Contents list available http://www.kinnaird.edu.pk/

Journal of Natural and Applied Sciences Pakistan

Journal homepage: http://jnasp.kinnaird.edu.pk/

ISOLATION AND IDENTIFICATION OF BACTERIA FROM THE TOOLS OF BARBERSHOPS AT UNIVERSITY OF PESHAWAR

Haider Ali[1], Fawad Ali[2*], Zarmish Javed[1], Javed khan[3], Muhammad Shafee[4], Haider Ali Khan[5]
[1]Centre of Biotechnology and Microbiology, University of Peshawar
[2]State Key Laboratory of Microbial Metabolism, and School of Life Sciences & Biotechnology, Shanghai Jiao Tong University, Shanghai, 200240, People's Republic of China
[3]Department of Zoology, Abdul Wali Khan University Mardan
[4]Centre for Advanced Studies in Vaccinology & Biotechnology, University of Balochistan, Quetta, Pakistan
[5]Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan

Article Info

*Corresponding Author
Email: fawada47@yahoo.com

Abstract

Continuous use of unsterilized tools and equipment in barbershops can lead to the transmission of microorganisms from infected individuals to healthy individuals, posing potential health risks to customers. The type of the service provided and the tools and equipment used in barbershops can define the health problems that may arise. Sharing tools in barbershops can result in eye and skin infections. In this study, samples were collected from various barbershops using wet cotton swabs to identify different bacterial species present on tools such as combs, trimmers, brushes, and blades. The samples were promptly transported to the Center of Biotechnology & Microbiology research laboratory for microbiological examination following standard protocols. No prior study has revealed the microbial contamination of barbershop tools within the university of Peshawar campus. The current research aimed to isolate and identify bacterial strains responsible for contaminating barber tools. A total of 30 samples were collected from 5 barbershops on the University of Peshawar campus. Among the samples, 93.33% (28) tested positive for pathogenic bacteria while 6.67% (2) were negative. The isolates consisted of 92% (46) Gram-positive cocci and 8% (4) Gram-negative rods. Mannitol fermenters accounted for 60% (30) of the isolates, while non-mannitol fermenters were 32% (16). Non-lactose fermenters made up 8% (4) of the isolates. The identified bacterial strains included *S. aureus* 32% (16), *S. epidermidis* 32% (16), *S. saprophyticus* 18% (9), *Enterococcus* 10% (5) and *Enterobacteriaceae* 8% (4). Various growth media were used for isolation and identification of bacteria such as blood agra, nutrient agar, Manitol Salt Agar (MSA) and MacConkey agar. The p-value $p < 0.001$) indicates a significant prevalence of bacterial infection in barber tools.

Keywords

Isolation, Identification, barbershops, tools, contamination and Infection.

1. Introduction

Barber tools can become contaminated due to a lack of proper sanitization systems and poor handling practices. Barbers use a variety of tools such as tweezers, scissors, blades, trimmers, brushes, and aprons for haircuts, shaves, and facial treatments (*Al Yousef*, 2021). These tools can also serve as a potential vectors for bacteria. Bacteria can accumulate on brushes, combs, scissors, and sharp blades when they are shared or used without proper sterilization. Repeated use of the same blade or shaving cream brush can increase the risk of facial infections. Skin infections resulting from the repeated use of barber tools have significantly increased in recent years (*Hay et al*, 2014). Microbiological research has identified barber shop tools as reservoirs and vehicles for the spread of potential pathogenic species. These microorganisms and debris from the tools can be transferred to the skin, where they can multiply and spread to new clients. This practice of using tools without proper sterilization creates ideal conditions for the capture, retention, and multiplication of microorganisms (*Enemuor et al*, 2012; *Khan et al*, 2010). Infectious organisms can be easily transmitted if proper precautions are not taken. For example, mishandling or poor hygiene practices can lead to the spread of these organisms. Piercing the skin of an infected person can contaminate tools like clippers, blades, and scissors, even if there are no visible signs of contamination (*Ibrahim et al*, 2007; *Mbajiuka et al*, 2014). Due to the use of contaminated razor blades, clients can be at risk of contracting deadly diseases such as Hepatitis B, C, and Human Immuno deficiency Virus (HIV) (*Mutocheluh & Kwarteng*, 2015; *Adjei-Gyamfi et al*, 2024; *Britsch et al*, 2024). It is crucial to take precautions against the harmful microbes that can be present on the blades (*Janjua & Nizamy*, 2004; *Zenbaba et al*, 2023). Razor blades should be disposed off after each use, and new blades should be used for each individual to prevent the transmission of infections. *Staphylococci* are commonly found in bloodstream infections, cuts and bruises can serve as a source of transmission (*Enemuor et al*, 2012; *Bassetti et al*, 2016; *Daka*, 2017). Folliculitis is the inflammation of the hair follicle generally produced by bacterial infections. It seems like small white pustules (loaded up with pus). The potential source for folliculitis infection is *Staph* species, which can spread through disinfected brushes, scissors, or blades (*Lin et al*, 2021; *Al Yousef*, 2021; *Zenbaba et al*, 2023). Tinea capitis is a disease condition that affects the scalp and can also lead to ringworm formation (red spot with scale around the border) or it can resemble a red flakey tickly patch. At the barber shop, it can spread through inadequately sterilized brushes or towels, and in serious cases, it can prompt perpetual scarring and baldness (*Takwale et al*, 2001; *Britsch et al*, 2024; *Addari et al*, 2025). Barbering procedures are also one of the sources for spreading infections among the individuals due to poor sanitation. A research was conducted in Kenya where 56 bacterial isolates were taken from the barber tools. The bacterial isolates identified were *S. aureus*, *E. coli*, *P. aeruginosa* and *Klebsiella* spp. (*Mwabu et al*, 2018). Another Study in 2010 evaluated 15 classes of skin illness from 1990 to 2010 for 187

countries. They found skin break out was in the top 10 major illnesses worldwide in 2010. Furthermore, worldwide, skin conditions stood as the fourth driving force for nonfatal infections. These outcomes are affecting range of people around the world where as one reason for it is the barber tools as well (*Hay et al*, 2014). Various tools were trimmer; scissors, Apron, comb and Brush etc. were used in five barbershops harbor different microorganisms that were identified. Our findings indicate that *S.aureus*, *S. epidermis*, and other harmful bacteria are generally present in all the barbershops we examined, suggesting extensive bacterial contamination. It is important for barbershops to follow strict protocols to reduce potential health risks, as demonstrated by the presence of both fermenting manitol and non-fermenting bacteria on tools.

