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Comparative Study of the Prevalence and Antibiogram of Bacterial Isolates from the Urinary and Genital Tracts of Antenatal Patients

Sylvia. O. Anyadoh-Nwadike [1], Sylvester. I. Okorondu[2], Ifeanyi .O.C. Obiajuru[3], Peter O. Nwadike[4], F.O Nwaokorie[5] and John .O. Akerele [6]

[1] *Department of Biotechnology, School of Science, Federal University of Technology, Owerri, Imo State Nigeria*
[2]*Department of Microbiology, School of Science, Federal University of Technology, Owerri, Imo State Nigeria*
[3]*Department of Microbiology and Parasitology, Faculty of Medicine, Imo State University, Owerri*
[4]*KNCV Nigeria, Central Area, Abuja, FCT Nigeria*
[5]*Department of Biotechnology and Molecular Biology, Nigeria Institute for Medical Research, Lagos, Nigeria*
[6] *Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Edo State Nigeria*

Abstract: *The study compared the prevalence and antibiogram of bacterial isolates from the urinary and genital tracts of pregnant women attending ante-natal clinics in Imo State. Urine and High vaginal swab (HVS) samples were collected from across the three geopolitical zones of Imo State (Owerri, Orlu and Okigwe). Federal Medical Centre (FMC) Owerri, Imo State University Teaching Hospital (IMSUTH) Orlu and General Hospital Okigwe (GHO) were used as focal points. A total of 1197 samples were obtained from women and used. Infection was significantly more with the urine samples than the HVS samples (P < 0.05) while polymicrobial growth was more observed with the HVS samples. Escherichia coli was the predominantly isolated organism (38.3%) from the urine samples while Staphylococcus aureus (29.1%) was the predominant bacterial isolates in HVS. Other commonly isolated bacterial species include; Enterococcus faecalis and Staphylococcus epidermidis, Klebsiella pneumoniae, Proteus mirabilis and Bacteriodes were solely isolated from urine while Lactobacillus was solely isolated from HVS. Overall antibiogram showed ciprofloxacin to be the most effective antibiotic followed by nalidixic acid and pefloxac in for both specimens. Generally, multi-drug resistance was more in urine isolates (55.7%) than vaginal isolates (53.6%) with many showing the same resistance patterns. The rate of multi/drug resistance in both samples is high (>50%) and worrisome. These call for routine HVS as well as urine culture to be carried out on all antenatal women to ensure holistic antenatal care/management.*

Keywords: *Prevalence, Antibiogram, Urinary tract, Genital tract, Pregnant Women.*

I. Introduction

Ante-natal patients are pregnant women who attend the clinic to ascertain their good health and those of their fetuses from time to time. They may not necessarily be presenting any ailment (Anyadoh et al., 2010). However, urogenital tract infections (mostly bacteriuria, vaginitis, cystitis and pyelonephritis) are frequently encountered medical complications of pregnancy (Onuh et al., 2006). Although the majority of infections in pregnancy maybe asymptomatic, studies have revealed that they pose high risk of low birth weight, preterm labour, hypertension, maternal anemia, thrombosis, still birth and abortion (Zinner, 1992; Akerele and Okonofua, 2002; Onuh et al., 2006). Serious infections such as pyelonephritis could cause significant maternal and fetal morbidity and mortality (Lucas and Cunningham, 1994; Akerele et al., 2001).

The structure and function of the urinary tract undergo a lot of changes during pregnancy. Blood-volume expansion is accompanied by increase in glomerular filtration rate and urinary output (Gilstrap and Ramin, 2001). These, predispose to microbial infection. The vagina of the pregnant women is richly provided with glycogen and moisture (Homeier, 2004) which favour Candidal and bacterial growth. The microbes present may not be pathogenic except in situations where their numbers outgrow competing microorganisms or where there is an abrasion. Frequent hospital visitations (antenatal visits) may also expose the pregnant women to contact with microorganisms especially those associated with nosocomial infections.

Antibiotic resistant microbes pose serious threats to both mother and foetus as treatment becomes more difficult with safe antibiotics. The development of anti-microbial resistance in many bacterial species constitutes one of the most serious problems in the control of infectious disease (Neu, 1992; Osuala et al., 2005; Todar, 2009). Antibiotic resistance in bacteria may be an inherent trait of an organism or it may be acquired by means of mutation in its own DNA or acquisition of resistance-conferring DNA from another source (Russel, 1994; Todar, 2009). Constant exposure of clinical bacterial isolates to hospital environment results to acquisition of resistance to numerous antibiotics by various mechanisms (Anyadoh et al., 2010).

