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Full Length Research Paper

Isolation and antibiotics sensitivity pattern of *Pseudomonas aeruginosa* species implicated with urinary tract infection among women patients visiting Federal Teaching Hospital, Abakaliki, Ebonyi State

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The isolation and antibacterial sensitivity pattern of *Pseudomonas aeruginosa* species implicated in urinary tract infections among women visiting Alex Ekwueme Federal Teaching Hospital, Abakaliki, Ebonyi State were evaluated. Forty midstream urine samples were collected from among women visiting the hospital. The samples were subjected to bacteriological screening by culturing on cysteine lactose and electrolyte-deficient (CLED) agar, nutrient agar, and Eosin MacConkey agar. Twelve *Pseudomonas* species were isolated, characterized and identified out of the forty urine samples analyzed. Results of antibiotics percentage resistance and susceptibility of *Pseudomonas* species isolated revealed that *Pseudomonas* species were 100% resistant to cefoxitin, *Pseudomonas* species were susceptible to Ciprofloxacin (75 %), oxacillin (20 %), vancomycin (60%) and gentamicin (90 %). In this study, the resistant nature of *Pseudomonas* species could be due to the presence of a resistant gene (plasmid mediated resistant gene) or the presence of efflux pump.

Keywords: Ciprofloxacin, antibacterial, Urinary tract infection, *Pseudomonas aeruginosa*

INTRODUCTION

Urinary tract infection (UTI) is an infection that affects part of the urinary tract. When it affects the lower urinary tract it is known as a bladder infection (cystitis) and when it affects the upper urinary tract it is known as kidney infection (pyelonephritis) (Gould *et al.,* 2010). Symptoms from a lower urinary tract include pain with urination, frequent urination, and feeling the need to urinate despite having an empty bladder (Döring *and Pier, 2008*). Symptoms of a kidney infection include fever and flank pain usually in addition to the symptoms of a lower UTI. Rarely, the urine may appear bloody. In the very old and the very young, symptoms may be vague or non-specific (Gould *et al*., 2010). One of the most common causes of infection is *Pseudomonas* and *Escherichia coli*, though other bacteria or fungi may rarely be the cause (Forestier *et al*., 2008). Risk factors include female anatomy, sexual intercourse, diabetes, obesity, and family history. Although, sexual intercourse is a risk factor, UTIs are not classified as sexually transmitted infections (STIs). *Pseudomonas aeruginosa* is a non-fermenter gram-negative bacilli with a large intrinsic resistance to multiple antibiotics (Hachem *e t al*., 2007). This organism has the ability rapidly acquire new antimicrobial resistance, makes this pathogen a growing problem in infectious disease pathology, especially when nosocomial in origin. There are no existing medical studies that explore possible factors associated with decreased survival in hospitalized patients

with urinary tract infections caused by *Pseudomonas aeruginosa* nor is the mortality of these patients known, except those associated with bacteremia, with an estimated mortality at 30 days from 5 to 33 %. Multiple factors have been associated with decreased survival in patients with bacteremia due to *Pseudomonas aeruginosa* such as age, low functional status, need for mechanical ventilation, central venous catheter, inadequate treatment with antibiotics and resistance to carbapenems. The influence of an appropriate empiric treatment on the mortality of patients with *P. aeruginosa* infections remains controversial, with mixed results in various studies in patients with bacteremia (Forestier *et al*., 2008). The aim of this work is to isolate, identify and determine the antibiotics susceptibility pattern of the isolated bacteria implicated in urinary tract infections among women visiting Alex Ekwueme Federal University teaching Hospital, Abakaliki, Ebonyi State.

MATERIALS AND METHODS

Study area

This research was carried out in the Department of Applied Microbiology Laboratory, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria. It lies approximately within the longitude 7° 30ˈand 8° 30ˈ E and 5° 40ˈ and 6° 45ˈ N. According to data from 2006 population and housing census, Ebonyi State has an estimated population of about 2.3 million and a land mass of 5,935 km^2.

Antibiotic used

The following Oxoid antibiotics disk were used: Ofloxacin (OFX, 5 µg), Vancomycin (VA 30 µg), Oxacillin (OX, 1 µg), Gentamicin (CN 10 µg), Ciprofloxacine (CIP, 5 µg).

