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Isolation and Diagnosis of Bacterial Species Found Under Artificial and Natural Long Fingernails in Women

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ABSTRACT

Background: Long, unclean nails may facilitate or serve as a conduit for bacterial spread. Even after thorough washing, bacteria may remain in the nail folds and under the nail.

Objectives: The purpose of the study was to identify pathogenic bacteria associated with short, long, natural, and artificial nails in women in Mosul. Additionally, the study aimed to assess the antibiotic susceptibility of the isolated bacteria.

Materials and Methods: Sterile dental swabs were used to collect 80 samples from 40 women in Mosul between November 2024 and February 2025.

Results: The study showed that all women (100%) were infected with bacteria. *Bacillus spp.* constituted 41.25% of the total, followed by *Staphylococcus aureus* (15%), *Escherichia coli* (13.75%), *Streptococcus* spp. (8.75%), *Klebsiella spp*. (5%), unidentified bacteria (5%), *Pseudomonas spp.* and *Shigella spp*. (3.75%), and *Salmonella spp*. (1.25%), which were the second most prevalent bacteria. The proportion of bacteria found on artificial nails was 65%, compared to 35% on natural nails. Higher bacterial counts (7×10^3) were observed on long nails (>2 cm) compared to short nails (<0.5 cm, 1.2×10^3). OfX, AZM, and AM were effective against *Bacillus spp.* and *Staphylococcus aureus*, while Gram-negative bacteria were more resistant.

Conclusions: These results suggested that nails may constitute a suitable environment for the proliferation of a number of bacteria and may contribute to the transmission of drug-resistant microorganisms.

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Introduction:

Unhygienic human practices cause a variety of infections via hands and nails [1]. Studies have shown that poor personal and household hygiene is associated with approximately 80% of diseases. Fecal contamination of hands is one of the most important ways through which the individuals are exposed to harmful bacteria, as nails can serve as a pathway for pathogens to enter food and cause hospital-acquired infections in patients [2, 3]. Individuals use their hands daily to perform a variety of tasks, making it relatively easy to come into contact with various bacteria and spread them to objects or people [4, 5, 6]. Over time, nails in humans have evolved from survival tools to decorative adornments [7]. The majority of bacteria found on human hands are found under the fingernails [8].

Due to the constant changes in the host's environment and surroundings, nails are more susceptible to the accumulation of various types of bacterial infections [9]. Compared to other areas of the hand, the area beneath the nails is the most contaminated and the most difficult to clean [10]. Nails are home to the majority of germs found on human hands [11]. The role of fecal-oral transmission from person to person is evident in that fingernail bacteria can be spread through food and water [12].

Bacteria carried by food handlers under their nails can contaminate food through their fingers during food preparation and serving and ultimately infect healthy individuals [13, 14]. Hands and nails are one of the primary routes through which children are exposed to harmful organisms, particularly through fecal contamination of their hands [15]. The condition of the hands and nails is also a variable influencing the microbiological effectiveness of hand hygiene [16]. Common nail-associated bacteria include Staphylococcus aureus, fungi such as Candida, and viral skin infections such as warts [17]. Studies indicate that artificial nails or nail tips may be colonized by pathogenic organisms, contributing to the spread of infection and the occurrence of epidemics [18]. Frequent use of nail polish and moisturizers to protect nails is an unresolved problem [19, 20]. According to the World Health Organization, improper hand hygiene practices are a major contributor to infectious diseases and healthcare-associated infections [21, 22]. Long nails are also more susceptible to infection [23, 24]. Artificial nails harbor more microorganisms and are more difficult to clean than natural nails [25]. Artificial nails are associated with the transmission of pathogens and fungi, and artificial nails and their enhancements are associated with poor hand hygiene practices [26]. A study by Wachukwu et al. 2007 [8] demonstrated that artificial nails can act as a conduit for bacteria and cause hospital-acquired infections in patients. *Staphylococcus aureus, Escherichia coli, Proteus spp.,* and *Pseudomonas aeruginosa* were isolated, with *Staphylococcus aureus* being the most common and widespread, followed by *Escherichia coli* [27]. Compared to natural nails, artificial nails were more susceptible to infections, particularly Gram-negative bacilli and yeast. Students are also at greater risk of contracting intestinal bacterial diseases due to ingesting food and liquids without washing their hands, which increases the risk of infection [28].Whether applied in a salon or at home, artificial nails can cause bacterial and fungal infections [29].