2. Materials and Methods

2. 1 Study Design

In this study research was conducted on samples collected from five different barbershops located at the University of Peshawar. The aim was to isolate and identify bacteria growing on the tools in these barbershops, which may be due to unhygienic conditions and the use of unsterilized tools. These factors are known to contribute to infections that students may contact after visiting the university barbershops. The research was conducted in the laboratories of the Center of Biotechnology and Microbiology at the University of Peshawar, Khyber Pakhtunkhwa. The research was completed in approximately 5 months.

2. 2 Chemicals and Reagents

The media used were MacConkey agar, Mannitol salt agar (MSA), Blood agar, Nutrient agar, Simon citrate agar, Urease agar, Triple Sugar Iron (TSI) agar, Sulfide Indole Motility (S.I.M) agar and DNase agar. The reagents include Gram iodine, crystal violet, safranin, oxidase reagent, 1N HCL, hydrogen peroxide (H_2O_2), ethanol and emersion oil.

2. 3 Sample collection

Samples were collected from the tools of 5 different barbershops located on the University of Peshawar campus. The samples were screened to isolate and identify pathogenic bacteria that could cause infections among university students. Sampling was carried out carefully using gloves and masks under sterile conditions to avoid contamination. The barbers cooperated effectively and offered complete support during the sample collection process.

2. 4 Sample size

A total of 30 samples were collected from various tools used in barbershops. Only tools that were used by barbers all day long for different clients. The sampling was done under aseptic conditions using wet cotton swabs which are commercially available. They were rubbed on the surface of the tools and were labeled with time, date of sampling procedure and name of the tool from which sample was taken carefully. For further processing, Samples were immediately taken to the laboratory at the Center of Biotechnology and Microbiology, University of Peshawar.

2. 5 Media Used For Bacterial Growth

To identify bacteria in the samples, various types of media were used for isolation, such as nutrient agar, blood agar, MacConkey agar, and Mannitol Salt Agar (MSA). Selective and differential media were also used.

2. 6 Preparation of Media

For isolation and confirmation of different bacteria, the samples were cultured on different media according to the requirements of the specific bacteria. The differential and selective media used were MacConkey, Blood agar and MSA. The media was prepared according to the given instructions and autoclaved at 121℃ at 15 psi pressure. Once prepared the media was poured onto petri dishes and incubated at 37℃ for 24 hours to check for sterility. The next step is to sterilize the laminar flow hood by washing it with 70% ethanol and then turning on the UV light for 15 minutes. These steps were followed consistently throughout the 5 months research period to prevent contamination.

2. 7 Sample Inoculation

The samples collected from different barbershops were inoculated on nutrient agar plates to get primary cultures. Pure isolates were obtained through replica plating based on colony morphology. The pure isolates were then streaked on to petri plates containing MacConkey and MSA media. Growth on these two differential and selective media helps us to differentiate between Gram-negative and Gram-positive bacteria. MacConkey media specifically allows the growth of the Gram-negative *Enterobacteriaceae* whereas MSA favors the growth of Gram-positive *staphylococci*. After the streaking of inoculum on the media, the petri dishes are kept in the incubator for 24 hours at 37℃ to observe the growth of bacteria.

2. 8 Biochemical tests for identification of Microorganisms

2. 8. 1 Oxidase test

This test was used for identification of bacteria which produces the enzyme oxidase. The method used for oxidase test was filter paper spot mechanism. A dropper is used to add oxidase to the filter paper. The next step is to pick a bacterial colony from the sample with the help of sterilized inoculation loop and is rubbed on the side of filter paper which consists of the oxidase reagent. The result is indicated within a time span of 10 seconds, color change occurs from colorless to purple for a positive test. If the result is negative the color remains unchanged within the specified time range.

2. 8. 2 Citrate test

The citrate test was used to separate the microorganism which uses citrate as source of carbon. The media preparation process followed the instructions provided in the manual. The media was autoclaved at 121℃ and 15 psi pressure. Sterilized tubes were filled with the media; slants were prepared, and then inoculated. The tubes were incubated at 37℃ for 24 hours. A color change from green to blue indicated positive results.

2. 8.3 Citrate test Triple Sugar Iron (TSI) agar test

To determine the carbohydrate fermentation of microorganisms the TSI test is performed. The H_2S production is also confirmed from this test.

All the ingredients were mixed in the flask and the 3-4ml media was poured into the autoclaved test tubes. The slants were then prepared by keeping the tube in tilted position. The inoculation was carried out by using sterile inoculation loop. The tubes were kept in incubator for growth at 37℃ for 24 hours.

2. 8. 4 Urease test

This test was used to confirm the bacteria ability to cleave urease using the urease enzyme. In a flask all the ingredients were mixed in a flask holding 95 ml of distilled water. The ureases media was then poured into the tubes for slant formation and autoclaved at 121℃ and 15 psi pressure. Then the tubes were placed in a tilted position for formation of slants and were checked for sterility. After the 18 hours, slants were then inoculated with bacterial cultures which were freshly made and then slants were checked again for results after 34 hours were passed. The color change for a positive result was pink, while for a negative result it was yellow.

