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Isolation and Antibiogram of bacteria and fungi associated with tattoo equipment used in port Harcourt metropolis

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Abstract
A tattoo is a form of body modification made by using tools and equipment to insert ink, dyes and or pigments, either indelible or temporary into the dermis layer of the skin to form a design. This research was carried out to investigate the microorganisms associated with tattoo equipment used in Port Harcourt and to determine the antibiotics susceptibility of the bacterial isolates. A total of five swab samples from tattoo equipment within Port Harcourt metropolis were aseptically collected and cultured on appropriate Agar medium such as nutrient Agar, Mannitol salt Agar, Sabouraud dextrose Agar, using spread plate technique. The isolates were identified and characterized based on morphological and biochemical characteristics using standard tests. Results showed that the ranges of the total heterotrophic bacterial, *Staphylococcal* and fungal counts were 1.4×10^5 to 4.1×10^5, 1.3×10^3 to 5.0×10^3 and 0.0 to 1.6×10^3, respectively. Despite the disparity in the total heterotrophic bacterial and *Staphylococcal* counts in the tattoo equipment, no significant difference was recorded ($P>0.05$), but there was a significant difference ($P<0.05$) in the fungal count from tattoo equipment. Fifteen (15) bacteria species belonging to seven (7) genera were identified and include *Bacillus* sp. (7%), *Proteus* sp. (20%), *Staphylococcus* sp. (13%), *Micrococcus* sp. (7%), *Enterococcus* sp. (27%), *Streptococcus* sp. (13%) and *Pseudomonas* sp. (13%). The fungal isolates (7), were *Aspergillus* sp.(22.5%), *Mucor* sp. (12.5%) *Penicillium* sp. (18%), *Trichoderma* sp. (10%), *Rhizopus* sp. (17%), *Candida* sp. (12%) and *Microsporum* sp. (8%). The distribution of bacterial isolates showed that *Bacillus* sp. was the most distributed bacterial isolate while *Streptococcus* sp. and *Micrococcus* sp. were the least distributed isolates. *Aspergillus* sp was the most predominant fungal isolate followed by *Penicillium and Rhizopus* sp while *Microsporum* sp was the least fungal isolates from the tattoo equipment. Sensitivity results showed that Ciprofloxacin, gentamycin, levofloxacin and streptomycin were the most effective antibiotics against the isolated bacteria. Proper sterilization and good hygiene are recommended for both spa owners and patrons, to prevent any form of infection during tattooing.

Keywords: Tattoo equipment, antibiotic resistance, bacterial and fungal pathogens

Introduction
A tattoo refers to implantation of an exogenous pigment into the skin or mucous membranes (Isaacs *et al*., 2018) [11]. Graudenz *et al.* (2003) [7] stated that the term tattooing refers to a process of implantation of permanent pigment granules in the skin and the word was derived from “tattau”, a Tahitian word which means “to mark”. For thousands of years, cultures all over the world have used tattoos as a form of body modification and art to leave their mark on the human body. In recent years, tattoos have also commonly been mechanically etched onto the flesh in addition to being hand-drawn (Ojeda *et al.*, 2023) [18]. Tattooing may be done on purpose for cosmetic (decorative tattoos and permanent makeup), therapeutic (medical tattoos), or inadvertently (traumatic tattoos) to treat skin injuries that have been abraded. The popularity of tattooing among teenagers and young adults as a kind of aesthetic and decorative body art has dramatically increased recently, despite the fact that it is an ancient technique. The use of it in "permanent makeup" by salons and spas is another instance. Medical therapeutic tattooing has been used to conceal scars after plastic and reconstructive surgery, vitiligo scars, permanent hair loss after craniofacial surgery, and breast reconstruction after cancer surgery (Khunger *et al*., 2015) [13]. In a previous study, the total prevalence of10–20% of adults in industrialized countries are known to have tattoos (Kluger, 2015) [15]. According to the International Classification of Procedures in Medicine (ICPM), getting a tattoo equates to having surgery, which has its own Operations and Procedures (OPS) code number (5–890.0; see OPS version 2015).

