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# Antimicrobial Resistance Pattern of Pigmented and Non-Pigmented *Pseudomonas aeruginosa* Isolated from Urine in a Tertiary Care Hospital of North India

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**ABSTRACT**

***Background***: *Pseudomonas* spp is a clinically significant opportunistic pathogen frequently implicated in urinary tract infections. Differences in pigment production may indicate pathogenic variations and aid in optimizing treatment strategies.

***Methods:*** A total of 4208 samples were processed according to standard microbiological techniques. Pigment production was enhanced on nutrient agar and antimicrobial sensitivity was performed according to CLSI guidelines. This data was then analyzed, noted and reported.

***Results:*** 62 samples of *Pseudomonas* spp. were isolated, 29 (46.8%) samples did not produce any pigment, 19(30.6%) were green pyocyanin producers and 14 (22.6%) produced the pigment pyoverdine. Strains producing pigment were overall more resistant to tested antibiotics than strains not producing pigment. Pyoverdine producing strains demonstrated most resistance, but pyocyanin producing strains showed better sensitivity.

***Conclusion***: The findings underscore the need for routine pigment-based differentiation in clinical microbiology laboratories, as it may offer an early clue to resistance patterns and guide more effective empirical therapy.

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## Introduction

The genus *Pseudomonas* consists of around 300 species of ubiquitous, motile, non-fermentative, gram negative, saprophytic bacilli which thrive in moist environments (1.2). Out of these, only few are associated with human disease such as P. aeruginosa, *P. stutzeri*, *P. putida* and *P. flourescens* (3). The most commonly isolated human pathogen is *P. aeruginosa*, which is a common source of hospital acquired infections (4). It has been implicated in a myriad of infectious syndromes of the respiratory tract, the urinary tract, skin and soft tissue infections and even invasion of the vascular and central nervous system (5). Urinary tract infections (UTI) caused by *Pseudomonas* spp. are mainly documented as catheter associated UTIs, since they are easily acquired from catheters, instrumentation or irrigation solutions used in hospitals (6, 7). However, they are also an important cause of community acquired urinary tract infections in previously hospitalized patients, immunocompromised patients, patients with anatomical variations in the urinary system and so on (8-10). UTIs caused by *Pseudomonas* spp. lend to therapeutic difficulties, owing to their multidrug resistant nature. *Pseudomonas* spp. is innately resistant to the common oral and parenteral antibiotics used to treat UTIs via various mechanisms, such as enzymatic inactivation (β-lactamase production, extended spectrum β lactamase production, carbapenemase production), efflux pumps and biofilm production. They also acquire resistance to antibiotics via mutations and horizontal gene transfers, and emergence of small colony variants. (11-13).

*Pseudomonas* spp are associated with a number of virulence factors, such as flagella, pilli, lipopolysaccharides and pigment production (14). *Pseudomonas* spp. is traditionally associated with pigment production of four types: Pyocyanin (blue-green), pyoverdine (fluorescent yellow-green), pyomelanin (brown) and pyorubrin (reddish) (2). These pigments are associated with antioxidant like activities, iron acquisition, communication as part of quorum sensing and increased virulence of the organism, as demonstrated by various studies (15-17). While these pigments are well documented as a virulence factor, not much research exists as to which pigment is more associated with increased virulence and increased drug resistance (18).

The aims of this study are two-fold, to determine the co-relation between pigment colour and MDR nature of the organisms isolated from urine samples at GMC Srinagar, India, and to determine the most appropriate therapeutic approach towards these organisms.

To identify and characterize the pigments produced by *Pseudomonas* spp in urine from SMHS and other associated hospitals of GMC Srinagar. To determine the relationship between pigment color and antimicrobial susceptibility patterns. To determine the most appropriate antibiotics for *Pseudomonas* spp isolated from urine.

## Materials and Methods

A total of 4208 urine samples were received in the bacteriology laboratory the study period (7 months from 01 April 2024 to 31 October 2024). All the relevant clinical and epidemiological details were noted. Samples were processed as per standard microbiological techniques including gram staining, colony morphology on HiCrome TM UTI Agar, gram staining, motility (hanging drop method), and oxidase reaction (Oxidase disc, HiMedia DD018). Once positively identified as *Pseudomonas* spp, pigment production was noted by subculture on Nutrient agar (HIMEDIA) Blue–green colonies were considered pyocyanin producers, while–yellow colonies were considered as pyoverdine producers. Antimicrobial susceptibility testing (AST) was performed as per Kirby Bauer disk diffusion method on Mueller Hinton Agar (HIMEDIA). Results of AST were

interpreted as susceptible (S), Intermediate (I) and Resistant (R) according to the Clinical Laboratory Standard Institute (CLSI) guidelines.

## Results

Out of the 4208 samples received, 863 were culture positive and 62 samples of *Pseudomonas* spp. were isolated from them, putting the burden at 7.19%. 45 (72.5%) of the patients were either currently or recently hospitalized in the last one month (Figure 1).

