

## Antimicrobial Activity of Selected Aftershaves on *Staphylococcus aureus* and *Escherichia coli* Isolated from Barbers' Clippers and Brushes within Abakaliki Metropolis, South East Nigeria

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### ABSTRACT

This study was carried out to determine the antimicrobial activities of some aftershaves on bacteria isolated from barbers' clippers and brushes in barbers' shops in Abakaliki, Ebonyi State. Forty-five (45) samples were collected from the different shops. Sterile swab sticks were used to obtain samples from the surface of barbers' clippers and brushes and were immediately sealed to prevent contamination and drying. Isolation and identification of bacteria from barbers' clippers and brushes in barbing salons within Abakaliki metropolis was carried out using standard microbiological procedures. Test for antimicrobial activity of aftershaves was also carried out on the isolated bacteria using agar-well diffusion method. The result revealed the presence of *Staphylococcus aureus*, and *Escherichia coli* on barbers' clippers and brushes. *Staphylococcus aureus* (60%) was the more prevalent than the *Escherichia coli* (40%) isolate. The result also shows that the sterilization method used by most barbers with is the burning of the clippers with alcohol or methylated spirit had little effect on *Staphylococcus aureus* (12.5%) and had a greater effect on *Escherichia coli* (70%). The aftershaves' antimicrobial activities test results showed that the aftershaves had significant inhibitory effect on the isolates. This study has shown that barbers' clipper harbour bacterial organisms which can be potentially pathogenic and may promote the spread of infection to human. It is therefore recommended that barbers must properly sterilize their hair clippers and other barbing tools before been used on their customers, in order to reduce the spread of infection and that the use of aftershave be made mandatory.

Keywords: Antimicrobial, *Staphylococcus aureus*, *Escherichia coli* and Clippers.

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### INTRODUCTION

The human hair shaft is keratinized fibrous tissue that grows from follicles beyond the surface of the epidermis. Hair plays a key role in body temperature regulation, defense, protection from the environment, and aesthetics, as well as acting as a

sensory organ. Human skin nurtures an estimated one million bacteria per square centimeter of skin [1], and the scalp houses miscellaneous common scalp commensal microflora [2]; [3]; [4]. Scalp disorders including folliculitis types, fungal diseases,

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dandruff, and folliculitis decalvans, among others, are caused by or linked to microbes, which play a key role in disease predisposition and pathogenesis [5]; [6]; [7].

Barbers are important professionals in the society and in most cases barbershops are owned, cared and financed by individual members of the society. Barbers' shops are classified as personal services establishments and such services may pose potential health concerns to their clients including the risk of infection and sometimes injury [8]. It is a demand on their profession to utilize instruments such as knife, blades, clippers which makes it necessary to evaluate health hazards relating to their profession and practices and to identify professional practices linked with infection transmission [9]; [10]. Microorganisms are everywhere including skin surfaces and are continually introduced into the environment and could therefore easily spread between clients and operators and transferred by contact with unwashed hands, soiled equipment or contact with blood and other body substances [11].

These health risks vary depending on the nature of the service, the tools and equipment that are used, the health status of the clients and service providers as well as the infection control procedures [12]. Therefore the equipment used may clearly be associated with bacterial, viral and fungal organisms posing infection risks [13]. A significant proportion of population is enjoying the services of barbers in the society and their shops and professional practices may be a

#### MATERIALS AND METHODS

##### Media

The following media were used: Nutrient broth (Fluka, chemieindia),

Okonkwo *et al*  
source of transmission of various infections-directly or indirectly and some bacterial infections can occur without breaking the skin and for this reason equipment must be cleaned between each client [14]. The person at risk may be the next client on whom the contaminated instrument is used. Organisms that can cause potentially serious infections may be transmitted where appropriate precautions are not taken. It is believed that any service with the potential to break the skin's surface can be associated with infections which can then be transmitted to and between clients if proper infection control procedures are not implemented [15]. Unfortunately, there are no established regulations, guidelines and best practices for many of these salons in our environment.

