EVALUATING THE ANTIBIOGRAM IN *PSEUDOMONAS AERUGINOSA* WITH SPECIAL REFERENCE TO *EXO T* GENE WITH THE ABILITY OF BIOFILM FORMATION: A VIRULENCE FACTOR

Raees Ahmed, Sofiya Rabye, Saiqa R Shah, Nashra Afaq, Awadhesh Kumar,

Mukesh Kumar Patwa, Prerna Singh[*]

PhD Scholar[1], Department of Microbiology, Government Medical College, Kota, Rajasthan, India.

Associate Professor[2], Department of Microbiology, LNTC Indore, India.

Associate Professor[3], Department of Biochemistry, LNTC Indore, India.

Research Associate[4], Department of Microbiology and CRL, Rama Medical College Hospital and Research Centre, Lucknow, Uttar Pradesh, India.

Assistant Professor[5], Department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Uttar Pradesh, India

Junior Resident-3[6], Department of Microbiology, King George Medical University, Lucknow, India.

Junior Resident-2[*], Department of Microbiology, King George Medical University, Lucknow, India.

The corresponding author: Dr. Prerna Singh[*]

Email ID: prerna93singh@gmail.com

ABSTRACT

Introduction: Medical therapy has become more difficult as drug-resistant *Pseudomonas aeruginosa* strains have evolved. Biofilm development and antibiotic resistance are two virulence mechanisms that contribute to the long-term maintenance of chronic diseases. Clinical isolates of

P. aeruginosa have been shown to express the gene *ExoT*, which is a significant virulent element that contribute to the causation of infection.

Aim and Objective: To Study the Molecular characterization of *ExoT* Gene from *Pseudomonas aeruginosa* and its Antibiogram with the ability of Biofilm Formation in patients.

Material and Methods: This was a cross sectional study carried out in the Department of Microbiology at a tertiary care centre for a period of 12 months i.e, June 2023 to June 2024. A total of 150 *Pseudomonas aeruginosa* isolated from different clinical samples including urine, sputum, ear swab, wound swab , pus were identified by standard microbiological techniques according to the CLSI guidelines. The isolates were further tested for MBL by Imipenem – EDTA combined disc test and MBL E-test (Imipenem). The DNA was isolated by Qiagen DNA extraction kit as per the manufacture's guidelines and the virulent gene *Exo T* was detected by the conventional PCR.

Results: In the present study a total of 450 clinical samples were studied out of which 150 *P. aeruginosa* isolates were obtained. The prevalence rate of *Pseudomonas aeruginosa* was observed to be 33.3%. It was observed that the ratio of Males 96 (64.6%) were more affected with the infection as compared to the Females 54 (36%). It was also noted that the age group of 21-30 years (45.3%) of age followed by 31-40 (24%) was affected the most. In the age group of 0-10 years (4%) and above 71 years (2%) was the least affected with the *Pseudomonas aeruginosa* infection. It was observed that the Sensitivities of Colistin was (97.3%), Piperacillin tazobactam (77.6%), Amikacin (76.6 %), and cefepime (76.6 %) were found to be the most effective Antibiotics. The resistance to ciprofloxacin was (46.6%), Levofloxacin (53.3%), Gentamicin (63.3%), Imipenem (65.3%), Tobramycin (68%), Ceftazidime (68%).

There were 30 (20%) were MBL positive by Imipenem – EDTA combined disc test, and 29 (19.3%) by MBL E- test. In the present study it was found that 83 (55.3%) of the *Pseudomonas aeruginosa* isolates showed biofilm formation. The molecular characterization confirms 102 (68%) expression of exo T gene.

Conclusion: The established significance of *ExoT* virulence genes in the pathogenicity of *P. aeruginosa* would aid in the treatment and prognosis of Pseudomonas infections.

Keywords: Virulence, Biofilm, Molecular Characterization, DNA, PCR, Exo T

INTRODUCTION

Pseudomonas aeruginosa is a common cause of human illness, particularly in people with weaker immune systems [1]. This bacteria forms biofilms on biotic or abiotic surfaces, requires little feeding, and may live in a wide range of conditions [2]. Pseudomonas colonises catheters, skin wounds, ventilator-associated pneumonia, and respiratory infections in patients with cystic fibrosis (CF) in human hospitals, making it a leading source of nosocomial infections. Pseudomonas germs colonise an area when the fibronectin layer that surrounds the host cells is disrupted due to injury or illness. They are resistant to multiple antibiotics due to acquired or inherent determinants. It can cause acute and chronic infections .

The increasing prevalence of multidrug-resistant (MDR) *P. aeruginosa* strains has hampered medical care, which is a global concern. *P. aeruginosa* forms biofilms in the airways if not removed during the early infection phase [3]. The creation of biofilm, which are structured bacterial colonies embedded in an extracellular polymeric matrix adhering to surfaces, is one of the primary causes of persistent infections [4,5]. Biofilms offer bacteria a protective lifestyle and are very difficult and expensive to treat with antibiotics .The biofilm components of *P. aeruginosa* are composed of at least three distinct exopolysaccharides, including alginate, Psl and Pel [6].

P.aeruginosa frequently forms biofilms, which are complex bacterial communities that cling to a wide range of surfaces, including metals, plastics, medical implant materials, and tissues. Growth in biofilms enhances bacterial survival; once a biofilm forms, it is exceedingly difficult to remove [5]. *P. aeruginosa* pathogenicity is influenced by a variety of virulence factors that are responsible for bacterial adherence and colonisation, as well as host immune suppression and escape.

Pathogen virulence is defined as the ability of a pathogen to infect and cause clinical symptoms in the host through factors such as bacterial attachment to and colonisation and invasion of the host, disruption of host tissue integration, suppression of and escape from the host immune response, and nutrient depletion [7].
In recent years, numerous studies have been published that detail the potential association or co-occurrence of the antibiotic-resistant (or MDR) phenotype with biofilm development in various bacterial species.

Biofilm is a specific aggregated form of bacteria encased in extracellular polymeric substances (EPS), which can help pathogenic bacteria avoid unfavourable conditions such as temperature fluctuations, nutrient deficiency, and antibiotic killing, while increasing bacterial persistency on biotic and abiotic surfaces. P. aeruginosa's propensity to form biofilms is its most distinguishing feature, and highly organised biofilms are typically detected in chronically infected patients [8].

The virulence factors can be chemical or proteinaceous, and either cell-associated or secreted. Proteinaceous virulence factors are often secreted through one of the five protein secretion systems so far described as *P. aeruginosa*: type I, II, III, V [9] and the recently discovered type VI [10]. Especially the type III secretion system (TTSS), which injects effector proteins directly into the eukaryotic host cell cytoplasm, has been associated with high virulence. Infection with a type III secreting isolate has been shown to correlate with severe disease [11], and type III secretion (TTS) in lower respiratory and systemic infections is associated with an increased mortality rate.

The biofilm provides a form of "adaptive" resistance against antimicrobial drugs (resulting in lower capacity for diffusion, low oxygen tension and emergence of dormant phenotypes), in addition to some researchers suggesting common regulatory mechanisms behind biofilm-production and the expression of resistance genes.

