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Research Article

Detection of Antibiotic-Resistance Genes associated with *S. aureus* and *E. coli* from Patients with Diabetic Foot Infections from Selected Hospitals in Katsina Metropolis, Katsina State, Nigeria

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ABSTRACT

This study, conducted in Katsina, Nigeria, investigated antibiotic resistance in Staphylococcus *aureus* and *Escherichia coli* from patients with Diabetic Foot Infections (DFIs). 150 wound samples were collected, identifying 44 isolates (22 *S. aureus* and 22 *E. coli*). Results showed high resistance rates: *E. coli* was 100% resistant to cefuroxime, ciprofloxacin, ofloxacin, augmentin, ampicillin, and erythromycin. *S. aureus* showed 100% resistance to pefloxacin, 95% to ciprofloxacin, and 45% to cefoxitin. All *E. coli* and six *S. aureus* isolates were multidrug-resistant (MDR). PCR confirmed resistance genes: *bla*TEM and *bla*CTX in *E. coli*, and *mecA* and *parC* in *S. aureus.* The study highlights MDR pathogens in DFIs, emphasizing the need for surveillance and improved antimicrobial stewardship in Katsina State. DFIs are a significant concern, and antibiotic resistance is a major challenge. Effective treatment strategies and infection control measures are crucial to prevent further resistance.

Keywords: Antibiotics; Diabetic foot infection; *Escherichia coli;* Genes; Multi-drug resistance; *Staphyloccus aureus*

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INTRODUCTION

Antibiotic resistance occurs when bacteria develop mechanisms to withstand the effects of antibiotics, making these medications ineffective aga inst infections (WHO, 2022). This phenomenon is a growing global health concern, driven by the overuse and misuse of antibiotics (CDC,2022). Antibiotic resistance can arise and spread due to a number of factors, such as the quantity of antibiotics used and unsanitary conditions (Gibreel, 2011). Molecular techniques have been applied and found helpful in accurately diagnosing diseases with a great sensitivity and specificity than conventional methods such as biochemical and cultural approaches (Merza and Jubrael 2015; Al-Saffar and Al-Khayyat, 2020).

Multiple drug resistance (MDR) is exhibited when some microorganisms show resistance to more than one class of antimicrobial drugs. The MDR microorganisms are threats to public health because they withstand a couple of antibiotics (Magiorakos, 2014; WHO, 2018). Antimicrobial resistance makes it difficult to eradicate infections from the body as present day drugs become less effective (Andersson,2010). Foot infection for example occurs when bacteria enter the foot in some way, either through a wound or through the intact skin. An infected foot can be painful and hinder mobility, but it can be difficult to treat because the infections underlying cause of infections and can be difficult to identify (Pascale,2012).

Diabetes mellitus (DM) is a pandemic disease affecting over four hundred million humans worldwide, which is approximately 9% of the adult population (Pascale, 2012). The prevalence of diabetic foot ulcers (DFU) varies, ranging from 3–13%, with international average of 6.4% (Zhang *et al.*, 2017). Prevalence statistics from the International Diabetes Federation estimates that DFUs improve in 9.1–26.1 million people with diabetes per year globally (Armstrong *et al.*, 2017). Of these with DFU, 50–60% will grow to diabetic foot infection (DFI), 15% of whom will require amputation. The estimated annual incidence of DFI ranges from 2.2–6.3% amongst sufferers with DM (Lipsky *et al.*, 2012). Globally, DFIs are the most common skeletal and soft-tissue infection in patients with DM. The incidence increases with age and varies among extraordinary ethnic groups. There is no gender predominance. The DFIs are the commonest diabetic complications requiring hospital admission, and they are essential part of the workload of clinical infection specialists, accounting for 19% of all infectious disease consultations in a report by (Commons *et al.*, 2018). Specialist infection expertise may currently not be accessible in many parts of the world, which puts extra burden on generalists, especially in the context of community-based care, where many cases are managed. The mortality hazard from DFI is best in sufferers with chronic osteomyelitis and acute necrotizing tender tissue infections (Saeed*et al.*, 2018). Multiple classification systems have been used for DFIs since the 1980s for the functions of prognosis, wound description, audit, and to information therapy. The microbiology of DFIs varies via geographical location, as well as with severity of ailment and the features of the patient (e.g. preceding antibiotic course, ultra-modern hospitalization) (Lipsky *et al.*, 2012 and Noviello *et al.*, 2012).

MATERIALS AND METHODS

Study Design

The study adopted cross-sectional design, incorporating both phenotypic and genotypic approaches to characterize the resistance mechanisms and associated genetic elements in the isolates.

Study Area

The study was conducted in two selected hospitals in Katsina, Katsina state, Katsina state has a land area of approximately 24,194 Km², it is situated between longitudes 6052', 9O20'E and latitudes 1108', 13022'N. The National Population Commission (2006) estimates that the State has 5,800,672 inhabitants, of which 2,947,639 are men and 2,853,033 are women. The major occupation in Katsina State are farming, cattle rearing, trading, fishing, hunting, and crafts, among other things. Herbalists, crafts people, and traditionalists are few. The State has two distinct seasons: wet and dry. According to Dauda *et al*. (2016), the Sahel region experiences 300–400 mm of annual rainfall, the Sudan 600–800 mm, and the Northern Guinea Savannah 900–1100 mm of annual rainfall. The mean daily temperature ranges between 160C and 400C as shown in figure 1 (Dauda *et al*., 2016).