##### Aim

The aim of this research work is to determine the antibacterial activities of selected aftershaves on *Staphylococcus aureus* and *Escherichia coli* isolated from barbers' clippers and brushes.

##### Specific Objectives

The objective of this research is to determine

- The bacterial load of barbers' clippers and brushes.
- The character and identity of the isolates.
- The effectiveness of the sterilization method used by most barbers.
- The antibacterial activities of selected aftershaves on the isolates.

Nutrient agar (Fluka, chemieindia),  
MacConkey agar (Fluka, Chemieindia),  
Mannitol salt agar (Fluka,

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Chemieindia), and Mueller-Hinton agar (Oxoid Ltd, Basingstoke Hampshire, England). These media were prepared according to the manufacturer's instruction.

#### **Aftershaves**

The following aftershave sprays and cream were used: methylated spirit (Gap Integrated Global Venture), shave rite (Chaoba Nig. Ltd.), clin barber (Alade J Health Products Nig. Ltd.), chaoba (Chaoba Nig. Ltd.), and authentic herbal cream (BFN Nig. Ltd.). The aftershaves were purchased from barbers.

#### **Methods Study Area**

This research was carried out in the Department of Applied Microbiology, Ebonyi State University, Abakaliki, Ebonyi State. The state capital is Abakaliki. It lies approximately within the longitude 7° 30 and 8° 30 E and 5° 40 and 6° 45 N. According to data from 2006 population and housing census, Ebonyi state has an estimated population of 2.3 million and a land mass of 5,935 km<sup>2</sup> in Ebonyi State, Nigeria.

#### **Collection of Samples**

A total of forty five (45) samples (15 samples each for brushes, clipper after barbing and clipper after sterilization by burning) were collected from fifteen different barbing saloons in Abakaliki metropolis. Barbers' clippers and brushes were swabbed with a moistened sterile swab stick. The swab sticks were thereafter placed into their casing to avoid contamination and drying, and then the swabs were labeled appropriately. The samples were taken to Applied Microbiology Laboratory, Ebonyi State University in clean polythene bags for

Okonkwo *et al*

microbial analysis within 30 minutes of collection.

#### **Microbiological Analysis**

The samples were processed by first inoculating into nutrient broth by stabbing the broth with the sample swab sticks. This was to increase the microbial population. The broth with the sample swab sticks were then incubated at 37°C for 18-24 hours. MacConkey agar and Mannitol Salt agar were prepared and using a sterile wire loop a loopful of the turbid broth were taken from the test tubes and streaked on the agar plates and then incubated at 37°C for 18-24 hours. Discrete colonies from the MacConkey agar and Mannitol salt agar were picked using a sterile wire loop and streaked on nutrient agar to get pure culture used for biochemical tests. An antimicrobial activity of the selected aftershaves was carried out on the pure culture.

#### **Identification and Characterization of Bacterial Isolates**

The bacterial isolates were identified on the basis of colonial morphology followed by microscopic examination after gram-staining. Biochemical tests including catalase, oxidase, indole test, coagulase test and methyl red test was carried out to characterize the isolates.

#### **Gram-Staining**

In this test, a drop of distilled water was placed on a clean, grease free glass slide, after which a small portion of the isolates was transferred from a day-old culture and emulsified on the drop of distilled water for smear preparation. The smear was allowed to air dry and then heat-fixed by waving it over a burning flame. The heat fixed smear was covered with crystal violet stain for one minute and subsequently washed off with clean water. All the water was tipped off, after which the

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smear was again covered with lugol's iodine solution for another one minute subsequently rinsed off in slowly running tap. The smear was decolorized with acetone drop- wisely until no more dye went off from the smear, after rinsing off the acetone; the smear was covered with safranin for 2 minutes and then rinsed off. The smear was air- dried, after which a drop of immersion oil was placed on it. The smear was then viewed under the light microscope using X100 objective lens (oil immersion) [16].

