

Characterization and antibiogram of bacteria isolated from second-hand undergarments sold in Port Harcourt Metropolis, Nigeria

Ogbonna S. I.*, Ogbuleka N. A. C., Robinson V. K. and Ejiogu T. P.

Department of Microbiology, Rivers State University, P.M.B. 5080, Port Harcourt, Nigeria.

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ABSTRACT

Second-hand undergarments, or fairly used underwear, refer to clothing items such as panties and boxers imported from countries including the United States of America, the United Kingdom, Germany, the Netherlands, and various parts of Asia after prior use by the original owners. These garments are widely used but may harbor microbial contaminants. This study aimed to assess the microbial composition of fairly used underwear sold in the Port Harcourt metropolis. A total of six (6) swab samples were collected from measured areas of second-hand undergarments (male and female) sold in three different markets in Port Harcourt, Mile 1, Mile 3, and Rumuokoro, and analyzed for microbial composition using standard microbiological techniques. Results showed that the Total Heterotrophic Bacterial Count (THBC) ranged from 4.40×10^4 to 9.40×10^4 CFU/cm² for female underwear and from 2.40×10^4 to 9.77×10^4 CFU/cm² for male underwear. A total of eight (8) bacterial species belonging to six (6) main genera were identified: *Staphylococcus* spp. (42.8%), *Bacillus* spp. (23.8%), *Micrococcus terrus* (19.5%), *Pseudomonas* sp. (4.76%), *Klebsiella* sp. (4.7%), and *Paenibacillus* sp. (4.7%). *Staphylococcus* spp. had the highest percentage occurrence, followed by *Bacillus* spp. (23.8%) and *Micrococcus terrus* (19.5%), while *Paenibacillus* sp., *Pseudomonas* sp., and *Klebsiella* sp. each had 4.7%. Male and female underwear samples from Mile 1 Market exhibited higher microbial loads compared to those from Rumuokoro and Mile 3 Markets. Antibiotic susceptibility tests revealed that *Staphylococcus aureus* was susceptible to ciprofloxacin and cefuroxime but resistant to rifampin, streptomycin, and azithromycin. *Bacillus* spp. was susceptible to ciprofloxacin and rifampin but resistant to azithromycin, gentamicin, and ceftazidime. It is recommended that government regulatory agencies minimize the importation of second-hand underwear and ensure that approved items are properly disinfected and laundered before sale to prevent potential public health risks.

Keywords: Second-hand undergarment, bacteria, antibiogram.

*Corresponding author. E-mail: solomon.ogbonna@ust.edu.ng.

INTRODUCTION

Fairly used or second-hand clothes in Nigeria, commonly referred to as "Okrika", include clothing materials such as shirts, trousers, towels, socks, panties, pillowcases, curtains, and bed sheets imported from countries like the United States of America, the United Kingdom, Germany, the Netherlands, and various parts of Asia after prior use by the original owners (Agbulu et al., 2015).

Second-hand clothing items, due to their previous usage and widespread circulation, pose major public health risks. Recognition of these risks prompted the Rwanda Bureau of Standards to ban the importation of

second-hand undergarments in Rwanda (The New Times Rwanda, 2011). Diseases transmissible through contact, such as candidiasis, hepatitis A, B, and C, as well as skin infections like scabies and ringworm, may be spread through these commonly used clothing items (Sharifzada, 2011; NAN, 2012). Bloomfield et al. (2011) demonstrated that clothing materials can retain bacteria, fungi, and viruses for varying lengths of time. It is therefore evident that second-hand clothing has an inherent capacity to harbor and transmit microorganisms from the first user to subsequent ones. Although washing such garments with

detergents and antiseptics can help disinfect them, the effectiveness of this process depends on several factors (Muthaini et al., 2010).

Several studies have shown that clothes can be contaminated by both chemical and biological agents. Used clothing may facilitate the transmission of pathogens from one person to another, especially if the previous wearer had an infectious disease transmissible through body fluids such as sweat. Most fabric type's wool, nylon, and cotton, can serve as carriers, spreading pathogens from their point of contact with human skin, particularly when moisture is present from sweat, saliva, open wounds, pimples, or spilled liquids.

The bacterial flora, also known as the microbiota, refers to the community of microorganisms residing in or on the human body. These microorganisms, primarily bacteria, play a crucial role in maintaining overall human health by aiding digestion, nutrient absorption, immune regulation, and protection against pathogens. The bacterial flora associated with the vagina, known as the vaginal microbiota, consists of a diverse community of microorganisms, predominantly *Lactobacillus* species. These bacteria help maintain the acidic pH of the vagina and protect against harmful pathogens. However, the composition of the vaginal microbiota varies among individuals and can be influenced by factors such as hormonal changes, sexual activity, hygiene practices, and the use of certain medications (France et al., 2022).

