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Research Article

Isolation of Pathogenic Bacteria from some Male Barbershops in the City of Nasiriyah

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ABSTRACT
A study was conducted to evaluate bacterial contamination in hairdressing and beauty salons in Thi-Qar at Alnasiriyah city. Samples were collected from ten different salons. The samples were collected from scissors,comb, razor, dryer, sink, and table tools. The isolates obtained were examined and identified using microscopic examination, colonial morphology and biochemical characteristics. Six bacterial species were isolated and identified. The bacterial isolates include *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus* spp, *Micrococcus Spp,Enterococus*spp and *Enterobacter*. Isolated from some men's salons and shaving tools and the highest frequency of bacteria was in the salon of Nawras by 15%, Salon Ahmed by 12.5% , more bacterial isolation of shaving tools,sink,razor and the presence of this potential pathogen is an indication that hairdressing salons could be contributing to the spread of infection.

Keywords:pathogenic bacteria, barber shop

INTRODUCTION

In recent decades, visits to barbershops have increased significantly due to increased income, peer pressure, emergence of different hair styles,and taking athletes and actor as role models[1]. 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[2]. 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 implemented. While it is known that invasive procedures, such as piercing and tattooing are clearly associated with bacterial, viral and fungal infection risks, even non-invasive procedures such as pedicures can result in infection[3]. It has been observed that hairdressing operators and their clients are constantly being exposed to bacterial or fungal contamination during their services, since most users are not aware of how to use these substances, so that provision of continuing trainings on this issue seems essential[4].

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[5].

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. Infections that can be spread in hairdressing premises include skin infections on the scalp, face and neck such as impetigo and fungal infections such as tineacapitis and ring worm[6]. Burns can also occur during hair dressing procedures involving hot rollers, tongs and crimpers and when hair is being washed with contaminated water or when stationary or hand-held dryers are improperly used[7]. Unfortunately, there are no established regulations, guidelines and best practices for many of these salons in our environment.

Aims of the study

To evaluate the bacterial contamination on tools used in selected barbershops.

To isolate and identify pathogenic bacteria associated with hair dressing tools.

METHODS AND MATERIALS

Collection of samples

In this study, a total of 50 samples were randomly collected from 10 hair and beauty salons in Thi-Qar at Alnasiriyah city. To determine the types of microorganisms present, comb, brushes, rollers, hairpins and dryers were sampled with a moistened sterile cotton swab.

Preparation of culture media

All culture media used were prepared according to the manufacturer' instructions.

Determination of bacterial contamination

Each of the samples were inoculated on the media (Blood agar, Macconkey agar and Mannitol agar) appropriately with a wire-loop using streaking method. The plates were incubated at 37°C for 24hrs -48hrs (1 -2days) for bacteria

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Table 1:Total number of bacterial isolates from each hairdressing and beauty salons.

Salon	No. of samples collected	Bacterial isolate		Bacterial contamination percent
		Organism	No. of samples present	
Nawras	5	*S. aureus*	3	15%
		S. epidermidis	1	
		Enterococcus Spp.	1	
		E. coli	3	
		Pseudomonas aeruginosa	2	
		Micrococcus Spp.	2	
Alhaneen	5	*S. aureus*	3	11.25%
		Enterobacteria Spp.	1	
		S. epidermidis	2	
		E. coli	3	
Anwar	5	*S. aureus*	4	10%
		Streptococcus Spp.	1	
		Enterobacteria Spp.	1	
		Micrococcus Spp.	2	
Ahmed	5	*Staphylococcus Spp.*	1	12.5%
		Streptococcus Spp.	1	
		S. aureus	3	
		S. epidermidis	1	
		Pseudomonas aeruginosa	2	
		Micrococcus Spp.	2	
Beauty Center	5	*S. aureus*	2	6.25%
		Staphylococcus Spp.	2	
		Streptococcus Spp.	1	
Tareq	5	Staphylococcus Spp.	2	10%
		S. aureus	3	
		Streptococcus Spp.	1	
		E. coli	2	
Ogun	5	Enterococcus Spp.	1	6.25%
		Enterobacteria Spp.	1	
		Streptococcus Spp.	1	
Aziz	5	*S. aureus*	2	10%
		S. aureus	3	
		Staphylococcus Spp.	1	
		S. epidermidis	2	
		Micrococcus Spp.	2	
Hairdresser Adnan	5	*S. aureus*	2	8.75%
		Enterococcus Spp.	1	
		Staphylococcus Spp.	2	
		E. coli	2	
Shanab	5	*S. aureus*	3	10%
		Staphylococcus Spp.	2	
		Enterobacteria Spp.	1	
		E. coli	2	

Gram staining

Make a smear of the organism on a grease free glass slide. Allow it to air dry. Flood the smear with crystal violet and gently rinse off the crystal violet with tap water. Flood the smear with iodine, leave for at least three seconds and gently rinse off the iodine with tap water. Decolourize by adding alcohol or acetone to the smear while holding the slide at an angle to allow the decolourizer to drain and rinse off excess decolorizer with tap water. Flood the smear with safranin counter stain and allow it for 30seconds and gently rinse off excess Safranin with tap water. The drain slide is allow to be air dry and grain negative (From positive organisms are purple while gramm negative pink.

