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Antibiogram of Bacteria and Fungi Associated with Local Pedicure Equipment Used in Port Harcourt Metropolis

Ogbonna, S. I[1]*., Oparaodu, U. A.[2] and Barika, P. N[3].
[1&3]Department of Microbiology, Rivers State University,
P.M.B. 5080, Port Harcourt, Nigeria.
[2]Department of Ear, Nose and Throat Surgery.
Faculty of Clinical Sciences, College of Medical Sciences,
Rivers State University, P.M.B. 5080, Port Harcourt, Nigeria
***Corresponding Author:** *solomon.ogbonna@ust.edu.ng*

ABSTRACT

Local or traditional pedicure is a personal or unprofessional cosmetic treatment of the feet and toenails in Nigeria. Local pedicure is fairly cheap and unlike salon pedicures though posing more risk on its patrons. Local pedicure equipment like scissors and razors has been reported as sources of varieties of infections and diseases. This study assesses the presence of microorganisms in local pedicure equipment used in Port Harcourt. Swab samples were aseptically collected from the cutting edge of scissors and razors at three different locations. Organisms were enumerated and identified using standard microbiological techniques. Kirby Bauer disc method was used for antibiotic sensitivity. Total heterotrophic bacterial counts ranged from 3.39±1.58 to 5.05±3.17x10^6CFU/ml for Razor and Scissor 1 while total fungal counts ranged from 3.10±2.68 to 4.30±2.82x10^5CFU/ml for Razor and Scissor 2 respectively with no significant difference ($p \geq 0.05$). Bacterial isolates identified and their occurrence (%) were; *Escherichia coli* (7.1%), *Pseudomonas* sp. (7.1%), *Staphylococcus* sp. (64.4%), *Bacillus* sp. (21.4%). While fungal isolates were; *Candida albicans* (28.6%), *Trichophyton* sp. (14.3%), *Penicillium* sp. (14.3%), *Mucor* sp. (28.6%), *Aspergillus* sp (14.2%). Sensitivity results showed that *Staphylococcus* sp. was more susceptible to Ampiclox, Streptomycin and Rifampicin and showed more resistance to Zinnacef. Amoxicilin and Rocephin. *Pseudomonas* sp. was more susceptible to Tarvid, Reflacin, Gentamycin, Ciprofloxacin, Septrin, Steptomycin, and Amplicin but was resistant to Nalidixic acid and Ceporex. *Bacillus* sp. was more susceptible to Levofloxacin, Rifampicin, Streptomycin and Gentamicin, and resistant to Amoxicilin, Septrin, Erythromycin and Reflacin. *Escherichia coli* was susceptible to Tarvid, Gentamicin and was resistant to Ceporex, Reflacin and Augmentin. The presence of these antibiotic resistant organisms on pedicure equipment poses public health risks. It is recommended to properly sanitize and sterilize these equipments before and after use to prevent a public health outbreak.

Keywords: Traditional pedicure, feet and toenails, scissors and razors, microorganisms, antibiotic resistance.

Introduction

Pedicure services have gained significant popularity in recent years, offering relaxation and aesthetic benefits to individuals seeking foot and nail care. However, the shared use of pedicure equipment in salons and spas has raised concerns about the potential transmission of pathogenic microorganisms, including bacteria and fungi, from one client to another. These microorganisms can colonize and persist on pedicure equipment, posing a risk to the health and well-being of clients, particularly in regions with high population densities such as Port Harcourt Metropolis (Mbajiuka *et al*., 2015).

The determination of bacterial and fungal isolates from local pedicure equipment in Port Harcourt Metropolis is crucial for several reasons. First, the presence of pathogenic microorganisms on pedicure equipment can lead to skin, nail, and soft tissue infections, making it imperative to identify and mitigate these risks. Second, understanding the microbial communities on these surfaces can provide insights into the efficacy of current cleaning and disinfection practices in local salons and spas. Third, investigating the prevalence and diversity of microbial isolates can aid in the development of guidelines and best practices for the safe operation of pedicure services in the region (Mbajiuka *et al*., 2015).

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Several studies have highlighted the importance of assessing microbial contamination in salon and spa settings. For instance, a study conducted by Roberts *et al*. (2017) found that pedicure equipment, such as foot baths and nail files, can harbor a diverse range of bacteria, including potential pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Additionally, fungal species, particularly dermatophytes like *Trichophyton* spp., have been implicated in salon-related infections (Monod *et al*., 2014).