Yet because medical professionals hardly perform the tattoos, their epidemiological monitoring cannot be done with the aid of medical data systems (Dieckmann *et al.*, 2016) [6]. Since the process of tattooing requires the use of equipment and inks in making the designs on skin surfaces, there could arise complication which may orchestrate infection especially since the skin represents the first line of immunity is broken. According to a recent classification provided by Serup *et al*. (2016) [22], tattoo complications can be divided into five categories: primary infections, non-infectious inflammatory (allergic, non-allergic, urticaria), psychological, technique and treatment associated, and lots more. It was also reported that infectious problems are a result of poor procedure including personal cleanliness and that tattoos can get complicated by fungus, viruses, and bacteria. Both superficial and deep skin infections caused by bacteria are possible. Impetigo and ecthyma, which are brought on by unsterile equipment, commonly appear at the tattoo site in the first few days following tattooing (Isaacs *et al.*, 2018) [11]. The use of contaminated tattoo ink, inadequate disinfection of the skin area to be tattooed to avoid resident flora from entering the skinduring tattooing including pruritus and burning developed by patients after tattooing are origins of microbial contamination often develop (Klügl *et al.*, 2010) [14]. More so, scratching of the tattooed area could orchestrate microbial colonization and therefore increase the risk of superinfection of the tattooed skin area (Wenzel *et al.*, 2013) [25]. In Port Harcourt and other part of Rivers State, many shops/ spa carryout tattoo services. Many of these spas are not regulated or supervised and with the increased demand for tattoo, there is need to investigate the microbial contaminants in the equipment. Furthermore, there is dearth of information on microorganisms associated with tattoo equipment in Rivers State. Thus, this study therefore seeks to investigate the bacterial and fungal isolates in tattoo equipment as well as the antibiotic susceptibility pattern of bacteria isolates. This study would no doubt improve awareness of complications that could result in tattooing especially if adequate care is not given when tattooing and after tattoing.

Materials and Methods

Description of Study Area

Salon/SPA shops within the Obio-Akpor and Port Harcourt City local government area that carry out tattoo services were considered. Tattoo equipment from five salon/SPA shops were investigated. The five salons were selected amongst other areas based on the high patronage.

Sample Collection

Samples were collected from different tattoo equipment from the different tattoo parlours. The samples were collected using a sterile swab stick (which had been moistened with sterile normal saline) by swabbing the tip of tattoo machines that comes in direct contact with the skin of customers. After which, samples were labelled accordingly and transported in ice pack container to the microbiology laboratory, Rivers State University, for microbiological analysis.

Enumeration of Bacteria and Fungi from Tattoo Equipment

The total heterotrophic bacterial, *Staphylococcal* and fungal load on the different tattoo equipment were enumerated using standard plate count (Prescott *et al.*, 2011) [20]. In this method, the swab samples were immersed in 9mL sterile normal saline. Ten-fold serial dilution was later carried out by transferring 1mL from the initial stock with the aid of sterile 1mL pipette into test tubes containing sterile 9mL normal saline. This was repeated to obtain dilutions of $1:10^{-4}$. After which, aliquot from the 10^{-1} dilution was aseptically inoculated at the centre of well dried mannitol salt agar and Sabouraud dextrose agar plates in duplicates for enumeration of *Staphylococcus* and fungi, respectively. While aliquot from the 10^{-3} dilution was inoculated on nutrient agar plate in duplicates for enumeration of the total heterotrophic bacteria.

Identification of Bacterial Isolates

The colonies were subcultured to obtain pure isolates. The pure isolates were then 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 Bergy's Manual of Determinative Bacteriology for confirmation.

Identification of Fungal Isolates

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) [21].