#### **Biochemical Test**

##### **Catalase Test**

A 2 ml drop of  $H_2O_2$  solution was poured into a test-tube, and a sterile wooden stick was used to remove several colonies of the test organism and was immerse in  $H_2O_2$ , active bubbling showed a positive catalase test [17].

##### **Coagulase Test**

The slide test method described by [18] was used in this test .A grease pencil was used to mark a glass slide into two sections, after which a loopful of normal saline was placed on each of the sections. A colony of the isolate (previously checked by gram staining) was collected using a sterile wire loop and emulsified homogenously on each drop of the normal saline on the slide. After the above step, a drop of sheep plasma was added to only one of the suspensions and mixed gently for 5 seconds. The observation of clumping after few second indicated a positive result (that is, the presence of coagulase).

##### **Methyl Red Test**

A colony of the tested organism was inoculated in 0.5ml of sterile glucose phosphate broth and was incubated aerobically at 37°C for 24hrs.A drop of methyl red solution was added and

Okonkwo *et al*

immediately observed for a bright red color development [19].

##### **Indole Test**

The test organism was inoculated into a test-tube or Bijou bottle containing 3ml of sterile peptone water and incubated at 37°C for up to 48 hours. Then 0.5ml of kovac's indole reagent was added to the broth culture and shaken gently and examined for a red colour in the surface layer within 10 minutes. Red surface layer showed positive indole test and no red surface layer showed negative indole test.

##### **Citrate Utilization Test**

A light suspension of the organism was made in saline. This was inoculated by stabbing on Simmons citrate agar and it was incubated at 37°C for 24-72 hours using an incubator. A growth of blue colour in Simmons citrate agar indicates a positive result [20].

##### **Preparation of 0.5 McFarland Standards**

Firstly, a 1% v/v solution of sulphuric acid was prepared by adding 1 ml of concentrated sulphuric acid to 99 ml of water. Also, a 1% w/v solution of barium chloride was prepared by dissolving 0.5 g of dihydrate barium chloride ( $BaCl_2 \cdot 2H_2O$ ) in 50 ml of distilled water. Then, with slow agitation 0.6ml of the barium chloride solution was added to 99.4 ml of the sulphuric acid solution, and mixed [21] [22].

##### **Standardization of Inoculum**

Using a sterile wire loop, 3-5 well-isolated colonies of similar appearance to the test organism was picked and emulsify in 5 ml of sterile water. In a good light the turbidity of the suspension was matched to the turbidity standard (0.5 McFarland standards) [23] [24].

### **Preparation of Aftershaves**

Different concentrations of the aftershaves were made by serially diluting in sterile distilled water ( $5^0$ ,  $5^{-1}$ ,  $5^{-2}$ ,  $5^{-3}$ , and  $5^{-4}$ ). This was done by adding 1ml of each of the aftershaves to 4mls of sterile distilled water and mixing properly. From the first concentration (preparation), the 1ml of each of the aftershaves diluted was transferred to another 4mls of sterile distilled water. This process was repeated two more times to get the third and fourth dilution. This procedure was used to get the  $5^{-1}$ ,  $5^{-2}$ ,  $5^{-3}$ , and  $5^{-4}$  concentrations respectively. The  $5^0$  concentration was prepared by pipetting 1ml of the aftershaves and using it without diluting in water [25].

### **Aftershaves antimicrobial activities test**

The antimicrobial activities of the aftershaves were determined using the agar-well diffusion method. Mueller Hinton Agar (MHA) plates were prepared by pouring 15 ml of molten media into sterile Petri plates. The plates were allowed to solidify for 5

### **Result of Morphological and Biochemical Characteristics of the Bacteria species Isolated barbers' clippers and brushes Abakaliki.**

In this study, forty-five (45) samples were examined and a total of three bacteria were identified as: *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella species* [30]. This result shows that out of the three bacteria isolated, *Escherichia coli* is Gram negative, rod shaped, single arrangement, coagulase negative, catalase negative, citrate negative,

min. Then, with a sterile swab, the plates of Mueller Hinton agar was inoculated with 0.1 % inoculum suspensions prepared by comparing with McFarland standard [26]. Excess fluid was removed by pressing and rotating the swab against the side of the tube above the level of the suspension. The inoculums were allowed to dry for 5 min. The swab was streaked evenly over the surface of the medium in three directions, rotating the plate approximately  $60^\circ$  to ensure even distribution [27].