Motility test

This test was performed to determine motility. The pure isolates in the stock culture were first inoculated into nutrient broth to keep the organism inactive o phase. A hanging drop technique was carried out for motility. Drops of the cultures were placed on cover slips and plasticizes placed on slides enough to cover the cover slips. The slides were the gently placed over the drops of

the cultures without allowing contact. The slides were quickly inverted, are being taken not to allow drops, from the cover slips to touch the slide.

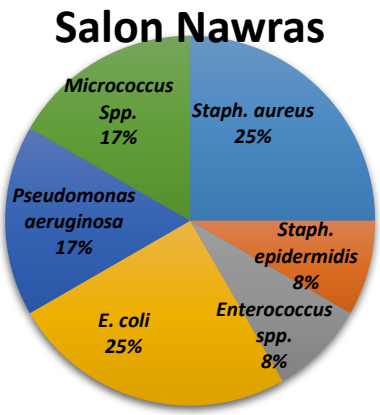

Figure 1: The percentage of the bacterial species isolated for Salon Nawras.

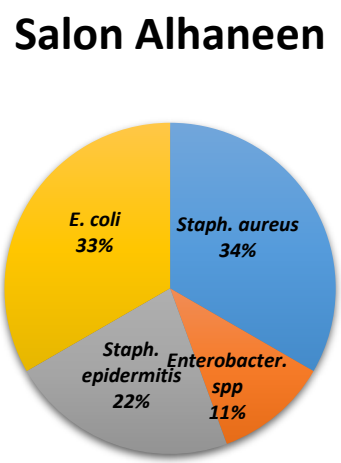

Figure 2: The percentage of the bacterial species isolated for Salon Alhaneen.

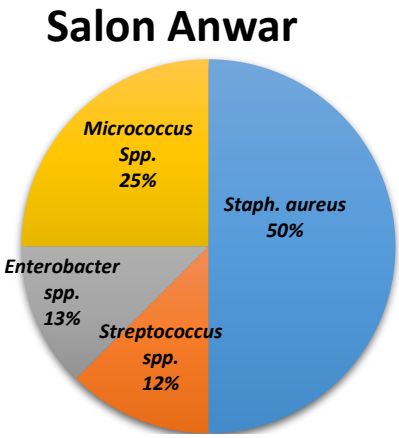

Figure 3: The percentage of the bacterial species isolated for Salon Anwar.

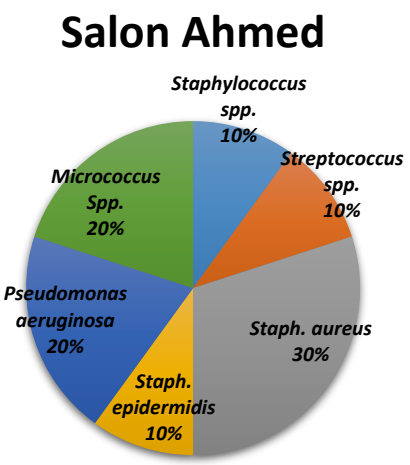

Figure 4: The percentage of the bacterial species isolated for Salon Ahmed.

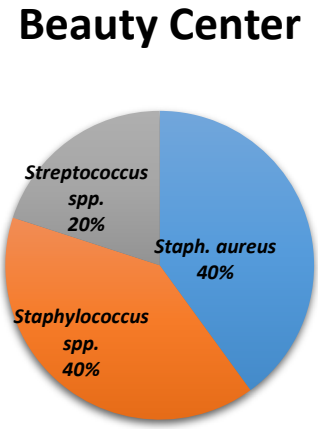

Figure 5: The percentage of the bacterial species isolated for Beauty Center.