28 of all patients were recently catheterized or had catheters in situ (45%), making it the single most important risk factor (Figure 2). However, it also indicated that a majority of patients could develop *Pseudomonas* UTI without this risk factor.

Based on pigment production on nutrient agar, 29 (46.8%) samples did not produce any pigment, 19(30.6%) were green pyocyanin producers and 14 (22.6%) produced the pigment pyoverdine. No strain was isolated during the study period which produced red or brown pigments. Green pigment producers were predominantly found amongst non-catheterized patients whereas yellow pigment was mainly seen in catheterized patients (Figure 3).

The next objective was an analysis of the difference in susceptibility patterns of the various pigment producers. Antimicrobial drugs used were as per CLSI guidelines M100 34th edition, with the exception of Tier 3 drugs (Cefiderocol, Ceftazidime- avibactam, Ceftolozane- tazobactam and Imipenem- relebactam). Strains producing pigment were overall more resistant to tested antibiotics than strains not producing pigment. Pyoverdine producing strains demonstrated most resistance, but surprisingly pyocyanin producing strains showed better sensitivity to some antibiotics even in comparison with non-pigment producing strains (Table 4).

Pyocyanin producers individually showed the highest susceptibility percentages, with maximum susceptibility to piperacillin tazobactam (Tier 1) and Aztreonam (Tier 4), both 100%. The least susceptibility was seen with Ciprofloxacin and Imipenem.

Pyoverdin producers were the most worrisome with strains showing 100% resistance to Ciprofloxacin and Imipenem, and showing significant resistance to other classes of antibiotics also. The only antibiotics showing good susceptibility for these strains were Piperacillin tazobactam (71.4%) and Amikacin (54.5%).

Non pigmented strains showed good sensitivity for most antibiotics, with most sensitivity seen to Piperacillin tazobactam (92%) and Aztreonam (68.75%) and least susceptibility to Imipenem and Ciprofloxacin.

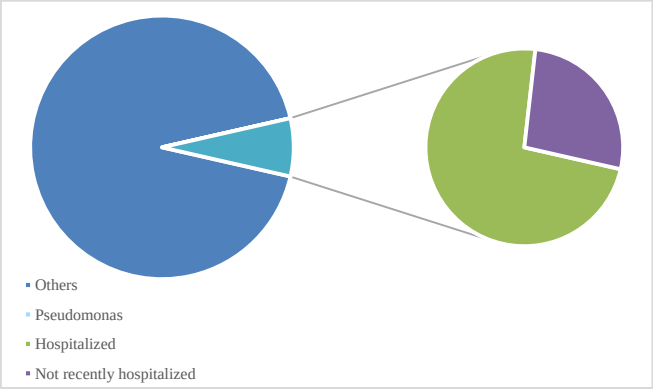


**Figure 1.** Prevalence of *Pseudomonas* spp in Urinary Tract Infections.

The final goal of the study was to select the most appropriate therapeutic approach to *Pseudomonas* UTI. Tier 1 drugs encouragingly demonstrated continued promise with maximum samples testing susceptible to Piperacillin Tazobactam (87.8%). Cephalosporins (Ceftazidime, Cefepime) also showed good susceptibility patterns, however the only orally active drugs i.e. fluroquinolones (Ciprofloxacin, Levofloxacin) did not provide with much hope, exhibiting high resistance patterns.

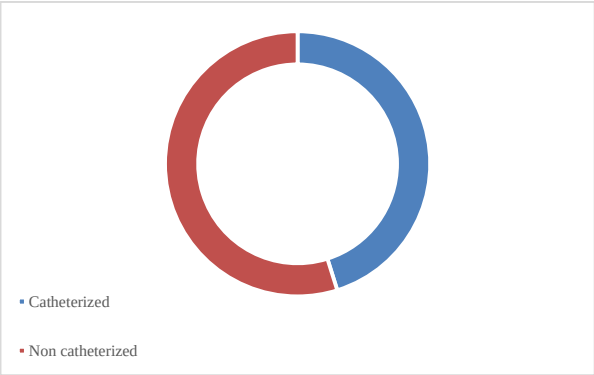


**Figure 2.** Prevalence of *Pseudomonas* UTI in catheterized vs non catheterized .

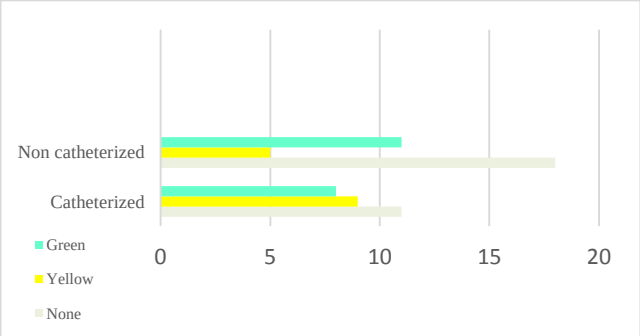


**Figure 3.** Difference in pigment production in catheterized vs non catheterized patients.

Aminoglycosides can be used as monotherapy in *Pseudomonas* UTI and Amikacin demonstrated better results than Tobramycin. Amongst carbapenems Meropenem displayed better efficacy than Imipenem. Aztreonam as a Tier 4 drug remains a hopeful option, however care needs to be taken so as not to induce more resistance to it. A reassuring result of this study was that no strain isolated was pan drug resistant.

