Original Research Article

Antibiogram and MAR indices of bacterial isolates from urine of diabetics and non-diabetics attending some Hospitals in Rivers State, Nigeria

ABSTRACT

There has been a constant rise in the number of cases of antimicrobial resistance with paucity in data from the developing nations. This study determined the antibiograms of uropathogens isolated from diabetics and non-diabetics subjects in three hospitals in Rivers State Port Harcourt, Nigeria. The Kirby-Bauer disc diffusion procedure was used to determine the antimicrobial susceptibility pattern of the uropathogens with the following antibiotics: vancomycin, cefotaxime, cefuroxime, imipenem, cefoxitin, ceftazidime, erythromycin, ciprofloxacin, tetracycline, amoxiclav and ofloxacin. *S. aureus* (20%), *E. coli* (9%), *K. aerogenes* (8%), and *K. ascobata* (7%) were the most prevalent bacterial isolates. The antibiotics with the highest sensitivities to the test bacteria were imipenem (18.6%), ofloxacin (13.8%) and ceftazidime (12.3%). On the other hand, the antibiotics with the highest resistance were cefotaxime (9.5%), tetracycline (14.1%), erythromycin (18.4%), and vancomycin (21.1%). The diabetic subjects showed significantly (P = <0.0001) lower prevalence of bacteria with MAR indices below 0.2 but significantly (P = <0.0001) higher prevalence of bacteria showing MAR indices ≥0.2. In the analyses of **≥**0.2 MAR indices of bacterial isolates according to location. Abonnema had highest percentage distribution, with higher number of diabetics 21 (81%) to non-diabetics 5 (19%), with P = <0.0001. This was followed by Mgbundukwu, which had higher number of diabetics 10 (77%) to non-diabetics 3 (23), with P = <0.0001, and then Eleme, with higher number of diabetics 8(80%) to non-diabetics 2 (20%), with P = <0.0001. This indicates a high level of antibiotic abuse and hence resistance to antibiotics among diabetic patients.

Keywords: Antimicrobial susceptibility, Imipenem, Ofloxacin, drug resistance, urinary tract infection

1. INTRODUCTION

Antimicrobial susceptibility testing (AST) is a laboratory procedure performed by the clinical microbiologist to identify which antimicrobial regimen is specifically effective for individual patients. It is also the measurement of the susceptibility of bacteria to antibiotics. Empirical therapy continues to be effective for some bacteria pathogens because resistance mechanisms have not been observed. Antimicrobial susceptibility testing is based on the principal that a standardized inoculum of bacteria (usually 0.5 McFarland) is dabbed unto the surface of a dish of Mueller-Hinton agar. Filter paper discs impregnated with antimicrobial agents are placed on the agar. Diameter of zone of inhibition around each disc was measured after overnight incubation [1]. The advent of antimicrobial resistance has added significantly to the impact of infectious diseases, in number of infections, as well as added healthcare costs. Even though we have a very large number of antimicrobial agents from which to choose for potential infection therapy, there is documented antimicrobial resistance to all of these, and this resistance occurs shortly after a new drug is okayed for use. These

concerns prompted the WHO to launch a Global Action Plan on antimicrobial resistance in 2015 [2].

Antimicrobial agents can be divided into groups based on the mechanism of antimicrobial activity. The main groups are agents that inhibit cell wall synthesis, depolarize the cell membrane, inhibit protein synthesis, inhibit nuclei acid synthesis, and inhibit metabolic pathways in bacteria. With a wide range of mechanisms, we would have better control over the organisms. Unfortunately, improper stewardship of antimicrobial agents has helped lead to the tremendous resistance issue that we now face. Factors that contributed to the growing resistance problem include increased consumption of antimicrobial drugs, both by humans and animals, and improper prescribing of antimicrobial therapy. Overuse of many common antimicrobials agents by physicians may occur because the choice of drug is based on a combination of low cost and low toxicity [3]. There may also be improper prescribing of antimicrobials drugs, such as the initial prescription of a broad-spectrum drug that is unnecessary, or ultimately found to be ineffective for the organism(s) causing the infection. The danger is that excessive use of antibiotics in humans leads to emergence of resistant organisms [4]. In addition, prior use of antimicrobial drugs puts a patient at risk for infection with a drug resistant organism, and those patients with the highest exposure to antimicrobials are most often those who are infected with resistant bacteria [5].

