



# EVALUATION OF MICROBIAL LOAD ON COMBS IN VARIOUS PLACES WITHIN THE TIRUPUR DISTRICT, TAMILNADU, AND SENSITIVITY PATTERN OF DISINFECTANTS

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## ABSTRACT

A study was conducted to evaluate bacterial and fungal contamination in hair combs. Samples were randomly collected from salons, school and college students, working women, children's, hospital workers and animal dispensaries to determine the type of microorganisms present in combs were sampled with a moistened sterile cotton swab. The sterile swab sticks were moistened normal saline first before they were used to swab the combs. All the samples collected were transported to the laboratory without delay for culture and treated according to standard method, a variety of selective and different microbial media were used for presumptive identification of contaminating microorganisms. Culture media used in the laboratory for the cultivation of microorganism supply the nutrients required for the growth and maintenance. The isolates obtained were examined using biochemical characteristics, colonial morphology and also identified using microscopic examination. Commonly isolated bacteria from frequently used hair combs include *Staphylococcus aureus*, *Staphylococcus epidermis*, *Bacillus* sp., *Shigella* sp., *Serratia* sp., *Streptococcus pyogenes*, while fungal isolates include *Aspergillus flavus*, *Aspergillus fumigates*, *Aspergillus niger*, *Penicillium* spp, *Rhizopus* sp., *Trichophyton*, *Candida albicans*, *Mucor* sp. An antifungal agent, is a biocide chemical or biological compound, used to kill or inhibit fungi or fungal spores. The present study reveals to assess the role of combs as reservoirs and vehicles in the transmission of potentially pathogenic bacterial and fungal species.

**Keywords:** Hair combs, Microbial contamination, disinfectant, percentage inhibition.

## INTRODUCTION:

The use of hair combs dates as 5,000 years ago! Combs are actually among the oldest tools found by archaeologists. Hair combs are used by humans to separate tangled hairs, to keep their hair clean. Hair combs were initially made of stone, wood or ivory from elephant tusks, but are now made almost exclusively from metal or plastics. The hair has been described as the crowning glory of a person. Daily care and maintenance of hair and hair combs is very important in order to prevent or minimize fungal infection of the hair (Mandy M .2013, Cruz C F *et al.*, 2016). Along with hair clumps and product residue, dust mites, dead skin cells, and oils can accumulate. The buildup of such on one's hair combs can serve as media for bacteria and fungi overgrowth, resulting in an infection risk. (Escobar S *et al.*, 2018, Grimalt R A.2007). Hair infection is common in both old and young and has been associated with fungal and bacterial contamination of beauty tools such as hair combs. (Enemour S C, *et al.*, 2013, Edward S M, 2015). Microorganisms are everywhere including skin surfaces and are continually introduced into the environment (De Souza and Shibu, 2004). Health care is one of the most important aspects of all human endeavors aimed at improving the quality of life. Many of the infectious diseases are preventable or treatable but have continued to be successful due to lack of personal and environmental hygiene.

Important routes of transmission of bacteria, fungal or viral infection include airborne, faecal-oral spread, vector borne and direct spread either through person to person contact or by direct inoculation (WHO, 2006). Besides the day to day interactions of people which constitute one way of spreading disease, the major source and spread of infections in communities are fomites. (Kramer A *et al.*, 2006). The environment plays an important role in transmission of microbial agents to humans, with many environmental materials serving as vehicles (WHO, 12 may 2018). The basic minerals, organic and inorganic compounds, growth factors on cosmetic tools, and moisture provide suitable environments to grow microbes (Enemuor, *et al.* 2013). Infections that can be spread hairdressing premises include skin infections on the scalp, and fungal infections. Salons are personal service establishments that provide services which may present potential health concerns to their client including the risk of infection (caused by microorganisms) and sometimes injury (Adeleye and Osidipo, 2004; Barn and Chen, 2011). These health risks may vary depending on the kind of the service, the tool and equipment that are used, physiological state of the client and the kind of the service provided (Stout *et al.*, 2011) which can be transmitted between clients if proper infection control procedure are not implemented. Other primary infections associated with barbing practices include skin infection of the scalp, neck, and face such as impetigo and fungal infection such as ringworm, and *tinea capitis* (Brown 2006; Amodio *et al.*, 2010; Barn and Chen 2011). Beauty tools such as hair combs are used in everyday life for the purpose of hair care. However, they are rarely kept clean and thus give rise to likely contamination and colonization of these items by microorganisms. Daily care and maintenance of hair combs is very important in order to prevent or minimize fungal infection of the hair (Mandy M .2013, Cruz C F *et al.*, 2016). Along with hair clumps and product residue, dust mites, dead

