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Socio-Demographic Distribution of Multidrug Resistant *Staphylococcus aureus* from Clinical Sources and their Comparative Control with White and Red Roselle (*Hibiscus sabdariffa)* Calyces

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Abstract

Background: Increasing resistance of bacterial infections to current treatment threatens to derail progress made to reduce the global burden of diseases. However, there is insufficient research on effective ways to target information or provision of alternative method of mitigating multidrug resistance that could increase public knowledge toward improvement of antibiotics stewardship. Identification, antimicrobial sensitivity pattern and antimicrobial activity was determine using standard microbiological method.

Results: Among the 50 Staphylococcus aureus isolated from different clinical samples, 38% and 62% were recovered from male and female patients respectively. largest proportions were from age-group 21-30 years (47.5%). Urine had the Highest number of occurrence of S. aureus 16 (32%). The isolates showed high resistance to Cefoxiitin (FOX), Ceftazidime (100%), Ciprofloxacin (100%), Cefolaxime (100%), Cloxacillin (100%), gentamicin (12%) and erythromycin (36.8%). White and red calyces contain the same type of phytochemicals except Tannin which was absent in the red calyces. the Red calyces (73.45) contain higher phenol than White calyces (38.60). White calyces (163.05) contain higher amount of Flavonoids than in Red calyces (98.30). DDPH% Inhibition observed in red calyces (76.50) was higher than white calyces (45.33). NO% Inhibition has higher value in the white calyces (162.13) than red calyces (148.35). Both white and red calyces conatin equal Tbars (0.136). % Iron chelation observed in white calyces (125.49) was higher than in the red calyces (110.43). Red calyces contain higher amount of Vitamin-C than in the white calyces. Red Hibiscus sabdariffa extract shows greater inhibitory property against isolates than the white Hibiscus sabdariffa extracts. Although, they showed less inhibitory potential on the studied isolates in comparison with commercial antibiotics.

Conclusions: To prevent further emergence and spread of MDR Staphylococcus aureus, rational use of antibiotics and regular monitoring of antimicrobial resistance patterns are made easily through social demographic investigation of the affected population.

1. Introduction

The inappropriate use of antimicrobials such as antibiotics accelerates the emergences of antibiotic-resistant pathogenic process and diminishes drug efficacy. Particularly, widespread misconceptions about the potency of antibiotics for various ailments have led to unnecessary use and self-medication practices in communities. Knowledge about antibiotics and resistant infections has been found to influence antibiotic use **(Fair and Tor, 2014; Gualano *et al.,* 2015).** Understanding the different factors that lead to inappropriate antibiotic use may help mitigate this global health problem through effective antibiotic stewardship programs to promote rational use of medicines in communities. Age, gender, education level, and socio-economic status have been hypothesized to predict typology of antibiotic knowledge and use **(Demoré et al., 2017).** For instance, studies have shown that younger individuals; those with lower educational attainment, rural residents, and men exhibited lower knowledge and were more likely to misuse antibiotics, although it is unclear the extent to which the conclusions are generalizable **(Pavydė*et al.,* 2015).** Identifying predictors of inappropriate antibiotic use could provide important information about the specific knowledge and behaviors to target during the development and implementation of public health interventions and will subsequently promote prudent use of antibiotics in communities.

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For several decades, the emergence of multidrug resistant *Staphylococcus aureus* has been an obstacle to the control of the disease. Early detection of drug resistance is crucial to prevent transmission of drug-resistant staphylococcus aureus and avoid mortality **(Erku *et al.,* 2017).** In low- and middle-income countries, the implementation of robust resistance diagnostic programs using molecular tools remains a challenge. *Staphylococcus aureus* are Gram-positive cocci ranging from 0.5 to 1.5 mm in diameter, which may or may not contain a polysaccharide capsule. They are non-motile, non-spore forming facultative anaerobes that produce catalase and coagulase enzymes **(Fisher and Paterson, 2020).** *Staphylococcus aureus* is a commensal of humans, it is also a frequent cause of human infections which may become serious if caused by antimicrobial resistant strains **(Lee, 2003).** Antibiotic resistant *S. aureus*, especially MRSA, are equally adopted to hospitals and outer environments evolving as major pathogens of public health concern **(Shah *et al.,* 2013).**

The fact that *S. aureus* is resistant to multiple classes of antimicrobial agents in the hospital environment is a challenge currently facing clinicians when treating *S. aureus* infections **(Saba *et al.,* 2017).** This resistance stems from a history of over 50 years of recurrent adaptation of *S. aureus* to different antibiotics introduced into clinical practice over the years. Abuse of as well as indiscriminate use of antimicrobials are contributing factors to the spread of resistance **(wang *et al.,* 2017).** Antibiotic-resistance genes are carried on plasmids and transposons, and can be transferred from one staphylococcal species to another and among other Gram-positive bacteria. Antimicrobials act by targeting important bacterial functions such as cell wall synthesis (beta-lactams and glycopeptides), protein synthesis (aminoglycosides, tetracyclines, macrolides, lincosamides, chloramphenicol, mupirocin and fusidic acid), nucleic acid synthesis (quinolones), RNA synthesis (rifampin), and metabolic pathways such as folic acid metabolism (sulphonamides and trimethoprim) **(Schnitt *et al.,* 2021).** The overuse of antimicrobials elicits resistance either by the emergence of point mutations or by the acquisition of foreign resistance genes, which leads to alteration of the antimicrobial target and the degradation of the antimicrobial or reduction of the cell's internal antimicrobial concentration **(Fisher and Paterson, 2020).**

Staphylococcus aureus. *S. aureus* is a potentially harmful human pathogen associated with both nosocomial and community-acquired infections, and it is increasingly becoming resistant to most antibiotics. Previous studies of *S. aureus* in marine environments have linked swimmers to the dissemination of *S. aureus*in marine water, via the shedding of the bacterium from their nose, skin, and respiratory tract. On recreational beaches, *S. aureus* has occasionally been found in high abundance in both water and sand, which can be directly associated with bather density and human activities around the beach **(Akanbi *et al.,* 2017).**

Plants are rich sources of several classes of bioactive compounds that have been responsible in the prevention and treatment of chronic health pathologoies such as hypertension, cardiovascular diseases, inflammation and cancer (Bresciani *et al*, 2015). Hibiscus sabdariffa (figure 1), a member of Malvaceae family, is a known medicinal plant with a worldwide fame **(Abbas et al, 2011)** and the plant can be found in almost all warm countries such as India, Saudi Arabia, Malaysia, Indonesia, Thailand, Philippines, Vietnam, Sudan, Egypt and Mexico.

Figure 1 *Hibiscus sabdariffa* Linn

There are health and nutritional claims that Hibiscus sabdariffa possess health benefits such as soothing colds, opening blocked nose, clearing up mucous, promoting proper kidney function, helping digestion and helping reduce fever. Roselle is known for its antibacterial, antifungal and anti-parasitic actions. Oil extract, Acqeous and ethanol extracts from seeds of Roselle has been shown to have an *in vitro* inhibitory effect on microorganisms. However, there are limited studies to substantiate these health/nutritional claims. There is need for scientific information to substantiate the claims and validate its applicability in the treatment of infectious diseases.

2. Material and methods

Sample Collection

Clinical samples**:**
Clinical isolates were collected from State Specialist Hospital. The isolates were isolated from fifty (50) patients with vary sources which include ear, hair, toe, high vaginal swab (HVS) and urine. The isolates were subcultured into slants and were transported to Laboratory of the Department of Microbiology, Federal university of technology Akure.

Plant samples
Hibiscus sabdariffa used for this project were gotten from the market in Ado-Ekiti (Oja-Bisi), Ekiti State, Nigeria.

Reagent used
Mercuric chlorate, 100ml distilled water, Biamuth nitrate, iodine, 2% Hydrochloric acid, 5% $Fecl_3$, 10% Lead acetate, 1N Sodium hydroxide, Concentrated Tetraoxosulphate(vi) acid (H_2SO_4), Ammonia solution, Methanol, Chloroform, Sulphuric acid, 10% Acetic acid, 10% Thio urea, 2,4-Dinitrophenyl Hydrazine, 85% Sulphuric acid, 5-Bromine water, 10% potassium iodide, 1M potassium iodate (KI03), Sodium thiosulphate and 3% Starch solution.

Materials
Test tube, Filter paper, Glass slide, Air oven, Desiccator, Porcelain crucible, Round bottom flask, pipette, weighing balance, Atomic Absorption Spectrophotometer, Apple-UV-Visible spectrophotometer.

Preparation of plant Materials for Extraction
The dried samples were grinded using electric blender. The powdered samples were then kept in an air-tight plastic container, further soxhlet extraction process using water as solvent of extraction was carried out using standard method as described by Ibisanmi and Aribisala, 2022.

Preparation of Culture Media
All glass wares and culture media were sterilized by autoclaving at 121°C for 15 minutes at 15psi Mannitol salt agar and Nutrient Agar usedin this study was all prepared according to manufacturer's instructions.

Morphological identification of isolates
The morphology of the clinical isolate was based on the physical appearance of the colonies on the Mannitol Salt Agar (MSA), the size, the color, the shape, the edge, the elevation, the texture and the transparency.

2.1 Biochemical identification of isolates

Gram staining
This was carried out to establish the Gram reflection of the isolates. A smear of each isolates was made on glass slides, crystal violet stain was added to the smear for 30-60 seconds after which the stain was washed off with sterile distilled water, then iodine was added to the smear for another 30-60 seconds to fix the stain and washed off with sterile distilled water. Alcohol (70%) was added to the smear for 60 seconds to decolorize the stain, safranin red was finally added to the smear as a counter stain. The glass slide was mounted on microscope and observed under oil immersion objectives lens (x100). A purple colour indicated the presence of Gram-positive microorganism while red/pink colour indicates presence of Gram –negative microorganism

Biochemical tests
The various biochemical tests were carried out on the organisms to aid in their identification. They include the following

Citrate test
This test screens a bacterial isolate for the ability to utilize citrate as its carbon and energy source. A positive diagnostic test rests on the generation of alkaline by-products of citrate metabolism. The subsequent increase in the pH of the medium is demonstrated by the color change of pH indicator. The isolates were inoculated into citrate slants and incubated at room temperature (28±2°C) for 24 hours. Organisms that are able to utilize citrate as their source will be indicated by a colour change of the agar from green to deep blue while microorganisms that cannot utilize citrate will be indicated y no colour change.

Indole test
The indole test screens for the ability of the organism to degrade the amino acid tryptophan and produce indole. It is used as part of the IMViC (indole, MR-VP citrate) procedures. A positive test indicates the formation of a pink to red color ("cherry-red ring") in the reagent layer on top of the medium within seconds of adding the reagent.

Oxidation test
This is a test that assays for the presence of cytochrome oxidase, an enzyme sometimes called indophenols oxidase. In the presence of an organism that contains the cytochrome oxidase enzyme, the reduced colorless reagent becomes an oxidized colored product. It is Oxidase positive when the colour changes to dark purple within 5 to 10 seconds and Oxidase negative if the colour does not change or it takes longer than 2 minutes.

Methyl red
This test demonstrates the ability of an organism to oxidize glucose with the production and stabilization of high concentrations of acid end products. The methyl red indicator will turn red throughout the tube, which is indicating of a positive test at pH6, still indicating the presence of acid but with a lower hydrogen ion concentration, the indicators turn yellow, which is indicating the negative test.

