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"STUDY OF ANTIBIOTIC SUSCEPTIBILITY PATTERNS IN CLINICAL ISOLATES PSEUDOMONAS SPECIES" IN A TERTIARY CARE HOSPITAL IN MEERUT CITY

Microbiology

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ABSTRACT

Background: Pseudomonas is a large group of aerobic, non – sporing, Gram – negative bacilli, motile by polar flagella. They are ubiquitous, mostly saprophytic, found in water, soil or other moist environments. Ps. aeruginosa is increasingly recognized as an emerging opportunistic pathogen of clinical relevance that causes infections in hospitalized patient particularly in burn patients, orthopedic related infections, respiratory diseases, immunosuppressed and catheterized patients.

Methods: The study was conducted in the Department of Allied Medical sciences, IIMT University, Meerut and associated IIMT Life Line Hospital, Meerut. The study included 200, randomly selected non- repeat clinical isolates of Pseudomonas species that were isolated from clinical samples such as urine, blood, endotracheal aspirate, pus, sputum and fluids received from various inpatient units (ICUs and wards) and outpatient departments in the Paramedical and Microbiology Laboratory.

Result: A total of 200 non repeat clinical isolates of Pseudomonas species received. Pseudomonas aeruginosa. 138/200 (69%) was isolated more frequently from the clinical samples than Pseudomonas species 62/200 (31%) form our hospital.

Conclusions: This study concluded that Pseudomonas aeruginosa is one of the commonly isolated organisms and it is becoming more resistant to commonly used antibiotics. Carbapenems and aminoglycosides were the two classes of drugs that showed best activity against Pseudomonas.

KEYWORDS

Pseudomonas aeruginosa, Antibiotic susceptibility, Sputum culture, Multi drug resistance, Clinical isolates.

INTRODUCTION

Pseudomonas is a large group of aerobic, non – sporing, Gram – negative bacilli, motile by polar flagella. They are ubiquitous, mostly saprophytic, found in water, soil or other moist environments. Some of them are pathogenic to plants, insects and reptiles. Pseudomonas species is increasingly recognized as an emerging opportunistic pathogen of clinical relevance that cause infections in hospitalized patients, most common infections being respiratory tract infection, urinary tract infection, wound infection, blood stream and central nervous system infection[1]. *Ps. aeruginosa* is increasingly recognized as an emerging opportunistic pathogen of clinical relevance that causes infections in hospitalized patient particularly in burn patients, orthopedic related infections, respiratory diseases, immunosuppressed and catheterized patients[2,3,4].

Pseudomonas aeruginosa plays an important role in hospital acquired infection. Within the hospital Pseudomonas aeruginosa can colonize moist surface of patients on the axilla ear and perineum and is also isolated from other moist in animate environments including water in sinks and drains, toilets, sewers.

A high level antibiotic resistance in Pseudomonas aeruginosa is occurring worldwide is of considerable impotence. Pseudomonas aeruginosa carries multiple genetically based resistance determinants, which may act independently or in concert with others. Among those of greatest concern are the chromosomal β- lactamases and spectrum β – lactamases which degrade carbapenem.[5]

Now days multiple antibiotic resistances in bacterial population are a growing clinical problem which is a threat to public health. Hence there is a need to conduct studies to profile different pathogens responsible for specific infections and their resistance patterns so as to generate data that would help clinicians to choose correct antibiotics for treatment. The more resistance of pseudomonas species are encountered in routine clinical practice is a serious problem, increasing morbidity and mortality and also cost of treatment.

MATERIAL & METHODS

The present study was carried out in the Department of Paramedical Sciences, IIMT University associated with Lifeline Hospital, Meerut, over a period of one year. The study period was from January 2020 - December 2020. The study included 200, randomly selected non-repeat clinical isolates of Pseudomonas species that were isolated from clinical samples such as urine, blood, endotracheal aspirate, pus, sputum and fluids received from various inpatient units (ICUs and wards) and outpatient departments in the Paramedical and Microbiology Laboratory.

All the isolates of Pseudomonas species were identified by colony morphology and biochemical reactions as per standard bacteriological techniques.[6]

The culture media, reagents and chemicals used in the study were purchased from Hi Media Laboratories Private Limited, Mumbai, India. Pseudomonas aeruginos ATCC 27853 was used as a control.

Pseudomonas Was Identified On Basis Of Fowling Characteristics

Gram Stain: Gram negative bacilli, 1.5 – 3 µm x 0.5 µm, non – capsulated, non – sporing, and is actively motile by a polar flagellum.