Cork borer was used to bore five (5) holes on the surface of the agar and 1 drop of the different aftershave concentration ( $5^0$ ,  $5^{-1}$ ,  $5^{-2}$ ,  $5^{-3}$ , and  $5^{-4}$ ) was pipetted into the holes on the agar surface.

The plates were allowed to standard in that form without inversion so that the aftershave solution would not pour out. They were incubated at  $37^\circ\text{C}$  for 24 hours [28] [29]. The diameter of the zone of inhibition was measured after incubation to the nearest millimetre (mm).

### **RESULTS**

*Staphylococcus aureus* is indole positive and methyl red positive; *Klebsiella species* is Gram negative, rod shaped, single arrangement, coagulase negative, catalase positive, citrate positive, indole negative and methyl red negative while *Staphylococcus aureus* is Gram positive, cocci shaped, group arrangement, coagulase positive Gram negative, rod shaped, single arrangement, coagulase negative, catalase positive, citrate positive, indole negative and methyl red positive (table 1).

**Table 1: Morphological and Biochemical Characteristics of the Bacteria Isolated from barbers' clipper and brushes within Abakaliki metropolis**

Colony Morphology	Gram Reaction	Arrange- Ment	Shape	CA	CO	CT	IND	MR	Suspected Bacteria
Yellow Colonies	+	Cluster (bunch)	Cocci	+	+	+	-	+	<i>Staphylococcus aureus</i>
Pink dry Colonies	-	Single	Rod	-	-	-	+	+	<i>Escherichia coli</i>
Pink mucoid colonies	-	Single	Rod	+	-	+	-	-	<i>Klebsiella species</i>

Key: CA = catalase test; CO = coagulase test; CT= citrate test; IND = indole test; MR = methyl Red test; (+) = positive; (-) = negative

**Percentage frequency of occurrence of Bacteria Species Isolated from barbers' clippers and brushes within Abakaliki Metropolis.**

Out of the forty-five (45) samples examined, thirty seven isolates were identified and their frequencies of occurrence are as follows; *Staphylococcus aureus* 17(46%), *Escherichia coli* 12 (32.4%) and *Klebsiella species* 8(21.6%) as shown in Table 2. From the result it was observed that the brushes used in barbing saloon contained more of Gram negative organisms while the clipper had more of Gram positive bacteria. It was observed that out of the 17 isolates suspected to be *Staphylococcus aureus*; 8 were isolated from clippers after barbing without sterilization by burning, 6 were isolated from clippers after sterilization by burning and 3 isolated from brushes.

Also, out of the 12 isolates suspected to be *Escherichia coli*; 4 were isolated from clippers after barbing without sterilization by burning, one (1) were isolated from clippers after sterilization by burning and 7 isolated from brushes [31].

Also, out of the 8 isolates suspected to be *Klebsiella species*; 3 were isolated from clippers after barbing without sterilization by burning, one (1) were isolated from clippers after sterilization by burning and 6 isolated from brushes.

