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Identification of genes associated with resistance to disinfectants in the most common resistant bacteria strain of Bacteria isolated from Salon Tools in Ishaka Town, Bushenyi District, Uganda

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ABSTRACT

This study identified the genes associated with resistance to disinfectants in the most common resistant bacteria strain of Bacteria isolated from Salon Tools in Ishaka Town, Bushenyi District, Uganda. Twenty-five (25) Salon owners/ Head operators were enrolled in this study to determine the level of awareness about the biosafety practice of salons operation using semi-structured questionnaire. Additionally, total of 125 swab samples were collected from different salons tools (combs, brushes, scissors, clippers and shaving machines) and processed using standard Microbiological methods for isolation of bacteria. The isolated bacteria were identified using standard phenotypic methods including analytical profile index (API). Quaternary ammonium compounds (qac) genes (qacA/B and C) associated with disinfectant resistances were detected from disinfectant resistant Staphylococcus aureus using Multiplex polymerase chain reaction (PCR) and Sanger sequencing methods. Results obtained from this study showed that a significant number of salon operators ($p \leq 0.05$) were aware about the important of disinfectant usage, disinfect tool(s) after use and washing hands /tools after use. Among the 125 swabbed samples collected from the salons, 78 (65.5%) were contaminated with different bacterial species. Clippers were found to have higher (25.6%) contamination among the salon tools from which samples were collected. Of all the tested disinfectants, most resistance was shown with Sodium hypochloride1%. Out of the eight (8) bacterial isolates that were analyzed for qac genes, 2(25%) isolates (STP6 and STP9) were found to be qac A/B positive while 2 (25%) isolates (STP8 and STP9) were found to be qac C gene positive. In conclusion, this study showed that, majority of the salon operators was aware about the biosafety practice of salon operation despite the higher contaminations of salons tools. qac A and qac C were detected among some S. aureus isolated. As recommendations, further study should be done and biosafety guideline should be reviewed.

Keywords: Identification, genes, resistance and disinfectants.

INTRODUCTION

Beauty salons are considered as one of the dangerous places for transmission of diseases, they may pose potential health risks to their clients and service providers such as skin infections on the scalp, face and neck or sometimes injuries [1; 2]. Some bacteria, viruses and fungi especially yeasts have been isolated from manicure, pedicure, hairdressing and barbering equipment and tools used in salons [3; 4; 5; 1; 6]. Studies done in United states of America by [7] and [5] on manicure and pedicure tools used on hands and feet of customers who acquired those services showed that they were contaminated by *Streptococcus sp., Enterococcus sp., Micrococcus sp., Bacillus sp., Enterobacter sp., Klebsiella sp., Acinetobacter sp., Citrobacter sp.,* and *Escherichiacoli*. Other studies on some tools also isolated; other bacteria such as *Mycobacterium fortuitum, Mycobacterium chelona*e and *Mycobacterium mageritense* [8; 9; 10]. Similarly studies done on in-use tools in hairdressing and barbering, showed contamination by bacteria like *Staphylococcus aureus*, *Pseudomonas aeruginosa, Staphylococcus epidermidis, Streptococcus sp, Enterococus sp* and *Enterobacteria* [11; 4; 1].

Furthermore, the proper use of disinfectants can help contain and

prevent the spread of harmful bacteria and viruses. Disinfectants are antimicrobial agents that are applied to the surface of non-living objects to destroy microorganisms that are living on the objects. However, studies have revealed resistance of some microorganisms on different type of disinfectants. A study done by [12], on different disinfectants revealed that, among 27 bacteria isolates used, all strains were susceptible to sodium hypochlorite, glutaraldehyde and to the association quaternary ammonium - formaldehyde - ethyl alcohol disinfectants. However, the susceptibility of strains to phenol and to one quaternary ammonium compound (QAC) was variable. Only eight (08) strains (one MRSA strain, two *S. epidermidis* strains, *E. cloacae*, *P. mirabilis*, *S. marcescens* and two strains of *P. aeruginosa* (38% of all drug resistant strains identified)) were resistant to the quaternary ammonium and phenol compounds, while of the six antibiotic susceptible strains demonstrated only two (33%) strains (*Proteus mirabilis* and *Staphylococcus epidermidis* strains) were resistant to the QAC and phenolic compounds, respectively. However, a number of factors may affect effectiveness of disinfectants among which are the concentration used, application method, contact time of the disinfectant and the safety considerations for operators to apply disinfectants [13; 14; 15]. Uganda National Bureau of Standard UNBS (2008) recommends testing for effectiveness of these disinfectants, since the role of salon in the spread of such infections in Uganda has not yet been elucidated, hence a need to explore the effectiveness of the commonly used disinfectants and Identify the genes associated with disinfectant resistance.

Aim of the study

The aim of this study was to identify genes that are associated with resistance to disinfectants in the most common resistant bacteria strain of Bacteria isolated from Salon Tools in Ishaka Town, Bushenyi District, Uganda.

Specific Objectives

i. To assess the level of awareness about biosafety practices among salon operators in Ishaka town.
ii. To identify the genes associated with disinfectant resistance in the most common resistant bacteria strain.

Research Questions

i. What is the salon operators' level of awareness on biosafety practices for salons?
ii. Are there genes associated with disinfectant resistance in the most common resistant bacteria to the different disinfectants tested?
iii. The identification of the genes associated with disinfectant resistance among common resistant bacteria strain identified.

