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ORIGINAL ARTICLE

Antibiogram Profile and Resistance Patterns of Microflora from Vaginal Discharge in Reproductive-Age Women at a Nigerian Teaching Hospital

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ABSTRACT

Background: The adult human vagina hosts a complex biota containing diverse communities of microorganisms. The occurrence of multi-drug-resistant strains of these microorganisms has persistently increased due to poor hygiene and misuse or abuse of antibiotics. The vaginal microflora may exhibit patterns of growth, biochemical expression, or response to the standard drugs which consequently lead to answer the complex questions of antimicrobial resistance.

Aim: The study aimed to quantify the susceptibility profile of microorganisms isolated from vaginal discharge and evaluate the minimum inhibitory concentration of diverse antimicrobial drugs.

Methods: Fifty vaginal swabs were collected from female students of Madonna University, Nigeria while two samples were collected each from a pregnant and a non-pregnant woman at the university's tertiary care teaching hospital. The isolates were grown in selective media and identified through Gram-staining and biochemical physiology for identification. The Kirby-Bauer disc diffusion method was used for microbial susceptibility testing, and the agar dilution method was used to determine the minimum inhibitory concentration of commonly prescribed antibiotics at the teaching hospital.

Results: Sixty-eight microorganisms comprising 17 Gram-positive (*Staphylococcus* sp.) and 31 Gram-negative (*Escherichia coli* and others) bacteria and 20 fungi (*Candida* sp.) were isolated. The bacteria showed a high resistance (>80%) to amoxicillin, cefuroxime, and cefixime but were relatively susceptible (35–100%) to levofloxacin and ofloxacin. Cefepime showed high activity with a minimum inhibitory concentration range of 25–50 µg/mL against the studied bacteria. The isolated fungi were susceptible to amphotericin B (35–40%) but resistant (>85%) to other antifungal drugs tested.

Conclusion: The study suggests that bacterial vaginosis prevalence at the university could best be treated with ofloxacin (second generation- fluoroquinlone), levofloxacin (third generation- fluoroquinolone), and cefepime (fourth generation- cephalosporin) due to their greater sensitivity, while candidiasis could best be treated with amphotericin B (a pyolene).

Keywords	*Address of Correspondence	Article info.
Antimicrobial Agents, Bacterial Vaginosis, Multi-Drug Resistance, Vaginal Discharge.	*charles.nnadi@unn.edu.ng*	*Received: February 14, 2024* *Accepted: April 17, 2024*

Cite this article: *Enemchukwu CM, Nwabueze C, Osuala OJ, Ikem CJ, Nwokeji MC, Nnadi CO. Antibiogram Profile and Resistance Patterns of Microflora from Vaginal Discharge in*

Reproductive-Age Women at a Nigerian Teaching Hospital. RADS J Biol Res Appl Sci. 2024; 15(1):9-19.

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INTRODUCTION

Vaginitis/vaginosis is a urinary tract infection (UTI) comprising an infection of the vagina resulting in the presence (vaginitis) or absence (vaginosis) of inflammation[1]. The severity of UTI symptoms is determined by fever, burning when urinating (dysuria), lower abdominal pain, itching, blisters and genital ulcers, pelvic pain, pyuria, the age of the infected person, and the part of the infected urinary tract [2,3]. Both Gram-negative and Gram-positive bacteria, as well as some specific fungi, are responsible for UTIs[4]. The most frequent gynecologic ailment seen in clinics is vaginal irritation. Its diagnosis is determined by the presence of abnormal discharge symptoms, vulvovaginal pain symptoms, or both. Cervicitis occasionally coexists with vaginitis and might result in a vaginal discharge, the flow of which is the body's approach to maintaining a normal and healthy environment[5].

The normal vaginal discharge is clear, flavorless, and non-irritating which keeps the vagina clean and moist. However, a departure from this pattern may indicate vaginitis. Bacterial vaginosis/vaginitis (BV) is caused by an abundance of pathogenic bacteria in the vagina and is distinguished by itching and a fishy odour that worsens during menstruation and sex, and fungal infection called vulvovaginal candidiasis. It also manifests as a thick and white clumpy discharge, and trichomoniasis, which is a common sexually transmitted infection and is brought on by the parasite[6]. In contrast to yeast infection, candidiasis is the major cause of nosocomial fungal UTIs and it refers to a candida infection[6,7]. The most frequent cause of both simple and complex UTIs is *Escherichia coli* and other uropathogens such as *Proteus mirabilis, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumonia*, and group B *streptococcus* (GBS)[8]. Vaginitis is more likely to cause infection in women who undergo surgery such as abortion or hysterectomy. Also, it increases a woman's susceptibility to other sexually transmitted diseases, such as chlamydia and gonorrhoea[9-11].

