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Antibiotic Susceptibility of High Vaginal Swab's Isolates Obtained from the University of Port Harcourt Teaching Hospital, Rivers State, Nigeria

E. Effiong[1], N. N. Ndukwe[2*] and Y. S. Wali[3]

[1]Faculty of Sciences, University of Port Harcourt, Rivers State, Nigeria.
[2]Faculty of Sciences, Federal University of Kashere, Gombe State, Nigeria.
[3]Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author EE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NNN and YSW managed the analyses of the study. Author YSW managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Bacterial vaginosis is caused by the invasion of the vagina by pathogenic microbiota with a unique adaptive strategy. Immunodeficiency and immune compromised female patients may have reported cases of this category of infections.
Aims: This study evaluated the susceptibility pattern of High vaginal swab (HVS) isolates using both Optudisc and Abtek antibiotics susceptibility disc obtained from the Department of Medical Microbiology and Parasitology Unit, University of Port Harcourt Teaching Hospital (UPTH); Rivers State, South-South Nigeria.
Study Design: Thirty (30) isolates with multidrug resistance were screened, selected and identified with frequencies of occurrence with 36.67% *E. coli*, 29.9% *Klebsiella* sp., 16.67% *Staphylococcus aureus*, 6.6% *Pseudomonas* sp. and 13.33% *Proteus* sp. The susceptibility of the isolates was assessed using Kirby Bauer disc diffusion method.

**Corresponding author: E-mail: nelson_ndukwe@yahoo.com;*

Results: Over 80% were susceptible to Gentamicin, 64% to Ofloxacin, using the Abtex Biological Limited while Optudisc revealed 72% to Peflacin, Ciprofloxacin, Streptomycin, 60% resistant to Ampicillin, 56% to Nalidixic Acid, Septrin. About 100% sensitivity was observed in the second generation Cephalosporins, Cefuroxime and Ceftazidime, whereas 75% were resistant to Oxacillin and Augmentin. While Optudisc for Gram-positive isolates were 100% susceptible to Levofloxacin and Ciprofloxacin. Statistical analysis using t-test at $p< 0.05$ showed that mean results using the different disc were significant.

Conclusion: The trend in the susceptibility of isolates was attributed to the spate of self-medication and abuse and misuse of herbal remedies. These findings underscore the need to enforce proper susceptibility testing prior to administration of therapeutic formulations.

Keywords: Susceptibility; high vaginal swab; Kirby-Bauer; Rivers State; Nigeria; L-agar slopes; antimicrobial.

1. INTRODUCTION

One of the major challenges facing reproductive health is the colonization of the genitourinary tract by invasive microbiota. Several reports, suggest 70-80% of the cases reported by peer review articles was attributed to sexual activity and that contributes to the presence of invasive microbiota. Bacterial vaginosis is defined as the invasion of the vagina by pathogenic microbiota with a unique adaptive strategy. It has been reported to occur as a result of drift from the normal eco-flora to novel microflora. Immunodeficiency and immune compromised female patients may have reported cases of this category of infections. Changes in pH from 4.5 for normal flora to pH >5.0 suggest cases of colonized surfaces [1]. One of the manifestations of vaginosis could range from pelvic inflammatory diseases to severe cases of infertility. Furthermore, Kumari *et al*. [2] suggested that PIDs is common amongst sexually active and in rare cases of gynaecological infection. Pathogens such as *E. coli, Klebsiella* sp., *Proteus* sp., *Staphylococcus* sp. and *Streptococcus* sp.

Antimicrobial therapies can be categorized into two major groups, technically, the effectiveness of such antibiotics to both Gram-negative is said to have a broad (wide) spectrum, but when it is effective against either the gram negatives or Gram-positive it regarded to have a narrow spectrum [3]. Bacterial resistance is a survival route in which bacterial groups react to either strange toxicant, biological substances or even to a novel ecosystem [4]. Antimicrobial susceptibility of pathogens has evolved and thereby creating numerous challenges to patients and health care delivery [5]. The resistance profile of several pathogens has been reported to be caused by a wide range of mutation and genetic transfers [6], chemical modification of target sites and efflux [7].