METHODOLOGY

Study Design

This was a cross-sectional study (because was observational study which analyzed data from a representative subset, at a specific point in time), where salons were selected using a purposive sampling strategy (also known as subjective). Therefore, samples were collected from salon service operators' tools such as combs, brushes, scissors, clippers and shaving machines purposively. ID codes were used to protect the privacy of salons. Information about how disinfectants are used on the tools and disinfectants used were also collected; after which samples were analyzed In the lab to determine the prevalence of bacterial contamination. This was followed by the determination of major bacteria contaminants. From the bacteria identified, all species identified were represented for the tested commonly used disinfectants.From the resistant *Staphylococcus aureus* isolates, disinfectant resistant genes (QAC) were determined and confirmed through sequencing using Sanger sequencing method.

Study area

The study was carried out on selected beauty salons in Ishaka town division located in Ishaka municipality, Bushenyi district, Uganda. Ishaka town has a human population of 41,217 according to the

population census 2014 [16]. Ishaka municipality has 20 villages including 4 villages which Ishaka town division covers. This study was conducted on beauty salons selected in 4 villages (Cell A, Cell B, Cell C and Cell D).

Sample size

The minimum sample size was determined by [17] formula which states as:

$$S = \frac{X^2 NP(1-P)}{d^2(N-1)+X^2P(1-P)}$$ Where;

S= required sample size;
X^2 = the table value of chi-square for 1 degree of freedom at the desired confidence level (1.96*1.96=3.841);
N = the population size (27 saloons listed from pilot study);
P=the population proportion (assumed to be 0.50 since this would provide the maximum sample size);
d=the degree of accuracy expressed as a proportion (5%=0.05).

$S = \frac{3.84*27*0.5(1-0.5)}{(0.05)^2 (27-1) +3.84*0.5(1-0.5)} = \frac{25.92}{1.02525} = 25$ Salons.

Twenty-five (25) minimum numbers of salons were used during this study.

Sampling strategy

The convenience sampling method was used to select Ishaka town out of other towns of Bushenyi district. Convenience sampling also known as availability sampling is a non-probability sampling technique where subjects are selected because of their convenient accessibility and proximity to the researcher. Ishaka town division has 27 beauty salons where 8 are barbershops, 9 are women salons only and 10 are Unisex beauty salons. From these salons, a total of 25 salons were selected purposively. The simple random sampling method which is a sampling technique where every item in the population has an even chance and likelihood of being selected in the sample was used to select 25 Beauty salons. Furthermore, swab samples were taken from tools such as Scissors, Brushes, Combs, Clippers and Shaving Machine selected purposively. The choice of selected tools was in accordance with literature which has shown that the tools selected for this study are the most commonly contaminated [3]. Furthermore, 25 salon operators were selected purposively from the selected salons participated in this study to obtain qualitative data such as their Knowledge, attitude and practice with respect to biosafety while carrying out their daily activities (like daily cleanliness practice used daily, how they disinfect equipment and tools used and infection controls measures implemented).

Sample collection and storage

Moisten sterile swabs with 0.85% normal saline were collected from salon tools such as combs, brushes, scissors, clippers and shaving machines. It was done by moving a pre moistened sterile swab two to three times, over the surface which gets in contact with the skin of customers or salon providers. The swabs collected were inserted into sterile 5 ml tubes containing 2 ml of Stuart transport medium. After taking sample, each tube was covered appropriately to avoid contamination and labeled, then were carried out in a cool box. The samples were transported to the Microbiology Laboratory, Kampala International University -Western Campus promptly. Samples were processed immediately when taken to the Microbiology Laboratory, Kampala International University -Western Campus and those that were not processed were stored at 2^0–8^0C and processed the next day [3].

Data collection

Both qualitative and quantitative data were collected in this study and the data collection methods are described below as per the objective.

Assessing the level of awareness about biosafety practices among Salon owners/ Head operators in Ishaka town

Data were collected from Salon owners/ Head operators using semi-structured questionnaire in order to generate useful information which helped to explain the quantitative data obtained. Information sought includes; knowledge, attitude and practice with respect to biosafety and socio-demographic information. Professional ethics were upheld at all the stages, including sample collection, processing and reporting results which will be in accordance with approved Standard operation procedures. All the equipment in the laboratory, such as

autoclave, microscope, incubator, etc, were used following the manufacturer's operating guidelines. Each test was done in duplicates, in order to minimize errors. Negative control was used in order to get precise and reliable results. For Polymerase Chain Reaction, some measures were taken in order to avoid contamination search as decontamination of surfaces, changing gloves, lab coats and testing of primer on positive and negative controls to avoid positive results.

Data analysis

The raw data was entered in excel after being edited and cleaned in case of any obvious errors. The data from objective 1 and 3 were then analyzed using statistical package for social sciences (SPSS) version 21 software. For prevalence of commonly bacteria contaminated beauty salon tools were presented in form of frequencies and percentages with their corresponding 95% confidence interval and *P- value* comparison among tools, it was done using Chi-square.

Ethical considerations

In order to make sure that the study is conducted ethically, several specific issues were addressed.

Institutional consent

Ethical clearance was sought from the Research Ethics Committee of KIU-WC (*ref* No.: SF201813; Nr UG-REC-023/201813).

Salon owners' approval

Permission to collect sample from tools and qualitative data from salon owners/Senior salon service providers was sought from salon owners of the selected salons and approval was obtained.

Informed consent

The salon owners of selected salons were informed of the study, using the best locally understood language. The purpose of the study, methods, possible risk(s) and benefits of participation were clearly spelt out. Involvement in the research was voluntary and participants were free to opt out at any time without penalty or loss of potential advantages. Individuals willing to be part of the study were requested to fill out and sign a pertinent Consent form, administered by the researcher, and in the presence of a witness.

Privacy and confidentiality

Privacy of participants was insured by protecting individual identity and information. For example, all data collected was used without names of the participants and kept safely and confidentially. Salon facilities were protected by using Identification Number to protect their anonymity.

