



Comparative Study of the Effects of Plant Extracts in Traditional Medicine and Antibiotics on *Staphylococcus aureus* Isolated from Patients in Hospital Medical Care in Ekiti State of Nigeria

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ABSTRACT

Aims: Hospitalized patients are often immunocompromised as a result of invasive medical examinations and treatments. Of course, the tendency to do care practices for these patients and the hospital environment may facilitate the transmission of pathogenic microorganisms among them.

Materials & Methods: The study population and health status of volunteer patients were collected using a pretested questionnaire and patients information available in hospital files. A total of 102 samples were collected from patients' wounds, noses, ears, and urine and microbiologically analyzed for the presence of *Staphylococcus aureus* species by plating on Mannitol Salt agar. Colonies were purified by streaking on Nutrient agar, Gram stained, and tested for the presence of coagulase and the capability of growing on 3–5% salt concentration.

Findings: Male patients (51.3%) were more infected by *S. aureus* strains than female patients (48.7%). In terms of age, *S. aureus* infection rate was higher in patients within the age ranges from 17-50 years (56.32%) and lesser in patients within the age ranges from 51-100 years (43.68%). Genogram of the isolates indicated two major groups based on the genotypic responses to the antibiotics and extracts (This means the possible separation of the isolates into family groups according to their responses to antimicrobial agents). The prevalence of *S. aureus* colonization was higher in male patients.

Conclusions: Observed indices suggest that sex could be considered as a risk factor for *S. aureus* infection in patients. In addition to antibiotics, plants extracts could be used as an effective alternative for the treatment of *S. aureus* infections to control resistant *S. aureus* species.

Keywords: Hospitals, *Staphylococcus aureus*, Patients, Epidemiology, Susceptibility, Nigeria

CITATION LINKS

[1] Self-disinfecting surfaces: Review of current methodologies and... [2] Hand hygiene: Back to the basics of infection... [3] Bacteria isolated from surgical infections and its... [4] Antimicrobial effect of mango (*Mangifera indica* Linn.) leaf extract against antibiotic sensitivity and multi-drug resistant *Salmonella typhi* ... [5] Species-specific uptake of DNA by... [6] Genetic fingerprinting and phylogenetic diversity of... [7] NTSYS-pc: numerical taxonomy and multivariable ... [8] Clinical and Laboratory Standards... [9] Urinary tract infections in adults. *Am Fam Physician*. [10] Uropathogens and their susceptibility ... [11] Antimicrobial resistance of *Staphylococcus aureus*... [12] Urinary tract infection in Okada village: Prevalence and antimicrobial [13] Incidence and antibiotic susceptibility pattern of *Staphylococcus*... [14] Antimicrobial resistance in *Escherichia coli* strains from... [15] Urinary pathogens and antimicrobial susceptibility: A retrospective... [16] Antibiotics sensitivity and resistance patterns of... [17] Antimicrobial susceptibility pattern of urinary tract ... [18] Antimicrobial resistance profile of *Staphylococcus*... [19] Frequency of MRSA isolates in mobile phones, ears, and... [20] Prevalence of *Staphylococcus aureus* nasal colonization in the... [21] Occurrence of *Staphylococcus aureus* in hospital environment and... [22] Antibiotic resistant *Staphylococcus aureus* in... [23] Antimicrobial constituents of the leaves o... [24] Postoperative nosocomial infections and antimicrobial resistance ... [25] Bacterial isolates and their antibiotic... [26] Antibigram of clinical isolates from ... [27] On expressing the antibacterial activity of ... [28] Honey, milk, and antibiotics. [29] Fungal infection in surgical patients. *Am J* ... [30] Risk factors for fungemia in children infected with human ... [31] A survey of antibiotic resistant *Staphylococcus aureus* strains from ... [32] The use and abuse of antibiotics. *Nig Med Pract*. [33] Associated risk factors and pulsed field gel electrophoresis of nasal isolates of... [34] Phage typing, PCR amplification for *mecA* gene, and antibiotic resistance patterns...

Introduction

Patients and people with poor hygiene practices are the major sources of pathogens causing pathogenic and opportunistic infections, which commonly populate any hospital environment and cause nosocomial infections in hospitalized patients. Hospitalized patients are often immunocompromised as a result of invasive medical examinations and treatments. The care practices and microbial environment of hospitals are among the factors that could enhance pathogenic cross infections amidst hospitalized patients [1].

The high population of nosocomial pathogens in hospitalized patients is certainly associated with the poor quality of health services delivery. In this regard, many factors such as overcrowding, poverty, and poor sanitary conditions have been reported to contribute immensely to the regularity and continuity of nosocomial infections [2]. Hospitalized patients with poor health status as a result of microbial infections are responsible for the occurrence of cross infections among newly admitted patients and hospitals staff. Overcrowding of hospitals, frequent transmission of sick patients from one unit to another one, and the aggregation of patients who are highly susceptible to infection in some parts of hospitals are among the factors facilitating the proliferation of nosocomial infections [3]. Microbial flora may contaminate objects, devices, and materials, which may subsequently be contacted with exposed body sites of patients.

Staphylococci are a major group of bacteria inhabiting the skin. Most of which are harmless and reside normally on the skin glands and mucous membrane of humans, mammals, birds, and other animals [4].

Since the rate of disease progression around us demands immediate attention, there is a need for advancement in alternative control measures.

Objectives: As most synthetic antibiotics are currently ineffective against a large number of pathogenic microorganisms, the aims of this research were centered on *Staphylococcus aureus* strains isolated from hospitalized patients' clinical samples in a comparative study on the effects of plant extracts in traditional medicine and antibiotics in modern medicine.