In Port Harcourt Metropolis, where the demand for pedicure services is on the increase, and the climate can foster microbial growth, investigating the microbial flora on pedicure equipment is essential for public health and safety. This study aims to determine the bacterial and fungal isolates present on local pedicure equipment within Port Harcourt metropolis. By shedding light on the microbial ecology of these surfaces, this research seeks to contribute valuable insights to the enhancement of pedicure service quality and the reduction of associated health risks in Port Harcourt Metropolis. The determination of bacterial and fungal isolates from local pedicure equipment in Port Harcourt Metropolis is a critical undertaking with implications for public health and safety. This research will build upon existing knowledge and provide valuable data for the development of guidelines and interventions aimed at ensuring the hygienic operation of pedicure services in the region.

Materials and Methods

Description of Study Area

The study was carried out in Mile 3, Mile 1 and Town in Port Harcourt, Rivers State, Nigeria. The local pedicure samples were obtained from three different locations: The location coordinates of the study areas are as follows; Mile 3, 4^{o}48’19” N 6^{o}58’29” E; Mile 1, 4^{o}47’19” N 7^{o}2’7” E and Town 4^{o}45’43” N 7^{o}1’36”E

Sample Collection

Samples were collected randomly from the different pedicure equipment at the different locations. The samples were collected using a sterile swab stick (which had been moistened with sterile normal saline) by swabbing the cutting edge of the scissors and razor. After which, samples were labelled accordingly and transported in ice pack container to the microbiology laboratory of the Rivers State University for microbiological analysis.

Sample Preparation

The two-fold serial dilution was adopted. In this method, a sterile swab was moistened normal saline and used to swab the cutting edge of the scissors and razor samples, and transferred into test tubes containing sterile 2ml diluent (normal saline) which gave an initial diluent of 1:2ml. Subsequent dilutions were carried out by transferring 1ml from the initial dilution to other test tubes containing 1ml sterile diluent. This was repeated until a dilution of $1:2^{-4}$ was reached (Ogbonna *et al*., 2022)

Enumeration of Bacteria and Fungi

The total heterotrophic bacterial and fungal load on the different equipment was enumerated using standard plate count (Prescott *et al*., 2011). After the serial dilutions, aliquot of 2^{-3} and 2^{-4} dilutions were transferred in duplicates into prepared Nutrient agar and Mannitol Salt agar, aliquot of 2^{-2} and 2^{-4} were transferred in duplicate on prepared Sabouraud Dextrose agar which had been fortified with tetracyclin antibiotics for the inhibition of bacterial growth (Ogbonna *et al*., 2022). Plates were incubated at 37^{o}C for 24 – 48 hours for the Nutrient Agar and Mannitol Salt Agar. Sabouraud Dextrose Agar was incubated 37^{o}C for 5 days.

After incubation, plates were observed for microbial growth and ensuing colonies on Nutrient Agar, Mannitol Salt Agar and Sabouraud Dextrose Agar plate were counted and discrete colonies were sub-cultured onto sterile nutrient agar for colonies on Nutrient Agar, Mannitol Salt Agar and Sabouraud Dextrose Agar plates to obtain pure isolates (Cheesebrough, 2000).

Identification of Bacterial and Fungal Isolates

The pure bacteria isolates were characterized by Gram's staining and Biochemical tests such as catalase test, indole test, methyl red test, citrate test, coagulase test, Voges Proskauer test and sugar fermentation tests. Identity of the isolates was matched with the Bergey's Manual of Determinative Bacteriology and ABIS online for confirmation.

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Fungal Isolates were identified using their morphological features such as colony color, shape, texture and size of colony followed by microscopic examination (conidial shape, arrangement of hyphae and type of spore) of their wet mounts prepared with lactophenol cotton blue. The results were later compared by referencing fungal characteristics in the book of fungi identification manual (Sarah *et al*., 2016).

Antibiotics Sensitivity Test

The disk diffusion method of antibiotics testing according to the Clinical laboratory standard institute was adopted. Twenty-four (24) hours old cultures was standardized using the 0.5 McFarland standard (CLSI, 2020). This was done by matching the turbidity of the isolates in sterile 4mL normal saline to the 0.5McFarland standard. Sterile swab sticks were then dipped into the standardized isolates and swabbed uniformly on the surface of the dried Mueller-Hinton agar plates. The bacterial isolates were tested against already prepared commercial antibiotics: Ciproflox (10µg), Augmentin (30µg), Tarivid (10µg), Streptomycin (30µg), Reflacine (10µg) Nalididixic Acid (30µg), Ceporex (10µg), Septrin (30µg), Norfloxacin (10µg), Levofloxacin (20µg), Ampiclox (20µg) Chloramphenicol (30µg), Amoxil (20µg), Rifampicin (20µg), Erythromycin (30µg) and Ampicilin (30µg).