Justice in selection

The salons were selected equitably and fairly by using simple random sampling technique, where we listed all 27 salons' names on a piece of paper and choose 25 salons needed in this study randomly in order to give all salons an equal opportunity to be involved. Besides, justice was involved in the application of fairness to individuals in choosing interviewees to participate in this study. Every respondent was given equal opportunity to participate in the study. No particular priority was given to anyone.

Respect for rights of respondents

Each respondent had an entitlement to his/her opinion, response and comments. The researcher ensured that each and every response provided during the course of the study is respected.

Protection of research personnel and environment

Protective wear, including gloves and laboratory coats, was used to protect research personnel against the test organism. Inoculation of samples was carried out in a safety cabinet to prevent environmental contamination and infection to research personnel. All plates and any disposable materials used were properly disposed of or burnt after being autoclaved. Reusable glass wares were autoclaved so as to prevent the risk of infection; any washing was done in a sink; and the runoff disposed of in a septic tank. The surfaces of the working benches were decontaminated with disinfectants.

The genes responsible for bacteria to commonly used disinfectants will be determined by Multiplex polymerase chain reaction (PCR)

Plasmid from bacterial isolates was prepared by boiling method [18,19]. The samples were centrifuged at 15000 rpm (rotation per minute) for 15min. The

supernatant was eliminated, and pellet was suspended in molecular biology-grade water and centrifuged at 15000 rpm for 10 min. The supernatant was eliminated again and the pellet was suspended in 40 µl of molecular biology-grade water, which was subjected to boiling at 100°C in a water bath for 10 min and was cooled on ice, and was centrifuged at 15000 rpm for 10 seconds before it was stored at 20°C. Ultra-pure plasmids were ready for use for PCR.

The primers used are qacA/B: forward primer5'CTATGGCAATAGGAGATATGGTGT ,reverse primer 5'-CCACTACAGATTCTTCAGCTACATG-3'; qacC: forward primer 5'-AAACAATGCAACACCTACCACT, reverse primer 5'-AACGAAACTACGCCGACTATG-3' [20].

PCR Amplification

PCR was performed by using 50ng of bacteria plasmid extracted from resistant species and add each primer (10 pmol), Taq DNA polymerase (1·25 U; Promega, Madison, WI, USA), dNTPs (200 µm,Promega), and $MgCl^2$ (1.25mM; Promega) in 50 µl of reaction buffer using a DNA Thermal Cycler (Model480, Perkin-Elmer Cetus, Foster City, CA, USA). The conditions for PCR were: initial denaturation of Plasmid at 95°C for 3 min for bacteria, then 35cycles of three-step PCR amplifications consisting of denaturation at 94°C for 1 min, primer reannealing, and extension at 72°C for 10 min. [20].

Analyzing PCR product size using PFGE (Purified field gel electrophoresis

Purified field gel electrophoresis was performed by using 10 µl of treated product and analyse it by using gel electrophoresis in 3% agarose gel in 1X Tris-borate-EDTA buffer (TBE, pH 8.3). The amplification products were photographed and their size was determined using a 25 bp molecular size marker (Promega, USA). Bands were observed with expected sizes. As control, we were standard PCR with same primers (the same used in multiplex PCR amplification) [20]. Negative controls *(S. aureus* ATCC 29213) were used. *Staphylococcus aureus* isolates (STP 9 and STP 6) that were Qac A gene positive were further partially sequenced using Sanger sequencing method for confirmation, this was done at inqaba biotec laboratory /South Africa.

RESULTS

Participants demographic characteristics

Data was collected from 25 participants who included salon owners and service providers the response rate was 100%. Of the participants respondent's gender; there were slightly more male 60% were male while 40% were females. The average age of the respondents was 25±0.6 years. Majority of the respondents (64%) had secondary school level education while 36% had no formal education. Fifty-two percent (52%) of the respondents had Barbershops within Ishaka town Among the 25 salons visited in this study, 13(52%) were Barbershops, 6(24%) were Hairdressing/Women salon and 6(24%) were unisex salon. The rest of the details are shown in Table 1.

Table 1: Participants demographic characteristic

Characteristics	Frequencies n (%)	*P-value*
Sex		
Male	15(60)	
Female	10(40)	**0.05***
Education		
None	3(12.0)	
Primary	6(24.0)	
Secondary	16(64.0)	0.89
Location		
Outside town	14(56.0)	
In town	11(44.0)	0.42
Type of saloon		
Barbershop	13(52.0)	
Hairdressing/Ladies salon	6(24.0)	**0.013***
Unisex Salon	6(24.0)	

Key: ***S**tatistically significant

Level of awareness about biosafety practices among salon operators

Based on information obtained from respondents about disinfectants uses, majority of the respondents (88%) were aware about the importance of using disinfectant. Eighty-four (84.0%) percent of respondents acknowledged the purchase of disinfectants from supermarkets and knew the important of using the different tools in salon. All the studied participants (100%) agreed on cleaning of tool(s) after use. Fifty-two (52%) and Ninety-two (92%) of participants were not aware about biosafety guideline and usage of Methylated Spirit and Sodium hypochloride (JIK) respectively, the eighty-four (84%) of the respondents were un-aware of biosafety guidelines and usage of Surgical Spirit and Sodium hypochloride (JIK) combination in salons shops, so were for those who were un-aware of biosafety guideline and usage of Surgical spirit & shampoo/savlon combination. Use of after shave as disinfectants on tools was not known by 92% of respondents. Majority of the participants (96%) were un-aware of the use of heat on clipper as a disinfectant but were aware of the washing hands /tools after shaving to prevent infections transmission. Disinfecting of the shaving machines before using was known by 54% of the participants. Most of the studied participant 80%, 92%, 100% and 96% were aware of the disinfecting the Clippers, Scissors, Combs and Brushes respectively before use. The rest of the details are shown in Table 2.