For many years, antibiotics have been used for treating or preventing disease in raising food animals. The animal feed often contains antibiotics in amounts that range from below therapeutic levels to full therapeutic levels, and the antibiotics used come from most of the antimicrobial classes used in humans. There is evidence to support the idea that feeding antibiotics to animals may result in development of antimicrobial resistant organisms, and that those resistant organisms may be transferred to the humans who consume those animals [6]. The antimicrobial resistance patterns seen in the animals reflects the types and amounts of antibiotics given to the animals. The transmission of antimicrobial resistance from the animals to humans may occur in various ways, with the direct oral route being the most common (includes eating meat and ingestion of faeces in contaminated food or water). Another common route is from direct contact with the animals by humans [7].

Continued increases in antimicrobial resistance have led to fewer treatment options for patients, and an associated increase in morbidity and mortality. The result is that now we are facing more severe infections needing more extensive treatment, and longer courses of illness often requiring extended hospitalization. This has dramatically increased the healthcare costs associated with these infections. The centre for disease control has reported that a conservative estimate is that over 2 million people in the U.S become ill each year with antimicrobial resistant infections, resulting in more than 23,000 deaths [8]. Various methods of antimicrobial stewardship have been suggested to stem the increases in resistance. One method involves the use of diversity in antimicrobial use. This refers to various components such as not giving a single drug, but using two or more drugs, either alternatively or concurrently, preferably using drugs with different mechanisms of action [9]. Hence, this study hopes to investigate the antibiogram of bacteria isolated from diabetic and non-diabetic individuals.

2. MATERIAL AND METHODS

2.1 Study design

A cross-sectional design was used in this study. Random sampling methods were used to sample 300 participants in different three Hospitals in the three (3) senatorial districts of Rivers State, Nigeria.

2.2 Sample collection

The study population included diabetic patients attending Model Primary Healthcare centre, Mgbundukwu, General Hospital, Ogale, Nchia-Eleme, and Model Primary Healthcare centre, Abonnema as well as non-diabetic patients attending these three hospitals who were used as controls. Early morning mid-stream urine samples of diabetic and non-diabetic subjects (male and female) totalling three hundred (300) were collected aseptically in sterile universal containers using case control study design. These were transported in an ice pack to the clinical microbiology laboratory. Subjects that had been on antibiotic during the previous month were excluded from the study. Multi drug resistant (MDR) bacteria were defined as isolates resistant to ≥ 2 antimicrobial agents.

2.3 Identification of bacterial isolates

Using sterile wire-loop, urine samples were inoculated only mannitol salt agar MacConkey agar (oxoid, UK), CHROMagar Orientation (TM) plates, using the streak plate method according to the method of Miles and Misra [10]. Inoculated plates were incubated aerobically at 37^{o}C for 24-48 hours. Inspection of discrete colonies was done based on colour, size, morphology, shape, etc. Isolation of pure cultures was done by aseptically subculturing bacteria colonies obtained from a previous study onto freshly prepared nutrient agar plates. These were incubated at 37^{o}C for 24 hours. Pure cultures of bacterial isolates were Gram stained and confirmed by biochemical tests to enhance the identification of isolates [11].

2.4 Antimicrobial susceptibility testing of bacterial isolates

Using the Kirby-Bauer disc diffusion procedure under Clinical Laboratory Standard Institute [12] antimicrobial susceptibility test was done using the following antimicrobials: vancomycin, cefotaxime**,** cefuroxime, imipenem, cefoxitin, ceftazidime, erythromycin, ciprofloxacin, tetracycline, amoxiclav, ofloxacin. The choice of antibiotics was according to the CLSI guideline of 2013. The cultures were standardized by serially diluting with sterile normal saline to achieve a MacFarland standard of 0.5 corresponding to a cell density of 1.5×10^{8} cfu/µl. Mueller-Hinton agar (Oxoid, Cambridge, UK) plates were then inoculated uniformly using sterile swab sticks and incubated at 37^{o}C. Zones of inhibition were determined by measuring the size of the clear zones with a graduated ruler. Results of resistance (R) and sensitive (S) were recorded and compared with CLSI standards for interpretation.