2. 8. 5 DNase test

To determine the ability of the microorganisms to produce deoxyribonuclease (DNase) enzyme. All the materials were mixed in a flask and autoclaved. The autoclaved media was then poured onto petri dishes and incubated for 18-24 hours to check for sterility. After the incubation period, the plate was divided into compartments, and an inoculating loop was used to inoculate each compartment. The smear formation method was used for inoculation, and the plates were incubated for 18 hours at 37℃. Subsequently, 1N HCL was poured onto the plates to obtain results. Positive results were indicated by the presence of clear zones around the smears (*Kateete et al*, 2010).

2. 8. 6 Sulfide Indole Motility (S.I.M) test

This test was used to discriminate bacteria on the basis of sulfide production, indole formation and motility. The ingredients were mixed in the flask as per the instructions and autoclaved. The media was then poured into the tubes and incubated. All the ingredients were mixed in the flask according to the given instructions and were autoclaved. The media was then poured into the tubes and incubated at 37℃ for 24 hours. Then the colony was picked from fresh cultures and inoculated into the media. The tubes were then incubated again at 37℃ for 24 hours. Positive H_2S production was indicated by the blackening of the media, while a positive motility test was confirmed by the formation of diffusion zones. A positive indole test was confirmed by the appearance of a red to pink band on top of the media after the addition of Kovacs Reagent.

3. Results

3. 1 Sample size

A total of 30 samples were collected from 5 different barbershops located on the University of Peshawar campus. The samples were taken from various tools used in the shops, including trimmers, brushes, combs, blades, scissors and aprons. Six samples were obtained from each barber shop within the campus (Fig. 3.1).

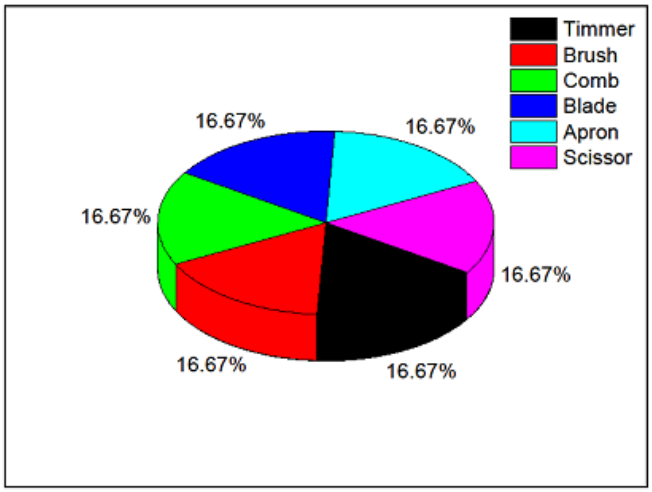


Figure 3.1: Showing percentage of samples collected from different barber tools

3. 2 Samples Screening

The samples were initially streaked onto sterile nutrient agar plates and subsequently cultured on various selective and differential media to isolate and identify bacterial species. A total of 50 bacterial isolates were obtained from the 30 samples on Mannitol salt agar (MSA) and MacConkey agar.

3. 2. 1 Growth on Mannitol salt agar

Mannitol salt agar (MSA) was used for the isolatation Gram-positive bacteria, particularly for the selection and differentiation of *Staphylococcus* species. Among the 50 bacterial isolates tested, 46 (92%) exhibited growth on Mannitol salt agar. Out of 46 bacterial isolates, 30 (65.22%) were Mannitol fermenters while 16 (34.78%) were non-mannitol fermenter on MSA (Table 3.1). *S. aureus* has the ability to ferment mannitol, producing acidic by-products that cause the pH indicator phenol red to change from red to yellow. *S. aureus* changed the media color to yellow, while *S. epidermidis* was identified by the pink color as a non-Mannitol fermenter. *S. saprophyticus* and *Enterococcus* also fermented mannitol, turning the media color yellow.

Table 3.1: Bacterial species isolated and identified on Mannitol salt agar (MSA)

S. No.	Barbershops (S. No)	Tools	Mannitol Fermenter (F)	Mannitol Non-fermenters (NF)	Bacteria identified
1	**1.**	Trimmer	F		*S. aureus*
2		Trimmer		NF	*S. epidermidis*
3		Apron		NF	*S. epidermidis*
4		Comb	F		*S. aureus*
5		Comb		NF	*S. epidermidis*
6		Brush	F		*Enterococcus*

7		Scissor	F			*S. saprophyticus*
8	**2.**	Comb	F			*Enterococcus*
9		Brush	F			*S. saprophyticus*
10		Brush			NF	*S. epidermidis*
11		Brush	F			*S. aureus*
12		Blade			NF	*S. epidermidis*
13		Apron	F			*S. saprophyticus*
14		Apron			NF	*S. epidermidis*
15		Trimmer			NF	*S. epidermidis*
16		Trimmer	F			*S. aureus*
17		Scissor	F			*S. aureus*
18		Scissor	F			*S. saprophyticus*
19	**3.**	Scissor	F			*Enterococcus*
20		Scissor	F			*S. aureus*
21		Comb			NF	*S. epidermidis*
22		Comb	F			*S. saprophyticus*
23		Trimmer	F			*S. aureus*
24		Trimmer			NF	*S. epidermidis*
25		Brush	F			*S. aureus*
26		Brush	F			*Enterococcus*
27		Blade			NF	*S. epidermidis*
28		Blade	F			*S. saprophyticus*
29		Apron	F			*S. aureus*
30	**4.**	Trimmer	F			*S. aureus*
31		Trimmer			NF	*S. epidermidis*
32		Comb	F			*S. aureus*
33		Comb	F			*S. saprophyticus*
34		Scissor	F			*S. aureus*
35		Scissor			NF	*S. epidermidis*

36		Brush	F			*Enterococcus*
37		Brush	F			*S. saprophyticus*
38		Apron	F			*S. aureus*
39		Apron			NF	*S. epidermidis*
40	**5.**	Brush	F			*S. aureus*
41		Brush			NF	*S. epidermidis*
42		Trimmer	F			*S. aureus*
43		Blade	F			*S. saprophyticus*
44		Scissor			NF	*S. epidermidis*
45		Comb	F			*S. aureus*
46		Apron			NF	*S. epidermidis*

3. 2. 2 Growth on the MacConkey media

MacConkey media was utilized to isolate and differentiate Gram-negative rods based on lactose fermentation. Among the 50 bacterial isolates tested, only 4 (8%) were able to grow on MacConkey agar, and all of them were non-lactose fermenters, as indicated by the yellow color of the media and the colony (Table 3.2).