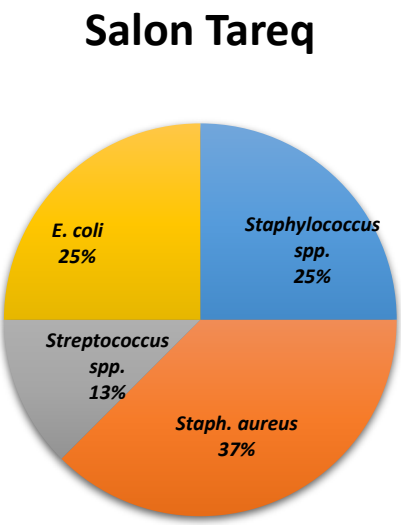

Figure 6: The percentage of the bacterial species isolated for Salon Tareq.

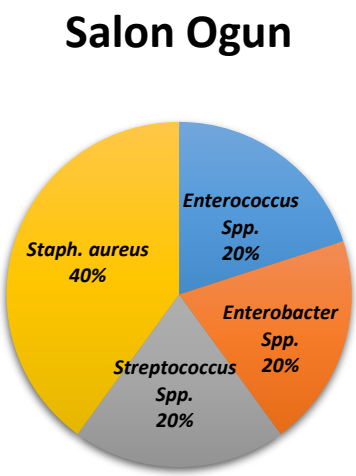

Figure 7: The percentage of the bacterial species isolated for Salon Ogun.

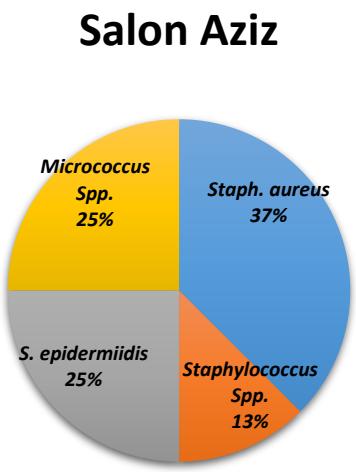

Figure 8: The percentage of the bacterial species isolated for Salon Aziz.

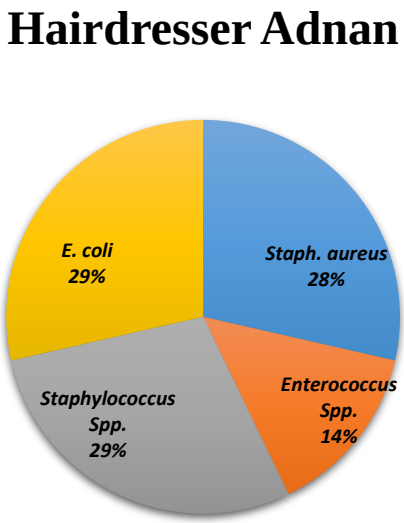

Figure 9: The percentage of the bacterial species isolated for Hairdresser Adnan.

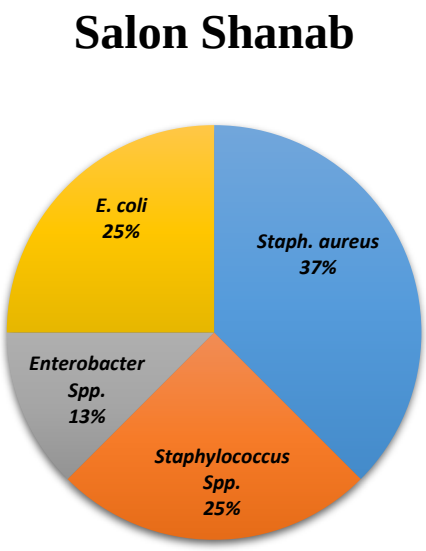

Figure 10: The percentage of the bacterial species isolated for Salon Shanab.

The preparations were observed under the microscope. Motile organisms were seen, moving from one corner of the cover slip to another while non-motile organisms did not move about[8].

Biochemical test for characterization of the isolates

Biochemical test refers to the chemical identification of unknown substances within a living thing. The test quantitatively and qualitatively determines a particular substance lie bacteria and enzyme with the blood. A biochemical test can be used to diagnose a given disease. It can also be used to find the metabolic properties of bacteria.

Catalase test

Three (3) millitres of hydrogen peroxide (3%v/v) solution was disposed into a series of test tubes labeled with the isolate numbers. Using sterile wooden spatula, good growths of the test organisms were removed from the plates and immersed into the respective test tubes appropriately.

Budding was observed in organisms that catalase was produced. The absence of bubbles showed catalase negative result[9].

Methyl red –vogesproskauer (mrvp) test

The medium is used to test for the end-product of glucose metabolism in bacteria and the method used was adapted from[9]. The MR positive organism produces acids as their end-products of sugar metabolism. The VP organism produce 2,3 – butanediol or acetyl methyl carbihol from fermentation of pyruvic acid.