2.5 Statistical Analyses

Data obtained from this study presented in percentages and were analysed using chi-square where necessary.

3. RESULTS

Out of the 19 different species of bacteria isolated the 4 most prevalent bacteria were: *S. aureus* (20%), *E. coli* (9%), *K. aerogenes* (8%), and *K. ascobata* (7%) (Fig. 1). The antibiotics with the highest sensitivities to the test bacteria were imipenem (18.6%), ofloxacin (13.8%) and ceftazidime (12.3%). On the other hand, the antibiotics with the highest

resistance were cefotaxime (9.5%), tetracycline (14.1%), erythromycin (18.4%), and vancomycin (21.1%) (Table 1). Bacteria of *P. fontium, E. coli, S. aureus and Klebsiella* sp. showed the highest resistance to the different antibiotics tested for diabetic subjects (Table 2)

Table 1. Percentage prevalence of resistance and susceptibility to antibiotics of isolated bacteria

Antibiotic	Percentage Resistance (%)	Percentage Susceptibility (%)
Imipenem	0.6	18.6
Ofloxacin	3	13.8
Ceftazidime	3.9	12.3
Amoxiclav	6	6.9
Cefuroxime	7.3	10.7
Cefoxitin	7.5	11
Ciprofloxacin	8.7	9
Cefotaxime	9.5	4.3
Tetracycline	14.1	6.5
Erythromycin	18.4	2.1
Vancomycin	21.1	4.4

Table 2. Percentage prevalence of resistance to antibiotics of isolated bacteria in diabetic and non-diabetic subjects

Antibiotics	*Enterobacter sp.*		*E. coli*		*Kleb. sp*		*Kluyvera sp.*		*Serratia sp.*		*P. fontium*		*P. mirabilis*		*Rauotella planticola*		*S. aureus*		*Tatumella terrea*		*P. Asymbiotica*	
	D	**ND**	**D**	**ND**	**D**	**ND**	**D**	**ND**	**D**	**ND**	**D**	**ND**	**D**	**ND**	**D**	**ND**	**D**	**ND**	**D**	**ND**	**D**	**ND**
Vancomycin	14	14	33	22	20	0	0	0	54	31	100	0	50	0	100	0	26	37	75	0	100	0
Cefotaxin	36	14	11	33	10	20	0	0	15	54	80	100	0	50	50	100	11	27	75	75	50	100
Cefuroxime	21	7	22	22	10	0	0	13	23	8	60	0	0	0	0	0	11	11	0	0	50	0
Imipenem	14	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0	5	0	0	0	0	0
Cefoxitin	7	7	11	11	10	0	13	0	8	0	40	0	0	0	0	0	16	37	0	0	50	0
Ceftazidime	21	7	11	0	11	0	13	13	8	8	40	0	0	0	0	0	5	5	0	0	0	0
Erythromycin	36	14	44	33	20	0	25	13	31	16	40	0	100	0	75	0	21	11	0	0	0	0
Ciprofloxacin	21	7	11	0	10	0	25	0	8	0	40	0	100	0	75	0	11	5	0	0	0	0
Tetracycline	21	7	22	22	20	0	50	25	54	31	20	0	50	0	25	0	32	16	25	0	50	0
Amoxiclav	21	7	33	0	10	0	0	0	15	8	40	0	50	0	25	0	47	53	100	0	100	0
Ofloxacin	14	0	11	0	0	0	0	0	8	0	20	0	0	0	50	0	11	0	0	0	0	0

