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Prevalence of Multidrug Resistance among Staphylococcus Species Isolated from Clinical Samples

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AAA and JAA design the concept. Author CEU collected the samples, carried out the laboratory works and gathered the data. Authors AAA and JAA oversaw the laboratory works. Authors CEU prepared the draft manuscript. Author JAA carried out the statistical analysis. Authors AAA and JAA finalized the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: One major thing that promotes antimicrobial resistance among bacteria is their potential to produce enzymes and biofilms, which remain noteworthy elements in their pathogenicity. This study aimed to determine the prevalence of multidrug resistance *Staphylococcus* species isolated from different clinical samples.
Study Design: The study employed independent measures experimental study design.

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Place and Duration of Study: *Staphylococcus* species originating from clinical samples were obtained from the microbiology laboratory of two teaching hospitals in Ogbomosho, Oyo State, between April and October, 2023.
Methodology: Twenty-six (26) pre-identified (by the hospital Laboratories) *Staphylococcus* spp. were obtained from the microbiology laboratories of two teaching hospitals in Ogbomoso, Nigeria. The isolates were subjected to microscopic, and biochemical tests to confirm their identities. The testing for antibacterial susceptibility was carried out using the Kirky-Bauer disk diffusion technique. A modified crystal violet biofilm assay was used to determined the ability of the isolates to produce biofilm. Molecular characterization was carried out to identify bacteria with very high resistance to the used antibiotics using 16S rRNA. The isolates were subjected to enzyme production assay like pectinase, protease, keratinase, cellulose and collagenase assay.
Results: All the *Staphylococcus* species in this study showed varied degrees of prevalence. All the clinical bacterial isolates also showed 100% resistance to Amoxicillin/Clavulanate, Cefuroxime, Cloxicillin Meropenem, and Doxycycline, while the slightest resistance was observed for Gentamicin with 29.17%. The multiple antibiotic resistance index (MARI) for all the isolates was between 0.3 to 1.0, which is higher than the safe limit of 0.2, with a high percent (95.8%) of the bacteria being MAR. The result shows that 77.8% of these isolates could produce one or more enzymes.
Conclusion: The biofilm and enzyme production abilities of the clinical bacteria were major factors that led to expanded resistance, as observed in this study.

Keywords: Staphylococcus; multidrug resistance; clinical samples; AMR; biofilm.

1. INTRODUCTION

Resistance to multiple antimicrobials is referred to as multidrug resistance (MDR), and various studies have shown that there are different resistance mechanisms in these bacteria, while their distribution and interaction are primarily complex and unknown. Resistance among bacteria can occur naturally through genetic mutation or if one species acquires resistance from another. However, extended use of antimicrobials encourages mutation selection, rendering antimicrobials ineffective (Amenu, 2014). Antimicrobial resistance is induced by the overuse of antimicrobials, thus leading to microorganisms evolving a defense against drugs or certain strains of microbes developing a natural resistance to antimicrobials, which become prevailing than the ones that are easy to defeat with medication (Jiregna & Nesrie, 2017).

Bacteria have the ability to acquire and transmit resistance to antimicrobial agents. Following the massive use of antibiotics in human treatments, bacteria have evolved several resistance mechanisms, including expression of the efflux pumps, target site modification, and metabolic inactivation, which contribute to drug resistance in multidrug-resistant bacteria (Shaik et al*.,* 2014). The role of coagulase-negative Staphylococci in causing antibiotic resistance infections has been shown in recent reports. Presently, some relevant clinical species such as *Staphylococcus epidermidis*, *Staphylococcus heamolyticus*, *Staphylococcus saprophyticus* and *Staphylococcus lugdunensis* have been reported (Franca et al., 2021).

Staphylococcus aureus, a coagulase-positive bacterium, is known to cause many infections, even life-threatening diseases like bacteremia, endocarditis and necrotizing pneumonia. This is because of its virulence, which enables it to escape the immune system and cause severe harm to the host (Mahmood et al., 2021). Some *Staphylococcus* species live in normal microflora on human skin and mucous membranes, where they are associated with wound and urinary tract infections. Infectious diseases remain the leading mortality and morbidity cause around the world, especially in developing countries.