Table 3.2: Bacterial species isolated and identified on MacConkey agar

S.NO.	Barbershop (S. NO.)	Tools	Lactose fermenters (LF)	Non-Lactose fermenters (NLF)	Bacteria identified
1	**1.**	Blade		NLF	Enterobacteriaceae
2	**2.**	Comb		NLF	Enterobacteriaceae
3	**4.**	Brush		NLF	Enterobacteriaceae
4	**5.**	Blade		NLF	Enterobacteriaceae

3. 2. 3 Growth on Blood agar media

Blood agar media was used to differentiate bacterial species based on hemolysis. *S. aureus* exhibited beta (β) hemolysis on blood agar, characterized by transparent zones around its colonies indicating complete lysis of red blood cells (RBCs). In contrast, *S. epidermidis* and *S. saprophyticus* showed gamma (γ) hemolysis, with no color change around their colonies. *Enterococcus spp*. displayed alpha (α) hemolysis, with a greenish color around its colonies, indicating partial breakdown of RBCs (Table 3.3) (Fig. 3.2).

Table 3.3: Showing results of tests performed for Gram-positive Cocci

S.NO	Mannitol fermenter (F)	Mannitol Non-fermenter (NF)	Hemolysis on BAP	Catalase	DNase	Bacteria identified
1	F		β-hemolytic	Positive	positive	*S. aureus*
2		NF	α-hemolytic	Positive	negative	*S. epidermidis*
3	F		α-hemolytic	Positive	negative	*S. saprophyticus*
4	F		α-hemolytic	Negative	negative	*Enterococcus*

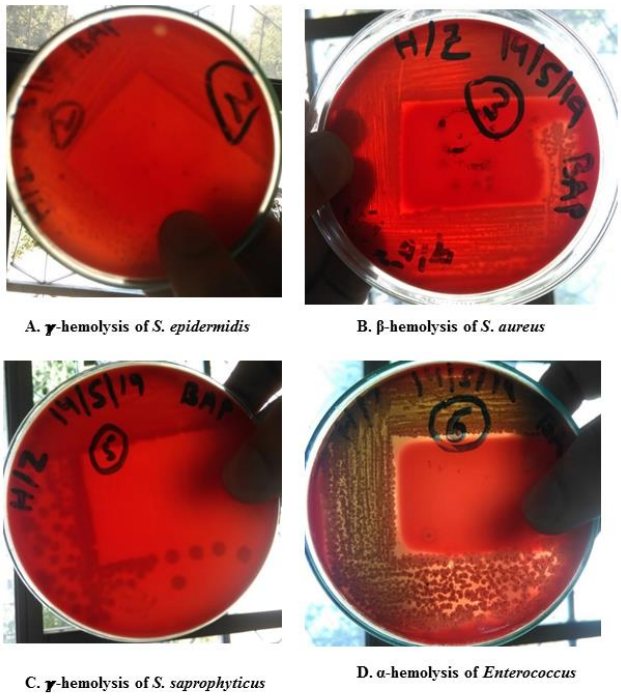


Figure 3.2: Growth of pure bacterial isolates on Blood agar, A. *S. epidermis*, B. *S. aureus*, C. *S. saprophyticus*, D. *Enterococcus spp*.

Fifty bacterial isolates were identified from 30 samples, including both Gram-positive and Gram-negative bacteria. Among the Gram-positive bacteria, 53.33% were mannitol fermenters, while 46.67% were non-mannitol fermenters. In the Gram-negative group, 20% of the bacteria fermented lactose, while the remaining 80% did not. This study provides information on the distribution and fermentation properties of the bacterial isolates obtained from the samples (Fig. 3.3).

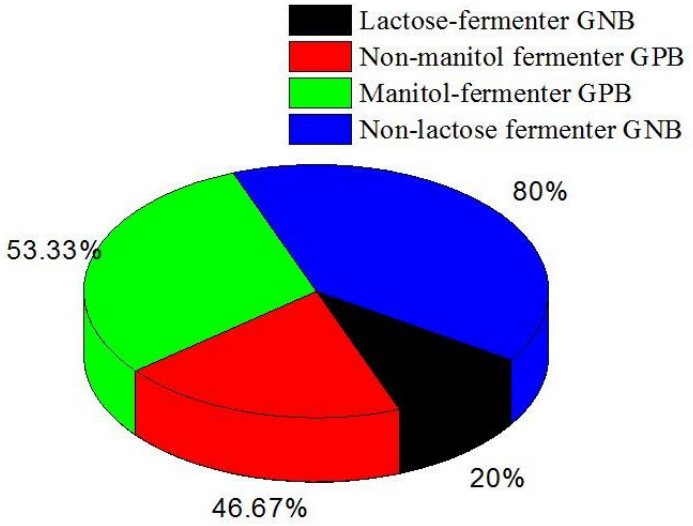


Figure 3.3: Percentage of bacterial isolates based on Mannitol fermentation, Non-mannitol fermentation and Non-lactose fermentation.

In this study, a total of fifty isolates were analyzed. The most common bacterial species were *S. epidermis* and *S. aureus* , accounting for approximately 32% of all isolates. *S. saprohyticus* made up 18% of the isolates, while *Enterococcus* represented 10% and *Enterobactericea* had the lowest representation at 8%. This distribution sheds light on the prevalence of different bacterial species in the studied population, which is crucial for determining effective treatment strategies and understanding infections (Table. 3.4).