Key: D – diabetic subjects, ND – non-diabetic subjects*, P. mirabilis = Proteus mirabilis, P. fontium = Pragia fontium, P. asymbiotica =Photorhabdus asymbiotica, S. aureus= Staphylococcus aureus, E. coli= Escherichia coli*

3.1 Multiple antimicrobial resistance indices of the isolates

The percentage comparison of MAR indices of bacteria <0.2 and ≥0.2 for diabetic and non-diabetic subjects are shown in Fig 2. The diabetic subjects showed significantly (*P* = <0.0001) lower prevalence of bacteria with MAR indices below 0.2 but significantly (*P* = <0.0001) higher prevalence of bacteria showing MAR indices ≥0.2. Table 3 shows analyses of **≥**0.2 MAR indices of bacterial isolates according to location. Abonnema had highest percentage distribution, with higher number of diabetics 21 (81%) to non-diabetics 5 (19%), with *P* = <0.0001. This was followed by Mgbundukwu, which had higher number of diabetics 10 (77%) to non-diabetics 3 (23), with *P* = <0.0001, and then Eleme, with higher number of diabetics 8(80%) to non-diabetics 2 (20%), with *P* = <0.0001. Statistical significance was observed.

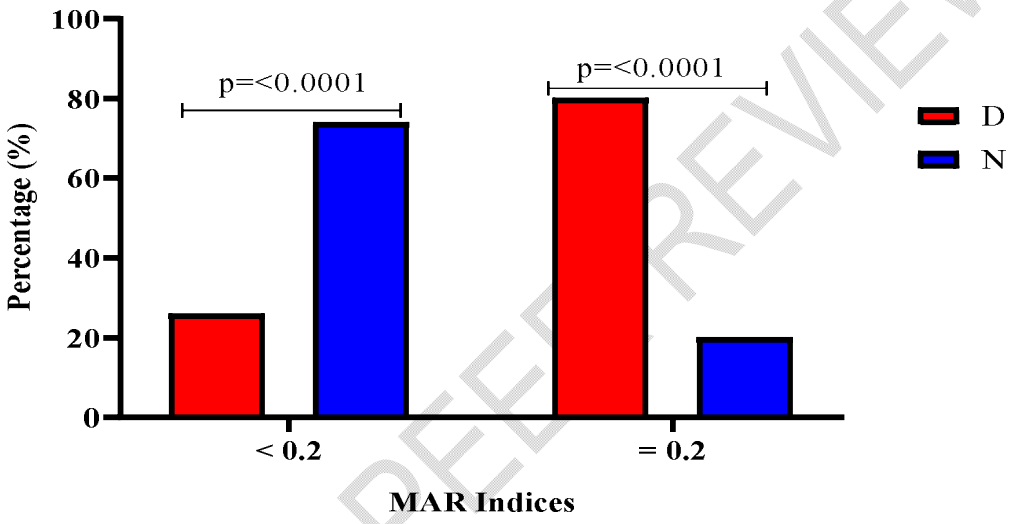

Fig. 1. Distribution Pattern of MAR Indices. N - Non-diabetic and D – diabetic. χ=25.21, *P* = <0.0001 Cramer's V = 0.5363.

Table 3. Analyses of ≥0.2 MAR Indices of the Bacterial Isolates According to Location

MAR Indices	Non-diabetic subjects n (%)	Diabetic subjects n (%)	Total	*P*-value
Abonnema	5 (19)	21 (81)	26	<0.0001
Mgbundukwu	3 (30)	10 (26)	13	<0.0001
Eleme	2 (20)	8 (21)	10	<0.0001
Total	**10**	**39**	**49**	

n represents the number of cases of MAR Indices. Prevalence (%) is calculated based on the overall of the respective groups (non-diabetic and diabetic subjects).