Acrylic nails have gained significant popularity, with approximately $265 million spent annually on artificial nails and related products in the United States [30]. The effectiveness of removing microorganisms from under nails is influenced by nail length and texture [31, 32]. Long polished nails typically retain more microbes after hand washing than short unpolished nails. Since artificial nails are typically longer than natural nails, wearing them may affect the quality of hand hygiene [33]. Studies indicated that artificial nails harbor higher numbers of bacteria than natural nails, making effective hand-washing techniques essential to reducing the spread of disease [34, 35]. Because they can host a wide variety of bacteria, nails are a significant health concern [36]. Infection can occur when bacteria from the area around the nails are transferred to the body [37]. However, the effect of nail polish on the growth of potentially harmful bacteria on the hands is not fully understood [38]. The American Academy of Dermatology indicated that 99% of people will experience a nail problem at some point in their lives [39]. Women often seek medical care for nail diseases, which may be related to age, the use of nail cosmetics, or difficulties with nail care [40, 41]. It has long been known that hand washing is an essential practice for preventing the spread of infectious diseases [42].

MATERIAS AND METHODS

The purpose of this study was to isolate and identify the bacteria present under artificial nails compared to natural nails. Given women's habit of wearing artificial nails and their typical nail length, women were selected for this study.

Data Collection:

A standardized questionnaire was used to collect information from participants. The questionnaire included questions about age, education, occupation, nail condition (natural or artificial), nail length, nail polish type (polish or non-polish), and dominant hand (left or right).

Sample Collection and Transport:

Using sterile dental swabs, 80 swab samples were randomly taken from 40 women. A swab was taken from under the nails of both hands for each participant. The samples were then stored in test tubes containing normal saline until culture (Figure 1). This study was conducted in Mosul City between November 2024 and February 2025.

Culture and Identification

To isolate bacteria, fingernail swabs were cultured on various selective media, including nutrient agar, MacConkey agar (Difco), mannitol salt agar, blood agar, and Salmonella-Shigella agar (Oxid). After culture, the plates were incubated at 37°C for 24–48 hours. Bacterial colonies grown on nutrient agar were presumptively identified using Gram staining and colony morphology, as well as a series of biochemical tests, including oxidase, catalase, Simon citrate, indole production, urease test, nitrate reduction test, and hydrogen sulphide (H_2S) production [43].

Antibiotic Susceptibility Testing:

Antibiotic susceptibility tests were performed using the disk diffusion method on Mueller-Hinton (Oxid) agar. As shown in Table 1, a set of commercial antibiotic disks was used in the study. To determine the resistance of the isolated bacteria, an antibiotic susceptibility test was performed, where samples were incubated for 24 hours at 37°C. Susceptibility results were interpreted according to the National Committee for Clinical Laboratory Standards [44].

STATISTICAL ANALYSIS

The independent t-test for continuous variables was used for statistical analysis. To compare categorical variables like nail length (long vs. short), the chi-square test was employed. Statistical significance was defined as a p-value of less than 0.05.

RESULTS

Microbial contamination under the nails has emerged as a major global health problem, with subungual microorganisms being one of the most common health problems today. Using sterile dental swabs, 80 swabs were taken from under the fingernails of 40 women's right and left hands. The data showed that the largest age group was between 21 and 30 years old (45%), followed by those between 31 and 40 years old (22.5%). In terms of educational attainment, housewives were the most prevalent occupational group (35%), while university graduates constituted the largest proportion of participants (50%). Regarding nail health, 80% of the individuals had long nails (over 2 cm), while 65% had artificial nails. Furthermore, as shown in Table 2, the majority of participants (90%) were right-handed, and 72.5% of them applied nail polish.

