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Prevalence and antimicrobial susceptibility of *Pseudomonas aeruginosa* isolated from urine samples of pregnant women presenting with urinary tract infections with asymptomatic bacteriuria in different hospitals in Abakaliki, Ebonyi State

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ABSTRACT

***Background**: Pseudomonas aeruginosa* organisms are notorious for their intrinsic resistance to multiple antibiotics and have been seriously implicated in urinary tract infections (UTIs). In this study, the prevalence and antibiogram of *Pseudomonas aeruginosa* isolated from the urine samples of pregnant women hospitalized at different clinics was investigated.

Methods: A total of fifty (50) urine samples obtained by informed consent from pregnant women were cultured on Centrimide agar for the isolation of *P. aeruginosa*. Significant growth was sub-cultured on Centrimide agar for microbiological analysis. Various physical and biochemical tests as well as antimicrobial susceptibility test were carried out on the isolates. **Results**: A prevalence rate of 45% observed for *P. aeruginosa* was considered high (n=22). The prevalence of *P. aeruginosa* among pregnant women in relation to age and educational status revealed that the observed changes are likely to have occurred by chance at $\alpha = 0.05$. The antimicrobial susceptibility test revealed that drugs found most effective against *P. aeruginosa* were Gentamicin, Imipenem and Ciprofloxacin, while the other antibiotics used showed little or no effect.

Conclusion: Routine check of UTIs due to *Pseudomonas aeruginosa* is advocated in pregnant women especially in hospitalized cases.

1. Introduction

The quick ability of *P. aeruginosa* to acquire a new antimicrobial resistance makes it a growing problem in infectious disease pathology, especially when nosocomial in origin[1]. There are reports associated with decreased survival in hospitalized patients with bacteremia due to *P. aeruginosa*. This is of a great concern as UTIs remain one of the most prevalent diseases in hospitalized patients, accounting for between 20 and 49% of all nosocomial infections[2, 3]. Also, *P. aeruginosa* remains a common pathogen associated with hospital-acquired catheter-associated UTIs in female pregnant patients[4,5].

P. aeruginosa most commonly exists in environments like water, plants and soil. It also exists in moist or wet areas, like bathtubs or sinks as well as body surfaces[6, 7]. *P. aeruginosa* can cause sepsis, septicemia, ear and eye

infection, lung, skin and urinary tract infections mostly in immunocompromised individuals[8]. Transmission is known to be partly endogenous and exogenous. Human digestive flora has been described as the main source of endogenous transmission mostly due to multi-drug resistant (MDR) Pseudomonas. Hydric environment as well as medical staff has been identified as the key factor for exogenous transmission[9].

Pseudomonas plays a dual role as beneficial environmental bacteria, and potential human and animal pathogens[10]. The persistence and recurrent sources of infection in hospitalized pregnant women due to *P. aeruginosa* have been attributed to its ability to form biofilms on the surfaces of urinary catheters[11]. Formation of biofilm is an important mechanism for increased antibiotic resistance as well as resistance to host immune system[12] .

The organism has a tendency to cause multi-site infections, of which bacteremia is fetal, with a mortality rate ranging from 18% to 61% globally[13]. *P. aeruginosa* bacteremia could trigger severe septic shock and multiple organ failure, and result in high mortality rate and substantial medical cost[14].

Pseudomonas has been highlighted as an antibiotic-resistant pathogen of concern by the World Health Organization (WHO). The isolates are known to display higher levels of antibiotic resistance than *Escherichia coli,* the most common UTI pathogen[15]. The organism can invade corneal cells, lung epithelia cells and recently urinary epithelial cells[8]. Current evidence suggests that patients suffering from *P. aeruginosa* UTI will be infected by a single clone type rather than multiple distinct strains. Consequently, antibiotic combination therapy has no beneficial influence on clinical outcomes of therapy, but rather, monotherapy has proven more valuable[16,17].

P.aeruginosa has been an important uropathogen that causes complicated urinary tract infections. This is basically on the fact that the organism can invade bladder epithelial cells in an *in vitro* model[17]. It also has a high capacity to adapt to adverse conditions such as pH and osmoregularity of urine. *P. aeruginosa* urinary tract infections are highly antibiotic resistant and require costly and intensive treatment[18].