Antibiotics sensitivity test

The disk diffusion method of antibiotics testing according to the Clinical laboratory standard institute. First the isolates (24 hours old) were 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. After which, sterile swab sticks were 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 (10 µg), Augmentin (30 µg), Tarivid (10 µg), Streptomycin (30 µg), Reflacine (10 µg) Nalididixic Acid (30 µg), Ceporex (10 µg), Septrin (30 µg), Norfloxacin (10 µg), Levofloxacin (20 µg), Ampiclox (20 µg) Chloramphenicol (30 µg), Amoxil (20 µg), Rifampicin (20 µg), Erythromycin (30 µg) and Ampicilin (30 µg). 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 of the various tattoo equipment from five different SPA is presented in Table1. Results showed that the ranges of the total heterotrophic bacteria, *Staphylococcal* and fungal counts were 1.4×10^5 to 4.1×10^5, 1.3×10^3 to 5.0×10^3 and 0.0 to 1.6×10^3, respectively. Results further showed that the microbial counts in the different tattoo equipment varied. Despite the disparity in the total heterotrophic bacterial and *Staphylococcal* counts in the tattoo equipment, there was no significant differences recorded ($P>0.05$) while there was a

significant difference (P<0.05) in the fungal count of tattoo equipment especially with saloon equipment in shop 5 showing no fungal contaminant. The bacterial isolates associated with the different tattoo equipment were *Bacillus* sp, *Enterobacter* sp, *Proteus* sp, coagulase positive and negative *Staphylococcus* sp, *Micrococcus* sp, *Enterococcus* sp, *Streptococcus* sp and *Pseudomonas* sp. The fungal isolates were *Aspergillus* sp, *Mucor* sp, *Penicillium* sp, *Trichoderma* sp, *Rhizopus* sp, *Candida* sp and *Microsporum* sp.

The percentage occurrence of the bacterial isolates of tattoo equipment are as follows; *Bacillus* sp (7%), *Proteus* sp (20%), *Staphylococcus* sp (13%), *Micrococcus* sp (7%), *Enterococcus* sp (27%), *Streptococcus* sp (13%) and *Pseudomonas* sp (13%) (fig. 1). Results showing the percentage distribution of bacterial isolates in the various tattoo palour/SPA is presented in fig. 2. Results however, showed that *Bacillus* sp was the most distributed bacterial isolate as its prevalence cut across all the tattoo parlours under study while *Staphylococcus* sp was the second most distributed isolate with prevalence in four out of the five locations. *Enterococcus* sp was isolated in three tattoo parlours while *Proteus* sp and *Pseudomonas* sp were isolated from two tattoo palours. Although *Streptococcus* sp and *Micrococcus* sp were isolated from tattoo equipment, their prevalence rate was only in one tattoo parlour each. The percentage distribution of fungal isolates is presented in fig. 3. *Aspergillus* sp was the most predominant fungal isolates followed by *Penicillium and Rhizopus* sp while *Microsporum* sp was the least fungal isolates from the tattoo equipment.

Table 1: Microbial Population Count of Bacterial Isolates in cfu/ml

Total heterotrophic bacteria (x10^5)	***Staphylococcal count*** (×10^3)	**Fungal count** (×10^3)
2.3±0.1^a	1.3±0.0^a	1.6±0.1^b
2.7±0.1^a	5.0±0.0^a	1.2±0.1^b
1.4±0.7^{5a}	4.0±0.0^a	2.0±0.1^b
4.1±0.0^{5a}	2.0±0.0^a	0.0±0.0^a
2.08±0.2^{5a}	2.1±0.0^a	1.0±0.2^b

*Means with similar superscript (alphabets) showed no significant difference (P<0.05)