The results showed that the bacterial isolates formed round colonies with various colours and textures. Salmonella-Shigella agar showed colonies with different shapes, with black centres, wavy edges, and dry surfaces, while MacConkey agar colonies were colorless and non-lactose fermenters, while nutrient agar colonies were white and had a mucous surface (Table 3).

Bacillus spp. was the most common bacteria found under women's nails, accounting for 41.25% of the total (33 samples). *Staphylococcus aureus* was also isolated in 15% of cases, *Escherichia coli* in 13.75%, Streptococcus spp. in 8.75%, *Klebsiella spp.* in 5%, *Pseudomonas spp.* and *Shigella spp.* in 3.75% each, *Salmonella spp.* in 1.25%, and 5% of the isolated samples were unidentified bacterial species. A chi-square test showed a statistically significant difference ($P < 0.05$) between women with artificial nails and women with natural nails regarding the number of bacterial isolates. For example, *Bacillus spp.* was identified in 27 cases of artificial nails compared to only six cases in women with natural nails. The rate of bacterial contamination was significantly higher in women with artificial nails (65%) compared to women with natural nails (35%), as shown in Table 4.

The different bacterial species listed in the table exhibit a range of biochemical test results. For example, *Staphylococcus aureus* exhibits positive results only for the production of catalase, coagulase, and nitrate reductive activity, while Bacillus species exhibit positive results for the production of oxidase, catalase, and hydrogen sulphide. Gram-negative bacteria, such as *Salmonella spp., Pseudomonas spp.,* and *Klebsiella spp*., also differ in how they utilize citrate and produce hydrogen sulphide. These differences aid in the identification and classification of bacterial species (Table 5).

Artificial nails had higher levels of bacterial contamination than natural nails, with the difference between the two being statistically significant ($p < 0.05$) according to a T test, as shown in Figure 4. According to the study, the average number of bacterial colonies in long nail samples (>2 cm) was 7 × 103 CFU/ml, while the average number in short nail samples (≤0.5 cm) was 1.2×10^3 CFU/ml. The T test showed a highly significant difference ($P < 0.05$) between the two groups, as shown in Figure 5.

Nail polish may increase the likelihood of some bacteria multiplying, which can pose a health problem (Figure 6). Painted nails were most prevalent with *Bacillus spp*., *Staphylococcus aureus* , *Klebsiella spp*., and *Pseudomonas spp*., while unpainted nails had higher levels of *Streptococcus spp. Salmonella spp*., and *Shigella spp.,* were only present when nail polish was applied. Painted and unpainted nails showed a significant difference in bacterial levels, according to chi-square analysis ($P < 0.05$).

The disk diffusion agar method was used to perform antimicrobial susceptibility testing in order to ascertain the resistance to various antimicrobial medications. The growth (or lack thereof) in the area surrounding the disk is an indirect indicator of the antibiotic's ability to inhibit the organism (figure 7).

Fifeteen antimicrobial discs were employed, and the area was measured in millimetres, as indicated Table 6. The majority of bacteria, particularly *Klebsiella spp.* and *Pseudomonas* spp., demonstrated significant resistance to antibiotics, including ampicillin (AM), chloramphenicol (C), and spectinomycin (SP), according to the results. *Pseudomonas spp.* were the most resistant to almost all antibiotics except ciprofloxacin and meropenem, while *Klebsiella spp.* were mostly resistant to most antibiotics. Ofloxacin (OFX), azithromycin (AZM), and ciprofloxacin (CIP) were the most widely effective antibiotics, demonstrating activity against most species, particularly *Bacillus spp., Streptococci spp.,* and *E. coli.*