Strategies for overcoming multidrug resistance includes targeting resistance mechanisms, developing novel drug targets, mining microbial genomes, targeting essential genes, vaccines and immunomodulators and the use of normal microbiota limiting the spread of drug-resistant bacteria [8,4]. The progressive increase in resistance rates of most pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant Enterococcus (VRE), multidrug-resistant (MDR) *Pseudomonas aeruginosa*, Imipenem-resistant *Acinetobacter baumannii* and third-generation Cephalosporin-resistant *Escherichia coli* and *Klebsiella pneumonia*, constitute a serious concern to public health. Sadly, most bacterial groups are evolving with more resistance mechanisms and posing a serious threat to humans.

Disc-diffusion method is the most popular technique employed in the health facilities in Nigeria. The locally produced disc has been identified to gain grounds especially in the cottage and private laboratories while the patronage of foreign Disc has been credited to enhance the accuracy of laboratory findings. The aim of this study is to isolate and compare the susceptibility using the local and foreign disc. The objective of the study was designed to isolate and compare the antibiotic susceptibility of High Vaginal Swab's isolates obtained from the University of Port Harcourt Teaching Hospital, Rivers State, Nigeria.

2. MATERIALS AND METHODS

Research design: Randomized sampling.

Target population. Adult women within the age brackets of 16 years and above.

Area of study: The study was designed to evaluate the incidence of pelvic inflammatory

diseases among women that presented symptoms of sexually transmitted diseases in and around Rivers State, Niger Delta region, Nigeria.

2.1 Sample Size

This study was investigating the proportion of swab samples in the total hospital attendees with clinical infection. Therefore, the sample size was determined using a qualitative variable (Charan and Biswas, 2013) employing the following equation:

$$Samplesize = \frac{Z_{1-\alpha/2}{}^2 p(1-p)}{d^2}$$

Where:

$Z_{1-œ/2}$ = is the standard normal variate (at 5% type 1 error (P<0.05) it is 1.96 and at 1% type error (p<0.01) it is considered significant below 0.05 hence 1.96 is used in formula.

P = expected variation in a population based on previous studies or pilot studies.

d = absolute error or precision (has to be decided by a researcher).

The proportion of patients hypothetically with possible infections collected from clinical swabs specimens in the hospital with bacterial origin among all age group according to laboratory statistics was estimated to be 32%. Using an absolute error of 5% therefore,

$$\text{Sample size} = \frac{1.96X(1.96)\ X\ (0.02)}{(0.05)X(0.05)} = 30.8$$

Sampling technique: Trained physicians at the University of Port Harcourt Teaching hospital were employed in the aseptic collection of the samples using a speculum device.

2.2 Sample Collection

Swab isolates were obtained from the patients by trained laboratory scientist at the same hospital and were transported in nutrient agar slopes in L-agar slopes from the Department of Microbiology and Parasitology, University of Port Harcourt teaching hospital to the Medical Microbiology Laboratory University of Port Harcourt for analysis.

2.3 Isolation and Characterization of Bacterial Pathogens

The method described by Cheesebrough [9] was adopted in the processing of specimens. Then Morphological examination was conducted along with the analysis. All the media were prepared according to manufacturers' instruction, sterilized by autoclaving at 121ºC, for 15mins at 15Psi. The swab sticks were used to make a smear on Blood agar (BA), Chocolate Agar (Choc), MacConkey agar (MA) and Nutrient Agar (NA). A sterile wire loop (sterilized using Bunsen burner flame) was used to streak on the media in a pattern enabling the collection of discrete colonies. Pure cultures obtained from the swab-sticks were purified by continuous sub-culturing. An 18h old pure culture was used for the biochemical characterization. The schemes of Madigan and Martinko [10] was employed in the characterization of the isolates while Manual of deterministic bacteriology was employed to identify the isolates.