Study Population

The study subjects were total of 150 diabetic foot-ulcer patients (comprise of 75 from General Hospital Katsina and 75 from Federal Teaching Hospital Katsina) between from patients attending General Hospital Katsina and Federal Teaching Hospital Katsina, Katsina Metropolis from January to April 2024.

Sample Size Determination

The sample size of 150 was determined using the formula $n = Z^2 p (1-p)/d^2$ where; Z= Z-score for 95% confidence interval = 1.96, p = previous prevalence in Nigeria = 8% and d = acceptable error (5%).

$$n = \frac{(1.96)^2 \times (0.08) \times (1-0.08)}{(0.05)^2} = 113.1$$

which was rounded up to 150.

Inclusion Criteria

This study only included patients who were diagnosed with diabetic foot infection and attending either Federal Teaching Hospital or General Hospital Katsina. *Escherichia coli and Staphylococcus aureus* isolates were only considered in this study. Age bracket of ≥30 and 81≤years were only considered (Torres-Sangiao *et al.,* 2022).

Ethical Approval

Ethical approval was obtained from the research ethical committee before commencement of the study from Katsina State Ministry of Health and Federal Teaching Hospital Katsina with Health Research Ethical Review Committee (HREC) assigned Number MOH/ADM/SUB/1152/1/850 and FTHKTHREC: REG.24/06/22C/130, respectively Informed consent was obtained from all patients before collection of samples Abdulkadir and Aisha, 2018; Abdu *et al.,* 2018).

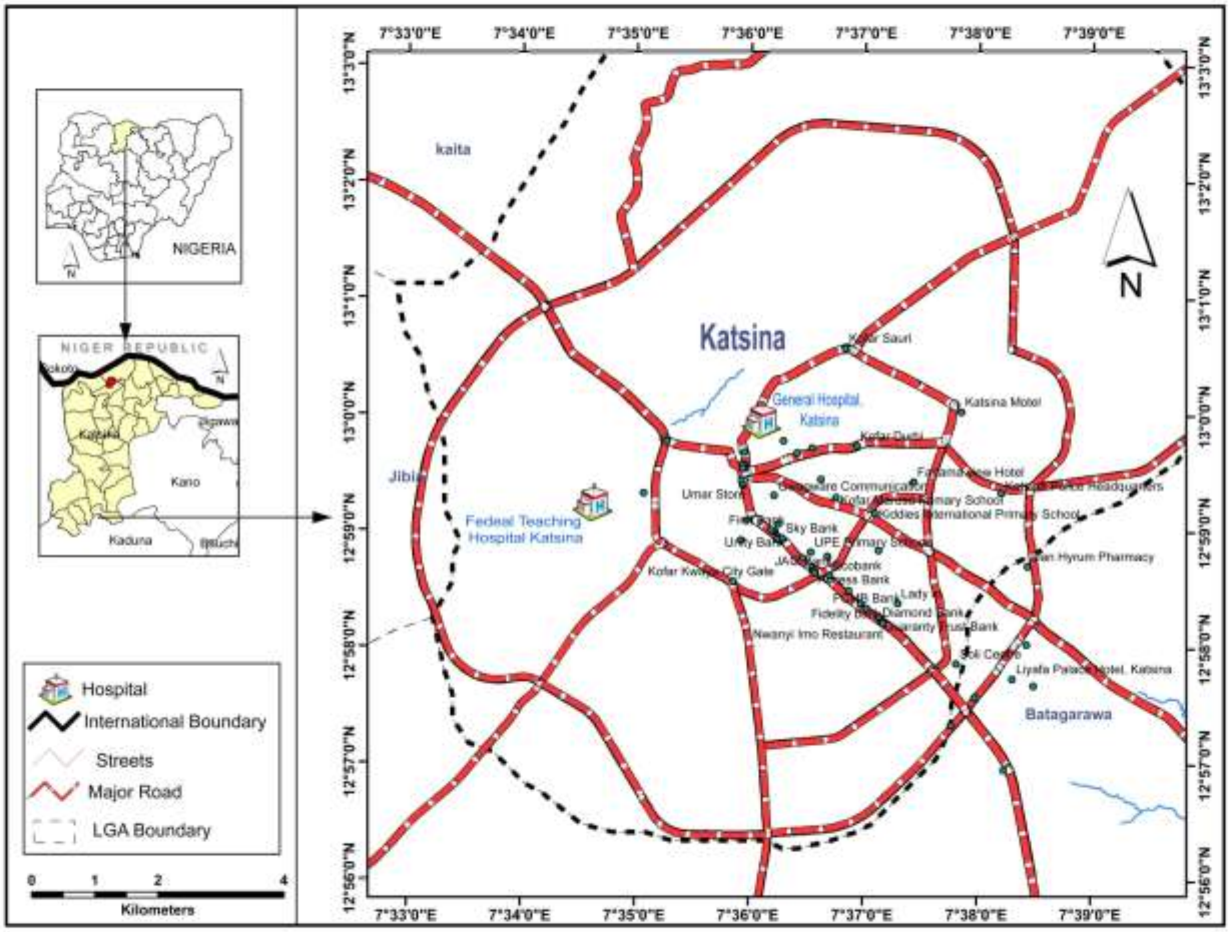

Figure 1: Map of Katsina State showing the Study Sites

Sample Collection and Handling

A total of 150 (i.e. 75 samples from each of General Hospital Katsina and Federal Teaching Hospital Katsina). Pus samples were obtained by swabbing the base of ulcer or the deep portion of the wound edge of diabetic's patients presenting with foot ulcer using a sterile swab stick and placed in a well labeled test tubes containing 10 mL of sterile peptone water (Appapalam*et al.,* 2021) and transported to Microbiology laboratory at Federal Teaching Hospital Katsina and processed for bacterial isolation. Samples arrived at the laboratory after the collection within 30 minutes.