Voges-Proskauer
This test determines the ability of the organism to produce acetone (acetyl methyl carbinol) during fermentation of glucose. The reagent used in this test, Barrett's reagent, consists of a mixture of alcoholic alpha-naphthol and 40% potassium hydroxide solution. Development of a deep rose colour in culture within a minute following the addition of Barrett's reagent is indicative of presence of the acetyl methyl carbinol and represents a positive result. The absence of rose colouration is a negative result.

Sugar fermentation test
This test is used to determine the ability of the organisms to degrade and ferment carbohydrate with the production of acid and gas. In fermentation, substrate and alcohols undergo anaerobic dissimilation and produce an organic acid. The pH indicator red is used to detect the production of acid, which is red at a neutral pH 7 and changes to yellow at a slightly acidic pH of 6.8. this indicates a positive reaction. The following ssugars were used for the experiment: Galactose, Lactose, Siucrose and Maltose.

Triple sugar iron test
The triple sugar- iron test is designed to differentiate among the different groups of organisms capable of fermenting glucose with the production of acid. Carbohydrate fermentation is indicated by the presence of gas and visible colour change of the pH indicator, phenol red. The production of hydrogen sulphide in the medium is indicated by the formation of a black precipitate that will blacken the medium in the butt of the tube.

Coagulase test
A drop of sterile distilled water was placed on clean slides, a sterile loop was used to pick colonies and a hick suspension was made. A loopful of plasma was added to the suspension. Formation of clumps within 10 seconds indicates positive reaction and absence of clumps indicates negative reaction.

Mannitol motility test
This test is used to detect if an organism is motile and also mannitol is fermenting or not. A motile organism typically diffused, hazy growth that spreads throughout the medium rendering it slightly opaque. This test also helps to identify weather the organisms ferment mannitol or not. It produces acidic end products which in turn change the red colour of phenolred indicator to yellow.

2.2 Phytochemical analysis (Fresh samples)

Detection of alkaloids

Mayer's test
A fraction of the extract is treated with Mayer's reagent (1.36g of mercuric chlorate and 5g of potassium iodide in 100ml distilled water and noted for a cream-coloured precipitate).

Dragendroff's test
A fraction of the extract is treated with Dragendroff's reagent and observed for the formation of reddish orange precipitate. (Bismuth nitrate 1.7g, glacial acetic acid 20ml, water 80ml, and 100ml of 50% solution of KI in water, mix together and keep as stock solution, 10ml of stock, 20ml of glacial acetic acid make up for 100ml in water for working solution)

Wagner's test
A fraction of the extract is treated with Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 100ml of distilled water) and observed for the formation of reddish-brown precipitate.

Alternative test for Alkaloids
For the purpose of phytochemical analysis of the selected plants, 0.2g of the selected plant samples were added in each test tube and 3ml of hexane were mixed in it, shaken well and filtered. Then took 5ml of 2% HCL and poured in a test tube having the mixture of plant and hexane. Heated the test tube having the mixture, filtered it and poured few drops of picric acid in a mixture. Formation of yellow colour precipitate indicates the presence of alkaloids.

Detection of phenolic compounds

Ferric chloride test
A fraction of the extract was treated with 5% $FeCl_3$ solution and observed for the formation of deep blue colour.

Lead acetate test
A fraction of the extract was treated with 10% lead acetate solution and observed for the formation of white precipitate.

Detection of Flavonoids

Aqueous NaOH test
To a fraction of the extract, 1N acqeous NaOH was added and observed for the formation of yellow-orange colour

Concentrated H2SO4 test
To a small fraction of the extract, concentrated H_2SO_4 was added and observed for the formation of orange colour.

Schinodo's test
To a small fraction of the extract, a piece of magnesium turnings was added, followed by concentrated HCL and then heated slightly and the formation of dark pink colour was observed.

Alternative test for flavonoids
For the confirmation of flavonoid in the selected plants, 0.5g of each selected plant extract were added in a test tube and 10ml of distilled water, 5ml of dilute ammonia solution were added to a portion of the acqeous filtrate of each plant extract followed by addition of 1ml concentrated H2SO4. Indication of yellow colour shows the presence of flavonoid in each extract.

Detection of saponins

Foam test
A fraction of the extract was vigorously shaken with water and observed for persistent foam.

Haemolytic test
A fraction of the extract will be added to a drop of blood placed on a glass slide and observed for the haemolytic zone.

Test for terpenoids
An amount of 0.8g of selected plant sample will be taken into a test tube, then 10ml of methanol will be poured into it, shaken well and filtered to take 5ml extract of plant sample. Then 2ml of chloroform is mixed with the extract of selected plant sample followed by 3ml of sulphuric acid in selected sample extract. Formation of reddish brown colour indicates the presence of terpenoids in the selected plants.

Test for tannins
0.5g of the dried powdered samples is boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride is added and observed for brownish green or a blue-black colouration.

2.3 Some in-vitro antioxidant analyses of the plants samples

Determination of DPPH free radical scavenging ability

The 1, 1- diphenyl-2-picryhydrazyl (DDPH) free radical scavenging ability of the extract was determined using the modified method of (Gyamfi *et al*., 1999). Briefly, 1.0 mL of different concentrations (20, 40 and 80 mg/mL) of the extracts was placed in respective test tubes. 1.0 ML of 0.1Mm methanolic DPPH solution was added to the samples. These samples were vortexed, and incubated in dark at room temperature for 30 minutes before absorbance measured at 516cm. Decreased absorbance of the sample indicates DDPH free radical scavenging capability. Further calculation was carried out according to standard method **(Ibisanmi and Aribisala, 2022).**

Determination of Nitric oxide (NO) scavenging ability

The modified methods of (Jagetia and Baliga, 2004) was used to determine the Nitric oxide radical scavenging ability. Sodium Nitroprusside in acqeous solution at physiological PH 7.0 spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent (1.0mL sulfanilic acid reagent (0.33% prepared in 20% glacial acetic acid at room temperature for 5 minutes with 1mL of naphthylethylenediamine dichloride (0.1% w/v). Further calculation was carried out according to standard method **(Ibisanmi and Aribisala, 2022)**.

Determination of ferric reducing antioxidant power

The reducing property of the extract was determined by the modified method of (Pulido *et al*., 2002). This method is based on the reduction of (Fe3+) ferricyanide in stoichiometric excess relative to the antioxidants. Different concentrations of the methanolic extract of the sample and its various fractions (10-50 g/Ml) was added to 1.0mL of 200Mm of sodium phosphate buffer PH 6.6 and 1.0mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50^0C for 20 minutes, thereafter 1.0mL of freshly prepared 10% TCA was quickly added and centrifuged at 2000rpm for 10 minutes, 1.0mL of the supernatant was mixed with 1.0mL of distilled water and 0.25ml 0f 0.1% of $FeCl_3$ solution was added. Distilled water was used for blank without the test sample while control solution contained all other reagents except the 0.1% potassium ferricyanide. Absorbances of these mixtures were measured at 700nm using a spectrophotometer. Decreased absorbance indicates ferric reducing power capability of sample. Further calculation was carried out according to standard method **(Ibisanmi and Aribisala, 2022)**.

Determination of Fe^{2+} Chelation

The ability of the extract to chelate Fe^{2+} was determined using a modified method of Minotti and Aust (1987) by (Puntel *et al*., 2005). Freshly prepared 500 M $FeSO_4$ (150 L) was added to a reaction mixture containing 168 L of 0.1M Tris- HCL (PH 7.4), 218 L saline and the different concentrations of extracts (0-25 L). The reaction mixture was incubated for 5 minutes before the addition of 13 L of 0.25% 1, 10- phenanthroline (w/v). The absorbance was subsequently measured at 510nm in a

spectrophotometer. Further calculation was carried out according to standard method **(Ibisanmi and Aribisala, 2022)**.

Quantification of Total Phenolic Content and flavonoids

Estimation of Total Phenolic Content

The extractable phenol content was determined on the extracts using the method reported by Singleton et al., (1999). 0.2ml of the extract was mix with 1.5mL 0f 10% Folinciococalteau's reagent and 2ml of 7.5% Sodium carbonate. The reaction mixture will be subsequently incubated at 45 C for 40 minutes, and the absorbance was measure at 700nm in the spectrophotometer, garlic acid would be used as standard phenol. Further calculation was carried out according to standard method **(Ibisanmi and Aribisala, 2022)**.

Determination of total flavonoid

The total flavonoid content of the extract was determined using a colorimeter assay developed by (Bao, 2005). 0.2 ml of the extract was added to 0.3mL of 5 % NaNO3 at zero time. After 5 minutes, 0.6ml of 10% $AlCl_3$ was added and after 6 minutes, 2mL of distilled water. Absorbance was read at 510nm against the reagent blank and flavonoid content was expressed as galic acid equivalent. Further calculation was carried out according to standard method **(Ibisanmi and Aribisala, 2022)**.

Determination of Vitamin C (ascorbic acid) Contents in various fruit and vegetable by UV- spectrophotometry and titration methods

Determination of Vitamin C (ascorbic acid) Contents in various fruit and vegetable by UV- spectrophotometry and titration methods carried out according to standard method as described by ibisanmi and Aribisala, 2022.

Antibiotic Sensitivity Test Using Agar Diffusion Method (discs)

The disk diffusion method was used and after 16-18 hours of incubation at 37°C zone of inhibition was measured and interpreted as recommended by the Clinical and Laboratory Standards Institute **(CLSI, 2018).** Using a sterile wire loop, 3-5 pure colonies were picked from Nutrient agar for Gram positive then emulsified in nutrient broth. Standard inoculums adjusted to 0.5 McFarland standard using McFarland Densitometer was swabbed onto Muller-Hinton agar (dispensed on 100mm plate). Accordingly, detailed CLSI guidelines for each category of Gram-positive bacteria, isolates were tested against. The zone of inhibition was measured to the nearest millimeter and all bacterial isolates were classified as sensitive, intermediate and resistant according to the standardized table supplied by **CLSI (2018).**

Antimicrobial Activity of White and Red Roselle Sun-Dried Calyces (*Hibiscus Sabdariffa)* Extracts

Agar well diffusion method was used to test the antimicrobial activity of the extracts. By making used of fresh medium, bacterial suspensions were streaked on petri dishes containing freshly prepared Muller Hinton Agar using a sterile swab stick. Five 6mm wells were bored on the already inoculated MHA plates using a sterile cork borer, the susceptibility test was carried out in triplicate. Each of the wells were filled up with 200mg/ml and 100mg/ml of the reconstituted extracts respectively. The plates were incubated at 25 ºC for 18 to 48 hours and zones of inhibition were measured with milliliter ruler at 24hours interval.

3. Results

3.1 Identification of Staphylococcus aureus

Fifty (50) target organisms (*Staphylococcus aureus*) were isolated from different clinical sources were identified by standard microbiological methods. Results obtained for biochemical test is shown in Table 1.