The biggest challenges facing the management of hospital and community-acquired illnesses today are their asymptomatic nature and the global rise of antibiotic resistance[12,13]. Antibiotic resistance can be due to the incorrect prescription of antibiotics or their indiscriminate use[14]. Extended-spectrum beta-lactamases (ESBLs) and multi-drug resistant *S. aureus* (MRSA), which have few effective treatments available, have become a serious threat to human medicine globally[13-15]. Therefore, as the antibiotic susceptibility profile might change over time, ongoing susceptibility testing and control measures are required to further understand their prevalence and management strategies[16]. The vaginal discharge can be another source of biological specimen to quantitatively monitor the growth kinetics of microbiota, physico-chemical parameters, and antibiotic susceptibility to infer something new for the antimicrobial resistance. This study was meant to evaluate the susceptibility profile of microorganisms isolated from vaginal discharge of asymptomatic community of the Madonna Teaching Hospital, Nigeria; and to narrate the minimum inhibitory concentration of diverse antimicrobial drugs.

MATERIALS AND METHODS

Microbiological Media and Consumables

The standard growth media such as nutrient agar, mannitol salt agar (MSA), Mueller-Hinton agar (MHA), nutrient broth, Salmonella Shigella agar (SSA), eosin methylene blue (EMB) agar, Sabouraud dextrose agar (SDA), cetrimide agar, MacConkey agar and glucose were procured from the Titan Biotech Ltd., Delhi, India.

Study Location and Sample Size

The study was carried out at the Madonna University Teaching Hospital, of Madonna University, Nigeria.

Volunteers considered for the study included young women of reproductive age (15 – 49 years), with no confirmed case of urogenital infection or abnormal vaginal discharge. The facility serves as primary, secondary and tertiary hospital for the students, workers and other residents of the University community which is located within the South-South of Nigeria.

According to the prevalence research carried out in a Tertiary care Hospital in Port- Harcourt, Nigeria, the sample size was estimated from the formula given below[17].

$$Sample\ size, n = \frac{Z^2 p(1-p)}{d^2} \qquad \text{Equation (1)}$$

Where Z = confidence level, p =expected prevalence obtained from a pilot study and, d = precision

Samples Collection

A total of fifty-two (52) vaginal discharge samples were collected of which fifty (50) samples were from female students of Madonna University of reproductive age. Two samples were collected from a pregnant and a non-pregnant woman who visited visited Madonna University Teaching Hospital. The research ethics committee of Madonna University Nigeria reviewed and approved the protocol involving the human volunteers and issued the ethical clearance (Approval number: MAU/DRC/HD/E/09) on the 15th of August 2022 after the volunteers' consent was obtained. The volunteers were provided with sterile transport swabs with Cary Blair medium and given instructions on how to collect the vaginal discharge. Microbiological isolation was made through standard microbiological procedures on the aseptically collected samples and the isolates were stored at 4ºC in an agar slants until their further use.

Identification of the Isolates

The cultured microorganisms were identified using standard microbiological techniques including Grams' staining, colony morphology, and biochemical tests[18].

Preliminary Sensitivity Test for the Isolated Organisms

Disc Diffusion Test

The National Committee for Clinical Laboratory Standards (NCCLS) institute's disc diffusion test method was used to determine the antibiotic susceptibility patterns of the isolates of *S. aureus, E. coli, K. pneumoniae, S. typhi, P. aeruginosa, and S. dysenteriae*. Mueller-Hinton agar in a 20 ml sterile volume was added to sterile Petri dishes and left to gel[19]. The 24 h cultures of the isolates were diluted in sterile nutrient broth using a microliter syringe at 2 µL, and the diluted organisms were then swabbed on the MHA plates that had already been prepared, according to the McFarland method of swabbing[19]. It was made sure that the entire antibiotic disc hit the agar when the discs were administered. To prevent contamination, the plates were kept at room temperature for 1 h in laminar flow before being incubated inverted at 37 °C for 18–24 h. According to the Clinical and Laboratory Standards Institute (CLSI) interpretive chart for zone sizes, the inhibitory zone diameters (IZDs) were measured using a transparent plastic rule to the nearest millimetre and categorized as sensitive, moderate, or resistant[20]. Each isolate had two plates, and the average readings were computed.

Using the disc diffusion method, the antifungal drug sensitivity pattern was evaluated against the *Candida* isolates[19]. Aseptically poured into sterile Petri dishes, 20 ml of modified MHA with 2% glucose and methylene blue was left to gel. A sterile swab was used to swab the diluted organisms on the MHA + 2% glucose and methylene blue that had already been prepared after diluting the 48 h cultures of the *Candida* isolates to 10^{-5}. Fluconazole, ketoconazole, amphotericin B, cycloheximide, griseofulvin, and clotrimazole are the antifungal medications utilized, each at a concentration of 50 µg/ml; making sure that every area of the antifungal discs touched the agar, they were applied. Agar plates were then incubated at 37 °C for 24 h after the setup was kept at room temperature for 1 h to allow the agents to diffuse into the agar medium. A plastic, clear ruler was used to measure the millimetre-sized zone of inhibition. Calculations and records of the mean of IZD were made[21].

