

MICROBIAL EVALUATION OF MANICURE AND PEDICURE SHOPS ALONG ADEWOLE ESTATE, ILORIN KWARA, NIGERIA

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Abstract: In this study, isolation and characterization of microorganisms associated with trimmed human nails were investigated. The trimmed nails were collected from three Manicure and Pedicure shops along Adewole Estate, Ilorin, Kwara State over a period of nine weeks. Microbial isolation was done using a standard serial dilution technique. The colonial morphology and biochemical study of the isolates indicated two gram positive cocci and three gram negative rod bacteria. These bacteria were *Staphylococcus aureus*, *Staphylococcus epidermis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterobacter* species. Fungi isolated were *Aspergillus flavus*, *Aspergillus fumigates*, *Alternaria* species, *Cladosporium* species, *Geotrichum candidum*, and *Rhizopus nigricans*. The study reveals that hygienic practices of both the attendants and the patrons at the manicure and pedicure shops may have not been adequate enough and hence both are advised to improve their hygienic level in order to prevent transmission of the associated diseases.

Keywords: Microorganisms, hygienic level, colonial morphology, manicure, pedicure.

INTRODUCTION

Human nails are one of the most fascinating and cosmetically important components of the skin. The nail plate has been described as a laminated structure microscopically layered as sheets of cells and macroscopically consisting of two principal strata, the dorsal and intermediate plates (Walters and Flynn, 1983). The function of the human nail is to assist in picking up small objects, to protect the distal digit, to improve fine-touch sensation and to enhance the

aesthetic appearance of the hands (Rich and Scher, 2003).

The component parts of the nail are collectively called the nail unit. The nail unit consists of the nail matrix, the nail bed (Bharat *et al.*, 2012), hyponychium, proximal and lateral nail folds. Anatomic structures of the nail include, from distal to proximal, hyponychium, onychodermal band, nail bed, nail plate, lateral nail folds, lunula, cuticle, nail matrix, and proximal nail fold (Rich and Scher, 2003).

The nail plate is made up of keratin protein, could be digested by fungus. This could be compromised by

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other infectious organisms and if left untreated, the nail plate may separate from the nail bed and crumble off (Bharat *et al.*, 2012). Onychomycosis, a fungal or yeast infection can invade through a tear in the proximal and lateral nail folds as well as the Eponychium which often result to nail plate separation.

Nail disorder comprises of approximately 10% of all dermatological conditions and affect a high percentage of the entire population. Various physiological diseases and disorders are associated with the ageing nails (Rich and Scher, 2003 and Draelos, 2000). A recent epidemiological survey by (Orton and Wilkinson, 2004) as cited by (Pelenita, 2006) revealed that about 23% of women experience some sort of adverse reaction to nail cosmetics products and the likes over a period of time which could be as a result of subjective sensory irritations. Causative products include deodorants and perfumes, skin care products, hair care products, and nail cosmetics (Orton and Wilkinson, 2004).

Complications associated with nail health are not only a resultant effect of chemicals in nail cosmetic products, but also with cosmetic tools used in nail shops (Pelenita, 2006). Improper sanitized cuticle cutters had been attributed to cause varying serious complications, ranging from an inflamed cuticle to hepatitis (Kurtzweil, 1995).

Kurtzweil (1995) cited in Pelenita, 2006) stated that dirty instruments also contribute to infection by blood borne diseases such as HIV or hepatitis. Also, unclean equipment is dangerous to broken nail area around the skin, which could allow the entrance of Infectious

agents. Dirt as well as bacteria and fungi can also grow in between the nails, if the nail is not properly cleaned. Bacterial and fungal infections are common problems associated with nails.

Nail bacterial and fungal infections are common causes of nail disorders (Szepietowski and Salomon, 2007; Rockwell, 2001). The prevention and management of these conditions require periodic cutting of the nails and appropriate medical care. Manicure is a cosmetic treatment for the fingernails performed at home or a saloon. It consists of filling, shaping of the free edge, treatment, massage of the hand and the application of polish. The condition of nails cannot be over emphasized in assessing the individual hygiene (Moossavi and Scher, 2001). In the light of this, the importance of personal cleanliness and aesthetic beauty of nails, we examine the microorganisms associated with nail samples obtained from manicure and pedicure shops along Adewole Estate, Ilorin, Kwara State, Nigeria.

MATERIALS AND METHODS

Sample Collection

Three Manicure and Pedicure shops along Adewole Estate, Ilorin, Kwara State were selected for nail sample collection for the duration of nine weeks. In each shop three samples were collected within the period. These were carefully transferred into sterile sample tubes, and immediately carried to the laboratory and aseptically stored prior to the evaluation.