3.2 Socio-demographic characteristics

A total of fifty (n=50) eligible out-patients attending Image Diagnostic Centre were investigated during this study period. Of these patients who developed infections from different clinical sources 38% (n=19/50) of them were male and 62% (n=31/50) were female shown in Figure 1. The majority of these patient 30% (n=15/50) were between the ages of 21-30 years as shown in Figure 2. Among all study participants, the highest rate of occurrence of target organism 32% (n=16/50) were urine, 24% (n=12/50) HVS, 22% (n=11/50) ear, 12% (n=6/50) hair, 10% (n=5/50) were toe as shown in Figure 3.

3.3 Antibiotic Sensitivity testing for *Staphylococcus aureus* from clinical sources

The frequency of occurrence of antibiotics susceptibility and multidrug-resistant *Staphylococcus aureus* from different clinical sources are indicated in figure 4 to figure 7. Among the urine samples Ofloxacin (OFL) (100%) was found to have a very good efficacy on almost all the isolates whereas the isolates were able to completely resist the effect of Cefoxiitin (FOX), Amoxillin (AUG), Ceftazidime (CAZ), Ciprofloxacin (ORX), Ceftriaxone (CTR) and Cloxacillin (CXC). Among the Toe samples Ofloxacin (OFL) (100%) and Gentamicin (GEN) (100%) was found to have a very good efficacy on almost all the isolates whereas the effect of others antibiotics varied as shown in figure 4. Among the hair samples Ofloxacin (OFL) (100%) was found to have a very good efficacy on almost all the isolates whereas the effect of others antibiotics varied. Result obtained from HVS sample shows that the isolates were able to resist the effect of Cefoxiitin (FOX), Amoxillin (AUG), Ceftazidime (CAZ), Ciprofloxacin (ORX), Ceftriaxone (CTR) and Cloxacillin (CXC) whereas Gentamicin (GEN) (100%) were found to be most effective as shown in figure 5. Among the ear samples one of the isolates 17% resistance was observed for Ofloxacin (OFL), the result shows that effect of antibiotics used varied among the isolates as shown in figure 6. Among all the clinical samples Ofloxacin (OFL) (94%) was found to have a very good efficacy on almost all the isolates, followed by Gentamicin (GEN) (73%) and Erythromycin (ERY) (42%) (figure 7). The resistance patterns of these isolates were little bit low to OFL, GEN and ERY. The antibiotic susceptibility profile showed that the isolates were most resistant to FOX, CAZ, ORX, CTR, and CXC. Multi drug resistant pattern of *Staphylococcus aureus* from clinical sources is shown in Table 2.

Table 1 Biochemical characteristics of *Staphylococcus aureus* from different clinical sources

S/N	Catalase	Oxidase	Citrate	Fructose	Galactose	Maltose	Indole	MR	VP	Motility	Butt	Slant	Gas	H_2S	Coagulase	Shape	Gram	Suspected organism
U1	+	-	+	-	+	-	-	-	-	-	A	A	-	+	+	COCCI	+	*Staphylococcus aurues*
U2	+	-	+	-	+	+	-	-	-	-	B	B	-	+	+	COCCI	+	*Staphylococcus aurues*
U3	+	-	+	-	+	-	-	+	-	-	B	A	-	-	+	COCCI	+	*Staphylococcus aurues*
U4	+	-	-	-	-	-	-	-	-	-	A	B	-	+	+	COCCI	+	*Staphylococcus aurues*
U5	+	-	+	-	+	+	-	+	-	+	A	A	-	-	+	COCCI	+	*Staphylococcus aurues*
U6	+	-	-	-	+	+	-	-	-	+	A	B	-	+	+	COCCI	+	*Staphylococcus aurues*
U7	+	-	-	-	-	-	-	-	-	-	A	B	-	+	+	COCCI	+	*Staphylococcus aurues*
U8	+	-	-	-	+	+	-	+	-	-	B	A	+	-	+	COCCI	+	*Staphylococcus aurues*
U9	+	-	-	+	+	+	-	+	-	-	A	A	-	-	+	COCCI	+	*Staphylococcus aurues*
U10	+	-	-	-	+	-	-	+	-	+	A	A	-	+	+	COCCI	+	*Staphylococcus aurues*
U11	+	-	+	+	-	+	-	+	-	+	A	A	-	+	+	COCCI	+	*Staphylococcus aurues*
U12	+	-	+	+	+	+	-	+	-	+	A	A	-	+	+	COCCI	+	*Staphylococcus aurues*
U13	+	-	+	-	+	+	-	+	-	+	A	B	+	+	+	COCCI	+	*Staphylococcus aurues*
U14	+	-	+	+	+	-	-	+	-	+	A	A	+	+	+	COCCI	+	*Staphylococcus aurues*
U15	+	-	_	+	+	+	-	-	-	+	A	B	-	-	+	COCCI	+	*Staphylococcus aurues*
U16	+	-	+	+	+	+	-	+	-	+	A	B	-	+	+	COCCI	+	*Staphylococcus aurues*
HV1	+	-	+	+	+	+	-	_	-	+	A	B	-	+	+	COCCI	+	*Staphylococcus aurues*
HV2	+	-	_	-	-	-	-	+	-	+	B	B	-	-	+	COCCI	+	*Staphylococcus aurues*
HV3	+	-	+	+	+	+	-	-	-	+	A	B	-	+	+	COCCI	+	*Staphylococcus aurues*
HV4	+	-	-	-	-	-	-	-	-	-	A	B	-	+	+	COCCI	+	*Staphylococcus aurues*
HV5	+	-	-	-	+	+	-	+	-	-	B	A	+	-	+	COCCI	+	*Staphylococcus aurues*
HV6	+	-	-	+	+	+	-	+	-	-	A	A	-	-	+	COCCI	+	*Staphylococcus aurues*
HV7	+	-	-	-	+	-	-	+	-	+	A	A	-	+	+	COCCI	+	*Staphylococcus aurues*
HV8	+	-	+	+	-	+	-	+	-	+	A	A	-	+	+	COCCI	+	*Staphylococcus aurues*
HV9	+	-	+	+	+	+	-	+	-	+	A	A	-	+	+	COCCI	+	*Staphylococcus aurues*
HV10	+	-	+	-	+	+	-	+	-	+	A	B	+	+	+	COCCI	+	*Staphylococcus aurues*
HV11	+	-	+	+	+	-	-	+	-	+	A	A	+	+	+	COCCI	+	*Staphylococcus aurues*
HV12	+	-	_	+	+	+	-	-	-	+	A	B	-	-	+	COCCI	+	*Staphylococcus aurues*
E1	+	-	-	-	-	-	-	-	-	-	A	B	-	+	+	COCCI	+	*Staphylococcus aurues*
E2	+	-	-	-	+	+	-	+	-	-	B	A	+	-	+	COCCI	+	*Staphylococcus aurues*

E3	+	-	-	+	+	+	-	+	-	-	A	A	-	-	+	COCCI	+	*Staphylococcus aurues*
E4	+	-	-	-	+	-	-	+	-	+	A	A	-	+	+	COCCI	+	*Staphylococcus aurues*
E5	+	-	+	+	-	+	-	+	-	+	A	A	-	+	+	COCCI	+	*Staphylococcus aurues*
E6	+	-	-	-	+	+	-	+	-	-	B	A	+	-	+	COCCI	+	*Staphylococcus aurues*
E7	+	-	-	+	+	+	-	+	-	-	A	A	-	-	+	COCCI	+	*Staphylococcus aurues*
E8	+	-	-	-	+	-	-	+	-	+	A	A	-	+	+	COCCI	+	*Staphylococcus aurues*
E9	+	-	+	+	-	+	-	+	-	+	A	A	-	+	+	COCCI	+	*Staphylococcus aurues*
E10	+	-	+	+	+	+	-	+	-	+	A	A	-	+	+	COCCI	+	*Staphylococcus aurues*
E11	+	-	+	-	+	-	-	-	-	-	A	A	-	+	+	COCCI	+	*Staphylococcus aurues*
T1	+	-	+	-	+	+	-	-	-	-	B	B	-	+	+	COCCI	+	*Staphylococcus aurues*
T2	+	-	+	-	+	+	-	+	-	+	A	A	-	-	+	COCCI	+	*Staphylococcus aurues*
T3	+	-	-	-	+	+	-	-	-	+	A	B	-	+	+	COCCI	+	*Staphylococcus aurues*
T4	+	-	-	-	-	-	-	-	-	-	A	B	-	+	+	COCCI	+	*Staphylococcus aurues*
T5	+	-	-	-	+	+	-	+	-	-	B	A	+	-	+	COCCI	+	*Staphylococcus aurues*
H1	+	-	+	-	+	+	-	+	-	+	A	A	-	-	+	COCCI	+	*Staphylococcus aurues*
H2	+	-	-	-	+	+	-	-	-	+	A	B	-	+	+	COCCI	+	*Staphylococcus aurues*
H3	+	-	-	-	-	-	-	-	-	-	A	B	-	+	+	COCCI	+	*Staphylococcus aurues*
H4	+	-	-	-	+	+	-	+	-	-	B	A	+	-	+	COCCI	+	*Staphylococcus aurues*
H5	+	-	+	-	+	+	-	+	-	+	A	A	-	-	+	COCCI	+	*Staphylococcus aurues*
H6	+	-	-	-	+	+	-	-	-	+	A	B	-	+	+	COCCI	+	*Staphylococcus aurues*