Multiple Antibiotic Resistance (MAR) Index

The MAR index was determined by dividing the number of antibiotics that an organism is resistant to by the total number of antibiotics that the organism has been exposed to. Indicators of increased risk of contamination where antibiotics are often used include MAR index values greater than 0.2. Using the following formula, the previous study computed and interpreted the MAR index[22].

$$MAR\ index = \frac{a}{b} \qquad \text{Equation (2)}$$

Where "a" represents the number of drugs a particular isolate proved resistant to, and "b" denotes the number of different antibiotics tested in the study.

Determination of Minimum Inhibition Concentration (MIC)

The MIC was determined using the agar dilution method[23]. A loopful of individual colonies of the bacteria isolates were

immersed in a sterile 10 ml nutrient broth and incubated for 24 h at 37 °C. The overnight cultures of the isolates were diluted to 10^5 units[24]. The antibiotics used were nitrofurantoin, cefepime, and cephalexin. Sterilized 20 ml of MHA were used to dilute the antibiotics to five double-fold serial dilutions in sterile Petri dishes to obtain the final concentrations as follows: (nitrofurantoin: 1000, 500, 250, 125, 62.5 µg/ml; cefepime: 400, 200, 100, 50, 25 µg/ml and cephalexin: 500, 250, 125, 62.5, 31.25 µg/ml) which was allowed to set. The diluted culture was streaked on the already prepared Mueller Hinton-drug agar plates. It was allowed to stand for 1 h, then inverted and incubated at 37 °C for 18-20 h[25].

RESULTS

Sample Identification

Out of the 52 vaginal samples collected, bacteria were detected in 35 samples which represented 67.3% of the population and were identified as *E. coli*, *K. pneumonia*, *S. aureus*, *S. dysenteriae*, *P. aeruginosa,* and *S. typhi*. Fungal growth, identified as *Candida* species, was detected in 20 samples representing 38.4% of the sample population.

Susceptibility of Isolates to Antimicrobial Agents

The mean Inhibitory Zone Diameter (IZD) of selected antimicrobial agents against the test isolates in the antibiotics susceptibility test are shown in Tables **1 – 6**.

Susceptibility of Isolated *S. aureus* to Selected Antibiotics

The seventeen *Staphylococci* (Sa1 – Sa17) were found to have varying degrees of susceptibility to different antibiotics (Table **1**). The isolates, Sa1 and Sa3 were most susceptible to ceftriaxone sulbactam, levofloxacin, ciprofloxacin, ofloxacin, erythromycin, and gentamycin.

Susceptibility of Isolated *P. aeruginosa*, *S. dysenteriae,* and *K. pneumonia* to Selected Antibiotics

The *P. aeruginosa* isolate showed the highest susceptibility to levofloxacin (IZD, 23.0 mm). The two isolates of *S. dysenteriae* showed different susceptibility patterns, however, the Sh1 isolate showed higher susceptibility to all the antibiotics except for nitrofurantoin (Table **2**). The seven isolates of *K. pneumonia* were found to be more susceptible to ofloxacin, levofloxacin, and ceftriaxone sulbactam.

Susceptibility of Isolated *E. coli* to Selected Antibiotics

The different isolates of *Escherichia* species showed varying susceptibility patterns to the antibiotics tested (Table **3**). Isolates 1 and 7 were more susceptible to ceftriaxone sulbactam; isolates 2, 8 and, 12 to levofloxacin; isolates 3 and 11 to imipenem, isolates 4, 5, 7, 9 and, 11 to ofloxacin, isolates 6 to amoxicillin-clavulanate, isolates 9 and 10 to gentamicin, isolates 13 to cefotaxime.

Table 1. Inhibitory Zone Diameter (IZD) of Selected Antibiotics Against *S. aureus*.

Drugs/ Isolates	Ceftriaxone Sulbactam (C)	Levofloxacin (F)	Ofloxacin (J)	Gentamycin (K)	Ciprofloxacin (M)	Erythromycin (N)	Azithromycin (O)
*Sa*1	16.5±0.5	21.0±1.0	14.5±0.5	12.5±0.5	20.0±1.0	12.5±0.5	10.5±0.5
*Sa*2	16.5±0.5	18.5±0.5	12.0±1.0	-	11.5±0.5	8.5±0.5	15.5±0.5
*Sa*3	12.5±0.5	20.5±0.5	20.5±0.5	-	22.0±1.0	18.5±0.5	-
*Sa*4	-	15.5±0.5	11.0±1.0	12.5±0.5	-	9.0±1.0	10.5±0.5
*Sa*5	11.0±1.0	21.0±1.0	11.5±0.5	-	10.5±0.5	-	-
*Sa*6	-	17.0±1.0	10.5±0.5	-	16.5±0.5	-	-
*Sa*7	-	-	-	-	10.0±1.0	-	-
*Sa*8	-	18.0±1.0	10.0±1.0	-	-	19.0±1.0	-
*Sa*9	-	8.5±0.1	13.5±0.5	-	8.5±0.5	-	-
*Sa*10	-	16.0±1.0	11.5±0.1	-	11.0±1.0	23.0±1.0	-
*Sa*11	-	15.0±1.0	19.0±1.0	18.5±0.5	10.5±0.5	-	19.0±1.0
*Sa*12	-	6.5±0.5	8.5±0.5	-	-	-	-
*Sa*13	14.5±0.5	14.0±1.0	11.5±0.5	-	-	-	-
*Sa*14	-	16.5±0.5	11.5±0.1	-	11.5±0.5	-	-
*Sa*15	-	13.5±0.5	15.5±0.5	-	18.0±1.0	-	-
*Sa*16	-	12.5±0.5	-	-	8.5±0.5	-	-
*Sa*17	-	12.5±0.5	15.0±1.0	-	9.0±1.0	13.0±1.0	-

S. aureus (Sa); not determined (-);data are expressed as IZD ± SEM

Table 2. IZD of Selected Antibiotics Against *P. aeruginosa*, *S. dysenteriae* and *K. pneumonia*.