skin cells, and oils can accumulate. The buildup of such on one's hair combs can serve as media for bacteria and fungi overgrowth, resulting in an infection risk. (Escobar S *et al.*, 2018, Grimalt R A.2007).

Hair combs may serve as potential vehicles for fungi capable of causing infections of the hair and other parts of the human body. Hair infection is common in both old and young and has been associated with fungal and bacterial contamination of beauty tools such as hair combs. (Enemour S C, *et al.*, 2013, Edward S M, 2015). Fungi are able to grow in indoor environments where there is sufficient moisture and a nutrition source, such as wood, paint and insulation and release spores as part of their reproductive process (Clean air society of Australia and New Zealand, Sydney, 2002). 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 (De Souza and Shibu, 2004). Many of the infectious diseases are preventable or treatable but have continued to be successful due to lack of personal and environmental hygiene; ignorance and poor political commitment; from the government.

There are reports of people who have been infected with head lice from direct hair-to-hair contact with someone who has head lice (Ruddy *et al.*, 2011). The Australian Mould Guideline is commonly adopted by industry and recommends damp wiping with a detergent, hydrogen peroxide, vinegar solution or alcohol solution for removing fungi from contamination. It also lists antifungal agents such as bleach, alcohol and formaldehyde as chemicals that are used in the treatment of fungi. Hydrogen peroxide was discovered by Louis Thenard in 1818 and its use as a disinfectant first proposed by B.W.Richardson in 1891. The Australian Mould Guideline is commonly adopted by industry and recommends damp wiping with a detergent, hydrogen peroxide, vinegar solution or alcohol solution for removing fungi from contamination. It also lists antifungal agents such as bleach, alcohol and formaldehyde as chemicals that are used in the treatment of fungi. The present study reveals to assess the role of combs as reservoirs and vehicles in the transmission of potentially pathogenic bacterial and fungal species.

## **MATERIALS AND METHODS:**

**COLLECTION OF SAMPLES:** A total of 60 hair comb samples were randomly collected from salons, school, and college students, working women, children's, hospital workers and animal dispensary (in Tirupur). To determine the types of microorganisms present in combs were sampled with a moistened sterile cotton swab. A total count of the different colonies isolated on each plate was determined and was significant when bacterial count is  $\geq 10^5$ . Gram staining, microscopic examination and confirmatory biochemical tests were performed to further identify bacteria and fungi (Adeleye and Osidipo, 2004).

**MICROSCOPIC OBSERVATION:** Gram staining and Capsule staining were performed to demonstrate the morphology and Capsule possession of the organism. The culture was subjected to lacto phenol blue staining method. The identification of molds based on the shape, method of production and arrangement of spores.

**MOTILITY DETERMINATION:** Motility by bacterium is demonstrated in semisolid agar medium. The medium mainly used for this purpose is SIM medium (Sulphide indole motility medium) which is a combination differential medium that tests three different parameters, Sulfur reduction, indole production and motility. With a sterile straight needle, touch a colony of a young (18 to 24hours) culture growing on agar medium. Single stab down the centre of the tube to about half the depth of the medium. Incubate at 35°-37°C and observe it.