Biofilm production plays a vital role in the virulence of *Staphylococcus species* by allowing the cells to persist in the human body and evade the host immune defense system (Szczuka et al., 2016). Some *Staphylococcus* species form biofilms on or around medical equipment, such as central venous catheters and prosthetic heart valves (Giampero et al., 2022). Biofilm protects bacteria from the effect of antibiotics; low metabolic activity of bacteria in biofilm lowers the uptake of antibiotics, making them develop a high level of antibiotic resistance and increased pathogenicity. *Staphylococcus* species produce enzymes such as cellulases, pectinases, protease, collagenase, and keratinase, which, once on the surface, interact with host

components like fibronectin and plasminogen to trigger signal transduction and thereby enable the pathogens to colonize, persist and invade the host tissue (Berne et al*.,* 2015).

Enzymes facilitate the penetration spread of the pathogen in the host and cause the collapse and disintegration of the cellular structure, thereby aiding the pathogen in the production of disease (Pirvanescu et al*.,* 2014). These enzymes convert fibrinogen into fibrin, which forms the threads of a blood clot and contributes to its pathogenicity (John et al*.*, 2018). Lipases are crucial in lipid metabolism, which includes digestion, transport, and the processing of dietary lipids. Lipase activity is required for the colonization and persistence of bacterial pathogens like *Staphylococcus epidermidis* on human skin, the lipases of *S. aureus* have been shown to interfere with the host cell immune response (Jaeger et al., 1994). Collagenase breaks down the peptide bond in collagen and assists in destroying extracellular structures, thereby encouraging the spread of infection and pathogenesis (Gerald et al*.,* 2007). This study aimed to find out the predominance of multidrug resistance *Staphylococcus* species isolated from clinical samples.

2. MATERIALS AND METHODS

2.1 Isolates

Staphylococcus species of clinical samples origin were obtained from the microbiology laboratory of two teaching hospitals in Ogbomosho, Oyo State, between April and October, 2023. The clinical origins of the isolates were of diverse sites, as reported by the laboratory. The bacteria were obtained as pure culture and were already identified by the hospital microbiology laboratories. These were collected on sterile nutrient agar slants and then transported to the microbiology laboratory of the Department of Pure and Applied Biology, LAUTECH, Ogbomoso, for further study**.**

2.2 Identification of the Clinical Isolates

The obtained bacterial isolates were earlier identified by the source hospital microbiology laboratory, but they were subjected to microscopic and some biochemical characteristics according to standard conventional procedures (Bergey's Manual, 2000), to ascertain their identity. These include microscopic, colonial and biochemical tests.

2.3 Antibiotic Susceptibility Testing

The antimicrobial susceptibility test of the bacteria was determined using the Kirby-Bauer disk diffusion method on Muller Hinton Agar (MHA) plates (Patel et al*.,* 2017). Standardized inoculums were swabbed on the prepared MHA plates, and antibiotic disks were aseptically placed on the swabbed plates. Antibiotics used (CM-12-8PR100, product of Rapid Labs, UK) include Ceftazidime (30µg), Cefuroxime (30µg), Ceftriaxone (30µg), Gentamicin (10µg), Erythromycin (5µg), Cloxacillin (5µg), Ofloxacin (5µg), and Amoxicillin/Clavulanate (30µg). Bacterial isolates that showed 75% to the first eight (8) antibiotics used were further subjected to another four antibiotics namely; Meropenem (10µg), Doxycycline (30µg), Imipenem (10µg), Levofloxacin (5µg), products of BIO RAD (California, USA). The plates were incubated at 37°C overnight. Zones of inhibition were measured to determine susceptibility patterns, and results were compared with the Clinical and Laboratory Standard Institute (CLSI, 2018).

The multiple antibiotic resistance index (MARI) was calculated as:

$$\text{MAR Index} = \frac{a}{b} \quad \text{(i)}$$

Where a is the number of antibiotics an isolate is resistant to, and b is the total number of antibiotics used in the study.