Drugs/ Isolates	*P. aeruginosa*	*S. dysenteriae*		*K. pneumonia*						
	Pa	*Sh*1	*Sh*2	*Kb*1	*Kb*2	*Kb*3	*Kb*4	*Kb*5	*Kb*6	*Kb*7
A	12.5±0.5	10.5±0.5	-	-	9.0±1.0	-	-	-	8.5±0.5	8.0±1.0
B	-	12.5±0.5	10.5±0.5	-	12.5±0.5	-	-	-	-	-
C	12.5±0.5	25.0±1.0	20.5±0.5	20.0±1.0	14.5±0.5	22.5±0.5	31.0±1.0	13.0±1.0	17.5±0.5	10.5±0.5
D	-	13.0±1.0	-	-	-	-	-	-	11.5±0.5	8.5±0.5
E	10.5±0.5	22.5±0.5	12.5±0.5	10.5±0.5	24.5±0.5	-	12.5±0.5	12.0±1.0	-	9.0±1.0
F	23.0±1.0	31.0±1.0	10.5±0.5	10.5±0.5	20.5±0.5	20.5±0.5	18.5±0.5	25.0±1.0	21.0±1.0	30.0±1.0
G	-	30.5±0.5	-	-	-	-	-	-	-	12.5±0.5
H	-	20.5±0.5	10.5±0.5	-	13.5±0.5	-	-	9.5±0.5	11.5±0.5	8.5±0.5
I	15.5±0.5	14.0±1.0	-	-	-	-	17.5±0.5	-	-	-
J	21.5±0.5	32.5±0.5	17.5±0.5	-	18.5±0.5	18.5±0.5	19.0±1.0	25.0±1.0	20.5±0.5	28.5±0.5
K	8.5±0.5	20.5±0.5	15.5±0.5	-	8.5±0.5	12.5±0.5	10.5±0.1	13.0±1.0	-	16.5±0.5
L	-	15.0±1.0	20.5±0.5	-	-	10.5±0.5	-	11.0±1.0	-	19.0±1.0

P. aeruginosa (Pa); *S. dysenteriae* (Sh)*; K. pneumonia* (Kb)*; not determined* (-)*;* A (nalidixic acid)*;* B (cefuroxime); C (ceftriaxone sulbactam); *D (amoxicilli*n); E (cefixime); F (levofloxacin); G (amoxicillin-clavulanate); H (cefotaxime); I (imipenem/cilastatin); J (ofloxacin); K (gentamycin); L (nitrofurantoin); data are expressed as IZD ± SEM.

Table 3. IZD of Selected Antibiotics Against *E. coli*.

Drugs/ Isolates	*Escherichia coli*												
	1	2	3	4	5	6	7	8	9	10	11	12	13
A	10.5±0.5	17.0±1.0	11.5±0.5	4.5±0.5	4.5±0.5	8.5±0.5	12.5±0.5	17.0±1.0	11.0±0.5	2.5±0.5	10.5±0.5	6.5±0.5	10.5±0.5
B	-	-	-	-	-	-	-	-	4.5±0.5	-	-	-	-
C	28.0±1.0	11.5±0.5	17.5±0.5	18.5±0.5	12.5±0.5	17.0±1.0	16.5±0.5	19.0±1.0	21.0±1.0	11.0±1.0	15.5±0.5	15.0±1.0	11.0±1.0
D	-	-	-	-	-	9.5±0.5	-	-	-	-	10.5±0.5	-	17.5±0.5
E	-	21.0±1.0	13.5±0.5	9.5±0.5	-	-	-	16.5±0.5	-	-	-	-	-
F	21.0±0.0	26.0±1.0	13.0±1.0	22.0±0.5	10.0±1.0	16.0±2.0	16.0±2.5	20.0±1.0	24.0±1.0	12.0±3.0	16.0±0.0	14.0±1.0	16.0±0.0
G	-	-	-	4.0±2.5	12.0±1.5	20.0±1.5	-	14.0±0.0	-	-	-	-	-
H	-	-	9.0±3.0	-	-	-	10.0±2.5	16.0±1.5	-	-	11.0±3.0	-	25.0±0.0
I	21.5±0.5	15.5±0.5	21.5±0.5	18.5±0.5	15.5±0.5	15.5±0.5	15.5±0.1	12.5±0.5	7.0±1.0	-	20.5±0.5	-	6.5±0.5
J	16.5±0.5	21.5±0.5	12.5±0.5	30.5±0.5	17.5±0.5	15.5±0.5	16.5±0.5	16.5±0.5	35.0±1.0	13.0±1.0	20.5±0.5	14.0±1.0	15.0±1.0
K	15.5±0.5	12.5±0.5	10.5±0.5	10.5±0.5	15.0±1.0	3.0±1.0	-	13.0±1.0	35.0±1.0	14.0±1.0	18.5±0.5	10.0±1.0	10.5±0.5
L	10.5±0.5	16.5±0.5	11.5±0.5	4.5±0.5	4.5±0.4	8.5±0.5	12.5±0.5	16.5±0.5	11.0±1.0	3.0±1.0	11.0±1.0	7.0±1.0	10.5±0.5