Materials and Methods

Samples collection and preparation: The study population and health status of volunteer patients were collected using a pretested questionnaire and patients' information available in hospital files, including age, sex, ward of admission, location, HIV serostatus, plants used for treatment, frequently eaten foods, and length of hospital stay.

A total of 102 samples were collected from wounds, noses, ears, and urine of both male and female patients with different age groups, hospitalized in different wards of Ekiti State University Teaching Hospital (EKSUTH), State Specialist Hospital Ikere-Ekiti (SSHIE) and General Hospital Iyin-Ekiti (GHIE) in Ekiti State Nigeria. The samples were transported on ice packs within a period of 2 hours to the Microbiology Research Laboratory in the Department of Biological Sciences, Afe Babalola University, Ado Ekiti, Nigeria, for microbiological analysis. Patients' urine samples were aseptically collected into the sterile bottles suitable for sampling, while ear, nose, and wound swabs were collected using a sterile swab stick. The samples were rinsed in physiological saline, and 1 mL of each was plated on Manittol Salt agar. Resultant colonies were purified, Gram stained, and tested for the presence of coagulase and the capability of growing on 3-5% salt concentration.

Extraction and purification of Genomic DNA: About 51 to 100 mg (wet weight)

of bacterial (*S. aureus*) cells previously suspended in 200 μ L of isotonic buffer were added to a ZR Bashing Bead™ Lysis tube, and 750 μ L of lysis solution was also added to the tube. Tube was secured in a bead beater fitted with a 2 mL tube holder assembly and processed at maximum speed for 5 min. The ZR Bashing Bead™ Lysis tube was centrifuged at $\geq 10,000\times g$ for 1 min in a micro centrifuge. Then 400 μ L of supernatant was transferred to a Zymo-spin™ IV Spin Filter (orange top) in a collection tube and centrifuged at 7,000 rpm for 1 min, and 1200 μ L of bacterial DNA binding buffer was added to the filtrate in the collection tube. Next, 800 μ L of the mixture was transferred to a Zymo-spin™ IIC Column in a collection tube and centrifuged at $10,000\times g$ for 1 min, flow from the collection tube was discarded, and the step was repeated. About 200 μ L of DNA pre-wash buffer was added to the Zymo-spin IIC in a new collection tube and centrifuged at $10,000\times g$ for 1 min. The Zymo-spin™ IIC Column was transferred to a clean 1.5 mL micro centrifuge tube, and 100 μ L DNA Elution buffer was added directly to the column matrix. It was centrifuged at $10,000\times g$ for 30 sec to elute the DNA. Ultrapure DNA was obtained and used [5].

RAPD PCR analysis: Random Amplified Polymorphic DNA (RAPD) is a multiplex marker system, in which a single- primer PCR is conventionally used for the amplification of random DNA fragments. RAPD PCR analysis used in this research was performed according to Onasanya et al. (2003) [6]. Data were analyzed using NTSYS-pc statistical software version 2.02i (Rohlf, 1989) [7]. DNA primers used in this study were purchased from Operon Technologies (Alemada, California, USA). DNA primers tested were with 10 nucleotide long. In order to test reproducibility and eliminate sporadic amplification products, two concentrations of each DNA (24 and

96 ng per reaction) were used. Also, the ability of 13 primers in amplifying the *S. aureus* isolates DNAs was evaluated. Of 13 screened primers, 6 were found to be useful because they showed polymorphism. They were used to amplify the DNA of 20 *S. aureus* species isolated and identified from the human clinical samples. Amplification was performed in a 25 μ L reaction mixture containing genomic DNA; IX reaction buffer (Promega); 100 μ M of each dATP, dCTP, dGTP, and dTTP; 0.2 μ M Operon random primer; 2.5 μ M MgCl₂; and 0.2 μ L (1 unit) of Taq polymerase (Boehringer, Germany). Each reaction was performed with a single primer. The reaction mixture was overset with 50 μ L of mineral oil to prevent from the evaporation of the mixture. Amplification was performed in a thermowell microtiter plate (Costa Corporation) using a (Perkin Elmer) programmable Thermal Controller model 9600. The cycling program was set as follows: an initial denaturation step at 94 °C for 3 min, followed by 45 cycles at 94 °C for 1 min (denaturation), annealing at 40 °C for 1 min, extension at 72 °C for 2 min, and a final extension at 72 °C for 7 min. Amplified DNA products were analyzed by electrophoresis method in 1.4% agarose gel using TAE buffer (45 Mm Tris-acetate, 1Mm EDTA, pH 8.0) at 100 V for 2 hrs. A 1 kb ladder (Life Technologies, Gaithersburg MD USA) was used as a molecular size marker. Gels were stained with ethidium bromide solution (0.5 μ g/mL) and visualized using UV light and photographed for banding patterns.

Plant Extracts: The leaves of *Terminalia catappa* (Almond), *Mangifera indica* (Mango), and *Acalypha wekesiana* (*Acalypha*) obtained from the campus of Afe Babalola University, Ado Ekiti were authenticated by Mr. Esimekhuai Donatus from the university of Ibadan, Department of Botany. The voucher specimens of the leaves samples were T. cattapa (UIH 22567),

M. indica UIH 22568, and *A. wekesiena* (UIH 22569), deposited at the University of Ibadan Herbarium. The leaf samples were washed with water and rinsed in deionized water. Therefore, the leaves were ground into fine infusion to obtain crude aqueous extract of the leaf samples. The obtained crude extracts were subjected into filtration and kept in sterile bottles. The filtrates were tested for sterility by streaking on freshly prepared nutrient agar plates before use in sensitivity test.

