

# ISOALATION OF MULTIDRUG RESISTANT BACTERIAL PATHOGENS FROM HUMAN HAIR

## ABSTRACT

This study was carried out to investigate the antibiotic susceptibility/resistivity 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  $2.80 \times 10^4 \pm 0.8$  cfu/g to  $6.13 \times 10^4 \pm 0.21$  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. Microbial contaminants evaluated in hospital operating rooms have been associated largely with humans, rather than dust and soil particles. 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 and their

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

## 51 **MATERIALS AND METHODS**

### 52 **Samples collection**

53 Hair samples were randomly collected from ten (10) different barbing salons within Benin  
54 metropolis, Edo State, Nigeria. The samples were collected with sterile spatula and placed in a  
55 sterile universal container to avoid contamination. Samples were then transported to the  
56 laboratory for microbial analysis without delay.

### 57 **Culture medium and Isolation of Bacteria**

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

### 67 **Procedure for identification of the organisms**

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

### 70 **Antibiotic Susceptibility pattern of the isolates:**

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

## 80 **RESULTS**

81 Table 1 show total bacterial counts of the different hair samples. Value ranged from  
82  $2.80 \times 10^3 \pm 0.8 \text{ cfu/g}$  to  $6.13 \times 10^3 \pm 0.21 \text{ cfu/g}$ . Table two describes the cultural, morphological and

83 biochemical characterization of the bacterial isolated. The isolates identified include *Escherichia*  
 84 *coli*, *Proteus vulgaris*, *Streptococcus viridians* and *Corynebacterium* sp. Table 3 presents  
 85 percentage distribution of the bacteria species among the different samples with the most  
 86 prevalent being *Corynebacterium* sp (80%) while the least was *Escherichia coli* (40%) table 4  
 87 explains the antibiotic sensitivity pattern of bacterial isolates. All identified bacterial strain were  
 88 observed to be multiple drug resistant.

89  
 90 Table 1: Total viable bacterial counts in hair samples from dilution of  $10^{-2}$

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

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

92 SE = Standard error; P<0.05

93

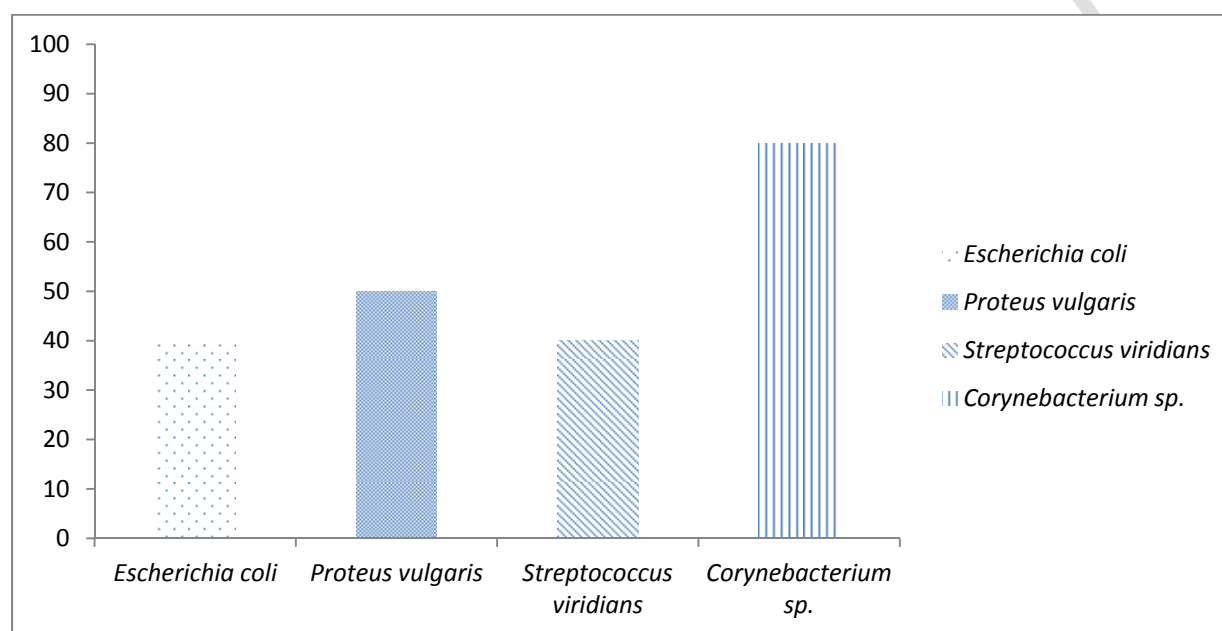
94

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

Characteristics	Isolates			
	B1	B2	B3	B4
<b>Cultural</b>				
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
<b>Morphological</b>				
Gram staining	-	-	+	+
Cell type	Rod	Rod	Cocci	Rod
Cell arrangement	Single	Single	Chains	Single
<b>Biochemical</b>				
Catalase	+	+	-	+
Oxidase	-	-	-	-
Coagulase	-	-	-	-
Urease	-	+	-	+
Indole	+	+	-	-

Citrate	-	+	+	+
Sugar fermentation				
Glucose	+	+	+	+
Lactose	+	-	-	-
<b>Possible isolates</b>	<i>Escherichia coli</i>	<i>Proteus vulgaris</i>	<i>Streptococcus viridians</i>	<i>Corynebacterium sp.</i>