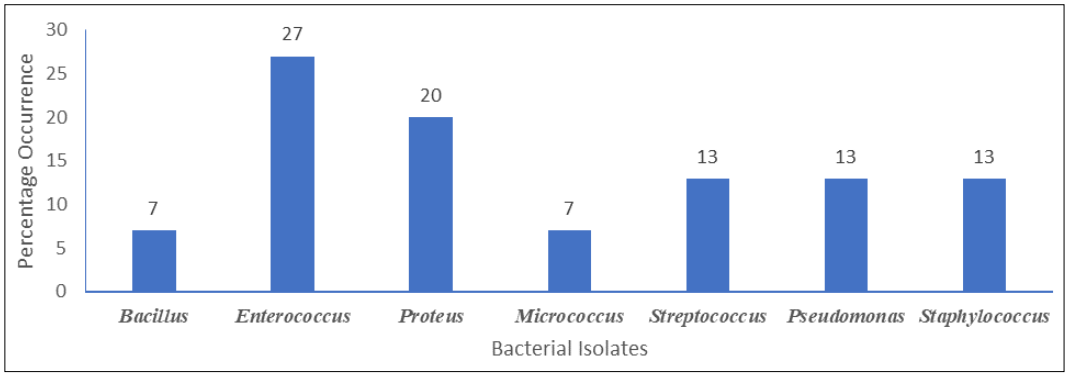

Fig 1: Percentage Occurrence of bacterial isolates from tattoo equipment

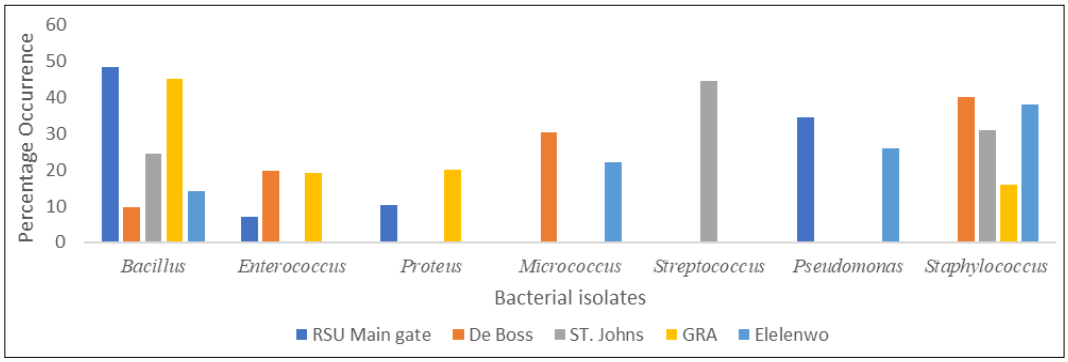

Fig 2: Percentage distribution of bacterial Isolates from Tattoo palours/SPA

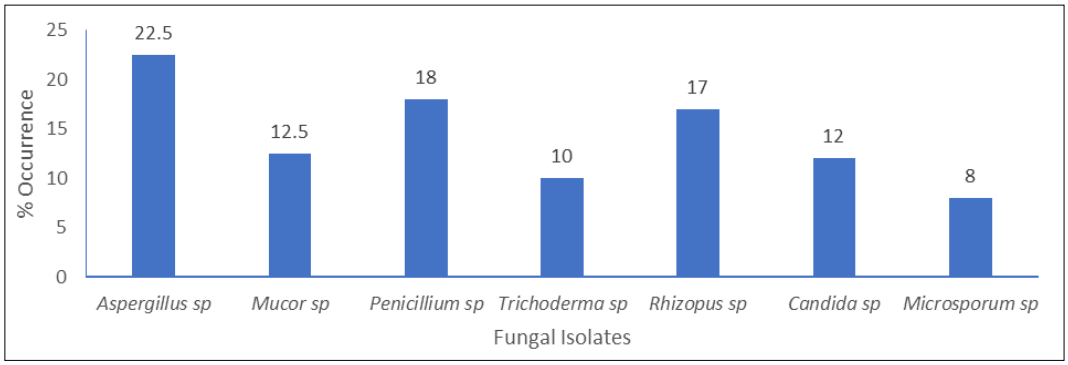

Fig 3: Percentage Occurrence of fungal Isolates from tattoo equipment

Results of the susceptibility pattern of Gram-positive and Gram-negative bacterial Isolates is presented in figs. 4 and 5, respectively. Results showed that the susceptibility of *Bacillus* sp to levofloxacin, gentamycin ciprofloxacin and septrin was 100% while being highly resistant to ampiclox, erythromycin, streptomycin, Amoxil and pefloxacin. Susceptibility of *Enterococcus* sp to chloramphenicol, erythromycin, gentamycin, ciprofloxacin, streptomycin, Amoxil and pefloxacin was 75%, 50%, 25%, 50%, 50%, 100and 50%, respectively. The *Micrococcus* isolates were 100% susceptible to ampiclox, erythromycin, gentamycin, ciprofloxacin, streptomycin and Amoxil, while the susceptibility pattern of *Staphylococcus* sp to chloramphenicol, levofloxacin, ciprofloxacin, streptomycin, pefloxacin and septrin was 100, 100, 50, 100, 50 and 100%, respectively. Results of the antibiotics susceptibility pattern of gram-negative bacterial isolates showed that the percentage susceptibility pattern of *Proteus* sp to Augmentin, ciprofloxacin, septrin, Ceporex, Streptomycin, Nalidixic acid, Gentamycin and Pefloxacin was 75, 100, 75, 25, 75, 75, 100 and 25%, respectively, while the susceptibility pattern of *Pseudomonas* sp to Augmentin, ciprofloxacin, Ceporex, Streptomycin, Gentamycin and Pefloxacin was 50, 100, 100, 50, 100, 100 and 100%, respectively.