P. aeruginosa infections are problematic due to their intrinsic and acquired resistance to many effective antimicrobial classes [2]. *P. aeruginosa* naturally shows resistance to penicillin and most beta-lactam antibacterial. Therefore, carbapenems are the drug of choice for MDR- *P.*

aeruginosa, however, the increasing frequency of carbapenem-resistant P. aeruginosahas recently become a serious concern globally [8]

MDR, XDR, and PDR variations exhibit significant levels of intrinsic resistance to antimicrobial medicines via efflux pumps, biofilm development, aminoglycoside modifying enzymes, and, in certain cases, chromosomal gene mutation (ESBL and AmpC hyper expression). Pseudomonas spp. can also develop resistance through the horizontal gene transfer pathway, which is responsible for class B carbapenamase (MBL) [12].

Genes responsible for drug resistance are located on integrons, which are usually found in plasmids or transposons, and these genes can move extremely regularly, contributing to the dissemination of resistance mechanisms over the world [13].

P. aeruginosa also has a large number of other virulence factors such as exotoxin A (*exoA*), alkaline protease (*aprA*), exoenzyme S, U, and T (*exoS*, *exoU*, *exoT*), elastase and sialidase, which are *exoA* gene and virulence factor *exoS* secretions by a type III section system [14,15].

P. aeruginosa pathogenesis is linked with the production of different virulence factors; rhamnolipids, pyocyanin, exotoxin A, elastase, phospholipase C, and Type-III Secretion System (T3SS) [15]. Through T3SS P. aeruginosa injects 04 effector proteins into host cytosol: Exo-enzyme Y, Exo-enzyme S, Exo-enzyme T, and Exo-enzyme U encoded by the genes exoY, exoS, exoT, and exoU, respectively. exoY and exoT play a minor role in virulence, exoY and exoT are present in most of the clinical isolates , while exoU are generally variably distributed and exoS are the more prevalent ones among the isolates .

P. aeruginosa secretes four known effector proteins via the type III secretion system: *exoS*, *exoT, exoU*, and *exoY* [16]. These proteins modulate host cell functions which are important in cytoskeletal organization and signal transduction. *ExoS* and *exoT* are bifunctional toxins exhibiting ADP-ribosyltransferase and GTPase-activating activity [17].

ExoT is a bi-functional protein possessing an N-terminal GTPase-activating protein (GAP) domain, which inhibits RhoA, Rac1, and Cdc42, small GTPases, and a C-terminal ADP-ribosyltransferase (ADPRT) domain, which targets Crk adaptor proteins and PGK1 glycolytic enzyme [16]. Due to its multiple targets, ExoT performs a number of distinct virulence functions for *P. aeruginosa* [17-20].

One of the causes of poor clinical outcomes in P. aeruginosa infections is assumed to be virulence factors, particularly the Type III secretion system (T3SS), which is thought to contribute significantly to cytotoxicity and invasion. This technique enables the bacteria to inject effector proteins directly into eukaryotic cells. Currently, four effector proteins have been identified: ExoU, a phospholipase; ExoY, an adenylate cyclase; and ExoS and ExoT, which are bifunctional proteins. ExoT and ExoY are encoded by almost all strains, therefore might be considered an inevitable component of *P. aeruginosa* virulence [19,20].

P.aeruginosa harbors at least one or more exoS, exoT, exoU and exoY genes that translated into protein products related to type III secretion systems (TTSS). ExoS and exoT are biofunctional enzymes that 75% amino acid identity and encode Gtpase-activating protein (GAP) and ADP-ribosyltransferase (ADP-RT) activities. ExoY is Adenylate Cyclase [11-13]. In addition to its ability to grow in biofilms, P.aeruginosa possesses other important virulence factors. Exos transfer the ADP-ribose moiety from NAD+ to many eukaryotic cellular proteins. *P.aeruginosa* exoS is an adenosinediphosphate ribosyltransferasethat is distinct from Pseudomonas toxin A [14, 20].

Drug-resistant phenotypes have emerged as a result of Pseudomonas spp.'s ability to create a diverse spectrum of drug resistance mechanisms. Our clinician is at a loss because he is treating a dangerous infection. This type of scenario results in the formation of phenotypes that produce a variety of pharmaceutical resistance mechanisms in order to avoid treatment failure and hospital-acquired infections [7].

Therefore the present study was undertaken to study the antibiogram in *Pseudomonas aeruginosa* with special reference to *EXO T* gene with the ability of biofilm formation as virulence factor in patients attending a tertiary care centre.

MATERIAL AND METHODS

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 09, 2024

The present study was a Cross sectional study carried out in the Department of Microbiology, for a period of 1 year i.e, between June 2023 to June 2024. A Total of 450 clinical isolates from different clinical samples including urine, sputum, ear swab, wound swab , pus were identified by Standard microbiological techniques and biochemical methods, including pigment production in agar, oxidase and catalase tests, reactions in triple sugar iron (TSI) agar, SIM (sulfide, indole, motility), and oxidative-fermentative (OF) media (Merck, Darmstadt, Germany), and finally, growth at 42 °C [21]. Samples were processed as soon as received in laboratory. In cases where a delay was expected, the sample was refrigerated for up to 4 hours at 4°C.

All clinical samples were inoculated onto MacConkey agar, Nutrient agar and Blood agar plates. Inoculated plates was incubated at 37 ^{0}C for 24 hours. After obtaining the growth, *P.aeruginosa* was identified by studying colony characteristics, production of pyocyanin pigments, grape like odour, growth at 42^{o}C, motility test, Gram staining, and positive oxidase, citrate, and catalase tests was performed according to the latest CLSI guidelines . And the isolates were further tested for MBL by Imipenem – EDTA combined disc test and MBL E-test (Imipenem) according to the CLSI guidelines (CLSI, 2023) [22].

INCLUSION CRITERIA:-

1. All patients from both IPD and OPD who had a positive Pseudomonas culture.
2. Patients of all ages and genders participated in our study.
3. This investigation comprised samples in which *P. aeruginosa* was the sole cause of infection.

EXCLUSION CRITERIA:-

Samples showing mixed growth was excluded from this study.

The patients demographic profile and consent was taken, and the Ethical clearance was duly obtained from the Institutional Ethical Committee

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 09, 2024

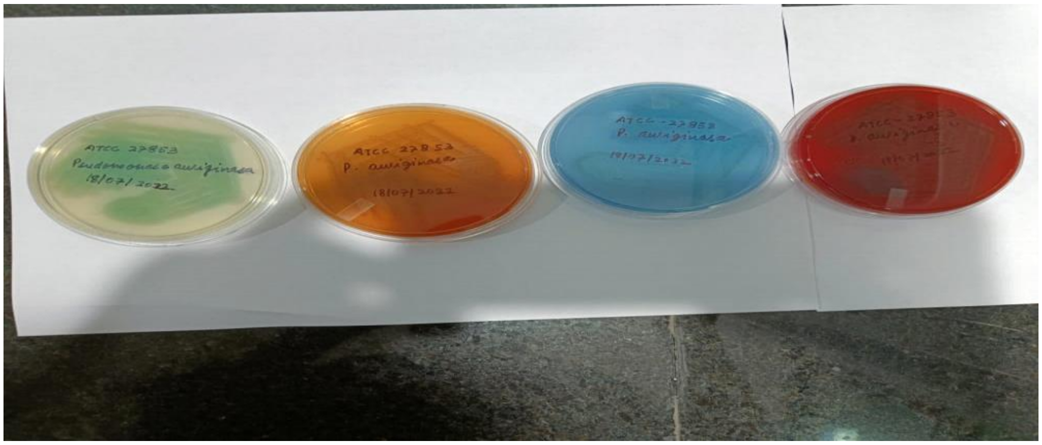


Fig No. 1 : *Pseudomonas aeruginosa* ATCC Strain (27853) Used for Quality Control, which was Subculture on Nutrient Agar Plate, MacConkey Agar, Cled Agar and Blood Agar

The Susceptibility of isolates to different antibiotics was determined by disk diffusion agar method on cation-adjusted Mueller–Hinton agar (Merck, Darmstadt, Germany) according to the Clinical and Laboratory Standards Institute (CLSI) recommendations 2023 [22]. Antibiotic disks (MAST Diagnostics, Merseyside, UK) tested were ceftazidime (CAZ, 30 µg), piperacillin/tazobactam (PTZ, 100 µg/10 µg), ciprofloxacin (CIP, 5 µg), levofloxacin (LEV, 5 µg), gent amicin (GM, 10 µg), amikacin (AK, 30 µg), tobramycin (TOB, 10 µg), imipenem (IMI, 10 µg), and meropenem (MEM, 10 µg) [22].