## Discussion

*Pseudomonas* spp has historically always been an organism easy to identify but difficult to treat, owing to both its intrinsic resistance as well as MDR nature. This study highlights the antimicrobial resistance patterns among pigmented and non-pigmented *Pseudomonas* spp isolates obtained from urine samples at SMHS and affiliated hospitals of GMC Srinagar. Pigment production, a hallmark of *Pseudomonas* , was observed in a majority of isolates, with pyocyanin being the most commonly isolated. Similar results were seen by Kothari et al. (2022) (18) as well as Sarkheili et al. (2025) (19). However, both were able to isolate strains demonstrating pyorubrin and pyomelanin pigment, which was not seen in our study.

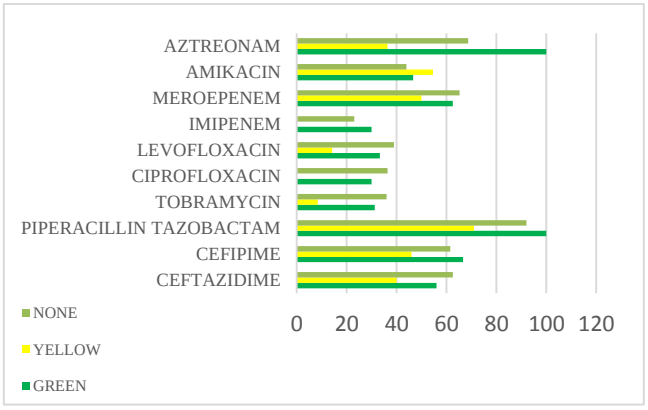


**Figure 4.** Comparison of sensitivity patterns of various strains of *Pseudomonas* spp.

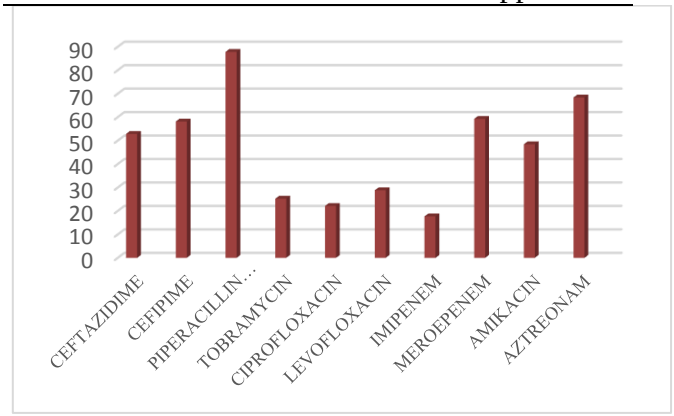


**Figure 5.** Average susceptibility percentage of *Pseudomonas* spp to various antibiotics used for testing and treatment.

Not much data is available as to the co-relation between pigment production and antimicrobial sensitivity. A significant relationship was observed between pyoverdin pigment production and antimicrobial susceptibility in our study, with these strains being significantly resistant to almost

all classes of antibiotics. Pyocyanin producing strains however did not show the same results, with it being more sensitive to certain classes of antibiotics as compared to even non pigmented strains. This was corroborated by Kang et al. (2019) (20) as well as Kothari et al. (2022) (18). Finlayson et al (2011) (21), while not establishing a clear relationship between pigment production and MDR nature, did draw a co-relation between expression of more virulence factors by pyoverdine producing strains, which indirectly may contribute to antimicrobial resistance.

Among the antibiotics tested, piperacillin-tazobactam and aztreonam were found to be the most effective across both pigmented and non-pigmented isolates, indicating their continued relevance in treating *P. aeruginosa* urinary tract infections. Similar results were observed by Yadav A. et al (2024) (22) for Piperacillin Tazobactam and Pina-Sánchez et al (2025) (23) in the case of Aztreonam. Increased trend of resistance to Carbapenems was noted by Chatterjee N. et al (2023) (24) . However, Yadav et al (2024) (22) noted a high susceptibility to levofloxacin, and higher rates of resistance to cephalosporins was noted by Kumar D et al. (2024) (25).

## Conclusion

Overall, this study reinforces the importance of local surveillance in informing antibiotic stewardship, especially in high-burden healthcare settings.

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## Ethics approval and consent to participate

None.

## Conflict of interest

The authors declare no conflict of interest.

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