Not determined (-); A (nalidixic acid); B (cefuroxime); C (ceftriaxone sulbactam); D (amoxicillin); E (cefixime); F (levofloxacin); G (amoxicillin-clavulanate); H (cefotaxime); I (imipenem/cilastatin); J (ofloxacin); K (gentamycin); L (nitrofurantoin); data are expressed as IZD ± SEM.

Table 4. IZD of Selected Antibiotics Against *S. typhi*.

Drugs/Isolates	A	C	F	I	J	K	L
*Sal*1	10.5±0.5	12.5±0.5	18.0±1.0	-	12.5±0.5	11.0±1.0	-
*Sal*2	-	-	11.0±1.0	-	-	-	-
*Sal*3	-	18.0±1.0	30.5±0.5	-	29.5±0.5	8.5±0.5	10.5±0.5
*Sal*4	-	-	17.5±0.5	10.5±0.5	11.0±1.0	-	-
*Sal*5	-	-	20.5±0.5	-	19.5±0.5	-	-
*Sal*6	-	-	20.0±1.0	-	-	-	-
*Sal*7	-	-	21.0±1.0	-	16.0±1.0	-	-
*Sal*8	-	-	18.0±1.0	-	-	-	-

Data are expressed as IZD ± SEM; Sal- *Salmonella typhi*; all the isolates were not sensitive to cefuroxime, amoxicillin, cefixime, amoxicillin-clavulanate, and cefotaxime; not determined (-); A (nalidixic acid); L (nitrofurantoin); C (ceftriaxone sulbactam); F (levofloxacin); I (imipenem/cilastatin); J (ofloxacin); K (gentamycin).

Susceptibility of Isolated *S. typhi* to Selected Antibiotics

Of the eight isolates of *S. aeruginosa* from the vaginal swabs, only isolate 1 was more sensitive to ofloxacin as ceftriaxone sulbactam while other isolates were more sensitive to levofloxacin (Table **4**).

Susceptibility of Isolated *Candida* Species to Selected Antifungals

Of the 20 isolates of *Candida* species, the 13 isolates tested against different antifungal agents elicited varying IZDs (Table **5**).

Susceptibility/Resistance Patterns of Isolates to Test Antimicrobials

The results of the 68 isolates' susceptibility tests, which included 48 bacteria (*E. coli*, *K. pneumonia, S. aureus, S. dysenteriae, P. aeruginosa, S. typhi*) are shown in Figure **1**

The prevalence of resistance of *K. pneumonia* against nalidixic acid, cefuroxime, amoxicillin, amoxicillin-clavulanate, and cefotaxime was higher (100%) than 85.7% recorded in cefixime, imipenem/cilastatin, gentamycin, and nitrofurantoin as shown in Figure **1-1.** The organism also showed varying degrees of susceptibility (40-75%) to levofloxacin and ofloxacin. The thirteen *E. coli* isolates were found to show high prevalence resistance of 100% to cefuroxime, 92.3% resistance to cefotaxime, amoxicillin, and amoxicillin-clavulanate, 84.6% to nalidixic acid, nitrofurantoin and cefixime as well as 76.9 % to gentamycin (Figure **1-2).** There was a 30-40 % susceptibility of *E. coli* to levofloxacin and ofloxacin. The *P. aeruginosa* isolated were found to show 100% resistance to nalidixic acid, cefuroxime, ceftriaxone sulbactam, amoxicillin, cefixime, amoxicillin-clavulanate, cefotaxime, gentamycin, and nitrofurantoin as well as 100% susceptible to levofloxacin and ofloxacin (Figure **1-3**). The isolated *S. typhi* were 100 resistant to nalidixic acid, cefuroxime, amoxicillin, cefixime, amoxicillin-clavulanate, cefotaxime, imipenem/cilastatin, gentamycin, and nitrofurantoin. There was 87.5% resistance to ceftriaxone sulbactam and 10-40% susceptibility to levofloxacin and ofloxacin (Figure **1-5**).

The *S. dysenteriae* and *S. aureus* isolates were found to have high prevalence resistance to cefuroxime, cefixime and imipenem/cilastatin. The *S. aureus* was found to be 17-35% susceptible to levofloxacin and ofloxacin (Figure **2X**). The susceptibility profiles of the 20 *Candida* species isolates are shown in Figure **2Y.** The isolated *Candida* sp was found to have high prevalence resistance (> 80%) to fluconazole, clotrimazole, ketoconazole, griseofulvin and cycloheximide with 20-40% susceptibility to amphotericin B.

