

Microbial Contamination of Applied Instrument in Female Hair-Dressers Salon in Wukari, Taraba State, Nigeria

R.E. Aso, C. Hammuel, T.I. Ade, J. Briska, and C.S. Hyelnaya

ABSTRACT

Hairdresser's salons are public places that can contribute to the spread of viral, fungi and bacterial pathogens. However, little is known about the contamination of hairdressing tools by bacterial and fungal pathogens. Hence, this study was conducted to determine bacterial and fungal contaminants of tools used in hairdressing salons within Wukari metropolis, Taraba State. Eighty (80) different samples were collected from combs, brushes, rollers, and hairdryers used in hairdressing salons using sterile swab stick moistened with normal saline. Samples were cultured aerobically on nutrient agar, MacConkey agar, and sheep blood agar for bacterial isolation and potato dextrose agar for fungal isolation. Bacterial isolates were identified using conventional biochemical tests while fungal isolates were identified on the basis of their cell wall structure using the lactophenol cotton blue stain. Antibiotic sensitivity pattern of bacterial isolates was tested using the modified Kirby-Bauer disc diffusion method. Sixty-seven (83.75%) of the collected sample were positive for bacterial and/or fungal contamination, yielding twenty-two (22) and eighteen (18) isolates each of bacteria and fungi. The bacterial isolates were *Staphylococcus aureus* (81.82%), *Staphylococcus epidermidis* (13.64%), and *Escherichia coli* (4.64%) while the fungal isolates were *Aspergillus fumigatus* (31.25%), *Aspergillus flavus* (50%), *Aspergillus niger* (6.25%), *Madurella grisea* (6.25%), and *Rhizopus stolonifera* (6.25%). Bacterial isolates were generally sensitive to ciprofloxacin, gentamicin, rifampicin, ofloxacin, and streptomycin. The highest resistances were against cefuroxime, trimethoprim-sulfamethoxazole, ampicillin, and augmentin. The presence of these microorganisms on hairdressing tools is an indication of poor hygienic practices among hairstylists in Wukari and these tools can serve as vehicles for the transmission of bacterial pathogens. Hence, appropriate measures should be taken to reduce the microbial load from hairdressing salons instruments.

Keywords: Hair-dresser salons, hairdressing tools, microbial contamination, Wukari metropolis.

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I. INTRODUCTION

Salons are private business enterprises responsible for improving beauty, dealing with cosmetic products and tools to improve hair, face, and body of individuals. They provide a broad range of services including hairdressing, nail care (manicures and pedicures), hair removal by waxing and threading, mud baths, among others (Alharbi & Alhasim, 2021a). Despite their immense significance in the maintenance of beauty, salons have been shown to constitute a risk to public health (Enemuor *et al.*, 2013; Stanley *et al.*, 2014). The risks posed by salons are a function of the products and tools which are used within, the nature of the business, and the service provider themselves (Stout *et al.*, 2011).

Hairdressing tools contribute to the spread of viral, fungal, and bacterial infections because they serve as fomites that support the transmission of pathogenic

organisms (Dadashi & Dehghanzadeh, 2016). Fomites act as environmental reservoirs, ultimately enhancing the transmission of pathogens from one host to the other (Kraay *et al.*, 2018). Several tools used within salons have been reported to harbor microbial contaminations including brushes, combs, cosmetic tools, wax, lipstick, eyeliner, mascara, foundation, cosmetic powders, hairdryers, hair stretchers, rollers, hairpins, cosmetic creams, nail care tools, facial creams and hand and body lotions (Ebuara *et al.*, 2020; Enemuor *et al.*, 2013; Stanley *et al.*, 2014; Dadashi & Dehghanzadeh, 2016; Hassan *et al.*, 2018). According to Alharbi and Alhashim (2021b), microbial contamination of tools used in salons arise due to improper implementation of acceptable levels of sterilization for used tools.

Bacterial contaminants of tools used in salons are potential pathogens that can cause diseases when they come in contact with susceptible hosts. Bacterial contaminants can be transmitted to the host through traumatic breakage of the

anatomical skin barrier. Hence, this present study was aimed at identifying bacterial and fungal contaminants of tools used in hairdressing salons within Wukari metropolis.

II. MATERIALS AND METHODS

A. Study Area

This study was carried out in Wukari, the headquarter of Wukari Local Government Area of Taraba State, North Eastern part of Nigeria. Wukari is the home of Federal University Wukari and Jubilee University (Kwararafa University). The major languages spoken are Junkun, Tiv, Hausa and Fulani.

