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Microbial Analysis and Antibiogram of Workers Clothes from Building Construction Sites in Ikot Ekpene L.G.A

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Abstract: *The microbial assessment and antibiogram of workers clothes at building construction sites was carried out. Swabbed samples of clothes worn by workers were collected at building construction site in Ikot Ekpene L.G.A. The samples were analyzed following standard microbiological procedures. Antibiogram of the bacterial isolates was carried out using standard antibiotics for Gram positive and Gram negative bacteria. The results obtained revealed the isolation of five bacterial and four fungal species from the samples. The bacterial count was high in sample D (262 Cfu/cm^2) followed by sample E (202 Cfu/cm^2), sample A (96 Cfu/cm^2), sample B (88 Cfu/cm^2) and sample C (63 Cfu/cm^2). The bacterial species isolated were; Klebsiella sp., Pseudomonas sp., Staphylococcus sp., Bacillus sp. and Streptococcus sp. The percentage occurrence of the bacterial isolates were; Klebsiella sp. (18.7%), Pseudomonas sp. (20.0%), Staphylococcus sp. (22.2%), Bacillus sp. (22.5%) and Streptococcus sp. (16.6%). Total fungal counts was high in sample A (17 Cfu/cm^2), followed by sample C and D (14 Cfu/cm^2), sample B (11 Cfu/cm^2) and then sample A (10 Cfu/cm^2). Fungal isolates were; Aspergillus sp., Candida sp., Penicillium sp. and Trichophyton sp. Among the fungal isolates, Aspergillus sp. was the most occurring isolate with percentage occurrence of 31.8%, followed by Candida sp. (25.8%), Penicillium sp. (24.2%) and then Trichophyton sp. (18.2%). Antibiotics susceptibility profile of bacteria showed that 85 % of the bacterial isolates were sensitive to the commonly used antibiotics. The presence of bacterial and fungal isolates on the workers clothe samples is an indication of potential risk of bacterial and fungal infections pose to the workers. However, the antibiogram points to the efficacy of the antibiotics in the treatment of bacterial infections that may arise due to the repeated wearing of the work clothe at building construction sites. Workers at building construction sites should practice good personal hygiene and ensures that their work clothes are washed on daily basis and disinfected before being use again. However, in case of bacterial infection, the effective antibiotic should be used for treatment.*

Keywords: Microbial, Antibiogram, building, construction sites, analysis

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1.0 Introduction

Clothing is defined as items worn to enclose or cover the body and does not include body modifications (Madigan *et al.*, 2019). Clothing provides a barrier between the skin and the external environment and serves a variety of functions, which include protection from harmful UV rays, social and cultural purposes and thermoregulation.

The transport of microorganisms from a working environment with a high exposure to microorganisms or with pathogenic or allergenic microorganisms to another environment may constitute a problem and

may be a source of exposure to other people. In spite of this, transport of microorganisms via work clothes is generally not well studied, though it is well established that microorganisms can accumulate on hospital uniforms as reviewed (Heth *et al.,* 2015).

A pilot study from the U.S., where the wearing of hospital uniforms outside of work is not regulated, showed accumulation of bacteria on sterilized uniforms worn by nurses (Kong, 2016). Bacteria were found on all 10 uniforms after use, and 7 out of the 10 uniforms were positive for antibiotic-resistant *Staphylococcus aureus*. However, the study did not investigate the transport of bacterial pathogens via clothes but highlighted the need for investigations and policies on wearing health care uniforms outside the working environment. Another study from Korea has similarly found high concentrations of microorganisms on the work clothes of waste collection workers (Park *et al.*, 2011), but did not study transport of these microorganisms to other environments. These studies highlight the importance of hygiene in relation to work clothes. Para-occupational exposure, or so called 'take-home' exposure, by means of bringing home contaminated work clothes, has been studied for chemical agents such as lead pesticides, and polychlorinated biphenyls (PCB) (Park *et al*., 2011). Moreover, exposure to allergenic biological particles from clothes has been studied for cat and dust mites, where these allergens were transported from private homes to schools and workplaces. Furthermore, pollen has been shown to accumulate on and be transported with clothes.