4. DISCUSSION

In this current study, eleven (11) genera of bacteria were isolated from the urine specimens of diabetic and non-diabetic subjects (Table 2). Nine (9) were Gram-negative bacteria while only one (1) namely *Staphylococcus aureus* was Gram positive. The antimicrobial susceptibility pattern was similar in both diabetic and non-diabetic groups with maximum susceptibility to imipenem (a carbapenem) followed by ofloxacin and least susceptibility was vancomycin, followed closely by erythromycin as shown in Tables 1 and 2. This agrees with the report of Aswani *et al.* [13] were meropenem which is carbapenem showed maximum sensitivity to bacteria isolates from both diabetic and non-diabetic subjects studied. It was also observed in this study that the isolate *S. aureus* showed resistance to vancomycin in diabetics and non-diabetics while *Kluyvera ascorbata* also showed resistance to tetracycline in diabetics and non-diabetics as shown in Tables 2.

Distribution pattern of MAR indices among diabetic and non-diabetic subjects in this study showed that in the <0.2 group, the percentage prevalence of the non-diabetic subjects was significantly higher than the diabetic subjects with P=<0.0001 while in the ≥ 0.2 group, the diabetic subjects had significantly higher percentage prevalence when compared to the non-diabetic subjects with P=0.0001. This indicates a higher antibiotic usage among diabetic subjects and hence more antibiotic resistance as compared to their non-diabetic counterparts as shown in figure 1. It was also discovered in this current study that *Enterobacter sp.* showed 100% sensitivity to imipenem and ofloxacin among non-diabetics (Table 2) and minimum resistant among their diabetic counterparts as indicated in Table 2. *Escherichia coli* showed 100% sensitivity in both diabetic and non-diabetic subjects to imipenem and ofloxacin.

However, *E. coli* showed intermediate resistance to amoxiclav (33.3%) among the diabetic subjects which is same as in Hamdan *et al.* [14, 15]. There was 100% resistance of *Photorhabdus asymbiotica* isolate among diabetic subjects to vancomycin and amoxiclav (Table 2) though it was the second least isolated organism in this study and was found only among diabetic subjects. *Serratia* sp. showed 100% sensitivity to Imipenem in both diabetic and non-diabetic subjects. The findings of this study are also in agreement with the study conducted by Khorshidi and Shariff [16] where imipenem had maximum susceptibility of 96.7% in urine samples and that conducted by Boroumand *et al.* [17] with 92%. Imipenem is a broad- spectrum, beta-lactam antibiotic and it acts by inactivating penicillin binding proteins thereby causing lysis of the bacterial cell. Ofloxacin, a quinolone which was found to be the second most susceptible antibiotic in this study is a synthetic, broad-spectrum antibiotic which mechanism of action is the inhibition of DNA gyrase and topoisomerase iv, with the consequent DNA breakdown and cell death due to genetic damage.

The resistance of *Staphylococcus aureu*s to vancomycin as discovered in this study agrees with the findings of Cox and Wright [18] 2013. Resistance is mediated through the acquisition of van genes which results in changes in the italic structure of peptidoglycan precursors that cause a decrease in the binding ability of vancomycin. The ineffectiveness of vancomycin against Gram negative isolates is because of its large molecular size and inability to penetrate the outer bacterial membrane (thick lipopolysaccharide layer), thereby making Gram negative bacteria to have intrinsic resistance to vancomycin and glycopeptides [19]. Resistance in *Staph. aureus* is often acquired by horizontal transfer to genes from outside sources and because of chromosomal mutation and antibiotic selection. Members of the Enterobacteriaceae generally reduce porin number as a mechanism for resistance to carbapenems.

4. CONCLUSION

S. aureus, *E. coli*, *K. aerogenes*, and *K. ascobata* were the most prevalent bacterial isolates. The antibiotics with the highest sensitivities to the test bacteria were imipenem, ofloxacin and ceftazidime while those with the highest resistance were cefotaxime, tetracycline, erythromycin, and vancomycin. Abonnema had highest percentage distribution, with higher number of diabetics to non-diabetics. This was followed by Mgbundukwu, which had higher number of diabetics to non-diabetics and then Eleme, with higher number of diabetics to non-diabetics. These results indicate higher levels of antibiotic abuse and hence resistance to antibiotics among diabetic patients.

CONSENT

All authors declare that written informed consent was obtained from the patient.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki."

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