## **BIOCHEMICAL TESTS:**

**INDOLE TEST:** The indole test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole. The medium was sterilized by autoclaving at 121°C for 15 minutes. The peptone water broth tubes were inoculated with the stock cultures of the isolates and incubated at 37°C for 48 hrs. After incubation, 0.5ml of Kovacs reagent were added and shaken gently. A red ring formation at the surface of the tube was an indication of a positive test while yellow colouration of the surface layer indicated a negative result.

**METHYL RED VOGES PROSKAUER (MRVP) TEST:** The medium is used to test for the end product of glucose metabolism in bacteria. The MR positive organism produces acids as their end products. The VP organism produce 2, 3 -butanediol from fermentation of pyruvic acid.

**CITRATE:** Citrate test is used to differentiate among enteric bacteria on the basis of their ability to utilize as their soul carbon source. The utilization of citrate depends upon the presence of enzyme “citrate permease” produced by organism that helps its transport into the cell. Prepare the citrate slant according to the given composition and dispensed into the test tube and autoclaved at 121°C for 15 lbs. inoculate the tubes with the given culture and then inoculate at 37°C for 48 hrs. Observe the tubes for colour change from green to Prussian blue colour.

**COAGULASE TEST:** This test was used to distinguish *Staphylococcus aureus* from *Staphylococcus epidermis*. In the tube coagulate method, human plasma was diluted 1 in 10 using normal saline as diluent. one millimeter of the diluted plasma was added to 0.1ml of a 24hr nutrient broth culture of the organism. The mixture was indicated by the formation of a solid clot. Examine the clotting after 1hour. If no clotting formation in the coagulase negative isolates.

**CATALASE PRODUCTION TEST:** This test is done to check whether the test organism produce catalase or not. The organism is subjected to 3% H<sub>2</sub>O<sub>2</sub> solution and catalase enzyme acts on it. This test is done to check whether the test organism produce catalase or not. The organism is subjected to 3% H<sub>2</sub>O<sub>2</sub> solution and catalase enzyme acts on it. Take a drop of reagent on a clean slide and transfer bacterial colonies into it. Observe the effervescence formation.

**TRIPLE SUGAR IRON TEST:** This test is used to differentiate among the members of Enterobacteriaceae according to their ability to ferment lactose, sucrose and glucose and production of hydrogen Sulphide.

**FERMENTATION OF CARBOHYDRATE:** Carbohydrate fermentation patterns are useful in differentiating among bacterial groups or species. Fermentation broth contains a nutrient broth with specific carbohydrates such as glucose, lactose, mannitol, sucrose may be altered during autoclaving by heat in the presence of other ingredients.

### **CULTURAL CHARACTERISTICS:**

- A) **NUTRIENT AGAR:** Nutrient agar media was used for preparation of pure culture. 50 ml nutrient agar was prepared, sterilized in the autoclave and transferred into petri plates. In dilution plates the good number of colonies are selected, a colony from it was streaked on nutrient agar plate and incubated at 37°C for pure culture.
- B) **BLOOD AGAR:** Blood agar are enriched medium. It is required to detect and differentiate haemolytic bacteria, especially *Streptococcus* species. It is also a differential media in allowing the detection of hemolysis by cytolytic toxins.
- C) **MANNITOL SALT AGAR:** Mannitol salt agar (MSA) is used as a selective and differential medium for the isolation and identification of *Staphylococcus aureus* from specimens.
- D) **EOSINE METHYLENE BLUE AGAR:** EMB agar is a differential medium, which inhibits the growth of gram positive bacteria. Helps in isolation and differentiation of enteric bacilli and gram negative bacilli.
- E) **ROSE BENGAL AGAR:** It is a selective media for isolation and enumeration of yeasts and molds from environmental materials.
- F) **BAIRD PARKER AGAR:** Baird parker agar is a selective medium for the enumeration of *Staphylococcus aureus*. Suspend 6 grams in 100ml of distilled water. Heat to boiling, to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 minutes. Mix well and pour into sterile petri plates. Incubate and examine it
- G) **XLD AGAR:** Xylose Lysine Deoxycholate (XLD) agar is a selective medium for the isolation of *Salmonella* and *Shigella* spp.