In a study conducted by Briones et al. (2016) to determine the prevalence of bacterial and fungal pathogens on different types of second-hand clothing, *Staphylococcus epidermidis* was the only bacterium isolated, occurring in samples from bras, briefs, and the perianal regions of long pants. Similarly, Awe and Abuh (2016) isolated seven bacterial species including *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* sp., *Pseudomonas aeruginosa*, and *Proteus vulgaris* from second-hand garments purchased from the International Market in Lokoja, Kogi State, Nigeria.

This study, therefore, aims to investigate the bacterial flora associated with fairly used undergarments sold within the Port Harcourt metropolis and to determine their susceptibility to commonly used antibiotics.

MATERIALS AND METHODS

Study area

The study was carried out in three major markets within the Port Harcourt metropolis Mile 1, Mile 3, and Rumuokoro in Rivers State, Nigeria. Table 1 shows the sample collection location and coordinates.

Sample collection

Second-hand underwear samples (male and female)

Table 1. Sample collection location and coordinates.

Mile 3	4°47'53" N 6°59'33" E
Mile 1	4°48'16" N 6°59'23" E
Rumuokoro	4°52'11"N 6°59'56"E

were purchased from Mile 1, Mile 3, and Rumuokoro markets. Each sample was placed in a sterile zip-lock bag and transported to the Microbiology Laboratory of Rivers State University for analysis. Samples were collected by swabbing the measured areas of the underwear that come into direct contact with the genital region.

Enumeration of bacteria

The total heterotrophic bacterial load on the various undergarments was enumerated using the standard plate count method (Prescott et al., 2011). A ten-fold serial dilution technique was employed. Sterile swabs moistened with normal saline were used to swab the areas of the underwear that come into contact with the genitals. The swabs were transferred into test tubes containing 9 mL of sterile normal saline, resulting in an initial dilution of 1:9. Subsequent dilutions were made by transferring 1 mL from the initial tube into another tube containing 9 mL of sterile diluent. This process was repeated until a dilution of 10^{-4} was achieved.

Aliquots from the 10^{-3} dilution were aseptically inoculated onto nutrient agar plates in duplicates for enumeration of total heterotrophic bacteria (Ogbonna et al., 2022). The plates were incubated at 37°C for 24 hours, after which colony counts were recorded as colony-forming units per square centimeter (CFU/cm²).

Identification of bacterial isolates

Distinct colonies were sub-cultured to obtain pure isolates. The pure isolates were characterized using Gram staining and standard biochemical tests, including catalase, indole, methyl red, citrate, coagulase, Voges-Proskauer, and sugar fermentation tests. The identity of each isolate was confirmed using Bergey's Manual of Determinative Bacteriology (Cheesbrough, 2000).

Antibiotic sensitivity test

Antibiotic susceptibility testing was carried out using the disk diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI, 2025). Twenty-four-hour-old bacterial cultures were standardized using the 0.5 McFarland standard by adjusting the turbidity of the bacterial suspension in 4 mL of sterile normal saline to match the standard.

Sterile swab sticks were dipped into the standardized suspension and evenly spread across the surface of dried Mueller-Hinton agar plates. The plates were allowed to dry for 3–5 minutes at room temperature before the application of commercially prepared antibiotic discs using sterile forceps. The plates were then incubated at 37°C for 18–24 hours.

After incubation, the diameters of inhibition zones were measured in millimeters and interpreted as resistant (R), intermediate (I), or susceptible (S) based on the CLSI interpretive chart (CLSI, 2025).

RESULTS

At the end of the study, the bacterial load from second-hand underwear samples showed Total Heterotrophic Bacterial Counts (THBC) ranging from 4.40×10^4 to 9.40×10^4 CFU/cm² for female underwear and 2.40×10^4 to 9.77×10^4 CFU/cm² for male underwear (Table 2). Table 2 summarizes the THBC values obtained from male and female underwear samples collected from the three different markets within Port Harcourt metropolis, Mile 1,

Mile 3, and Rumuokoro. The highest microbial loads were observed in samples obtained from Mile 1 Market.

Table 2. Population Count of Bacterial Isolates (Cfu/cm²).

Underwear	THB ($\times 10^4$)
Female Pant 1	9.41×10^4
Female Pant 2	4.48×10^4
Female Pant 3	4.40×10^4
Male Boxer 1	9.77×10^4
Male Boxer 2	7.65×10^4
Male Boxer 3	2.40×10^4

Keys: THB = Total Heterotrophic Bacteria.