The plates were held at room temperature for 3-5mins to allow drying. The antibiotics discs were placed on the plates and the plates were incubated for 18-24 hours at 37ºC. The diameters of zone of inhibition were recorded to millimeter and classified as resistant (R), intermediate (I) and susceptible (S) according to published interpretive chart (CLSI, 2020).

Results

Results of the microbial counts from the local pedicure equipment are presented in Table 1. Results of the total heterotrophic bacterial counts ranged from 3.39±1.58 to 5.05±3.17x10^6CFU/ml for Razor and Scissor1 and the total fungal counts ranged from 3.10±2.68 to 4.30±2.82x10^5CFU/ml for Razor and Scissor2 respectively. Results also showed that the microbial counts in the different equipment varied. Despite the disparity in the total heterotrophic bacterial and fungal counts in the various pedicure equipment, there was no significant differences recorded (P≥0.05). Identified bacterial isolates associated with the different local pedicure equipment include; *Bacillus* sp., *Staphylococcus* sp., *Pseudomonas* sp. and *Escherichia coli*. The fungal isolates were *Candida albicans*, *Mucor* sp., *Aspergillus* sp., *Penicillum* sp., *Aspergillus niger*, *Aspergillus flavus*, and *Trichophyton rubrum*.

Table 1: Microbial Population (CFU/ml) of the Local Pedicure Equipment

Samples	Total heterotrophic bacterial (×10^6CFU/ml)	Total fungal (×10^5CFU/ml)
Razor	3.39±1.58	3.10±2.68
Scissors 1	5.05±3.17	4.25±2.89
Scissors 2	4.10±2.68	4.30±2.82
P-value	0.863	0.930

*T-test showed that there was no significant difference (p≥0.05)

Results showing the percentage distribution of bacterial isolates from the local pedicure equipment is presented in Fig. 1 and are as follows; *Bacillus* sp., (21.4%), *Staphylococcus* sp., (64.4%), *Pseudomonas* sp.(7.1%) and *Escherichia coli* (7.1%). Results show that *Staphylococcus* sp. was the most distributed bacterial isolate as its prevalence cut across all the equipment under study while *Pseudomonas* sp. and *Escherichia coli* were the least distributed isolates within the study locations.

The percentage distribution of fungal isolates is presented in Fig. 2. *Candida albicans* (28.6%) and *Mucor* sp. (28.6%) were the most predominant fungal isolates followed by *Trichophyton* sp. (14.3), *Penicillium* sp. (14.3%), and *Aspergillus* spp. (14.2%).

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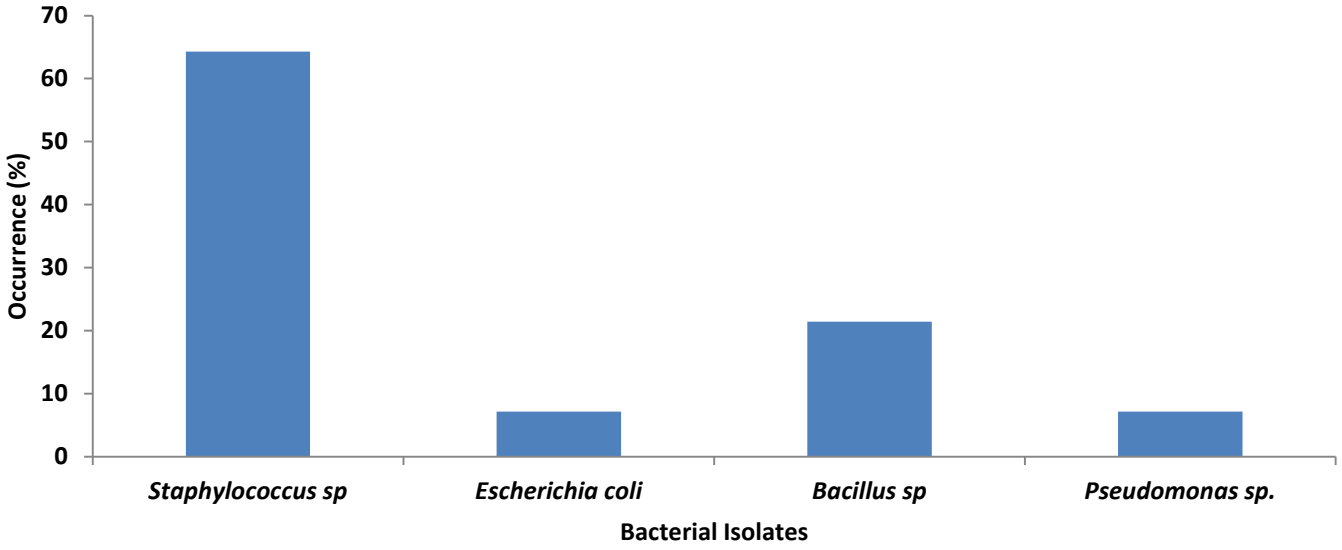