Much emphasis have been laid on urinary tract infections recently with quite little attention being given to genital tract infections especially during pregnancy, this work therefore sought to compare prevalence and antibiogram of bacterial isolates between the urinary and genital tracts of pregnant women with a view to proposing a holistic antenatal care.

II. Materials And Methods

Study location: This study was carried out in Imo State Nigeria. Antenatal clinics of Government owned hospitals located in the three zones of the State were used as focal points. The Hospitals include: Federal Medical Centre (FMC) Owerri, Imo State Teaching Hospital (IMSUTH) Orlu and General Hospital (GH) Okigwe.

Study subjects: Pregnant women attending antenatal clinics in the focal points were recruited for the study following ethical permission from the Ethics committees of the respective hospitals and signing of informed consent forms by the subjects. In all, 625 pregnant women were recruited and used as study subjects.

Specimen collection and preparation: Urine and high vaginal swab (HVS) specimens were collected from the women. Mid-stream urine specimens were then collected by the subjects into wide capped sterile specimen containers while HVS were collected by Obstetricians using cuscos' specula. The specimens were carried to the laboratories of the respective hospitals within 25 mins of collection for microbiological analysis.

Isolation and characterization of microbes: Isolation of microorganisms was done using standard materials and microbiological cultural methods; Streaking on solid agar media (Nutrient, blood and Chocolate) as described by Cheesbrough (2000). This was done aseptically and in duplicates. Incubation was done for 24 hrs at 37^{o}C. Isolates were characterized/identified using Gram Staining and other standard biochemical methods as described by Cheesbrough (2000).

Antibiotic susceptibility/sensitivity testing: Mueller Hinton Agar (MHA) medium and commercial multidisc sensitivity discs were used for the susceptibility test following the method described by [5]. The multidisc contained the following antibiotics; augmentin® (AUG) 30 µg, ceftriazone (CRO) 30 µg, nitrofurantoin (NIT) 200 µg, gentamicin (GEN) 10 µg, cotrimoxazole (COT) 25 µg, ofloxacin (OFL) 5 µg, amoxicillin (AMX) 25 µg, ciprofloxacin (CPX) 10 µg, tetracycline (TET) 30 µg and pefloxacin (PFL) 5 µg. Single discs containing nalidixic acid (NAL) 30 µg were also added alongside the multidisc.
Zones of inhibition were used to extrapolate the level of susceptibility of the isolates to the test antibiotics.

Result presentation and analysis: Results are presented using Charts, tables and analyzed using chi square (χ^2) statistical test, percentages and averages**.**

III. Results

A total of 619 urine samples and 578 HVS respectively were collected from the subjects out of which, 296 and 232 respectively had microbial growth giving a prevalence rate of 47.8% and 40.1% respectively. Out of the positive cultures, a total of 241(81.4%) and 182(78.4%) yielded single microbial isolates while 55(18.6%) and 50(21.6%) yielded polymicrobial growth for urine and HVS respectively. Table 1 shows prevalence of individual microbial species isolated from both specimens while Tables 2 and 3 show the antibiogram of bacterial isolates from Urine and HVS respectively. Tables 4 and 5 show the prevalence of multi-drug resistant isolates from both specimens respectively.

Table 1: Prevalence of various isolates from Urine and HVS of pregnant women in Imo State

Organism	URINE		HVS		TOTAL	
	Freq. of occurrence	%age prevalence	Freq. of occurrence	%age prevalence	Freq. of occurrence	%age prevalence
E. coli	132	38.3	50	16.7	182	28.3
S. aureus	121	35.1	87	29.1	208	32.3
Kl. pneumoniae	11	3.2	-	-	11	1.7
Ent. faecalis	15	4.4	14	4.7	29	3.7
S. epidermidis	27	7.8	27	9.0	54	8.4
Bacteroides	05	1.4	-	-	05	0.8
P. mirabilis	05	1.4	-	-	05	0.8
Lactobacillus species	-	-	03	1.0	03	0.5
C. albicans	29	8.4	118	39.5	147	22.8
Total	**345**	**100**	**299**	**100**	**644**	**100**

Table 2: Antibiogram of bacteria isolates from urine of pregnant women in Imo State.