Escherichia coli ATCC 25922 was used as a control for susceptibility testing. Multidrug-resistant *P. aeruginosa* (MDR-PA) was defined as resistant to more than one antimicrobial agent in three or more antimicrobial categories [23,24].

The detection of Biofilm formation in *Pseudomonas aeruginosa:*

The Biofilm Formation Steps:

1. A loopful of test organism was added to 10 ml of Trypticase Soy Broth with 1% Glucose in the test tubes.
2. Tubes were incubated at 37 ^{0}C for 24 hours.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 09, 2024

3. Following incubation, tubes were decanted, washed with phosphate buffer (normal saline), and dried.
4. The tubes were stained with 0.1% crystal violet.
5. Remove excess discoloration with distilled water.
6. Tubes were dried in an inverted orientation.
7. Scoring for tube method was based on control strain results.

• Positive biofilm production occurs when a visible thick film forms between the tube's wall and bottom [25]

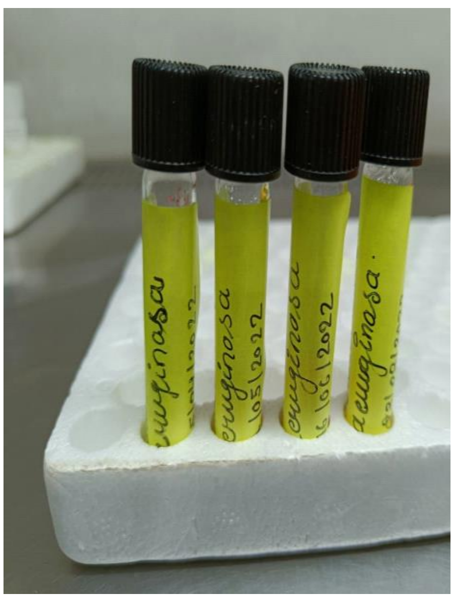


Fig No. 2 : The purified isolated colony of *Pseudomonas aeruginosa* was stabbed in the BHI agar with 10 % glycerol and stored at -4^{o} C for further Analysis of *Pseudomonas aeruginosa*

GENOTYPIC METHOD

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 09, 2024

The Molecular Detection of DNA extraction was done to detect the presence of exo T gene in *Pseudomonas aeruginosa* from the clinical isolates.

Molecular Identification of *Exo U* gene

The DNA was isolated using the Qiagen DNA Extraction Kit as per the manufactures guidelines. The DNA was eluted in 60 μl elution buffer and preserve at -20 °C till PCR analysis. For amplification of the target gene, PCR was carried out in a 50 μL reaction mixture. The primers were purchased from "**Saha gene"** and was reconstituted with sterile double distilled water based on the manufacturer's instruction.

The kit works on the principle of selective binding of DNA/RNA to silica membrane in a spin column micro-centrifuge tube. DNA/RNA is rapidly purified by centrifugation procedure. Briefly, the specimen is lysed in the lysis bufler, then the released nucleic acid is adsorbed to a silica membrane in a spin subsequently released using the elution buffer.

Fig No. 3: The *Exo T* primer from the Saha gene

Polymerase Chain Reaction (PCR): After the DNA Extraction, the PCR was done for the gene detection. The sequences of the primers used in PCR for detection of exoT gene and its molecular weight are mentioned in the Table no. 1.

Gene	**Primer sequence**	**Amplicon size**	**Length (bp)**
ExoT	Forward 5'-AATCGCCGTCCAACTGCATGCG-3'	22	152
	Reverse 5'-TGTTCGCCGAGGTACTGCTC-3'	20	

Table No. 1: The Primer sequence used for the detection of *Exo T* gene

ATCC 27853 was used as Positive Control (PC), while nuclease free water will be used as Negative control (NC).

Gene	**Initial Denaturation**	**No.of Cycles**	**Denaturation in Each Cycle**	**Annealing**	**Primer Extention**	**Final Extention**
ExoT	95ºC, 2 min	36	95ºC, 30 sec	58ºC,30 sec	72ºC, 30 sec	72ºC,5 min

Table No. 2 : The Polymerase Chain Reaction (PCR) conditions.

The above was the cycling conditions used for the PCR.

Gel electrophoresis : The Agarose Gel Electrophoresis was performed in order to identify the Purified PCR Product which was previously identified by its amplified DNA fragments. The resulting PCR product was subjected to 1 % agarose gel electrophoresis and visualized by Gel Doc™ EZ Gel Documentation System (Bio-Rad Laboratories Inc., Hercules, CA, USA). A 1 kb DNA Ladder (Thermo Fisher Scientific ™, Waltham, MA, USA) was used as the marker to evaluate the PCR product of the sample [26].

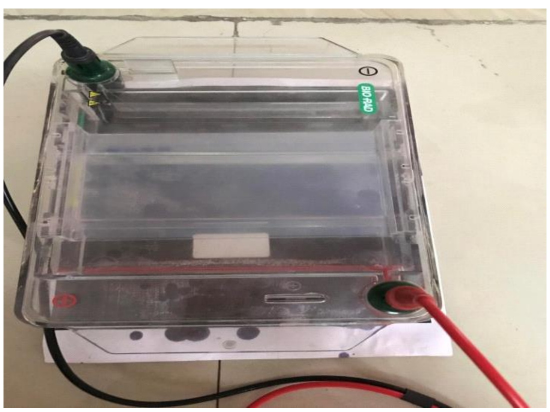


Fig No.4: Gel Electrophoresis for the DNA Extraction

Statistical analysis

The statistical analysis was done by *t*-test using SPSS 20 software in order to find the independence of the variables or whether they had similarities in their MIC values with $P < 0.005$.

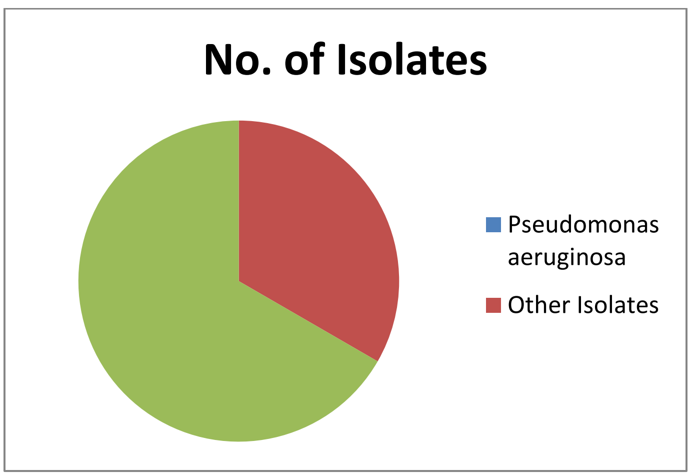
RESULTS

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 09, 2024

A total of 450 clinically suspected cases from both IPD & OPD were included out of which 150 were culture positive for *Pseudomonas* infection. [Table No. 3]. Therefore, the prevalence rate of *Pseudomonas aeruginosa* was found to be 33.3%.