**H) HEAKTON ENTERIC AGAR:** Heakton enteric agar is a selective and differential medium to isolate *Salmonella* and *Shigella* species. HE agar allows a good growth of *Shigella* because the inhibition of these organisms by bile salts is reduced by addition of peptone and carbohydrates.

### **PREPARATION OF EACH DISINFECTANTS:**

The used disinfectants are Hydrogen peroxide, alcohol and detergent. Disinfectant concentrations were prepared at 2% to perform *in vitro* tests for the effectiveness against the bacterial and fungal strains.

### **DISINFECTANT EVALUATION:**

Efficacy of the disinfectant was evaluated at concentrations against the established microorganism, according to (Uchikawa.M, *etal.*2013). The disinfectants were previously prepared and 2ml of each disinfectant was evaluated. The used detergents were Dettol.

Subsequently, the suspension with the disinfectant and the microorganisms were dispensed in petri plates with Muller Hinton agar for 24-48 hrs, and a plate count was carried out to verify the inhibition percentages from the initial count (Uchikawa.M, 2013 and Kohli R, 2013).

### **WELL DIFFUSION METHOD:**

In this method, Muller-Hinton agar was prepared by equally cutting spaced well (6 mm). Then the plates were inoculated with a cotton swab dipping into screw tube containing a bacterial and fungal suspension and streaked over the surface of the plates. Muller-Hinton agar well was filled with 0.1 mL of prepared concentration solutions for each well and incubated the plates at 37°C for 24 hours. The susceptibility to this mixture was determined by measuring inhibition zone around well for concentration.

### **RESULTS AND DISCUSSION:**

The present study assessed the mycological quality of hair combs. Contamination retention and contamination transfer are two of the most significant risk factors associated with cosmetic products. Of the 60 samples obtained from the different hair combs. The isolated organisms from the hair comb samples are *Staphylococcus aureus*, *Staphylococcus epidermis*, *Bacillus subtilis*, *Micrococcus*, *Serratia sp*, and *Shigella sp.*, And fungi includes *Aspergillus flavus*, *Aspergillus fumigates*, *Penicillium sp*, *Candida albicans*, *Trichophyton sp*, and *Microsporium*. It appears that the most of the contamination is caused by bacteria and fungi.

The results of bacterial and fungal load obtained from combs was shown (Table 2). From the results of the current study, it appears that the most of the contamination is caused by bacteria and fungi. The percentage of the bacterial contamination was (79%), but the proportion of fungal contamination was (21%). The percentage of *Staphylococcus* was (55%), while the proportion of *Streptococcus* and *Enterococcus sp* were (18%), respectively. The outcome of this study show that majority of the hair combs (92.5%) examined, had fungal contaminants, similar to the works of Edward et al.

The percentage occurrences of dermatophytes and non dermatophytes fungal contamination of hair combs of the study participants were 52.8% and 39.7%, respectively. The presence of potential pathogens as well as infection control practices of personal service establishments such as salons so as to better understand and characterized potential hazards in Salons. Due to the presence of these potential pathogens, the authors concluded that current disinfection techniques used at each salon were inadequate in preventing health among clients. *Staphylococcus epidermidis* which was also isolated from most of all the samples is a normal habitat of the skin but can occasionally cause endocarditis. Isolation of the organisms from the sample indicate that the sterilization methods employed by the operators are not effective if at all they sterilize items between clients. *Trichophyton* sp. isolated from some of the samples also indicates that ringworm or dermatophytosis can also be spread via salons.

*Staphylococcus* sp, due to this bacterium is capable of causing various diseases in humans such as scaly skin abscess, which is the most common agent responsible for skin and soft tissue infections (Biadgelegn, *et al.* 2012). The ratio of *Candida albicans* was (39%) of the samples. Explaining this ratio for *Staphylococcus* sp, due to this bacterium is capable of causing various diseases in humans such as scaly skin abscess, which is the most common agent responsible for skin and soft tissue infections (Biadgelegn, *et al.* 2012).