Whether microorganisms are transported and accumulated from work clothes is of particular importance in the meeting with e.g. immunocompromised or allergic persons and should therefore influence workers' hygiene and change of clothes at the end of the workday. The relationship between microbes and clothing is of great significance to fashion, medicine and public health. Its consequences ramify. Considering the fact that workers clothing in building construction sites are often reused day-to-day sometimes without washing, it is therefore of health importance that study be conducted to assess the microbiological status of workers' clothes in such work environment. The skin is a complex human organ functioning to prevent loss of moisture and confine the entry of pathogens. It also provides an environment for part of the human flora. There are approximately one million resident bacteria per square centimeter of skin, for a total of about 1010 skin microorganisms covering the average adult (Madigan *et al.*, 2019). Normal human skin is colonized by huge quantities of microbiota that live on its surface. The normal flora of the skin, composed primarily of gram-positive cocci and diphtheroid, may represent a selective barrier against the proliferation of potentially pathogenic organisms. Moreover, small numbers of gram-negative bacteria or yeasts may also include in the normal human skin flora. Skin sets a good example of microenvironments of the varying temperature, pH, moisture, and sebum content. The organization of the microflora varies from site to site as indicated by the character of the microenvironment.

The types of microbes recoverable from clothing items are strongly linked to the microbiome of the adjacent skin, which hosts an enormous diversity of bacteria, fungi, and viruses. For instance, depending on the skin properties (i.e., oily, moist, dry), and anatomical location (e.g., foot), between 102 to 107 bacterial cells per cm2 skin surface may be present, representing up to 1000 different species from 19 different bacterial phyla (Kong and Segre 2016). Clothing can alter skin and its microhabitat, but it can also form a microhabitat in and of itself. Those fibres can provide habitat to microbes (and their effects) has long been known. Arguably, aspects of this effect were understood before microbes had even been discovered. For example, the idea that clothes of sick individuals could, themselves, cause disease

was understood before the elaboration of germ theory (Sherman, 2017).

It is well known that textiles can support the growth of bacteria and fungi as they have been found to degrade natural fibres, and to a lesser extent, synthetic fibres. Textile-degrading taxa include the fungal genera *Aspergillus, Penicillium* and *Microsporum* and bacterial genera *Bacillus,* Streptomyces and *Pseudomonas* (Szostak-Kotowa, 2004). Microbial growth on textiles can cause unpleasant odours, physical irritation, and the loss of tensile strength and decolourization of the fabric (Gutarowska and Michalski, 2012). Therefore, this study is aimed at assessing the of microbial analysis and antibiogram of workers' clothes from building construction sites in Ikot Ekpene metropolis.

2.0 Materials and Methods

2.1 Materials

Filter paper, beaker, petri dishes, slides, spatula, swab sticks, Durham's tube, weighing balance, microscope, incubator, autoclave, water, distilled water, nutrient agar (NA), Sabouraudextrose agar (SDA), Blood agar, peptone, phenol, normal saline, etc.

2.2 Methods

2.2.1 Collection of Samples

Work clothes of five workers at building construction site in Ikot Ekpene L.G.A were swabbed using sterile swab sticks, samples were stored in sterile containers and transported to the microbiological laboratory, for microbial analysis.

2.2.2 Sterilization of Materials

All the media would be autoclaved at 121 °C, diluents at (121 °) and glassware (Petri plates, Bijou bottles, test tubes, pipette) would be sterilized using hot air oven at 160 ^{0}C for 1 hr. Inoculating loop was sterilized by flaming using dry heat, while 70 % alcohol was used in sterilization of work workbench using cotton wool.

2.2.3 Media Preparation

Culture media were prepared according to the manufacturer's instructions by dissolving 2.8 g of agar into 100 ml of water contained in a conical flask. The prepared culture media was sterilized by moist air using an autoclave at 121 ^{o}C for 15 minutes.

2.2.4 Inoculation of Test Organisms

The sample swabbed sticks on arrival to the laboratory were immediately inoculated on an already prepared nutrient agar, blood agar and Sabouraud Dextrose Agar (SDA) using the streak-plate technique. The plates were accurately labelled according to the sample ID and agar type. The nutrient agar and blood agar plates were incubated at 37 ^{o}C for 24 hours for the visible growth of bacteria, while the SDA plates were incubated at 35^{o}C for 48 hours for the fungal growth.