Table 3.4: Number and Percentage of bacterial species identified from the barber tools

S.NO.	Bacterial species	Number of isolates	Percentage
1	*S. aureus*	16	32%
2	*S. epidermidis*	16	32%
3	*Enterococcus*	5	10%
4	*S. saprophyticus*	9	18%
5	*Enterobacteriaceae*	4	8%

3. 2. 4 Bacterial growths on Nutrient agar

Nutrient agar is a media that supports bacterial growth and is used for culturing and studying bacterial colonies. Figure. 3.4 show five petri dishes with different types of bacteria labeled with sample type and inoculation date. Petri dish labeled “A” represents *S.epidermis* characterized by small, round, white colonies. Petri dish labeled “B” represents *S. aureus* appearing golden yellow and larger in size. *Enterobactericea* are shown in petri dish labeled “C” which was mucoid, larger with irregular edges. The colonies in petri plates labeled “D” were mucoid, large, opaque and ranging in color from white to yellow and are identified as *S. saphrophyticus*. The colonies in petri dish labeled “E” were identified as

Enterococcus species which are clear, greyish, small in size with thin edges (Fig. 3.4).

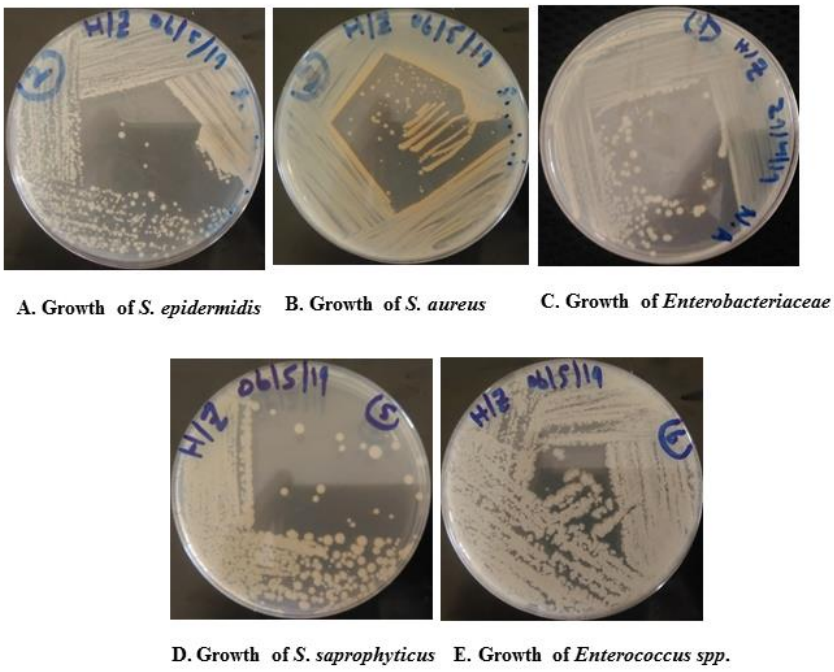


Figure 3.4: Growth of pure bacterial isolates on nutrient agar, A. *S. epidermis*, B. *S. aureus*, C. *Enterobactericeae*, D. *S. saprophyticus*, E. *Enterococcus spp*.

3. 2. 5 Bacterial growths on Mannitol salt agar

Mannitol salt agar is a selective media used to isolate and differentiate *Staphylococcus* bacteria. Four different bacteria including *Staphylococcus aureus*, *Staphylococcus epidermis*, *Enterococcus* and *Staphylococcus saphrophyticus* were observed on Mannitol salt agar plates. Each figure illustrates mannitol fermentation capabilities and growth characteristics of these bacteria on MSA. *Staphylococcus epidermis* is unable to ferment mannitol, but it can withstand high concentration of salt in MSA. As a result, no acid is produce, and the phenol red indicator remains red. Small visible colonies with thin edges were observed on the plates (Fig. 3.5 A). Mannitol was fermented by *Staphylococcus aureus*, which thrives on MSA. This fermentation produces acid, reducing the pH of the medium and causing the phenol red indicator to yellow and also turn the colonies to yellow (Fig. 3.5 B). *Staphylococcus aureus* and *Staphylococcus saphrophyticus* have the capability to ferment mannitol, leading to the production of acid that turns the media yellow as well as colonies (Fig. 3.5 C). The media color does not change because the *enterococcus* does not ferment mannitol, despite its ability to withstand high salt concentrations. The phenol red remains unchanged (Fig. 3.5 D).

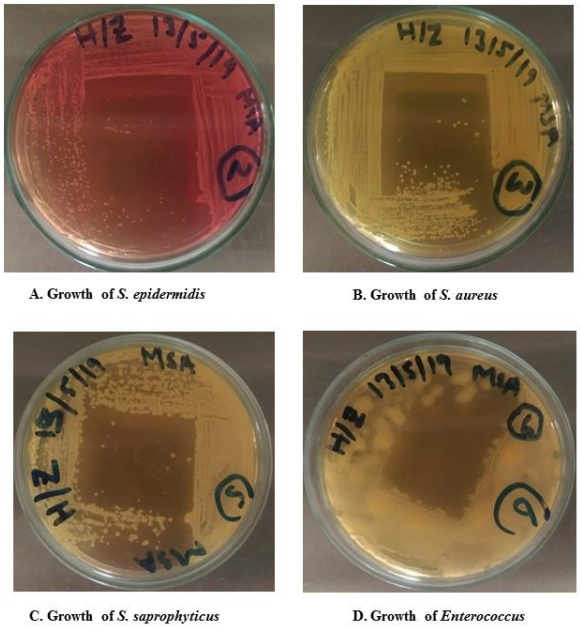


Figure 3.5: Growth of pure bacterial isolates on Mannitol salt agar, A. *S. epidermis*, B. *S. aureus*,C. *S. saprophyticus*, D. *Enterococcus spp*.