2.4 Standardization of Bacterial Isolates

The procedure was carried out to standardize the inoculum turbidity. A barium sulphate standard ($BaSO_4$) and 0.5 Mcfarland standard were used. Under the aseptic condition, a loopful of the culture colony was inoculated into a test tube containing 5 ml of freshly prepared and sterilized physiological saline. The broth was left to stand for 5 to10 minutes and its turbidity compared to the 0.5 Mcfarland standard within 15 minutes of adjusting turbidity of inoculums suspension.

2.5 Antibiotic Susceptibility Test

A total of 50 antibiotics discs (Abtek technological limited and Optudisc) were used for the investigation as presented in Tables 1-4. It was used in determining the resistance or susceptibility of the isolates to the antibiotics. The results were obtained by measuring the diameter of zones of inhibition in millimetres (mm) and the test organism was classified as either sensitive or resistance after comparison with the standards for antimicrobial disc susceptibility test. Kirby Bauer method was employed for susceptibility testing. Here, a standard concentration of bacterial strain was prepared using Mcfarland standard. A sterile swab stick was dipped into the adjusted suspension and rotated several times inside the walls of the test tube and inoculated by spreading on the surface of the ISO sensitivity agar (LAB M). The plates were covered for 4 minutes for excess surface moisture to be absorbed before placing the antibiotics disc on the surface of the medium with sterile forceps. It was ensured that the disc does not move once

placed on the agar plate. The plates were inverted and incubated at 37^0C for 18 hours. Finally, the diameter of the zones of inhibition was measured.

2.6 Interpretation of the Zones and Diameters

The zones of inhibition were measured using a pair of the divider and a ruler. The zones of inhibition obtained for the isolates were compared to the breakpoints as recommended by the Clinical and Laboratory Standards Institute [11]. The susceptibility was interpreted as either Resistant (R) or Sensitive (S).

3. RESULTS

Tables 5-7 revealed *Escherichia coli* had 18.18% resistance and 81.18% sensitive to Gentamicin and Nitrofurantoin respectively. 72% sensitive for Ofloxacin and 27% resistant. Cloxacillin, Ceftazidime and Cefuroxime had low susceptibility. *Klebsiella* sp. was 78% susceptible to Gentamicin, 22% resistant; Ofloxacin had 44.44% susceptibility and 55.56% resistance. *Proteus* sp. had 80% sensitivity for Gentamicin and 20% resistance; 50% sensitive to Ofloxacin, 70% susceptibility to Nitrofurantoin, 5% sensitive to Cloxacillin and 95% resistant. About 100% resistant to Cefuroxime and Ceftazidime.

Table 1. Concentrations of gram-negative antibiotics (Using Optudisc)

Antibiotics	Abbreviations	Concentration (mcg)
Ciprofloxacin	CPX	10
Streptomycin	S	30
Peflacine	PEF	10
Septrin	SXT	30
Ampicillin	PN	30
Tarivid	OFX	10
Ceporex	CEP	10
Gentamycin	CN	10
Augmentin	AU	30
Nalidixic acid	NA	30

Table 2. Concentrations of gram positive antibiotics (Using Optudisc)

Antibiotics	Abbreviation	Concentration (mcg)
Ciprofloxacin	CPX	10
Norfloxacin	NF	10
Gentamycin	CN	10
Amoxicilin	AMX	20
Streptomycin	S	30
Rifampicin	RD	20
Erythromycin	E	30
Ampiclox	APX	20
Levofloxacin	LEV	20

Table 3. Concentrations of gram negative antibiotics (Using Abtek)

Antibiotics	Abbreviation	Concentration (µg)
Gentamycin	GEN	10
Cefotaxime	CTX	30
Ofloxacin	OFL	30
Augumentin	AUG	30
Nitrofurantoin	NIT	300
Cloxacillin	CXC	5
Ceftaxidime	CAZ	30
Cefuroxime	CAX	30