U= urine sample, HV= HVS sample, T = toe sample, E= ear sample, H = hair sample

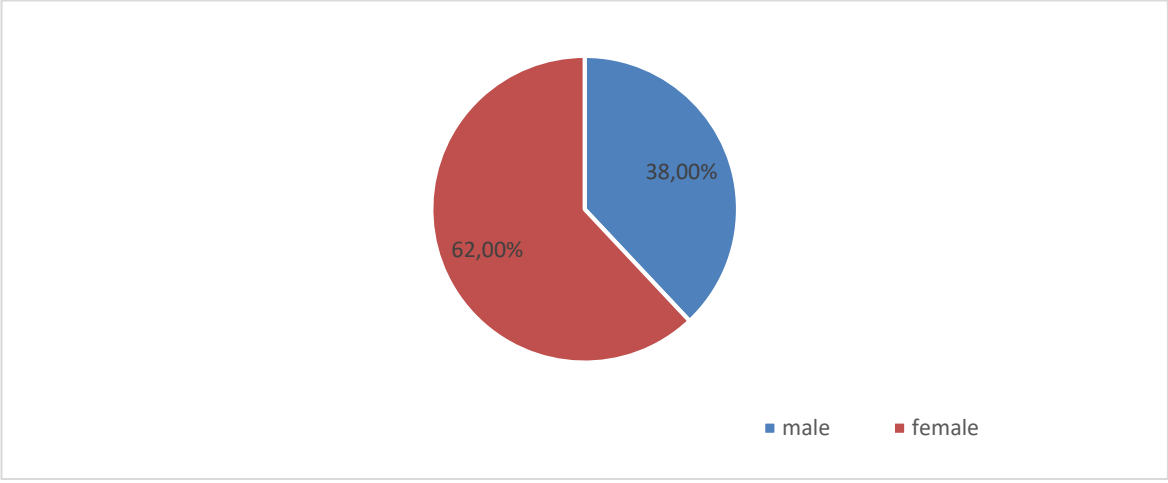

Figure 1 Frequency of *Staphylococcus aureus* among genders

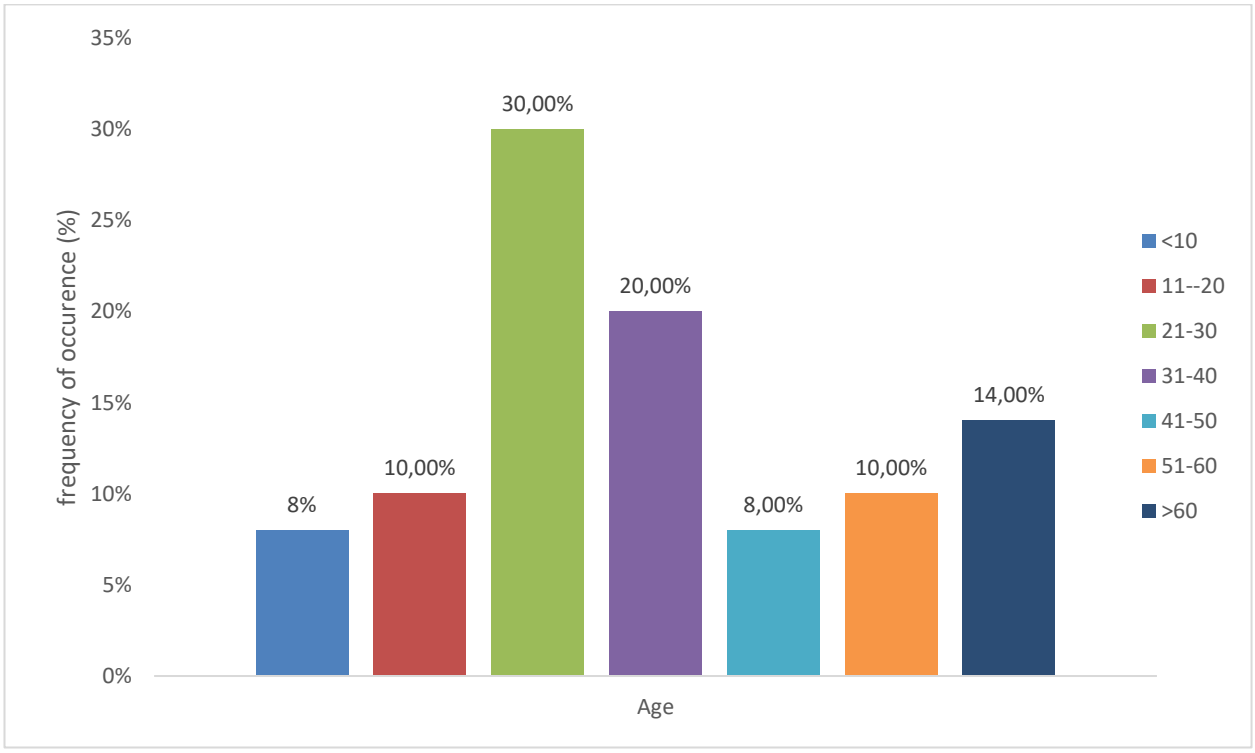

Figure 2 Frequency of *Staphylococcus aureus* among age in year of patients

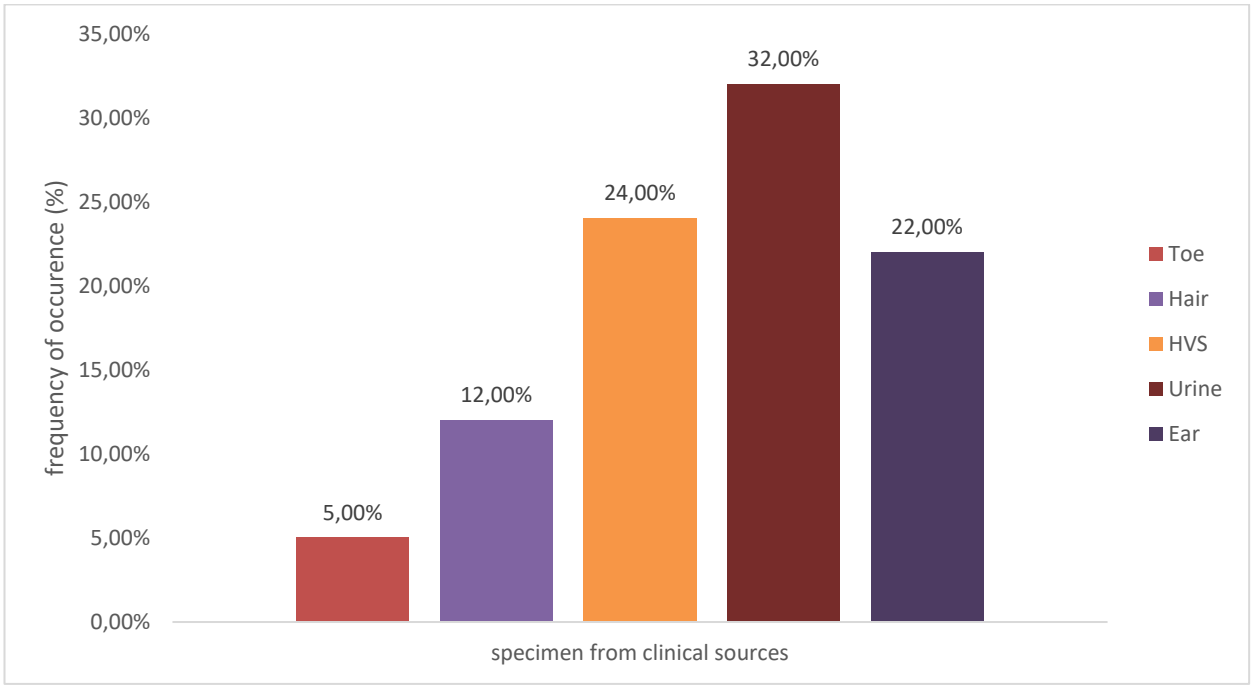

Figure 3 Frequency of *Staphylococcus aureus* among the different types of specimen collected

Table 2 Multidrug resistant pattern of *Staphylococcus aureus* from clinical sources

S/N	Source	Antibiotics	No of MDR *S. aureus* (n=50)	Percentage of MDR *S. aureus*
	Urine			
1		AUG-CAZ-ORX-FOX-CTR-ERY-CXC	7	43.8
2		AUG-CAZ-ORX-FOX-CTR-CXC	7	43.8
3		AUG-CAZ-ORX-GEN-FOX-CTR-CXC	1	6.3
4		OFL-AUG-CAZ-ORX-FOX-CTR-ERY-CXC	1	6.3
			N=16	
	HVS			
1		AUG-CAZ-ORX-GEN-FOX-CTR-CXC	3	25
2		AUG-CAZ-ORX-FOX-CTR-ERY-CXC	2	16.7
3		AUG-CAZ-ORX-GEN-FOX-CTR-ERY-CXC	1	8.3
4		AUG-CAZ-ORX-FOX-CTR-CXC	5	41.7
			N=12	
	Ear			
1		AUG-CAZ-ORX-FOX-CTR-ERY-CXC	4	36.4
2		AUG-CAZ-ORX-GEN-FOX-CTR-ERY-CXC	1	9.1
3		AUG-CAZ-ORX-FOX-CTR-CXC	1	9.1
4		AUG-CAZ-ORX-FOX-CTR-CXC	5	45.5
			N=11	
	Toe			
1		AUG-CAZ-ORX-FOX-CTR-ERY-CXC	3	60
2		AUG-CAZ-ORX-GEN-FOX-CTR-CXC	1	20
3		OFL-AUG-CAZ-ORX-FOX-CTR-CXC	1	20
4		AUG-CAZ-ORX-FOX-CTR-CXC	1	20
			N=5	
	Hair			
1		AUG-CAZ-ORX-FOX-CTR-ERY-CXC	1	16.7
2		AUG-CAZ-ORX-GEN-FOX-CTR-CXC	2	15
3		AUG-CAZ-ORX-FOX-CTR-CXC	4	66.7
			N=6	

Ceftazidime=CAZ(30 mg/ml), Amoxicillin =AUG (30 mg/ml), Gentamicin=GEN(10 mg/ml), Cloxacillin=CXC (3.8-6.4 mg/ml), Ofloxacin=OFL(200-800 mg/ml), Ciprofloxacin= ORX(5mg/ml), Erythromycin=ERY(5 mg/ml), Cefoxiitin = Fox samples (30 mg/ml), Cefolaxime=CTR (81-102 mg/ml).

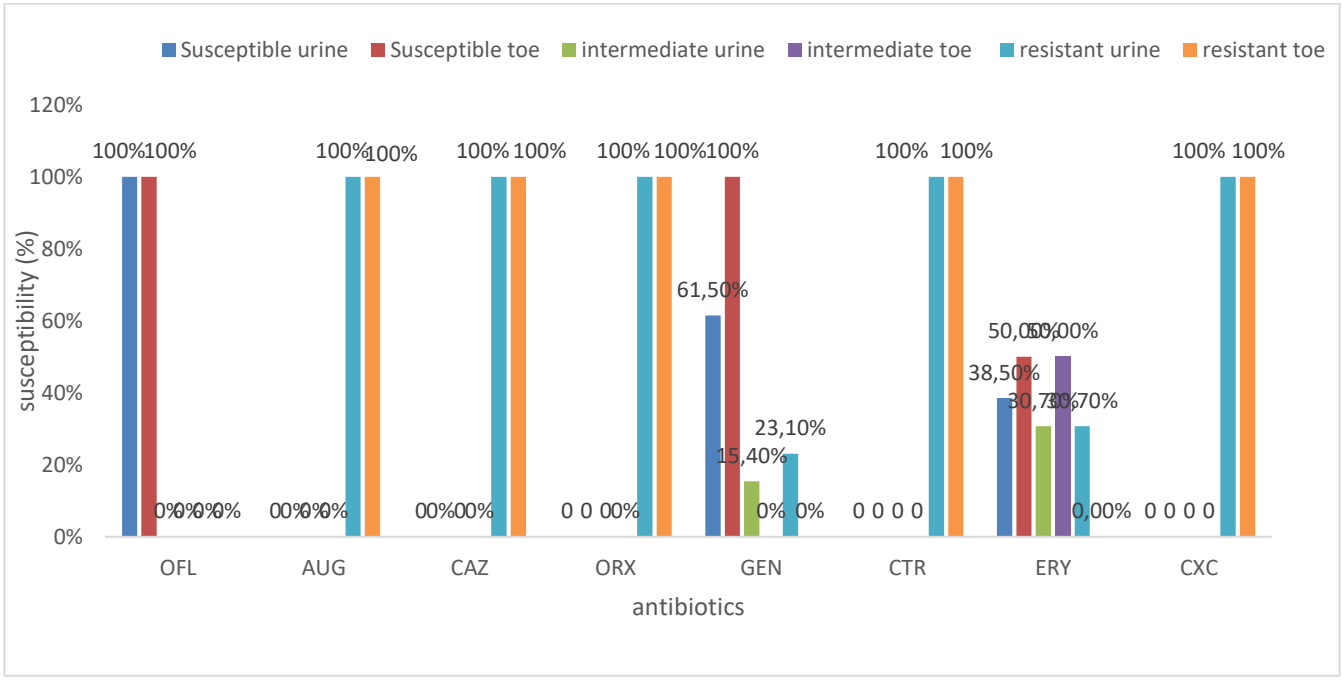

Figure 4 frequency of occurrence of antibiotics susceptibility and multidrug-resistant *Staphylococcus aureus* from urine and toe