DISCUSSION

Despite careful hand washing, the areas under the nails may harbor more bacteria than the rest of the hand. Bacterial growth in nails can impair nail health, making them thin and brittle, making them a health concern [45]. Many pathogenic bacteria can be found on fingernails, and unclean nails can serve as a conduit for their spread to healthy individuals [46]. In daily life, nail hygiene has become increasingly important, and skilled medical practitioners are mindful of nail hygiene etiquette. Because nails are home to many bacterial pathogens, it is essential to keep them and the area under them clean. However, cleaning this area is more difficult than other parts of the body. Nails are also more susceptible to changes in host and environmental factors. Bacteria identified from nails have been considered important public health organisms [47]. *Bacillus spp., Staphylococcus aureus , Streptococcus spp., Klebsiella spp., Pseudomonas spp., Escherichia coli, Shigella spp., and Salmonella spp.* were among the bacteria isolated from nails. Research has shown that several species of bacteria can grow under nails, suggesting they are a potential source of ineradicable bacteria [44]. All women (100%) were found to have bacteria on their nails. Since intestinal bacteria are found in the gastrointestinal tract, their presence under the nails suggests a fecal infection. Compared to other intestinal bacteria, *Klebsiella spp.* have a higher survival rate on fingertips, possibly because they are part of the normal skin flora. The adaptability of human skin bacteria to a dry environment may also be a reason why Klebsiella multiplies better on fingertips than *E. coli* [48]. Klebsiella bacteria are not considered significant in foodborne or waterborne diseases [49]. According to Tambekar and Shirsat, significant bacterial growth was found in students' hand swabs, including *Escherichia coli, Pseudomonas aeruginosa, Proteus, Citrobacter spp., Klebsiella spp., Salmonella spp., Enterobacter,* and *Staphylococcus aureus [21].*

Tambekar *et al*., (2009) also isolated *Escherichia coli, Staphylococcus aureus, Enterobacter, Klebsiella, Enterococcus, Pseudomonas aeruginosa, Shigella, and Corynebacterium* from under fingernails longer than 1 mm. Students with short, properly trimmed fingernails were found to have 64% bacterial contamination, while those with long fingernails showed a higher incidence (67%) [9]. Since *Staphylococcus aureus produces enterotoxins* that cause food poisoning, its presence in students' fingernails could pose a significant health risk [50, 51]. According to a study by Risan [52], fingernails carried the highest level of *Bacillus spp.* infection. Ali *et al*. (45) also showed that males recorded the highest percentage of isolated bacteria (61%), while females recorded 10%. The predominant bacterial pathogens included *E. coli*, *Staphylococcus aureus*, *Pseudomonas spp*., *Enterobacter spp*., *Streptococcus spp*., *Proteus spp*., *Klebsiella spp*., *Micrococcus spp*., and *Citrobacter spp*. [53]. Microbial infection of artificial nails has also emerged in women as a global health concern, as artificial nails are frequently used to decorate nails or extend the length of natural nails [54].

More pathogens were recovered from artificial nail samples than from natural nails (65% vs. 35%; P < 0.005). Figure 4. According to one study, nurses with artificial nails had higher counts of Gram-negative bacteria before and after handwashing than those with natural nails. The higher counts of bacteria on artificial nails are attributed to nail length, as most artificial nails are longer than 2 mm. The properties of acrylic or the increased focus on the exposed surface during hand washing rather than the area beneath the nail may also be a factor. [55]. Healthcare workers with artificial nails were found to isolate pathogens from their hands more frequently than those with natural nails (92% vs. 62%). [56].