2.4 Biofilm Production Ability of the Bacterial Isolates

The bacterial isolates were assessed on their ability to produce biofilm, using a modified crystal violet assay according to a method described by Shukla et al*.* (2017). Bacteria were grown in nutrient broth (NB) overnight and diluted at a ratio 1:10 with fresh sterile NB in microtiter plates, then incubated for 48 hours at 37^{o}C (Amao et al., 2019). The microtiter plates were turned over, washed and then, 0.01% of crystal violet solution was introduced. The plates were washed and vigorously blotted after 15 minutes incubation and then allowed to dry overnight. The quantification of the formed biofilms was performed at 492 nm on a HALO MPR-96 visible microplate reader after adding 125µL of 30% acetic acid solution, followed by incubation at room temperature for 15 mins. Results were interpreted as weak, moderate, and strong biofilm formers groups, according to Singh (2017).

2.5 Molecular Characterization of Isolates

Molecular characterization based on 16S rRNA was carried out on the isolates that showed greater than 75% resistance to the second set of antibiotics used, with the primer pair 27F (AGAGTTTGATCMTGGCTCAG) and 1525R (AAGGAGGTGWTCCARCCGCA). Assembled nucleotide sequences were analyzed for similarities on the National Centre for Biotechnology Information site (NCBI) using the BLASTN tool. The information was used for phylogenetic analysis on MEGA X (USA), and the data were submitted to the NCBI data bank.

2.6 Determination of Enzyme Production Abilities of Bacterial Isolates

2.6.1 Pectinase activity assay

Assay for pectinase activity was carried out for the bacteria using the method of Kavuthodi et al. (2015). Clearance zone on agar plates were determined and recorded as positive results.

2.6.2 Protease activity assay

Casein agar medium was prepared as described by Larone (1993). The bacteria isolates were inoculated on casein agar plates and incubated overnight at 37^{o} C. A clear zone indicates a positive test result.

2.6.3 Keratinase activity assay

The assay for keratinase was carried out according to the method described by Alwakeel et al. (2021). The halo zones were measured and recorded as an indicator of keratinase activity, with the presence of a zone confirming a positive result.

2.6.4 Cellulase activity assay

This assay was done to determine the ability of bacteria isolates to break down cellulose, employing the method of Miller (1959). The halo zones were measured and recorded as an indicator of keratinase activity, with the presence of a zone confirming a positive result.

2.6.5 Collagenase assay

Production medium for collagenase contained in 500 ml distilled water includes gelatin- 10 g: NaCl-0.05 g, H2PO4; 0.25 g, Mg SO4- 0.1 g, Peptone- 2.5 g, Agar- 8 g. The production medium was sterilized, and 20 ml of each was dispensed into the petri dish and allowed to solidify. Overnight pure culture of 0.5 ml was inoculated into the collagenase medium and incubated at 37 ^{o}C; the clearance zone was taken as a positive test. The wider the zone, the higher the potential to produce collagenase enzyme.

2.7 Statistical Analyses

Data analyses were based on the average of three replicates from independent studies. Statistical analyses of these averages were analyzed using One-way analysis of variance (ANOVA) in SPSS version 20 software at a 95% significance level.

3. RESULTS AND DISCUSSION

The confirmatory test for collected bacterial isolates showed that 24 (92%) of the 26 isolates were Gram positive as reported by the hospital laboratories, while 2 (8%) showed different Gram reaction status (Table 1). Table 2 shows the results of antibacterial susceptibility testing of bacterial isolates to selected antibiotics. *Staphylococcus* species resisted Amoxicillin/ Clavulanate, Cloxacillin, Cefuroxime (100% resistance), and Ceftazidime (92%). In this study, the vast majority of the bacterial isolates showed a multidrug resistance pattern, which is similar to the study conducted by Ehssan et al. (2022), who reported 89.2% resistance. Omaba et al. (2021) reported that Ofloxacin and Gentamicin displayed high percentage of sensitivity against the *Staphylococus* isolates in their study. Our result was different from that of Ong'era et al. (2023) and Onyeka et al. (2021), who also reported 100% resistance among *Staphylococcus* species to Ceftazidime. The differences in resistance profile may be due to differences in infection epidemiology, prescription patterns, and the population's socio-demographic features (Kim et al., 2015).