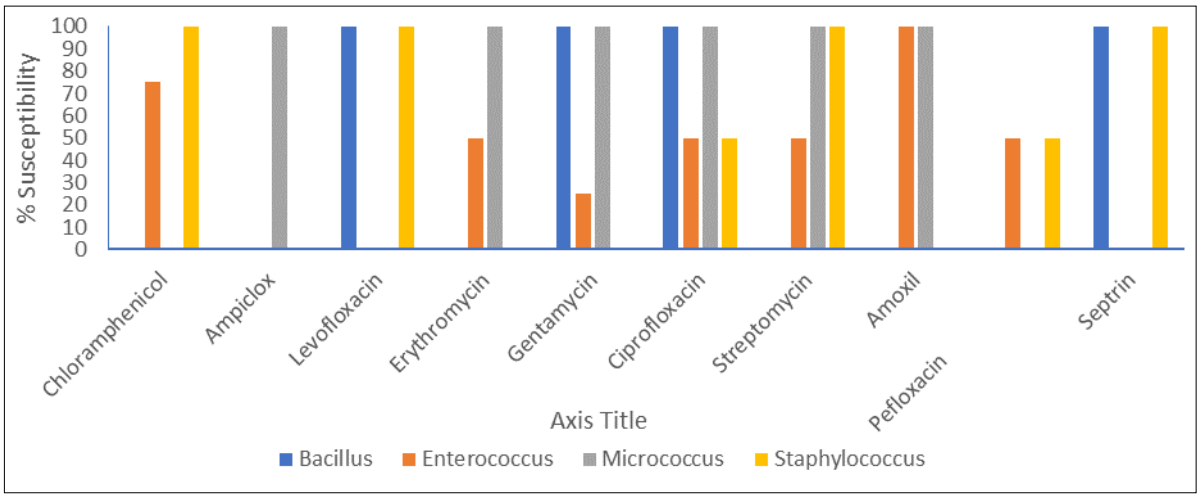

Fig 4: Percentage Susceptibility of Gram-Positive bacterial Isolates

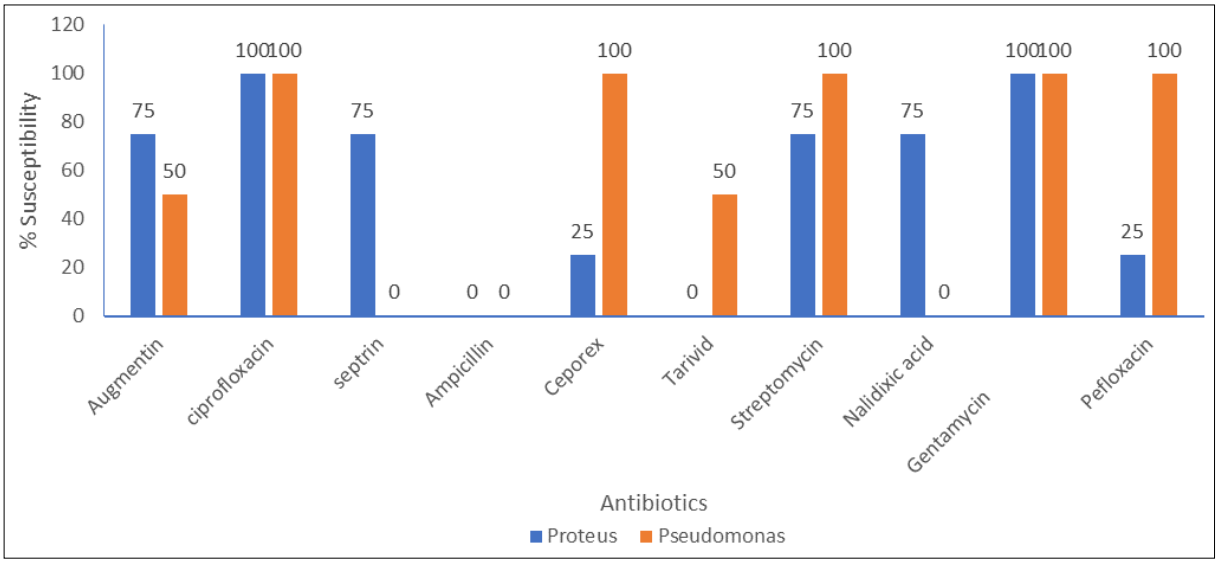

Fig 5: Percentage Susceptibility of Gram-Negative Isolates

Discussion

The microorganisms associated with tattoo equipment and the susceptibility to commonly used antibiotics was investigated. The microbial load of the tattoo equipment from the respective tattoo SPA/parlour varied and this variation could be due to contaminating microorganisms from the environment (within and outside the SPA), or from skin surfaces. This is supported by the microbial distribution in the respective tattoo equipment in the respective parlour of the present study. Since most of these tattoo parlours are within the barber's shop, there could be a possibility of microorganisms from barbing, cleaning material and other anthropogenic activities to settle on equipment surfaces. In a previous study, tattoo inks were reportedly contaminated by non-sterile water which was used as diluent (Høgsberg *et al.*, 2013) [8]. Several microorganisms such as *Staphylococcus*, *Bacillus*, *Streptococcus*, *Trichophyton*, *Aspergillus*, *Mucor* and *Microsporum* sp have been reported in beauty salons (Alharbi and Alhashim, 2021) [2]. While other routes of contamination could be from the resident and normal skin flora of the tattooed individual or from the needles as well as the inks used for tattooing. Improper use