Compared with women in the control group, women wearing artificial nails had a higher chance of being infected with isolated pathogens [57, 58]. A shock blow or other forceful impact can lift the natural nail at the base and allow dirt and bacteria to enter the artificial nail. Failure to clean the reattached nail properly can also lead to the spread of bacteria and fungi from the inter-nail spaces to the original nail [59]. According to the results of the current and other research, people with artificial nails had higher levels of germs and pathogens than those with natural nails [60]. It should be noted that higher bacterial concentrations and difficulty cleaning artificial nails are associated with an increased risk of infection [61]. The results also showed that artificial nails recorded a higher frequency of isolated bacterial genera than natural nails. Salmonella was the least prevalent, while *Escherichia coli* was the most common

(12.7%) in both natural and artificial nails, followed by *Klebsiella spp.* (11.4%), [62]. The results of the present investigation also showed that short nails contained fewer bacteria than long nails (Figure 5). In today's world, nail care products such as acrylics and nail polish are becoming more popular, especially among young women. The area under the nails is the main place for germs to be found [63]. Long nails are more likely to harbor bacteria than short ones [64]. Nail length is directly associated with increased bacterial loads, and the World Health Organization recommends that nails should not exceed 0.5 cm in length [65]. Nail length should not exceed 2 mm, as its effect on the spread of bacteria is greater than that of nail polish or artificial nails [66, 67].

The results of our study showed differences in the growth and prevalence of specific bacterial species, such as *Bacillus spp*., *Klebsiella spp*., *Staphylococcus aureus,* and *Pseudomonas spp*., between painted and unpainted nails, with the latter being more prevalent on painted nails, suggesting that the polish may facilitate the growth of these organisms by trapping moisture or making cleaning difficult. These results emphasize the importance of practicing good nail hygiene, especially when applying nail polish, to reduce bacterial build up and associated health problems (Figure 6). Nail polish, also known as varnish or enamel, is used to beautify nails, and some brands may contain preservatives that may support bacterial growth. Using the same bottle in beauty salons also increases the likelihood of cross-infection between individuals [68].

Antibiotic susceptibility testing results (Table 6) showed varying responses among bacterial isolates. Gram-positive bacteria, such as *streptococci spp*., *Staphylococcus aureus*, and bacilli, showed significant resistance to chloramphenicol (C), clindamycin (CL), and sulfamethoxazole/trimethoprim (SP), but were susceptible to most other antibiotics, particularly ofloxacin (OFX), azithromycin (AZM), and amoxicillin (AM). Gram-negative bacteria, such as *Salmonella spp*., *Escherichia coli*, *Pseudomonas spp*., Shigella spp., and *Klebsiella spp.,* have shown more widespread resistance patterns to amoxicillin (AM), chloramphenicol (C), ceftriaxone (CRO), and sulfamethoxazole/trimethoprim (SP). Some isolates, including Shigella and Salmonella, have also shown significant susceptibility to azithromycin (AZM), meropenem (MEM), and ofloxacin (OFX). The most resistant isolate was discovered to be *Pseudomonas spp*., which exhibited resistance to practically every antibiotic examined except for limited susceptibility to meropenem (MEM) and ciprofloxacin (CIP). Given that resistance patterns differ greatly among bacterial spp., these results emphasized the significance of doing antibiotic susceptibility testing before beginning therapy. This bolsters the necessity of precise diagnosis and focused antibiotic treatment [69].

CONCLUSION

This study suggested that women's nails may harbor microorganisms that pose a public health risk, highlighting the importance of nail hygiene. The results showed that harmful germs accumulate under nails and require care to maintain good health, and artificial nails were more susceptible to infection.

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TABLES

Table 1: Types of antibiotics used and their concentrations.

Number	Full Name of Antibiotic	Abbreviation	Disk Concentration (µg)
1	Ofloxacin	OFX	5
2	Azithromycin	AZM	15
3	Ampicillin	AM	10
4	Chloramphenicol	C	30
5	Clindamycin	CL	2
6	Spiramycin	SP	15
7	Streptomycin	S	10
8	Trimethoprim	TMP	5
9	Ciprofloxacin	CIP	5
10	Ceftriaxone	CRO	30
11	Cefixime	CFM	5
12	Meropenem	MEM	10
13	Nitrofurantoin	F	300
14	Moxifloxacin	MOR	5
15	Neomycin	N	30

Table 2: data obtained by questionnaire.

