ORIGINAL RESEARCH PAPER
Volume - 13 | Issue - 05 | May - 2024 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

PRODUCTION OF HEMOLYSIN AND GELATINASE BY PSEUDOMONAS AERUGINOSA IN VARIOUS CLINICAL SAMPLES AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN AT TERTIARY CARE HOSPITAL.

Medical Microbiology

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ABSTRACT

Introduction:- Pseudomonas aeruginosa has emerged as a significant opportunistic bacterial pathogen that causes nosocomial infection in healthcare settings resulting in treatment failure throughout the world. This study was carried out to compare the relatedness between virulence characteristics and antimicrobial susceptibility pattern in isolated Pseudomonas aeruginosa. **Objective:** To detect Hemolysin and Gelatinase Production in isolated P. aeruginosa and its correlation with antimicrobial susceptibility pattern. **Material and methods:** The present study was conducted in the department of Microbiology, GMC, Kota (Rajasthan), India. 100 non-duplicate isolates of Pseudomonas aeruginosa from various clinical samples such as pus, urine, sputum, ET and body fluids taken as sample for study. All isolates were subjected to routine antibiotic susceptibility testing by Kirby Bauer Disc Diffusion method. Phenotypic detection of virulence factors like Hemolysin, Gelatinase were done for the same. **Results:** The highest number of Pseudomonas aeruginosa were isolated in urine, followed by sputum and pus and out of 100 isolates of Pseudomonas aeruginosa, Virulence factors like Hemolysin, Gelatinase, were shown in 73%, 65% respectively. Pseudomonas species demonstrated marked resistance against monotherapy of Penicillin, 1st,2nd generation Cephalosporins, Tetracyclines and Macrolides. Only Carbapenems and combination drug like Piperacillin + Tazobactam showed higher sensitivity to Pseudomonas infections; however, the maximum sensitivity was shown by the Amikacin and Gentamicin. **Conclusion:** Hemolysin and Gelatinase producing P. aeruginosa were found to be resistance to Ciprofloxacin, Ceftazidime, Cefepime antibiotics hence antimicrobial susceptibility testing should be performed so as guide to clinician for proper initiation treatment.

KEYWORDS

Hospital acquired infections (HAI), antimicrobial susceptibility testing (AST), CLSI

BACKGROUND

Pseudomonas aeruginosa is a gram negative, rod-shaped bacterium known for its versatility and resistance to various environmental conditions. widely distributed in diverse environments, it is one of the species that can grow in the nutrient-deficient condition and live in a broad temperature range from 6 to 42°C.[1,2]

The tenacious adaptability and survivability of *P. aeruginosa* allows itself to survive on dry, abiotic surface in hospitals for up to 6 months.[3] It is an opportunistic pathogen causing infections in humans, particularly in immunocompromised individuals. *P. aeruginosa* is often associated with hospital-acquired infections and is notorious for its intrinsic and acquired antibiotic resistance mechanisms. In the hospital, commonly caused nosocomial infection particularly in immunosuppressed, burns and cystic fibrosis patients are due to *P. aeruginosa.*[4,5]

Pseudomonas aeruginosa releases various types of virulence factors and the pathogenesis is linked with several cell mediated and extracellular factors which are responsible for the severe therapeutic problem. Infectious diseases caused by *P. aeruginosa* are problematic to treat due to resistance development and produce multiple virulence factors.[6]

Several extracellular toxin and enzymes virulence factors such as exotoxins A, hemolysin and Protease are liable for blood steam invasion, diffusion and tissue damage after colonization.[7]

Hemolysis and protease are known to be important virulence factors of *P.aeruginosa*. The formation of hemolysin production in *Pseudomonas aeruginosa* is dependent on the isolate specimens and antigenic structure.[8]Gelatinase is a group of Protease which can hydrolyze gelatine into a polypeptide, amino acid, peptides.[9]

Pseudomonas aeruginosa has emerged as a significant opportunistic bacterial pathogen causes nosocomial infections in healthcare settings resulting in treatment failure throughout the world.