Methodology

Study area and population:

This study was carried out at Abakaliki sub-urban Areas, in Ebonyi State, southeastern Nigeria. According to the United Nation World Urbanization Prospects, Abakaliki's 2023 population is now estimated at 662,202. Abakaliki is predominantly inhibited by civil servants, students, traders, artisans and cohort miners. The sub-urban hospitals where the study was conducted are mostly patronized by indigenes and few settlers. Female who are pregnant and admitted in such hospitals were enrolled for the study. Samples were collected between the month of October and November, 2023.

Sample collection and processing:

Hospitals and individual consents were obtained before samples were collected with the anonymity and confidentiality of the results being assured. Sterile urine bottles were labelled and participants instructed to aseptically collect mid-stream urine. A total of fifty samples were collected, packed in ice-bag, and transported to the microbiology laboratory unit of Ebonyi State University for analysis.

Laboratory Procedure (Cheesbrough, 2006)[19]

Two miles (2 ml) of agitated urine sample was cultured at 37^{o}C for 24 hours in a test tube containing 5 ml of sterile Nutrient broth. Thereafter, the overnight culture was plated on freshly prepared Centrimide agar and incubated at 37^{o}C for 24 hours. Significant colonies were sub- to obtain pure isolates which were subjected to gram staining, motility and biochemical tests. Antibiotic susceptible profile was carried out on Mueller-Hinton agar plates using the Kirby-Bauer disc diffusion technique according to the guidelines of the Clinical and Laboratory Standard Institute (2008)[20]. Susceptibility test plates were incubated at 37^{o}C for 24 hours. Inhibition zones were measured and interpreted as either sensitive or resistant by comparison to the Standard breakpoint of the Clinical and Laboratory Standard Institute (CLSI).

Data Analysis:

Data analysis was performed in Microsoft Excel 2021 using Chi-square.

Result

Table 1: Morphological and Biochemical identification of *Pseudomonas aeruginosa*

Gram Stain	Centrimide on agar	Colour on Centrimide agar	Oxidase test	Catalase test	Indole test	Methyle-red test	Nitrate test	organism
-ve rod	Round and flat colonies	Green	+ve	+ve	-ve	-ve	+ve	*Pseudomonas aeruginosa*

Table 2: Prevalence of *Pseudomonas aeruginosa* in urine samples of pregnant women in relation to age

Age	Number screened	Number infected	% infected
15-24	14	6	43
25-34	11	4	36
35-44	25	12	48
Total	50	22	
df = 2	**X^2** = 4.73	**CV** = 5.99	**α** = 0.05

Table 3: Prevalence of *Pseudomonas aeruginosa* in pregnant women in relation to Educational Status

Educational **status**	**Number screened**	**Number infected**	**% infected**
Illiterate	12	6	50
Primary	15	7	47
Secondary	15	6	40
Tertiary	8	3	38
Total	50	22	
df = 3	**X^2 = 2.38**	**CV = 7.82**	**α = 0.05**

Table 4: Antibiogram of *Pseudomonas aeruginosa* from urine of pregnant women

Antibiotic	Potency	Resistance	%	Susceptibility	%
Gentamicin	30	2	9.1	20	90.9
Imipenem	10	0	0.0	22	100
Ampicillin	30	18	81.8	4	18.2
Ciprofloxacin	10	4	18.2	18	81.8
Meropenem	30	0	0.0	22	100
Levofloxacin	10	22	100	0	0.0
Bacitracin	10	0	0.0	22	100
Perfloxacin	30	0	0.0	22	100
Amikacin	30	19	86.6	3	13.6

DISCUSSION

Pseudomonas aeruginosa is an opportunistic Gram-negative nosocomial pathogen that is capable of causing a variety of infections in both immunocompetent and immunocompromised hosts[2]. Its predilection to cause infection among immunocompromised hosts, extreme versatility, antibiotic resistance, and a wide range of dynamic defenses makes it an extremely challenging organism to treat in modern-day medicine[6, 21]. The cases of antibiotic resistance are increasing in clinical isolates of *P. aeruginosa* and the organism has been associated with infective antibiotic treatment concern to the WHO[15].