Preparation Of Culture Media

Manitol salt agar (MSA) (L:S-BIOTEC, USA), Eosin Methylene blue (EMB) (TM MEDIA, INDIA), Nutrient agar (NA) (HiMedia, USA), Simmons Citrate Agar (LIFESAFE BIOTECH, USA), Methyl Red VogesProskaur (MRVP) agar (HKM, CHINA), Triple Sugar Iron Agar (TSI) (HKM, CHINA), peptone (ZAYO SIGMA, GERMANY) were prepared according to the manufacturer's instructions and autoclaved at 121°C for 15 minutes.

Isolation of Bacteria and Identification (Gram stain and Biochemical profile)

The collected pus samples contained in peptone water were streaked on blood agar using sterile inoculation loop. The inoculated plates were inverted and incubated at 37°C for 24 hours. Thereafter, the cultural characteristics i.e. (size, shape, consistency, colour, and elevation) and the differential characteristics such as pigmentation were recorded, *S. aureus* appears creamy white while *E. coli* appears pale on blood agar. Thereafter, discrete colonies of *S. aureus and E. coli* were sub-cultured by re-streaking on MSA plates and EMB, respectively to obtain pure culture. Distinct well-separated yellow colonies and creamy white colonies on MSA were presumed *S. aureus* and blue-black colonies on EMB with a green metallic sheen were presumed *E. coli.* Afterwards, isolates were re-streaked out on sterile nutrient agar in Petri dish to obtain a pure culture and stored on nutrient agar slant at 4^0C for further biochemical test and analysis. Stored bacteria were subjected to selected preliminary biochemical tests such as gram staining, catalase, mannitol fermentation, and slide coagulase test, indole, methyl red and Voges-Proskauer, and citrate utilization test (Gladys, 2019).

Antibiotic Susceptibility Testing

The antibiotic susceptibility testing followed the modified single disc diffusion techniques, interpreted accordance to Clinical and Laboratory Standard Institute (CLSI, 2024) guidelines. standardized overnight culture of each isolate (containing approximately 10^6cfu/ml) equivalent to 0.5 McFarland Standard was used to flood the surface of Müeller-Hinton agar plates and allowed to dry while the Petri dish lid was in place. The following standard antimicrobial discs namely; ciprofloxacin (CIP, 5µg), erythromycin (ERY, 15µg), gentamysin (GEN, 10µg), imipenem (IMI, 10µg), amoxicillin (AMP, 20µg), ceftazidime (CAZ, 30µg), cefuroxime (CRX, 30µg), ofloxacin (OFL, 5 µg), augumentin (AUG, 30µg), cefoxitin (FOX, 10µg), pefloxacin (PEF,10ug,), streptomycin (S, 30ug), ampicillin, (AMP, 30µg) obtained from MASTDISCS^{R}AST, UK, were aseptically placed at reasonable equidistance on the inoculated plates. The plates were incubated at 37^0C for 24 hours. The diameter of the zones of inhibition produced by each antibiotic disc were measured with a graduated ruler in millimeters (CSLI, 2023). Those that shows resistance to 2 to 3 classes of antibiotics are the one that are selected for molecular characterization.

Molecular characterization of *S. aureus and E. coli* Isolates

DNA extraction of *S. aureus* and *E. coli*

The DNA of the *S. aureus* was extracted using the QIAGEN QIAamp mini extraction kit protocol. The pure culture of the isolates that have been maintained on nutrient agar plate were harvested into 200μL of tris EDTA (TE) buffer. Subsequently, 25μL of proteinase K solution was added to the broth culture and vortexed slightly. After which 200μL of bacterial lysis solution (AL buffer) was added to the mix and incubation was done in a thermomixer at 56℃ for 30 minutes followed by 5 minutes incubation at 95℃. About 200μL of absolute ethanol was added and pause vortexed for 15 seconds. Thereafter, the mix was transferred into a column and centrifuged at 8000 xg for 60 seconds. The flow through with the collection tube was discarded and the column was transferred into a new collection tube. In addition, 500 μL of AW1 buffer was added to the column and spun at 8 000 X g for 60 seconds. The flow through was discarded and 500μL of AW2 buffer was added to the column and spun for 12 000 xg for 3 minutes. The column was transferred into a new microcentrifuge tube (Eppendorf) and DNA was eluted using 100μL of elution buffer. The DNA was quantified using the nanopore spectrometer (Jneid *et al.,* 2019).

Identification of *S. aureus* by PCR amplification of*tuf* and *nuc* genes

The extracted DNA were used in the amplification of the *Staphylococcus* elongation factor gene, *tuf,* (genus specific primer) and the thermonuclease gene, *nuc* (*S. aureus* specific primer) for the identification of the Gram-positive isolates. A duplex PCR was carried out in a 15 μL final volume reaction containing 7.5μL of mastermix, 1 μL for each of the *tuf* and *nuc* forward and reverse primers. 5μL of PCR grade water and 1μL of DNA. The reaction was carried out using the following PCR conditions: initial denaturation at 94℃ for 3 minutes followed by 30 cycles of denaturation at 94℃ for 30 seconds, annealing at 51℃ for 30 seconds and elongation at 72℃ for 60 seconds. This was followed by a final elongation at 72℃ for 7 minutes. *S. aureus* ATCC 43300 was used as a positive control (Jouhar *et al*., 2020).