Collection of samples

Forty samples of urine were collected from patients at Smile specialist hospital, Abakaliki between the month of May and June, 2019. The samples were collected with sterile container and labeled accordingly. In some instance, the samples were collected from different patients. The urine amples were transported to the Department of Applied Microbiology Laboratory, Ebonyi State University, Abakaliki, Nigeria for bacteriological analysis.

Cultivation of the Samples for Isolation

Urine samples were inoculated into nutrient broth and the swab stick was inserted into urine on already prepared nutrient agar and incubated aerobically at 37°C for 18-24 h. A loopful of inoculated nutrient broth and nutrient agar were streaked on MacConkey agar, CLED agar and Eosin medium. The inoculated medium were incubated aerobically at 37°C for 24-48 hours and then examined for bacterial growth. The colonies were sub-cultured on MacConkey and nutrient agar and incubated at 37°C for 24 h to obtain the pure cultures of the isolate.

Identification of isolates

The bacteria isolates were primarily characterized and identified by microscopic examination, Gram staining, Sugar fermentation and other biochemical test (Cheesbrough, 2006).

Standardization of test bacteria

All the test bacteria isolated and purified were standardized before use by inoculating 5ml normal saline in sterile test tubes with loopful of a 24 h culture of the test organism from a nutrient agar slant. The dilutions using loopful of the test organism was done to obtain microbial population of 10^5colony forming unit per ml (CFU/ml) by comparing it with 0.5 McFarland turbidity standard (Cheesbrough, 2006).

Antibiotics susceptibility test

The susceptibility and resistant pattern of isolates were determined by the Kirby-Bauer susceptibility test method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2002). The following Oxoid antibiotics disk were used: Ofloxacin (OFX, 5 µg), Vancomycin (VA 30 µg), Oxacillin (OX, 1 µg), Gentamicin (CN 10 µg), Ciprofloxacine (CIP, 5 µg).Test antibiotics listed above were aseptically placed on the inoculated Muller Hinton agar plates and incubated at 37^{o}C for 18-24 h. Inhibition zone diameters were measured and the organisms were identify as either susceptible or resistance based on National Committee for Clinical Laboratory Standards (NCCLS, 2002).

RESULTS

The morphological and biochemical characteristics of the *Pseudomonas* species isolated from urine samples showed that *Pseudomonas* species were rod shaped, light green in colour, single in arrangement, positive in Gram reaction and Motility negative (Table 1). The demographic distribution of samples base on the age range of patients and sex and percentage frequency of

Table 1. Morphological and Biochemical Characteristics of the *Pseudomonas* species.

Shape	Colour	Arrangement	Gram rxn	CA	CO	OX	IND	MR	Motility	Suspected organism
Rod	Light green	Single	+	+	-	-	+	+	-	*Pseudomonas* species

Table 2. Demographic Distribution and Percentage frequency of *Pseudomonas* species Isolated from female urine of patients attending Alex Ekwueme Federal teaching hospital Abakaliki.

Age	Sex	Number of samples collected	Number of *Pseudomonas* species Isolated	Percentage % of the *Pseudomonas* species isolated
17-25	Female	10	3	3(25.1 %)
26-34	Female	14	5	5(41.7 %)
35- 43	Female	10	2	2(16.6 %)
44-52	Female	6	2	2(16.6%)
Total		40	12	16(100%)