The medium was prepared by dissolving 15g in 1 litre of deionized water. The medium was then dispensed in 10ml, aliquots into tubes. Sterilization was done by autoclaving at 121°C for 15 minutes. On cooling at room temperature, isolates were used to inoculate the medium and incubated at 37°C to 48hours. Some of the tubes inoculated with the bacterial isolates were labeled MR and VP respectively.

The MR test was done by delivering 5 drops of methyl red indicator to broth tubes labeled MR. These gently shaken so that the indicator can be dispensed in the broth. Cherry pink (red) color formation was taken as MR positive while yellow coloration of methyl red in the broth within seconds will be taken as a negative result.

In the VP test, 6 drops of Baritt reagent (alphanaphthol) were added to the broth tubes labeled VP, followed by the addition of drops of 40% potassium hydroxide. These were gently shaken and left to stand for 10minutes. Positive organisms changed the colour of the medium to pink red while those that did not show colour change were VP negative.

Indole test

The test demonstrates the ability of certain bacteria to decompose the anuro acid tryptophane to indole which accumulates in the medium[9].

The medium was sterilized by autoclaving at 121°C for 1 5minutes. The peptone water broth tubes were inoculated with the stock cultures of the isolates and incubated at 37°C for 48hours. After incubation, 0.5ml of Koval's reagent comprising 130 amyl alcohol, 150ml; P-Dimethyl-amino benzoaldehyde, 10g and concentrated hydrochloride, 50ml were added and shaken gently.

A red ring formation at the surface of the tubes was an indication of a positive test while yellow colouration of the surface layer indicated a negative result.

Citrate test

This is a test for the ability of the organism especially the members of the enterobaceteriaceae to utilize citrate as the sole carbon and energy source for growth and ammonium slat as the sole source of nitrogen and also Simmon's citrate agar was used[9]. Slants of Simmon's citrate agar medium in tubes were inoculated with sterile wire loops containing the test organisms. The tubes were incubated at 37°C for 96 hours. A positive result was indicated by a change in color from green to blue while

Table 2: Distribution of role of organisms in the samples.

Sample	Bacterial isolate					
S/No.	Staph	Strep	Micro	Enterobacter Spp.	*pseudomonas aeruginosa*	Enterococcus Spp.
Scissors	+	+	+	-	-	+
Comb	+	-	-	+	+	+
Razor	+	-	+	+	+	+
Dryer	-	-	-	-	+	-
Sink	+	+	+	+	+	+
Table of tools	+	+	+	+	+	+

Staph means Staphylococcus Spp.
Strep means Streptococcus Spp.
Micro means Micrococcus Spp.

the retention of the original green color showed citrate negative reaction.

Coagulase test

This test was used to distinguish staphylococcus aureus (coagulase positive) from Staphylococcus epidermidis and Staphylococcus saprophyticus (coagulase negative). The test was carried out using the tube coagulase technique (DIFCO) capable of demonstrating both free and bound coagulase.

Tube coagulase: In the tube coagulate method, human plasma was diluted 1 in 10 using normal saline as diluent. One milliliter of the diluted plasma was added to 0.1ml of a 24 hour nutrient broth culture of the organism. The mixture was indicated by the formation of a solid clot within the tube while there was no clot formation in the coagulase negative isolates.

RESULTS

Table 1 show the total number of bacterial isolates and bacterial contamination percent from each hairdressing and beauty salons for 50 samples taken from 10 different salons in Alnasiriyah. In Salon Nawras and Ahmed six bacterial species has been isolated which gives 15% bacterial contamination for Salon Nawras and 12.5% for Salon Ahmed. While the other salons four bacterial species isolated except Beauty Center three bacterial species isolated. The minimum bacterial contamination has been appeared in Beauty Center and Salon Ogun with 6.25% percent.

Figure 1 shows the percent of the bacterial species isolated in Salon Nawras, it has been noticed that the maximum percent is for *Staphylococcus aureus* and *E. coli* with 25% for each one. While the minimum bacteria percent 8% for each *Staphylococcus epidermidis* and *Enterococcus spp.*

Staphylococcus aureus found to be in the most samples in Salon Alhaneen with 34%, while the minimum percent is *Enterobacter. spp.* with 11% percent from all samples isolated, as shown in figure 2.

The maximum bacteria noticed in Salon Anwar is *Staphylococcus aureus* with 50% percent from all samples isolated, while the lowest bacteria appeared is *Streptococcus spp.* with 12% percent, as seen in figure 3.