Social and demographic characteristics	Frequency	Percentage (%)
Women age in year ≤20	7	17.5
21-30	18	45
31-40	9	22.5
41-50	6	15
Total 40		
Educational level		
Primary	6	15
Secondary	12	30
University graduates	20	50
uneducated	2	5
Job		
housewives	14	35
Beauty salon workers	11	27.5
healthcare workers	5	12.5
female employees	10	25
Finger nail status		
Natural	14	35
Artificial	26	65
Finger nail length		
Short than 0.5 cm	8	20
Long than 2 cm	32	80
Finger nail Polish		
Non-Polish	11	27.5
Polish	29	72.5
Dominant hand		
right	36	90
left	4	10
Total 40		

Table 3. Bacterial colonies morphological features on various culture media

Media	Form	Colour	Margin	surface
	Circular	White	Entire	Mucoid
MaCconky agar	Circular	Colourless colonies (lactose fermented)	smooth	Mucoid
Salmonella-shigalla agar	Circular	Colourless colonies with black centre	Wavy edges	Dry Non-mucous

Table 4. The proportion of microorganisms recovered from nail-substrate samples.

Bacterial isolates (spp.)	No	(%)	Natural nails	(%)	Artificial	(%)
Bacillus spp.	33	41.25	6	21.4	27	51.9
Staph. aureus	12	15	2	7.14	10	19.2

Streptococcus spp.	7	8.75	2	7.14	5	9.61
Klebsiella spp.	4	5	1	3.57	3	5.76
Pseudomonas spp.	3	3.75	1	3.57	2	3.84
Escherichia coli	11	13,75	2	7.14	9	17.3
Shigella spp.	3	3.75	0	0	3	5.76
Salmonella spp.	1	1.25	0	0	1	1.9
Unknown	4	5	1	3.57	3	5.76
Total	80	100	28	35	52	65

Table 5: Morphological and biochemical traits of the bacterial isolates found in samples taken under the nails.

Bacterial isolates (Spp.)	Gram stain	Shape	Indole Test,	Oxidase test	Catalase test,.	Citrate Utilization	Urease Test	Coagulase Test	Nitrate reduction test	hydrogen sulfide (H_2S) production
Bacillus spp.	+	Bacilli	-	+	+	+	-	-	+	+
Staphylococcus aureus	+	cocci	-	-	+	-	+	+	+	-
Streptococcus spp.	+	cocci	-	-	-	-	-	-	+	-
Klebsiella spp.	-	Bacilli	-	-	+	+	+	-	+	-
Pseudomonas spp.	-	Bacilli	-	+	+	+	-	-	+	+
Escherichia coli	-	Bacilli	+	-	+	-	-	-	-	+
Shigella spp.	-	Bacilli	+	-	+	-	-	-	+	-
Salmonella spp.	-	Bacilli	-	-	+	+	-	-	+	+

Table 6: Antibiotic susceptibility of bacteria isolated from nails.

Antibiotic	OFX	AZM	AM	C	CL	SP	S	TMP	CIP	CRO	CFM	MEM	F	MOR	N
Bacillus spp.	S	S	S	S	S	R	R	S	S	R	S	S	S	S	R
S. aureus	S	S	S	R	S	R	R	S	S	R	S	S	S	S	R
Streptococcus	S	S	S	R	S	S	R	S	S	R	S	S	S	S	R
Klebsiella	R	S	R	R	R	R	R	S	R	R	R	S	R	R	R
Pseudomonas	R	R	R	R	R	R	R	R	S	R	R	S	R	S	R
E. coli	S	S	R	R	R	R	R	R	S	R	R	S	S	S	R
Shigella spp.	S	S	R	R	R	R	R	R	S	R	S	S	R	S	R
Salmonella	S	S	R	R	R	R	S	S	S	R	S	S	R	S	R

S = Sensitive , R = Resistant

FIGURES

Figure 1: Using sterilized toothpicks to extract a sample from the subungual area of the fingernails.

Figure 2: Growth of bacteria isolated from nails on different culture media.