Table 2: Level of awareness on biosafety practices used in beauty salons

Variable	Frequency (Percentage)	p value
Disinfectants usage		
Yes	22 (88)	0.006*
No	3 (12)	
Source of disinfectant		
Pharmacy	4 (16)	0.03*
Supermarkets	21 (84)	
Important of having more than one tool in use		
Yes	21 (84)	0.03*
No	4 (16)	
Cleaning instruments after use		0.001*
Agree	25 (100)	
Disagree	0 (0)	
Use of Methylated Spirit		0.02*
Yes	12 (48)	
No	13 (52)	
Use of Sodium hypochloride (JIK) only		0.006*
Yes	2 (8)	
No	23 (92)	
Use of Surgical Spirit and Sodium hypochloride JIK		
Yes	4 (16)	0.03*
No	21 (84)	
Use of Surgical spirit &shampoo/savlon		0.03*
Yes	4 (16)	
No	21 (84)	
Usage of after shave as disinfectants on tools		0.006*
Yes	2 (8)	
No	23 (92)	
Usage of heat on clipper as a Disinfectant		0.004*
Yes	1(4)	
No	24 (96)	
Importance of washing hands /tools		0.004*
Yes	24 (96)	
No	1(4)	

The following tools must be disinfected before use		
Shaving machine		
Yes	14 (56)	0.71
No	11(44)	
Clipper disinfection before use Yes		0.047*
Yes	20 (80)	
No	5 (20)	
Scissor disinfection before use		0.006*
Yes	23 (92)	
No	2 (8)	
Combs disinfection before use		0.001*
Yes	25 (100)	
No	0 (0)	
Brushes disinfection before use		0.004*
Yes	24 (96)	
No	1 (4)	

Key: * Statistically significant.

Identification of genes associated with resistance to disinfectants in the most common resistant bacteria strain

Eight isolates of *Staphylococcus aureus* that showed resistant to disinfectant was subjected to Multiplex PCR for detection of QacA/B and QacC genes associated with disinfectant resistance. Approximately, 380 base pair (bp) and 500 base pair (bp) amplicon size was obtained for QacA and QacC genes respectively. Out of the eight (8) bacterial isolates that were analyzed for Qac genes, 2(25%) isolates (STP6 and STP9) were found to be Qac A gene positive while 2 (25%) isolates (STP8 and STP9) were found to be Qac C gene positive (**Fig. 1**).

A pairwise comparison between STP 9 and STP 6 isolates yield 100% identity (Appendix 4). Both isolates were further partially sequenced using Sanger sequencing method and bout 400 sequences were obtained for each isolate. The sequences obtained were blasted in NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using BLASTN search. The results of the search showed that, the antiseptic resistance protein (QacA) gene sequence of the two *Staphylococcus aureus* were 100% similar to about 17 *Staphylococcus* spp which included *Staphylococcus aureus, Staphylococcus epidermidis* and *Staphylococcus hominis* strains (**Table 3**) and these disinfectant resistance protein (QacA) gene sequences of the above *Staphylococcus species* were downloaded and compared with *Staphylococcus aureus* antiseptic resistance protein (QacA) genes identified in this study using Phylogenetic tree in order to obtained a significance similarities. Phylogenetic tree was constructed using maximum Likelihood statistical method at 100 number of bootstrap replications (**Fig. 2**). Results from the phylogenetic tree didn't yield any significance differences bootstrap values, which means that the results of QacA gene seen in this study is similar to the other *Staphylococcus* species carried QacA genes downloaded from Gene Bank.

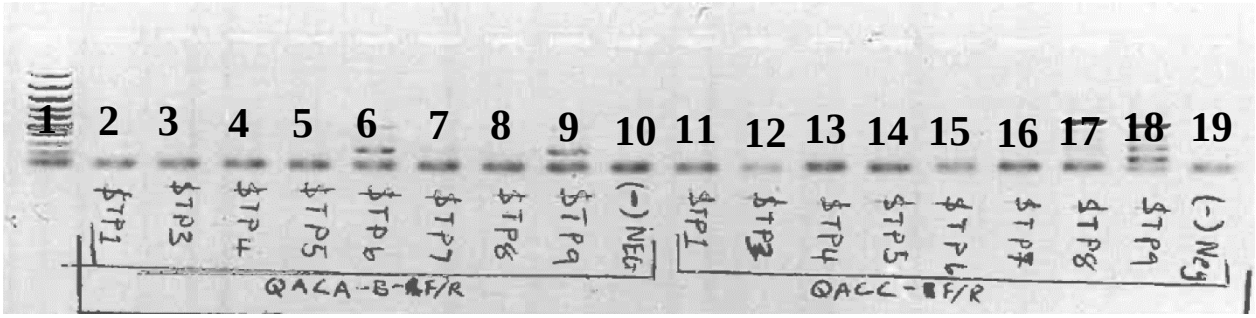

Figure 1: Virtual gel image showing PCR results of both qacA/B

Virtual gel image showing PCR results of both qacA/B (Line 2-10) and qacC (Line 11-20), Line 1 ladder 1000 bp (Fermentas), Line 2 STP1, line 3 STP 3, line 4 STP4, line 5 STP 5, line 6 STP 6, line 7, STP 7, line 8 STP 8, line 9 STP 9, line 10 Negative control, line 11: STP1, line 12 STP 3, line 13 STP4, line 14 STP 6, line 15 STP 7, line 16 STP 8, line 17 STP 9, line 19 Negative control.

Table 3: Blast comparison between Staphylococcus aureus disinfectant resistance protein (QacA) genes identified and others Staphylococcus species in Gene Bank using NCBI Blast.