Microbial Analysis

Culture media used were the Nutrient Agar, MaConkey Agar, *Salmonella Shigella* Agar, Sabouraud

Dextrose Agar (SDA) and peptone water. All media were prepared according to manufacturers' instructions. 5.0mg of nail samples was weighed and transferred into properly labelled test tubes. 5ml of sterilized peptone water was aseptically added to each sample and incubated overnight at 37°C. One millilitre (1 ml) each of overnight culture was transferred into 9 ml of freshly prepared sterile peptone water in a series of test tubes and each of the test tubes was diluted up to 10^{-3} (Fawole and Oso, 2001). About 0.1 ml from the each 10^{-3} aliquot was plated out on Nutrient agar, MaConkey agar, and *Salmonella Shigella* agar and incubated for 48 hours at 37°C in an inverted position. Also, 0.1 ml from the 10^{-2} aliquot was plated out on the Sabouraud Dextrose Agar (SDA) plates and the plates were incubated for 3 to 7 days at temperature 28°C in normal positions. All the plates were examined daily for possible bacterial and fungal growth. The colonial count was computed and recorded following the technique described by (Benson, 2002).

The isolates were subcultured repeatedly in order to obtain pure cultures. The colonial morphology was later examined and recorded and the pure cultures were stored on agar slants for proper maintenance and further experimentation. Biochemical analysis and microscopy were later carried out on each of the isolates following the

methods described by (Willey, et al., 2009; Fawole and Oso, 2001; Olutiola and Famurewa, 2000; Moore, 1990).

RESULTS

Bacteria isolated were *Staphylococcus aureus*, *Staphylococcus epidermis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterobacter* species. The characteristics of the bacterial isolate are presented in table 2. Fungal isolates were *Aspergillus flavus*, *Aspergillus fumigates*, *Alternaria* species, *Cladosporium* species, *Geotrichum candidum*, and *Rhizopus nigricans*. The results suggested that hygienic practices of the attendants in the manicure and pedicure shops were not adequate enough and the patrons need to engage in more hygienic practices by taking good care of their nails. The cfu/ml of the broth culture prepared from the nail samples is presented in table 1 and 3. Out of the selected shops, shop 3 had the highest number of bacterial colonies 16.4×10^4 cfu/ml, followed by shop 2 with 9.6×10^4 cfu/ml, while shop 1 exhibited the least number of bacterial colony 3.7×10^4 cfu/ml as shown in table 1. The number of bacterial colonies progressively increases weekly in shop 2 and 3. In table 3, shop 2 had the highest number of fungal colonies 20.1×10^3 cfu/ml, this was closely followed by shop 3 with 19.4×10^3 cfu/ml, while shop 1 showed the lowest colony number 2.6×10^3 cfu/ml.

Table 1: Bacterial Colony Count

S/N	Shop	Sampling	Average colony counts
1	Shop 1	Week 1	5.2 x 10 ⁴
2		Week 2	3.7 x 10 ⁴
3		Week 3	6.4 x 10 ⁴
4	Shop 2	Week 4	3.3 x 10 ⁴
5		Week 5	6.1 x 10 ⁴
6		Week 6	9.6 x 10 ⁴
7	Shop 3	Week 7	9.2 x 10 ⁴
8		Week 8	10.4 x 10 ⁴
9		Week 9	16.4 x 10 ⁴

Table 2: Characterization of Bacterial Isolates

Isolates	Shape	Gram Reaction	Spore Formation	Citrate Test	Catalase Test	Oxidase Test	Indole test	Sugar Fermentation test			Possible isolates
								Glucose	Monitol	Lactose	
A1	Cocci	+	-	+	+	-	-	+	-	+	<i>Staphylococcus epidermis</i>
A2	Rod	-	-	+	+	-	-	+	+	+	<i>Klebsiella sp.</i>
A3	Rod	-	-	+	+	+	-	+	+	-	<i>Pseudomonas aeruginosa</i>
A4	Cocci	+	-	-	-	-	-	+	+	-	<i>Staphylococcus aureus</i>
A5	Rod	-	-	+	-	-	-	+	+	+	<i>Enterobacter sp.</i>

KEYS: + = positive, - = negative

Table 3: Fungal Colony Count

S/N	Shop	Sampling	Average colony counts
1	Shop 1	Week 1	2.6 x 10 ³
2		Week 2	6.4 x 10 ³
3		Week 3	12.2 x 10 ³
4	Shop 2	Week 4	9.1 x 10 ³
5		Week 5	20.1 x 10 ³
6		Week 6	11.6 x 10 ³
7	Shop 3	Week 7	15.3 x 10 ³
8		Week 8	19.4 x 10 ³
9		Week 9	17.7 x 10 ³

Characterization and Identification of Fungal Isolates

The total number of fungi identified during the course of this study was six

and they include the following: *Alternaria sp.*, *Geotrichum candidum*, *Rhizopus nigricans*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigate*

and *Cladosporium* sp. The descriptions of the isolates are presented in the paragraphs below:

Isolate 1

Colonies matured within 3-4 days with the wooly growth on its surface which appeared as white at first, but later turned to gray then to brown and finally to dark green. The reverse side appeared black. Macroconidia were longitudinally and transversely septate, brown and produced in chains directly on the sides of the hyphal conidiophores. This isolate was designated as *Alternaria* sp.