All the bacterial isolates were most sensitive to Gentamicin (70%) and least sensitive to Ofloxacin (54%). The antibiotic susceptibility profile results for the bacteria showed >75% resistance, as represented in Table 3. Many studies have shown that Meropenem has greater efficacy than Imipenem (Ahmed Hunjra et al., 2022). However, other researchers have shown different findings with this antibiotics, a resistance of 62.5% was reported from Nepal (Parajuli et al., 2017), 79.3% from Vietnam (Tran

et al., 2017), and 69.68% from Mexico (Andres et al., 2019). These bacterial isolates have a high resistance rate to the standard antibiotics used singly or combined.

The findings on Imipenem and Meropenem are disturbing since they are the last line of drugs used in the treatment of infections caused by multidrug-resistant bacteria. Imipenem-resistant strains are always resistant to other antimicrobial drugs, and the outcome of their resistance appears worse in the area of mortality and morbidity (Reissier et al., 2023, WARNING, 2023). The total resistance of the *Staphylococcus* species was observed for Meropenem and Doxycycline, followed by imipenem (94%) and Levofloxacin (6 3%). Table 4 summarizes the sensitivity of the bacterial isolates to different antibiotics. Fig. 1 shows the resistance pattern of the *Staphylococcus* species to the different antibiotics used in this study; 100% resistance was observed for Amoxicillin/Clavulanate, Cloxacillin, Cefuroxime, Meropenem, and Doxycycline. Most bacteria were resistant to four or five different antibiotics, proving them to be multidrug-resistant (Table 5). The multiple antibiotic resistance index (MARI) for all bacteria ranges from 0.3 to 1.0, higher than the acceptable limit of 0.2 (Table 5). The antibiotic resistance index for all the bacterial isolates is high, ranging from 0.3 to 1.0 and higher than the recommended safe limit of 0.2. Ibanga et al. (2019) reported that the MAR indices of their study showed that 85.7% of the isolates had confirmed multi-drug resistance status, with 60.7% of the isolates showing resistance to between four or more of the tested antimicrobials. The reason for resistance to these carbapenems may result from carbapenemase enzymes, which are clinically important because of their ability to hydrolyze all or most of the beta-lactam drugs.

Table 1. Biochemical Tests of all obtained Staphylococcus Isolates

Isolate	Gram Reaction	Catalase Test	Oxidase Test	Coagulase Test	Haemolysis Test	Gas Prd Test	Nitrate Test	Glucose Prd Test
A1	+	+	-	-	-	+	+	+
A2	+	+	-	+	+	-	+	+
A3	+	+	-	-	+	+	+	+
A4	+	+	-	-	-	+	+	+
A5	+	+	-	-	-	+	+	+
B3	+	+	-	+	-	-	+	+
B5	+	+	-	-	+	+	+	+
B9	+	+	-	-	-	+	+	+
B12	+	+	-	+	+	+	+	+
B14	+	+	-	-	+	+	+	+
C2	+	+	-	-	+	+	-	+
C3	+	+	-	-	+	+	-	+
C4	+	+	-	-	+	+	-	+
C5	+	+	-	-	-	-	-	+
C8	+	+	-	-	-	+	+	+
C10	+	+	-	+	-	+	+	+
C11	+	+	-	-	+	+	+	+
C16	+	+	-	-	+	+	+	+
A14	+	+	-	-	+	+	+	+
A15	+	+	-	-	+	+	+	+
A16	+	+	-	-	+	+	+	+
B16	+	+	-	-	+	+	+	+
B17	+	+	-	-	+	+	-	+
A13	+	+	-	-	+	+	+	+
C6	-	+	-	-	-	-	+	+
C7	-	+	-	-	+	-	+	-

Key: Positive (+); Negative (-)