Fig. 1 Percentage Occurrence of Bacterial Isolates from all Samples

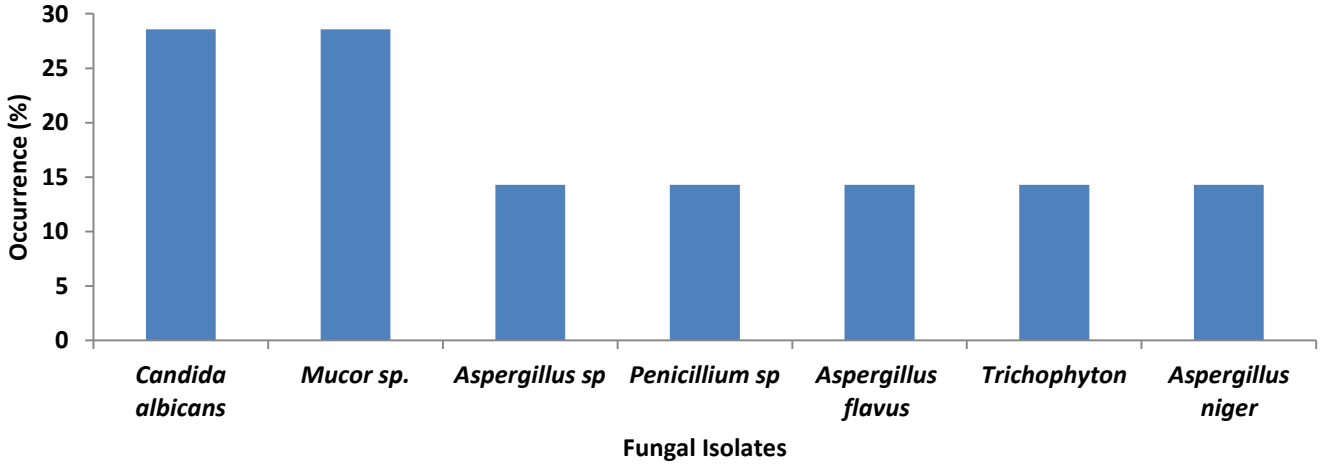

Fig. 2: Percentage Occurrence of Fungi Isolates from the Samples

Results of the susceptibility pattern of Gram-positive and Gram-negative bacteria isolates are presented in Tables 2 and 3 respectively.

Results showed that *Bacillus* sp, was sensitive to Streptomycin, Rifampicin and Levofloxacin (100%), while being highly resistant (100%), to Septrin, Erythromycin, Reflacin and Amoxicillin. *Staphylococcus* sp was susceptible to Ampiclox and Streptomycin was 66.7%, while being resistant (100%), to Zinnacef, Amoxicillin.

Results of the antibiotics susceptibility pattern of gram-negative bacterial isolates showed that the percentage susceptibility pattern of *Pseudomonas* sp to Tarvid, Reflacin, Gentamicin, Ciprofloxacin, Septrin, Streptomycin and Ampicilin was 100%, respectively.

While being resistant (100%), to Nalidixic acid and Ceporex respectively. The *Escherichia coli* isolate was 100% susceptible to Tarvid, Reflacin, Gentamicin, Ciprofloxacin, Septrin and Streptomycin while being resistant Nalidixic acid, Augmentin and Ceporex.