**Table 2: Percentage frequency of occurrence of Bacteria Species Isolated from barbers' clippers and brushes within Abakaliki Metropolis.**

Total Number of samples collected	Total Number of Bacteria Isolated	Type Of Bacteria Isolated	Source			Total Number and % of Bacteria Isolated
			CB	CA	BR	
Forty-Five (45)	Thirty seven (37)	<i>Staphylococcus aureus</i>	8	6	3	17 (46.0%)
		<i>Escherichia coli</i>	4	1	7	12 (32.4%)
		<i>Klebsiella species</i>	3	1	4	8 (21.6%)
<b>Total</b>			14	9	14	37(100%)

Key:

CB = Clipper after barbing without sterilization by burning

CA = Clipper after sterilization by burning

BR = Brushes

**Effect of sterilization by burning with alcohol and other substances on the bacterial isolates in percentage**

The sterilization technique used by barbers which involves the burning of the clipper with alcohol or any other substance had a very little effect on *Staphylococcus aureus* isolates as 87.5% of the *Staphylococcus aureus* isolates survived while 12.5% were killed. The sterilization method had

greater effect on *Escherichia coli* isolates as 25% of the *Escherichia coli* isolates survived while 75% were killed. Significant cidal effect was also observed in the case of *Klebsiella species* as 33.3% of the organism survived while 66.7% were killed [32]. This result was obtainable because the samples that had growth for clippers before sterilization and after sterilization were compared to check for corresponding results.

**Table 3: Effect of sterilization by burning with alcohol and other substances on the bacterial isolates in percentage**

Suspected bacteria	CB	CA	Survival (%)	Effect (%)
<i>Staphylococcus aureus</i>	8	7	87.5%	12.5%
<i>Escherichia coli</i>	4	1	25.0%	75.0%
<i>Klebsiella species</i>	3	1	33.3%	66.7%

Key:

CB = Clipper after barbing without sterilization by burning

CA = Clipper after sterilization by burning

**Antimicrobial activities of selected aftershaves against *Staphylococcus aureus* isolated from barbers' clipper and brushes within Abakaliki Metropolis.**

Out of the 5 aftershaves used clin barber had the highest inhibitory

effect which ranged from 12 to 30 mm for the concentrations of  $5^{-4}$  and  $5^0$  respectively [33]. It was seconded by chaoba aftershave spray. Shave rite, methylated spirit and authentic herbal cream followed respectively according to their inhibitory effect.

**Table 4: Antimicrobial activities of selected aftershaves against *Staphylococcus aureus* isolated from barbers' clipper and brushes within Abakaliki Metropolis. Inhibition zone diameter (mm)**

Aftershaves	$5^0$	$5^{-1}$	$5^{-2}$	$5^{-3}$	$5^{-4}$
MHS	17	15	14	11	11
CHB	25	27	17	14	11
CLB	30	20	14	15	12
SHR	18	12	11	10	10
AHC	13	15	11	11	10

Keys: MHS: Methylated spirit; CHB: Chaoba Aftershaves; CLB: Clin Barber Aftershaves; SHR: Shave Rite Aftershave; AHC: Authentic Herbal Cream Aftershave.

**Antimicrobial activities of selected aftershaves against *Escherichia coli* isolated from barbers' clipper and brushes within Abakaliki Metropolis.**

Out of the 5 aftershaves used clin barber had the highest inhibitory effect which ranged from 10 to 18 mm

for the concentrations of  $5^{-4}$  and  $5^0$  respectively [34]. It was seconded by methylated spirit aftershave spray. Chaoba, authentic herbal cream and shave rite aftershaves followed respectively according to their inhibitory effect.

**Table 5: Antimicrobial activities of selected aftershaves against *Escherichia coli* isolated from barbers' clipper and brushes within Abakaliki Metropolis.**

**Inhibition zone diameter (mm)**

Aftershaves	5 <sup>0</sup>	5 <sup>-1</sup>	5 <sup>-2</sup>	5 <sup>-3</sup>	5 <sup>-4</sup>
MHS	16	12	10	10	10
CHB	14	14	13	12	12
CLB	18	15	13	10	10
SHR	12	10	10	9	8
AHC	13	14	11	11	10

**Keys:** MHS: Methylated spirit; CHB: Chaoba Aftershaves; CLB: Clin Barber Aftershaves; SHR: Shave Rite Aftershave; AHC: Authentic Herbal Cream Aftershave.