2.2.5 Enumeration of Bacterial Isolates

The emerging visible disease colonies in the inoculated plate were counted and expressed in colony-forming unit (CFU) per surface area of the skin. Colony appearance examinations for all different growth in each plate were carried out in order to identify their growth characteristics such as shape, and optical characteristics, pigmentations and colony surface.

2.2.6 Purification of Bacterial Isolates

Subculture of all the grown colonies were done by the streak method on freshly prepared agar plates. These were incubated for 24 hours at 37 ^{o}C for pure colonies. The stock culture was prepared by using a non-selective medium (nutrient agar) prepared and aseptically poured into sterile MacConkey bottles and allowed to set on a slant position using a sterile inoculating loop flamed till red hot. A loopful of pure bacterial colony was taken from a subculture plate and aseptically transferred into the MacConkey bottles and inoculated by direct streaking on the surface of the slant medium. The bottles were covered and incubated at 37 ^{o}C for 24hours and was stored in a refrigerator at 4 °C for further analysis(Cheesbrough 2004).

2.2.7 Identification and Characterization of Isolates

Bacterial isolates were characterized and identified based on their morphological and culture characteristics. Visual inspections of colonial appearance of colonies were carried out to identify the characteristics. The inspection was done by observing the colony surface, appearance, shape, edge, pigmentation, elevation, consistency and optical features using the method of Cheesbrough (2006).

The following biochemical tests were carried out to further characterize bacterial isolates; Gram staining, motility, urease, citrate, oxidase, catalase, coagulase and sugar fermentation as shown in the appendix using the methods of Cheesbrough, (2006). Identification was done by comparing the colonial and biochemical features of each with those of a known taxonomy in the Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). Fungal isolates were identified and characterized using cultural morphology and microscopic characteristics.

2.2.8 Antibiotics susceptibility Test

A fresh nutrient agar was prepared according to manufacturer's instruction and was poured into two petri dishes for Gram-positive and Gram-negative bacteria and was allowed to solidify. With the aid of a sterile wire loop, the bacterial isolates were picked and inoculated on the nutrient agar by streak method. The respective antibiotic disc for Gram-positive bacterial was placed on the inoculated plate labeled Gram-positive while the Gram-negative antibiotic disc was also placed on the inoculated plate labeled G-negative. The plates were covered and wrapped in foil paper and then incubated at 37^{o}C for 48 hours.

After incubation, the plates were observed for zones of inhibition. Established zones of inhibition were measured using meter rule calibrated in millimeter and were recorded for respectively for each bacterial species with respect to the antibiotics(CLSI, 2018).

3.0 Results and Discussion

The results of the microbial analysis and antibiogram of workers cloths of building construction site are presented in Table 1 – 7 and are described as follows:

3.1 Total Bacterial Colony Counts from the Samples

Table 1 shows the bacterial colony counts from the samples. From the table, the bacterial colony counts for samples ranged from 63 Cfu/cm^2 to 262 Cfu/cm^2.

2.2 Total Fungal Colony Counts from the Samples

Table 2 shows the fungal colony counts from the samples. From the table, the fungal colony counts ranged from 10 Cfu/cm^2 to 17 Cfu/cm^2.

2.3 Cultural, Morphological and Biochemical Characterization of the Bacterial Isolates

Table 3 shows the cultural, morphological and biochemical characteristic of the bacterial isolates. Based on these characteristics, five bacterial species were identified as *Klebsiella* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Bacillus* sp. and *Streptococcus* sp.

2.4 Cultural and Microscopical Characterization of the Fungal Isolates

Table 4.4 shows the cultural and microscopical characteristics of the fungal isolates, which revealed four fungal species: *Aspergillus* sp., *Trichophyton* sp., *Candida* sp., and *Penicillium* sp.

Table 1: Total Bacterial Colony Counts from the Samples

Sample	Colony Forming Units per Surface Area (CFU/cm^2)
A	96
B	88
C	63
D	262

E	202

Table 2: Total Fungal Colony Counts from the Samples

Sample	Colony Forming Units per Surface Area (CFU/cm²)
A	17
B	11
C	14
D	14
E	10

2. 5 Frequency and Percentage Distribution of the Bacterial Isolates

Table 5 shows the frequency and percentage distribution of the bacterial isolates, which revealed the highest percentage occurrence of 22.5% for *Bacillus* sp. followed by *Staphylococcus* sp. (22.2%), *Pseudomonas* sp (20%), *Klebsiella* sp. (18.7%) and the least percentage occurrence of 16.6% for *Streptococcus* sp.

