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Bacteriological and Mycological Assessments of Second-Hand Wears Procured From Girei And Jimeta Markets, Adamawa State, Nigeria

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Abstract

Used clothes can potentially be an element in the chain of infection transmission during normal daily activities. Second-hand wear is among such used clothes purchased by many people and used directly without considering any attending health implications. This study, therefore, examined the level of microbial contamination of second-hand clothes and the association between the different categories of the clothes and microbial contaminants. Second-hand clothes such as shirts, trousers, vests, socks, and shoes were bought from three Markets in Girei, Yola North and Yola South. Isolation of organisms was carried out via Standard Plate Count. Isolates were identified using morphology and appropriate biochemical methods. A chi-square test (P ≤ 0.05 significant) was done to analyze some of the data obtained. Antibiotic susceptibility of the bacterial isolates was also determined using the Kirby-Bauer method. The most frequently isolated bacteria were Staphylococcus aureus. Other bacteria isolated were Pseudomonas spp and E. coli. A total of four fungal species were isolated. These are Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger and Trichophyton rubrum. Trousers had the highest bacterial count (1.10 x10^3 CFU/mL) and fungal count (8.00 x10^4 CFU/mL). The data obtained shows no significant association between fungal species and the categories of clothes with (P = 0.15), but there is a significant association between bacterial species isolated and the categories of clothes analyzed (P = 0.02). S. aureus was susceptible to ciprofloxacin (100%) and erythromycin (92.8%), while Pseudomonas sp and E. coli were most susceptible to ciprofloxacin and sparfloxacin (100%). The least susceptibility was observed with amoxicillin and sulfamethoxazole-trimethoprim. It became obvious that these clothes are possible carriers of antibiotic-resistant pathogens for skin and other infections. Washing and disinfecting second-hand wear before use may be important in minimizing the potential health implications associated with the isolated pathogens.

Keywords: Antibiotic, Susceptibility, Clothes, Second-hand, Sabouraud Dextrose Agar

INTRODUCTION

Second-hand clothes are clothing that one person has used before the present user. Examples are used shirts, socks, skirts, undergarments, household linens, towels, pillowcases, bedding, tablecloths, and curtains (Didymus, 2012). Some of the used clothes imported into the country are male wear, such as trousers in denim jeans, corduroy, blazers, shirts, jackets, suits, boxers, stockings, and face caps. Female clothes such as skirts, trousers, blazers, blouses, shirts, skirt suits, trouser suits, evening wears, wedding gowns, underwear (comprising of brassieres, pants, leggings, panty hose, camisoles and tights blouses), sweaters, jeans, blazers, and ties. Children's second-hand clothing includes: baby dresses, layettes, shawls, stockings, trousers, dungarees, baby bonnets, christening gowns, and party wears (Areo & Areo, 2015).

Second-hand wear locally called gwanjo, or bend-down select, are popular in Nigeria. Business men/women and charitable organizations import them into the country. In the past, second-hand wears are tantamount with poverty, but today, all classes of people source them because they are cheaper and are thought to be more durable than the new ones. Nigeria comprises over 200 million people, with more than half the population under 30 years of age (National Bureau of Statistics, NBS 2022). This group has a tight budget (mostly youths, unemployed, self-employed, young parents) but still wants to look good and so shop for these wears. Some of these wears could be contaminated with spores from the air, domestic animals and previous users (Hammer et al., 2011).

Nigerian's craving for foreign merchandise (including wears) is on the increases daily. The driving force for the desire for such wear is the quality of the products adjudged better than the new ones despite being cheaper. These are purchased without considering any attending health implications. Clothes have the potential, to be a component in the chain of infection transmission during normal daily activities just as any other hand contact site, (Malnick et al., 2008, Olajubu et al., 2017).

Recently, second-hand clothing skyrocketed as they entered into businesses and clothing stalls. Many buyers start buying them because of their good quality and low prices. Being drawn by the low prices, most buyers would not consider the possible risks they can acquire from these clothing, even as some are concerned about their safety (Abimola, 2012).