Table 2. Antibiotic Susceptibility Test *Staphylococcus* Isolates

Isolate	AUG	CAZ	CRX	GEN	CTR	OFL	ERY	CXC	% Resistance
B9	R	R	R	S	R	R	R	R	87.5
C10	R	R	R	R	R	S	R	R	87.5
C2	R	R	R	S	R	R	R	R	87.5
B16	R	R	R	R	R	R	R	R	100
B14	R	R	R	S	R	R	R	R	87.5
C11	R	R	R	S	R	S	R	R	75
A15	R	I	R	S	S	R	R	R	62.5
A5	R	R	R	S	R	S	I	R	62.5
B17	R	R	R	S	R	S	I	R	62.5
C4	R	R	R	S	S	S	R	R	62.5
B3	R	I	R	R	S	R	R	R	75
A1	R	R	R	S	R	I	I	R	62.5
C16	R	R	R	S	R	R	R	R	87.5
A2	R	R	R	S	R	S	R	R	75
A3	R	R	R	S	R	S	R	R	75
A14	R	R	R	S	R	S	I	R	62.5
B5	R	R	R	S	R	S	R	R	75
A4	R	R	R	S	R	S	S	R	62 5
C5	R	R	R	R	R	R	R	R	100
C8	R	R	R	R	R	R	R	R	100
B12	R	R	R	S	R	R	R	R	87.5
A16	R	R	R	R	R	R	R	R	100
A13	R	R	R	S	R	S	S	R	62.5
C3	R	R	R	R	R	S	R	R	87.5
% of Resistance	100	91.67	100	29.17	87.5	45.83	75	100	

KEYS: AUG: Amoxicillin/ Clavulanate (30µg), CAZ: Ceftazidime (30µg); CRX:Cefuroxime (30µg); GEN: Gentamicin (10µg); CTR : Ceftriazone (30µg); OFL: Ofloxacin (5µg); ERY:Erythromycin (5µg); CXC: Cloxicillin (5µg) R:Resistance; S: Sensitive; I:Intermediate

Table 3. Antibiotic Susceptibility Test for *Staphylococcus* Isolates with Resistance >75%

Isolate	MERO	DOXY	IMI	LEVO	% Resistance
B14	R	R	R	R	100
B16	R	R	I	R	75
B9	R	R	R	R	100
CI6	R	R	R	R	100
B5	R	R	R	S	75
B3	R	R	R	R	100
A3	R	R	R	R	100
C1	R	R	R	S	75
C10	R	R	R	R	100
C5	R	R	R	R	100
C2	R	R	R	S	75
A16	R	R	R	S	75
B12	R	R	R	R	100
A2	R	R	R	R	100
C3	R	R	R	S	75
C8	R	R	R	S	75

Key: MERO: Meropenem (10µg); DOXY: Doxycycline (30µg); IMI: Imipenem (10µg); LEVO: Levofloxacin (5µg); R: Resistance; S: Sensitive; I: Intermediate

Table 4. Summary of the Sensitivity Pattern of *Staphylococcus* Isolates to different Antibiotics

Antibiotics	Sensitive	Intermediate	Resistance
Ceftazidime	0	2 (8%)	22 (92%)
Cefuroxime	0	0	24 (100%)
Gentamicin	17 (71%)	0	7 (29%)
Ceftirazone	3 (12%)	0	21 (88%)
Erythromycin	2 (8%)	4 (17%)	18 (75%)
Cloxicillin	0	0	24 (100%)
Ofloxacin	12 (50%)	1 (4%)	11 (46%)
Amoxicillin/ Clavulanate	0	0	24 (100%)
Meropenem	0	0	16 (100%)
Doxycycline	0	0	16 (100%)
Imipenem	0	1 (6%)	15 (94%)
Levofloxacin	6 (37%)	0	10 (63%)

Table 5. Antibiotypes of selected antibiotics on *Staphylococcus* isolates and their multiple antibiotic resistance index