*Staphylococcus aureus* was isolated from all the samples and are among the most important bacteria that cause disease in humans. *Candida albicans* is the most common fungal pathogen in humans, and can grow as yeast and filamentous forms in the host. *Staphylococcus* sp was the most abundant of all bacterial isolates while *Bacillus* sp. was the least abundant because *Staphylococcus* sp exist as normal flora on the skin while *Bacillus* sp is found in soil. Among the fungal species, *Aspergillus* sp was the most abundant while *Rhizopus* sp was the least abundant.

The outcome of this study shows that 51 out of the 60 hair combs examined had fungi contaminants, while the remaining 9 were fungi-free. Also, out of the 60 hair combs examined, 11 had one fungi isolate (mono fungi contamination), 28 had two fungi isolates (dual-fungi contamination), while 21 had more than two fungi isolates.

Figure -1 shows the occurrence of bacterial isolates in hair combs by the percentage of sharing hair combs, frequency of changing hair combs, dandruff hair combs, and animal dispensary. Figure-2 shows the occurrence of fungal isolates in hair combs by the same category. The category of participants with high level of fungal contaminants on their hair combs need to give more serious attention to their personal hygiene including that of their hair combs. The activities of various disinfectants on the microbial isolates from hair combs are shown in the figure. It results that the disinfectants of hydrogen peroxide, alcohol and detergent were exhibited microbial activity against isolated microorganisms. (Table -3) shows the antimicrobial activity of hydrogen peroxide. (Table -4) shows the antimicrobial activity of alcohol. (Table-5) shows the antimicrobial activity of detergent (Dettol).

It shows that the hydrogen peroxide is 92% effective against these microorganisms. Detergent shows (79%) effective and alcohol shows (65%) against isolated microorganisms. Several hygiene measures were reported to prevent cross contamination. The types of fungal contaminants present on the hair combs examined in this study (*Microsporium* sp., *Aspergillus* sp., *Candida* sp. and *Trichophyton* sp.) are the same with those isolated by Enemuor *et al* (2013) except for *Penicillium* sp., *Mucor* sp., *Rhizopus* sp., and *Cephalosporium* sp., Stanley *et al* (2019) except for *Mucor* spp and *Rhizopus* spp., as well as Edward *et al*, (2015) except for *Penicillium* and *Fonseceae* species on hair brushes and/combs.

Regarding the nature of materials used for washing of hair combs, most of the participants use water and shampoo (33%) to wash their hair combs, some use water and detergent (7%), some use water and soap (31%), while some use water only (27%). The implication of this is that their hair combs are not properly disinfected as the detergent does not possess fungicidal properties, hence upon washing created a wet, moist condition that favors fungi colonization and growth. Even though most people are of the opinion that using water and shampoo were the best option, but that was put in doubt as fungi were isolated from hair combs of the individuals who used water and shampoo as seen in this study. Hence the need for proper and regular washing of the hair combs.

TABLE- 1

## ANTIMICROBIAL ACTIVITY OF HYDROGEN PEROXIDE

Bacterial isolates	Zone of inhibition (mm)	Fungal isolates	Zone of inhibition (mm)
<i>Staphylococcus aureus</i>	30mm	<i>Aspergillus flavus</i>	23mm
<i>Staphylococcus epidermis</i>	28mm	<i>Aspergillus fumigates</i>	21mm
<i>Bacillus subtilis</i>	15mm	<i>Penicillium sp</i>	19mm
<i>Micrococcus sp</i>	20mm	<i>Trichophyton sp</i>	16mm
<i>Serratia sp</i>	18mm	<i>Microsporium sp</i>	16mm
<i>Shigella sp</i>	10mm	<i>Candida albicans</i>	11mm
		<i>Mucor sp</i>	14mm
		<i>Rhizopus sp</i>	11mm