Bacteria	No. (%) susceptible to											
	No. tested	AUG (30µg)	CRO (30µg)	NIT (200µg)	GEN (10µg)	COT (25µg)	OFL (5µg)	AMX (25µg)	CPX (10µg)	TET (30µg)	PFL (5µg)	NAL (30µg)
E. coli	132	75 (54.5)	68 (51.5)	76 (57.6)	51 (38.6)	63 (47.7)	62 (47)	4 (3.0)	103 (78)	35 (26.5)	89 (67.4)	104 (78.8)
S. aureus	121	48 (39.7)	41 (33.9)	74 (61.2)	35 (28.9)	105 (86.8)	114 (94.2)	57 (47.1)	118 (97.5)	55 (45.5)	108 (89.3)	115 (95)
Kl. pneumoniae	11	5 (45.5)	9 (81.8)	7 (63.6)	5 (45.5)	11 (100)	8 (72.7)	2 (18.2)	10 (90.9)	2 (18.2)	9 (81.8)	11 (100)
Ent. faecalis	15	13 (86.7)	15 (100)	6 (40.0)	10 (66.7)	13 (86.7)	12 (80.0)	4 (26.7)	14 (93.3)	5 (33.3)	13 (86.7)	13 (86.7)
S. epidermidis	27	22 (81.5)	20 (74.1)	17 (63)	22 (81.5)	25 (92.6)	24 (88.9)	12 (44.4)	26 (96.3)	12 (44.4)	24 (88.9)	26 (96.3)
Bacteroides	05	4 (80)	2 (100)	3 (60)	4 (80)	3 (60)	5 (100)	4 (80)	5 (100)	0 (0)	4 (80)	5 (100)
P. mirabilis	05	4 (80)	5 (100)	2 (40)	2 (40)	5 (100)	5 (100)	2 (40)	5 (100)	1 (20)	4 (80)	5 (100)
Total	**316**	**168 (53.2)**	**162 (51.3)**	**185 (58.5)**	**129 (40.8)**	**225 (71.2)**	**230 (72.8)**	**90 (28.5)**	**280 (88.6)**	**110 (34.8)**	**251 (79.4)**	**279 (88.3)**

Table 3: Antibiogram of bacterial isolates from HVS of pregnant women in Imo State

Bacteria	No. (%) susceptible to											
	No. tested	AUG (30µg)	CRO (30µg	NIT (200µg	GEN (10µg)	COT (25µg)	OFL (5µg)	AMX (25µg)	CPX (10µg)	TET (30µg)	PFL (5µg)	NAL (30µg)
E. coli	50	23 (46)	25 (50)	28 (56)	18 (36)	28 (56)	23 (46)	3 (6)	39 (78)	14 (28)	32 (64)	39 (78)
S. aureus	87	48 (55.2)	35 (70)	42 (48.3)	42 (48.3)	46 (52.9)	78 (89.7)	36 (41.4)	80 (92)	35 (40.2)	80 (92)	75 (86.2)
Ent. faecalis	14	10 (71.4)	13 (92.9)	6 (42.9)	11 (78.6)	13 (92.9)	10 (71.4)	4 (28.6)	12 (85.7)	5 (35.7)	11 (78.6)	13 (92.9)
S. epidermidis	27	21 (77.8)	21 (77.8)	17 (63)	17 (63)	24 (88.9)	25 (92.6)	13 (48.1)	26 (96.3)	14 (51.9)	22 (81.5)	26 (96.3)
Lactobacillus species	03	2 (66.7)	2 (66.7)	2 (66.7)	3 (100)	3 (100)	3 (100)	1 (33.3)	3 (100)	2 (66.7)	3 (100)	3 (100)
Total	**181**	**104 (57.5)**	**96 (53.0)**	**95 (52.5)**	**91 (50.3)**	**114 (63.0)**	**139 (76.8)**	**57 (31.5)**	**160 (88.4)**	**70 (38.7)**	**148 (81.8)**	**156 (86.2)**

Table 4: Prevalence of MDR Bacteria Isolates in Urine Samples of Pregnant Women in Imo State