Isolate	Antibiotypes	MARI	Resistance Status
A1	Aug-Caz-Crx-Ctr-Ery-Cxc	0.5	MDR
A2	Aug-Caz-Crx-Ctr-Ery-Cxc-Mero-Doxy-Imi-levo	0.8	MDR
A3	Aug-Caz-Crx-Ctr-Ery-Cxc Mero-Doxy-Imi-levo	0.8	MDR
A4	Aug-Caz-Crx-Ctr-Ery-Cxc	0.5	MDR
A5	Aug-Caz-Crx-Ctr-Ery-Cxc	0.5	MDR
A14	Aug-Caz-Crx-Ctr-Cxc	0.4	MDR
A13	Aug-Caz-Crx-Ctr-Cxc	0.4	MDR
A15	Aug-Crx-Ctr-Ery-Cxc	0.4	MDR
A16	Aug-Caz-Crx-Gen-Ctr-Ofl-Ery-Cxc-Mero-Doxy-levo	0.9	MDR
B3	Aug-Caz-Crx-Ctr-Ery-Cxc- Mero-Doxy-Imi-levo	0.8	MDR
B5	Aug-Caz-Crx-Ctr-Ery-Cxc- Mero-Doxy-Imi-levo	0.8	MDR
B9	Aug-Cax-Crx-Ctr-Ofl-Ery-Cxc-Mero-Doxy-Levo	0.8	MDR
B12	Aug-Caz-Crx-Ctr-Ery-Cxc- Mero-Doxy-Imi-levo	0.8	MDR
B14	Aug-Caz-Crx-Ctr-Ery-Cxc- Mero-Doxy-Imi-levo	0.8	MDR
B16	Aug-Caz-Crx-Gen-Ctr-Ofl-Ery-Cxc-Mero-Doxy-levo	0.9	MDR
B17	Aug-Caz-Crx-Ery-Cxc	0.4	MDR
C2	Aug-Caz-Crx-Ctr-Ery-Cxc- Mero-Doxy-Imi-levo	0.8	MDR
C3	Aug-Caz-Crx-Ctr-Ery-Cxc- Mero-Doxy-Imi-levo	0.8	MDR
C4	Aug-Caz-Crx-Ctr-Ery-Cxc	0.5	MDR
C5	Aug-Caz-Crx-Gen-Ctr-Ofl-Ery-Cxc- Mero-Doxy-Imi-levo	1.0	MDR
C8	Aug-Caz-Crx-Ctr-Ery-Cxc	0.5	MDR
C10	Aug-Caz-Crx-Ctr-Ery-Cxc Mero-Doxy-Imi-Levo	0.8	MDR
C11	Aug-Caz-Crx-Ctr-Ery-Cxc- Mero-Doxy-Imi-Levo	0.8	MDR
C16	Aug-Caz-Crx-Ctr-Ery-Cxc-Mero-Doxy-Imi-Levo	0.8	MDR

KEYS: AUG: Amoxicillin/Clavulanate (30 µg); CAZ: Ceftazidime (30 µg); CRX: Cefuroxime (30 µg); GEN: Gentamicin (10µg); CTR: Ceftriazone (30µg); OFL: Ofloxacin (5 µg); ERY: Erythromycin (5 µg); CXC: Cloxicillin (5 µg); MERO: Meropenem (10 µg); DOXY: Doxycycline (30 µg); IMI: Imipenem (10 µg); LEVO: Levofloxacin (5 µg); AMP: Ampicillin (10 µg), NIT: Ntrofurantoin(30 µg); CPR: Cprofloxacin (5µg); MARI: Multi-Antibiotic Resistance Index, MDR: Multidrug resistant.

