ISOALATION OF MULTIDRUG RESISTANT BACTERIAL PATHOGENS FROM HUMAN HAIR OBTAINED FROM BARBING SALONS LOCATED WITHIN BENIN CITY, NIGERIA.

ABSTRACT

This study was carried out to investigate the antibiotic susceptibility pattern of bacterial pathogens isolated from human hair in barbing salon. Hair samples were collected from ten different barbing saloons in Benin City and immediately transported to the laboratory for microbiological analysis using pour plate isolation method. Isolated bacteria were identified based on their cultural, morphological and biochemical characteristic. Antibiotics sensitivity was carried out using commercially available antibiotic disks. Total bacteria counts ranged from $2.80x10^4$±0.8cfu/g to $6.13x10^4$±0.21 cfu/g. Bacterial isolated included *Escherichia coli*, *Proteus vulgaris*, *Streptococcus viridians* and *Corynebacterium* sp. The least occurring bacteria were *Escherichia coli* and *Proteus vulgaris* with percentage distribution of 40% each while the most widely distributed was *Corynebacterium* sp. (80%). All the bacterial isolates were observed to be multiple drug resistant. The most effective drugs were sparfloxacin, perfloxacin, gentamicin, erythromycin and ciprofloxacin. This study has shown that high densities of multiple drug resistant pathogenic bacteria are usually associated with human hair.

Keywords

Transmission, resistance, pathogen, infection, disease, antibiotics

INTRODUCTION

Hair is a protein filament that grows from follicles found in the dermis, or skin. Hair is one of the defining characteristics of mammals. Each strand of hair is made up of the medulla, cortex, and cuticle (Abbasi, 2011). Each has specific characteristics that determine the length of the hair. The hair found on the head serves as primary sources of heat insulation and cooling (when sweat evaporates from soaked hair) as well as protection from ultra-violet radiation exposure (Summers *et al*., 1995). Attitudes towards hair, such as hairstyles and hair removal, vary widely across different cultures and historical periods, but it is often used to indicate a person's personal beliefs or social position, such as their age, gender, or religion (Yun *et al*., 2010). Shaving is accomplished with bladed instruments, such as razors. The blade is brought close to the skin and stroked over the hair in the desired area to cut the terminal hairs and leave the skin feeling smooth. The majority of airborne contaminants containing bacteria have been associated with the hair, skin, and respiratory tracts of humans. Human hairs may function as an air-collecting agent for micro-contaminants, because the hairs are constantly exposed to air and can readily adsorb a variety of airborne particles via electrostatic attraction, grooved surfaces, thin and long structures, and biochemical affinity.

Hairdressing and beauty salons are classified as personal service establishments and such services may pose potential health concerns to their clients including the risk of infection and sometimes injury (Adeleye and Osidipo, 2004; Barn and Chen, 2011). It is believed that any service with the potential to break the skin's surface can be associated with infections that can then be transmitted to and between clients if proper infection control procedures are not implemented (Stout *et al*., 2011). It has been observed that hairdressing operators including

barbers and their clients are constantly being exposed to bacterial or fungal contamination during their services (Tharmila *et al*., 2012). Naturally human hair harbours many pathogenic bacteria, and also it acts as a potential source of cross infections. Bacteria such as *Staphylococcus aureus, Escherichia coli, Streptococcus viridians,* β haemolytic streptococci, the *Proteus* group, *Pseudomonas pyocyanea* and *Streptococcus faecalis* have been reported to be present in the hair (Tharmila *et al*., 2012). This therefore represent a potential risk factor for customers and visitors to the salon. Due to these problems, in this study, we aimed to investigate the antibiotic susceptibility pattern of bacterial pathogens isolated from hair in barbing salons, within Benin metropolis.

MATERIALS AND METHODS

Samples collection

Hair samples were randomly collected from ten (10) different barbing salons within Benin metropolis, Edo State, Nigeria. The ten salons were carefully selected based on population of clients that always go there. Before sampling was carried out, appropriate arrangement was ensured to disinfect the perimeter inside the barber's chop in order to prevent contamination. The samples were collected with sterile spatula and placed in a sterile universal container to avoid contamination. Samples were then transported to the laboratory for microbial analysis without delay.