Isolates	No Isolated	No Resistant To:			Total MDR	% MDR
		3 Drugs	4 Drugs	≥5Drugs		
E. coli	132	39	22	14	75	56.8
S. aureus	121	39	21	13	73	60.3
Kl. pneumoniae	11	3	2	1	6	54.5
Ent. faecalis	15	5	1	2	8	53.3
S. epidermidis	27	7	0	1	8	29.6
Bacteroides	05	1	1	0	2	40.0
P. mirabilis	05	1	2	0	3	60.0
Total	316	95(30.1)	49(15.5)	31(9.8)	175	55.4

Table 5: Prevalence of MDR Bacteria Isolates in HVS Samples of Pregnant Women in Imo state

Isolates	No Isolated	No Resistant To:			Total MDR	% MDR
		3 Drugs	4 Drugs	≥5 Drugs		
E. coli	50	10	9	9	28	56.0
S. aureus	87	30	13	10	53	60.9
Ent. faecalis	14	4	0	3	7	50.0
S. epidermidis	27	6	1	2	9	33.3
Lactobacillus sp.	03	0	0	0	0	0
Total	181	50(29.3)	23(12.7)	24(13.3)	97	53.4

For this study multidrug resistance (MDR) is described as the capacity of an isolate to be resistant to at least three groups of the antibiotics tested at their in-use concentrations. There were more MDR isolates from urine samples (55.4%) than from HVS (53.4%). This difference proved to be statistically significant (at 1df; $P < 0.05$).

IV. Discussion And Conclusion

From the prevalence results, microbial infections were significantly higher in Urine samples than in HVS ($\chi^2 = 7.162$; $P < 0.05$) and could be related to the site of collection. However, mixed (polymicrobial) growth was more in the cases of HVS than within urine samples. Many of the polymicrobial growths of the

HVS samples were found to be a mixture of Candida albicans (C.albicans) with other microorganisms hence the very high prevalence of Candida albicans (39.5 %) in HVS samples. This corroborates reports that the female genital tract has diverse microenvironments optimal for growth of different types of aerobic and anaerobic bacteria as well as C. albicans (Uneke and Alo, 2007). The implication of the occurrence of polybacterial cultures in this study is that bacterial synergism and antibiotic resistance make the selection of an optimal antibiotic regimen difficult (Melvin, 1990), especially in the developing countries including Nigeria, where inadequate health services, inadequate drug supplies, non-adherence to treatment strategies and dubious drug quality all favour the emergence of microbial resistance.

The overall prevalence of 47.8% for Urine and 40.1% for HVS reported in this study is high though similar to 48.0% reported by Nworie and Eze (2009) and 45.3% reported by Imade and Eghafona (2010) with urine samples. The HVS prevalence is similar to the 42.9% reported among HVS of Nigerian women in Abakaliki by Uneke and Alo (2007). The prevalence however, does not agree with Onyemelukwe et al., (2003) who reported a prevalence of 12.7%. This difference may be due to the inclusion of both symptomatic and asymptomatic pregnant women in this study or as a result of differences in geographical location or socioeconomic status of the pregnant women.

In the entire state, for Urine samples, E. coli was the most prevalent organism followed closely by Staphylococcus aureus while for HVS samples, Staphylococcus aureus was the most prevalent bacterial isolate followed closely by E. coli. (Table 1) corroborating Akerele and Okonofua (2002). Other organisms isolated have been described as less common causes of genitourinary tract infections (Cheesbrough, 2000; Onyemelukwe et al., 2003).

The overall antimicrobial susceptibility pattern showed ciprofloxacin to be the most effective antibiotic showing 88.6% and 87.8% efficacy against the isolates from Urine and HVS samples respectively in the state (Tables 2 and 3). This was followed by nalidixic acid (88.3% and 86.2% respectively) and pefloxacin (79.4% and 81.8% respectively). Ofloxacin also showed high antibiotic activity eliciting 72.8% and 76.8% efficacy against the isolates from the respective samples. There was no significant difference in the susceptibility patterns showed by these antibiotics for both specimens. These antibiotics will therefore be highly efficacious in combating infections caused by the isolated bacteria from both specimens.

The susceptibility rate of the isolates to the fluoroquinolones used in this is remarkable and somewhat consistent between the specimens (Tables 2 and 3). This could be because the fluoroquinolones are newer drugs with mode of action central on inhibition of the DNA replication which stops the multiplication of the bacteria cells (Knobler, 2003; Kaplowitz, 2005). The flouroquinolones are also relatively expensive therefore they are more likely less available for abuse. They may though have some safety issues in pregnancy such as spontaneous abortion hence are only recommended for life threatening infections in pregnancy.