**Antimicrobial activities of selected aftershaves against *Klebsiella species* isolated from barbers' clipper and brushes within Abakaliki Metropolis.**

Out of the 5 aftershaves used methylated spirit and clin barber had the highest inhibitory effect which

ranged from 9 to 18 mm and 9 to 15 mm respectively for the concentrations of 5<sup>-4</sup> and 5<sup>0</sup> respectively. Chaoba, authentic herbal cream and shave rite aftershaves followed respectively according to their inhibitory effect [35].

**Table 5: Antimicrobial activities of selected aftershaves against *Klebsiella species* isolated from barbers' clipper and brushes within Abakaliki Metropolis.**

**Inhibition zone diameter (mm)**

Aftershaves	5 <sup>0</sup>	5 <sup>-1</sup>	5 <sup>-2</sup>	5 <sup>-3</sup>	5 <sup>-4</sup>
MHS	18	13	11	9	9
CHB	13	10	10	9	10
CLB	15	13	12	10	9
SHR	12	10	11	10	10
AHC	12	10	11	11	10

**Keys:** MHS: Methylated spirit; CHB: Chaoba Aftershaves; CLB: Clin Barber Aftershaves; SHR: Shave Rite Aftershave; AHC: Authentic Herbal Cream Aftershave.

## DISCUSSION

The result showed the presence and prevalence of bacteria on barbers' clippers and brushes from the different barbers' shop as presented in table 1 and 2. *Staphylococcus aureus* (46.0%) was more widely distributed than *Escherichia coli* (32.4%) while *Klebsiella species* had the least percentage prevalence of 21.6%. The presence of bacteria organisms such as *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella species* in barbers' clippers and brushes

sampled in this study conforms to the study of [36] isolated *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* in barbers' clippers. It also conforms to the study of [37] [38] who isolated *Staphylococcus aureus*, *Streptococcus* sp. and *Micrococcus* spp. in barbers' tools in their study. Their findings also correlate with those obtained in this study. The result also shows that the normal sterilization technique used by

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barbers which involves the burning of the clipper with alcohol or any other substance had a very little effect on *Staphylococcus aureus* isolates as 87.5% of the *Staphylococcus aureus* isolates survived while 12.5% were killed. The sterilization method had greater effect on *Escherichia coli* isolates as 25% of the *Escherichia coli* isolates survived while 75% were killed. Significant cidal effect was also observed in the case of *Klebsiella species* as 33.3% of the organism survived while 66.7% were killed [39]. Consequently, this study has revealed that barbering procedures and tools particularly in the study area present the risk for bacterial infections even after sterilization by burning with

#### CONCLUSION

This study has shown that barbers' clippers and brushes harbour bacteria which can be potentially pathogenic and possibly promote the spread of infections to human and that the major sterilization methods used in

#### RECOMMENDATIONS

I recommended that enough attention should be given to the hygienic practices in barbershops through routine supervision and monitoring by agencies of the government. In addition, practical-oriented training on how to carry out decontamination with emphasis on the use of correct procedure and potent disinfectants

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Okonkwo *et al*

alcohol, methylated spirit and other substances.

This project also shows that the aftershaves sprays and creams had inhibitory effects on the bacterial isolates more than the sterilization techniques used by most barbers do. The five (5) different concentrations of the different aftershaves used showed zones of inhibition that was corresponding to the concentration (that is; higher concentration of aftershave had greater zone of inhibition). The clin barber aftershave showed greater inhibitory effect on both isolates [40]. The aftershaves also had more effects on the *Staphylococcus aureus* isolates than on the *Escherichia coli* isolates and *Klebsiella species*.

the area this study was carried out in was futile against some isolates. This study has also shown that aftershaves are very effective against these organisms.

should be organized for barbers through barbers' union and peer education approach. I also recommend that every person that gets a hair cut or shave should ensure that aftershaves are applied immediately after barbering or shaving to help eliminate some of these organisms.

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