Figure 1 presents the percentage occurrence of bacterial isolates obtained from the analyzed samples. The results show that *Staphylococcus* spp. (42.8%) and *Bacillus* spp. (23.8%) were the most frequently isolated organisms, followed by *Micrococcus terrus* (14.5%), *Pseudomonas* sp. (4.76%), *Klebsiella* sp. (4.76%), and *Paenibacillus* sp. (4.76%).

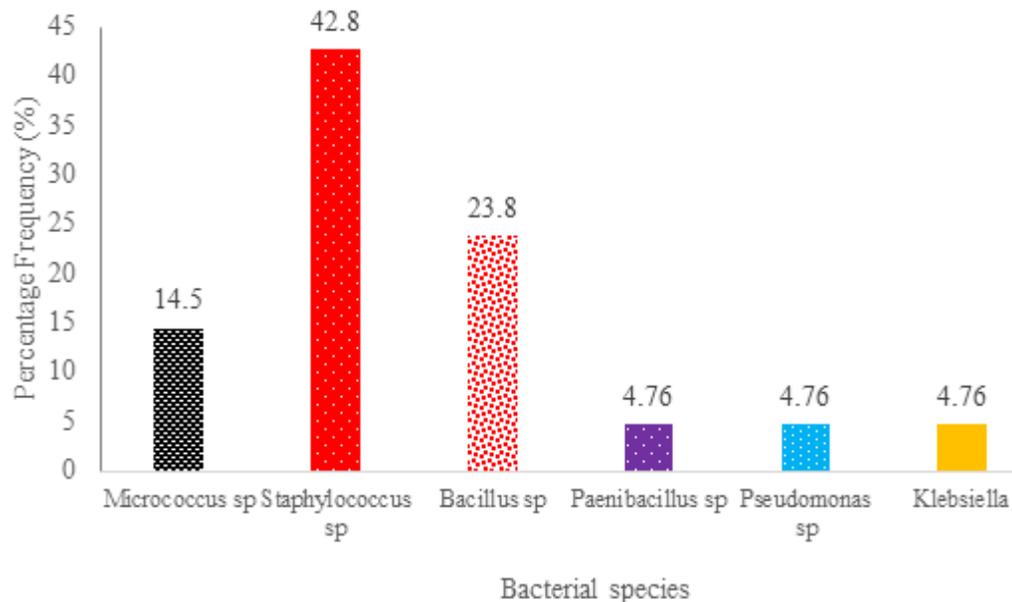


Figure 1. Percentage frequencies of the bacteria isolated.

The results of the antibiotic susceptibility patterns of both Gram-positive and Gram-negative bacterial isolates are presented in Tables 3 to 5.

Table 3 shows that *Staphylococcus* spp. were most susceptible to ciprofloxacin (55.6%), amoxicillin (22.2%), and ceftazidime (22.2%), but displayed high resistance to rifampin and streptomycin (88.9% each).

Table 4 illustrates the antibiotic susceptibility of other

Gram-positive isolates. *Bacillus* spp. showed susceptibility to ciprofloxacin (60%) and rifampin (60%), and moderate susceptibility to erythromycin, levofloxacin, and cefuroxime (40% each). However, the isolates were resistant to azithromycin, gentamicin, and ceftazidime (80% each). *Paenibacillus* sp. demonstrated complete susceptibility to levofloxacin (100%) but total resistance to azithromycin, amoxicillin, erythromycin, and

Table 3. Antibiotics sensitivity pattern of *Staphylococcus* spp. and *Bacillus* spp.

Antibiotics	<i>Staphylococcus</i> spp. (n=9)			<i>Bacillus</i> spp. (n=5)		
	R(%)	I(%)	S(%)	R(%)	I(%)	S(%)
Azithromycin 10µg	7(77.8)	1(11.1)	1(11.1)	4(80)	1(20)	0(0)
Amoxil 20µg	6(66.7)	1(11.1)	2(22.2)	3(60)	1(20)	1(20)
Ciprofloxacin 10µg	3(33.3)	1(11.1)	5(55.6)	0(0)	1(20)	3(60)
Erythromycin 30µg	7(77.8)	1(11.1)	1(11.1)	2(40)	1(20)	2(40)
Levofloxacin 20µg	6(66.7)	1(11.1)	2(22.2)	2(40)	1(20)	2(40)
Gentamycin 10µg	7(77.8)	1(11.1)	1(11.1)	4(80)	0(0)	1(20)
Cefuroxime 30µg	5(55.6)	1(11.1)	3(33.3)	3(60)	0(0)	2(40)
Rifampin 20µg	8(88.9)	0(0)	1(11.1)	2(40)	0(0)	3(60)
Ceftazidime 30µg	7(77.8)	0(0)	2(22.2)	4(80)	0(0)	1(20)
Streptomycin 30µg	8(88.9)	0(0)	1(11.1)	2(40)	2(40)	1(20)

R = Resistance, I = Intermediate, S = Susceptibility.