Users in various parts of Nigeria do not have adequate access to high quality cloths, so they depend heavily on second-hand wears, which may be contaminated with bacteria from previous users, storage methods, handling or transportation. Second-hand clothes are purchased and worn without properly considering the health implications. Thereby subjecting the users to possible infections by pathogenic bacteria such as *Escherichia coli, Staphylococcus epidermidis, Staphylococcus aureus,* and the coliform bacteria such as *Enterococcus spp*. (Agbulu, et al., 2015, Olajubu et al., 2017). Many forms of skin infections can be attributed to wearing contaminated second-hand cloths (Olajubu et al., 2017). Bloomfield et al., (2011) indicated that clothes have the ability to retain bacteria, fungi and viruses for varied lengths of time.

The need for regular monitoring of used cloths is supported by findings from previous studies. Agbulu et al., (2015) demonstrated the presence of some pathogenic bacteria and fungi from fairly used clothes. *Enterococci spp* and *Staphylococcus aureus* have been isolated from materials regularly worn by patients and healthcare workers (Neely and Maley, 2000). Also, Pilonetto et al. (2004) and Neely and Orloff (2001) isolated *Staphylococcus aureus, Acinetobacter baumani, Klebsiella pneumonia, Candida spp, Aspergillus spp, Mucor spp*. and *Fusarium spp* from fabrics which were linked to nosocomial infections. Olajubu et al. (2017) also isolated some Pathogenic fungi and bacteria from second-hand wear.

Some diligent buyers wash these with different detergents, whereas others wear them unwashed as new clothes. The potential for the spread of infectious agents from infected sources (people, foods, domestic animals) to clothing is moderately high (Bloomfield et al., 2011). Nigerians highly patronize Gwanjo and yet, there is very little information on its health implications and remedy. Thus, thoroughly evaluating the contamination level of these pathogenic organisms found on second-hand wear is paramount. Therefore, this study aims to determine the microbial load on second-hand wear and the association between microbial species and the category of these wear.

MATERIALS AND METHODS

Study Area

The study was conducted in three local Government Areas of Adamawa state: Girei, Yola North and Yola South. Three different markets where second-hand clothes are sold were randomly selected for the study (Jimeta Modern market, Girei Market and Yola town Market).

Sample Collection

A total of fifty-four (54) samples of different categories of second-hand clothes, 30 comprising of shirts, trousers, socks, vests and shoes, (6 each) for bacterial isolation and 24 samples comprising of shirts, trousers, socks, and shoes (6 each) for fungal isolation were obtained randomly from the three selected markets. The samples were labeled appropriately and packaged in sterile resalable polythene bags as described in a previous study (Olajubu et al., 2017) and transported to the laboratory for processing.

Sample Processing

The entire area of each sample was swabbed using a sterile moistened cotton swab, and the swab was immersed in 5ml of sterile distilled water in a test tube to obtain a stock solution, from which serial dilutions were made (Al-Easawi & Emran, 2017).

Bacterial and Fungal Isolation

The different dilutions made were aseptically inoculated in Sabouraud Dextrose Agar (SDA) using the pour plate method and incubated at 20 - 300C for five days for fungal isolation and on Nutrient and MacConkey agar, incubated for 24hr at 370C for bacterial isolation (Cheesbrough, 2006).

Determination of Microbial Load

Numbers of colonies formed on countable plates were counted and the microbial load in colony forming unit [cfu] per ml was determine using the formular by (Owuama, 2015) as thus;

$$\text{Cfu/ml} = \frac{\text{colonies counted on plate}}{\text{volume of inoculum used in ml x dilution times}}$$

Identification of Fungi

Fungal colonies were identified macroscopically by using Lactophenol Cotton Blue and morphologically by observing the colonies' morphology on a Sabouraud dextrose agar and comparing with a standard fungal atlas as described by (Winn et al., 2006).