3. 2. 6 Bacterial growths on MacConkey Agar

MacConkey agar is a selective and differential medium used to isolate and identify enteric bacteria based on their capability to ferment lactose. The agar here retained its natural color, ranging from pale pink to crimson, indicating that the pH of the medium has remained relatively same, limiting lactose fermentation (Fig 3.6).

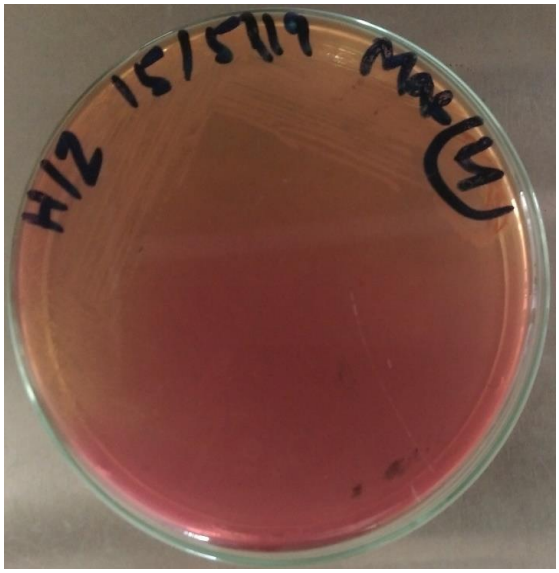


Figure 3.6: Growth of *Enterobacteriaceae* on MacConkey agar (Non-lactose fermenter)

3. 2. 7 Biochemical test results

In additin to Gram staining, a variety of biochemical tests were conducted to confirm the identification of bacterial strains isolated from the samples collected from the tools. These tests include catalase, DNase, citrate, urease, triple Sugar Iron (TSI), oxidase and sulfide Indole motility (S.I.M) were performed on the pure isolates.

3. 2. 8 Microscopic Identification of Bacteria

Gram staining was performed to confirm the presence of Gram-positive and Gram-negative bacteria. Microscopic identification of different bacteria is shown in Figure. 3.7. *Staphylococcus. epidermis* is observed as small, circular clusters, indicating Gram-positive cocci with a thick peptidoglycan layer (Fig. 3.7A). *Staphylococcus aureus* appears as purple clusters of Gram-positive cocci in grape like clusters (Fig. 3.7B). The red/pink rod- shaped bacteria were identified as Gram-negative bacteria, showing the absorption of counterstain due to their thinner peptidoglycan coating and outer membrane (Fig. 3.7C).

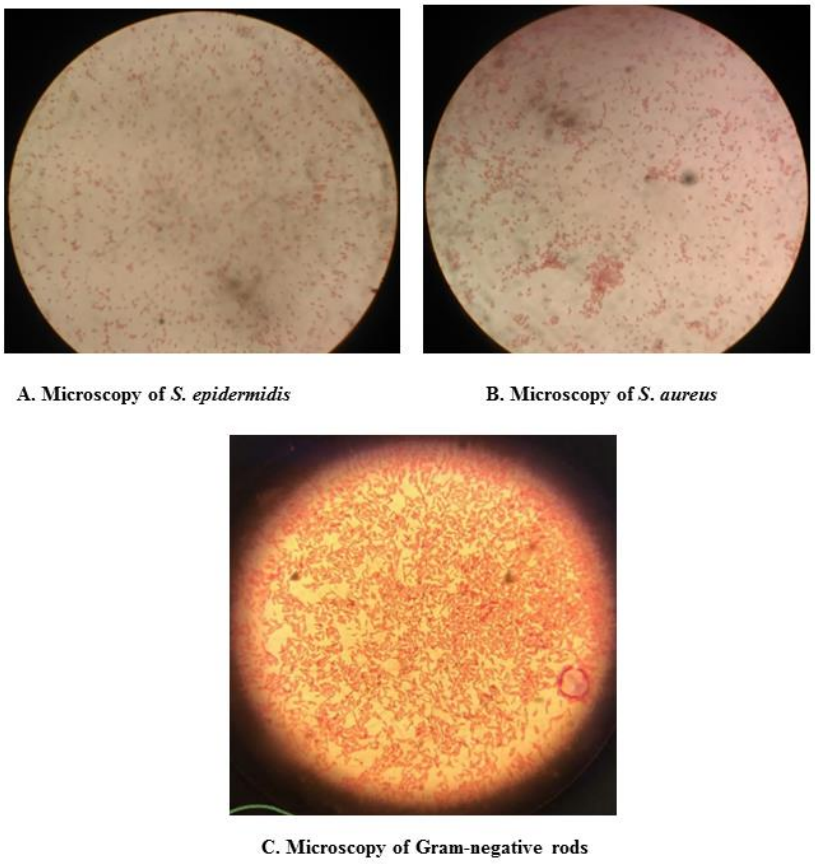
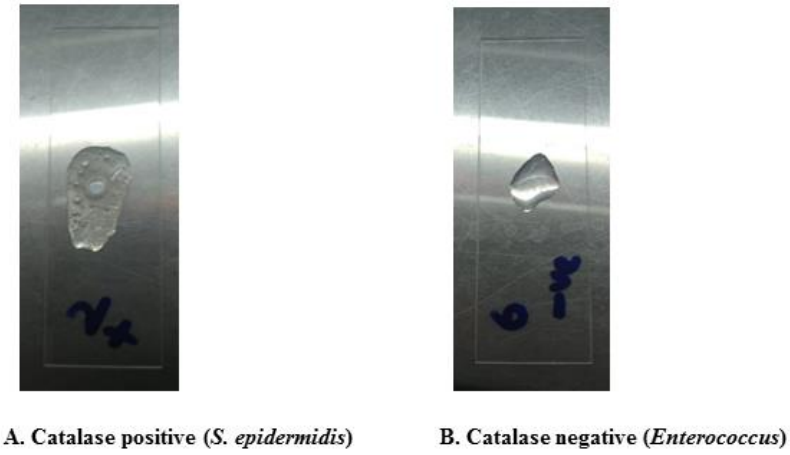


Figure 3.7: Microscopic identification of bacteria A. *S. epidermis*, B. *S. aureus*, C. Gram-negative rods.