S.No.	**Type of Isolates**	**Total Number of Isolates (n=450)**	**Percentage**
1.	*Pseudomonas aeruginosa*	150	33.3%
2.	Other Isolates	300	66.6%

Table No. 3: Total Number of Cases



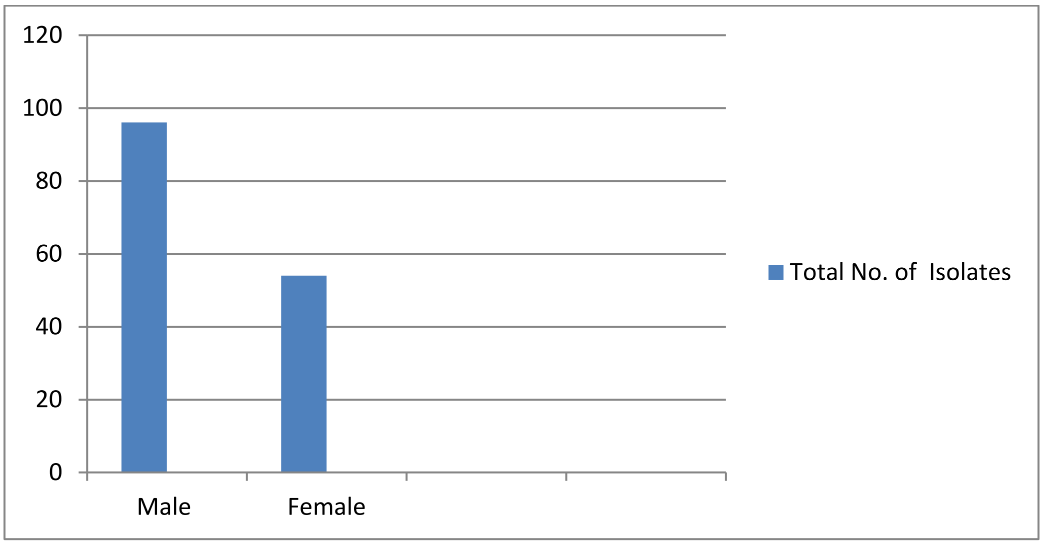
Graph No. 1: Graphical Representation of Total Number of Cases

From the present study it was observed that the Males 96 (64.6%) were more affected with the infection as compared to the Females 54 (36%). It was also noted that the age group of 21-30 years (45.3%) of age followed by 31-40 (24%) was affected the most. In the age group of 0-10 years (4%) and above 71 years (2%) was the least affected with the *Pseudomonas aeruginosa* infection.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 09, 2024

S.NO.	GENDER	TOTAL NO. OF ISOLATES (N=150)	PERCENTAGE
1.	Male	96	64.6%
2.	Female	54	36%

Table No. 4: Genderwise distribution of the *Pseudomonas aeruginosa* Isolates



Graph No. 2: Graphical Representation of genderwise distribution of the *Pseudomonas aeruginosa* Isolates

S.NO.	Age	No. of Isolates (n=150)	Percentage
1.	0-10	6	4%
2.	11-20	18	12%
3.	**21-30**	**68**	**45.3%**
4.	**31-40**	**37**	**24%**
5.	41-50	11	7.3%
6.	51-60	1	0.6%
7.	61-70	6	4%

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 09, 2024

8.	≤ 71	3	2%

Table No. 5: Agewise distribution of the *Pseudomonas aeruginosa* Isolates

It was also noted that the age group of 21-30(45%) years of age followed by 31-40(24%) was affected the most. In the age group of 0-10 years and above 71 years was the least affected with the *Pseudomonas aeruginosa* infection 4% and 2% respectively.

It was also observed that the maximum number of isolates observed were from the Urine sample (50.66%) followed by the pus (21.3%) and sputum (16%), Et- secreation (6%) and throat swab (4%), and least was from the blood (2%).

S.No.	Type of Isolates	Number of Isolates (N=150)	Percentage
1.	Pus	32	21.3%
2.	**Urine**	**76**	**50.66%**
3.	Sputum	24	16%
4.	Throat Swab	6	4%
5.	Et-Secretion	9	6%
6.	Blood	3	2 %

Table No. 6: Type of Clinical Isolates Detected from *P.aeruginosa*

Antibiotic susceptibility testing was performed by Kirby bauer Disk diffusion method as per the CLSI guidelines 2023. The antibiotic discs was placed on the Muller–Hinton agar which were previously inoculated with test strains and incubated at 37 °C for 16–18 h. After incubation,

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 09, 2024

inhibition zones was recorded as the diameter of the clear zones around the disc and interpreted according to performance standard for antimicrobial disk susceptibility test as per the CLSI guidelines.

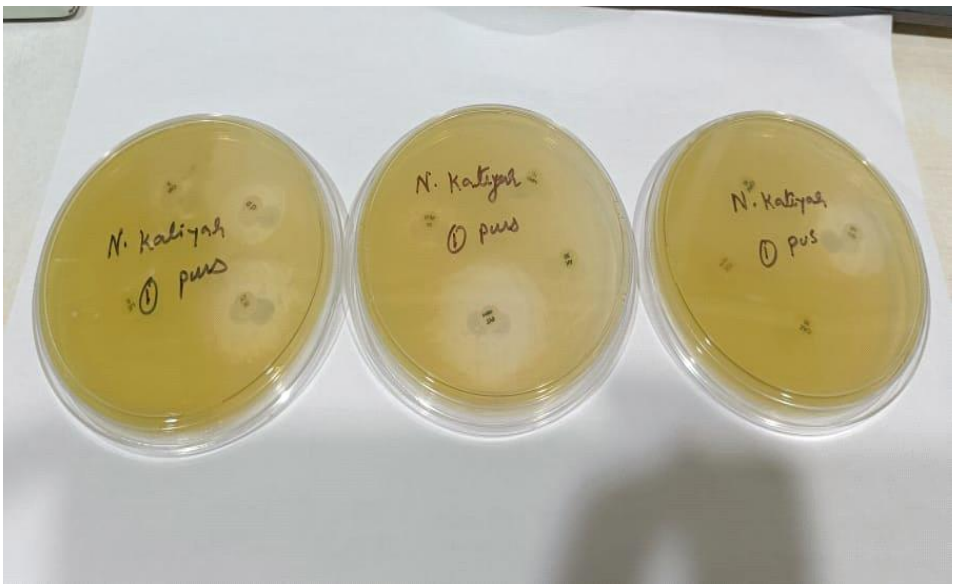


Fig No. : 5 : The Antimicrobial Disk Susceptibility Test

It was observed that the Sensitivities of Colistin was (97.3%), Piperacillin tazobactam (77.6%), Amikacin (76.6 %), and cefepime (76.6 %) were found to be the most effective Antibiotics. The resistance to ciprofloxacin was (46.6%), Levofloxacin (53.3%), Gentamicin (63.3%), Imipenem (65.3%), Tobramycin (68%), Ceftazidime (68%).