Identification of Bacteria

Bacterial identification was done macroscopically, where the colony characteristics on Nutrient and MacConkey agar were observed, microscopically by Gram reaction and Biochemically by carrying out the catalase test, oxidase test, motility test, coagulase test, and indole test as described by Cheesbrough (2006).

Statistical Analysis

Data obtained were analyzed using Stata 11 Statistical software and chi-square test analysis to determine the association between species of fungi/bacteria and the categories of cloth, where a P value of ≤ 0.05 was considered statistically significant.

Antibiotic susceptibility test on bacteria isolated from second-hand wears

Bacteria isolates were subjected to an antimicrobial susceptibility test by the standard Kirby. Bauer's disc diffusion method. Inoculum was adjusted to 0.5 McFarland, inoculated on Muller Hinton agar, and allowed to soak for 2 to 5 minutes. Antibiotic discs, i.e., ciprofloxacin (10mcg), Ofloxacin (30ug), Chloramphenicol (30mcg), Pefloxacin (10ug), Gentamycin (10mcg), Sparfloxacin (10ug), Ampiclox (20mcg), Amoxicillin (20mcg), Streptomycin (30mcg), Erythromycin (30mcg), Sulfamethoxazole-trimethoprim (30ug) were placed on the surface of media and pressed gently, and the plates were incubated at 370C for 24h, and the inhibition zones were measured and interpreted by the recommendations of clinical and laboratory standards (CLSI, 2021).

RESULTS

Isolation and Determination of Microbial Load

Fungal and Bacterial growth exhibited a mixture of colonies on SDA, Nutrient and MacConkey Agar respectively (Figures 1a and b). Table 1a shows the fungal and bacterial count in CFU/mL obtained from different categories of second-hand wears, while Table 1b shows the bacterial count in CFU/mL obtained from vest samples. Shoes samples showed higher fungal load and trousers showed higher bacterial load. Microbial count was carried out to assess the level of microbial contamination of the second-hand wears. It indicates the quantity of bacteria and fungi present on each type of clothe.

Identification of fungal and Bacterial Isolates

The fungi and bacteria isolates' characteristic colony appearance on different media, and microscopic as well as biochemical identification of the isolates obtained from the different categories of second-hand wear revealed four fungal and three bacterial species as shown in Tables 2a and b.

Determination of Association between Category of Wear and Microbial Species Isolated

Distribution of bacteria/fungi species isolated based on samples used as presented in Tables 3(a) and (b) shows that there is a significant association between bacterial species and category of wears (P = 0.02), but there is no significant association between fungal species and category of wears (P = 0.16).

Antibiotic susceptibility profile of bacteria isolated from second-hand wears

The antibiotic susceptibility test revealed that S. aureus was susceptible to ciprofloxacin (100%) and erythromycin (92.8%), while Pseudomonas spp and E. coli were most susceptible to ciprofloxacin (100%) and sparfloxacin (100%). The least susceptibility pattern was observed with sulfamethoxazole-trimethoprim. I was resisted by (66.6%) of E.coli, (54.5%) of Pseudomonas spp, and (64.3%) of S. aureus. Amoxicillin was also resisted by (63.6%) of Pseudomonas spp and (57.1%) of S. aureus, as shown in Table 4.