In this study, the frequency and antibiogram of *P. aeruginosa* isolated from urine samples of hospitalized pregnant women was investigated. A determined study population of 50 participants yielded a total of 22 (44%) isolates of *P. aeruginosa* as shown in Table 1. This result is an indication of relatively high prevalence of *P. aeruginosa* amongst hospitalized pregnant women in this zone of our study. The UTIs are one of the most common bacterial infections affecting humans throughout their life span. The infection accounts for more than 8 million visits to physician's office, 1.5 million emergency room visits, and 300,000 hospital admissions in the United States annually[22, 23]. Within the hospital, *P. aeruginosa* finds numerous reservoirs: disinfectant, respiratory equipment, food, sinks, taps, and mops. Also, the organism is constantly reintroduced into the hospital environment on fruits, plants, vegetables, patient to patient transfer and contamination from hospital personnel[24]. Various works done on UTIs showed that *P. aeruginosa* gets alongside other bacteria pathogens[24,15].

In a study conducted on UTIs in Bayelsa state, *P. aeruginosa* showed a prevalence of 23% and ranked second most common after *Escherichia coli,* showing the significance of *P. aeruginosa* as an agent of UTI[25]. However, there is paucity of information on *P. aeruginosa*-associated UTIs due to lack of knowledge on its effect and significance.

Studies have shown *P. aeruginosa*'s ability to bind to, invade, and injure wounded epithelial cells. The organism possesses a widely conserved ability to invade 5637 cells to initiate UTI infection. This rare ability correlates well with the high prevalence of *P. aeruginosa* UTI in hospitalized elderly or pregnant populations who may be exposed to catheter and other invasive equipments[8].

Our study recorded the highest incidence of infection amongst the most elderly pregnant patients aged 35 and above with a prevalence of 48%, followed by 43 and 36 with age of 25-34 and 15-24, respectively (Table 2). The observed differences in the prevalence of *Pseudomonas aeruginosa* among pregnant women in relation to age range did not differ from what would have been expected theoretically. Therefore, the differences are statistically is significant.

This result is in agreement with the report of Lawan *et al*[26] who also noted that mutiparity could be an added predisposing factor for acquiring UTIs in women in aside short distance of the urethal meatus, wet environment of the urethral opening and sexual activities[27].

The results of frequency of the UTIs in the hospitalized pregnant women in relation to educational status in our study showed that illiteracy had highest value of 50% with 47, 40 and 38% for primary, secondary and tertiary, respectively (Table 3). The observed changes in the prevalence of *Pseudomonas aeruginosa* among pregnant women in relation to educational levels did not differ from what would have been expected theoretically. The rural setting where this work was conducted could be a contributory factor for the high prevalence. The sub-urban region is mostly inhabited by uneducated or averagely educated persons who may not have enough awareness on UTIs. Education offers awareness on cleanliness, hygiene and knowledge on control of infections. Our result is in agreement with the finding of Ahmed and Avasara[27] whose work in rural setting supported that malnutrition, poor hygiene, low Socio-economic status are associated with UTIs.

P. aeruginosa isolates recorded from the pregnant women in this study revealed high level of resistance to clinically relevant antibiotics such as Ampicillin (81.8%), Amikacin (86.6%), Levofloxacin (100%) etc. However, the Carbapenems (Imipenem and Meropenem), Bacitracin and Perfloxacin were highly effective on the isolates with 100% susceptibility (Table 4). The high antibiotic resistance observed in various bacterial strains could be attributed to prescription of antibiotics without laboratory guidance as well as over the counter sales of antibiotics which is a common practice in Nigeria[28].

The emergence of resistant *P. aeruginosa* strains due to the intrinsic and acquired resistance mechanism had increasingly drawn more attention especially as a high resistance level might be a therapeutic challenge for clinicians[29]. Thus, an appropriate choice of effective antibiotics according to the antimicrobial sensitivity test *in vitro* is critical to improving poor outcome for patients with *P. aeruginosa*.

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