MATERIALS AND METHODS

A cross-sectional study, conducted in Department of Microbiology, GMC, Kota from February 2023 to June 2023, included 100 samples of isolated *P.aeruginosa* from various clinical samples at microbiology laboratory, GMC Kota. The isolated *P.aeruginosa* was further conformed by subculture on Nutrient agar for demonstration of pigment and MacConkey's agar to demonstrate non- lactose fermented colonies, Oxidase test, Citrate, OF test, arginase hydrolysis.

Inclusion Criteria-

only isolates of *P. aeruginosa* from various clinical sample, patients above 18 years of age and male, female and transgender were included.

Phenotypic detection of virulence factors-

phenotypic detection of Hemolysin and Gelatinase production were done by the following methods.

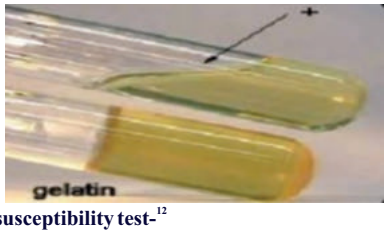
1. Detection of Hemolysin production-

Blood agar plates inoculated with the colonies was incubated at 37°C for 24 hours and then checked for zone of hemolysin around them. The results were recorded as α- hemolysis (greenish zone), β-hemolysis (clear zone) or no hemolysin.[10]

BETA-HEMOLYSIN

2. Detection of Gelatinase Production-

Gelatin production was determined by inoculating tubes containing nutrient-gelatin medium. The tubes were incubated for 48hrs at 37°C. Uninoculated tubes were kept as negative control. At the end of incubation period the culture tubes were kept at 4°C overnight was observed for liquefaction which is positive for gelatinase production.[11]



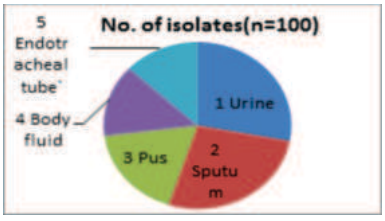
Antibiotic susceptibility test-[12]

A routine antibiotic susceptibility test was performed for *P.aeruginosa* by Kirby-Bauer disk diffusion standard method as per CLSI 2022 guidelines.0.5 McFarland standard was used to compare the inoculum turbidity. The inoculated plates were incubated overnight at 37◦c.various antibiotics such as Amikacin-AK 30mcg, Gentamicin-GEN 10mcg, Tazobactam 10mcg/ piperacillin75mcg-TZP, Ciprofloxacin-CIP 5mcg, Imipenem-IPM10 mcg, Cefepime-CPM 30mcg, Aztreonam-AT 30mcg, Ceftazidime-CAZ 30mcg, and Meropenem-MRP 10mcg were used. The results were recorded by measuring the inhibition zone as sensitive(S), intermediate(I) and resistant(R), as per CLSI 2022 guidelines.[12] As a quality control, we were used *P.aeruginosa* ATCC 27853.

RESULTS-

100 isolated *P.aeruginosa* from various sources such as Urine, Sputum, Pus, Body fluid, Endotracheal tube were used in this study. The percentage of the distribution pattern in clinical samples is shown in Table 1.

Sample	Specimen type	No. of isolates (n=100)	(%)
1	Urine	28	28%
2	Sputum	27	27%
3	Pus	18	18%
4	Body fluid	14	14%
5	Endotracheal tube	13	13%



Virulence factors play a major role in antimicrobial resistance pattern. In this study, 73% isolates were positive for hemolysin and 65% isolates were positive for Gelatinase producer.