Antibiotics: The multiple antibiotic discs (Oxoid, (England) used in this study contained Ofloxacin (OFL), Augmentin (AUG), ceftazidime (CAZ), Cefuroxime (CRX), Gentamicin (GENT), Ceftriaxone (CTR), Erythromycin (ERY), and Cloxacillin (CXC).

Inhibitory Tests: Inhibitory assay was performed for both the plants extracts and antibiotic discs using agar diffusion method. A 50 µL volume of each extract with 100 mg/mL concentration was filled into the wells to evaluate their inhibitory effect.

Effectiveness of the antibacterial agents was shown by zone of inhibition, which was interpreted as either resistance, intermediate, or susceptible as described by Clinical Laboratory Standards Institute (CLSI) [8].

Findings

Collected Samples: A total of 102 human clinical specimen were collected from both male and female patients with different age groups, admitted to the studied hospitals. About 33 urine samples, 14 ear swabs, 4 nose swabs, and 5 wound swabs were obtained from the male patients, while 25 urine samples, 10 ear swabs, 6 nose swabs, and 5 wound swabs were obtained from the female patients. Ekiti State University Teaching Hospital (EKSUTH) had admitted the highest number of patients colonized with *S. aureus*; hence, the population

of patients admitted in this hospital for treatment was more than that of the State Specialist Hospital Ikere-Ekiti (SSHIE) and General Hospital Iyin-Ekiti (GHIE).

As shown in Figure 1, the results indicated that out of 56 urine samples analyzed, *S. aureus* infection rate was higher in male (57.1%) than in female (43.1%) patients. In terms of age, 51.7% of patients with the age ranges from 17-50 years were more at risk of staphylococcal infections, followed by 44.7% of patients with the age ranges from 51-100 years, having *S. aureus* colonization in their urine. The number of *S. aureus* strains causing ear infection were recorded to be higher in male (58.3%) than in female (41.7%) patients. In terms of age, *S. aureus* infection was more in 15-50 year old patients (60%) than in 51-100 year old patients (40%). Nose colonization with *S. aureus* strains was observed in 60 and 40% of female and male patients, respectively. However, patients with the age ranges from 17-50 years had *S. aureus* infection in their noses (60%) more than patients with the age ranges from 51-100 years (40%). *S. aureus* infection in wounds was observed in both male and female patients equally (50%). However, wound colonization with *S. aureus* was observed more in 17-50 year old patients (60%) than in 51-100 year old patients (40%).

Phenotypic grouping was performed by transforming the positions of unequivocally scorable RAPD bands into a binary character matrix. Compilation of pairwise distance matrices was made possible by the Numerical Taxonomy System (NTSYS) software ver. 2.0 and using the simple matching coefficient constructed similarity matrix. By this, cluster and dendrogram were produced (Table 1). These results made it possible to deduce the population structure across the studied areas in Ekiti State and the *S. aureus* isolates responses to

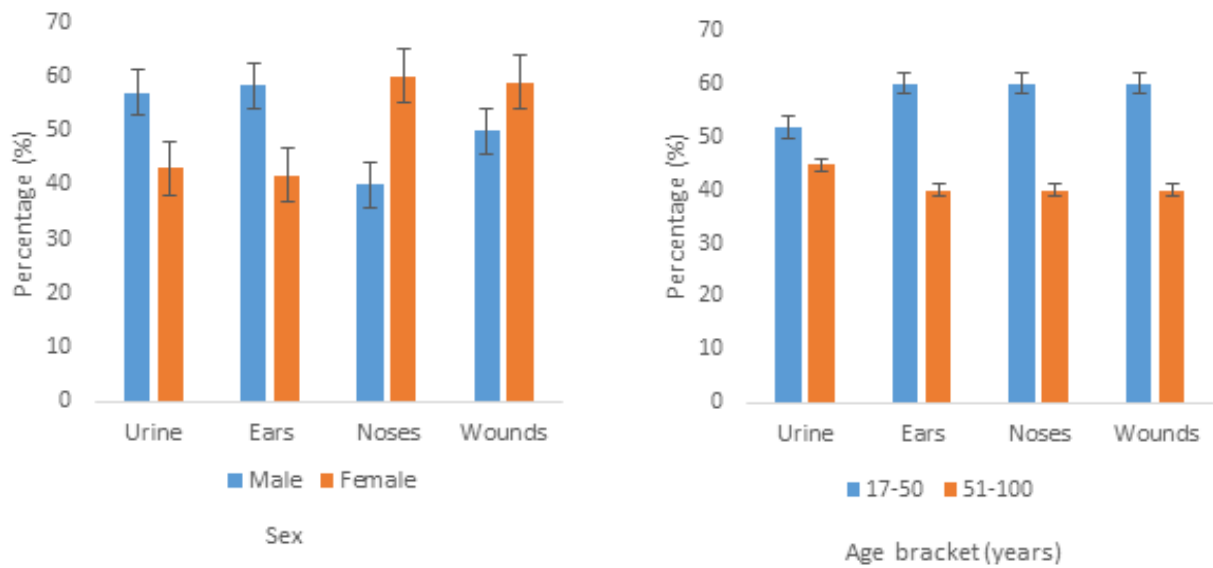


Figure 1) *S. aureus* infection rates in relationship to the patients' sex and age

