**Prevalence and Antibiotic Sensitivity Pattern of Pseudomonas Aeruginosa Isolates from Urine Samples in A Tertiary Care Hospital**

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**Abstract**

**Background:** Urinary tract infection (UTI) is one of the leading causes of infection worldwide. *P. aeruginosa* is a versatile opportunistic pathogen and it is the third most common organism causing nosocomial urinary tract infections. With the widespread use of antibiotics, multidrug resistance pseudomonas continues to go up rapidly. This multidrug resistance pseudomonas increases the risk of mortality and morbidity.

**Objective:** To determine the prevalence of *P. aeruginosa* and to understand the current statistics of its antimicrobial resistance pattern.

**Methods:** This retrospective study was conducted from January 2019 to December 2020 in the Department of Microbiology, Bangladesh Medical College Hospital, Dhaka. Clean catch mid-stream urine samples were collected in sterile containers. The samples were cultured on CLED agar media with a standard calibrated loop and incubated at 37°C overnight. *P. aeruginosa* grows well at 25°C to 37°C, and its ability to grow at 42°C helped to distinguish it from other Pseudomonas species. Antibiotic sensitivity test was done according to Clinical and Laboratory Standard Institute (CLSI) guideline.

**Results:** A total of 10427 urine sample were received in the department of Microbiology of BMCH during these two years. Isolation rate of *P. aeruginosa* was 4% in 2019 and it increased to 6% in 2020. Piperacillin/Tazobactam showed sensitivity which was 63.33% in 2019 and 82% in 2020, followed by imipenem, meropenem, amikacin, ciprofloxacin, ceftazidime and aztreonam; which were 50% to 60% on an average. Sensitivity of the organism to Gentamycin (13.33% in 2019 & 30.3% in 2020), Netilmicin (20% in 2019 & 30.3% in 2020) and Ceftriaxone (13.33% in 2019 & 12.3% in 2020) was found very low.

**Conclusion:** *P. aeruginosa* isolates in urine culture is increasing in hospital admitted patient and becoming resistant to multiple antibiotics which is frightening.

**Keywords:** *P. aeruginosa*, urine, sensitivity.

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Introduction
Urinary tract infection (UTI) is one of the leading causes of infection worldwide. Escherichia coli is predominantly associated with UTI followed by Klebsiella, Proteus, Enterobacter, Citrobacter, Enterococci etc. However non fermenting Gram-negative bacilli such as Pseudomonas is now an emerging pathogen which is an observation of our laboratory and from others. Pseudomonas is a ubiquitous, Gram-negative bacillus that can survive in myriad of environment such aquatic and terrestrial. It is versatile opportunistic pathogen associated with nosocomial infection. The capability of surviving in variety of environmental conditions make it is ubiquitous pathogen, allowing it to persist on numerous living and nonliving surface due to minimum nutritional requirements. According to the report of nosocomial infection surveillance system of center for disease control and prevention P. aeruginosa is the third most common organism causing nosocomial urinary tract infections. Recently this bacterium has acquired resistance to various antibiotics. With the widespread use of antibiotics such as quinolones both in the hospital and community settings, multidrug resistance pseudomonas continues to go up rapidly. This multidrug resistance pseudomonas increases risk of mortality and morbidity. Therefore, the aim of the present study was to determine the prevalence of P. aeruginosa in a tertiary care hospital and to understand the current statistics of the antimicrobial resistance pattern of this Gram-negative opportunistic pathogen.