B. Ethical Approval

Consent and approval were obtained from owners of hairdressing salons before samples were collected.

C. Sample Collection and Culture

A total of eighty (80) samples from combs, hair brushes, rollers and dryers were collected from twenty (20) randomly selected hairdressing salons within Wukari metropolis. Samples were collected aseptically using sterile swab sticks moistened in normal saline. Samples were cultured aerobically on Nutrient agar, MacConkey agar, sheep blood agar, and potato dextrose agar supplemented with Chloramphenicol (500mg). Inoculated nutrient agar, MacConkey agar, and sheep blood agar plates were incubated aerobically at 37 °C for 18-24 hours while inoculated potato dextrose agar plates were incubated for 5-7 days at 27 °C.

D. Identification of Microorganisms

Distinct bacterial colonies were first identified on the basis of colonial morphologies and were further identified using Gram staining, catalase test, coagulase test, citrate utilization test, indole production test, and triple sugar iron agar test. Fungal isolates were identified using lactophenol cotton blue stain (Hemraj *et al.*, 2013).

E. Catalase Test

A drop of 3% hydrogen peroxide was placed on a grease-free glass slide. A sterile glass rod was then used to pick a distinct colony from an overnight culture and immersed into the drop of hydrogen peroxide. The development of bubbles is positive for catalase production while the absence of bubbles is negative for catalase production (Hemraj *et al.*, 2013).

F. Coagulase Test

Peptone water (10% w/v) was prepared and dispensed in 5 mLs into sterile test tubes. The peptone water was then inoculated with distinct colonies of the test organism before adding 3 – 4 drops of EDTA-treated plasma. Inoculated tubes were then incubated at 37 °C and observed every two hours for clumping. If after 8 hours of incubation, clumping is not observed, the tube is further incubated till 18 – 24 hours. Development of clumps in tubes after incubation is positive for coagulase production (Hemraj *et al.*, 2013).

G. Citrate Utilization Test

Simmons citrate agar (Oxoid, UK) was prepared according to manufacturer's instructions, sterilized and

allowed to solidify into agar slants. Distinct and morphologically similar colonies from an overnight culture of the test organism were picked and used to inoculate the surface of the freshly prepared agar slants. Inoculated slants were then incubated aerobically for 18-24 hours at 37 °C. After incubation, culture slants were observed for colour change. Change in the colour of the medium from green to blue is considered positive citrate test (Hemraj *et al.*, 2013).

H. Indole Production Test

Peptone water (10% w/v) was prepared and dispensed in 5 mL test tubes. Peptone water were then inoculated with distinct and morphologically similar colonies from an overnight culture of the test isolate. Inoculated broth was incubated aerobically for 18 – 24 hours at 37 °C. After incubation, 2 – 3 drops of Kovac's reagent were added into the culture tubes and allowed to stand for 15 – 30 seconds. The development of a bright red or pink ring at the top of the tube is considered a positive indole test (Hemraj *et al.*, 2013).

I. Triple Sugar Iron (TSI) agar test

Triple sugar iron agar (Oxoid, UK) was prepared according to manufacturer's instructions, autoclaved and allowed to solidify into agar slants. The molten agar was dispensed into sterile tubes such that the slants have significant base and slant. Distinct and morphologically similar colonies from an overnight culture of the test isolate were picked and used to inoculate the surface of the freshly prepared TSI agar slants. Inoculated slants were then incubated aerobically at 37°C for 18 – 24 hours. After incubation, culture slants were observed for the sugar fermentation, hydrogen sulfide production, and gas production. Observations were then compared with charts containing expected reactions in the identification of bacterial isolates (Hemraj *et al.*, 2013).

J. Lactophenol Cotton Blue Stain

A drop of 70% ethanol was made on the surface of a grease-free glass slide. The fungal specimen was then picked with the aid of a teasing pin to mix well. Two drops of lactophenol cotton blue solution was then added to the teased specimen before covering with a clean sterile coverslip without making air bubbles. Slides were then examined under the X40 objective lens of a compound microscope (Jain *et al.*, 2020).