Table 1) Relationship between the ERG and ARG *S. aureus* isolates population structure across the three locations in Ekiti States

Typing	Main Group	Subgroup	Resistant Isolates	Isolates Origin and Distribution			% Occurrence
				EKSUTH	SSHIE	GHIE	
ERG	ERG1	ERG1a	12	11	1	-	60
		ERG1b	6	6	-	-	30
	ERG2	-	2	1	1	-	10
ARG	ARG1	ARG1a	6	5	1	-	30
		ARG1b	4	3	1	-	20
		ARG1c	1	1	-	-	05
	ARG2	ARG2a	7	5	2	-	35
		ARG2b	2	2	-	-	10

Legend: ERG = Extract resistant group, ARG =Antibiotic Resistant group, EKSUTH =Ekiti State University Teaching Hospital, SSHIE = State Specialist Hospital Ikere-Ekiti, GHIE = General Hospital Iyin-Ekit

the antibiotics and plant extracts. According to the isolates phenotypic grouping, the extract-resistant group (ERG) was divided into two main groups (ERG1 and ERG2). ERG1 was further sub-grouped into ERG1a and ERG1b. ERG1a had 12 *S. aureus* isolates resistant to the employed plant extracts, of which 11 isolates were from EKSUTH, and 1 isolate was from SSHE, accounting for 60% occurrence. ERG1b had 6 identified resistant isolates, which were recorded to be only from EKSUTH samples, accounting for 30% occurrence. ERG2 had 2 identified resistant isolates (one from EKSUTH and

one from SSHIE samples), accounting for 10% occurrence. The antibiotic-resistant group (ARG) had two main groups (ARG1 and ARG2). ARG1 was further sub-grouped into ARG1a, b, c, and d. In ARG1a, 6 isolates were resistant to the employed antibiotics, of which 5 isolates were from EKSUTH, and only one isolate was from SSHIE. ARG1b had 4 resistant isolates, of which 3 isolates were from EKSUTH, and 1 isolates was from SSHIE. ARG1c had only 1 resistant isolate isolated from EKSUTH, totally accounting for 55% occurrence. ARG2 had 2 subgroups (ARG2a and ARG2b). ARG2a had 7 resistant isolates,

of which 5 isolates were from EKSUTH samples, and 2 isolates were from SSHIE samples. ARG2b had 2 resistant isolates, which were only from EKSUTH samples with a total occurrence of 45%.

Antibiotics inhibition: Figure 2 shows the similarity between the antibiotics reactions with respect to the resistance behavior of 20 *S. aureus* isolates. The clusters were made up of 3 different antibiotic groups, namely Group 1 (Grp1), Group 2 (Grp2), and Group 3 (Grp3) based on the reaction of the 8 antibiotics against the 20 *S. aureus* isolates. Antibiotics of Group 1 included OFL, GENT, and ERY. Antibiotics of Group 2 included CRX and CTR; and antibiotics of Group 3 included AUG, CAZ, and CXC. Antibiotics OFL and GENT which were both of Group 1 behaved in the same way in their reaction

so that a high level of isolates susceptibility was observed to these antibiotics; however, isolates expressed a similar resistance behavior in their reaction to CAZ and CXC antibiotics both of Group 3. Antibiotics 1 and 2 of Grp1 and Antibiotics 3 and 4 of Grp3 were identical in their reaction on the 20 *S. aureus* isolates with 25% similarity.

Plants' extracts inhibition: Figure 3 presenting cluster E shows how the leaves extracts of *T. catappa*, *M. indica*, and *A. wikesiena* inhibited 20 isolated *S. aureus* strains. Two major groups of ERG1 and ERG2 were formed by 55% coefficient of similarity. From the first group, 2 subgroups were emerged, including ERG1a and ERG1b. ERG1a consisted of 12 isolates including 1-MSWB5U, 2-FSWB4U, 3-FSWB4iiU, 4-MMWB1iU, 17-FMWB3i, 18-MSWB6iiWS,