No.	Organisms	Sequence	Accession Number	References	Percentage Identity (%)	E-value
1	*Staphylococcus aureus*	STP6	NA	This study	100.00	0.027
2	*Staphylococcus aureus*	STP9	NA	This study	100.00	0.027
3	*Staphylococcus aureus*	NSNJ5	LC335721.1	Noor *et al.*,2019	100.00	0.027
4	*Staphylococcus aureus*	NSNJ4	LC335720.1	Noor *et al.*,2019	100.00	0.027
5	*Staphylococcus aureus*	NSNJ3	LC335719.1	Noor *et al.*,2019	100.00	0.027
6	*Staphylococcus aureus*	NSNJ2	LC335718.1	Noor *et al.*,2019	100.00	0.027
7	*Staphylococcus aureus*	NSNJ1	LC335717.1	Noor *et al.*,2019	100.00	0.027
8	*Staphylococcus aureus*	TPS89 pTZ2089	NG_048037.1	Nakaminami *et al.*2010	100.00	0.027
9	*Staphylococcus aureus*	Teh11	KP687798.1	Hassanzadeh et al.2017	100.00	0.027
10	*Staphylococcus aureus*	FJ857944.1	FJ857944.1	Zoung et al.,2009	100.00	0.027
11	*Staphylococcus Epidermidis*	MK040371.1 Strain 20.1	MK040371.1	Addetia *et al.*,2018	100.00	0.027
12	*Staphylococcus Epidermidis*	MK040366.1 Strain 106.1	MK040366.1	Addetia *et al.*,2018	100.00	0.027
13	*Staphylococcus Epidermidis*	MK040365.1 Strain 99.1	MK040365.1	Addetia *et al.*,2018	100.00	0.027
14	*Staphylococcus Epidermidis*	MK040364.1 Strain 68.5	MK040364.1	Addetia *et al.*,2018	100.00	0.027
15	*Staphylococcus Epidermidis*	MK040363.1 Strain 66.3	MK040363.1	Addetia *et al.*,2018	100.00	0.027
16	*Staphylococcus hominis*	MK040362.1 Strain 125.1	MK040362.1	Addetia *et al.*,2018	100.00	0.027
17	*Staphylococcus Epidermidis*	MK040362.1 Strain 36.5	MK040361.1	Addetia *et al.*,2018	100.00	0.027

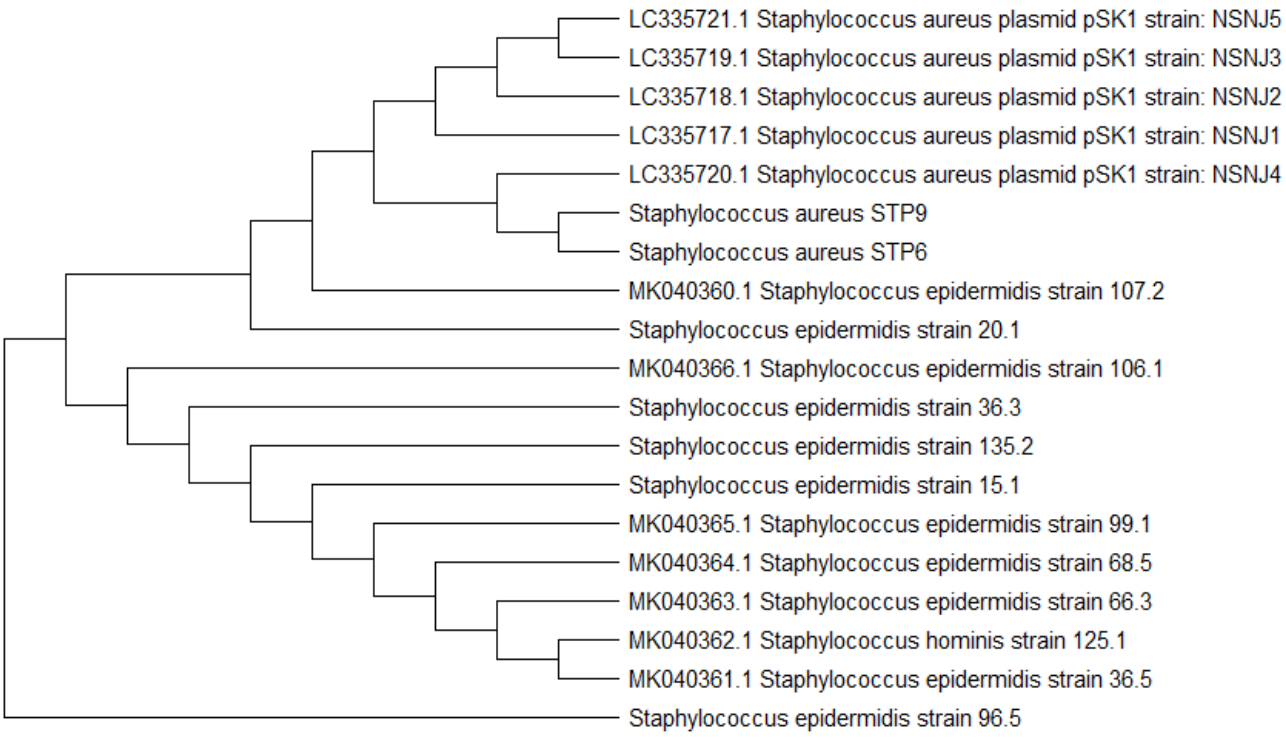

Figure 2: **Phylogenetic tree constructed for 17 different QacA proteins extracted from**

GenBank, together with two *Staphylococcus aureus* isolates (STP 9 and STP 6) that were QacA gene positive in this study. It showed that there were no significant bootstrap differences between both QacA genes.