Detection of Virulence Gene (Panton Valentine Leukocidin (PVL) of *S. aureus*

Furthermore, the Panton Valentine Leukocidin (PVL) gene of *S. aureus* was detected in the isolates by PCR amplification of the PVL gene. This was carried out in a 15 μL final reaction containing 7.5μL of mastermix, 1 μL each for the PVL forward and reverse primers, 4.5μL of PCR grade water and 1μL of the DNA template. The PCR reaction was carried out in a thermocycler using the following conditions: initial denaturation at 94℃ for 3 minutes followed by 30 cycles of denaturation at 94℃ for 30 seconds, annealing at 56℃ for 30 seconds and elongation at 72℃ for 45 seconds. Final elongation was carried out at 72℃ for 7 minutes. *S. aureus* USA 300 (control strain) was used as a positive control (Mukherjee *et al.,* 2011).

Detection of Antibiotic Resistance Gene (*mecA* gene) among the *Staphylococcus spp*

In addition, the antibiotic resistance genes encoding methicillin resistance (*mecA*) was tested for the isolates by PCR. The *mecA* gene was detected in a PCR reaction containing 7.5μL mastermix, 1μL each of the *mecA* forward and reverse primers (Table1), 4.5μL of PCR grade water and 1μL of the DNA template. The reaction was carried out in a cycler using the following conditions: initial denaturation at 94℃ for 3 minutes, 30 cycles of denaturation at 94℃ for 30 seconds, annealing at 55℃ for 30 seconds and elongation at 72℃ for 45 seconds. A final elongation at 72℃ for 7 minutes was also carried out, this was carried out using a 15 microlitre final reaction (Merza and Jubrael,2015).

Gel Electrophoresis of each PCR amplification products

Agarose (1.5%) was prepared using the agarose powder and tris borate EDTA (TBE) buffer. The agarose was homogenized in a microwave till clear and allowed to cool to about 50℃ before the incorporation of the intercalating agent (gel red) at 10μL in 100 mL of agarose. The agarose was poured in a casting tray having combs at appropriate positions and allowed to solidify. The combs were removed and the gel was placed in an electrophoresis chamber. Amplicons (7μL) were loaded into different wells and a 100 bp ladder (5μL) was loaded. The set-up was subjected to electrophoresis at 160 volt for 40 minutes. Thereafter, the gel was viewed in a gel documentation system (Shinkafi *et al.,* 2023).

Multiplex PCR Amplification of *bla*$_{\text{TEM}}$ and *bla*$_{\text{CTX-M}}$

Multiplex PCR was standardized to identify two different DNA targets (*bla*$_{\text{TEM}}$ and *bla*$_{\text{CTX-M}}$) within the bacterial isolates simultaneously. The extracted genomic DNA was used as the template in the PCR reaction. Primers used to detect *bla*$_{\text{TEM}}$ specific gene were 5’-3’. Accordingly, primers used were *bla*$_{\text{CTX-M}}$ 5’-3’. Final reaction volume of 20µl was prepared with 5 µl nuclease free water, 3 µl each of primers, 10 µl of premix (dNTP, Taq polymerase and 2 µl of PCR buffer containing 1.5 mM $MgCl_2$) and 2 µl DNA template. Amplification reactions were carried out in a thermocycler (PTC-100^{TM}, USA) under the following conditions: initial denaturation, 30 cycles of denaturation, annealing at and elongation at. The final elongation step was extended to 72°C for 1 min. The amplified products were separated in 1.5% agarose gel containing ethidium bromide (0.5 µg/ml). A 100 bp DNA ladder was used to measure the molecular weights of the amplified products. The images of ethidium bromide-stained DNA bands were visualized and documented using a gel documentation system (Mukherjee *et al.,* 2011).

Agarose Gel Electrophoresis of PCR Products

A mixture of 1.5g of agarose (Seakem, USA) and 100ml of 1X Tris-acaetate ethylene diaminetetra acete (TAE) buffer (Bio-Rad, USA) were used to make up a medium size of 1.5% agarose gel. The resulting mixture was heated for 3 minutes in a microwave oven to dissolve the agarose, it was allowed to cool to about 50°C at room temperature where 1µl/ml ethidium bromide was poured into a gel casting tray containing combs and allowed to solidify. After 30 min, the gel comb was carefully removed and the gel casting tray containing the gel was placed into a gel electrophoresis tank filled with TAE buffer (40mM Tris, 20 mM acetic acid and 100 mM EDTA pH 8). For each run, 5 µl DNA ladder was added to one of the wells to estimate the band sizes and 5 µl of negative control (Nuclease free water) was added to another well. The PCR products, 5 µl each, were carefully loaded into the remaining wells. An electric current of 80V; 400 mA (mini-Ampere) was run through the gel for 35 minutes and the gel was distained. The gel was then visualized and photographed using gel documentation system (FISHER BIOTECH FB 105, USA) (Gudjonsdottir, 2015; Shinkafi *et al.,* 2023).