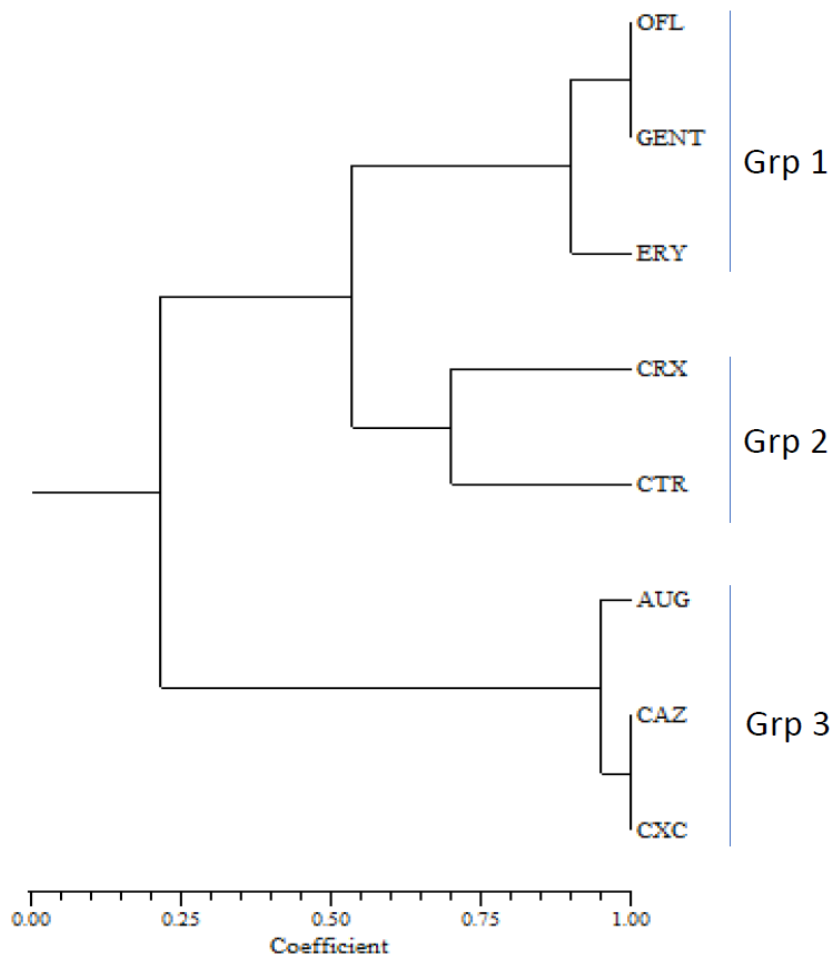


Figure 2) Similarity between the antibiotics reactions with respect to the resistance behavior of 20 *S. aureus* isolates

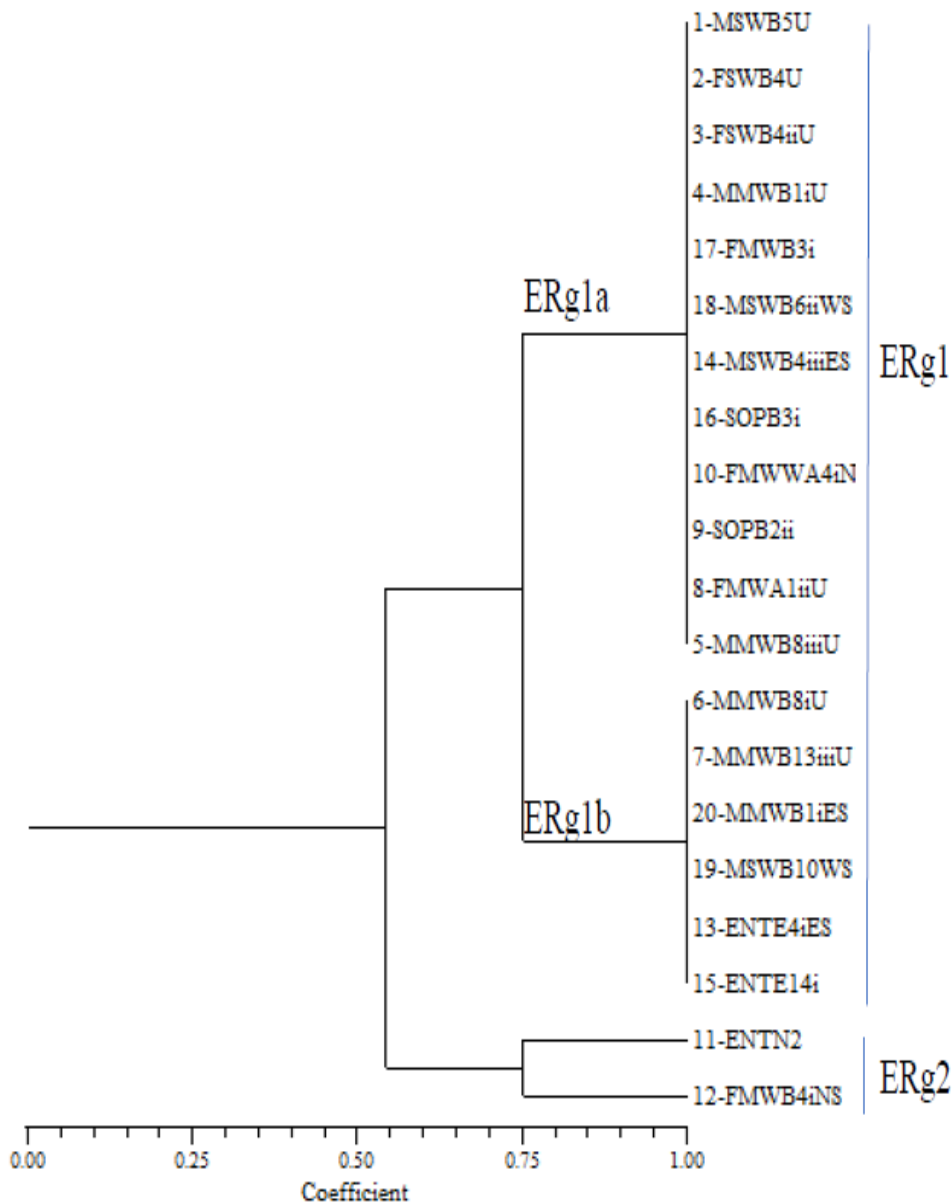


Figure 3) Cluster E showing the reaction of 20 *S. aureus* isolates against the three plant leaves extracts

14-MSWB4iiiES, 16-SOPB3i, 10-FMWW A4iNS, 9- SOPB2ii, 8- FMWA1iiU, and 5-MMWB8iiiU). Aqueous leaves extracts exhibited the same inhibitory effects on these isolates. ERG1b consisted of 6 isolates including 6-MMWB8iU, 7-MMWB13iiiU, 20-MMWB1iES, 19-MSWB10WS, 13-ENTE4 iES and 15-ENTE14i, reacting in the same way to the extracts. ERG2 consisting of two isolates of 11-ENT N2 and 12-FMWB4iNS) reacted differently to the extracts. ERg1b consisting of 6 isolates (6-MMWB8iU,

7-MMWB13iiiU, 20-MMWB1iES, 19-MSWB1 0WS, 13-ENTE4iES and 15-ENTE14i) also reacted in the same way to the extracts. ERg2 consisting of two isolates of 11-ENT N2 and 12-FMWB4iNS reacted differently to the extracts.