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Table 2: Susceptibility Pattern of *Bacillus* spp and *Staphylococcus* spp from Local Pedicure Equipment

Antibiotics/ Conc	*Bacillus* spp			*Staphylococcus* spp		
	R n (%)	I n(%)	S n(%)	R n(%)	I n(%)	S n(%)
Septrin 30µg	3(100)	0(0.00)	0(0.00)	8(88.9)	1(11.1)	0 (0.00)
Erythromycin 30µg	3(100)	0(0.00)	0(0.00)	8(88.9)	0(0.00)	1(11.1)
Reflacin 10µg	3(100)	0(0.00)	0(0.00)	5(55.5)	0(0.00)	4(44.4)
Gentamicin 10µg	0(0.00)	1(33.3)	2(66.7)	8(88.9)	0(0.00)	1(11.1)
Ampiclox 20µg	0(0.00)	3(100)	0(0.00)	3(33.3)	0(0.00)	6(66.7)
Zinnacef 20µg	0(0.00)	3(100)	0(0.00)	9(100)	0(0.00)	0(0.00)
Amoxicillin 30µg	3(100)	0(0.00)	0(0.00)	9(100)	0(0.00)	0(0.00)
Rocephin 25µg	1(33.3)	0(0.00)	2(66.7)	9(100)	0(0.00)	0(0.00)
Ciprofloxacin 10µg	2(66.7)	0(0.00)	1(33.3)	8(88.9)	1(11.1)	0(0.00)
Streptomycin 30µg	0(0.00)	0(0.00)	3(100)	3(33.3)	0(0.00)	6(66.7)
Rifampicin 20µg	0(0.00)	0(0.00)	3(100)	0(0.00)	3(33.3)	6(66.7)
Levofloxacin 20µg	0(0.00)	0(0.00	3(100)	0(0.00)	4(44.4)	5(55.5)

Table 3: Susceptibility Pattern of *Pseudomonas* spp and *Escherichia coli* from Local Pedicure Equipment

Antibiotics	*Pseudomonas* sp.			*Escherichia coli*		
	R n(%)	I n(%)	S n(%)	R n(%)	I n(%)	S n(%)
Tarvid (10µg)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)
Nalidixic acid (30µg)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)
Reflacin (10µg)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)
Gentamycin 10µg	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)
Augumentin 30µg	0(0.00)	1(100)	0(0.00)	1(100)	0(0.00)	0(0.00)
Ciprofloxacin 10µg	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)
Septrin 30µg	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)
Streptomycin 30µg	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)
Amplicin 10µg	0(0.00)	0(0.00)	1(100)	0(0.00)	1(100)	0(0.00)
Ceporex 10µg	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)

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Discussion

Local pedicure is very cheap and affordable; people tend to patronize them more without considering the health implications and can also be a vehicle in the transmission of some life threatening diseases (Redbord *et al*., 2006).

The microbial population was higher in the scissors than the razor from the various location this could be due to non-sterilization of the equipment and contamination may be due to the dirt in the hands of the pedicurist or by the mishandling of the tool, as well as the environment of these local pedicurist. Lack of knowledge among technicians and the public about infection prevention, absence of proper sanitation practices in pedicure establishments, and shaving one's legs prior to a pedicure have been cited as risk factors for infections. This corroborate with the work of Trevino and Weissfeld. (2006). Ogbonna *et al*. (2022) also recorded higher counts in salon equipments.

Staphylococcus sp., *Bacillus* sp., *Candida albicans*, *Trichophyton* sp., *Mucor* sp. and *Aspergillus* sp. were the most predominant bacteria and fungi in this study. This could be due to the reuse of contaminated and disposable instruments, the inadequate disinfection of equipment, and the inadequate management of blood or body fluid exposure. The increase in the potential of spreading of the microorganisms is becoming a concern to the public (Johnson *et al*., 2001). Procedure-related problems include trauma to the skin, cuticles, or the protective seal around the nail plate. These lesions can act as a portal through which bacteria can enter the body. Inadequate sterilization of tools is common and can also lead to transfer of the bacteria.

The organisms where resistant to Nalidixic acid, Septrin, Augmentin and Ceporex while being sensitive to Streptomycin, Gentamicin, and Ciprofloxacin.

The bacterial isolates demonstrated varying susceptibility to the antibiotics which should be one of the major reasons to maintain sanitary and hygienic conditions in pedicure equipment. This is in agreement with the work of Zahraa *et al*. (2020) who reported that *Staphylococcus*, *Pseudomonas* and *Bacillus* were sensitive to Gentamicin and resistant to Augmentin, and Septrin.

In conclusion, this study showed high microbial load in the local pedicure equipment and presence of potentially pathogenic bacteria and fungi which were more resistant to multiple antibiotics but sensitive to Streptomycin, Gentamicin, Ciprofloxacin. Increased public awareness of pedicure infection risks is needed to prevent such infections from occurring.

More specifically, local pedicure clients should be strongly discouraged from shaving their legs prior to this service as it has been identified as a frequent risk for infection. Information on what to observe during procedures such as the cleaning and disinfection of footbaths and tools will help the public make informed choices to protect their health.

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