Table 5. IZD of Selected Antifungal Agents Against *Candida* sp.

Drugs/Isolates	P	Q	R	S	T	U
2	-	-	10.5±0.5	-	10.5±0.5	-
3	-	-	-	-	22.5±0.5	-
5	-	22.5±0.5	11.0±1.0	-	15.5±0.5	-
6	-	-	-	-	14.0±0.5	-
7	-	-	-	-	15.5±0.5	11.0±1.0
11	-	8.5±0.5	-	-	18.0±1.0	25.5±0.5
12	-	-	8.0±1.0	-	20.5±0.5	8.5±0.5
13	25.5±0.5	10.5±0.5	-	-	21.0±1.0	11.0±1.0
14	11.5±0.5	9.5±0.5	9.5±0.5	8.5±0.5	15.5±0.5	9.0±1.0
15	-	-	-	-	10.0±1.0	9.5±0.5
17	22.5±0.5	20.5±0.5	-	41.0±1.0	22.5±0.5	31.0±1.0
18	12.5±0.5	12.0±1.0	12.5±0.5	12.5±0.5	13.0±1.0	12.5±0.5
19	16.5±0.	-	11.5±0.5	-	-	9.5±0.5

Data are expressed as IZD ± SEM; P (fluconazole); Q (clotrimazole); R (ketoconazole); S (griseofulvin); T (amphotericin B); U (cycloheximide).

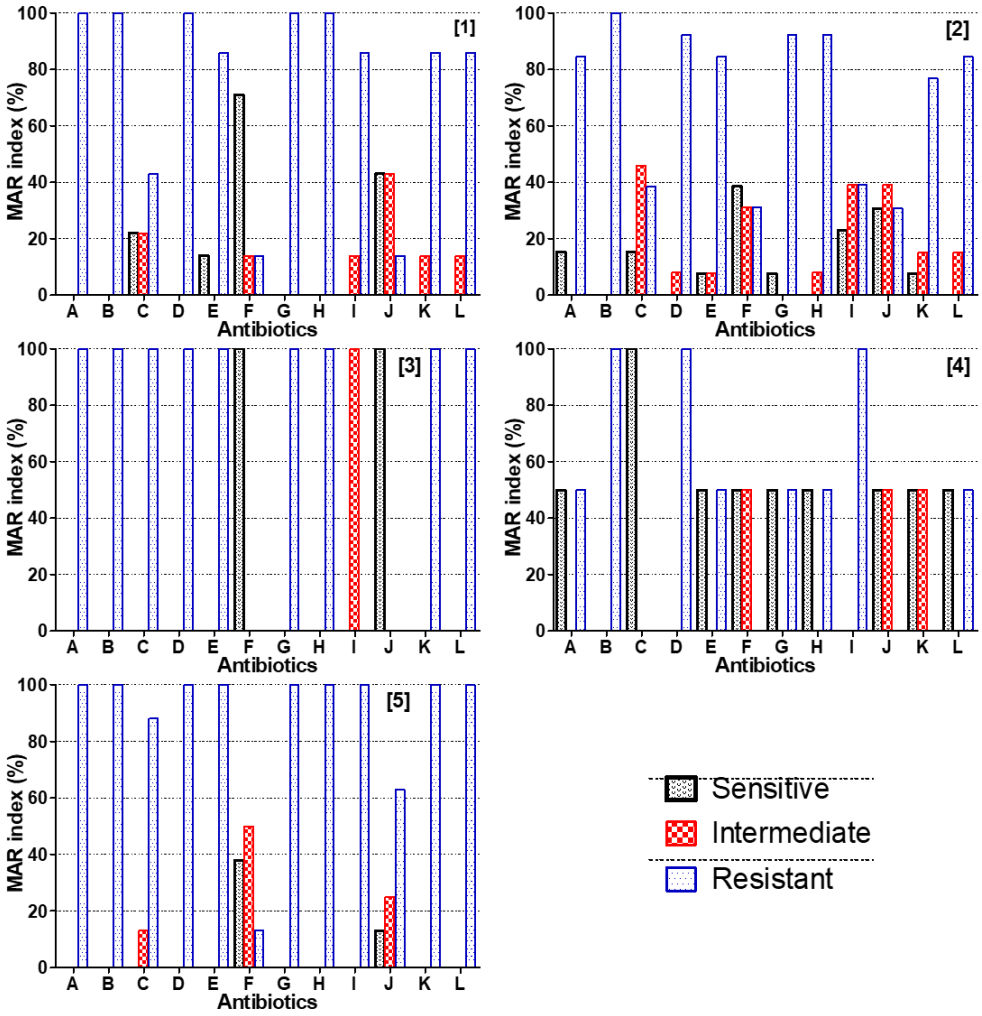

Figure 1. Sensitivity pattern of *Klebsiella* (**1**), *Escherichia* (**2**), *Pseudomonas* (**3**) *Shigella* (**4**) and *Salmonella* (**5**) species isolates to nalidixic acid (A), cefuroxime (B), ceftriaxone sulbactam (C), amoxicillin (D), cefixime (E), levofloxacin (F), amoxicillin-clavulanate (G), cefotaxime (H), imipenem/cilastatin (I), ofloxacin (J), gentamycin (K) and nitrofurantoin (L); sensitive, intermediate or resistant (SIR)