DISCUSSION

Detection of genes associated with disinfectant resistance is clinically important in the treatment of these infections as these genes were reported to vary and confer reduced susceptibility to commonly used antiseptics and disinfectants [21]. This study was therefore designed to identify the genes associated with resistance to disinfectants in the most common resistant bacteria strain of Bacteria isolated from Salon Tools in Ishaka Town, Bushenyi District, Uganda. The majority of the participant being aware of the usage of disinfectants in salons is in line with the findings of [22] from Rome, who reported that, more than 95% of the centers use more than one method of decontamination. This was contrary to the findings of [23] who reported higher percentage 58% (n=50) of barbers that were not aware of any health hazards associated with their profession from Kharian in Pakistan. The higher percentage of barbers who knew about usage of disinfectants in salons reported in this study is not surprising because most of the participants (64%) had secondary school certificate which is consider as an ordinary level of education in Uganda. At that level, a student is expected to be able to read, write and understand common guideline or rules within the community. Understanding guideline on the usage of disinfectants in salons is very important, as careless use of salons equipment without disinfecting between one client to another can leads to the transmission of infectious diseases [24] within the community including resistant organisms. Obtaining disinfectants from supermarket by most of the participants could be due to the easy access or the disinfectants being sold at a cheaper price. But the implication of this is, some of the disinfectant in the super market could be of low standard or expired compare to the one bought in Pharmacies which could leads to in-effectiveness. This could lead to in appropriate decontamination of

salons tools which increases the risk of spreading the infectious diseases including resistant organisms. This confirmed the report of [16], who reported that, there is an increase of purchase of over the counter medicine in Uganda which fuels the spread of resistant organisms within the community. Majority of the participants responded to had more than one salon tools in their shops. This could reduce the risk of immediate transfer of contaminated equipment between one client to another. All participants interviewed (100%) responded to have clean or disinfect the instrument after used, this also showed a good level of hygiene practice by these studied participants. Furthermore, most of the respondents were aware of the guideline of usage of different disinfectants such as Methylated Spirit, Sodium hypochloride (JIK) only, Surgical Spirit and Sodium hypochloride, Surgical spirit & shampoo/savlon, after shave as disinfectants on tools and heat on clipper. This was contrary to the findings of Wazir *et al.*, 2008 who reported that 90% (n=50) of barbers did not wash hands, 80% did not change the apron, 66% did not change towel during barbering services to different customers, which may be another way of transmitting resistant organisms from one client to another.

Out of eight isolates of *S. aureus* isolated from Barbershops/Hairdressing/ladies salons and subjected to multiplex PCR to detect the presence of qacA/B and qacC associated with disinfectant resistant, 2(25%) isolates (STP6 and STP9) were found to harbored qacA/B genes while 2 (25%) isolates (STP8 and STP9) were found to be qcC gene positive. Presence of bacterial pathogens within the community is chained from animal industries and food industries to community or hospitals to community [21]. These sectors used disinfectant in their day to day activities. Therefore, due to lack of enough literature on the detection of qac genes associated with disinfectant resistant in *S. aureus* isolated from the solons, the results of this study were compared with previous study done on qac genes associated with disinfectant resistant detected from *S. aureus* isolated from both community and Hospital settings. The prevalence qacA/B and qacC genes associated with disinfectant reported in this study is lower than the prevalence reported by a study conducted within the community of Hong Kong, China on an automated teller machine, in which 11% of the isolated *S. aureus* carried *qacA/B* and less than 2% had *qacC/smr*, while *qacC/smr* was found in 14% and *qacA/B* in 26% of the CNS isolates [25]. This was contrary to the findings of [26] who reported zero prevalence of qacC/smr *and* qacA/B genes associated with disinfectant genes in methicillin-resistant *S. aureus* isolated in porcine although other gene associated with disinfectant resistant such as qacG was detected. The genes qacA/B, qacC/smr have all been detected from beta-lactam antibiotics in bovine, caprine and other food-related Coagulase Negative *Staphylococcus spp.* such as *S. epidermidis*, *S. saprophyticus, S. cohnii and S. hominis* [27; 28; 21].

However, the presence of qac genes associated with disinfectant resistant was also reported in many studies involving hospital or clinical samples. Study on qac genes associated with disinfectant resistant in African countries seem to be scarce and was reported to be higher in Asian [21]. For example, [29], reported that qacA/B and qacC/ smr were detected in 44% and 31%, respectively, in MRSA isolated between 1998 and 1999 from Asian countries. [30] reported 7.5% of qacA/B genes in clinical isolates of 32% MRSA in Japan. [31] also reported higher prevalence of qacA/B (83%) in clinical MRSA isolates from Malaysia, even though qacC/smr was reported to be negative. Prevalence of qac genes were reported to be lower from European continent compare to Asian countries, for instance, [32], in their study carried out in Toronto and Canada on clinical MRSA isolated from intensive care unit from 2005-2009 detected 2% and 7% of qacA/B and qacC/smr genes respectively. Similarly, McDane *et al.*, reported 0.6% qacA/B genes from over 800 clinical samples.

In Africa, study conducted by Conceição *et al.*, 2016 from three African countries (Angola, São Tomé and Príncipe, and Cape Verde) among 82 methicillin-

resistant *Staphylococcus aureus* (MRSA) and 219 methicillin-susceptible *S. aureus* (MSSA) isolated from previous nasal carriage showed 40.5% qacA/B genes. Mahomed *et al*., 2018 also reported the prevalence of qac genes of 13/17 (76%) and 10/16 (63%) from both MRSA and MSSA respectively, isolated from cystic fibrosis patients at a tertiary academic hospital in Pretoria, South Africa. Presence of disinfectant resistant *S. aureus* harboring qac genes from this studied salons from the study area showed that, there is need for frequent proper sensitization of these salons operators on the biosafety guideline of salon operation especially on the implication of miss used of disinfectant as this can lead to the increase of acquiring disinfectant resistant genes between the bacterial community [33,34] of salons which can spread within the community.