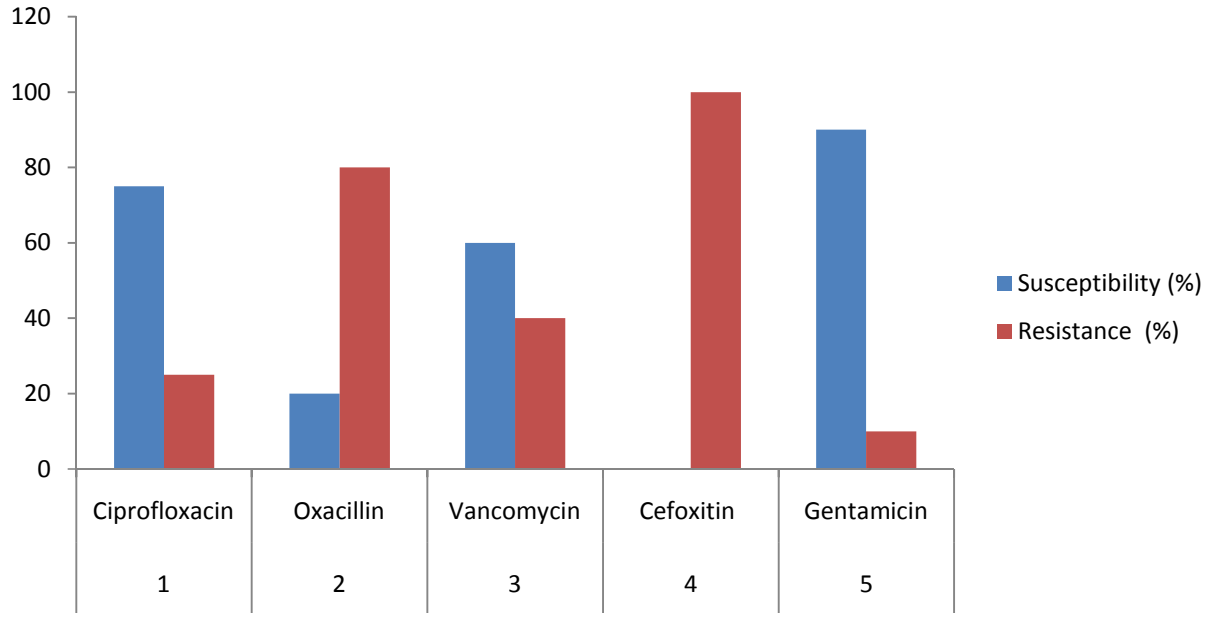

Figure 1. Percentage resistance and susceptibility of *Pseudomonas* species against different antibiotics.

Salmonella species isolated from urine samples of patients attending Alex Ekwueme Federal Teaching Hospital Abakaliki showed that out of 40 urine sample were analyzed, twelve (12) *Pseudomonas* species were identified which includes age 3(25.1 %) 17-25 and 5(41.7 %) 26-34, while 35-43 and 44-52 were 2(16.6 %) respectively (Table 2). The results of antibiotics percentage resistance and susceptibility of *Pseudomonas* species isolated from urine of female patients attending Alex Ekwueme Federal Teaching Hospital Abakaliki, showed that *Pseudomonas* species were 100% resistant to cefoxitin while *Pseudomonas* species were susceptibility to Ciprofloxacin (75 %), oxacillin (20 %), vancomycin (60%) and gentamicin (90 %) (Figure 1).

DISCUSSION

A total of 40 mid stream urine samples were collected from women patients visiting Alex Ekwueme Federal Teaching Hospital, Abakaliki, Ebonyi State. Twelve *Pseudomonas* species were characterized and identified on the basis of their morphology,Gram staining and biochemical tests. The demographic distribution of samples base on the age range of patients, sex and Percentage frequency of *Salmonella* species from 40 urine samples analyzed twelve *Pseudomonas* species were identified which included age 3(25.1 %) 17-25 and 5(41.7 %) 26-34, while 35-43 and 44-52 were 2(16.6 %) respectively (Table 2). The high prevalence in this study

might be due to women in whom UTIs develop, the urethra is colonized and the uropathogen gains entry to the bladder, presumably by means of the urethral massage that accompanies sexual intercourse. Once the bacteria ascend into the bladder, they may multiply and then pass up the ureters, particularly if vesico ureteral reflux is present, to the renal parenchyma. This is in agreement with the previous studies of Abraham *et al*., 2015 and Flores-Mireles *et al*.,2015. The results of antibiotics percentage resistance and susceptibility of *Pseudomonas* species isolated from female patients attending Alex Ekwueme Federal Teaching Hospital Abakaliki under the age of 1-17 showed that *Pseudomonas* species were 100% resistant to cefoxitin and *Pseudomonas* species were susceptibility to Ciprofloxacin (75 %), oxacillin (20 %), vancomycin (60%) and gentamicin (90 %) (Figure 1).In this study the resistant nature of *Pseudomonas* species could be due to the presence of a resistant gene encoded in the plasmid (plasmid mediated resistant gene) or the presence of efflux pump.

Conclusion

In conclusion, the risk of contracting *P. aeruginosa* can be reduced by avoiding pools, hot tubs, and other stagnant water bodies, regularly disinfecting and/or replacing equipment that regularly encounters moisture (such as contact lens and solutions) and washing one's hands regularly (which is protective against other pathogens as well). However, even the best hygiene practices cannot totally protect an individual against *P. aeruginosa*, considering its abundance in the environment.

Authors' declaration

We declared that this study is an original research by our research team and we agree to publish it in the journal.

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