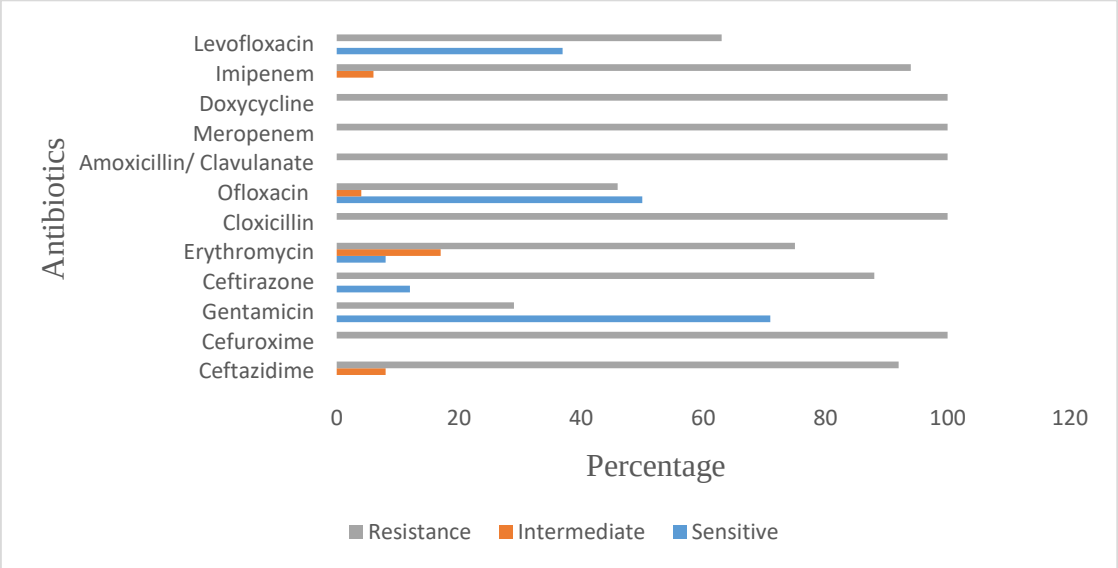

Fig. 1. Percentage of the Resistance Pattern of *Staphylococcus* Isolates to different Antibiotics

Table 6. Biofilm Production ability of Isolated Bacteria

Isolate	Mean	Biofilm Former Group
C11	0.064±0.01	Non biofilm producer
C16	0.061±0.11	Non biofilm producer
A15	0.061±0.03	Non biofilm producer
B3	0.061±0.01	Non biofilm producer
B5	0.060±0.01	Non biofilm producer
C4	0.070±0.01	Non biofilm producer
A3	0.081±0.04	Weak biofilm former
A4	0.094±0.02	Weak biofilm former
B12	0.097±0.03	Weak biofilm former
B14	0.078±0.04	Weak biofilm former
C3	0.081±0.02	Weak biofilm former
A1	0.119±0.03	Moderate biofilm former
A2	0.123±0.01	Moderate biofilm former
A5	0.103±0.04	Moderate biofilm former
A13	0.126±0.02	Moderate biofilm former
A14	0.120±0.01	Moderate biofilm former
A16	0.151±0.03	Moderate biofilm former
B9	0.117±0.01	Moderate biofilm former
B16	0.119±0.04	Moderate biofilm former
B17	0.103±0.04	Moderate biofilm former
C2	0.121±0.03	Moderate biofilm former
C5	0.119±0.02	Moderate biofilm former
C7	0.117±0.02	Moderate biofilm former
C10	0.119±0.02	Moderate biofilm former

Key: Value = mean ± Standard deviation.

The isolates' ability to produce biofilm is presented in Table 6. Thirteen (13) isolates (54.2%) were moderate biofilm producers, five isolates (20.8%) were weak biofilm formers, and six isolates, representing 25%, were non-biofilm former. The production of biofilms by (75%) of these bacteria might have aided their multiple antibiotic tolerance mechanisms like impermeability, rapid growth and influence drug resistance (Liu et al., 2024). Biofilm production supports gene transfer among microorganisms through their various connection

channels, thereby increasing antibiotic resistance (Bowler et al., 2020). The mutation also facilitates adjacent microcolonies in a biofilm, taking up free DNA and making the biofilms more antibiotic-resistant (Usui et al., 2023). Bacteria in biofilms produce potential virulence factors to prolong infections to a more chronic disease state (Cohen et al., 2022) by suppressing immune responses.