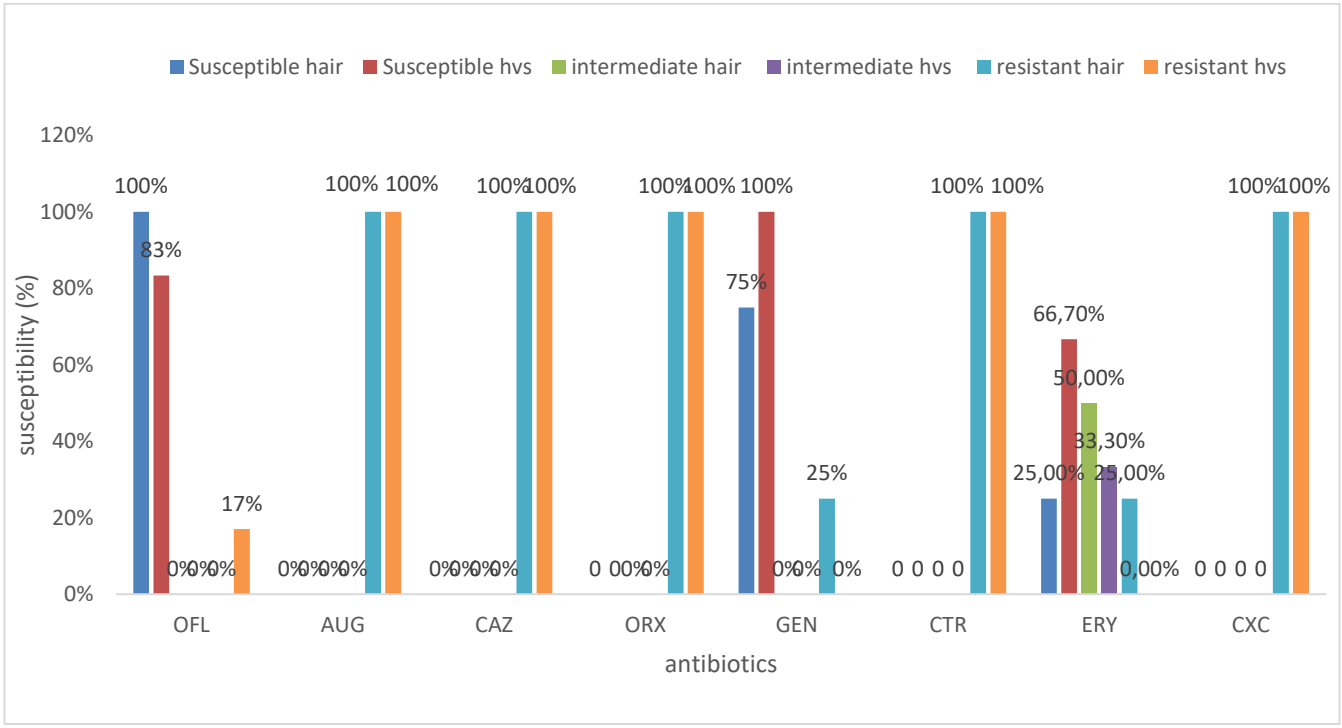

Figure 5 frequency of occurrence of antibiotics susceptibility and multidrug-resistant *Staphylococcus aureus* from hair and hvs samples

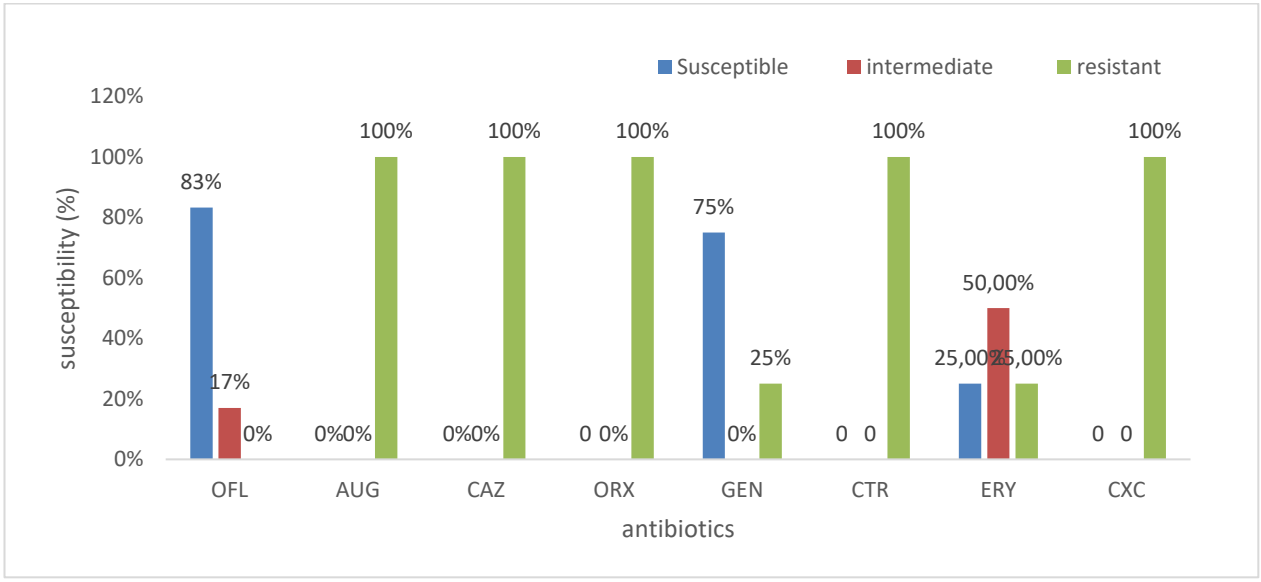

Figure 6 frequency of occurrence of antibiotics susceptibility and multidrug-resistant *Staphylococcus aureus* from ear samples

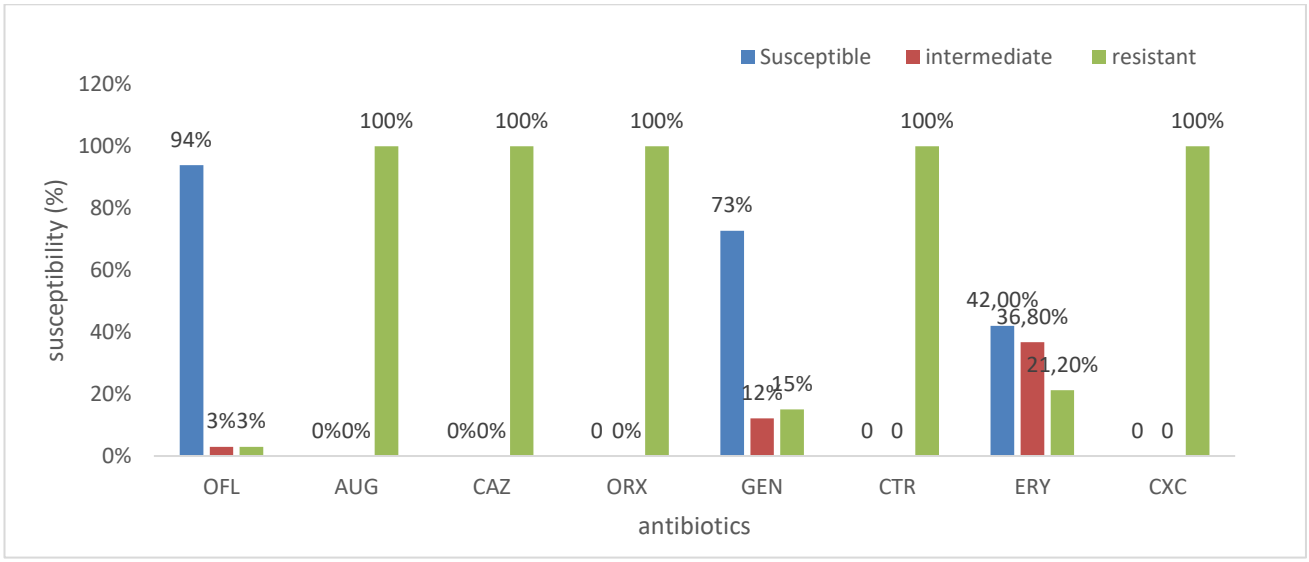

Figure 7 frequency of occurrence of antibiotics susceptibility and multidrug-resistant *Staphylococcus aureus* from all the clinical samples

3.4 Extract Yield

After the extraction process, the white *Hibiscus sabdariffa showed* yields of 25.23% while the yields from red *Hibiscus sabdariffa* is 24.85%.

3.5 Phytochemicals composition of *Hibiscus sabdariffa*

Table 3 shows the Qualitative analysis of *Hibiscus sabdariffa* (Sun dried calyces of white and red roselle). All the phytochemicals were present in the white and red calyces except Tannin which was absent in the red calyces.

3.6 Quantitative Phenolics and Flavonoids composition

Table 4 shows the quantitative composition of Total Phenolics and Flavonoid in the sun dried white and red calyces of *Hibiscus sabdariffa*. Higher Total Phenolics was observed in the Red calyces (73.45) while lower value was recorded in the White calyces (38.60). However, Flavonoids has higher value in the White calyces (163.05) and lower value in the Red calyces (98.30).

Table 3 Qualitative phytochemicals composition of *Hibiscus sabdariffa*

Phytochemicals	Red calyx	White calyx
Saponin	++	+++
Alkaloid	++	++
Flavonoid	++	++
Tanin	-	+
Phenolic	++	+
Terpenoids	+	+

+++ = Present in an appreciable amount ++ = Present in a moderate amount + = Present in a minute amount - = Completely absent

Table 4 Quantitative Phenolics and Flavonoids composition of calyces of *Hibiscus sabdariffa* at 100mg/ml

Parameters	Total Phenolics	Flavonoids
Red calyx	73.45a	98.30b
White calyx	38.60b	163.05a

Means with the same letter within a column are not significantly different at P≤0.05

3.7 Quantitative composition of DDPH% Inhibition, NO% Inhibition, TBars and % Iron chelation analysis

Table 5 shows the quantitative composition of DDPH% Inhibition, NO% Inhibition, TBars and % Iron chelation analysis in the sun dried white and red calyces of *Hibiscus sabdariffa.* DDPH% Inhibition has higher value in the red calyces (76.50) while lower value was recorded in the white calyces (45.33). NO% Inhibition has higher value in the white calyces (162.13) while lower value was recorded in the red calyces (148.35). For TBars, the same result was recorded both in the white and red calyces (0.136). % Iron chelation has higher value in the white calyces (125.49) while lower value was recorded in the red calyces (110.43).

Table 5 Quantitative composition of DDPH% Inhibition, NO% Inhibition, TBars and %Iron chelation of sun dried red and white calyces of *Hibiscus sabdariffa* at 100mg/ml

Phytochemicals	Red calyx	White calyx
DPPH% Inhibition	76.50a	45.33b
N0% Inhibition	148.35b	162.13a
TBars	0.136a	0.136a
% Iron chelation	110.43b	125.49a

Means with the same letter within rows are not significantly different at P≤0.05; DPPH - Diphenyl Picryl Hydrazyl Radical; NO - Nitric oxide TBars - Thiobarbituric acid reactive species

3.8 Concentration of vitamin C in *Hibiscus sabdariffa*

Table 6 presents the concentration of vitamin C in *Hibiscus sabdariffa.* In all the concentration, vitamin C was higher in the red calyces than in the white calyces (Figure 8).