The AST was performed, and it was discovered that colistin and polymixin-B were the most sensitive medicines. Amikacin is the most sensitive aminoglycoside against *P. aeruginosa.* Amikacin was meant to be a poor substrate for enzymes that cause inactivation by phosphorylation, adenylation, or acetylation, but certain organisms have evolved enzymes that also inactivate this drug. Amikacin appears to be a viable treatment for Pseudomonal infections. As a result, its usage

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 09, 2024

should be limited to severe nosocomial infections in order to prevent the rapid establishment of resistance strains.

The detection of Biofilm formation in *Pseudomonas aeruginosa*

In the present study it was observed that 83 (55.3%) of the *Pseudomonas aeruginosa* isolates showed biofilm formation.

Isolates	Biofilm Producer	Percentage	Non Biofilm Producer	Percentage
Pseudomonas aeruginosa	83	55.3%	67	44.6%

Table No. 7: The Biofilm producer in *Pseudomonas aeruginosa*

There were 30(20%) were MBL positive by Imipenem – EDTA combined disc test, and 29 (19.3%) by MBL E- test.

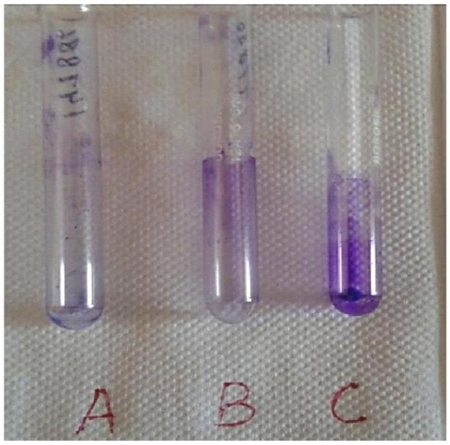


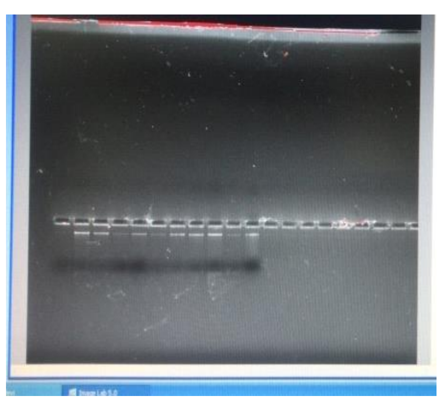
Fig No. 6: The tube Method for the Biofilm formation in *pseudomonas aeruginosa*

A: Sample Negative for the Biofilm Formation

B: Sample Positive for the Biofilm

C: Positive Control for the Biofilm formation

The reference strain *P. aeruginosa* (PA01) was also used as a positive control of the test because this strain has been characterized as a biofilm producer.



ISSN: 0975-3583,0976-2833 VOL15, ISSUE 09, 2024

Fig No. 7 The DNA Extraction in Agarose gel

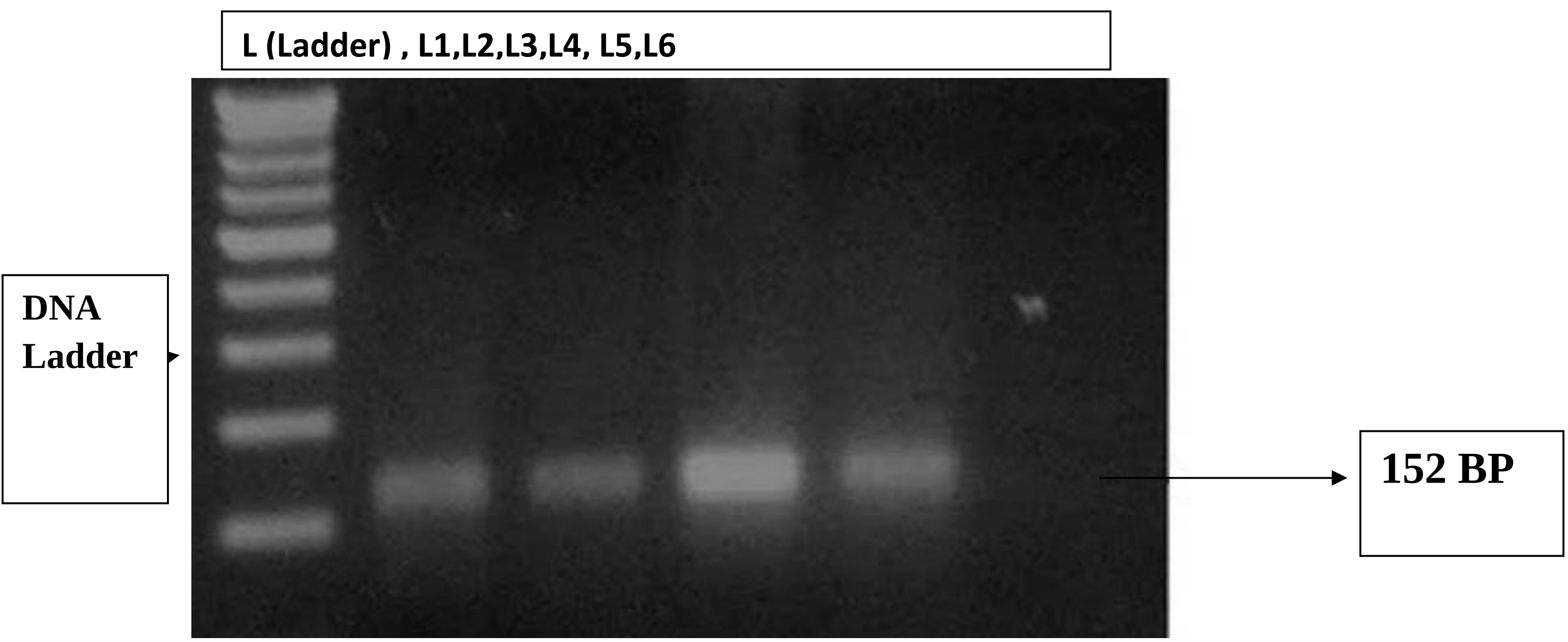


Fig No. 8: The Gene Exctraction ExoT gene:

L corresponds to the DNA Ladder; L1- L2 are the sample positive for ExoT gene; L3 corresponds to the Positive Control for ExoT gene; L4 corresponds to the sample positive for ExoT gene; L5 is the Negative Control gene for ExoT gene, L6 corresponds to the sample negative for Exo T gene

In the current study there were 102 (68%) which expressed Exo T gene.

DISCUSSION

Pseudomonas aeruginosa is a significant cause of human infection, particularly in hosts with weak defence mechanisms. *P. aeruginosa* is a nosocomial pathogen with MDR that can cause fatal infections in critically unwell individuals.

It is a frequent nosocomial pathogen known for its multidrug resistance (MDR) and potentially fatal infections in critically unwell patients. Carbapenems are increasingly being used as a last resort antibiotic treatment for serious infections caused by MDR *P. aeruginosa* [27].

In the current study a total of 450 clinically suspected cases from both IPD & OPD were included out of which 150 were culture positive for *Pseudomonas* infection. Therefore, the prevalence rate of *Pseudomonas aeruginosa* was found to be 33.3%. This study was similar to the study performed by the other author where the prevalence of *Pseudomonas aeruginosa* was found to be 33.8% [28].

In India, prevalence rate of *P.aeruginosa* infection varies from 10.5% to 30%. There was another study conducted by other research investigator [29] where the prevalence was found to be 2.76% which was in contrast to our study.