Table 7. Selected *Staphylococcus* Isolates with their Accession Number

Isolate Code	Isolate Identity	Accession Number
B14	*Staphylococcus haemolyticus*	OR367738
C16	*Staphylococcus xylosus*	OR367735
A3	*Staphylococcus epidermidis*	OR367734
C10	*Staphylococcus aureus*	OR367732
C5	*Staphylococcus warneri*	OR367730
B9	*Staphylococcus aureus*	OR367729
B12	*Staphylococcus haemolyticus*	OR367728
A2	*Staphylococcus aureus*	OR367727
B3	*Staphylococcus aureus*	OR367726
C2	*Staphylococcus aureus*	PQ643445

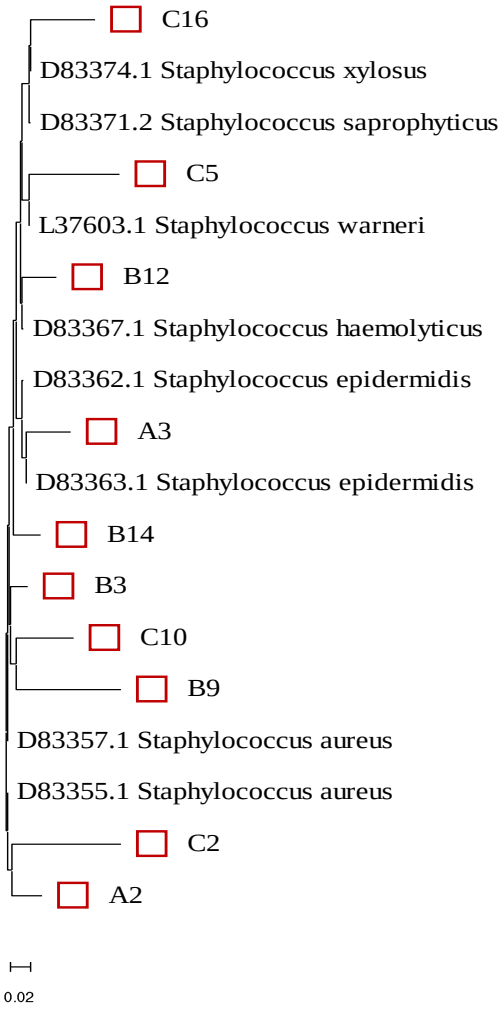

Fig. 2. Phylogenetic relationship of selected *Staphylococcus* isolates

Table 8. Enzyme Assay for Selected Bacteria Isolates

Isolate Code	Accession Number	Pectinase	Protease	Keratinase	Cellulase	Collagenase
A2	OR367727	+	-	-	+	-
B9	OR367729	-	-	-	-	+
B12	OR367728	-	-	-	-	-
C10	OR367732	+	-	+	-	-
B3	OR367726	-	-	-	+	+
C16	OR367735	+	-	-	+	+
C5	OR367730	-	-	+	-	-
B14	OR367738	-	-	-	-	-
A3	OR367734	+	-	+	+	-
C2	PQ643445	-	-	-	-	-

KEY: + = Positive reaction; - = Negative reaction

The phylogenetic relatedness of the selected bacterial isolates, identifying the closest identity for each of the isolates, is presented in Fig. 2. *Staphylococcus* species in this study showed similarity with *Staphylococcus* species from the gene bank database. The accession numbers of the isolates submitted to the Gene bank were presented in Table 7. The result shows that 77.8% of these isolates could produce one or more enzymes, while 22.2% of bacterial isolates proved otherwise (Table 8). Many of these bacteria isolates (77.8%) have the potential to produce one or more enzymes, which make them metabolically active, possibly influencing their colonization of infection sites, pathogenicity and resistance to antimicrobial agents (Reissier et al*.,* 2023).

4. CONCLUSION

Resistance to the carbapenems class of antibiotics by multidrug-resistant clinical isolates is a growing concern in healthcare. *Staphylococcus species* are a major rising threat to public health and modern medicine due to their vast resistance to multiple antibiotics. Most of the bacteria collected from different clinical site samples in the two teaching Hospitals from Ogbomoso showed multiple resistance to all the antibiotics tested. The production of certain enzymes and biofilm formation by these microorganisms are essential factors that enhance their virulence, hence leading to multiple drug resistance. At times, errors from the Hospital laboratory scientists may occur in the area of bacteria identification, which affects antimicrobial stewardship and may contribute to multidrug resistance. There is need to employ real-time bacterial identification like real-time PCR and digital inline holography methods among others, in the Nigerian health care system for real-time identification and reduced error.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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