Discussion

S. aureus may colonize in any part of human body, but in this study, the particular attention was on its colonization in patients' noses, wounds, ears, and urine.

The pathogens commonly isolated from urine are *Klebsiella* sp, *Escherichia coli*, *S. saprophyticus*, *Enterococcus* sp, and *Proteus mirabilis* [9-10]. Of all the specimens collected from male patients, urine was ranked as the second most site of *S. aureus* colonization by 51.7%, while in female patients, urine was ranked as the third site of *S. aureus* colonization by 43.1%. In general, males' urine samples were more colonized with *S. aureus* strains than that of females. The reason could be the particular health challenges which may be related to more *S. aureus* colonization. However, increasing level of *S. aureus* prevalence among men could result in its easy transfer to women due to the relationship between men and women. Urine samples of 17–50 year old patients were more prone to *S. aureus* infection than that of the 51–70 year old patients. Onanuga et al. (2012) reported that subjects in the age groups of 21–30 years were more at risk of infection [11]. However, *S. aureus* complications in urine samples have recently been reported by other researchers [12-14]. Consistent with the present study findings are the reports of Akerele et al. (2000), recording 35.6% *S. aureus* infection in Benin City, Nigeria [15]. Also, consistent with the present study findings are the findings of Okonko et al. (2009) in Ibadan and Nigeria and Manikandan et al. (2001) in India, emphasizing that *S. aureus* is the second most prevalent pathogen in Urinary Tract Infections (UTIs) [16-17].

The ears of male patients had the highest prevalence (58.3%) of *S. aureus* infection among the human clinical samples collected. Deyno et al. (2017) reported 31.8% *S. aureus* infection in ear among the male subjects [18]. Almagadam et al. (2013) as well recorded 100% in their study [19]. Among the patients, 15–50 year old male patients were more at risk of *S. aureus* infection in their ears.

The obtained results in this study showed

that 60 and 40% of the female and male patients had nasal infection caused by *S. aureus*, respectively. This study showed that the prevalence of *S. aureus* infection in the nostrils of 17-50 year old patients was 60%. This finding is in agreement with previous reports by Kuehnert et al. (2006) [20]. Meanwhile, Plat and Rai (2007) reported 43.8% *S. aureus* nasal colonization rate among the staff of a teaching hospital in Nepal, a value closer to what was obtained in this study [21]. Also, in Abia State of Nigeria, Chigbu and Ezeronye (2003) reported a rate of 50% for nasal colonization in both hospital and non-hospital subjects [22], while Adesina et al. (2000) reported a much lower infection rate (14.0%) for nasal colonization in medical students in Lagos State, Nigeria [23]. These variations may be attributed to the characteristics of the population under study and the attributes of *S. aureus* distribution in different locations. Other factors causing variations in the results may be the type of sampling and culture techniques used, as reported by Chigbu and Ezeronye (2003) [22]. In this study, *S. aureus* infection in wounds was significant in no sex as equal rate (50%) of infection was observed. However, in age distribution, *S. aureus* infection was observed more in the age ranges from 17–50 years (60%) than in the age ranges from 51–100 years (40%). The high rate of infection in 17–50 year old patients could be due to their working conditions, where a part of their daily activities in the active phase of life could expose them to *S. aureus* colonization. The nutrient present in wounds and *S. aureus* pyogenic nature in wound sepsis are among the factors contributing to their high number in wound. High isolation rate of *S. aureus* from surgical sites and patients with wound pus discharge have also been reported [24-26]. The observed findings in this study about the distribution of *S. aureus* infection in two sexes are consistent with

the findings of Onanuga et al. (2012) [11]. Modern science and technology have achieved success in new drug discovery; nevertheless, pathogens with high frequency develop means to resist against some synthesized drugs used for their treatment. Extensive use of broad-spectrum antibiotics results in the widespread prevalence of nosocomial infections caused by multidrug resistant pathogens (Chikere et al. (2008)[26]. Therefore, the high prevalence of *S. aureus* strains in this study and their resistance pattern to most of the antibiotics used make it necessary to pay needed attention in order to aware the world about the adverse effects of multidrug resistant microorganisms. This aim could be achieved by finding new measures to combat them. However, some ineffective synthesized drugs are of more recognition than those faced with in traditional folklore. The prescription and use of these ineffective drugs may favor the emergence of hospital and community acquired resistant strains of pathogens. Studied have revealed that resistance has been almost observed to all antibiotic groups [27]. In addition, adverse side effects of some antibiotics on the host have been reported in many studies, including hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immunosuppression, and allergic reactions [28], those which have not been reported for plant extracts. The number of multi-drug resistant microbial strains and the emergence of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to the indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation, and the epidemics of HIV infection [29, 30]. Among the 20 isolated *S. aureus* species, some were found to be resistant only to *A. wikesiena*, among the employed plant extracts. This suggests that the isolates

need to be exposed to a wide range of plant extracts instead of only 3, compared to the 8 antibiotics employed. Doing so would produce a better result in comparison with the existing antibiotics and would be a solution for discovering alternatives to many antibiotics that are ineffective on *S. aureus* origin infections.