TABLE – 2

## ANTIMICROBIAL ACTIVITY OF ALCOHOL

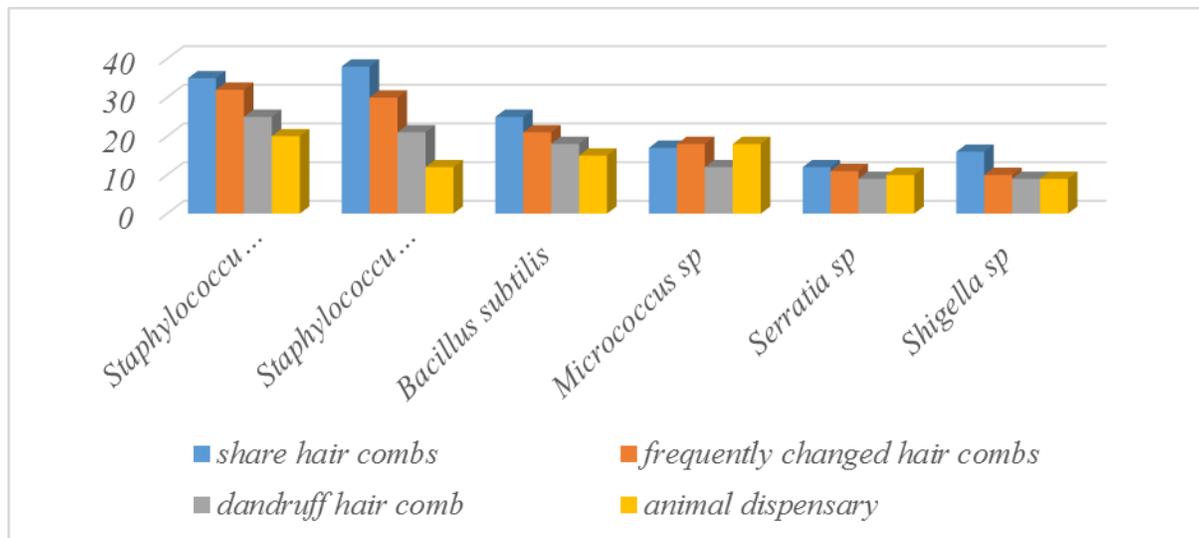
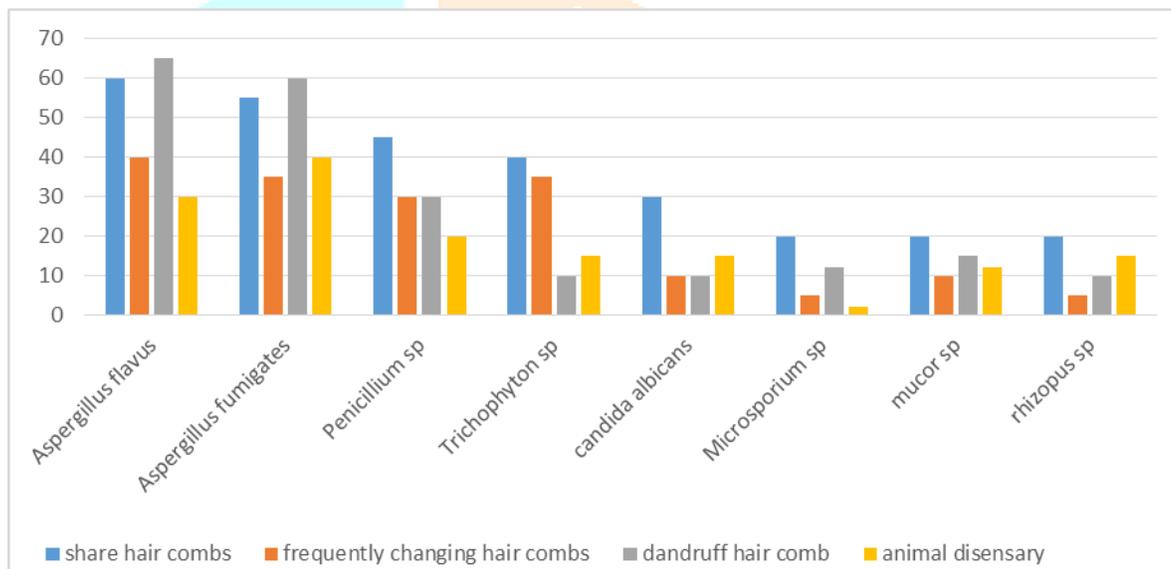
Bacterial isolates	Zone of inhibition (mm)	Fungal isolates	Zone of inhibition (mm)
<i>Staphylococcus aureus</i>	22mm	<i>Aspergillus flavus</i>	19mm
<i>Staphylococcus epidermis</i>	22mm	<i>Aspergillus fumigates</i>	16mm
<i>Bacillus subtilis</i>	18mm	<i>Penicillium sp</i>	16mm
<i>Micrococcus sp</i>	11mm	<i>Trichophyton sp</i>	08mm
<i>Serratia sp</i>	07mm	<i>Microsporium sp</i>	13mm
<i>Shigella sp</i>	10mm	<i>Candida albicans</i>	21mm
		<i>Mucor sp</i>	15mm
		<i>Rhizopus sp</i>	12mm