Table 1: Primers employed for the detection of specific genes among *Staphylococcus* spp and *E. coli* in this study

Primers	Descriptions	Sequences	Amplicon Sizes	References
Tuf	*Staphylococcus* elongation factor gene	F: GGCCGTGTTGAACGTGGTCAAATCA R: TIACCATTTCAGTACCTTCTGGTAA	370 bp	Martineau *et al*., 2001
Nuc	Thermonucleasegene	F: GCGATTGATGGTGATACGGTT R: GCCAAGCCTTGACGAACTAAAGC	279 bp	Brakstad*et al.,* 1992
PVL	Panton Valentine Leukocidin gene	F: ATCATTAGGTAAAATGTCTGGACATGATCCA R: GCATCAASTGTATTGGATAGCAAAAGC	433 bp	Lina*et al.,* 1999
mecA	Methicillin resistance gene	F: AAAATCGATGGTAAAGGTTGGC R: AGTTCTGCAGTACCGGATTTGC	533 bp	Murakami *et al*., 1991
*Pac*C	Quonolones resistance gene	F: GACCTCGGTTTAGTTCACAGA R: CACACGCTGACGTGACCA	585 bp	Mamun*et al*., 2016
UidA		F:'-TGGTAATTACCGACGAAAACGGC- R: 5'-ACGCGTGGTTACAGTCTTGCG	168bp	Merza and Jubrael (2015)
bla_{TEM}	ESBL resistance gene	F:GTATCCGCTCATGAGACAATAACCCTG R:CCAATGCTTAATCAGTGAGGCACC	918bp	Merza and Jubrael (2015)
bla_{CTX}	ESBL resistance	F:CGCTTTGCGATGTGCAG R:ACCGCGATATCGTTGGT	550bp	Merza and Jubrael (2015)

RESULT

Out of one hundred and fifty (150) wound samples analyzed 75 from each hospital, both gram positive and negative were present, among which 22 were phenotypically confirmed to be *E. coli* and 22 *S. aureus* (Table 2).

Antibiograms of *S. aureus* and *E. coli* and from Diabetic Foot infection

Out of 22 isolates of *E. coli,* it showed 100% resistance to all antibiotic tested except impenem and gentamycin, while in *S. aureus* only 45% showed resistant to cefoxitin, all the 22(100%) *S. aureus* showed resistance to fluoroquinolones class, 27% showed resistance to gentamycin, 4% showed resistance to erytromycin, 9% showed resistance augmentin, while 100% showed sensitive to streptomycin (Table 3).

Molecular detection and amplification of virulence and antibiotics resistance genes in *S. aureus* from Diabetic Foot Ulcer from GHK and FTHK

The *tuf* and *nuc* genes are positive in most (83.3%) of the *S. aureus* isolates selected for PCR amplication, only one isolate (16.7%) is positive for *mec*A, indicating that it is methicillin-resistant. All isolates (0%) were negative for the *PVL* gene, one isolate (16%) was positive for *Par*C, indicating that this isolate may have acquired resistance to fluoroquinolones. All the (0%) isolates were negative for *qnr*A. (Table 5).

The *uid*A gene is positive in all (100%) the tested isolates, all (100%) of these isolates are positive for the bla_{TEM} gene, indicating that they all harbor TEM-type beta-lactamases, only two isolates are positive (50%) for the bla_{CTX} gene (Table 6).

Table 2: **Distribution of *S. aureus* and *E. coli* from diabetic wound of patients attending two selected hospital GHK and FTHK**

Hospital	Isolates	No. Positive	Morphology	Gram reaction	Ct	Coa	Prevalence (%) (n=75)
GHK	*E. coli*	12	Moist, round, and pale on blood agar and Green Metallic Sheen on EMB	-			16
	S. aureus	11	Golden yellow colonies on MSA and creamy white colonies blood agar	+	+	+	14.7
Total		23 (15.3%)					
FTHK	*E. coli*	10	Moist, round snd pale colonies on blood agar and Green Metallic Sheen on EMB	-			13.3
	S. aureus	11	Golden yellow colonies on MSA and creamy white colonies blood agar	+	+		14.7
Total	1 (14%)				T=150		

Keys: GHK=General Hospital Katsina, FTHK: Federal Teaching Hospital Katsina, +: Positive, -: Negative.

Table 3: Antibiogram of *S. aureus and E. coli* from Diabetic Foot Infection

S. aureus				***E. coli***			
Antibiotics (Potency)	**Resistance No (%)**	**Intermediate No (%)**	**Susceptible No (%)**	**Antibiotics (Potency)**	**Resistance No (%)**	**Intermediate No (%)**	**Susceptible No (%)**
FOX (10µg)	10 (45)	6 (27)	6 (27)	CAZ (30µg)	22 (100)	0 (0)	0 (0)
CPR (5µg)	21 (95)	0 (0)	1 (4)	CRX (30µg)	22 (100)	0 (0)	0 (0)
GEN (10µg)	6 (27)	0 (0)	15 (68)	GEN (10µg)	0 (0)	0 (0)	22 (100)
ERY (15µg)	1 (4)	4 (18)	17 (77)	CPR (5µg)	22 (100)	0 (0)	0 (0)
AUG (10µg)	2 (9)	0 (0)	20 (91)	OFL (5µg)	22 (100)	0 (0)	0 (0)
S (10µg)	0 (0)	13 (59)	9 (41)	AUG (10µg)	22 (100)	0 (0)	0 (0)
PEF (30µg)	22 (100)	0 (0)	0 (0)	IMP (10µg)	0 (0)	0 (0)	22 (100)
				AMP (15µg)	22 (100)	0 (0)	0 (0)
				ERY (15µg)	22 (100)	0(0)	0 (0)

KEYS: CAZ: Ceftazidine, CRX=Cefuroxime, GEN= Gentamycin, CPR= Ciprofloxacin, OFL= Ofloxacin, AUG= Augmentin, IMP= Imepenem, AMP= Ampicillin, FOX=Cefoxitin, ERY= Erytromycin, S=Streptomycin, PEF= Pefloxacin

Table 4: Antibiotic Resistance Phenotypes of *S. aureus* and *E. coli* from Diabetic Foot Ulcers