Table 4. Concentration of gram positive antibiotics (Using Abtek)

Antibiotics	Abbreviation	Concentration (µg)
Gentamycin	GEN	10
Lincomycin	LIN	20
Oxacillin	OXC	20
Cloxacillin	CXC	5
Ofloxacin	OFL	5
Augumentin	AUG	30
Ceftazidime	CAZ	30
Cefuroxime	CRX	30

Pseudomonas aeruginosa was 66% sensitive to Augmentin, 33% resistant; same as Gentamicin and 100% sensitive to Ofloxacin and 90% resistant.

4. DISCUSSION

In this study, 36.67% of the bacterial isolates were *E. coli*, 29.9% *Klebsiella* sp, 16.67% *Staphylococcus aureus*, 6.6% *Pseudomonas* sp and 13.33% *Proteus* sp. were observed. This agrees with the report of Kumari *et al*. (2016) whose report isolated in PID was *E. coli* (30.30%), CONS (22.72%), *Staphylococcus* (15.15%) followed by *Klebsiella* (13.63%) and *Pseudomonas* (9.09%). Among infertility cases caused by Gram-positive isolates, *Staphylococcus* (24.32%) was most predominant followed by Gram-negative isolate *Klebsiella* (21.62%) and then the *E. coli* (13.51%), *Candida* (21.62%) and *Trichomonas* (10.81%) were also reported in significant cases. The result corroborates with the findings of Kumar and Singh [1] who reported *Escherichia coli* (63%), *Enterococcus faecalis* (26%), and *Candida* sp (7.3%) and *Staphylococcus aureus* (3.7%). These suggest that the surface colonization of the vagina has been a cause of worldwide concern to reproductive health. Epidemiological survey of pathogens of clinical importance for both resistance and multidrug resistance is critical for a robust curative and preventive measure for limiting the spread of these microorganisms [12].

Antimicrobial susceptibility of the patient to broad-spectrum antibiotics has gone abysmally low because of the abuse of broad-spectrum antibiotics and also the extended spectrum beta-lactam (ESBL). The thirty isolates collected from University of Port Harcourt teaching hospital, has shown a high level of resistance to Augmentin, and the second generation Cephalosporins line, the Ceftazidime and Cefuroxime were noticed among the Gram-negative microorganisms; and this can be linked to pharmacists and doctors who administer these broad-spectrum antibiotics without appropriate caution. This resistance in antibiotics especially Oxacillin in Gram-positive organisms could be attributed to suggest that beta-lactamase is present in the gene pool of the resistant organisms in environment, homes and hospitals today. Macrolides are a better option for resistant penicillin derivatives, the emergence of strains resistant to macrolides has been observed in several countries. Resistance to drugs like Lincomycin is on the high trend. This was seen in 20% susceptibility to the organism *Staphylococcus* sp. These support the concepts that drugs should be used more in combination to achieve a broader spectrum and area of coverage for treatment. Gentamicin could be said to be a drug of choice for *Escherichia coli, Proteus* sp. and *Klebsiella* infections. The second generations of Cephalosporins were also good for the treatment of *Staphylococcus aureus* infection.

In this study, 100% susceptibility to Ceftazidime, Cefuroxime and Levofloxacin were seen in *Pseudomonas aeruginosa*. This organism is naturally resistant to beta-lactam antibiotics while sensitive to colistins. Resistivity and susceptibility to antibiotics is purely a host factor related problem; most people go on self-medication from parents, family and friends, when the treatment is seemingly not effective or curative they run to the hospital and at that develop resistance to antibiotics, this attitude should be strongly discouraged. Aibinu et al., [13] and Stelling et al., [14] reported a 100% amoxicillin. Also resisted Ceftrioxin (76%), Augmentin (60%), Cefixime (59%) while the most susceptible antibiotics were Erythromycin (71%), Nitrofurantoin (70%), Ofloxacin (58%), Gentamicin (52%) and Cefuroxime (50%) whereas. The use of Nitrofurantoin is currently discouraged due to its toxicity and side effects, but in extreme cases of urinary tract infections it is limited for use for children, Which Jombo et al., [15] reported that