Culture medium and Isolation of Bacteria

Commercially available Nutrient agar medium was obtained and prepared following the manufacturer's instructions. Ten gram (10g) of each sample was weighed and aseptically introduced into 90ml of sterile distilled water, properly shaken before a 10 fold serial dilution, up to 10^{-3}, was performed. Pour plate isolation method was used for microbial enumeration. In this method, 0.1ml from each dilution was pipetted into sterile Petri dish and labelled. About 20ml of prepared agar medium was dispensed into the various Petri plates and mixed. The nutrient agar plates were allowed to solidify and then incubated at 37^{o}C for 24 hours, after which the developed colonies were counted to obtain total viable count. Discrete distinct colonies were purified by subculturing into nutrient agar plates using the streak plate method.

Procedure for identification of the organisms

The bacterial isolates were characterized and identified based on their cultural characteristics and biochemical reaction as presented in table 2.

Antibiotic Susceptibility pattern of the isolates:

Antimicrobial disc tests were performed on the isolates using the following antibiotic discs: perfloxacin, gentamicin, ampiclox, zinnacef, amoxicillin, rocephin, ciprofloxacin, streptomycin, erythromycin, gentamycin, septrin, chloramphenicol, sparfloxacin, and ofloxacin. The organism was inoculated into nutrient broth in test tube and incubated for 24hours. Measured 0.1 ml of liquid culture was added to solidified nutrient agar in Petri dish and a glass spreader was used to even spreading on the agar surface. The plates were allowed to dry for 5-10 minutes, after which standard antibiotics disks was layered on the inoculated agar. The plates were incubated at 37^{o}C for 24hours. Clear zones around each disk were measured and interpreted as either resistance or sensitive.

RESULTS

Table 1 show total bacterial counts of the different hair samples. Value ranged from $2.80x10^3$±0.8cfu/g to $6.13x10^3$±0.21 cfu/g. Table two describes the cultural, morphological and biochemical characterization of the bacterial isolated. The isolates identified include *Escherichia coli*, *Proteus vulgaris*, *Streptococcus viridians* and *Corynebacterium* sp. Figure 1 presents percentage distribution of the bacteria species among the different samples with the most prevalent being *Corynebacterium* sp (80%) while the least was *Escherichia coli* (40%). Table 3 explains the antibiotic sensitivity pattern of bacterial isolates. All identified bacterial strain were observed to be multiple drug resistant.

Table 1: Total viable bacterial counts in hair samples from dilution of 10^{-2}

Samples	**mean± SE($x10^3$cfu/g)**	**P-value**
A	$2.80±0.8^a$	
B	$5.77±0.31^b$	
C	$5.80±0.27^b$	
D	$6.07±0.21^b$	
E	$5.03±0.15^c$	0.000
F	$6.13±0.21^b$	
G	$5.10±0.20^c$	
H	$5.23±0.25^c$	
I	$5.53±0.68^b$	
J	$4.27±0.21^d$	

Key: A-J = Hair from barbing salon in ten different locations in Benin City

SE = Standard error; P<0.05

Table 2: Cultural, morphological and biochemical characteristics of the bacterial isolates

Characteristics	**Isolates**			
Cultural	B1	B2	B3	B4
Elevation	Low convex	Flat	Convex	Convex
Margin	Entire	Undulated	Entire	Entire
Colour	Cream	Cream	White	Cream
Shape	Circular	Irregular	Circular	Circular
Size	Small	Medium	Small	Medium
Morphological				
Gram staining	-	-	+	+
Cell type	Rod	Rod	Cocci	Rod
Cell arrangement	Single	Single	Chains	Single
Biochemical				
Catalase	+	+	-	+
Oxidase	-	-	-	-

Coagulase	-	-	-	-
Urease	-	+	-	+
Indole	+	+	-	-
Citrate	-	+	+	+
Sugar fermentation				
Glucose	+	+	+	+
Lactose	+	-	-	-
Possible isolates	*Escherichia coli*	*Proteus vulgaris*	*Streptococcus viridians*	*Corynebacterium* sp.