Bacteria	No of antibiotics	MDR Patterns	No of Isolates
S. aureus	1	PEF	1
	2	PEF, CPR	11
	3	PEF, CPR, FOX	3
	4	PEF, CPR, FOX, GEN	4
	5	PEF, CPR, FOX, GEN, ERY	1
	5	PEF, CPR, FOX, GEN, AUG	2
E. coli	7	CAZ, CRX, CPR, OFL, AUG, AMP, ERY	22

KEYS: CAZ= Betalactams, PEF= Fluoroquenolones, CPR= Fluoroquenolones, FOX= Betal ctams, GEN= Aminoglycoside, ERY= Macrolide, AUG= Betalactam, S= Aminoglycoside, CAZ= Betalactams, CRX= Betalactams, OFL= Fluoroquenolones, AMP= Penicillin

Table 5: Molecular detection of virulence and antibiotic resistance genes of *S.aureus* isolated from patients with Diabetic Foot Ulcers

Isolates ID	*Tuf*	*Nuc*	*mec A*	*PVL*	*qnrA*	*ParC*
3	+	+	-	-	-	-
20	+	+	-	-	-	-
21	+	+	+	-	-	+
44	+	+	-	-	-	-
46	-	-	-	-		
48	+	+	-	-		
%occurrence	5 (83.3)	5 (83.3)	1(16.7)	0 (0)	0 (0)	1 (16)

Keys: +: Indicates presence of resistance genes -: Indicates absence of resistance genes.

Molecular detection of antibiotics resistance genes in *E. coli*

Table 6: Molecular detection of antibiotics resistance genes in the *E. coli* isolates

Isolates ID	*UidA*	*bla*$_{TEM}$	*bla*$_{CTX}$
1	+	+	-
2	+	+	-
3	+	+	+
4	+	+	+
% occurrence	4 (100)	4 (100)	2 (50)