of hygiene regimens, particularly contaminated needles and contaminated pigments have been implicated as vehicles for microbial contamination of tattooed surfaces (Khunger *et al.*, 2015) [13]. The bacterial and fungal isolates in the present study could be pathogenic thereby resulting in bacterial or fungal infections. Kennedy *et al.* (2012) [12] in their study reported environmental and potentially pathogenic bacteria: *Pseudomonas* sp., *Staphylococcus* sp., *Enterococcus* sp, and *Streptococcus* sp from tattoo inks which agreed with the present study. Bacterial isolates belonging to the genus *Staphylococcus, Streptococcus*, and *Pseudomonas* have been reported as major concern owing to their implication in many common diseases and can cause respiratory problems including chronic diseases due to their toxicity (Behravan *et al.*, 2005; Huijsdens *et al.*, 2008) [3, 10]. Thus, the presence of these microorganisms on tattoo equipment could cause infections as well as health complications. In a previous study, prevalence of 67.5%, 6.6%,1.1% and 0.4% of skin problems, systemic reactions, fever directly after tattooing and pus-filled tattoo lesions was reported as health related problems associated with tattooing (Klügl *et al.*, 2010) [14]. Dieckmann *et al*. (2016) [6] in their study reported that tattooing could result to traumatization of the skin which could facilitate microbial pathogens to pass the epidermal barrier causing local skin infections. Although severe systemic mycoses are rarely transmitted by tattooing but *Candida* endophthalmitis in a 40-year-old asplenic man have been reported (Khunger *et al.*, 2015) [13].