From the present study it was observed that the Males 96 (64%) were more affected with the infection as compared to the Females 54 (36%). There was another study which was parallel to our study where the ratio of males being affected was more as compared to the females where 80 (66.6%) were from males and 40 (33.3%) were from females [30]. There were other studies performed by the other research investigator which were in support to the present study where the Male preponderance was found to be 55.76% [28]. Similar observations were made by (Anupurba

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 09, 2024

S *et al*, 2006) [31]. Outdoor activities, personal habits, nature of job, and exposure to soil, water, and other organism-inhabiting places could all be contributing factors to male dominance. It was also shown that the age range of 21-30 years, followed by 31-40, was the most affected. The age group 0-10 years and over 71 years was the least affected by Pseudomonas aeruginosa infection. This was consistent with another study reported by OkonK O et al., 2009 (45.88%) [32] where the common age group was between 21–40 years of age. It was also observed that the maximum number of isolates observed were from the Urine sample (50.66%) followed by the pus (21.3%) and sputum (16%), Et- secreation (6.1%) and throat swab (4%), and least was from the blood (2%). Similar results had been obtained in different studies in India reported by (Chander Anil *et al,* 2016) [33] , (Mohanasoundaram *et al*, 2011) [34] (Arora, D *et al*, 2011) [35] . A similar observation was reported in another study (Agarwal S *et al*, 2017) [36]. Most of the other studies showed the highest isolation from pus specimens (Bezalwar PM *et al,* 2019) [37] and Charde VN *et al*,2019) [38] (Koirala A *et al* ,2017) [39] . The high rate of isolation from the urine samples in our study may be due to its ability to cause urinary tract infections in most people (Forbes BA *et al*, 2007) [40].

It was observed that the Sensitivities of Colistin was (97.3%), Piperacillin tazobactam (77.6%), Amikacin (76.6 %), and cefepime (76.6 %) were found to be the most effective Antibiotics. The resistance to ciprofloxacin was (46.6%), Levofloxacin (53.3%), Gentamicin (63.3%), Imipenem (65.3%), Tobramycin (68%), Ceftazidime (68%).

This study was similar to the study performed by (Nabina Maharjan *et al,* 2022) [41] where , polymyxin B followed by imipenem were the two most effective drugs showing 92.6% and 89.7% sensitivity respectively. Ceftazidime was found to be the least effective drug showing 44.1% sensitivity. There studies also supported our work where similar results was found (Kaur A and Singh S *et al, 2*018) (Shrestha P *et al*, 2018) [42,43].

In the present study it was observed that 83 (55.3%) of the *Pseudomonas aeruginosa* isolates showed biofilm formation. The present study was in support to the results of other studies ,(Pournajaf A *et al*, 2018) (Vasiljević Z *et al*, 2014*)* where a significant number of included isolates (83.75%) formed biofilm [44,45].

P. aeruginosa secretes four recognised effector proteins through its type III secretion system: exoS, exoT, exoU, and exoY [46]. These proteins influence host cell processes involved in cytoskeletal organisation and signal transmission [47]. ExoS and exoT are bifunctional toxins that activate ADP-ribosyltransferase and GTPase, respectively [48]. ExoT exhibits lesser ADP-ribosyltransferase activity than exoS [48]. The majority of P. aeruginosa strains include exoT and exoY genes; however, the presence of exoS and exoU varies significantly among isolates and appears to be mutually exclusive [47].

In the present study it was observed that out of 150 Pseudomonas isolates there were 102 (68%) which expressed ExoT gene. Jabalameli et al. [48] report a rate of *exoT* as 64.5%, Fazeli et al. [49] in a study on isolates from Iranian hospital infections. There was another study which was in accordance to the current study where, among 18 isolates, 12 and 6 isolates showed presence of mutually exclusive virulence genes exo-S and exo-U respectively [50].

There were other studies in support with the current study by Dadmanesh et al. [51, 52] where *exoS* and *exoT* rate as 73.91% and 69.21% respectively.

There was another study which was in contrast to the present study where the rate of Exo T was 5% and exo U was 52% [53]. Another study stated that exoT gene occurred in 20 (66.67%) isolates of *P. aeruginosa*, while 10 (33.33%) showed negative amplification results [54].

In other similar study in southern India, the prevalence of exoT was recorded as 84% [55]. Many researches all over the world studied the prevalence of exoT genes as an epidemiological marker in pathogenic *P. aeruginosa* causing different type of infections. The prevalence of exoT genes has been found to be variable in *P. aeruginosa* isolates obtained from different infections in the world. Prevalence of exoT gene recorded in Iran was 36.27% [56] and in Egypt and Romania, prevalence of exoT in *P. aeruginosa* clinical isolates were recorded as 100% [57,58].

The study by Rooh Ullah et al in 2023 was also in accordance to the current study where 100% of the isolates carried exoT [59].

ExoT virulence genes have been shown to play a significant role in *P. aeruginosa* pathogenicity, which is important for understanding the prognosis of pseudomonas infections and developing a vaccine that will effectively prevent them. Biofilm formation has been identified as a significant factor of pathogenicity in *P. aeruginosa* infections. Biofilm development exacerbates persistent bacterial infections and lowers antimicrobial medication efficacy [60] .

The treatment for the infections caused by *P. aeruginosa* is frequently complicated due to limited susceptibility patterns to different antibiotics and the emergence of antibiotic resistance during therapy [61]. Eradication of MDR *P. aeruginosa* from hospital burn wards is a demanding task, therefore is preferred to use minimum inhibitory concentration and combination antibiotics therapy to provide broader spectrum antimicrobial effects and to prevent the rapid emergence of resistance in nosocomial infections caused by *P. aeruginosa* [62].

CONCLUSION

To prevent the formation of *P. aeruginosa* strains which may be MDR, an antimicrobial susceptibility test, especially MIC, should be performed before starting the treatment, and adequate supervision is required for the use of antibiotics. Future diagnostic microbiology trends should emphasise the development of rapid assays for detecting virulence factors, which are critical epidemiological markers. An enhanced understanding of virulence genes and biofilm production in P.aeruginosa may aid in the development of new vaccines and drugs.

Declarations:

Conflicts of interest: There is no any conflict of interest associated with this study

Consent to participate: We have consent to participate.

Consent for publication: We have consent for the publication of this paper.

Authors' contributions: All the authors equally contributed the work.

REFERENCES

1. Zhang Z., Zhang X . Evolution of Subfamily I.1 Lipases in Pseudomonas Aeruginosa. *Curr. Microbiol.* 2021; 78 (9), 3494–3504.

2. Florence CU, Abraham A, David AO, Adeyemi IA, Stella IS. Evaluation of efflux pump activity and biofilm formation in multidrug resistant clinical isolates of Pseudomonas aeruginosa isolated from a Federal Medical Center in Nigeria. Ann Clin Microbiol Antimicrob. 2021; 20:11.

3. Al-Obaidi RD, Al-Dahmoshi HOM. Biofilm and antibiotic resistance profile among Pseudomonas aeruginosa isolated from clinical samples. Eurasia J Biosci. 2020; 14:1135-39.

4. Zhang A., Veesenmeyer J. L., Hauser A. R., Payne S. M. Phosphatidylinositol 4,5-Bisphosphate-Dependent Oligomerization of the Pseudomonas Aeruginosa Cytotoxin ExoU. *Infect Immun.* 2018; 86 (1), e00402–e00417.

5. Motbainor H, Bereded F, Mulu W. Multi-drug resistance of blood stream, urinary tract and surgical site nosocomial infections of Acinetobacter baumannii and Pseudomonas aeruginosa among patients hospitalized at Felegehiwot referral hospital, Northwest Ethiopia: A cross-sectional study. BMC Infectious Diseases. 2020; 20(1):92.