Figure 4 shows the percent of the bacterial species isolated in Salon Ahmed, it has been noticed that the maximum percent is for *Staphylococcus aureus* with 30%. While the minimum bacteria percent 10% for each *Staphylococcus epidermidis, Staphylococcus spp. and Streptococcus spp.*

Figure 5 shows that in Beauty Center, three bacterial samples have been isolated *Staphylococcus aureus* and *Staphylococcus* spp. with 40% for each one. While the third bacteria *Streptococcus spp.* percent is 20%.

For the Salon Tareq, it has been noticed that the maximum percent is for *Staphylococcus aureus* with 37%. While the minimum bacteria is *Streptococcus spp*. with 13%, asseen in figure 6.

Figure 7 shows the percent of the bacterial species isolated in Salon Ogun, it has been noticed that the maximum percent is for *Staphylococcus aureus* with 40%. While the other bacteria *Enterococcus spp., Enterobacteria spp. and Streptococcus spp.* form 20% for each type from the total samples isolated.

For the Salon Aziz, it has been noticed that the maximum percent is for *Staphylococcus aureus* with 37%. While the minimum bacteria is *Staphylococcus spp*. with 13%, as seen in figure 8.

Staphylococcus spp. and E. coli are the maximum bacteria noticed in Hairdresser Adnan with 29% percent, as seen in figure 9. While the minimum bacteria presented is *Enterococcus spp.* with 14% percent.

Figure 10 shows that, 37% percent from *Staphylococcus aureus* has been presented, which is the maximum percent obtained in Salon Shanab. While the minimum bacteria is *Enterococcus spp*. with 13%.

The distribution of role of organisms in the samples has been concluded in table 2 for the scissors, comb, razor, dryer, sink, and table of tools.

DISCUSSION

Table 1 show the total number of bacterial isolates and bacterial contamination percent from each hairdressing and beauty salons for 50 samples taken from 10 different salons in Alnasiriyah. Only six bacterial species were isolated.Studies have examined the presence of potential pathogens as well as infection control practices of personal service establishments such as salon so as to better understand and characterize potential hazards in salons[10].

In our current study, we isolated six bacterial potential pathogenic species in the different salons. *Staphylococcus aureus* was isolated from all the salons and are among the most important bacteria that cause disease in humans. This bacterium has been shown to cause various pus-forming diseases in humans such as boils, carbuncles, foliculities ,impetigo contagiosa , scalded-skin syndrome. *Staphylococcus epidermidis* which was isolated from four salons is a normal habitat of the skin but can occasionally cause endocarditis. Isolation of the organisms from the equipment and items used in these salons indicate that the sterilization methods employed by the operators are not effective if at all they sterilize items between clients.This could imply that operators or workers in these salons are almost ignorant or less informed of the risks involved in their work. This situation calls for the agencies in public health sector to awake to their responsibilities in sensitizing and organizing lectures, training workshops and seminars for the operators and workers of hairdressing and beauty salons in our different communities. If the people are informed of the dangers or hazards associated with their profession they would help in improving on their practices and by so doing reduce the spread of these infections.

Each salon were inadequate in preventing health risks among clients. It has been observed that sterilization techniques differ between service providers with 38% reporting the use of ultraviolet (UV) light, 18% using glass beads and 1% using ultrasonic cleaners, all of which are not approved methods of sterilization in many jurisdictions[11].

Infection can occur during hair dressing 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. Infections that can be spread I hairdressing premises include skin infections on the scalp, face and neck such as impetigo and fungal infections such as *tinea capitits*[12].

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[13].

CONCLUSION

From table 1 it can be seen that the most bacterial contamination percent is in salon Nawras with 15% while the lower contamination is in Beauty Center and salon Ogun with 6.25%. Table 2, shows the distribution rate of the organisms in the samples. Here, six (6) bacterial potential pathogenic species were isolated from different hair dressing salons.

Most positive culture in the salons are table tools and sink followed by Razor and comb

RECOMMENDATIONS

For reduce bacterial infections, it is recommended to plan continuous and suitable trainings for barbers regarding the use of disinfectants and encourage clients to use personal tools at barbershops. All equipment must be cleaned (washed in hot soapy water) before disinfection or sterilization, to remove organic matter and other residue, which may cause a layer of buildup that prevents disinfection or sterilization. Equipment that comes into contact with the skin must be cleaned before re-use whether or not it looks dirty. Equipment should be thoroughly cleaned at least once a day and immediately after contamination with blood. A fresh disinfection solution should be prepared daily and the container thoroughly cleaned before refilling. Bench top sterilizers are the most effective means of sterilizing equipment. This will go a long way to reduce microbiological and other potential hazards associated with the services of hairdressing and beauty salons in the country.

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