K. Antibiotic Sensitivity Testing

Antibiotic sensitivity testing was carried out using the modified Kirby-Bauer disc diffusion method. Bacterial isolates were standardized to 0.5 McFarland before inoculating the surface of freshly prepared Mueller-Hinton agar plates. Bacterial isolates were tested against Ciprofloxacin (10µg), Augmentin (10µg), Streptomycin (30µg), Gentamicin (10µg), Ampicillin (30µg), Trimethoprim-Sulfamethoxazole (1.25µg/23.75µg), Ofloxacin (10µg), Rifampicin (10µg), and Cefuroxime (30µg). Observed zones of inhibition diameters were measured and compared with standard as stated in clinical laboratory standards institute (CLSI, 2021).

III. RESULTS

Of the eighty (80) collected swab samples, sixty-seven (67; 83.75%) yielded positive bacterial and/or fungal growth. A total of twenty-two (22) bacterial isolates and sixteen (16) fungal isolates were recovered from the fomites. *Staphylococcus aureus* (18; 81.82%) was the most prevalent bacterium, followed by *Staphylococcus epidermidis* (3; 13.64%), and *Escherichia coli* (1; 4.54%). Of the 16 fungal isolates, *Aspergillus flavus* (8; 50%) was the most prevalent, followed by *Aspergillus fumigatus* (5; 31.25%), while one isolate (6.25%) each of *Aspergillus niger*, *Madurella grisea*, and *Rhizopus stolonifera* were recovered. The fungal contamination is dominated by species of *Aspergillus* as well as *R. Stolonifera* and *M. grisea* (Table I). The statistical analysis showed that there was a significant association between the bacterial or fungal isolates and the hair-dressing salon fomites at $P < 0.05$.

TABLE I: PREVALENCE OF BACTERIAL AND FUNGAL ISOLATES FROM HAIRDRESSING SALOON FOMITES

	Organism	f (%)	p-value
Bacteria (n=22)	<i>Staphylococcus aureus</i>	18 (81.82)	0.00001
	<i>Staphylococcus epidermidis</i>	3 (13.64)	
	<i>Escherichia coli</i>	1 (4.54)	
	<i>Aspergillus fumigatus</i>	5 (31.25)	
Fungi (n=18)	<i>Aspergillus flavus</i>	8 (50.0)	0.01256
	<i>Aspergillus niger</i>	1 (6.25)	
	<i>Madurella grisea</i>	1 (6.25)	
	<i>Rhizopus stolonifera</i>	1 (6.25)	

The samples collected from the fomites all had bacterial and fungal contaminants as presented in Table II. *Staphylococcus aureus* and *Staphylococcus epidermidis* were contaminants of all sampled fomites and *E. coli* was only recovered as a contaminant of brushes. Furthermore, *A. flavus* and *A. fumigatus* were also contaminants of all sampled fomites. *Aspergillus niger* was only isolated from combs and rollers. *Madurella grisea* and *Rhizopus stolonifera* contaminated rollers and combs respectively.

Staphylococcus aureus isolates were highly resistant to Augmentin (55.56%), Trimethoprim-Sulfamethoxazole (33.33%), Cefuroxime (66.67%), and Ampicillin (100%). *Staphylococcus epidermidis* were resistant to Augmentin (66.67%), Trimethoprim-Sulfamethoxazole (66.67%), Ampicillin (100%), and Cefuroxime (100%) (Table III). For all tested antibiotics, there was no significant difference in the resistance pattern of *S. aureus* and *S. epidermidis* to the tested antibiotics. The statistical analysis showed that there was no significant association between the antibiotics resistance pattern and the *Staphylococcus* species at $P < 0.05$.

TABLE III: ANTIBIOTIC RESISTANCE PATTERN OF BACTERIAL ISOLATES FROM HAIRDRESSING SALOON FOMITES

Antibiotic	<i>S. aureus</i> n (%)	<i>S. epidermidis</i> n (%)	χ^2	p-value
Augmentin	10 (55.56)	2 (66.67)	0.1296	0.718816
Ampicillin	18 (100.0)	3 (100.0)	1.6212	0.202919
Cotrimoxazole	6 (33.33)	2 (66.67)	1.2115	0.271028
Cefuroxime	12 (66.67)	3 (100.0)	0.4136	0.520146

IV. DISCUSSION

One of the major ways by which microbial pathogens get transferred in nature is through vehicles, such as fomites. Fomites, being inanimate objects, commandeer the transmission of pathogens from person to persons. In this research, fomites used in hairdressing salons in Wukari metropolis were collected and assayed for bacterial and fungal contamination. Our study reported 83.75% microbial contamination of tools used in hairdressing shops. This is higher than 79% reported in Ranya, Iraq (Hassan *et al.*, 2018). However, high microbial contamination of tools as reported in this study can mostly be associated with poor hygiene among the hairdressers.