Table 5. Antibiogram for gram-negative isolates

S/n	CPX (mm)	S (mm)	PEF (mm)	SXT (mm)	PN (mm)	NA (mm)	AG (mm)	CN (mm)	OFX (mm)	CEP (mm)	GEN (mm)	CTX (mm)	OFL (mm)	AUG (mm)	NIT (mm)	CXC (mm)	CAZ (mm)	CRX (mm)
1	R	R	S	R	R	R	S	S	S	R	S	S	S	R	R	S	S	S
3	S	R	S	R	S	S	R	S	R	S	R	R	R	R	S	R	R	R
5	S	S	R	R	S	S	R	R	R	R	S	R	R	S	S	R	R	R
6	S	S	S	S	S	R	S	R	S	S	S	S	S	S	R	R	R	R
7	S	S	S	S	S	R	S	S	S	S	S	R	S	R	S	S	R	R
8	R	S	R	R	R	R	R	S	S	S	R	S	R	R	S	S	R	R
9	S	S	S	R	R	R	R	S	S	R	S	S	S	R	S	R	R	R
10	S	S	R	R	R	R	R	S	S	R	R	R	R	R	S	R	R	R
12	S	S	S	S	R	R	R	S	R	R	S	R	S	R	S	S	R	R
14	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	R	R	R
15	S	R	S	R	R	S	S	S	S	R	R	S	R	R	S	S	R	R
16	R	R	S	S	S	R	S	R	R	S	S	S	R	S	R	R	S	R
17	S	S	R	S	R	S	R	S	R	S	S	S	S	S	R	R	R	R
18	S	R	R	S	S	S	S	R	R	R	R	S	R	S	R	S	R	R
19	S	S	S	S	S	S	R	S	S	R	R	S	S	S	S	R	R	R
20	R	S	R	S	S	S	S	S	R	S	S	S	S	R	R	R	S	R
21	R	S	R	R	R	S	S	S	R	R	S	S	R	R	S	R	R	R
22	S	R	S	R	R	R	R	R	S	R	S	S	S	R	S	R	S	R
23	S	S	S	S	S	S	R	S	R	R	S	S	R	R	S	R	R	R
24	S	S	S	R	S	S	R	S	S	R	S	S	S	R	S	R	R	R
25	S	S	S	S	S	R	S	S	S	S	S	S	S	R	S	R	R	R
26	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	R	R	R
27	S	S	S	S	S	S	R	S	S	S	S	S	S	R	S	R	R	R
29	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R

Table 6. Comparison of the performance of common antibiotics present in two brands of antibiotics discs (Abtek and Optudisc) on gram-positive isolates

OPTUDISC (µg)											ABTEK(µg)						
NB (30)	CH (20)	CPX (10)	E (30)	LVE (10)	CN (10)	APX (30)	RD (10)	AMX (30)	S (30)	GEN (10)	LIN (10)	OXC (20)	CXC (5)	OFL (5)	AUG (30)	CAZ (30)	CRX (30)
S	S	S	S	S	R	R	S	R	S	R	R	R	R	R	R	S	S
R	S	S	R	S	S	R	R	R	S	S	S	R	R	S	R	S	S
S	R	S	R	S	S	S	R	R	S	S	R	R	R	S	R	S	S
R	S	S	R	S	S	S	S	R	R	S	R	R	R	R	R	S	S
R	R	S	S	S	R	S	R	R	S	S	R	R	R	S	R	S	S

Table 7. Comparison of the performance of common antibiotics present in two brands of antibiotics discs (Abtek and Optudisc) on gram-negative isolates