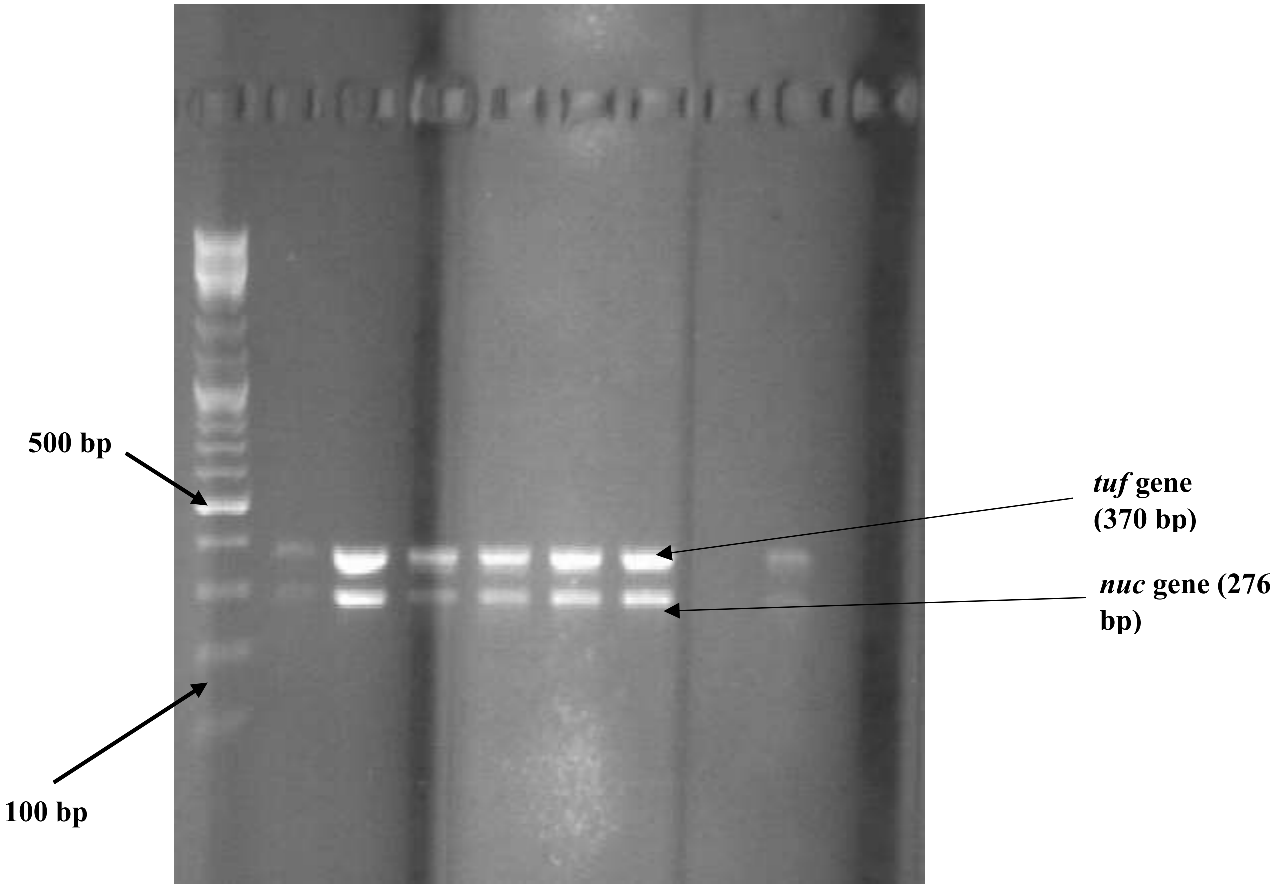

Plate 1: *tuf* and *nuc* housekeeping genes detected in *S. aureus*

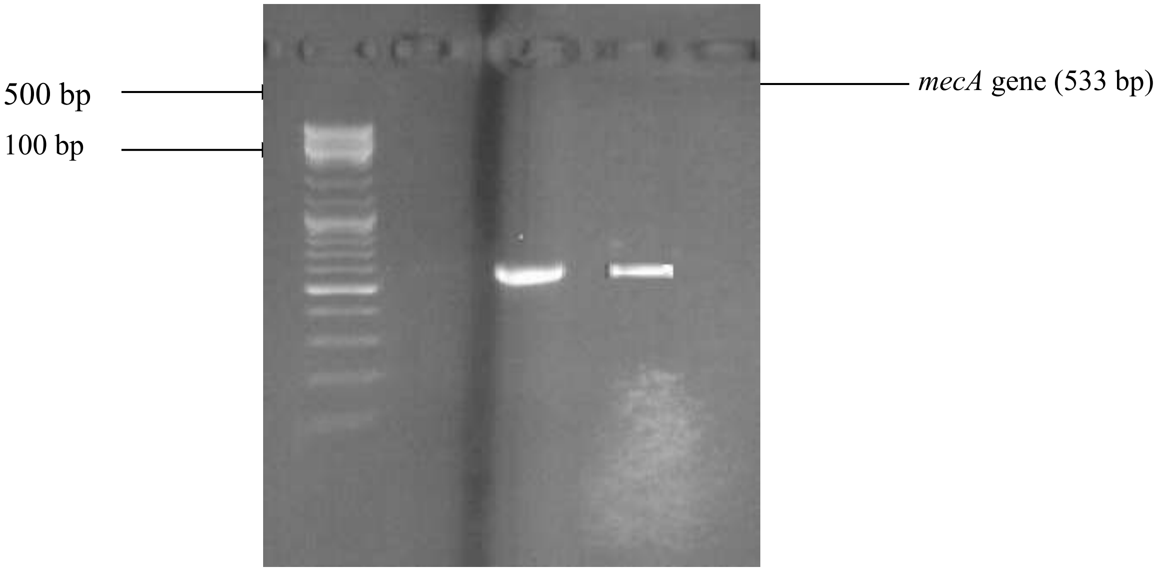

Plate 2: *mecA* gene detected in *S. aureus*

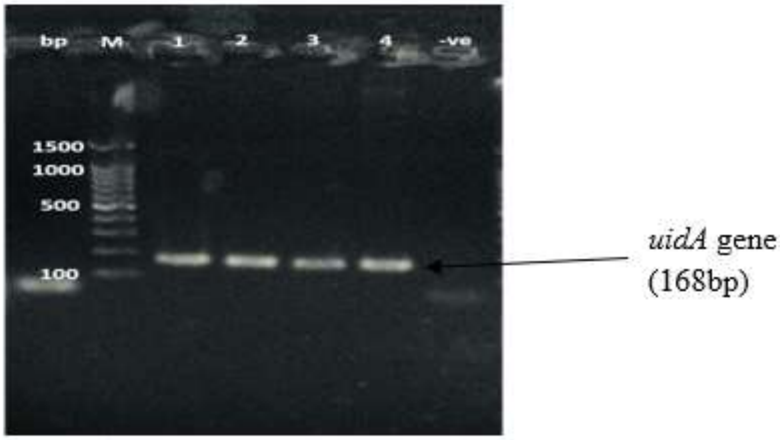

Plate 3: *uidA* gene detected in the *E. coli* isolates

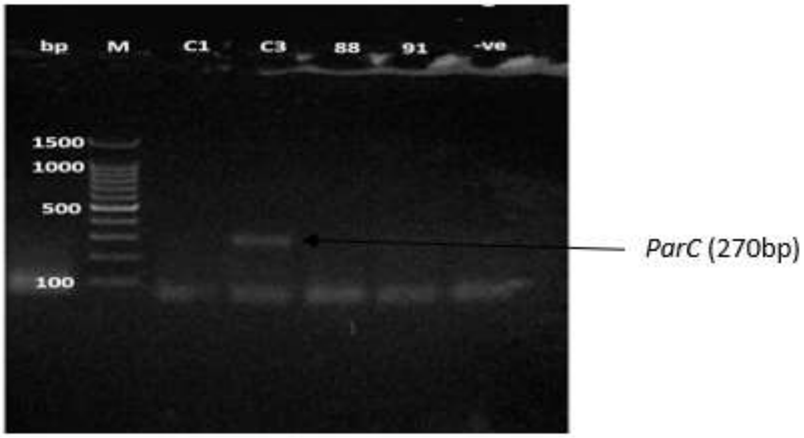

Plate 4: *parC* gene detected in the *S. aureus* isolates

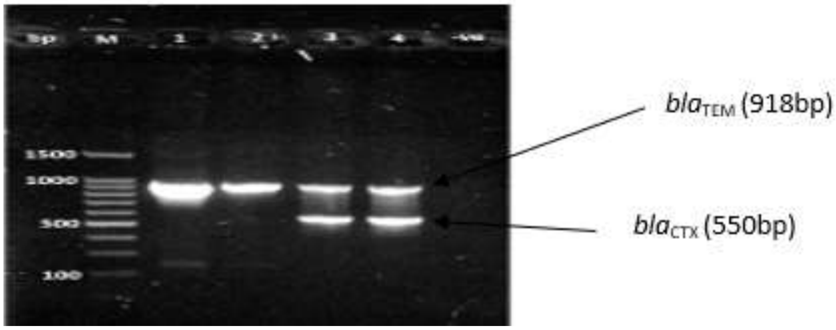

Plate 5: *bla*$_{\text{TEM}}$ and *bla*$_{\text{CTX}}$ genes detected in the *E. coli* isolates