2.6 Frequency and Percentage Occurrence of the Fungal Isolates

Table 6 shows the frequency and percentage occurrence of the fungal isolates which revealed the highest percentage occurrence of 31.8% for *Aspergillus* sp. followed by *Candida* sp. (25.8%), *Pencillium* sp. (24.2%) and *Trichophyton* sp. had least percentageoccurrence of 18.2%.

4.7 Antibiogram of the Bacterial isolates from the Samples

Table .7 shows the sensitivity of the bacterial isolates to antibiotics. *Staphylococcus* sp, *Bacillus* sp., and *Streptococcus* sp, klesialla sp, Pseudomonas sp were sensitive to riflacine, gentamycin, ciprofloxacin, amoxicillin, levofloxacin, erythromycin, azithromycin, rifampicin, ampiclox and streptomycin. with inhibition zones ranging from 19 – 33 mm.

Table 3: Cultural, Morphological and Biochemical Characterization of the Bacterial Isolates

Cultural Characteristics	Microscopy	Gram Reaction	Spore Test	Catalase	Coagulase	Motility	Citrate	Urease	Oxidase	Sugar Fermentation: Glucose	Maltose	Lactose	Sucrose	Most Probable Organisms
Irregular, milky, flat, undulate, opague	Rods	-	-	+	-	-	+	+	-	AO	AO	AG	AG	*Klebsiella* sp
Rhizoid, light green, crateriform, undulate translucent	Rods	-	-	+	-	+	+	-	+	AG	AG	AG	AG	*Pseudomonas* sp

Pin-point, milky, raised, entire, opaque	Cocci in clusters	+	-	+	+	-	+	+	-	AG	AG	AG	AG	*Staphylococcus* sp
Spindle, milky, convex, filform, translucent	Rods	+	+	+	-	+	+	-	+	AO	AG	AG	AG	*Bacillus* sp
Circular, pink, crateriform, entire, opaque	Cocci in chains	+	-	-	-	-	+	-	-	AG	AG	AG	AO	*Streptococcus* sp

Key: + = Positive, = Negative, A = Acid production, G = Gas production, O = No reaction

Table 4: Cultural and Microscopical Characterization of the Fungal Isolates

Colony Appearance	Surface Colony Colour	Reverse Side Colour	Microscopy	Most Probable Organisms
Powdery	Black	Brown	Non-septate hyphae and stalk-like conidiophores expanding at the apex into swollen dome-like vescicles bearing round and globose conidia	*Aspergillus* sp
Powdery	Yellow	Red	Septate hyphae and cigar-shaped macroconidia with about six cells or compartments, also with round and clustered micro conidia attached to the branched conidiophore.	*Trichophyton* sp
Moist	Creamy	White	Pseudo-hyphae surrounded by oval yeast cells	*Candida* sp
Velvetty	Blue-green with white boarder	White	Septate hyphae and erect, septate multi-branched conidiophores borne at the tips, flask-shaped phialides with round conidia in chain.	*Penicillium* sp

Table 5: Percentage frequency of Occurrence of the Bacterial Isolates

Isolates	Frequency Occurrence (F.O) (n=711)	Percentage of Occurrence (%)
Klebsiella sp.	133	18.7

Pseudomonas sp.	142	20.0
Staphylococcus sp	158	22.2
Bacillus sp	160	22.5
Streptococcus sp	118	16.6

Table 6:Percentage frequency Occurrence of the Fungal Isolates

Isolates	Frequency Occurrence (F.O)	Percentage of Occurrence (%)
Aspergillus sp.	21	31.8
Trichophyton sp	12	18.2
Candida sp.	17	25.8
Pencillium sp	16	24.2