In comparison, the inhibitions recorded with standard reference antibiotics were comparable to those observed with various leaves extracts; although some of the antibiotics such as OFL, GEN, ERY, CRX, GEN, and ERY exhibited higher inhibitory effect on the tested isolates, compared to *A. wikesiena* leaf extract.

Among the antibiotics used, OFL inhibited most the *S. aureus* isolates growth. This apparently high level of susceptibility to OFL suggests that it could be considered as a drug of choice for treating infections caused by *S. aureus* in the study area, especially at the present time that *S. aureus* strains are resistant to many commonly used antibiotics. Similar result about the high susceptibility of *S. aureus* strains to OFL was reported by Uwaezuoke and Aririatum (2004) [31].

The isolated *S. aureus* strains were resistant to CXC, CAZ, AUG, and ERY. The high level of resistance observed could be due to early exposure of these drugs to the isolates which may have enhanced resistance. This suggests that there is a high level of antibiotics abuse in the studied areas, which could be due to self-medication, inadequate dosage, and failure to comply with treatment, and availability of antibiotics to consumers across the counters with or without prescription, as reported by Odugbemi (1981) [32].

Identification of antibiotic resistant isolates using molecular techniques such as 16S rRNA, Random Amplification of Polymorphic DNA (RAPD), and plasmid profile causing infectious processes is usually essential for effective antimicrobial and supportive

therapy^[33]. Initial treatment may be empirical based on the microbiological epidemiology of the infection and the patient's symptoms^[34]. From the epidemiological point of view, in this study, it was observed that all the strains isolated from patients receiving treatment from EKSUTH exhibited a similar behavior in their resistance to the antibiotics used. There was a relationship between the ERG and ARG *S. aureus* isolates population structure across the three studied locations in Ekiti State.

The use of molecular methods (RAPD- PCR) confirmed the obtained results and that the isolates responding similarly to certain antibiotics and plant extracts shared similar genotypes and should therefore be grouped in the same clusters. By virtue of this analysis, it was possible to group isolates with similar genotypes and similar resistance behavior to the antibiotics and plants extracts in the same clusters.

Conclusions

Based on the obtained results in this study, it could be deduced that *S. aureus* infection is high and common among the patients. Infection rate was higher in the age groups below 50 years, and the risk factors were mainly poor hygiene and socio economic conditions. The differences observed in the colonization rates between the two sexes indicated that sex could be considered as a risk factor for *S. aureus* infection among patients. This was the first study reporting the epidemiology of *S. aureus* strains isolated from patients attending to hospital treatments in Ado Ekiti, Nigeria. This study provided information about the *S. aureus* infection rate in different age groups of patients receiving hospital treatments and also about the employed plant extracts for effective treatment of *S. aureus* infections, in addition to the existing traditional medication.

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Ethical Permissions: The protocol was confirmed in ethical committee, and the authors of this manuscript observed ethical issues (EKSUTH/A67/2016/10/021)

Conflict of interests: The authors declare that there is no conflict of interest.

Authors' contribution: MOO and FCA conceived the research idea, designed, and wrote the first draft of the manuscript. FCA managed the literature search. All authors read and approved the final manuscript.

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References

1. Weber DJ, Rutala WA. Self-disinfecting surfaces: Review of current methodologies and future prospects. *Am J Infect Control*. 2013; 41(5):S31–5.
2. Mathur P. Hand hygiene: Back to the basics of infection control. *Indian J Med Res*. 2011; 134(5):611–20.
3. Shinagawa N, Taniguchi M, Hirata K, Furuhashi T, Fukuhara K, Mizuguchi T. Bacteria isolated from surgical infections and its susceptibilities to antimicrobial agents-special references to bacteria isolated between April 2010 and March 2011. *Japan J Antibiot*. 2014; 67(5):293–334.
4. Harris L, Foster S, Richards S, An Hannan Abdul, Asghar Samra, Naeem Tahir Ullah, Muhammad Ikram. Medicinal effects of *Mangifera indica*. *Pak Jour Pharm Sci*. 2002;26:4-715.
5. Elkins C, Thomas CE, Selfert HS, Sparling PF. Species-specific uptake of DNA by Gonococci is mediated by