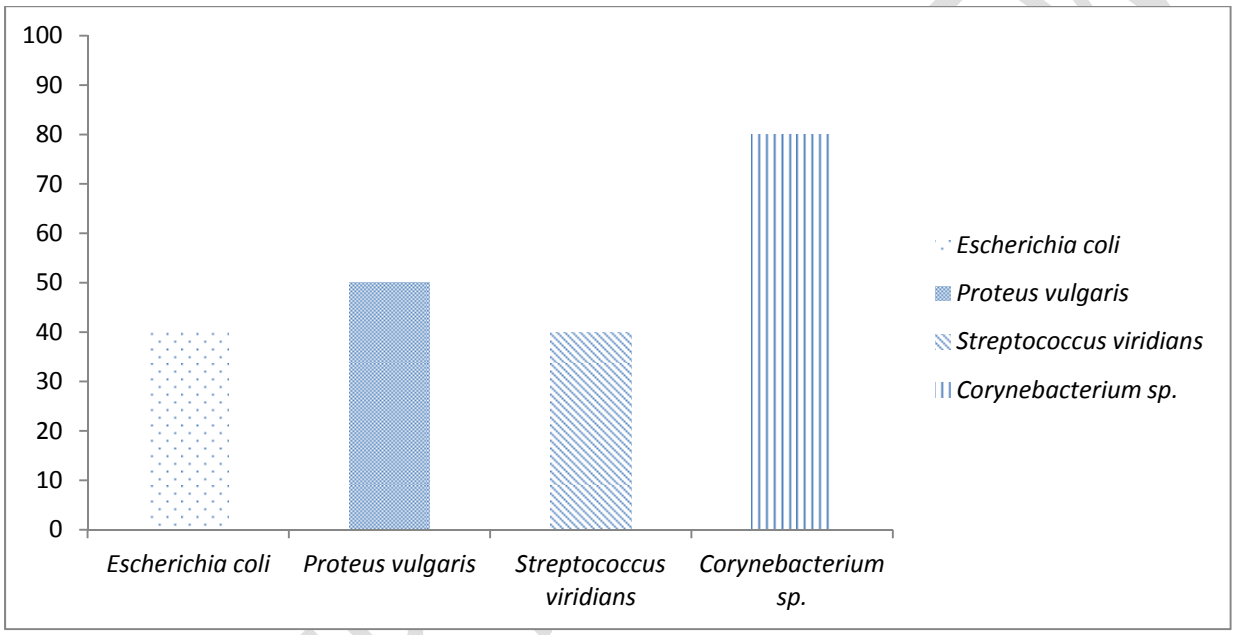

Fig. 1: Prevalence of bacterial isolated from hair

Table 3: Antibiotic susceptibility pattern of isolated bacteria

Bacteria	**No. I**	**Antibiotics**									
Gram +ve		**CPX**	**St**	**SXT**	**E**	**PEF**	**CN**	**APX**	**Z**	**AM**	**Ro**
S. viridians	4	2(50)	2(50)	3(75)	1(25)	4(100)	4(100)	3(75)	2(50)	3(75)	4(100)
Corynebacterim sp.	8	3(37.5)	4(50)	6(75)	3(37.5)	2(25)	2(25.0)	5(62.5)	1(12.5)	4(50.0)	3(37.5)
Gram –ve		**CH**	**SP**	**AU**	**OFX**	**SXT**	**PEF**	**AM**	**S**	**CN**	**CPX**
Escherichia coli	4	4(100)	1(25)	0(0.0)	0(0.0)	0(0.0)	2(50)	1(25)	3(75)	1(25)	0(0.0)
Proteus vulgaris	5	0(0.0)	0(0.0)	3(60)	2(40)	4(80)	1(20)	1(20)	3(60)	1(20)	1(20)

KEY:

No. I= Number of isoaltes; CPX-Ciprofloxacin, Ro-Rocephin, St-Streptomycin, AU-Augmentin, SXT-Septrin, SP- Sparfloxacin, E-Erythromycin, CH-Chloramphenicol, PEF-Pefloxacin, CPX-ciprofloxacin, CN-Gentamicin, APX-Apmpiclox, AM-Amoxacillin, Z-Zinnacef

Discussion

High bacteria load were observed in the different hair samples from different barbing salons. Total bacterial counts ranged from $2.80x10^3 \pm 0.8$cfu/g to $6.13x10^3 \pm 0.21$ cfu/g. Ajuzie and Osaghae (2011) reported high bacterial counts from salon waste water. The bacteria may have come from washed hair. Variations in bacterial counts from the different samples reflects the life style of the individual and the kind of hair treatment. These high bacteria counts shows that human hair is highly contaminated with diverse microorganisms especially bacterial, some of which can be potential pathogens of public health importance (Yun *et al*., 2010). This finding means that human hair in barbing salon represent potential source of bacterial contamination of either food or water. Also due to the light nature of the hair, it can be easily blown by wind to surrounding environment where it may deposit on food or water system, thereby leading to contamination.

Based on the cultural, morphological and biochemical characterization of the isolates, four different bacterial species were isolated and they included *Escherichia coli*, *Proteus vulgaris*, *Streptococcus viridians* and *Corynebacterium* sp. Enemuor *et al*. (2013) reported on the prevalence of these bacterial strains in hair dressing and beauty salons. Summers (1995) stated in his work that hair is a reservoir of *Staphylococcus aureus.* Although *S. aureus* was not detected in this work, the isolated bacterial strains from this work are potential pathogens implicated in various diseases of humans. *E. coli* is known to cause various gastrointestinal disorder such as diarrhoea; urinary tract infections and meningitis. *Proteus* spp. have been implicated in urinary tract infections. *Streptococcus* spp. are causative agents of several human diseases including pneumonia, caries and other pyogenic infections. The organism also produce super antigen which hyper regulate T-cell proliferation and activation, leading to autoimmune diseases. *Corynebacterium* sp. is a known human pathogen, causing diseases such as diphtheria. These pathogens can easily be transmitted from one person to another most especially when one clipper or comb is used for multiple customers. This calls for awareness on the part of customers, on the possibility of being infected. Tharmila *et al*. (2012) investigated the inhibitory effect of some traditional hair washing substances on hair borne bacteria, thus confirming the presence of bacterial pathogens on human hair. In another research study, five bacterial isolates including *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus* sp*, Enterococus* species and *Enterobacteria* were reported (Enemuor *et al*., 2013). The presence of these potential pathogens is an indication that hairdressing and beauty salons could be contributing to the spread of infection within the community (Enemuor *et al*., 2013). Infection can occur during hairdressing procedures since items such as razors, scissors, combs, clippers and hairpins can accidentally penetrate the skin. Blood and body fluids do not have to be visible on instruments, equipment or working surfaces for infection to be transmitted. Bacterial Infections that can be spread in hairdressing premises include skin infections on the scalp, face and neck such as impetigo (Brown, 2006; Amodio *et al*., 2010; Barn and Chen, 2011).

Summers *et al*. (1995) reported the presence of *Escherichia coli, Streptococcus viridans, Proteus* group, *Haemolytic streptococci, Pseudomonas pyocyanea*, *Streptococcus faecalis* and *Staphylococcus aureus* from the hair of the scalp.
The different bacterial strains from this study were observed to be variedly distributed among the different hair samples. The least occurring bacterial species were *Escherichia coli* and *Proteus vulgaris* with percentage distribution of 40% each while the most widely distributed was *Corynebacterium* sp. (80%).