Table 6 Analysis of Vitamin C in *Hibiscus sabdariffa*

	ABSORBANCE		
Conc.	Vit. C std	Red calyx	White calyx
20mg/ml	0.424	0.411	0.314
40mg/ml	0.572	0.456	0.329
80mg/ml	0.644	0.472	0.457
100mg/ml	0.730	0.486	0.475

3.9 Antimicrobial activity of white and red Hibiscus sabdariffa on multidrug resistance Staphylococcus aureus from clinical sources

According to the result obtained from this study, *Hibiscus sabdariffa* extract shows a great antimicrobial activity against 50 *Staphylococcus aureus* isolated from different clinical sources, only one of the isolates was able to resist the effect of red *Hibiscus sabdariffa* at 200mg/ml whereas 2 different isolates were able to resist the effect of both red and white hibiscus sabdariffa at 100mg/ml. The trend of the results shows that red Hibiscus sabdariffa extract shows a greater inhibitory property against isolates used than the white *Hibiscus sabdariffa* extracts. (Figure 9 & 10)

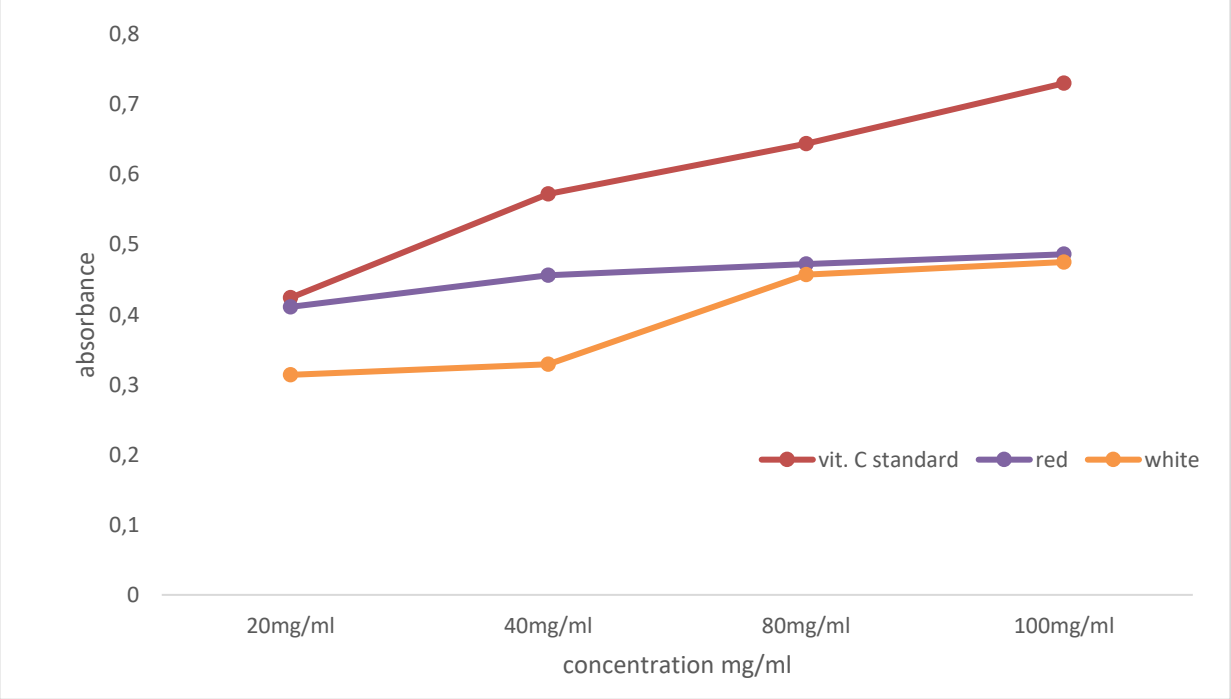

Figure 8 Graphical Representation of Vitamin C in *Hibiscus sabdariffa*

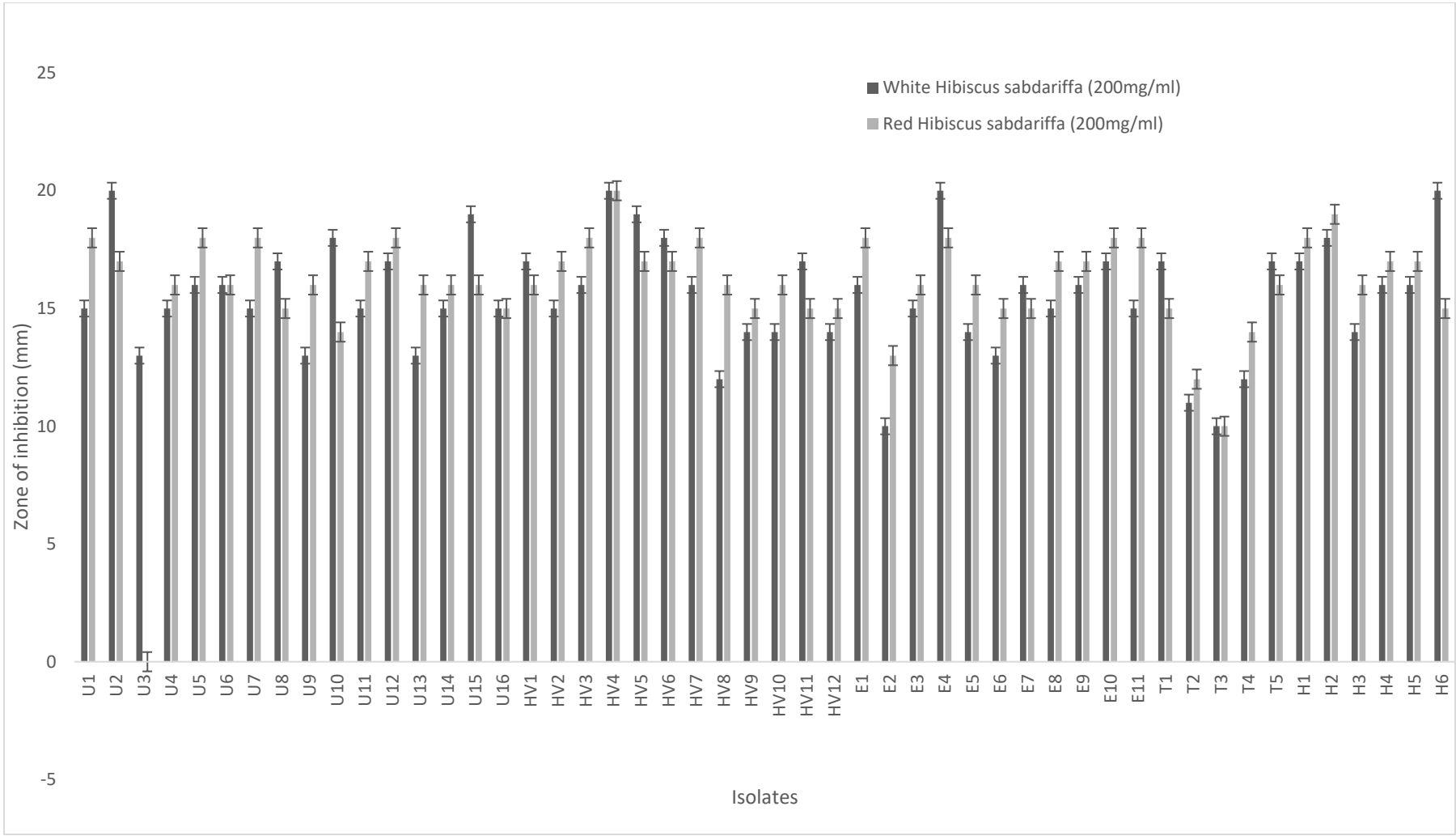

Figure 9 Zone of inhibition (mm) of ethanol extracts of white and red roselle (*Hibiscus sabdariffa)* calyces (200mg/ml) on *Staphylococcus aureus* from clinical sources

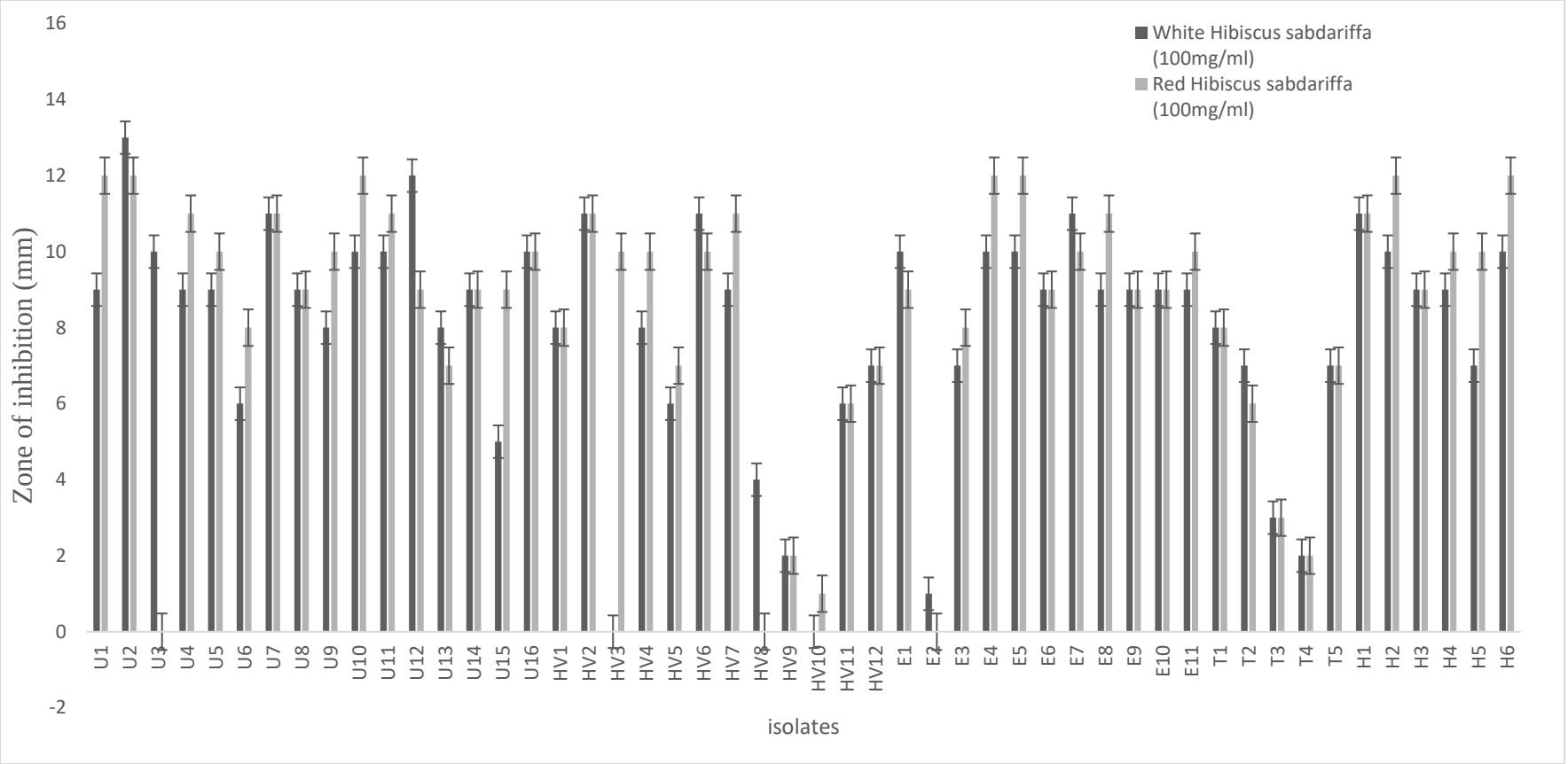

Figure 10 Zone of inhibition (mm) of ethanol extracts of white and red roselle (*Hibiscus sabdariffa)* calyces (100mg/ml) on *Staphylococcus aureus* from clinical sources