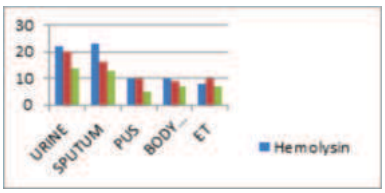
Phenotypic detection of various virulence factor of *P.aeruginosa* Table2

No. of isolated P.aeruginosa	Hemolysin production			Gelatinase production		Both Hemolysin &Gelatinase	
100	α	β	γ	Liquefaction	Non-liquefaction	Pos	Neg
	11	62	27	67	33	46	54

As the sample wise distribution Urine and Sputum samples showed maximum production of Hemolysin, Gelatinase and both Hemolysin and Gelatinase in isolated *Pseudomonas aeruginosa*.

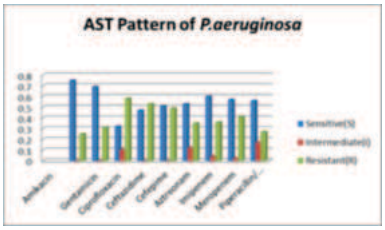
Sample wise distribution of virulence factors in isolated *Pseudomonas aeruginosa* is shown in Table3

No. of samples (N=100)	Hemolysin		Gelatinase		Both Hemolysin &Gelatinase	
	Pos %	Neg %	Pos %	Neg %	Pos%	Neg%
URINE (N=28)	22 (79%)	6 (21%)	20 (71%)	8 (29%)	14 (50%)	14 (50%)
SPUTUM (N=27)	23 (85%)	4 (15%)	16 (59%)	11 (41%)	13 (48%)	14 (52%)
PUS (N=18)	10 (56%)	8 (44%)	10 (56%)	8 (44%)	5 (28%)	13 (72%)
Body Fluid (N=14)	10 (71%)	4 (29%)	9 (65%)	5 (35%)	7 (50%)	7 (50%)
ET (N=13)	8 (61%)	5 (39%)	10 (79%)	3 (%)	7 (53%)	6 (47%)



Out of 100 isolated *P.aeruginosa* 75%, 69% susceptibility was found for Amikacin and gentamicin respectively followed by Imipenem 60%, Meropenem 57% , Piperacillin/ tazobactam 56% and 58% resistant was found for Ciprofloxacin followed by Ceftazidime 53%, Cefepime 43% and Aztreonam 35%. (Table 4)

Antibiotics	Sensitive (S)	Intermediate (I)	Resistant (R)
Amikacin	75%	0	25%
Gentamicin	69%	0	31%
Ciprofloxacin	32%	10%	58%
Ceftazidime	47%	0	53%
Cefepime	51%	0	49%
Aztreonam	53%	12%	35%
Imipenem	60%	4%	36%
Meropenem	57%	2%	41%
Piperacillin/ tazobactam	56%	17%	27%



Antibiotic resistance pattern due to virulence factors Table5

Virulence Factors	Antibiotics resistance pattern								
Hemolysin production N=73	AK	GEN	CIP	CAZ	CPM	AT	IPM	MRP	PIT
	21	16	42	31	36	25	26	31	17
Gelatinase production N=65	15	29	41	40	39	28	28	36	23
Both Hemolysin andGelatinase production N=46	17	19	29	25	28	21	21	28	15

P.aeruginosa that produced only Hemolysin showed the same antibiotic susceptibility pattern as the routine antibiotic susceptibility pattern where as those strains that produced Gelatinase and both Hemolysin and Gelatinase were found relatively more resistance to Meropenem, Gentamicin, Cefepime, Piperacillin/ tazobactam, Aztreonam, Ceftazidime, Amikacin, Imipenem as compared to routine antibiotic susceptibility pattern

DISCUSSION-

P.aeruginosa is known for its versatile virulence factors, including hemolysin, gelatinase and resistance mechanisms. These factors contribute to its pathogenicity in various infections.[13] As for antimicrobial susceptibility, *P.aeruginosa* is often resistant to multi drugs, making treatment challenging. Commonly used antibiotics like carbapenems, fluoroquinolones and Cephalosporins may develop resistance due to hemolysin and gelatinase. *P.aeruginosa* that produce virulence factors are difficult to treat with routinely used antibiotics as the strains shows resistance to various antibiotics. [14]

A total of 100 isolated *P.aeruginosa* from various clinical samples such as Urine, Sputum, Pus, Endotracheal tube, Body fluid were used in this study and virulence factors was observed in them.