Nitrofurantoin, augmentin®, ceftriazone and gentamicin were moderately effective against the isolates with susceptibility of isolates slightly above 50%. While there was no significant difference between susceptibility of isolates from Urine and HVS respectively to augmentin® and ceftriazone, nitrofurantoin showed significantly higher activity against Urinary isolates than vaginal isolates. This may have some medical implication in that while nitrofurantoin may be efficacious in treating urinary tract infections (UTIs) it may not be quite efficacious in vaginal infections. In contrast gentamicin showed significant higher activity (50.3%) against vaginal isolates as that against urinary isolates (40.8%) hence may be more indicated in treatment of vaginal infection than UTIs. Generally, amoxicillin and tetracycline showed very low efficacy (< 40%) hence should not be indicated in treatment of infections by isolates from both specimens. The high resistance ≥ 50.0% to gentamicin is worrisome as it mostly forms the firstline treatment (being the most common prescription) for most people with symptoms of staphylococcal infection and has been reported to be used to treat many types of bacterial infections, particularly those caused by Staphylococcus species and Gram-negative organisms (Moulds and Jeyasingham, 2010). It is also found to be safe in pregnancy. High resistance to amoxicillin and tetracycline has been documented (Anyadoh et al., 2010; Nworie and Eze, 2010

Our observation here appears to be related to inappropriate use of antimicrobial agents which leads to selective pressure. This can possibly be explained by the fact that the urine isolates were exposed to consistently higher urine concentrations of the antimicrobial agents, compared with the systemic lower concentrations to which the HVS isolates were exposed. Furthermore the urine isolates showed more multidrug resistance compared with the HVS isolates (55.7% and 53.4% respectively). This may possibly be related to location of specimen. This difference was though not statistically significant as $P>0.05$ ($\chi^2 = 0.149$; df =1).

There was no specific pattern of resistance in both specimens across the State. However, S.aureus from both specimen interestingly showed the highest multidrug resistance of 60.3% and 60.9% (mean= 60.6%), followed very closely by Proteus mirabilis showing 60% multidrug resistance and E. coli (56.4%). The high resistance of Proteus mirabilis is not surprising because the bacterium is a documented recalcitrant organism; its high resistance has also been reported by Akerele et al., (2001). The resistance of S. aureus is particularly interesting because it is a common bacterium found within the hospital environment as well as the community

(Prescott et al., 2005) and has been reported to be quite susceptible to known common antibiotics (Akerele et al., 2001).. The prevalence of multidrug resistant S. aureus in this study depicts a resistance profile of >50% to almost half of the antibiotics tested, some of which form the first line treatment for most patients in cases of self medication. The resistance pattern is however in conformity with previous observations that most isolates of S. aureus are resistant to a large number of commonly prescribed antibiotics (Olukoya et al., 1995). E.coli, Kl. pneumoniae and Ent. faecalis also showed a resistance pattern >50%. This contrasts the report of 75% and 74% sensitivities of E. coli and Klebsiella to antibiotics by Akerele et al., (2001). These clinical isolates are constantly exposed to the hospital environment where they acquire resistance to numerous antibiotics by various mechanisms.

Generally, the level of multi-drug resistance shown by these isolates from these mostly asymptomatic pregnant women is of great concern. The rate at which most antibiotics are losing the battle against resistant organisms should be of immense concern to the health professionals and calls for effective measures to promote rational use of antibiotics and thereby prolong 'life expectancy' (Onanuga et al., 2005).

Though a high percentage (78.08%) of the women was made up of asymptomatic from questionnaire analysis, it is worthy of note that asymptomatic infections predisposes to symptomatic infections. Hence, these infections if not identified early and properly treated may become symptomatic leading to infant mortality / morbidity (Nicolle et al., 2005).

From the discussions, it is obvious that though there are similarities in the prevalence and antibiogram of microbial isolates from urine and HVS samples, the differences observed may have some public health import. These therefore call for frequent and consistent evaluation of the prevalence, aetiologic agents and predisposing factors of genitourinary tract infections during pregnancy in Nigeria and other developing countries. Routine HVS as well as urine culture should be carried out on all antenatal women to ensure holistic antenatal care/management. This will enhance reduction of the devastating effects of microbial infections in pregnancy on both maternal and foetal health.

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