Prevalence of *Pseudomonas* sp. in urine samples of female students and their anti-drug potential against some common antibiotics

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ABSTRACT

Urinary tract infections (UTIs) are one of the most common bacterial infections in women. Total 40 urine samples were collected from female students of school and colleges and screened for the presence of *Pseudomonas* sp. The bacterial isolates were identified as *Pseudomonas* sp. on the basis of their morphological, cultural and biochemical characteristics. The isolates were subjected to the antibiotic susceptibility test using Kirby Bauer Disc Diffusion Method against 10 antibiotics. The results of the present study revealed that 35% of the total samples were shown the presence of *Pseudomonas* sp. However, prevalence of *Pseudomonas* sp. was 25% of the 20 samples of school going female students whereas 45% of college going students. Most of the common antibiotics were ineffective against the isolates, of which Amoxicillin, Cephalexin and Tetracycline exhibited complete resistance in both categories of students whereas Azithromycin and Imipenem shown sensitivity against 60% isolates found in the samples of school going students. Besides this 80-100% isolates were found to be resistant against the rest of the antibiotics. Hence, the results indicated the prevalence and antibiotic resistance potential of *Pseudomonas* sp. in older females is higher than that of their younger counterparts.

Keywords: Urinary tract infections, Female students, *Pseudomonas* sp., Anti-drug potential

INTRODUCTION

Urinary tract infection (UTI) is any infection which involves any part of the urinary tract, namely the kidneys, ureters, bladder and urethra (Tan and Chlebicki, 2016). Common pathogens that involve UTI are *Escherichia coli*, *Klebsiella* sp., *Proteus mirabilis*, *Pseudomonas* sp., *Streptococci*, *Staphylococcus* sp. such as *S. saprophyticus*, *S. epidermidis*, Coagulase-negative *Staphylococci*, *Serratia* sp., *Enterobacter* sp., Gram-negative enteric bacteria and yeasts (Calvin, 1994; Atlas, 1995; Mark and Gordon, 1994).

The normal female urinary tract has a comparatively short urethra, and therefore, it has an intrinsic vulnerability to proximal seeding of bacteria. This anatomy increases the frequency of infections (Bekele et al., 2015). Hence, females are more susceptible to UTI than males. In general, a woman experiences UTI at

least once in her lifetime. It essentially occurs when germs infect the bladder, kidneys or tubes, in other words the tract that carries urine out of a woman's body. UTI is not a serious threat if treated well in time. However, if the infection spreads to the kidney it can be lethal and can even cause death in some unfortunate cases (Kohli, 2011).

Generally urine of healthy individual does not contain bacteria. However, small number of bacteria may be found in it. Normally, urinary tract urine mostly dominated by *E. coli* 75%- 80%, followed by *S. saprophyticus* 10-15% (Balakrishnan and Hill, 2010; Farajnia et al., 2009). *Pseudomonas* is a one of the common uropathogens that cause asymptomatic bacteriuria (a condition in which bacteria are present in the urine without the signs of UTI) and responsible for the various types of UTI (Givler and Givler, 2018).

Pseudomonas sp. is a gram-negative bacilli having large inherent resistance to various antibiotics. This property along with its instant ability makes this pathogen a growing problem in infectious disease pathology, especially of nosocomial origin. There is increase in the incidences of the infections caused by *Pseudomonas* sp. both in hospital and in general community (Todar, 2008).

High rates of mortality and morbidity associated with *Pseudomonas* sp. induced UTIs in spite of the developments in antimicrobial therapy may be due to the inadequate literature in relation to pathogenesis of UTIs caused by it and ability of this organism to acquire new antimicrobial resistance along with its inherent resistance to various antibiotics (Ferreiro et al., 2017). Thus, the present work aims to study the prevalence of *Pseudomonas* sp. in urine samples of school and college going female students and its antibiotic resistance.

MATERIALS AND METHODS

Study was performed at Nagpur (Maharashtra) in which a total of 40 urine samples of school going (20 samples) and college going (20 samples) female students were collected for the study. All the microbiological media and antibiotics used in the present study were purchased from Himedia Laboratories Pvt. Ltd., Mumbai, M.S., India.

Sample Collection:

Midstream urine samples were collected from the school (5 to 12 years of age) and college going female students (18 to 23 years of age) in sterile sample collection bottles.

Isolation of *Pseudomonas* sp. :

A loopful of urine sample was inoculated on *Pseudomonas* Isolation agar and the isolated bacteria were identified on the basis of morphological, cultural and biochemical characteristics (Collee and Marr, 1996).