Table 7: Antibiogram of the bacterial isolates from the samples

Antibiotics	Klebsiella sp	Pseudomonas sp	Staphylococcus sp	Bacillus sp	Streptococcus sp
PEF	26	31	28	30	31
CN	-	21	25	-	24
S	-	-	-	-	-
CEP	-	-	-	-	-
SXT	-	30	28	28	33
CPX	31	21	-	-	-
OFX	21	28	-	-	-
AU	32	18	-	-	-
PN	-	-	20	22	29
NA	-	-	31	31	31
AMX	-	-	21	22	25
LEV	-	-	33	33	33
Z	-	-	31	31	31
E	-	-	22	24	27
AZM	-	-	25	19	32
RD	-	-	-	-	-
APX	-	-	-	-	-

Keys: Sensitive = ≥18mm; Resistance = 0–12mm; Intermediate = 13–17mm; SXT - Septrin; CH - Chloramphenicol; S - Streptomycin; AMX - Amoxil; PN - Ampicillin; APX - Ampiclox; CEP - Ceporex; RD - Rifampicin; OFX - Taravid; LEV - Levofloxacin; AU - Augmentin; NB - Norfloxacin; CPX - Ciprofloxacin; E - Erythromycin; CN - Gentamycin; S - Streptomycin.NA - Nalidixic Acid CPX-Ciprofloxacin, PEF - Reflcine, CN -Gentamycin

The study indicated that the potential for clothing items to serve as reservoirs for commensal and pathogenic bacteria and fungi has received attention in the past two decades, as it is relevant to infection prevention and control. The microbial transfer from clothing is possible in two directions, i.e., from the wearer to the clothing and from the clothing to the wearer (Roach-Higgains & Eicher, 2002).. Knowledge of the abundances and species of microbes being transferred in these processes is needed in order to determine any

potential health implications, as well as to determine strategies for mitigating any potential risk. Workers' clothes may act as a vector for the transport of microorganisms, leading to second-hand exposure.

In this study, microbial analysis and antibiogram of workers' clothes in building construction sites were carried out and the results obtained revealed varying degrees of bacterial and fungal colony count from the respective clothes samples based on the region of the body that makes contact with the cloth. The highest bacterial load was recorded for sample D (262 Cfu/cm^2), followed by sample E (202 Cfu/cm^2), sample A (96 Cfu/cm^2), sample B (88 Cfu/cm^2) and the least for sample C (63 Cfu/cm^2).

On the other hand, the highest fungal load was recorded for by sample A (17 Cfu/cm^2), followed by sample C (14 Cfu/cm^2) and sample D (14 Cfu/cm^2), then sample B (11 Cfu/cm^2) and the least for sample E (10 Cfu/cm^2). This result indicates that the microflora of the skin varies according to an individual's hygiene state and the environmental factors. It has been reported that repeatedly wearing of already worn clothing poses a risk of transmitting infectious diseases. Zhai & Maibach (2002), reported that bacterial levels on the surface of clothes worn by men were consistently higher with the neck, armpit, wrist and waist regions, harbouring a diverse and greater number of microorganisms. This is in line with the result of the present study, where higher bacterial counts were recorded for swabbed samples from the armpit and waist regions. There is a risk of developing a highly serious infectious disease from bacteria on the clothing.

The bacterial species isolated and their percentage distribution were: *Klebsiella* sp. (18.7%), *Pseudomonas* sp. (20.0%), *Staphylococcus* sp. (22.2%), *Bacillus* sp. (22.5%) and *Streptococcus* sp. (16.6%). This showed that *Bacillus* was the most predominant bacterial species, followed by *Staphylococcus*, *Pseudomonas*, *Klebsiella* and then *Streptococcus* species. *Klebsiella* is a type of bacteria normally found in human stool (feces) that can cause healthcare-associated infections (HAIs). *Klebsiella* can cause pneumonia, bloodstream infections, wound or surgical site infections, and meningitis. Some *Klebsiella* bacteria are becoming increasingly resistant to antibiotics. *Pseudomonas* is a clinically significant and opportunistic pathogen, often causing nosocomial infections. In addition to causing serious and often life-threatening diseases, these organisms exhibit innate resistance to many antibiotics and can develop new resistance after exposure to antimicrobial agents (Eklöf *et al*., 2022). *Staphylococcus* spp., are Gram-positive bacteria, some of which cause suppurative disease processes in animals and humans. Staph bacteria can cause a variety of infections, including: skin infections, which lead to open sores; infection of the bloodstream, known as bacteremia (Powers and Wardenburg, 2014).