Materials and Methods
The study was conducted in the department of Microbiology, Bangladesh Medical College Hospital (BMCH), Dhaka. This retrospective observational study was conducted over a period of two years from January 2019 to December 2020. Samples were received from both inpatient and outpatient department of this hospital. Age and gender of the patients were noted. Clean catch mid-stream urine samples were collected in sterile containers. The samples were cultured on CLED agar (Cystine Lactose Electrolyte Deficient) media with a standard calibrated loop and incubated at 37°C overnight. All the culture and sensitivity reports of urine with positive Pseudomonas aeruginosa showing ≥10^5 colony forming units /ml were considered as significant bacteriuria. The P. aeruginosa isolates were identified by conventional biochemical test. P. aeruginosa grows well at 25°C to 37°C, and its ability to grow at 42°C helps to distinguish it from many other Pseudomonas species. Antibiotic sensitivity test was done by Kirby-Bauer disc diffusion method on Mueller Hinton agar media and interpretation were done according to Clinical and Laboratory Standard Institute (CLSI) guideline. Antibiotics against which susceptibility tested were Ceftriaxone (30µg), Amikacin(30µg), Ciprofloxacin (5µg), ceftazidime(30µg), Cefuroxime (30µg), Imipenem (10µg), Meropenem (10µg), Piperacillin/Tazobactam (110µg), Aztreonam (30µg), Netilmicin (30µg), Gentamycin (10µg) and Colistin (10µg).

Results
A total of 10,427 urine sample were received in the department of Microbiology of BMCH. During these two years out of 10,427, 1337(13%) sample showed growth after culture. Among the positive urine cultures P. aeruginosa were identified in 63 (4.7%) cases. Isolation rate of P. aeruginosa was 4% in 2019 and it increased to 6% in 2020 (Table I). Out of 63 positive P. aeruginosa culture, 51 (81%) was from male. Majority of the samples (84%) were from indoor patient (Table II).
Table II

| Distribution of sample according to the age, sex & site of collection (N=65) |
|-----------------|-----------------|-----------------|
|                  | 2019            | 2020            | Total (%) |
| Age              |                 |                 |           |
| Child            | 2               | 7               | 9 (14.29) |
| Adult            | 28              | 26              | 54 (85.71)|
| Sex              |                 |                 |           |
| Male             | 26              | 25              | 51 (80.95)|
| Female           | 4               | 8               | 12 (19.05)|
| Site             |                 |                 |           |
| Indoor           | 29              | 24              | 53 (84.13)|
| Outdoor          | 1               | 9               | 10 (15.87)|

Table III

| Antibiotic sensitivity pattern of Pseudomonas species |
|---------------------------------|-------------|-------------|
| Drugs                           | 2019        | 2020        |
|                                 | N=30        | N=33        |
| Ceftriaxone (CRO)               | 4 (13.33%)  | 4 (12.12%)  |
| Amikacin (AK)                   | 17 (56.66%) | 19 (57.57%) |
| Ciprofloxacin (CIP)             | 17 (56.66%) | 13 (39.39%) |
| Ceftazidime (CAZ)               | 17 (56.66%) | 14 (42.42%) |
| Imipenem (IPM)                  | 20 (66.66%) | 18 (54.54%) |
| Cefuroxime (CXM)                | 0           | 1 (3.03%)   |
| Piperacillin/Tazobactam (TZP)   | 19 (63.33%) | 27 (81.81%) |
| Meropenem (MEM)                 | 15 (50%)    | 21 (63.63%) |
| Aztreonam (ATM)                 | 16 (53.33%) | 14 (42.42%) |
| Netilmicin (NET)                | 6 (20%)     | 10 (30.30%) |
| Gentamicin (GN)                 | 4 (13.33%)  | 10 (30.30%) |
| Colistin (CT)                   | 4 (13.33%)  | 11 (33.33%) |

Piperacillin/Tazobactam showed higher sensitivity which was 63.33% in 2019 and 82% in 2020, followed by Imipenem, Meropenem, Amikacin, Ciprofloxacin, Ceftazidime and Aztreonam, which were 50% to 60% on an average. Sensitivity of Gentamicin (13.33% in 2019 & 30.3% in 2020), Netilmicin (20% in 2019 & 30.3% in 2020) and Ceftriaxone (13.33% in 2019 & 12.3% in 2020) against P. aeruginosa was found very low (Fig.-1).