4. Discussion

The overall prevalence of bacterial isolates from out-patients attending the attending Image Diagnostic Laborstory at Rumuola from different clinical sources was 100% (n=50/50). The result was closely related to the one obtained from other localities of Port Harcourt **(Agbagwa, O and Jiriqwa, C 2015),** in their study the frequency of *Staphylococcus aureus* was 92%. On the contrary, the frequency is higher than report from previous study of Al-Hamdani and Hamadin (2012). The observed variation in proportion of *S. aureus* isolates has been attributed to differences in study design and study population (Khanal *et al.,* 2018) also the varying bacterial etiology and infection prevention practices in diverse geographical settings and at different sampling times **(Nwoire *et al.,* 2013).**

The incidence of bacterial infection was higher in female 62% (n=31/50) than in male 38% (n=19/50). Our study found that *Staphylococcus aureus* infection was common among female patients. This might be explained by the fact that traditionally, in Nigeria, females are predominantly involved in contracting bacterial infections due to their exposure to the outside environment than males. It is also speculated that male sex hormones may modify the immune response and impact contracting infection **(Soe et al., 2021)**.

According to our study association between *Staphylococcus aureus* infection and age was observed, the frequency of occurrence was higher in age of 21 to 30 years of age and a systematic literature review from India also showed similar evidence. Older age groups are more prone to get infected due to decreased host resistance and increased exposure to healthcare settings (Ghia *et al.,* 2020). Among the different clinical isolates, the highest proportion of *Staphylococcus aureus* in urine specimens with frequency of 32% (n=16/50) whereas toe had the lowest occurrence of the target organism 10% (n=5/50) the result was in harmony to the one obtained by Soe *et al.* in 2021. This may be due to urinary catheterization practice and the colonization by MRSA of indwelling urinary catheters **(Soe et al., 2021)**.

The overall multidrug resistance level of all *Staphylococcus aureus* isolates from different clinical samples was 100% (n=50/50). Among the urine samples Ofloxacin (OFL) (100%) was found to have a very good efficacy on almost all the isolates whereas 100% resistant was recorded for Cefoxiitin (FOX), Amoxillin (AUG), Ceftazidime (CAZ), Ciprofloxacin (ORX), Ceftriaxone (CTR) and Cloxacillin (CXC). Resistance to these antibiotics. Among isolates isolate from this source has been reported in two previous studies, one of which Daniyan *et al.* (2011) reported a complete resistance to Ceftriaxone (CTR) and Cloxacillin (CXC). Among the Toe samples Ofloxacin (OFL) (100%) and Gentamicin (GEN) (100%) was found to have a very good efficacy on almost all the isolates whereas the effect of others antibiotics varied. Among the hair samples Ofloxacin (OFL) (100%) was found to have a very good efficacy on almost all the isolates whereas the effect of others antibiotics varied. Result obtained from HVS sample shows that the isolates were able to resist the effect of Cefoxiitin (FOX), Amoxillin (AUG), Ceftazidime (CAZ), Ciprofloxacin (ORX), Ceftriaxone (CTR) and Cloxacillin (CXC) whereas Gentamicin (GEN) (100%) were found to be most effective. Among the ear samples one of the isolates 17% resistance was observed for Ofloxacin (OFL), the result also shows that effect of antibiotics used varied among isolates from this source. This high rate of antibiotic resistance might reflect the inappropriate use of antibiotic, lack of laboratory diagnostic tests for appropriate antibiotic selection, unavailability of guideline for the selection of antibiotics, unskilled practitioners, expired antibiotics, self-medication, counterfeit drugs, or inadequate hospital control measures.

All *Staphylococcus aureus* isolates tested in this study were completely resistant to Cefoxiitin (FOX), Ceftazidime (CAZ), Ciprofloxacin (ORX), Cefolaxime (CTR), and Cloxacillin (CXC). A similar result was also reported in a study where complete resistance of S. aureus to ceftazidime was observed. According to the result from these studies the isolates were susceptible moderately to erythromycin (ERY) and gentamicin (GEN) this result is in harmony to the one obtained by **Agbagwa and Jirigwa (2015)** there result showed that gentamicin and erythromycin was effective in the management of S. aureus from surgical wound in that locality. The high resistance trend in the sub region is indicative of high antibiotic selection pressure largely due to relatively cheap and easy availability of these agents, mostly used as first line or common choice of treatment in many healthcare settings in the sub region **(Ocan *et al.,* 2014).**

In the present study, S. aureus susceptibility to ofloxacin was 94%. This result is in agreement with reports from **Agbagwa and Jirigwa (2015)** who reported 95.65%. susceptibility to ofloxacin. Similar result was also obtained by **Abdelghafar *et al.* (2020).** Their work was in agreement with the study carried out by **Hanif *et al.* (2019),** where results obtained suggested ceftriaxone, ciprofloxacin, augumentin, ofloxacin and gentamicin as drugs of choice for *Staphylococcus aureus.*

The type of sample used had significant effect on the percentage recovery in the studied *Hibiscus sabdariffa* extracts. The yield of 25.23% was obtained for the white while the yields from red *Hibiscus sabdariffa* is 24.85%.

Phytochemicals present in red and white *Hibiscus sabdariffa* are Saponin, Alkaloid, Flavonoid, Tanin Flavonoid and Terpenoids whereas Tanin was only foundin the white *Hibiscus sabdariffa,* similar result was obtained by Adegunloye *et al*., 1996. Plant bioactive compounds/ Phytochemicals are known to for their ability protect the plant against bacterial and fungi from causing harming to the plant, they are known to play important roles in bioactivity of medicinal plants. **(Ibisanmi and Aribisala, 2022)**. Flavonoids were, however, more in the red calyces than in the white calyces. Flavonoids which are part of the phytochemical constituents of *Hibiscus sabdariffa* exhibit a wide range of biological activities, one of which is their ability to scavenge for hydroxyl radicals, and superoxide anion radicals, and thus health-promoting action. Flavonoids also exhibit anti-inflammatory, antiangionic, antiallergic, analgesic and antioxidant properties **(Hodek *et al*., 2002**). In summary, these phytochemicals were present as the concentration increases.

According to the result obtained DPPH% Inhibition poentional was noted in the *Hibiscus sabdariffa* samples used, although higher value was recorded in the red calyces than in the white calyces. Similar results were obtained by previous studoes DPPH% Inhibition (2,2 - diphenyl - 1 – Picryl – hydrazyl – hydrate) are well known antioxidant for their scavanging activities **(Subhaswaraj et al., 2017).** The higher the value the higher the antioxidant scavenging. Higher value of NO% Inhibition was recorded in white calyces, this NO% Inhibition is an important cellular signaling molecule, it also helps in modulating vascular tone, insulin secretion, airway tone and neural development while lower value was obtained in the red calyces. The result obtained from TBars (Thiobarbituric acid reactive specie) examination of the samples showns that equal value of Tbars were present in both red and white calyces. TBars as a reactive substance measures lipid peroxidation product in cells, tissues and body fluids, they are specific in their own destructive pattern of the body in which they destroy the membrane of the cells, red and white calyces have the ability to reduce Tbars **(Ibisanmi and Aribisala, 2022)**.. The lower the value, the higher the radical scavenging ability. Higher vallue of % Iron chelation was noticed in white calyces than in the other.

% Iron chelation is a therapy that prevents the accumulation of iron reaching harmful levels by matching iron intake from blood transfusion with iron excreted by iron chelation. The higher, the better. Therefore, white calyces are highly recommended for iron chelation. All the roselle calyces samples used were good source of Vitamin C, which make them a good sources of disease combating coumpounds for the body.

The effectiveness of antimicrobial agent varies with organism and type of extract used. Since microorganisms differ markedly in their susceptibility. The presence of the active principles in plants is influenced by several factors such as age of the plants, method of extraction and extracting solvent **(Ibisanmi and Aribisala, 2022)**. It was observed that phytochemical constituents of extract of both white and *Hibiscus sabdariffa* are almost the same varied. Table 1 shows the Qualitative analysis of *Hibiscus sabdariffa* (Sun dried calyces of white and red roselle). All the phytochemicals were present in the white and red calyces except Tannin which was absent in the red calyces. These bioactive compounds that are known for thei ability to protect the plant against bacterial and fungi in combating bacteria were confirmed in the extracts of sample after phytochemical screening **(Amit and Ranjeeta, 2018).** According to the result obtained from this studyHibiscus sabdariffa extract shows a great antimicrobial activity against 50 staphylococcus aureus isolated from diffrent clinical sources, only one of the isolates was able to resist the effect of red Hibiscus sabdariffa at 200mg/ml whereas 2 diffrent isolates were able to resist the effect of both red and white hibiscus sabdariffa at 100mg/ml, similar results was reported by Lim et al., 2020 red *Hibiscus sabdariffa* extract shows a greater inhibitory property against isolates used than the white *Hibiscus sabdariffa* extracts. This investigation had shown that the white and red *Hibiscus sabdariffa* extracts exhibited a potent inhibitory ability agaisnt studied isolates, although they showned less inhibitory potential on the studied in cmparison with commercial antibiotcics this might be because commercial antibioics have high degree of purity since they are prepared from synthetic materials by means of procedures expressing purity and reproducible manufacturing techniques and high fractionation which certainly will enhance antimicrobial effect than crude extracts.

5. Conclusion

The overall prevalence of *Staphylococcus aureus* from different clinical sources was high. In this study, it was observed that S. aureus was a major pathogenic agent prevalent in clinical samples. *Staphylococcus aureus* isolates tested in this study were completely resistant to Cefoxiitin (FOX), Ceftazidime (CAZ), Ciprofloxacin (ORX), Cefolaxime (CTR), and Cloxacillin (CXC), while a fewer of them showed resistance to erythromycin (ERY) and gentamicin (GEN). The high level of resistance could be associated with exposure of these drugs to isolates which may have enhanced development of resistance. There is a high level of antibiotic abuse in developing countries such as Nigeria arising from self-medication associated with inadequate dosage and failure to comply with treatment and availability of antibiotics to consumers across the counters with or without prescription. Hence, appropriate action is needed to enhance the infection control programs in healthcare settings and to focus more on the appropriate use of antibiotics. Although complete eradication of *Staphylococcus aureus* infections is not possible, proper precautions should be taken to minimize the occurrence by strictly adhering to the choice of drugs for the treatment of *Staphylococcus aureus* from *Staphylococcus aureus* infections was quite narrow especially for strains which were resistant to most classes of antibiotics which have been used previously. To prevent further emergence and spread of multi drug resistant *Staphylococcus aureus,* rational use of antibiotics and regular monitoring of antimicrobial resistance patterns are essential and mandatory.