Table 4. Antibiotics sensitivity pattern of *Paenibacillus* sp. and *Micrococcus terrus*.

Antibiotics	<i>Paenibacillus</i> sp (n=1)			<i>Micrococcus terrus</i> (n=4)		
	R(%)	I(%)	S(%)	R(%)	I(%)	S(%)
Azithromycin 10µg	1(100)	0(0)	0(0)	3(75)	1(25)	0(0)
Amoxil 20µg	1(100)	0(0)	0(0)	1(25)	1(25)	2(50)
Ciprofloxacin 10µg	0(0)	1(100)	0(0)	3(75)	0(0)	1(25)
Erythromycin 30µg	1(100)	0(0)	0(0)	3(75)	0(0)	1(25)
Levofloxacin 20µg	0(0)	0(0)	1(100)	1(25)	1(25)	2(50)
Gentamycin 10µg	0(0)	1(100)	0(0)	2(50)	2(50)	0(0)
Cefuroxime 30µg	1(100)	0(0)	0(0)	2(50)	1(25)	1(25)
Rifampin 20µg	0(0)	1(100)	0(0)	2(50)	0(0)	2(50)
Ceftazidime 30µg	1(100)	0(0)	0(0)	4(100)	0(0)	0(0)
Streptomycin 30µg	1(100)	0(0)	0(0)	3(75)	1(0)	0(0)

R = Resistance, I = Intermediate, S = Susceptibility.

Table 5. Antibiotics sensitivity pattern of gram negative bacteria from all samples.

Antibiotics	<i>Pseudomonas</i> sp (n=1)			<i>Klebsiella</i> sp (n=1)		
	R(%)	I(%)	S(%)	R(%)	I(%)	S(%)
Augmentin 30 µg	1(100)	0	0	1(100)	0(0)	0(0)
Peflacin 10 µg	1(100)	0	0	1(100)	0(0)	0(0)
Ceftazidime 30µg	1(100)	0	0	1(100)	0(0)	0(0)
Gentamycin 10 µg	1(100)	0	0	1(100)	0(0)	0(0)
Ciprofloxacin 10 µg	1(100)	0	0	0(0)	0(0)	1(100)
Ceporex 10 µg	1(100)	0	0	0(0)	1(100)	0(0)
Ceftriaxone 30 µg	1(100)	0	0	1(100)	0(0)	0(0)
Streptomycin 30 µg	1(100)	0	0	1(100)	0(0)	0(0)
Cefuroxime 30 µg	1(100)	0	0	1(100)	0(0)	0(0)
Ofloxacin 10 µg	1(100)	0	0	0(0)	0(0)	1(100)

R = Resistance, I = Intermediate, S = Susceptibility.

streptomycin (100% each). *Micrococcus terrus* showed moderate susceptibility to amoxicillin, levofloxacin, and rifampin (50% each), but was completely resistant to ceftazidime (100%).

Table 5 summarizes the susceptibility profiles of Gram-negative bacterial isolates. *Pseudomonas* sp. exhibited complete (100%) resistance to all antibiotics tested. In contrast, *Klebsiella* sp. showed full susceptibility (100%)

to ciprofloxacin and ofloxacin, but total resistance (100%) to all other antibiotics tested.

DISCUSSION

This study revealed a significant microbial load on

second-hand undergarments sold within the Port Harcourt metropolis. The Total Heterotrophic Bacterial Count (THBC) ranged from 4.40×10^4 to 9.40×10^4 CFU/cm² for female underwear and 2.40×10^4 to 9.77×10^4 CFU/cm² for male underwear (Table 2). These findings contrast with those of Odum and Idise (2022), who reported lower total aerobic counts (4.6×10^4 CFU/mL) and coliform counts (3.2×10^2 CFU/mL). Conversely, Agbulu et al. (2015) reported higher bacterial counts, ranging from 1.9×10^6 to 6.16×10^6 CFU/mL, in second-hand clothing. The observed variations in microbial load among studies could be attributed to factors such as geographic location, hygiene practices of the original users, packaging methods, sampling techniques, and the environmental conditions under which the garments were displayed and sold.