6. Juan, C., Peña, C. & Oliver, A. Host and pathogen biomarkers for severe *Pseudomonas aeruginosa* infections. *J. Infect. Dis.* 2017; 215(S1), S44–S51.

7. Banar M, Emaneini M, Satarzadeh M, Abdellahi N, Beigverdi R, van Leeuwen WB, et al. Evaluation of mannosidase and trypsin enzymes effects on biofilm production of *Pseudomonas aeruginosa* isolated from burn wound infections. *PLoS ONE.* 2016; 11(10):e016462

8. Rutherford, V. *et al*. Environmental reservoirs for *exoS*+ and *exoU*+ strains of *Pseudomonas aeruginosa*. *Environ. Microbiol. Rep.* 2018; 10, 485–492

9. Qu J, Cai Z, Liu Y, Duan X, Han S, Liu J, et al. Persistent bacterial coinfection of a COVID-19 patient caused by Pseudomonas aeruginosa chronic colonizer. Front Cell Infect Microbiol. 2021;11:641920.

10. Yaeger L. N., Coles V. E., Chan D. C., Burrows L. L . How to Kill Pseudomonas—emerging Therapies for a Challenging Pathogen. *Ann. New York Acad. Sci.* 2021; 1496 (1), 59–81.

11. Morales-Espinosa, R. *et al*. Fingerprint analysis and identification of strains ST309 as a potential high risk clone in a *Pseudomonas aeruginosa* population isolated from children with bacteremia in Mexico City. *Front. Microbiol.* 2017; 8, 313

12. Webster S. S., Lee C. K., Schmidt W. C., Wong G., O'Toole G. A . Interaction Between the Type 4 Pili Machinery and a Diguanylate Cyclase Fine-Tune C-Di-GMP Levels During Early Biofilm Formation. *Proc. Natl. Acad. Sci. United States America* . 2021; 118 (26), e2105566118

13. Viegas A., Dollinger P., Verma N., Kubiak J., Viennet T., Seidel C. A., et al.. . Structural and Dynamic Insights Revealing How Lipase Binding Domain MD1 of Pseudomonas Aeruginosa Foldase Affects Lipase Activation. *Sci. Rep.* 2020; 10 (1), 1–15.

14. Queenan AM, Bush K. Carbapenemases: The versatile beta-lactamases. *Clin Microbiol Rev* 2007; 20:440–458

15. Rezzoagli C., Archetti M., Mignot I., Baumgartner M., Kümmerli R . Combining Antibiotics With Antivirulence Compounds can Have Synergistic Effects and Reverse Selection for Antibiotic Resistance in Pseudomonas Aeruginosa. 2020; *PloS Biol.* 18 (8), e3000805.

16. Ferreira ML, Dantas RC, Faria AL, Goncalves IR, Silveira de Brito C, Queiroz LL, et al. Molecular epidemiological survey of the quinolone- and carbapenem-resistant genotype and its association with the type III secretion system in Pseudomonas aeruginosa. J Med Microbiol. 2015; 64(Pt 3):262–71.

17. Khattab MA, Nour MS, El Sheshtawy NM. Genetic identification of Pseudomonas aeruginosa virulence genes among different isolates. J Microb Biochem Technol. 2015;7:274

18.Goldufsky J., Wood S., Hajihossainlou B., Rehman T., Majdobeh O., Kaufman H. L., Ruby C. E., and Shafikhani S. H. *Pseudomonas aeruginosa* exotoxin T induces potent cytotoxicity against a variety of murine and human cancer cell lines. *J. Med. Microbiol*. 2015; 64, 164–173.

19. Tsukayama DT, van Loon HJ, Cartwright C, et al.. The evolution of *Pseudomonas aeruginosa* during antibiotic rotation in a medical intensive care unit: The RADAR-trial. *Int J Antimicrob Agents* 2004; 24:339–345

20. Engel, J. & Balachandran, P. Role of *Pseudomonas aeruginosa* type III effectors in disease. *Curr. Opin. Microbiol.* 2009; 12; 61–66.

21. Dogonchi AA, Ghaemi EA, Ardebili A, Yazdansetad S, Pournajaf A. Metallo-β-lactamase-mediated resistance among clinical carbapenem-resistant *Pseudomonas aeruginosa* isolates in northern Iran: a potential threat to clinical therapeutics. *Tzu Chi Med J*. 2018; 30(2):90–96.

22. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 28th ed. M100. Wayne: Clinical and Laboratory Standards Institute (CLSI); 2023. p. 3835–238.

23. Magiorakos AP, Srinivasan A, Carey R, Carmeli Y, Falagas M, Giske C, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*. 2012; 18(3):268–281

24. Spagnolo, Anna Maria; Sartini, Marina; Cristina, Maria Luisa. Pseudomonas aeruginosa in the health care facility setting. Reviews in Medical Microbiology: 2021; 32 - 3 - p 169-175.

25. Fritsch EFTM, Sambrook J. Molecular cloning-A laboratory manual, 7th edition, Cold Spring Harbor Laboratory, Principles of Gene Manipulation.

26. Leila Firouzi-Dalvand , Mehdi Pooladi , Jamileh Nowroozi , Abbas Akhvan-Sepahi , Mansooreh Hooshiyar. Presence of exoU and exoS Genes in Pseudomonas aeruginosa Isolated from Urinary Tract Infections. Infect Epidemiol Med. 2016 ; 2(2): 8-11.

27. Barbieri JT. Pseudomonas aeruginosa exoenzyme S, a bifunctional type-III secreted cytotoxin. *Int J Med Microbiol.* 2000 ;290 (4-5):381–387.

28. Barros LM, Bento JNC, Caetano JA, Moreira RAN, Pereira FGF, Frota NM, et al. Prevalência de micro-organismos e sensibilidade antimicrobiana de infecções hospitalares em unidade de terapia intensiva de hospital público no Brasil. *Rev Ciênc Farm Básica e Apl.* 2012; 33(3):429–4352.

29. Senthamarai S et al. Resistance Pattern of *Pseudomonas aeruginosa* in a Tertiary Care Hospital of Kanchipuram, Tamilnadu, India. J Clin Diagn Res. 2014 ; 8(5): DC30–DC32.

30.Saroj Golia. Isolation of Pseudomonas aeruginosa from various Clinical Isolates and it Antimicrobial Resistance Pattern in a Tertiary Care Hospital. International Journal of Current Microbiology and Applied Sciences ISSN: 2319-7706. 2016; 5 (3) : 247-253.

31.Anupurba S, Battacharjee A, Garg A, Ranjansen M. The antimicrobial susceptibility of *Psuedomonas aeruginosa*isolated from wound infections. *Indian J Dermatol.* 2006; 51(4):286–88

32. OkonK O, Aguwe PC, Oladosu W, Balogun, Uba A. Antibiotic resistance patterns of *Pseudomonas aeruginosa isolated from clinical specimens in* a tertiary care hospital in Northeastern Nigeria. *Journal of microbiology and antimicrobials.* 2009; 1(2):019–026

33. Charde VN. Study of Prevalence of Pseudomonas aeruginosa in various Bezalwar clinical samples and investigation of its antibiotic sensitivity pattern. *Int J Curr Microbiol Appl Sci.* 2019; 8(11):1825–31.

34.Mohanasoundaram, K.M. The antibiotic resistance pattern in the clinical isolates of Pseudomonas aeruginosa in a tertairy care hospital; 2008–2010 (A 3 year study). J. Clin. Diagn. Res. 2011; 5(3): 491–94.