Bacillus species are aerobic, sporulating, rod-shaped bacteria that are ubiquitous in nature. Several other *Bacillus* spp, in particular B cereus and to a lesser extent *B subtilis* and *B. licheniformis*, are periodically associated with bacteremia/septicemia, endocarditis, meningitis, and infections of wounds, the ears, eyes, respiratory tract, urinary tract, and gastrointestinal tract (Kramer and Gilbert, 2019). In humans, diseases associated with the streptococci occur chiefly in the respiratory tract, bloodstream, or as skin infections. Commonly, it causes throat infection (pharyngitis), tonsil infection (tonsillitis), scarlet fever, skin sores (impetigo) and skin infection (cellulitis) (Heth 2015).

Antibiotics are the first line of treatment for infections caused by these bacterial species. Although some isolates showed strong resistance to some of the antibiotics, 85 % of the bacteria isolates were sensitive to the commonly used antibiotics that were used for the research. These results point to the efficacy of the antibiotics in the treatment bacterial infections that may arise due to the

repeated wearing of work clothes at building sites without proper washing and disinfection.

Besides the bacterial isolates, four fungal species namely; *Aspergillus* sp., *Trichophyton* sp., *Candida* sp. and *Penicillium* sp were isolated from the workers clothing samples. Among these fungal species, *Aspergillus* sp. was the most occurring isolate with a percentage occurrence of 31.8%, followed by *Candida* sp. (25.8%), *Penicillium* sp. (24.2%) and then *Trichophyton* sp. (18.2%). These isolates have been implicated in various fungal infections. For instance, Aspergillosis is a group of illnesses caused by *Aspergillus* fungi. Some types include allergic bronchopulmonary aspergillosis (ABPA), aspergilloma, chronic pulmonary aspergillosis and invasive aspergillosis. They usually affect people with weakened immune systems or lung conditions (Thompson and Young, 2021). Trichophyton is a dermatophyte filamentous fungus. It is a common cause of superficial infections such as onychomycosis and various kinds of tinea (ringworm). The fungi can easily spread to other areas of the body as well and to the host's home environs (socks, shoes, clothes, showers, bathtubs, counters, floors, carpets, etc.). They can be transmitted by direct contact, by contact with infested particles (of dead skin, nails, hair) shed by the host, and by contact with the fungi's spores (Gupta , 2007). Candida species for example, *Candida albicans* is an opportunistic pathogenic yeast that is a common member of the human gut flora. Candidiasis is a fungal infection the predominantly caused by *Candida albicans*. *Penicillium* species can cause various diseases in humans, such as keratitis, otomycosis, urinary tract infections, allergic pulmonary disease and peritonitis etc. (Li and Browning, 2010).

The presence of the isolated organisms on the workers' clothing samples is attributed to the contact between the clothing samples and the wearer's body; hence, the transfer of skin microflora to the clothing.

4.0 Conclusion

The results of this study have established the presence of diverse pathogenic microorganisms on workers' clothes at building construction sites. The microorganisms isolated were comprised of five bacterial species; *Klebsiella* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Bacillus* sp. and *Streptococcus* sp., and four fungal *Aspergillus* sp., *Trichophyton* sp., *Candida* sp. and *Penicillium* sp. The presence of these organisms on the workers' clothing samples is an indication of the potential risk of bacterial and fungal infections posed to the workers. The results also point to the efficacy of the antibiotics in the treatment bacterial infections that may arise due to the repeated wearing of work clothe at building construction sites.

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Declarations

Consent for Publication

Not applicable.

Availability of Data and Materials

The publisher reserves the right to make the data publicly accessible.

Ethical Statement

This research was conducted entirely through computational simulations based on first-principles and many-body perturbation theory (MBPT) methods. No human participants or animals were involved in the study. All authors participated voluntarily, upheld scientific integrity, and have been properly acknowledged.

Competing Interests

The authors declare no conflicts of interest. This work represents a collective and collaborative effort among all contributors.

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Authors' Contributions
E.U. and M.P.U. designed the study. E.U. collected samples and performed laboratory analyses, while both authors identified and characterized microbes, conducted antibiogram analysis, reviewed literature, interpreted data, drafted and edited the manuscript, provided supervision, and approved the final version.