Antibiotic susceptibility of the bacterial isolates revealed varying degree of resistance to conventionally used antibiotics. All the isolates were observed to be multiple drug resistant. Result revealed that isolated bacterial from hair, were resistant to multiple antibiotics. There were variations in their degree of antibiotic resistance. Of the four isolates of *Streptococcus viridans*, 2(50%) were resistant to ciprofloxacin, streptomycin, zinnacef, 3(75%) were resistant to septrin, ampiclox and amoxicillin, while 4(100%) where resistant to perfloxain, gentamicin and rocephin. Antibiotic resistant pattern of *Corynebacterium* sp revealed that 5(62.5%) were resistant to ampiclox while 6(75%) were resistant to septrin. Perfloxacin, gentamicin and zinnacef were highly effective against *Corynebacterium* sp in this study. The 4(100%) of *Escherichia coli* were sensitive to augmentin, ofloxacin, septrin and ciprofloxacin. However, they were resistant to chloramphenicol, perfloxacin and streptomycin. *Proteus vulgaris* was also sensitive to chloramphenicol, sparfloxacin and resistant to augmentin, septrin and streptomycin

Antibiotic resistant genes in bacterial have been shown to be borne on either plasmid or chromosomally mediated. Bacterial pathogens have been reported to use various mechanisms to resist antibiotics, such mechanisms include use of efflux pumps, drug inactivating enzymes, drug modifying enzymes among others.

Conclusion

Hair samples from barbing salons have been shown to be highly contaminated with bacterial isolates. The isolated bacteria were found to be bacterial pathogens that are implicated in many human and animal diseases. These pathogens were also observed to be multidrug resistant. It is highly recommended that individual that goes to barbing salons should have their own clipper and always disinfect it to reduce the microbial load. People should also be aware of the potential possibility of pathogen transmission in barbing salon especially when such salon is situated near water or food canteens.

Competing Interests

All authors have declared that no competing interests exist.

References

Abbasi AA. Molecular Evolution of HR, a Gene that Regulates the Postnatal Cycle of the Hair Follicle. *Scientific Reports.* 2011; 1: 32-39.

184 Adeleye IA and Osidip OO. Isolation and Characterization of Microorganism from Instruments
185 Used by Pedicurists Operating within Lagos Metropholis, Nigeria. *Western Indian*
186 *Medical Journal.* 2004; 53:413-415.
187 Ajuzie CU and Osaghae BA. The Bacterial and Physico-chemical Properties of Hair Salon
188 Wastewater and Contaminated Soil in Benin Metropolis. *African Journal of*
189 *Biotechnology* 2011; 10(11): 2066-2069.

190 Amodio E, Benedetto MA, Gennaro L, Maida CM and Romano N. Knowledge, Attitudes and
191 Risk of HIV, HBV and HCV Infections in Hairdressings of Palermo City (South Italy).
192 *European Journal of Public Health* 2010; 20:433-437.
193 Barn P and Chen T. Infections associated with personal service establishments: aesthetics.
194 National Collaborating Centre for Environment Health ISBN: 978-1-926933-29-0 2011;
195 pp. 1-10.
196 Brown NJ. Guideline for public health standards of practice for hairdressing 2nd ed. ISBN:
197 073895521 Australia 2006; pp. 1-4.
198 Enemuor SC, Ojih MI, Isah S and Oguntibeju OO. Evaluation of Bacterial and Fungal
199 Contamination in Hairdressing and Beauty Salons. *African Journal of Microbiology*
200 *Research* 2013; **7**(14): 1222-1225.
201 Stout JE, Gadkowski LB, Rath S, Alspaugh JA, Miller MB and Cox GM. pedicure Associated
202 Rapidly Growing Mycobacterial Infections: an Academic Disease. *Clinical Infectious*
203 *Diseases* 2011; 53:787-792

204 Summers MM, Lynch PF and Black T. Hair as a Reservoir of *Staphylococci*. *Journal of Clinical*
205 *Pathology* 1995; 28: 230-238.
206 Tharmila S, Jeyaseelan EC and Thavaranjit AC. Inhibitory Effect of Some Traditional Hair
207 Washing Substances on Hair Borne Bacteria. *Der Pharmacia Lettre* 2012; 4(1):199-204.

208 Yun A, Yang EJ, Lee YM, Chae SC, Seo HN and Park DH. Quantitative and qualitative
209 estimation of bacteria contaminating human hairs. *Journal of Bacteriology and Virology*
210 2010; 40(1): 11 – 18.
211