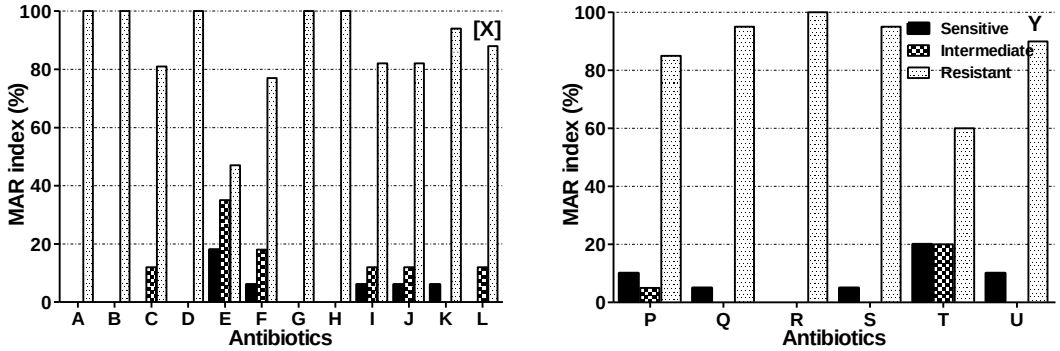

Figure 2. Sensitivity pattern of *Staphylococcus* (**X**) species isolates to amoxicillin-clavulanate (A), cefotaxime (B), ceftriaxone sulbactam (C), cefixime (D), levofloxacin (E), ciprofloxacin (F), imipenem/cilastatin (G), cefuroxime (H), ofloxacin (I), erythromycin (J), gentamycin (K) and azithromycin (L) and *Candida* (**Y**) species isolate to fluconazole (P), clotrimazole (Q), ketoconazole (R), griseofulvin (S), amphotericin B (T) and cycloheximide (U); sensitive, intermediate or resistant (SIR).

Table 6. MAR Indices of Microbial Isolates.

Features	*Isolates, a, MAR indices*																
Sa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
a	5	6	7	7	8	9	11	9	9	8	7	10	9	9	9	10	8
*MARI	0.42	0.50	0.60	0.60	0.67	0.75	0.92	0.75	0.75	0.70	0.60	0.80	0.75	0.75	0.75	0.80	0.67
Ec/Sh/Pa	Ec1	Ec2	Ec3	Ec4	Ec5	Ec6	Ec7	Ec8	Ec9	Ec10	Ec11	Ec12	Ec13	Sh1	Sh2	Pa1	-
a	5	4	3	3	4	3	5	2	4	6	3	6	3	0	4	5	-
*MARI	0.42	0.33	0.25	0.25	0.33	0.25	0.42	0.20	0.33	0.50	0.25	0.50	0.25	0	0.33	0.42	-
Kp/St	Kp1	Kp2	Kp3	Kp4	Kp5	Kp6	Kp7	St1	St2	St3	St4	St5	St6	St7	St8	-	-
a	9	4	7	6	5	6	2	7	11	7	9	10	11	10	11	-	-
*MARI	0.75	0.33	0.60	0.50	0.42	0.50	0.20	0.60	0.90	0.60	0.75	0.83	0.92	0.83	0.92	-	-
Cs	1	2	3	4	5	6	7	8-10	11-12	13	14	15	16	17	18	19	20
a	6	4	5	6	3	5	4	6	3	2	0	4	6	1	0	3	6
*MARI	1.0	0.7	0.8	1.0	0.5	0.8	0.7	1.0	0.5	0.3	0	0.7	1.0	0.2	0	0.5	1.0

*Ratio of the number of antimicrobials the isolates were exposed to (a) to the number of antimicrobials the isolate was resistant to (b = 12; ⁑b = 6). *S. aureus* (Sa); *E. coli* (EC); *Shigella* species (Sh); *P. aeruginosa* (Pa); *K. pneumoniae* (Kp); *S. typhi* (St); *Candida* species (Cs).

To further understand the sensitivity or resistance of the isolated microorganisms to various antimicrobial agents, the multiple antibiotic resistance (MAR) indices of the isolated microorganisms were calculated (Table **6**).