Colony Morphology:

1) **Nutrient Agar:** colonies are smooth, large, translucent, low convex 2 – 4mm in diameter. The organism produces a sweetish aromatic odour. There is greenish blue pigment which diffuses into the medium.

2) **Blood Agar:** colony characters are similar to those on nutrient agar. Many strains are haemolytic on blood agar.

3)**MacConkey Agar:** colonies are pale or colorless (non – lactose fermenters, NLF).

Rapid Tests:
Catalase: Positive

Oxidase: Positive

Hanging Drop Motility Test: Motile

The most common Pseudomonas species are Pseudomonas aeruginosa, *Pseudomonas maltophilia, P. mallei. P. pseudomallei, P. Cepacia, P. fluorescens, P. multivorans. P. stutzeri and P. Putida.*

Antibiotic Susceptibility Testing
Antimicrobial susceptibility testing was performed by the disk diffusion method according to Clinical and laboratory Standards Institute (CLSI) recommendations by using Muller Hinton Agar.

Carbapenems Detection of Carbapenemase production.
A) Screening For Carbapenems Resistance By Disc Diffusion:

All The Meropenem Resiatant Isolates Were Further Subjected To Modified Hodge Test (MHT) And Combined Disk Test (CDT).
Production of carbapenemase was confirmed by MHT [8,9] and MBL production was detected by performing CDT[10,11].

I) **Modified Hodge Test**
The Modified Hodge test (MHT) is recommended by CLSI for identification of carbapenemase producing Pseudomonas species.

2) Detection Of MBL Producers: Combined Disc Test (CDT) Using Imipenem (IPM) & ethylene diamine tetra acetic acid (EDTA), Detection of MBL producers was done by performing CDT using imipenem & EDTA.

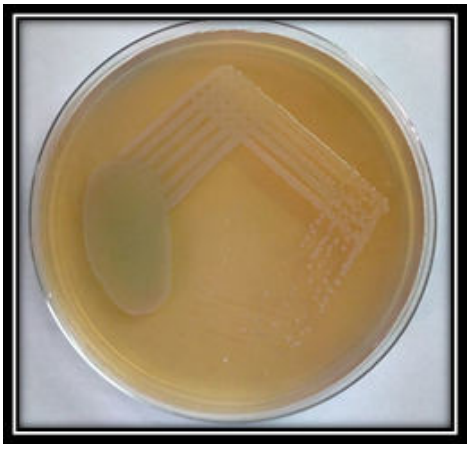

Fig.1: Growth of Pseudomonas species on Nutrient Agar showing colonies are smooth mucoid, colonies.

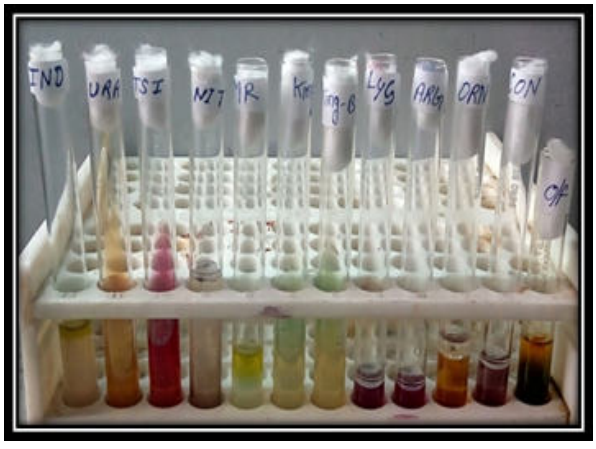

Fig. 2: Biochemical reaction of Pseudomonas aeruginosa.
I+, U-, TSI- K/NC, NIT+, MR-, KINGS A +, KINGS B+, LYSINE -, ARGININE+, ORNGINE -.

I= indole, U= ureases, TSI= triple sugar iron agar, MR=methyl red, NIT= nitrate.

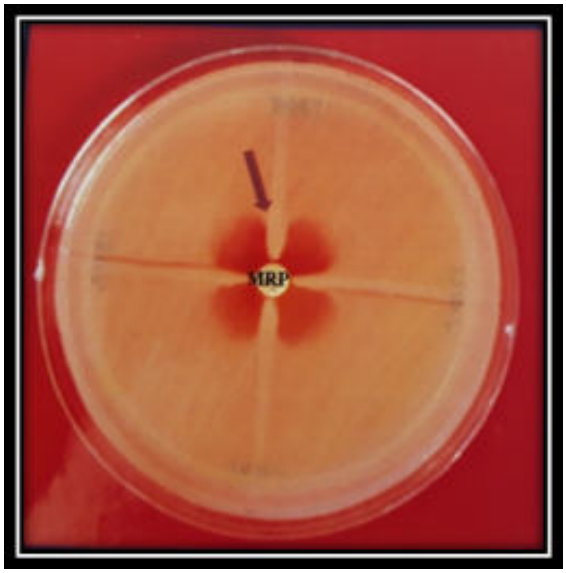

Fig.3: Modified Hodge Test Positive

Fig.4: Combined disc test positive

RESULTS
A total of 200 non repeat clinical isolates of Pseudomonas species received in Clinical Microbiological Laboratory from various inpatient units and outpatient department were studied.