- a 10-base-pair sequence. *J Bacteriol.* 1999; 173(12):3911-3.
6. Onasanya A, Mignouna HD, Thottappilly G. Genetic fingerprinting and phylogenetic diversity of isolates of *Staphylococcus aureus* from Nigeria. *Afr J Biotechnol.* 2003; 2(8):246-50.
 7. Rohlf FJ. NTSYS-pc: numerical taxonomy and multivariable analysis system. State university of New York: Stony brooks; 1989.
 8. Clinical and Laboratory Standards Institute (CLSI). M02-A11: Performance standards for antimicrobial disc susceptibility tests; Approved standard. 7th Edition. Wayne: Clinical and Laboratory Standards Institute; 2012.
 9. Orenstein R, Wong ES. Urinary tract infections in adults. *Am Fam Physician.* 1999; 59(5):1225-34.
 10. Khan SW, Ahmed A. Uropathogens and their susceptibility pattern: A retrospective analysis. *J Pak Med Assoc.* 2001; 51(2):98-9.
 11. Onanuga A, Awhowho GO. Antimicrobial resistance of *Staphylococcus aureus* strains from patients with urinary tract infections in Yenagoa, Nigeria. *J Pharm Bioallied Sci.* 2012; 4(3):226-30.
 12. Nwanze PI, Nwaru LM, Oranusi S, Dimkpa U, Okwu MU, Babatunde BB. Urinary tract infection in Okada village: Prevalence and antimicrobial susceptibility pattern. *Sci Res Essay.* 2007; 2(4):112-6.
 13. Akortha EE, Ibadin OK. Incidence and antibiotic susceptibility pattern of *Staphylococcus aureus* amongst patients with urinary tract infection (UTIS) in UBTH Benin City, Nigeria. *Afr J Biotechnol.* 2008; 7(11):1637-40
 14. Aboderin OA, Abdu A, Odetoyin BW, Lamikanra A. Antimicrobial resistance in *Escherichia coli* strains from urinary tract infections. *J Natl Med Assoc.* 2009; 101(12):1268-73.
 15. Akerele J, Akonkhai I, Isah A. Urinary pathogens and antimicrobial susceptibility: A retrospective study of private diagnostic laboratories in Benin City, Nigeria. *J Med Lab Sci.* 2000; 9:47-52
 16. Okonko IO, Donbraye-Emmanuel OB, Ijandipe LA, Ogun AA, Adedeji AO, Udeze AO. Antibiotics sensitivity and resistance patterns of uropathogens to nitrofurantoin and nalidixic acid in pregnant women with urinary tract infections in Ibadan, Nigeria. *Middle-East J Sci Res.* 2009; 4(2):105-9.
 17. Manikandan S, Ganesapandian S, Singh M, Kumaraguru AK. Antimicrobial susceptibility pattern of urinary tract infection causing human pathogenic bacteria. *Asian J Med Sci.* 2011; 3(2):56-60.
 18. Deyno S, Toma A, Worku M, Bekele M. Antimicrobial resistance profile of *Staphylococcus aureus* isolates isolated from ear discharges of patients at University of Hawassa comprehensive specialized hospital. *BMC Pharmacol Toxicol.* 2017; 18(1):35.
 19. Almagadam BS, Ahmed HM, Osman M.B, Omer SM. Frequency of MRSA isolates in mobile phones, ears, and hands of healthcare workers. (2018). *J Antimicrob Agents.* 2018; 4(1): 161.
 20. Kuehnert MJ, Deanna K, Hill HA. Prevalence of *Staphylococcus aureus* nasal colonization in the United States. *J Infect Dis.* 2006; 193(2):172-9.
 21. Plat J, Rai SK. Occurrence of *Staphylococcus aureus* in hospital environment and staffs in teaching hospital in Katmandu, Nepal. *J Nepal Assoc Med Lab Sci.* 2007; 8(1):72-3.
 22. Chigbu CO, Ezeronye OU. Antibiotic resistant *Staphylococcus aureus* in Abia State of Nigeria. *Afr J Biotechnol.* 2003; 2(10): 374-8.

23. Adesina SK, Idowu O, Ogundaini AO, Oladimeji H, Olugbade TA, Onawunmi GO. Antimicrobial constituents of the leaves of *Acalypha wilkesiana* and *Acalypha hispida*. *Phytother Res*. 2000; 14(5): 371-4.
24. Mulu W, Kibru G, Beyene G, Dامتie M. Postoperative nosocomial infections and antimicrobial resistance pattern of bacteria isolates among patients admitted at Felege Hiwot referral hospital, Bahirdar, Ethiopia. *Ethiop J Health Sci*. 2012; 22(1):7-18.
25. Muluye D, Wondimeneh Y, Ferede G, Nega T, Adane K, Biadgo B, et al. Bacterial isolates and their antibiotic susceptibility patterns among patients with pus and/or wound discharge at Gondar university hospital. *BMC Res Notes*. 2014; 7(1):619.
26. Chikere CB, Chikere BO, Omoni VT. Antibiogram of clinical isolates from a hospital in Nigeria. *Afr J Biotechnol*. 2008; 7(24): 4359-63.
27. Eloff JN. On expressing the antibacterial activity of plant extracts-a small first step in applying scientific knowledge to rural primary health care. *South Afr J Sci*. 2000; 96(3): 16-118.
28. Al-Jabri AA. Honey, milk, and antibiotics. *Afr J Biotechnol*. 2005; 4(13):1580-7.
29. Dean DA, Burchard KW. Fungal infection in surgical patients. *Am J Surg*. 1996; 171(3):374-82.
30. Gonzalez CE, Venzon D, Lee S, Mueller BU, Pizzo PA, Walsh TJ. Risk factors for fungemia in children infected with human immunodeficiency virus: A case-control study. *Clin Infect Dis*. 1996; 23(3): 515-21.
31. Uwaezuoke JC, Aririatu LE. A survey of antibiotic resistant *Staphylococcus aureus* strains from clinical sources in Owerri. *J Appl Sci Environ Managt*. 2004; 8(1):67-8.
32. Odugbemi T. The use and abuse of antibiotics. *Nig Med Pract*. 1981; 1(1):4-8.
33. Adesida SA, Abioye OA, Bamiro BS. Associated risk factors and pulsed field gel electrophoresis of nasal isolates of *Staphylococcus aureus* from medical students in a tertiary hospital in Lagos, Nigeria. *Braz J Infect Dis*. 2007; 11(1):63-9.
34. Al-Khulaifi M, Amin Aref M, Al Salamah A. Phage typing, PCR amplification for *mecA* gene, and antibiotic resistance patterns as epidemiologic markers in nosocomial outbreaks of methicillin resistant *Staphylococcus aureus*, Saudi J Biol Sci. 2009; 16 (1):37-49.