The results of sequences blastering of *Staphylococcus aureus* isolates (STP 9 and STP 6) that were qacA gene positive from NCBI(https://blast.ncbi.nlm.nih.gov/Blast.cgi) using BLASTN search showed that, the antiseptic resistance protein (qacA) gene sequences of the two *Staphylococcus aureus* were 100% similar to about 17 *Staphylococci* spp which included both *Staphylococcus aureus, Staphylococcus epidermidis* and *Staphylococcus hominis.*

The Phylogenetic tree analysis constructed using maximum Likelihood statistical method at 100 number of bootstrap replications showed that, the two *Staphylococcus aureus* isolates (STP 9 and STP 6) form a different clade from kwon *Staphylococcus* spp that harbored antiseptic resistance protein (qacA) gene extracted from Gene bank (NCBI). This indicates that, although these isolates harbored antiseptic resistance protein (qacA) gene, they could be new strains of *Staphylococcus aureus* since all the isolates used for comparisons were mostly isolated from clinical samples as compared to ours which was isolated from environmental sources (Salons) [35,36]. Secondly, most of the antiseptic resistance protein (qacA) gene sequences *Staphylococcus* spp extracted from Gene bank (NCBI) were whole genome sequences while our isolates were partial sequences. The results of this finding were in line with findings of [21] who reported variations in the phylogenetic analysis of antiseptic resistance protein (qacA) gene sequences of different species of *Staphylococci* sequences extracted from extracted from Gene bank.

CONCLUSION

Out of the 8 bacterial isolates that were analyzed for qac genes, 2(25%) isolates (STP6 and STP9) were found to be qacA positive while 2 (25%) isolates (STP8 and STP9) were found to be qacC gene positive. Furthermore, Phylogenetic analysis of the two *Staphylococci* spp sequences showed that they harbored qacA genes and they could be new strain (s).

REFERENCES

1. Stanley, C., Ifeanyi, O. E., Kingsley, O. and Onyekachi, I. S. (2014). Evaluation of Microbial Contamination of Tools Used In Hair Dressing Salons in Michael Okpara University of Agriculture , Umudike , Abia State.*Journal of Dental and Medical Sciences,13*(7): 22–27.
2. Barn, P.,and Chen, T. (2012). Infections Associated with Personal Service Establishments: Piercing and Tattooing.*Journal of the Canadian Institute of Public Health Inspectors*.
3. Enemuor, Mi., O., Isah, S. and Oguntibeju, O. O. (2013). Evaluation of bacterial and fungal contamination in hairdressing and beauty salons.*African journal of Microbiology Research7*(14):1222–1225.
4. Naz, S., Iqtedar, M., Ain, Q. U. and Aftab, K. (2012). Incidence of Human Skin Pathogens from Cosmetic Tools used in Beauty Saloons in Different Areas of Lahore, Pakistan. *Journal of Scientific Research*, *4*(2):2–6.
5. Sekula, S. A. (2002). Nail Salons Can Be Risky Business. *Arch dermatol*, *67*(6): 2009–2011.
6. Winthrop, K. L., Albridge, K., South, D., Albrecht, P., Abrams*, M.,*

*Samuel, M. C., Vugia, D. J. (2004). The c*linical management and outcome of nail salon-acquired Mycobacterium fortuitum skin infection. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *38*(1):38–44.

7. Hedderwick Sara A.;Shelly A.McNeil;Michael J.Lyons PA and carol A. Kauffman. (2011). Pathogenic Organisms Associated with artificial fingernails worn by Healthcare workers. *The University of Chicago Press /The Society for Healthcare Epidemiology of America*, *21*(8):505–509.
8. Gira, A. K., Reisenauer, A. H., Hammock, L., Nadiminti, U., Macy, J. T., Reeves, A. and Nolte, F. S. (2004). CASE REPORTS Furunculosis Due to Mycobacterium mageritense Associated with Footbaths at a Nail Salon.*Journal of clinical microbiology*, *42*(4):1813–1817.
9. Redbord, K. P., Shearer, D. A., Gloster, H., Younger, B., Connelly, B. L., Kindel, S. E. and Lucky, A. W. (2006). Atypical Mycobacterium furunculosis occurring after pedicures. *Journal of the American Academy of Dermatology*, *54*(3): 520–524.
10. Stout, J. E., Gadkowski, L. B., Rath, S., Alspaugh, J. A., Miller, M. B. and Cox, G. M. (2011). Pedicure-associated rapidly growing mycobacterial infection: An endemic disease. *Clinical Infectious Diseases*, *53*(8): 787–792.
11. David, D. L., Edward, A., Zaruwa, M. Z. and Addass, P. A. (2010). Barbing saloon associated fungal disease infection in Mubi, Adamawa State-Nigeria. *World Journal of Medical Sciences*, *5*(1): 17–21.
12. Marcia, J. E. (2002) Identity and Psychosocial Development in Adulthood, Identity, 2(1), 7-28. http://dx.doi.org/10.1207/S1532706XID0201_02
13. Awodele, O., Emeka, P. M., Agbamuche, H. C., and Akintonwa, A. (2007). The antimicrobial activities of some commonly used disinfectants on *Bacillus subtilis* , *Pseudomonas aeruginosa* and *Candida albicans*. *African Journal of Biotechnology*, *6*(8), 987–990.
14. Omoruyi and Idemudia. (2011). Comparative Analysis Of The Antiseptic Properties Of Some Disinfectants On Bacteria And Fungi Of Public Health Importance Isolated From Barbing Clippers. *Asian Scientific Research*, *1*(2): 65–68.
15. Oner, S. (2008). Disinfectants and antiseptics. *Journal of Pharmaceutical Sci. and Technology,* 30(6): 2108.
16. UNAS, CDDEP, GARP-Uganda, Mpairwe, Y. and Wamala, S. (2015). Antibiotic Resistance in Uganda: Situation Analysis and Recommendations (pp. 107). Kampala,Uganda:Uganda National Academy of Sciences; Center for Disease Dynamics, Economics & Policy.
17. Krejcie, R. V. and Morgan, D. W. (1970). Determining Sample Size for Research Activities. *Educational and Psychological Measurement.* Small-Sample Techniques (1960). *The NEA Research Bulletin*, Vol. 38.
18. Stoykova, A., Hatano, O., Gruss, P. and Gotz, M. (2003) Increase in reelin-positive cells in the marginal zone of Pax6 mutant mouse cortex. *Cereb Cortex* **13**: 560-571
19. Holmes, D. S. and Quigley, M. (1981). A rapid boiling method for the preparation of bacterial plasmids. Analyt Biochem 114:193–197
20. Sekiguchi, H., S. Aoi, R., Honda, N., Morikawa, T., Kunugi, A. and H. Fujiwara,
    Rupture process of the 2005 West Off Fukuoka Prefecture earthquake obtained from strong motion data of K-NET and KiK-net, Earth Planets Space, 58, 37-43, 2006.
21. Wassenaar, T. M., Wheatley, C. M. and Beale, N. (2019). Effects of a programme of vigorous physical activity during secondary school