Staphylococcus aureus, *Staphylococcus epidermidis*, and *Escherichia coli* were reported as the bacterial contaminants of hairdressing tools in Wukari. Enemuor *et al.* (2013), also reported similar bacterial contaminants in Anyigba, Kogi State except *E. coli*. However, in Umudike, Abia State, Stanley *et al.* (2014) reported *S. aureus*, *Streptococcus* species and *Micrococcus* species, *Staphylococcus aureus* and *S. epidermidis* are normal flora of the human skin, their high prevalence as reported in this study is a sign of poor hygienic practices in decontaminating and sterilizing used tools. The isolation of *Escherichia coli* in this study points towards faecal contamination and can be a significant pathogen especially in susceptible individuals.

The current study is dominated by species of *Aspergillus* as well as *R. Stolonifera* and *M. grisea*. In Jeddah City, high prevalence of *Aspergillus* sp. was also reported to contaminate hairdressing tools (Alharbi & Alhasim, 2021a). In Anyigba, Kogi State, and Umudike, Abia State, *Aspergillus* sp. and *Rhizopus* sp. were also reported as contaminants of hairdressing tools, but not *M. grisea* (Enemuor *et al.*, 2013; Stanley *et al.*, 2014). *M. grisea* is a fungal pathogen mostly associated with Madura foot, a persistent fungal infection of the skin. Hence, the presence of this organism on tools used in hairdressing salons is a cause for alarm as this organism is capable of infecting hands, feet, and other body parts.

Antibiotic resistance is one of the most important public health issues globally. The present research showed the bacterial isolates were sensitive to some of the selected antibiotics. All the bacterial isolates were sensitive to Ciprofloxacin, Streptomycin, Gentamicin, Ofloxacin, and Rifampicin. Bacterial antibiotic resistance was highest against Augmentin, Ampicillin, Cefuroxime, and Cotrimoxazole. The statistical analysis in this research showed that there was no association between antibiotic resistance and *Staphylococcus* species. The antibiotic resistance may be developed as result of some chemicals used in the Salon. However, this does not rule out the possibility of resistance as a result of indiscriminate use of antibiotics in the environment where these organisms were isolated. The high prevalence of resistance of *S. aureus* to β -lactam antibiotics could be as result of action of the enzyme, β -lactamase to inactivate the antibiotic as reported by Bush and Bradford, (2020).

TABLE III: OCCURRENCE OF MICROBIAL CONTAMINANTS ON HAIRDRESSING SALOON FOMITES

Samples	Bacterial Isolates				Fungal Isolates			
	<i>E. coli</i>	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>A. flavus</i>	<i>A. niger</i>	<i>R. stolonifera</i>	<i>A. fumigatus</i>	<i>M. grisea</i>
Comb	-	+	+	+	+	+	+	-
Dryer	-	+	+	+	-	-	+	-
Rollers	-	+	+	+	+	-	+	+
Brushes	+	+	+	+	-	-	+	-

+: Growth; -: No growth

V. CONCLUSION

Microorganisms are able to adhere to the surfaces of inanimate surfaces (fomites) and use these fomites to aid their transmission to susceptible human hosts. The present study evaluated and reported the microbial contaminants of applied instruments used in hairdressing salons within Wukari metropolis, Taraba State. All sampled applied instrument were positive for either/both of bacterial and fungal contaminants. Twenty-two bacterial isolates including *S. aureus* (18; 81.82%), *S. epidermidis* (3; 13.64%), and *E. coli* (4.54%) and Sixteen (16) fungal isolates including *A. fumigatus* (5; 31.25%), *A. niger* (1; 6.25%), *A. flavus* (8; 50%), *M. grisea* (1; 6.25%), and *R. Stolonifera* (1; 6.25%) were recovered in this study. All the bacterial isolates were sensitive to Ciprofloxacin, Streptomycin, Gentamicin, Ofloxacin, and Rifampicin. However, the highest resistance rates were against Trimethoprim-Sulfamethoxazole, Augmentin, Cefuroxime, and Ampicillin. High rate of bacterial and/or fungal contamination of used instrument in hairdressing salons is an indication of poor hygienic practices on the part of the service providers. Hence, proper infrastructures should be implemented to ensure the disinfection and/or sterilization of such instruments before and after use.

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