Discussion

Pseudomonas aeruginosa has become one of the leading causes of hospital acquired as well as community acquired infections due to significant changes in the microbial genetics. Also, the indiscriminate use of antibiotics has resulted rapid spread of acquired multidrug resistance (MDR) that has become global problem.

In our study the rate of isolation of Pseudomonas aeruginosa was 4.7% in urine samples. The isolation rate in the present study is compared to some recent studies from India and other countries. Regha et al\textsuperscript{10} from Kerala, India found 3.5% in 2018, Singh et al\textsuperscript{3} from Uttar Pradesh, India found 6.75% in 2017 and Shah et al\textsuperscript{5} found 5.4% from Karachi in 2015. Even though there is a slight variation in the prevalence, \textit{P. aeruginosa} continue to be an important uropathogen in majority of the studies.

When factors such as age, sex in patient and in and outpatient departments were considered, we found that the occurrence of \textit{P. aeruginosa} was higher in male (70%) in adult age. We have also found 85% isolation from indoor patient. Shobha et al\textsuperscript{11} from Karnataka, India showed 65% male patients in their urine sample had Pseudomonas growth and among them 84% patients were from indoor department. This male preponderance in Pseudomonas infection in urine also goes in concordance with different studies.\textsuperscript{12,13}

The highest sensitivity of \textit{P. aeruginosa} isolates was against piperacillin/tazobactam (63% in 2019 and 82% in 2020), imipenem (66.7% in 2019 & 54.5% in 2020), meropenem (50% in 2019 & 63.6% in 2020). Next to this amikacin (56.66% in 2019 and 57.57% in 2020). Sensitivity of ciprofloxacin, ceftazidime, aztreonam has been decreased from 2019 to 2020 which is 56.7%
to 39.45%, 56.7% to 42.4%, and 53.3% to 42.4% respectively. In our study imipenem sensitivity decreased from 2019 to 2020 which was 66.7% to 54.5%. Shobha et al. found 95.45% Imipenem sensitivity against *P. aeruginosa* in 2015 which reduced to 54.2% in 2017. Regha et al. from Kerala, India in 2018 found 72% sensitivity of imipenem which was high in comparison to us. Resistance to carbapenem may be due to the result of complex interaction of several mechanisms including production of carbapenemase, over production of efflux pumps that reduce intracellular concentration of the antibiotic.

Present study showed very low sensitivity of gentamycin (13.33% in 2019 & 30.3% in 2020), netilmicin (20% in 2019 & 30.3% in 2020) and ceftriaxone (13.33% in 2019 & 12.3% in 2020) against *P. aeruginosa*. Shobha et al. found 37.1% sensitivity of gentamycin in 2011 later in 2017 same group found sensitivity 48.6%. Regha et al. found 47% gentamycin sensitivity against *P. aeruginosa* in urine. Pseudomonas resistance to aminoglycosides is probably due to acquisition of resistance genes. Acquisition of aminoglycoside and âlactamresistance gene has been reported in *P. aeruginosa*.

Sensitivity of ciprofloxacin has been decreased from 56.7% in 2019 to 39.45% in 2020. Principal mode of fluoroquinolone resistance in *P. aeruginosa* is due to target modifications in DNA gyrase and topoisomerase IV or mutation of regulatory genes of efflux pumps that reduce intracellular concentration of the antibiotic.

Overall, this low sensitivity of urinary *P. aeruginosa* against commonly used antimicrobials is very alarming. The antibiotic resistance in *P. aeruginosa* is multifactorial in that it can occur through innate, acquired or adaptive mechanisms. The diversity of antibiotics mechanisms contributes to the development of multidrug resistance strains and makes conventional antibiotics ineffective for the treatment of *P. aeruginosa* infections.

**Conclusion**

*P. aeruginosa* isolates in urine culture is increasing in hospital admitted patient. This organism is becoming resistant to aminoglycoside, fluoroquinolone, carbapenem and â lactam group of antibiotics which is frightening.

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