physical education on academic performance, fitness, cognition, mental health and the brain of adolescents (fit to study): study protocol for a cluster-randomised trial. Trials, 20:189.

22. Mancini, L., Figliomeni, M., Puccinelli, C., Romanelli, C., Volpi, F., D'Angelo, A. M. and Marcheggiani, S. (2017). A descriptive survey on microbiological risk in beauty salons. *Microchemical Journal*, *4*(2):2–27
23. Wazir, M. S., Mehmood, S., Ahmed, A. and Jadoon, H. R. (2013). Awareness among barbers about health hazards associated with their profession. *Journal of Ayub Medical College, Abbottabad : JAMC*, *20*(2): 35–38.
24. Ataei, B., Shirani, K., Alavian, S. M. and Ataie, M. (2013). Evaluation of Knowledge and Practice of Hairdressers in Women's Beauty Salons in Isfahan about Hepatitis B, Hepatitis C, and AIDS in 2010 and 2011. *Hepatitis monthly*, *13*(3).
25. Zhang, B. C., Gao, J., Wang, J., Rao, Z. G. and Wang, B. C. (2011). Tumor-associated macrophages infiltration is associated with peritumoral lymphangiogenesis and poor prognosis in lung adenocarcinoma. Med Oncol 28:1447–1452.
26. Wong, C. A., Cummings, G. G. and Ducharme, L. (2013). The relationship between nursing leadership and patient outcomes: a systematic review update. *Journal of Nursing Management* **21**, 709–724.
27. Sidhu, M., Jani, H. and Ramesh, S. (2001). Critical Evaluation of Rich Pictures as a Pictorial Technique in SSM for Resolving Unstructured Problems. *National Conference on research and development in Computer Science*; 137– 143.
28. Bjorland, J., Steinum, T., Kvitle, B., Waage, S., Sunde, M. and Heir, E. (2005). Widespread distribution of disinfectant resistance genes among staphylococci of bovine and caprine origin in Norway. *J. Clin. Microbiol.* 43, 4363–4368
29. Noguchi, T., Ikeda, M., Ohmiya, Y. and Nakajima, Y. (2008). Simultaneous monitoring of independent gene expression patterns in two types of cocultured fibroblasts with different color-emitting luciferases. *BMC Biotechnol* 8:40.
30. Alam, A., Shi, J.P. and Harrison, R.M. (2003). Observations of new particle formation in urban air. Journal of Geophysical Research 108: doi: 10.1029/2001JD001417. issn: 0148-0227.
31. Shamsudin, S., Md Yusof, M. A. and Abdul Raof, A. H. (2010). Structural markers in British and Malaysian engineering lecture.
32. Longtin, Y., Sax, H., Allegranzi, B., Hugonnet, S. and Pittet, D. (2009). Patients' beliefs and perceptions of their participation to increase healthcare worker compliance with hand hygiene. *Infect Control Hosp Epidemiol.*, 30:830-839.
33. Corbett, J. A. and McDaniel, M. L. (1995) Intraislet release of interleukin 1 inhibits beta cell function by inducing beta cell expression of inducible nitric oxide synthase. J Exp Med 181: 559–568.
34. Conceição, I., González-Duarte, A., Obici, L. (2016). "Red-flag" symptom clusters in transthyretin familial amyloid polyneuropathy. J Peripher Nerv Syst., 21:5–9.
35. Mohamed Ishmael Belghazi ; Aristide Baratin ; Sai Rajeswar ; Sherjil Ozair ; Yoshua Bengio; Aaron Courville and Devon Hjelm (2021). Mutual Information Neural Estimation. arXiv:1801.04062v5 [cs.LG] 14 Aug 2021.
36. Gloria Nakalema, Yamile Ariaz Ortiz and Agwu Ezera (2015). Prevalence patterns of bacterial urinary tract infections among febrile children under-five years of age at Kampala International University Teaching Hospital. *IDOSR Journal of Biology,*

Chemistry and Pharmacy 7(1)41-55.

37. Petrus Baruti, Nzabandora Emmanuel and Agwu Ezera (2022). Evaluation of the bacterial agents associated with PID among women of reproductive age at Kampala International University Teaching Hospital. *IDOSR Journal of Biochemistry, Biotechnology And Allied Fields* 7(1): 64-74, 2022.