3. 2. 9 Catalase test

The catalase test was conducted on Gram-positive bacterial strains. Catalase positive strains were identified by observing froth formation upon the addition of 3% hydrogen peroxide (H_2O_2) to colony smears on sterile glass slides. *S. aureus, S. epidermidis* and *S. saprophyticus* tested positive for catalase, while *Enterococcus* tested catalase negative as no frothing was observed after the addition of 3% H_2O_2 (Fig. 3.8A & 3.8B).



A. Catalase positive (*S. epidermidis*) B. Catalase negative (*Enterococcus*)

Figure 3.8: Catalase test A. Catalase positive (*S. epidermis*) B. Catalase negative (*Enterococcus*).

3. 2. 10 DNase test

The DNase test was performed to differentiate *S. aureus* from *S. epidermidis* and *S. saprophyticus.* DNase positive strains were identified by the presence of clear zones around the colonies on DNase agar, while DNase negative bacteria did not show clear zones. *S. aureus* tested positive for DNase, whereas *S. epidermidis* and *S. saprophyticus* tested negative (Fig. 3.9).

Figure 3.9: (2) & (5) DNase negative, (3) DNase positive.

3. 2. 11 Citrate test

The citrate test was conducted on Gram-negative *Enterobacteriaceae*. A positive result was indicated by a color change in media from green to blue, while a negative result showed no color change. *Enterobacterium spp*. was identified as there was no observed color change in the media.

3. 2. 12 Urease test

The Urease test was conducted on Gram-negative *Enterobacteriaceae* to detect the presence of Urease enzyme. A positive result was confirmed

by a color change in the media from yellow to pink, caused by the production of ammonia from urea hydrolysis. The *Enterobacterium spp*. was identified as urease negative as it did not induce a color change to pink (Fig. 3.10).

Figure 3.10: Urease test result, *Enterobacteriaceae* (urease negative)

3. 2. 13 Triple Sugar Iron (TSI) Test

The TSI test was performed to detect carbohydrate fermentation by *Enterobacterium spp*. A positive result was indicated by a color change to yellow in the TSI media, with both the slant and the butt turning yellow. This confirmed fermentation of glucose, lactose and sucrose by the *Enterobacterium spp*. (Fig. 3.11).

Figure 3.11: TSI test result, *Enterobacteriaceae* (Carbohydrate fermenter).

3. 2. 14 Oxidase test

The oxidase test was performed to detect the presence of the oxidase enzyme in *Enterobacteriaceae.* A positive result was indicated by a color change, while a negative result showed no color change. The *Enterobacteriaceae* tested positive for oxidase as a color change was observed (Fig. 3.12).

Figure 3.12: Oxidase test result, *Enterobacteriaceae* (oxidase positive)

3. 2. 15 Sulfide Indole Motility (S.I.M) test

S.I.M is a multi-test medium used to detect motility, indole production, and H_2S production. Indole production is indicated by a color change in the Kovac's reagent from yellow to bright red, H_2S production by blackening around the inoculation line, and motility by clouding of the medium. The isolate being studied showed no changes, indicating it was negative for indole, H_2S, and motility (Fig. 3.13).

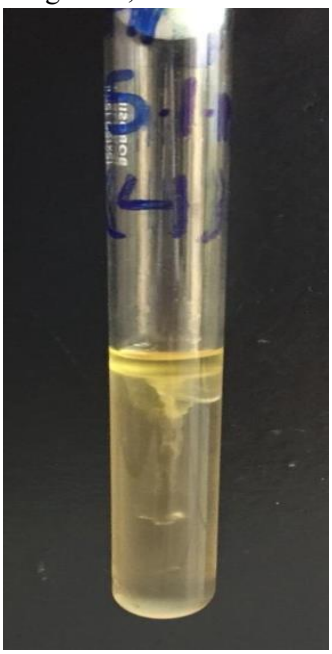


Figure 3.13: S.I.M test result, *Enterobacteriaceae* (Indole, motility and H_2S negative).

4. Discussion

Barbers are skilled professionals who provide hair and grooming services, including hair cutting, beard trimming, using tools like clippers and razors, styling hair and washing, coloring hair and dressing hair (*Enemour et al*, 2012). They also apply aesthetic preparation, hair tonics, antibacterial agents, oils, powders, clays, and moisturizers to the scalp, neck, or face for cosmetic purposes. These services are not intended to treat infections or mental health conditions (*Andrew & Rossana*, 2016). Skin microflora of humans is distinctive and could be spread to the others by utilizing barber tools, for example, brushes, scissors, aprons, trimmers and blades which could compromise the health of clients. Therefore, it is stated that barber tools have more decent variety and density of microorganisms (*Adjei-Gyamfi et al*, 2024; *Britsch et al*, 2024). Another study has shown the different operations performed by barbers; these include scalp massaging, hair-cutting, manicure and pedicure, shampooing and nail trimming. Different health complications and skin diseases are associated with the profession of barbers. These diseases also affect their clients (*Janjua et al*, 2004). Barber tools are the common risk factors for the spread of disease among individuals. A study was conducted to assess the microbiological perils related to barbershops in a Nigerian university. They found that barber clients were exposed to contaminated instruments. They found that ringworm could be caused by contact with tools and towels that are contaminated. The bacterial species found on the tools were *Staphylococcus aureus, Enterobacter spp*. and *Enterococcus* spp. (*Ibrahim et al*, 2007). In 2013 a study conducted in Kumasi, Ghana, included 200 barbershops in its research. These barbershops were surveyed consistently over an 8 – month period. The study found that barbers lacked awareness of the connection between blood borne diseases and their barbering practices. The study recommends the implementation of awareness programs targeted at barbers (*Mutocheluh et al*, 2015). The current research was conducted for the sole purpose to identify the bacteria that could potentially cause infections among visitors to barbershops on the University of Peshawar campus. A total of 50 bacterial isolates were identified, with 92% (46) being Gram-positive and 8% (4) being Gram negative. The result showed that 60 % (30) of the isolates were mannitol fermenters, while 32 % (16) were non-mannitol fermenter and 8 % (4) were non-lactose fermenters. The distribution of species among the isolates was as follows: *S. aureus* 32% (16), *S. epidermidis* 32% (16), *Enterococcus* 10% (5), *S. saprophyticus* 18% (9) and *Enterobacteriaceae* 8% (4). Statistical analysis ($p < 0.001$) shows a significantly high prevalence of bacterial infections in barbershop instruments. The presence of these pathogenic bacteria indicates that most of the campus students are prone to infection through the unsterilized barber's tools. This study examines bacterial contamination on various barbershop tools. The aim of the study was to identify the presence of bacteria that either ferment or do not ferment manitol on blades, brushes, aprons and trimmers from five different barbershops. The results revealed a significant level of microbial