A total of eight (8) bacterial species belonging to six (6) genera were identified in this study: *Staphylococcus aureus*, *Bacillus subtilis*, *Staphylococcus* sp., *Bacillus* sp., *Paenibacillus pectinilyticus*, *Micrococcus terrus*, *Pseudomonas* sp., and *Klebsiella* sp. These findings are consistent, to some extent, with those of Odum and Idise (2022), who isolated *Staphylococcus aureus*, *Pseudomonas* sp., and *Klebsiella pneumoniae* from second-hand undergarments sold in Abraka, Delta State. Similarly, Ikeh et al. (2024) reported the presence of *Staphylococcus aureus* and *Pseudomonas* sp. from fairly used clothing sold in Eke Awka Market, Anambra State. Differences in the bacterial profile among studies could result from variations in transportation, storage, display methods, and exposure to environmental contaminants. Although this study did not assess the pathogenicity of the isolates, similar organisms have been implicated in previous research as causative agents of diverse infectious diseases (Olajubu et al., 2017).

The percentage occurrence of bacterial isolates in this study was as follows: *Paenibacillus pectinilyticus* (4.76%), *Klebsiella* sp. (4.76%), *Pseudomonas* sp. (4.76%), *Micrococcus terrus* (19.5%), *Bacillus* sp. (23.8%), and *Staphylococcus* sp. (42.8%). These results differ from those of Olajubu et al. (2017), who reported a higher percentage occurrence of *Staphylococcus* (60.7%) and *Pseudomonas* (7.1%) from used clothing sold in Lagos markets. The differences may be attributed to sample size, environmental contamination due to frequent handling by customers and vendors, variations in sampling procedures, and differing market conditions. Similarly, Ikeh et al. (2024) reported a lower percentage occurrence of *Staphylococcus* (26.8%) and *Pseudomonas* (15.4%) compared to this study, indicating possible regional or methodological influences on bacterial prevalence.

Antibiotic resistance remains a major global public health concern, as pathogenic microorganisms increasingly develop resistance to commonly used antibiotics, including those of last resort. Although some bacterial isolates in this study exhibited susceptibility to

certain antibiotics, several multi-drug resistant strains were also identified. The emergence of resistant strains may be linked to the indiscriminate use of antibiotics and poor regulatory control, as suggested by Nwankwo and Nasiru (2011).

In this study, *Staphylococcus* isolates showed 55.6% susceptibility to ciprofloxacin, suggesting that this antibiotic could be effective against *Staphylococcus* related infections. However, resistance was observed against azithromycin, amoxicillin, erythromycin, levofloxacin, gentamicin, cefuroxime, rifampin, ceftazidime, and streptomycin. *Bacillus* isolates exhibited 60% susceptibility to both ciprofloxacin and rifampin, but lower susceptibility ($\leq 50\%$) to other antibiotics. *Paenibacillus* sp. was 100% susceptible to levofloxacin, while *Micrococcus terrus* exhibited complete resistance to all tested antibiotics. These findings are consistent with Mulu et al. (2015), who reported similar susceptibility patterns for *Staphylococcus* and *Bacillus* species. Moreover, *Klebsiella* sp. and *Staphylococcus* sp., which were isolated in this study, have been implicated in vaginitis and other genitourinary infections (Kaambo et al., 2018).

CONCLUSION

This study demonstrates that fairly used underwear can serve as a potential vector for microbial contamination, posing a significant public health risk. Given the widespread patronage of such garments in the Port Harcourt metropolis, these findings warrant serious public health consideration. The detection of antibiotic-resistant strains among the isolates is particularly concerning, as it suggests that second-hand underwear could facilitate the spread of drug-resistant pathogens.

Wearing contaminated undergarments without adequate laundering or sterilization increases the risk of skin and urinary tract infections. The high bacterial loads observed indicate that individuals using such underwear without proper disinfection may be exposed to pathogenic microorganisms. The presence of bacteria on these garments also reflects poor hygiene and handling practices among clothing vendors in Port Harcourt.

Variations in microbial load and species diversity across samples suggest that factors such as storage conditions, handling practices, and environmental exposure strongly influence the degree of contamination. Fairly used underwear provides a favorable environment for microbial survival and proliferation due to the warmth and moisture often retained in fabric, especially when stored improperly. The organisms identified likely originated from multiple sources, including inadequate washing or disinfection by previous users and exposure to unhygienic environments during transportation and display.

Proper disinfection procedures, regulatory oversight on

second-hand clothing importation, and public health awareness campaigns are therefore recommended to reduce the potential health risks associated with the use of fairly used undergarments.

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