96



97

98 Fig. 1: Prevalence of bacterial isolated from hair

99 Table 3: Antibiotic resistant pattern of isolated bacteria

Bacteria	No. I	Antibiotics									
		CPX	St	SXT	E	PEF	CN	APX	Z	AM	Ro
<b>Gram +ve</b>											
<i>S. viridians</i>	4	2(50)	2(50)	3(75)	1(25)	4(100)	4(100)	3(75)	2(50)	3(75)	4(100)
<i>Corynebacterim sp.</i>	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)
<b>Gram -ve</b>											
<i>Escherichia coli</i>	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)
<i>Proteus vulgaris</i>	5	0(0.0)	0(0.0)	3(60)	2(40)	4(80)	1(20)	1(20)	3(60)	1(20)	1(20)

100 **KEY:**

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

104

## 105 Discussion

106 High bacterial load were observed in the different hair samples from different barbing salons.  
107 Total bacteria counts ranged from  $2.80 \times 10^3 \pm 0.8$  cfu/g to  $6.13 \times 10^3 \pm 0.21$  cfu/g. Ajuzie and  
108 Osaghae (2011) reported high bacterial counts from salon waste water. The bacteria may have  
109 come from washed hair. Variations in bacterial counts from the different samples reflects the life  
110 style of the individual and the kind of hair treatment. These high **bacteria** counts shows that  
111 human hair is highly contaminated with diverse microorganisms especially **bacterial**, some of  
112 which can be potential pathogens of public health importance (Yun *et al.*, 2010). This finding  
113 means that human hair in barbing salon represent potential source of bacterial contamination of  
114 either food or water. Also due to the light nature of the hair, it can be easily blown by wind to  
115 surrounding environment where it may deposit on food or water system, thereby leading to  
116 contamination.

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

143 Summers *et al.* (1995) reported the presence of *Escherichia coli*, *Streptococcus viridans*,  
144 *Proteus* group, *Haemolytic streptococci*, *Pseudomonas pyocyanea*, *Streptococcus faecalis* and  
145 *Staphylococcus aureus* from the hair of the scalp.

146 The different bacterial strains from this study were observed to be variedly distributed among the  
147 different hair samples. The least occurring bacterial species were *Escherichia coli* and *Proteus*

148 *vulgaris* with percentage distribution of 40% each while the most widely distributed was  
149 *Corynebacterium* sp. (80%).

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

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

## 166 **Conclusion**

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

174

## 175 **Competing Interests**

176 All authors have declared that no competing interests exist.

## 177 **References**

- 178 Abbasi AA. Molecular Evolution of HR, a Gene that Regulates the Postnatal Cycle of the Hair  
179 Follicle. *Scientific Reports*. 2011; 1: 32-39.
- 180 Adeleye IA and Osidip OO. Isolation and Characterization of Microorganism from Instruments  
181 Used by Pedicurists Operating within Lagos Metropolis, Nigeria. *Western Indian*  
182 *Medical Journal*. 2004; 53:413-415.
- 183 Ajuzie CU and Osaghae BA. The Bacterial and Physico-chemical Properties of Hair Salon  
184 Wastewater and Contaminated Soil in Benin Metropolis. *African Journal of*  
185 *Biotechnology* 2011; 10(11): 2066-2069.

- 186 Amodio E, Benedetto MA, Gennaro L, Maida CM and Romano N. Knowledge, Attitudes and  
187 Risk of HIV, HBV and HCV Infections in Hairdressings of Palermo City (South Italy).  
188 *European Journal of Public Health* 2010; 20:433-437.
- 189 Barn P and Chen T. Infections associated with personal service establishments: aesthetics.  
190 National Collaborating Centre for Environment Health ISBN: 978-1-926933-29-0 2011;  
191 pp. 1-10.
- 192 Brown NJ. Guideline for public health standards of practice for hairdressing 2nd ed. ISBN:  
193 073895521 Australia 2006; pp. 1-4.
- 194 Enemuor SC, Ojih MI, Isah S and Oguntibeju OO. Evaluation of Bacterial and Fungal  
195 Contamination in Hairdressing and Beauty Salons. *African Journal of Microbiology*  
196 *Research* 2013; 7(14): 1222-1225.
- 197 Stout JE, Gadkowski LB, Rath S, Alspaugh JA, Miller MB and Cox GM. pedicure Associated  
198 Rapidly Growing Mycobacterial Infections: an Academic Disease. *Clinical Infectious*  
199 *Diseases* 2011; 53:787-792
- 200 Summers MM, Lynch PF and Black T. Hair as a Reservoir of *Staphylococci*. *Journal of Clinical*  
201 *Pathology* 1995; 28: 230-238.
- 202 Tharmila S, Jeyaseelan EC and Thavaranjit AC. Inhibitory Effect of Some Traditional Hair  
203 Washing Substances on Hair Borne Bacteria. *Der Pharmacia Lettre* 2012; 4(1):199-204.
- 204 Yun A, Yang EJ, Lee YM, Chae SC, Seo HN and Park DH. Quantitative and qualitative  
205 estimation of bacteria contaminating human hairs. *Journal of Bacteriology and Virology*  
206 2010; 40(1): 11 – 18.  
207