DISCUSSION

This study investigated antibiotic resistance genes associated with *S. aureus* and *E. coli* from patients with DFI attending GHK and FTHK with a view to provide baseline data for strategic mitigation of MDR strains of *S. aureus* and *E. coli* hindering accelerated healing of DFU. The identification of *E. coli* as a Gram-negative bacterium, along with its association with wound infections in diabetic patients, is consistent with its role as a prominent pathogen in wound colonization (Savoia, 2012). Furthermore, *S. aureus* continues to be a major threat in diabetic wound infections due to its ability to produce virulence factors that facilitate its persistence in the wound environment. Akinyemi *et al*. (2020) found that both *E. coli* and *S. aureus* were frequently isolated from diabetic foot ulcers, with *E. coli* being the most (75%) prevalent Gram-negative pathogen; while *S. aureus* was the most (87%) predominant Gram-positive pathogen, responsible for the persistence of infection due to their virulence factors such as biofilm formation and resistance to common antibiotics. Udo *et al.* (2019) in Southern Nigeria further confirmed the association of *E. coli* and *S. aureus* with diabetic foot infections. This study demonstrated that *S. aureus* had a higher incidence in patients with chronic wounds, while *E. coli* was more prevalent acute infections of diabetic wound. Contrary to the findings in this study, Okunade, (2020) revealed that *S. aureus* was responsible for a higher rate of DFI followed by *Pseudomonas aeruginosa.*

Resistance of *E. coli* (100%) in this study to a range of antibiotics, including ceftazidime, cefuroxime, ciprofloxacin, ofloxacin, augmentin, and, ampicillin demonstrated extensive resistance that is consistent with global concerns regarding *E. coli* resistance to commonly used antibiotics.

Similar to the study reported by Livermore, (2008), Olayinka *et al.* (2020) found that *E. coli* strains from wound of diabetic patients in Nigeria exhibited 100% resistance to ampicillin and high resistance to cephalosporins, with a significant proportion of these isolates producing ESBLs. In a more recent study by Akinmoladun *et al*. (2023), reported similar resistance with a high prevalence of ESBL production among *E. coli* isolates from both hospital and community-acquired infections in Lagos, Nigeria. Moreover, it was further noted by Akinmoladun *et al*. (2023) that ESBL-producing *E. coli* strains were resistant to multiple classes of antibiotics, including cefuroxime, ciprofloxacin, and augmenting, highlighting the challenges in treating such infections effectively in Nigerian healthcare settings.

Fluoroquinolones, once considered first-line agents for *E. coli* infections, are becoming increasingly ineffective due to mutations in the DNA gyrase and topoisomerase IV genes (Kahlmeter, 2003). Similar resistance patterns were observed (Okon *et al.*, 2020), where over 90% of *E. coli* isolates from both hospital and community-acquired infections were resistant to Ciprofloxacin. These differences may be due to regional variation and specific healthcare settings. However, carbapenems, particularly Imipenem, continue to show strong efficacy, with studies consistently reporting 95–100% susceptibility rates (Okon *et al*., 2023; Olayinka *et al*., 2021). In Nigeria, *Escherichia coli* isolates have demonstrated high susceptibility to gentamycin, with reports indicating 100% susceptibility in several studies, which is a promising finding for the treatment of infections, particularly when used in combination therapy (Adebayo *et al*., 2022). In the case of *S. aureus*, the findings showed 45% resistance to cefoxitin, an indicator of methicillin resistance in *S. aureus* (MRSA). The presence of intermediate resistance (27%) further supports the likelihood of MRSA in the hospital environment, as observed in other studies (Chung *et al*., 2016). These findings are consistent with trends observed in other parts of Africa, where the prevalence of MRSA has been rising, posing significant challenges to infection control and management (Sule *et al*., 2018).

The high resistance to ciprofloxacin (95%) observed in this study is consistent with findings from other healthcare settings, where fluoroquinolone resistance in *S. aureus* has been increasingly reported (Patel *et al*., 2006). All the isolates were found devoid of PVL gene, indicating that they may not possess this particular virulence factor. The absence of the PVL gene in the isolates could indicate that these are not community-associated virulent strains, which are often more aggressive. This finding is similar to Adeyemo (2021) who found that none of the MRSA isolates from Nigerian hospitals carried the PVL gene, suggesting that these strains may lack the aggressive virulence associated with PVL-positive MRSA strains. The absence of the *qnr*A gene in all the isolates suggests that these strains may still be susceptible to quinolones like ciprofloxacin and ofloxacin, though the absence of *qnr*A does not preclude other mechanisms of fluoroquinolone resistance, such as mutations in the *gyr*A or *par*C genes (Hooper, 2000). Presence of *par*C in one of the *S. aureus* isolates indicated that this isolate may have acquired resistance to fluoroquinolones through mutations in the *par*C gene, further corroborating the potential for reduced quinolone susceptibility.

CONCLUSION

Staphyloccus aureus and *E. coli* lacking of PVL genes were detected as prominent pathogens associated with DFI in diabetic patients attending GHK and FTHK. Whereas, occurrence of bla_{TEM} and $blac_{\text{CTX}}$ resistance genes in these pathogens informed threats to accelerated healing of the diabetic foot ulcer among the patients and higher chance of MDR phenomena with frequent resistance to commonly used beta-lactam and fluoroquinolones antibiotics.

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