Isolate 2

Colony appeared white, smooth often membranous, odour often sweet. Growth was thin, vigorous and waxy with radiating gray-white, cobwebby mycelia. Advancing hyphae septate, dichotomously branched (forked). Conidia were cylindrical, or barrel-shaped, mostly formed by breaking up fertile hyphae produced in chains with the youngest at the base. It was identified as *Geotrichum candidum*.

Isolate 3

Colonies matured within 3-4 days (rapid growth) which appeared cottony, dense, and filled the plate. The reverse was colourless, sporangioophores appeared in groups, collumella and sporangia were hemisphere. Rhizoids which developed properly appeared brown and endospores were of unequal shape size. This isolate was identified as *Rhizopus nigricans*.

Isolate 4

The fungus was slow-growing. Growth was velvety, folded, dark olive

green at first, but later turned gray, green and finally brown, reverse of the colony appeared black. Hyphae were dark and segmented; conidiophores were pigmented, branched or unbranched. Conidia were produced in chains, rough and single called scars referred to as disjunctors were observed at the attachment point. This identified the fungus as *Cladosporium* sp.

Isolate 5

The colony growth was fast, appeared rough, woolly, and yellowish-green at first, but later turned dark green with age. The reverse side changed from colourless to deep red-brown. Conidiophores were colourless and distinctly roughened, young vesicle and uniseriate. This isolate was identified as *Aspergillus flavus*.

Isolate 6

Colonies matured swiftly within 3-4 days. Early growth was white which turned blue-green and eventually gray, velvety in texture. The reverse appeared white to tan in colour. Conidiophores were short and smooth walled, vesicles flask-shaped and phialides were uniseriate and crowded together evenly. Conidial head and surface were globose and smooth, respectively. It was identified as *Aspergillus fumigates*.

DISCUSSION

Some of the microorganisms isolated in this study as presented in table 2 were reported by various authors to be pathogenic in nature. *Aspergillus* species have emerged as important causes of life-threatening infections, morbidity and mortality in immunocompromised patients as reported by (Denning 1998; Patterson et al., 2000; Marr et al., 2002; Walsh et al.,

2008). Walsh and Groll (2001), reported *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus terreus* among commonly recovered species from cases of invasive aspergillosis while some institutions may have a predominance of *A. flavus* or *A. terreus* as the most frequently recovered species of *Aspergillus* (Anaissie, 1992). Nilsson et al., (1998) stated that *Staphylococcus epidermis* were responsible for large wound infections, bloodstream infection, Catheter infections and meningitis infections. Alberti et al., (1996) reported that *Klebsiella* sp. was responsible for urinary infection and respiratory tract infections. Lederberg (2000), equally reported that *Pseudomonas aeruginosa* was responsible for bacteremia infection, bone and joint infection, urinary tract infection and gastrointestinal infections.

Considering the above stated, it becomes imperative that owners of manicure and pedicure shops and attendants should always maintain cleanliness. Patrons of these shops should also ensure that sterilized tool is used on their nails and feet. Dirt and bacteria can get trapped in nails that are not clean. Concerning the hands, nails are always carrier of microorganisms which can be passed from one person to another. Preventing the spread of bacteria will control the spread of illness and infections. Nails that are excessively long can by virtue of their length hold more dirt than shorter nails. Hangnails (loose skin near the base of the nail) should be carefully trimmed and kept clean in order to prevent infections.

The risk associated with artificial nails was reported by (Parry, et al., 2001 and McNeil et al., 2005) which strictly state the potential hazards to patients that might result from acrylic nails worn

by health care workers and 41 health care workers used to compare the reduction of microbial colonization, by either antimicrobial soap or alcohol based gel respectively were cited by (Pelenita, 2006).

A similar infectious pathogen *Mycobacterium fortuitum* was reported in an outbreak of furunculosis, a disease associated with rapidly growing bacteria occurred in salons, in the year 2000. The researchers studied infected customers who had used whirlpool footbaths at a nail salon (Vugia, et al., 2005 and Pelenita, 2006). Mycobacteria may pose an infectious risk for pedicure customers (Vugia et al., 2005). Getting a manicure or pedicure can break the skin, therefore creating openings that allow contagious germs to enter and infect the body (Kurtzweil, 1995).

CONCLUSION

The owners of manicure and pedicure shops and the attendants should always maintain absolute cleanness to avoid cross contamination among the patrons as observed in shop 2 and 3 in table 1. Patrons of these shops which are majorly adults should also protect themselves and should always engage in good hygiene practice. The idea of regulatory bodies to checkmate the manicure and pedicure shops in conforming to standards should be encouraged.

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