The incidence of bacterial infection was higher in female than in male. This might be explained by the fact that traditionally, in Nigeria, females are predominantly involved in contracting bacterial infections due to their exposure to the outside environment than males. It is also speculated that male sex hormones may modify the immune response and impact contracting infection. According to our study association between *Staphylococcus aureus* infection and age was observed, the frequency of occurrence was higher in age of 21 to 30 years of age although the new born and older age groups are more prone to get infected due to under developed immunity, decreased host resistance and increased exposure to healthcare settings. Among the different clinical isolates, the highest proportion of *Staphylococcus aureus* in urine specimens with frequency whereas toe had the lowest occurrence of the target organism. This may be due to urinary catheterization practice and the colonization by MRSA of indwelling urinary catheters.

The study shows that both red and white *Hibiscus sabdariffa* contain almost the same bioactive compound except tannin present in only white *Hibiscus sabdariffa.* According to the result obtained from this study, red *Hibiscus sabdariffa* extract shows a greater inhibitory property against isolates used than the white *Hibiscus sabdariffa* extracts. This investigation had shown that the white and red *Hibiscus sabdariffa* extracts exhibited a potent inhibitory ability agaisnt studied isolates, although they showed less inhibitory potential on the studied in comparison with commercial antibiotics.

Authors' contributions

TAI: conceived and designed the experiments contributed to sample preparation. TAI & EAO & OBO & PKO & UAP: carried out the experiment, processed the experimental data, performed the analysis, drafted the manuscript, designed the figures and contributed to the interpretation of the results. TAI: involved in planning and supervised the work, contributed to the interpretation of the results, other contribution. All authors discussed the results and commented on the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare no Competing interest.

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List of abbreviation

FUTA: Federal University of Technology Akure, RTE: Ready-to-eat, DMSO: Dimethylsulfoxide, NA: Nutrient agar.

References

1. Abbas, M., Shirin, M., Patricia, K. (2011). The effect of *Hibiscus sabdariffa* on lipid profile, creatinine, and serum electrolytes: a randomized clinical trial. International scholarly research network. ISRN gastroenterology, 1–4.
2. Abdelghafar, A., Yousef, N. and Askora, M. (2020). Prevalence and antimicrobial susceptibility patterns of *Staphylococcus aureus* isolated from different clinical sources. *Zagazig J. Pharm. Sci.* 29, 1–8.

3. Adegunloye, B. J., Omoniyi, J. O., Owolabi, O. A., Ajagbonna, O. P., Sofola, O. A. and Coker, H. A. (1996). Mechanism of the blood pressure lowering effect of the calyx extract of *Hibiscus sabdariffa* in rats. *African Journal of Medical Science*, 25(3): 235-238.
4. Akanbi, O., Njom, H., Fri, H. Anthony, C. and Anna, M. (2017). Antimicrobial Susceptibility of *Staphylococcus aureus* Isolated from RecreationalWaters and Beach Sand in Eastern Cape Province of South Africa. *Int. J. Environ. Res. Public Health* 2017, 14, 1001;
5. Agbagwa, O and Jiriqwa, C. (2015). Antibiotics resistance and plasmid profile swabs in port harcourt Nigeria.*current research in bacteriology*: 8;70-76
6. Amit, I, Ranjeeta, K (2018) Phytochemical Screening, anti-microbial activity of *M charantia* (Linn). *International Journal of Pharmacological Research* **8**(7): 63-65 https://doi.org/10.7439/ijpR
7. Bao, S. and Cagan, R. (2005). Preferential adhesion mediated by Hibris and Roughest regulates morphogenesis and patterning in the Drosophila eye. *Dev. Cell*, 8(6): 925-935.
8. Bresciani, L., Calani, L., M., Mena, P., Sayegh, M., Ray, S., and Del Rio, D. (2015). Poly phenolic characterization of three food supplements containing 36 different fruits, vegetables, and berries. PharmaNutrition, 3,11-19.
9. Clinical Laboratory Standard Institute (CLSI) (2018) Performance Standards for antimicrobial susceptibity tests Document M100-527 CLSI, Wagne, PA. *Clinical Microbiology*; **45**(1):199–205
10. Daniyan et al., (2011). Antibacterial avtivity of gassia occidentalis flower on selected bacteria: asian j biomed pharm sci 1 (1)
11. Demoré, B., Mangin, L., Tebano, G., Pulcini, C. and Thilly, N. (2017). Public knowledge and behaviours concerning antibiotic use and resistance in France: A cross-sectional survey. Infection. 45:513-520.
12. Erku, D. A., Mekuria, A. B. and Belachew, S. A. (2017). Inappropriate use of antibiotics among communities of Gondar town, Ethiopia: A threat to the development of antimicrobial resistance. Antimicrobial resistance and infection control. 6:112-.
13. Fair, R. J. and Tor, Y. (2014) Antibiotics and bacterial resistance in the 21st century. *Perspect Medicin Chem.;* 6:25-64.
14. Fisher, E. A., and Paterson, G. K. (2020). Prevalence and characterization of methicillin-resistant staphylococci from bovine bulk tank milk in England and Wales. *J. Glob. Antimicrob. Resist.* 22:139– 144.
15. Ghia, C., Waghela, S. and Rambhad, G. (2020). A Systemic Literature Review and Meta-Analysis Reporting the Prevalence and Impact of Methicillin-Resistant *Staphylococcus aureus* Infection in India. *Infect. Dis. Res. Treat.* 2:13
16. Gualano, M. R., Gili, R., Scaioli, G., Bert, F. and Siliquini, R. (2015) General population's knowledge and attitudes about antibiotics: A systematic review and meta-analysis. Pharmacoepidemiol Drug Saf. 24:2-10.
17. Hanif, E. John, A. and Hassan, S.A. (2019). Evaluation of antibiotic resistance pattern in clinical isolates of *Staphylococcus aureus*. *Pak. J. Pharm. Sci.* 32, 1749–1753.
18. Hodek, P., Trefil, P. and Stiborova, A. (2002). Flavonoids-potent and versatile biologically active compounds interacting with cytochrome P450. *Chemico Biological Interactions*, 139: 1-21.
19. Ibisanmi, T. and Aribisala. O. (2022). hevaluation of antioxidant, phytochemicals and antibacterial potential of *Mormordica charantia* (linn) against pathogenic bacteria isolated from ready to eat food sold in akure metropolis, nigeria. Bacterial empire: 5(1), e389
20. Jagetia, G. C., Venkatesh, P. and Baliga, M. S. (2004). Evaluation of the radio-protective effect of bael leaf (*Aegle marmelos*) extract in mice. *Int J Radiat Biol,* 80:281-290.
21. Khanal, L., Adhikari, R. and Guragain, A. (2018). Prevalence of Methicillin Resistant *Staphylococcus aureus* and Antibiotic Susceptibility Pattern in a Tertiary Hospital in Nepal. *J. Nepal Health Res. Counc.* 23:552
22. Lee, H. (2003). Methicillin (Oxacillin)-Resistant *Staphylococcus aureus* Strains Isolated from Major Food Animals and Their Potential Transmission to Humans. *Applied and Environmental Microbiology,* 69(11):6489–6494.
23. Minotti, G. and Aust, S. D. (1987). An investigation into the mechanism of citrate- Fe^{2+} dependent lipid peroxidation. *Free Radical Bio. Med,* 3: 379 387.
24. Nwoire, A., Madubuko E., Eze, U. A., Oti-Wilberforce, R., Azi S., Ibiam, G., Egwu, H., Okereke, E. and Obi, I. (2013). Incidence of *Staphylococcus aureus* in clinical specimens in Federal Teaching Hospital, Abakaliki, Ebonyi State. *Merit Research Journal of Medicine and Medical Sciences* Vol. 1(3) :043-046
25. Olusola-makinde, Olabanji, OB, Ibisanmi, TA (2021) Evaluation of the bioactive compounds of Vernonia amygdalina Delile extracts and their antibacterial potentials on water-related bacteria. Bulletin of the National Research Centre 45(1):191
26. Ocan, M, Bwanga, F, Bbosa, G.S, Bagenda, D, Waako, P, Ogwal-Okeng, J. and Obua, C. (2014). Patterns and predictors of self-medication in northern Uganda. *PLoS One*, 9:92323–30.
27. Pavydė, E., Veikutis, V., Mačiulienė, A., Mačiulis, V., Petrikonis, K. and Stankevičius, E. (2015). Public Knowledge, Beliefs and Behavior on Antibiotic Use and Self-Medication in Lithuania. International journal of environmental research and public health. 12:7002-7016.
28. Pulido, M. (2002). Estructuras de Coaliciones y Aplicaciones de la Teoría de Juegos. Tesis Doctoral, Universidad Miguel Hernández, Elche, Spain.
29. Puntel, R. L., Nogueira, C. W. and Rocha, J. B. T. (2005). Krebs cycle intermediates modulate thiobarbituric acid reactive species (TBARS) production in rat brain in vitro. *Neurochem. Res,* 30(2), 225 235.
30. Robert A, Nguyen Y, Bajolet O, Vuillemin B, Defoin B, Vernet-Garnier V, et al. (2017). Knowledge of antibiotics and antibiotic resistance in patients followed by family physicians. Med Mal Infect. 47:142-151.
31. Saba, C., Amenyona, J. and Kpordze, S. (2017). Prevalence and pattern of antibiotic resistance of *Staphylococcus aureus* isolated from door handles and other points of contact in public hospitals in Ghana. *Antimicrobial Resistance and Infection Control* (2017) 6:44
32. Schnitt, T., Lienen, H., Wichmann-Schauer, and Tenhagen, B. (2021). The occurrence of methicillin-resistant non-aureus staphylococci in samples from cows, young stock, and the environment on German dairy farms. *J. Dairy Sci.* 104:4604–4614
33. Shah, D., Wasim, S. and Farhan E. (2017). Comparison of Oxacillin and Cefoxitin for the Detection of mecA Gene to Determine Methicillin Resistance in Coagulase Negative Staphylococci (CoNs). *Journal of the College of Physicians and Surgeons Pakistan*. 27(8):520-522
34. Singleton, V. L., Orthofer, R., Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol,* 299: 152-179.
35. Soe, P., Han, W., Sagili, K., Satyanarayana, S., Shrestha, P. Htoon, T. and Tin, H. (2021). High Prevalence of Methicillin-Resistant *Staphylococcus aureus* among Healthcare Facilities and Its Related Factors in Myanmar (2018–2019). *Trop. Med. Infect. Dis*. 6:70.
36. Wang, L., Hu, C., and Shao, L. (2017). The antimicrobial activity of nanoparticles: present situation and prospects for the future. *Int. J. Nanomed.* 12, 1227–1249.
37. Weiner, M. A. (1980). Earth Medicine – Earth Food Remedies, Drugs and Natural foods of North American Indians.