Virulence factors plays a major role in antimicrobial resistance. In our study, 73% isolates were positive for Hemolysin and 65% were Gelatinase producer, which is similar to another studies done by Fazlul et al., Patel et al.[15,16] In contrast, the high percentage of hemolysin and gelatinase production was found in studies done by Khalil et al, Sonbol et al and Mittle et al, Pramodhini S. et al and Mohammand et al.[17,18,19,20] Reason being other studies assessing production of hemolysin and gelatinase were conducted over prolonged period and large sample size, indicating that larger groups studied over a long duration gives a better assessment of hemolysin and gelatinase production. Fazlul MKK, Farzana Y, Deepti S et al and Srikumar et al found that 48.33% were positive for Hemolysin and 31.66% were positive for Gelatinase, which was lower compared to our study.[21] This is because their sample size was small comparative to our study.

In our study, sample-wise Hemolysin production was mainly observed from sputum and urine and gelatinase production were observed in urine. Pramodhini S. et al[19], Mittal et al study showed highest hemolysin production in urine sample and gelatinase production was observed from urine, pus and sputum. Our study incorporated wide range of clinical samples in contrast to other studies which included one or two samples, hence the results cannot be compared

In our study, analysis of antimicrobial susceptibility pattern of *P.aeruginosa* isolates demonstrate high susceptibility rates to Amikacin (75%), Gentamicin(69%), Imipenem(60%), Meropenem (57%) and Piperacillin/tazobactam (56%).

Similar results were found in study conducted by khalil et al, Mohammad HH et al and Mittle et al.[17,20,18] In contrast, study done by S. Pramodhini et al. the results showed maximum sensitivity of 88.2% was for Imipenem, 80.2% for Piperacillin/ tazobactum.[19] In our study, resistance for Ciprofloxacin 58% which is similar to another study done by Fazlul, Zaini, Rashid & Nazlul 2011[15], In present study resistance to Ceftazidime 53%, Cefepime 49% which is higher as compared to the study done by Senthamarai2014 and Fazlul, Zaini, Rashid & Nazlul 2011[22,15] who reported 39%, 35%. The difference between these studies could be explained by the presence of intrinsic genetic differences between circulating isolates in specific location, which is corroborated by the presence of various resistance mechanisms and genes that contribute to virulence and resistance to various drug classes.[23] The percentage of carbapenem-resistance isolates in the present research is comparable to other studies globally. Eventually, the prevalence rates vary in different studies, which could be explained by differences in the sample size, distribution of risk factors and treatment regimen patterns.

Therefore Amikacin, Gentamicin, Meropenem and Imipenem are most active drug for the treatment of infection caused by *P.aeruginosa*. Followed by Piperacillin+ tazobactam and Aztreonam.[24]The association between resistance and virulence may have positive effect i.e increased resistance and increased virulence or it may have negative effect i.e, increased resistance correlated with diminished virulence.

CONCLUSION:

the production of Hemolysin and Gelatinase by *P.aeruginosa* plays a significant role in its pathogenicity, contributing to tissue damage and evasion of the host immune response. These virulence factors enhance the bacterial ability to establish infections. In terms of antimicrobial susceptibility, *P.aeruginosa* is notorious for developing resistance to a broad spectrum antibiotics. A comprehensive study on its susceptibility pattern is essential for effective treatment. Results may indicate resistance to commonly prescribed antibiotics, emphasizing the importance of alternative antimicrobial agents and a judicious approach for prescribing so as to prevent development of MDR. In conclusion, understanding the virulence factors and antimicrobial susceptibility pattern of *P.aeruginosa* is vital for devising targeted therapeutic strategies. Continuous surveillance, prudent antibiotic use and the exploration of novel treatment options are crucial in addressing the challenges posed by this opportunistic pathogen.

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