Antibiotic sensitivity test:

Antibiotic sensitivity test was performed by Kirby Bauer Disc Diffusion method (Bauer et al., 1966). Ten different types of antibiotics were used in the study (Table 1). Isolated bacterial pathogens were grown on nutrient agar at 37^0C for 24 hours and the colonies were suspended in sterile saline water equivalent to a 0.5 McFarland standard ($1.5X10^8$CFU/ml). Hi-sensitivity agar plate was uniformly seeded by adding 100µl inoculated broth and was spread by means of spreader. The discs were placed on each

inoculated Hi-sensitivity agar plate. The plates were incubated at 37^0C for 18 hours. The diameter of the zone of inhibition was observed in mm and the isolates were classified as "resistant" or "sensitive" based on the standard interpretative chart according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2007).

Table 1: Antibiotics Used in the Study

Antibiotics	Concentration	Antibiotics	Concentration
Amoxicillin	30ug	Cotrimoxazole	25ug
Azithromycin	15ug	Gentamycin	120ug
Aztreonam	30ug	Imipenem	10ug
Ciprofloxacin	5ug	Nalidixic Acid	30ug
Cephalexin	30ug	Tetracycline	30ug

RESULTS AND DISCUSSION:

Isolation of *Pseudomonas* sp.:

Urinary tract infection in females is among the most frequent infections in the community. Total 40 urine samples were collected, 20 each from school going and college going female students. Out of which 14 (35%) samples showed the presence of *Pseudomonas* sp. However, out of 20 samples of school going female students 5 (25%) samples were found to be positive with *Pseudomonas* sp. whereas 9 (45%) samples of college going students shown the presence of *Pseudomonas* sp (Table 2). Prevalence of high percentage of positive samples in college going students also indicates the low level sanitations practices among the washrooms in the colleges and lack of personal hygiene at this age (Calvin, 1994). In the similar study involving bacteriological analysis of urine samples of catheterized patients, 26.03% of females shown prevalence of *Pseudomonas aeruginosa* (Bekele et al., 2015).

In the work carried out by Ezeadila et al., (2015) urine samples of female students living in the hostels were analyzed for the presence of bacteria and the antibiotic sensitivity pattern of the isolates was studied. The 29% of the samples analyzed were positive for UTI. Substantial UTI was evidenced most among students of age 20-24 years (37.88%) and students studying non medical related courses (48.48%). These results are comparable with that of the present work. As in the present investigation the higher prevalence of *Pseudomonas* sp. was found in the older females as compared with their younger counterparts.

Table 2: Antibiotic Resistance Pattern of *Pseudomonas* sp. isolated from Urine Samples

Antibiotics	School going female students (No. of isolated *Pseudomonas* sp. =5)		College going female students (No. of isolated *Pseudomonas* sp. = 9)	
	S	R	S	R
Amoxicillin	-	5 (100%)	-	9 (100%)
Azithromycin	3 (60%)	2 (40%)	5 (56%)	4 (44%)
Aztreonam	1(20%)	4 (80%)	1 (11%)	8 (89%)
Ciprofloxacin	1 (20%)	4 (80%)	-	9 (100%)
Cephalexin	-	5 (100%)	-	9 (100%)
Cotrimoxazole	1 (20%)	4 (80%)	-	9 (100%)
Gentamycin	1 (20%)	4 (80%)	1 (11%)	8 (89%)
Imipenem	3 (60%)	2 (40%)	2 (22%)	7 (78%)
Nalidixic Acid	1 (20%)	4 (80%)	-	9 (100%)
Tetracycline	-	5 (100%)	-	9 (100%)

Where, R= Resistant, S= Sensitive

Antibiotic sensitivity of isolated strains of *Pseudomonas* sp.:

All the urinary isolates of *Pseudomonas* sp. exhibited complete resistance to Amoxicillin, Cephalexin and Tetracycline (100% each) in both types of female students. Azithromycin and Imipenem (60% each) were found to be effective against *Pseudomonas* sp. in school going students while Azithromycin (56%) was more effective against college going students. Besides this, *Pseudomonas* sp. isolated from college going students, were found to be completely resistant to Ciprofloxacin, Cotrimoxazole and Nalidixic acid (100% each). This indicates that resistance pattern of the college going female students was more as compared to that with school going female students (Table 2). This is might be due to the indiscriminate and incomplete use of antibiotics by the college going female students (Akter et al., 2013). In contrast to the present results, strains of *P. aeruginosa* isolated from urine samples of catheterized patients were found to be susceptible to the Ciprofloxacin. However, the urinary isolates of catheterized patients shown resistance to Gentamycin as evidenced in the present study (Bekele et al., 2015).

CONCLUSION

The results shown considerable prevalence of *Pseudomonas* sp. isolates among college and school going female students where the proportion of college going students detected positive for *Pseudomonas* sp. was higher than their school going counterparts. The antibiotic resistance potential of *Pseudomonas* sp. isolated from urine samples of younger females is relatively lower as compared to that of older females. Since the resistance pattern of pathogens are ever changing, dealing with a limited number of samples as in

case of the present study it is necessary to carry out a broad-based study that can reflect the authentic data and reliable information as treatment guidelines for UTIs.

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