mechanisms of bacterial resistance thus imperilling the selection of appropriate treatment\(^1\).

*Pseudomonas aeruginosa* is a multi-drug resistance (MDR) opportunistic pathogen, causing acute or chronic infection in immuncompromised individuals with chronic obstructive pulmonary disease (COPD), cystic fibrosis, cancer, traumas, burns, sepsis, and ventilator-associated pneumonia (VAP) including those caused by COVID-19\(^4\)-\(^6\). *P. aeruginosa* in biofilm states may survive in a hypoxic atmosphere or other extremely harsh environments\(^7\)-\(^8\). Broadly reviews the recent progress in *P. aeruginosa* research towards the regulatory and functional mechanisms of virulence factors, gene expression regulators, secretion systems, quorum sensing, and antibiotic resistance, as well as host-pathogen interaction, new technologic advances, and therapeutic development (Fig. 1)

**Fig-1:** broadly reviews

**ANTIMICROBIAL RESISTANCE MECHANISMS—**

Antimicrobial resistance in *P. aeruginosa* include outer membrane porins and permeability alterations, efflux pumps, antibiotic-inactivating enzymes, and target binding site mutations. Many resistance mechanisms are often present and expressed simultaneously in a given patient with a *P. aeruginosa* infection. The terms MDR, extensively drug resistant (XDR), and pandrug-resistant (PDR) are often used to characterize the different patterns of multidrug resistance exhibited by *P. aeruginosa*. An MDR isolate is nonsusceptible to at least one agent in three or more antibiotic classes with intrinsic activity. An XDR isolate is nonsusceptible to
Resistance mechanisms present in \( P. \) aeruginosa can be classified as intrinsic, acquired, or adaptive. Intrinsic resistance mechanisms stem from genes that encode the inherent properties of cell structures and composition that provide protection against toxic molecules and antimicrobials. Acquired resistance mechanisms result through mutation of intrinsic genes or horizontal acquisition from other bacteria through transferring plasmids carrying genetic materials encoding for antibiotic resistance. Acquired resistance typically occurs in response to selective antibiotic pressures. These mechanisms are stable and can be transferred vertically (e.g., upon bacterial replication) or horizontally (e.g., resistance genes by plasmids). Adaptive resistance is induced in the presence of specific antibiotics and other environmental stresses and is transient, given that susceptibility is restored upon removal of the stimuli. This type of resistance mainly relies on induced alterations in gene expression, resulting in increased protein production or alterations in antibiotic targets.

**Fig-2:** Intrinsic, acquired, and adaptive mechanisms confer antibiotic resistance in \( P. \) aeruginosa.

**Efflux Pump Systems**

\( P. \) aeruginosa has a robust efflux pump system. The primary purpose of these pumps is to expel toxic environmental compounds or metabolites from the cytoplasm that might
otherwise disorganize the cytoplasmic membrane. Substrates of these pump systems include many clinically relevant antibiotics such as β-lactams, fluoroquinolones, amino glycosides, macrolides, tetracyclines, sulfonamides, and chloramphenicol, among other compounds. Multidrug-resistant isolates are very likely to have efflux pump system up-regulation. P. aeruginosa has several multidrug efflux pump systems. Of the five protein efflux system families described to date, most of those expressed in P. aeruginosa are members of the same (i.e., resistance-nodulation-cell division) superfamily. These efflux systems usually have three components: a cytoplasmic membrane pump, a cytoplasmic membrane “exit” porin, and a linker protein. The best-described pump system in P. aeruginosa is MexAB-OprM, which is expressed in all isolates to varying degrees. Wild-type strains tend to have relatively low expression, but mutations in the mexR repressor gene can result in pump overexpression. Overexpression of MexAB-OprM results in high-level resistance (e.g., increases in MIC by 8-fold) to a range of antibiotics. Genetic deletion of this pump restores susceptibility to many agents that are not considered clinically active against P. aeruginosa such as amoxicillin, cefuroxime, and tetracycline. The antipseudomonal agents perhaps most affected by efflux pumps are β-lactams and aminoglycosides, with fluoroquinolones possibly less affected.

MATERIAL AND METHODS:
Fifty consecutive isolates of Pseudomonas aeruginosa obtained from various clinical specimens were processed at Central Microbiology Laboratory of Dhiraj Hospital between the periods of March 2024 to May 2024. Specimens were cultured on Blood agar, Mac Conkey agar and Nutrient agar and incubated at 37°C for 24 hours. Colonies were identified by its characteristics and biochemical reactions. The samples were further processed for antibiotic sensitivity testing using VITEK 2 automated system and interpreted using CLSI guidelines.

RESULTS:
A total of 50 isolates of Pseudomonas aeruginosa was found from clinical samples with 56% samples from pus, 24% from sputum, 5% from endotracheal tube secretion, 2% from CSF, and 8% from others. Most of samples were of male 72%. The result showed highest sensitivity to drug amikacin (54%), followed by Piperacillin/Tazobactam (50%), Ciprofloxacin(46%), Cefepime (42%), Cefosalbactam(40%), Levofloxacin, Doripenem, Meropenem, Gentamicin(38%), Ceftazidime(36%), Imipenem(34%), Ticarcillin/Clavulanic Acid (32%). The least sensitive drug was Tigecycline(8%).
Fig-3: male – female ratio

Fig-4: various samples
DISCUSSION:

P. aeruginosa is one of the most isolated health-care associated pathogen worldwide. A total of 50 isolates of Pseudomonas aeruginosa was found from clinical samples with 56% samples from pus, 24% from sputum, 5% from endotracheal tube secretion, 2% from CSF, and 8% from others. Most of samples were of male 72%.

Most of samples were from male patients (63%), which is same as reported by Sharma et.al, Viren et. al, Ali Hussein et al, Shampa et al. and Rakesh et al. Previous study show higher isolation of P.aeruginosa from intensive care unit.

The occurrence of P.aeruginosa is found to be higher in males, inpatients in age group >60,41 years and in surgery department, which is same as reported by Viren et. al10, Ali Hussein et al12, Shampa et al13 and Rakesh et al.10

P. aeruginosa rapidly acquires high-level resistance to these drugs which may have impacts on both clinical and economic outcomes. Sensitivity to Meropenem, Cefepime, and Piperacillin-Tazobactam was better (67%, 62%, and 59%). Amikacin, Meropenem, Cefepime, and Piperacillin-Tazobactam could be used to treat Pseudomonas infections.

CONCLUSION:

Healthcare settings largely contribute as reservoirs of pathogenic strains of Pseudomonas aeruginosa. They are known to utilize their highest levels of intrinsic and acquired resistance mechanisms against most antibiotics. Multidrug resistance is also increasing.

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