TABLE – 3

## ANTIMICROBIAL ACTIVITY OF DETERGENT

Bacterial isolates	Zone of inhibition (mm)	Fungal isolates	Zone of inhibition(mm)
<i>Staphylococcus aureus</i>	19 mm	<i>Aspergillus flavus</i>	15mm
<i>Staphylococcus epidermis</i>	20mm	<i>Aspergillus fumigates</i>	12mm
<i>Bacillus subtilis</i>	13mm	<i>Penicillium sp</i>	12mm
<i>Micrococcus sp</i>	08mm	<i>Trichophyton sp</i>	10mm
<i>Serratia sp</i>	05mm	<i>Microsporium sp</i>	08mm
<i>Shigella sp</i>	10mm	<i>Candida albicans</i>	14mm
		<i>Mucor sp</i>	10mm
		<i>Rhizopus sp</i>	10mm

## LIST OF CHARTS

**Figure: 1 OCCURRENCE OF BACTERIA IN HAIR COMBS****Figure: 2 OCCURRENCE OF FUNGI IN HAIR COMBS****CONCLUSION:**

This study further strengthens the earlier claim that beauty tools like hair combs may serve as potential vehicles for microbes, particularly fungal pathogens capable of causing hair infections. The microorganisms isolated from the hair comb can be inactivate by the disinfectant such as hydrogen peroxide, alcohol and detergent. Hydrogen peroxide shows high range of sensitivity against microorganisms. Candida plays the major role in the development of skin lesions, rashes, and dermatitis (Dadashi, & Dehghanzadeh, 2016). Because of the presence of these pathogens, we concluded that the current disinfection methods used in salons were not sufficient to prevent the health risks among clients

Microbial contamination among participants is caused by the use of none disinfection procedures. Equipment that comes into contact with the skin must be cleaned before re-use whether or not it looks dirty. Pathogenic fungi by nature are recalcitrant and difficult to treat. Treatment usually takes several weeks, and months and re-infection is common when antifungal therapy is stopped or discontinued. Once an individual is exposed to fungi infection, the propensity for re-occurrence to occur is high depending on the success of the initial treatment, the aggressiveness to cause infection and the resistance of the fungal pathogen to the available antifungal drugs, level of personal hygiene, the nutritional and immune status of the individual amongst others.

This shows that infections caused by these fungal pathogens are treatable by the available antifungal agents with varied levels of success rates. However, to prevent the development of resistance to the drugs, antifungal susceptibility testing using standard laboratory procedures must always precede prescription and medication.

Poor care and maintenance of hair combs including not removing hair strands from comb after use and not removing hair product or oil after use may as well favor fungal contamination and colonization of the hair combs examined. This is largely due to ignorance, negligence, laziness or procrastination on the part of the hair comb users. Poor personal hygiene amongst users of hair combs is a major factor contributing to the occurrence of fungal contaminants on the hair combs.

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## Conflicts of Interest

The authors have no conflicts of interest to publish this research article in this journal.

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