DISCUSSION

Antimicrobial resistance has been identified as one of the primary challenges of parasitic and infectious disease chemotherapies in the 21st century. Resistance from harmful bacteria typically emerges after the introduction of new forms of antimicrobial drugs[6]. It has been widely reported that isolates of *S. aureus, K. pneumonia, S. typhi, S. dysenteriae, P. aeruginosa, E. coli* and, *Candida* species are resistant to the commonly used antibiotics[26]. Studies on the antibiotic susceptibility pattern of the isolated microorganisms are, therefore, crucial for managing both hospital- and community-acquired illnesses and for recognizing new and developing trends in resistance[25]. The study isolated the bacterial and fungal species that were recovered from volunteers and the in-patient population at Madonna University Nigeria. The 52 vaginal discharge swabs of the volunteers were collected; from which seventeen *S. aureus*, thirteen *E. coli*, seven *K. pneumoniae*, two *S. dysenteriae*, one *P. aeruginosa*, eight *S. typhi,* and twenty fungi were isolated.

The results of the antibacterial susceptibility test showed that levofloxacin and ofloxacin (fluoroquinolones) are the most effective conventional antibacterial agents on the antibiotics disc when administered on the bacteria isolate recovered from this present study. This was in contrast to Sharma *et al*., (2019)[27] assertion that levofloxacin and ofloxacin, the most effective medications for the treatment of bacterial vaginitis/vaginosis, elicited a notable increase in resistance with patient ageing due to lowered immune function and general exposure to fluoroquinolones on a more frequent basis than in younger patients. Fluoroquinolone abuse and anarchic use were cited as contributing to the high rate of resistance[27].

The antifungal susceptibility test result showed that amphotericin B was the most effective antifungal agent on the antifungal disc. Fluconazole, clotrimazole, ketoconazole, griseofulvin, and cycloheximide showed a higher resistance rate, which also occurred in a similar study by Mendling *et al*., (2020)[28] which recorded high resistance to most antifungal agents in the treatment of vulvovaginal candidiasis asides from amphotericin B that has shown to have lesser resistance rate[28]. The isolates of vaginal discharge have very distinct MAR patterns. Except for *E. coli* (8), *K. pneumoniae* (7), and *Candida* sp. (17), all organisms had a MAR index ranging from 0.25 to 1.00.

The MIC is a parameter that also quantitatively describes the susceptibility of antimicrobials[29]. It was observed that the lowest concentrations of cefepime at 25 µg/ml and 50µg/ml, cephalexin at 62.5 and 31.5 µg/ml, and nitrofurantoin at 125 µg/ml were able to inhibit the growth of *S. aureus*. The lowest concentrations of cefepime at 25 µg/ml, cephalexin at 31.5 µg/ml, and nitrofurantoin at 125

µg/ml were able to inhibit the proliferation of *E. coli*. The lowest concentrations of cefepime at 50 µg/ml, cephalexin at 62.5µg/ml, and nitrofurantoin at 125 µg/ml were also able to stop the growth of *S. typhi*. The lowest concentrations of cefepime at 100 µg/ml, cephalexin at 62.5 and 31.5 µg/ml, and nitrofurantoin at 250 and 125 µg/ml inhibited the growth of *K. pneumonia*. The lowest concentrations of cefepime at 50 µg/ml, cephalexin at 31.5µg/ml, and nitrofurantoin at 125 µg/ml were able to stop the expansion of *P. aeruginosa*. The lowest concentrations of cefepime at 25 µg/ml, cephalexin at 62.5 and 31.5 µg/ml, and nitrofurantoin at 62.5 µg/ml were able to inhibit the growth of *S. dysenteriae*. While for the MIC also conducted on *Candida* sp, it was also found that the lowest concentration of amphotericin B and fluconazole were able to inhibit the growth of *Candida* species at 31.5 µg/ml and 62.5 µg/ml.

The study showed that cefepime is the most effective; in the treatment of diseases associated with bacteria vaginitis, Cephalexin which is the second most sensitive against the test isolate agrees with the previous work, and nitrofurantoin which was shown to be less effective elsewhere[30-32]. Amphotericin B appeared to be the most effective; as postulated by Kim and coworkers, (2019)[33] while fluconazole was shown to be less effective which is in parallel with the work done by Ejike *et al.,* (2018)[34].

CONCLUSION

The study showed that the bacteria and fungi isolated from the vaginal discharge of Madonna University residents were more susceptible to cefepime, ofloxacin, and levofloxacin and amphotericin B, respectively. To effectively treat bacterial vaginitis and vulvovaginal candidiasis in the area, it is advised that these antimicrobial medicines be prescribed as the drug of choice as a first-line option. The study has further provided prospects for the identification of resistant and/or virulent genes in these microorganisms to explore alternative preventive or curative therapies for vaginosis.

FUNDING

This research received no external funding.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ACKNOWLEDGMENT

None.

LIST OF ABBREVIATIONS

BV	Bacteria vaginosis/vaginitis
CLSI	Clinical and laboratory standards institute
DMSO	Dimethyl sulphoxide
ESBL	Extended-spectrum beta lactam
GBS	Group B Streptococcus
IZD	Inhibitory zone diameter
MAR	Multiple antibiotic resistant
MHA	Mueller-Hinton agar
MIC	Minimum inhibitory concentration
MRSA	Multi-drug resistant Staphylococcus aureus
NCCLS	National committee for clinical laboratory standards
SDA	Sabouraud dextrose agar
SIR	Sensitive, intermediate, or resistant
UTI	Urinary tract infection

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