35.Arora, D., Jindal, N., Kumar, R., Romit. Emerging antibiotic resistance in Pseudomonas aeruginosa. Int. J. Pharm. Sci. 2011; 3(2): 82–4

36.Agarwal S, Durlabhji P, Gupta S. Incidence of Metallo-ß-lactamase producing Pseudomonas aeruginosa isolates and their antimicrobial susceptibility pattern in clinical samples from a tertiary care hospital. *Int J of Res and Rev.* 2017; 4(1):92–6.

37.Bezalwar clinical samples and investigation of its antibiotic sensitivity pattern. *Int J Curr Microbiol Appl Sci.* 2019; 8(11):1825–31.

38.Charde VN. Study of Prevalence of Pseudomonas aeruginosa in various Bezalwar clinical samples and investigation of its antibiotic sensitivity pattern. *Int J Curr Microbiol Appl Sci.* 2019; 8(11):1825–31.

39.Koirala A, Agrahari G, Dahal N, Ghimire P, Rijal KR. ESBL and MBL mediated resistance in clinical isolates of nonfermentating Gram negative bacilli (NFGNB) in Nepal. *J Microb Antimicrob agents.* 2017; 3(1):18–24.

40.Forbes BA, Sahm DF, Weissfeld AS. *Bailey and Scott's diagnostic microbiology*. 12th ed. Elesvier; USA: 2007. p. 1031

41.Nabina Maharjan. *Pseudomonas aeruginosa* Isolates among Clinical Samples showing Growth in a Tertiary Care Centre: A Descriptive Cross-sectional Study. JNMA J Nepal Med Assoc. 2022 ; 60(252): 676–680.

42. Kaur A, Singh S. Prevalence of extended spectrum betalactamase (ESBL) and metallobetalactamase (MBL) producing pseudomonas aeruginosa and Acinetobacter baumannii isolated from various clinical samples. *J Pathog.* 2018; :6845985.

43. Shrestha P, Sharma S, Maharjan R. Extended spectrum beta lactamase and metallo-beta-lactamase producing pseudomonas aeruginosa at tertiary care hospital of Nepal. *Tribhuvan University Journal of Microbiology*. 2018; 5:45–50.

44.Pournajaf A, Razavi S, Irajian G, Ardebili A, Erfani Y, Solgi S, et al. Integron types, antimicrobial resistance genes, virulence gene profile, alginate production and biofilm formation in Iranian cystic fibrosis *Pseudomonas aeruginosa* isolates. Infez Med. 2018; 26(3):226–36.

45. Vasiljević Z, Jovčić B, Ćirković I, Đukić S. An examination of potential differences in biofilm production among different genotypes of *Pseudomonas aeruginosa*. Arch Biol Sci. 2014; 66(1):117–21.

46. Roy-Burman A, Savel RH, Racine S, Swanson BL, Revadigar NS, Fujimoto J, Sawa T, Frank DW, Wiener-Kronish JP. Type III protein secretion is associated with death in lower respiratory and systemic Pseudomonas aeruginosa infections. *J Infect Dis.* 2001 ;183(12):1767–1774.

47. Lee VT, Smith RS, Tümmler B, Lory S. Activities of Pseudomonas aeruginosa effectors secreted by the Type III secretion system in vitro and during infection. *Infect Immun.* 2005; 73(3):1695–1705.

48. Jabalameli F, Mirsalehian A, Khoramian B, Aligholi M, Khoramrooz SS, Asadollahi P, Taherikalani M, Emaneini M. Evaluation of biofilm production and characterization of genes encoding type III secretion system among Pseudomonas aeruginosa isolated from burn patients. *Burns.* 2012; 38(8):1192–1197.

49.Fazeli N, Momtaz H. Virulence Gene Profiles of Multidrug-Resistant Pseudomonas aeruginosa Isolated From Iranian Hospital Infections. *Iran Red Crescent Med J.* 2014; 16(10):e15722

50. Jangir, K et al. Virulence Characterization of exo-S, exo-U and algD Genes and Antibiogram Study of Pseudomonas aeruginosa Isolated from Goat Mastitis Milk in India. Israel Journal of Veterinary Medicine. 2021; 76 (3).

51. Hall-Stoodley L, Stoodley P. Evolving concepts in biofilm infections. *Cell Microbiol.* 2009 ; 11(7):1034–1043.

52.Dadmanesh M, Pilehvarzadeh M, Eramabadi M, Eramabadi P, Moghadam MB, Mashayekhi F. Community acquired Pseudomonas aeroginosa urinary tract infections in children hospitalized in a Baqiatallah hospital, Tehran, Iran: Virulence profile and antibiotic resistance properties. *Biosci Biotech Res Asia.* 2014; 11(2):417–426.

53. Jabalameli F, Mirsalehian A, Khoramian B, Aligholi M, Khoramrooz SS, Asadollahi P, Taherikalani M, Emaneini M. Evaluation of biofilm production and characterization of genes encoding type III secretion system among Pseudomonas aeruginosa isolated from burn patients. *Burns.* 2012 ;38(8):1192–1197.

54.Jagannath Dnyanoba Andhale1 , M Anjaneya Swamy2 , RN Misra. Prevalence of exoT Gene in Pseudomonas aeruginosa Isolated from Various Clinical Samples: A Cross-sectional Study. Journal of Clinical and Diagnostic Research. 202; 15(12): DC05-DC08.

55. Karthikeyan RS, Priya JL, Jr SML, Toska J, Rietsch A, Prajna V, et al. Host response and bacterial virulence factor expression in Pseudomonas aeruginosa and Streptococcus pneumoniae corneal ulcers. PLoS ONE. 2013; 8(6):e648667.
56. Fazeli N, Momtaz H. Virulence gene profiles of multidrug-resistant Pseudomonas aeruginosa isolated from Iranian hospital infections. Iran Red Crescent Med J. 2014; 16(10):e15722.
57. Gawish AA, Mohammed NA, El-Shennawy GA. An investigation of type 3 secretion toxins encoding-genes of Pseudomonas aeruginosa isolates in a University Hospital in Egypt. J Microbiol Infect Dis. 2013 ;3(3):116-22.
58. Georgescu M, Gheorghe I, Curutiu C, Lazar V, Bleotu C, Chifiriuc MC. Virulence and resistance features of Pseudomonas aeruginosa strains isolated from chronic leg ulcers. BMC Infect Dis. 2016; 16:92.
59. Rooh Ullah et al. Presence of T3SS (exoS, exoT, exoU and exoY), susceptibility pattern and MIC of MDR-Pseudomonas aeruginosa from burn wounds. J Infect Dev Ctries. 2023; 17(8):1130-1137.
60. Upadhaya S, Shenoy R, Shetty V, Lamsal A, Lamichhane P, Pokhrel S . Multi-drug resistant Pseudomonas aeruginosa isolated from intensive care burn unit. IJBR . 2014; 5: 271-273.
61. Qin, S., Xiao, W., Zhou, C., Pu, Q., Deng, X., Lan, L., et al. . *Pseudomonas aeruginosa*: pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. *Signal Transduct. Target. Ther.* 2022; 7:199.
62. Ahmed Elfadadny et al. Antimicrobial resistance of *Pseudomonas aeruginosa*: navigating clinical impacts, current resistance trends, and innovations in breaking therapies. Sec. Antimicrobials, Resistance and Chemotherapy. 2024; 15