contamination, with the identification of potentially harmful bacteria, highlighting the potential health risks associated with inadequate sterilization practices in these settings.

5. Conclusion

Samples from the tools of the barbers were collected from all the barbershops inside University of Peshawar campus which may infect many individuals visiting these barbershops. In the current research the identified species of the bacteria were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Enterococcus and Enterobacteriaceae*. This research revealed that Gram-positive bacteria were more prevalent than Gram-negative species.

6. Recommendations

- Proper sterilization methods should be used by all barbers to disinfect the tools to prevent its spread.
- Barbers must be licensed because their work is directly related to public health.
- Students visiting barbershops should make sure that the barber tools are properly sterilized prior to use.
- Tools of the barber shop inside the university campus should be tested for infectious agents on regular basis to protect the visitors from catching pathogenic bacteria.

References

Al Yousef, S. A. (2021). Degree of bacterial contamination in barbershops using hair dryers in Riyadh. *Saudi Journal of Biological Sciences*, 28(5), 2692-2694.

Adjei-Gyamfi, S., Korang, F. K., Asirifi, A., & Asobuno, C. (2024). Knowledge and occupational practices of beauticians and barbers in the transmission of viral hepatitis: a mixed-methods study in Voltage Region of Ghana. *Plos One*. https: //doi.org/10.1101/2024.06.28.24309659.

Andrew, M., Culomo & Rossana Rosado. (2016). Practice of Barbering License Law New York State, Department of State; Division of Licensing Service.

Addari, G., Corbeddu, M., Mugheddu, C., Chessa, M., Vivanet, G., Ferreli, C., & Atzori, L (2025). Tinea Capitis induced by barber shaving: Isolation of *Trichophyton tonsurans*. *Journal of Clinical Medicine*, 14(622).

Britsch, J. M., Bereswill, S., & Heimesaat, M. M. (2024). Infections acquired in barbershops-A review. *European Journal of Microbiology and Immunology*, 14, 366-372.

Bassetti, M., Righi, E., & Carnelutti, A. (2016). Bloodstream infections in the intensive care unit. *Virulence*, 7(3), 267-279.

Daka, D. (2017). Barbers knowledge and practice of biological hazards in relation to their occupation: A case of Hawassa Town, Southern Ethiopia. *Journal of Public Health and Epidemiology*, 9

Enemuor, S. C., Atabo, A. R., & Oguntibeju, O. (2012). Evaluation of microbiological hazards in barbershops in a university setting. http://hdl.handle.net/11189/6390, 1992-2248.8): 219-225.

Hay, R. J., Johns, N. E., Williams, H. C., Bolliger, I. W., Dellavalle, R. P., Margolis, D. J., & Michaud, C. (2014). The global burden of skin disease in 2010: an analysis of the prevalence and impact of skin conditions. *Journal of Investigative Dermatology*, 134(6): 1527-1534.

Ibrahim, M. T., Opara, W. E., & Tanimomo, T. (2007). Knowledge of HIV/AIDS, infection prevention practices and accidental skin cuts in barbing saloons in Sokoto, Nigeria. *Nigerian Medical Practitioner*, 51(6): 123-127.

Janjua, N. Z, Nizamy, M. A (2004). Knowledge and practices of barbers about hepatitis B and C transmission in Rawalpindi and Islamabad. *Journal of Pakistan Medical Association*, 54: 116-119.

Khan, G., Rizvi, T. A., Blair, I., & Adrian, T. E. (2010). Risk of blood-borne infections in barbershops. *Journal of infection and public health*, 3(2): 88-89.

Lin, H. S., Lin, P. T., Tsai, Y. S., & Wang, S. H., Chi CC. (2021). Interventions for bacterial folliculitis and boils (furuncles and carbuncles). *Cochrane Database of Systematic Reviews*. 26;2(2):CD013099.

Mwabu, A. K., Kimanga, A. N., & Bii, C. (2018). Evaluation of bacteriological risks of barbershops in Kenyatta Market, Nairobi County, Kenya. *East African Medical Journal*, 95(1): 1157-1167.

Mutocheluh, M., & Kwarteng, K. (2015). Knowledge and occupational hazards of barbers in the transmission of hepatitis B and C was low in Kumasi, Ghana. *The Pan African Medical Journal*, 20: 260.

Takwale, A., Agarwal, S., Holmes, S. C., & Berth-Jones, J. (2001). Tinea capitis in two elderly women: transmission at the hairdresser. *British Journal of Dermatology*, 144(4): 898-900.

Zenbaba, D., Sahiledengle, B., Yassin, A., & Geleta, E. N. (2023). Barbers compliance with biological hazard preventive measures and predictors in selected towns, Southeast Ethiopia: a cross-sectional study. *BMJ Open*, 13(10), e074495.