Isolates	ABTEK			OPTUDISC		
	AUG	GEN	OFL	AUG	GEN	OFL
Escherichia coli	R	S	S	S	S	S
Klebsiella sp.	R	R	R	R	S	R
Escherichia coli	S	S	R	R	R	R
Escherichia coli	S	S	S	S	R	S
Escherichia coli	R	S	S	S	S	S
Klebsiella sp.	R	R	R	R	S	S
Klebsiella sp.	R	S	S	R	S	S
Escherichia coli	R	R	R	R	S	S
Escherichia coli	R	S	S	R	S	R
Escherichia coli	R	S	S	S	S	S
Proteus sp.	R	R	R	S	S	S
Pseudomonas sp.	S	R	R	S	R	R
Klebsiella sp.	S	S	S	R	S	R
Pseudomonas sp.	S	R	S	S	R	R
Escherichia coli	S	R	R	R	S	S
Klebsiella sp.	R	S	S	S	S	R
Proteus sp.	R	S	R	S	R	R
Pseudomonas sp.	R	S	S	R	S	S
Klebsiella sp.	R	S	S	R	S	R

Isolates	ABTEK			OPTUDISC		
	AUG	GEN	OFL	AUG	GEN	OFL
Klebsiella sp.	R	S	S	R	S	S
Klebsiella sp.	R	S	S	S	S	S
Escherichia coli	R	S	S	S	S	S
Escherichia coli	R	S	S	R	S	S
Proteus sp.	R	S	S	S	S	S

bacteria have uncontrolled ability to produce the enzyme beta-lactamases in children which neutralize most penicillin group, further suggesting the use of Ofloxacin with a remarkable sensitivity of 58%. This agrees with the findings in this current study, cephalosporins like Erythromycin and Ciprofloxacin was slightly resistant. The weakest or poorly resistant Nitrofurantoin (27%) while the weakly sensitive Ciprofloxacin (9%) whereas other reports showed that ciprofloxacin is the most effective quinolone. In a study conducted among fresh students in Ahmadu Bello University, *Pseudomonas* showed a uniform susceptibility to ciprofloxacin [16] while contrary to this, the result suggests that the cephalosporin and beta-lactam were most resisted by the pathogens while Aminoglycoside, Azolidines and quinolones were susceptible although no certain group of antibiotics dominated the susceptibility profile. The susceptibility varied in that the standard and production of the disc may account for the sensitivity. The proximity of the discs, the production concentration may have wrongly sipped into the wrong disc; the quest to out-run or out-market the Abtek disc which is expensive by adding much more antibiotics and finally, not making a wrong stoichiometric value which accounts for some false sensitivity. The sensitive ones in the Optudisc could be as a result of diffusion of the sensitive antibiotics into the resistant ones during production. Laboratory physicians and scientists should always develop local antimicrobial susceptibility profiles (antibiograms) of local bacterial isolates, and the patterns regularly updated for ready consultations [17,18].

There is a need to strengthen decreased alcoholism, usage of illicit drugs and improved sanitary practices as modules to reduce the incidence of either nosocomial or home acquired the diseases, and to boost hygiene practices. People should be encouraged to shun false health practices so that the importance of the sensitivity test cannot be overemphasized at all levels. An antimicrobial sensitivity test should be carried out before drugs are prescribed and administered. Laws should be enacted for pharmaceutical stores and chemists not to prescribe drugs but only sell. This is because they contribute to this case of self-medication.

5. CONCLUSION

Drugs like Gentamicin, Nitrofurantoin, Peflacin and the second generation of Cephalosporin should not be administered without sensitivity testing. Some of the Penicillin and its derivatives like Cloxacillin should be regulated or controlled due to the incidence of high resistance shown by the isolates studied in this research. The variance with the locally produced disc could be attributed to the poor quality control processes during production, although being cheap and readily available. This sad observation needs to be checked and controlled.

CONSENT

As per international standard written participant consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard written ethical permission has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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