Antibiotics resistance have become a serious public health challenge owing to the fact that pathogenic microorganisms have developed resistance to commonly used antibiotics especially those of last resort. Although the present study showed that some of the isolates were very sensitive against the antibiotics, there were still presence of highly resistant (multi-drug resistant) isolates. The emergence of antibiotic resistant bacteria has been attributed to unrestricted use of antibiotics in a particular environment (Nwankwo and Nasiru, 2011) [17]. Resistance of *Staphylococcal* isolates to gentamycin, ampiclox, erythromycin and Amoxil antibiotics in the present study corroborates Aggarwal *et al.* (2019) [1] who reported a correlation between resistance of *S. aureus* to aminoglycosides and erythromycin. Although, the high resistance to chloramphenicol reported in their study do not agree with the present study. They also opined that resistance to gentamycin an aminoglycoside antibiotic was due to the possession of aminoglycoside-modifying enzyme, encoded by aac (6')-aph (2") gene. Although, the present study did not assay for resistant genes but previous study have shown that environmental factors as well as resistant genes play major roles in antibiotic resistance (Bengtsson-Palme *et al.*, 2018) [4]. Resistance to amoxicillin have been reported to be due to abuse of amoxicillin by the populace since antibiotics are still sold across the counter in some pharmaceutical and patent medicine stores in Nigeria (Mohammed *et al.*, 2013) [16]. Susceptibility of the isolates to levofloxacin, gentamycin, ciprofloxacin and septrin is an indication that these drugs are still potent and could be used in treating infections. Despite the resistance recorded in *Staphylococcus* and *Enterococcus* isolates to ciprofloxacin, the antibiotic showed complete sensitivity against the gram-negative bacterial isolates. Ciprofloxacin is a fluroquinolone antibiotic with broad spectrum activities. According to The American Society of Health-System Pharmacists (2015) [24], ciprofloxacin is used for the treatment of different forms of infections such as endocarditis, respiratory infections, urinary tract infections, cellulitis, gastroenteritis, and lots more. Thus, this statement agreed with the current findings which showed a broad-spectrum activity. The ciprofloxacin antibiotic like other fluoroquinolones function by inhibiting the DNA gyrase and type II topoisomerase and topoisomerase IV which is needed to unwind the DNA of the bacteria (Pommier *et al.*, 2010) [11]. Thus, resistance could also be either modification of binding site in the microorganisms. More so, resistance of Enterococci isolates to chloramphenicol, erythromycin, gentamycin and ciprofloxacin has been reported in a previous study (Suely *et al.*, 2007) [23].

Conclusion

Tattooing of body surfaces whether for medical or cosmetic reasons is a choice by the individual but careful attention on equipment or material involved in the process of tattooing should be given to avoid introduction or contamination of body parts or internal parts of the with microorganisms that could pose serious health issues. The microorganisms in the present study could be pathogenic and contamination with body surfaces could result to infection especially in convalescence or immune deficient individuals. More so, tattoo parlours should be kept clean and shouldn't be combined with barbing activities to avoid cross contamination of equipment with microorganisms from barbing activities. The continued misuse of antibiotics should be avoided and despite the effectiveness of some of the antibiotics in this study, proper laboratory tests is recommended for diagnosis before administration of antibiotics.

Conflict of Interest

Not available

Financial Support

Not available

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