Pseudomonas aeruginosa. 138/200 (69%) was isolated more frequently from the clinical samples than Pseudomonas species 62/200 (31%) form our hospital.

Pseudomonas species was isolated predominantly form pus samples received from both hospital 61(30.5 %) and community patients 5 (2.5 %).

Low level of resistance was seen with piperacillin – tazobactam 33.8 % in pseudomonas species in hospital isolates. Resistance to cefepime seen in 56.8 % and 35.2 % isolates of Pseudomonas species (Hospital and community) respectively.

Carbapenems resistance was seen in 44.8% (82/200) cases of Pseudomonas species isolated IPD and 23.5 % in OPD isolates.

Production of carbapenemases was confirmed by MHT and MBL production was detected by performing CDT.

Our study was intended to find out the prevalence of MBL in Pseudomonas species which we found to be 41 % (86/200) of total isolates. MBL was seen in 44.8 % of IPD isolates, and 23.5% in OPD isolates.

MHT could detect MBL production in 18.29 % of pseudomonas

species.

CDT could detect MBL production in more number of cases 21.95 % of Pseudomonas species. Overall, CDT could definitely detect more number of cases both in Pseudomonas species 21.95 % as compared to MTH 18.29 % in Pseudomonas species.

MHT as well as CDT, both were positive in 10.97 % of pseudomonas species.

In 48.78 % of Pseudomonas species which were resistant to meropenem on screening, both MHT & CDT were negative.

The Carbapenems resistant strains of Pseudomonas species were isolates more frequently from the hospital isolates (n= 183) than from community isolates (n=17) respectively. Isolation of Carbapenems resistant strains from the community is a matter of concern as such strains may spread into the community rapidly and may cause a therapeutic problem.

Pseudomonas species, including carbepenemase resistant Pseudomonas and MBL producers were 100 % susceptibility to colistin.

The emergence of multidrug resistant Pseudomonas aeruginosa due to the indiscriminate use of antibiotics is a challenging clinical problem which leads to the development of resistance to the routinely used antibiotics. There is a need to do surveillance to know the susceptibility pattern and to detect the MBL producers. MBL producers cause problems in treatment and in infection control. Revised guidelines on rational antibiotic usage are to be reinforced. Infection control measures must be intensified to minimize costs of patient care.

Frequency of isolates to hospital wards and outside patents. Pseudomonas aeruginosa is one of the most common causes of life – threatening and difficult – to – treat nosocomial infections. It was found that the most infections of this bacterium occur in the surgery pus patients in which it accounted for (61%) of these infections. Multidrug resistance has commonly been reported in nosocomial. P. aeruginosa infections, community acquired data have less frequently been reported. For this reason, epidemiological studies on the prevalence and antimicrobial susceptibility pattern of the resistant isolates in different geographical setting would provide useful information in order to guide clinicians in their choice of therapy and to contribute to the global picture of antimicrobial resistance.

DISCUSSION

The discovery and development of antibiotics is one of the greatest advances in modern medicine. Carbapenems are β- lactam antibiotics presently considered as potent agents to target multidrug resistant Gram negative infection, due to the stability of these agents many β lactamases. The prevalence of carbapenemase production among gram negative bacilli varies greatly from country to country and among different institutions within the country. Carbapenemase production is one of the mechanisms of resistance to Carbapenems, but other mechanisms such as porin loss, efflux of drug and target alteration also exist. However, it is well known that multiple mechanisms of resistance and enzyme production can co- exist in a single organism.[12]

The present study was carried out with the objective to study the antibiotic sensitivity Patten and to detect Carbapenems resistance in clinical isolates of Pseudomonas species in our hospital.

CONCLUSION

This study concluded that *Pseudomonas aeruginosa* is one of the commonly isolated organisms and it is becoming more resistant to commonly used antibiotics. Carbapenems and aminoglycosides were the two classes of drugs that showed best activity against *Pseudomonas*. The frequency of MDR strains in *Pseudomonas* is also on the rise. Since this study is limited to only one center in Peshawar, it is recommended to conduct a large scale study to find out the exact resistance pattern of our population. More rational use of antibiotics is required to counter the developing resistance among bacteria.

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