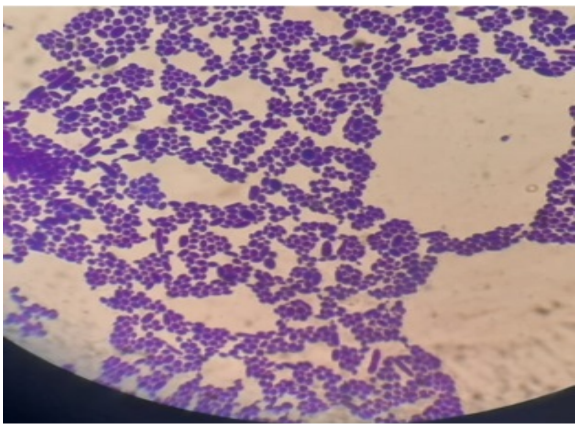

Figure 3: Microscopic examination of bacteria isolated from nails using Gram stain (100x).

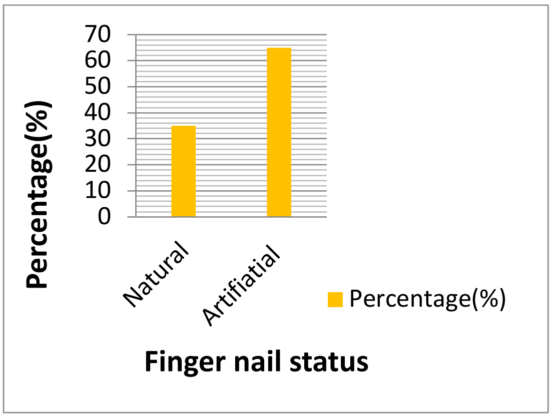

Figure 4: The percentage of bacteria in natural and artificial nails.

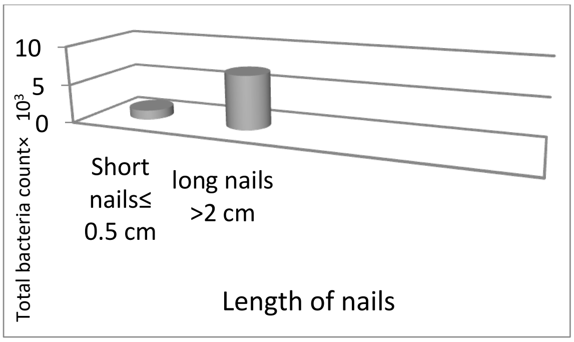

Figure 5: The total number of bacteria in both long and short nails.

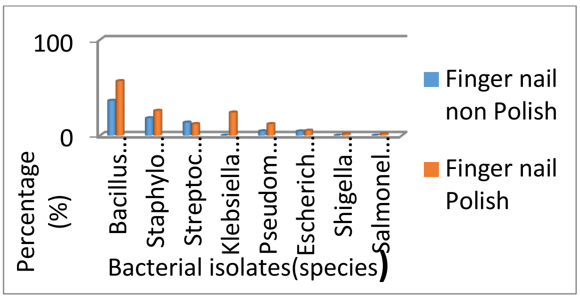

Figure 6: Bacterial species isolated from polished and non-polished nails

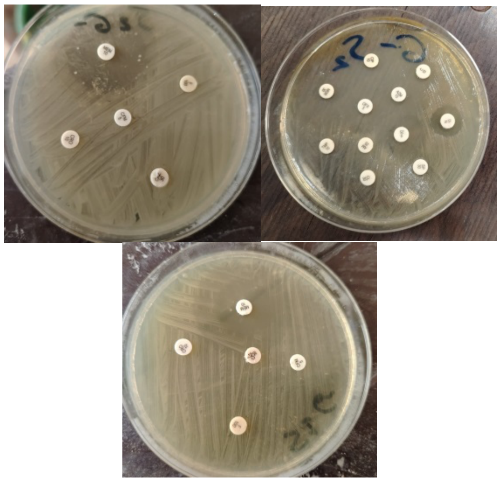

Figure 7: The antimicrobial susceptibility test plates