DISCUSSION

All fifty-four samples of second-hand wear analyzed in this study showed different contamination with pathogenic bacteria and fungi. The altitude of patronage given to these cloths in the society makes it a matter of public health importance. Although we have no evidence to show the link between wearing second-hand clothes and any infectious disease, the pathogens isolated have been incriminated in one disease condition or the other (Olajubu *et al.*, 2017). Trousers had the highest bacteria count from this study and are one of the most frequently purchased clothes. *Staphylococcus aureus* was the most frequently isolated bacterium from the analyzed cloth samples. Studies have revealed that Staphylococci spp. can survive in dust and soil particles for years. It can also resist desiccation conditions and tolerant high temperatures, and these bacterial characteristics favour its survival on fabric and clothes for a long time. Such clothes and undergarments are frequently in contact with the skin of the previous owner and are therefore, expected to be colonized with potentially pathogenic bacteria (Muthiani *et al.*, 2010). *S. aureus* was also isolated in previous studies (Al-Easawi & Emran, 2017; Olajubu *et al.*, 2017). *S. aureus* has been incriminated mainly in food poisoning, nosocomial infections with multiple drug-resistant strains, and skin infections such as furuncles, boils, and styles (Khadri & Alzohairy, 2010). This organism is also related to pneumonia, bacteremia, urinary tract infections, and endocarditis (Takashima *et al.*, 2004). Undoubtedly, *S. aureus* isolation from these cloth samples is of public health concern. The result of this study indicated that *Staphylococcus aureus* has the highest prevalence rate among bacteria isolated, which agrees with previous studies. Several bacteria, such as *Staphylococcus aureus, Pseudomonas Micrococcus*, and *Proteus,* were isolated from unwashed second-hand wear (panties, bras, towels, and socks). *Staphylococci* are widespread and can be cultured from shoes, clothes, and almost all environmental surfaces. The outcome of this research also agreed with those of Higaki et al., (2000) and Neely and Orloff (2001). In a similar study, *staphylococcus aureus was* the only bacteria isolated from used clothes samples (Agbulu *et al.*, 2015). But in this very study, *Staphylococcus aureus, E.coli,* and *Pseudomonas spp* were all present in the samples this may be due to differences in the environmental condition and the survival of these organisms.

Pseudomonas aeruginosa, also isolated in this study, has been associated with wound infections, and quite a lot are multidrug-resistant (Nkang *et al.*, 2009). Takashima et al. (2004) found that bound *S. aureus* and *P. aeruginosa have* a high affinity to polyester or acrylic fibers (> 80%) but low to cotton fibers (< 10%).

Figure 1(a). Fungal isolates on SDA.

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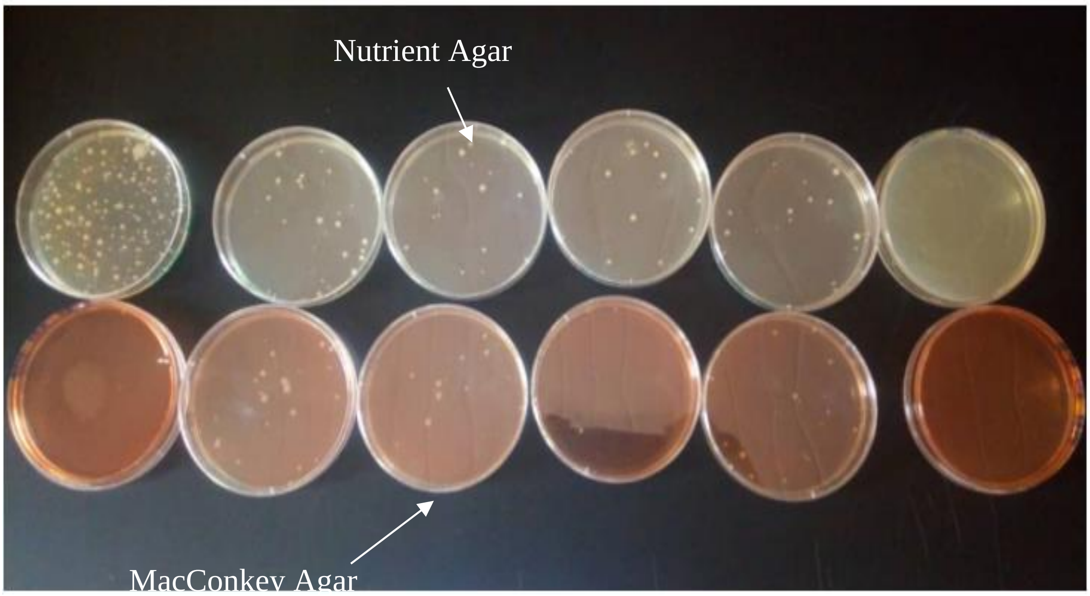

Figure 1(b). Bacterial Colonies Isolated on Nutrient and MacConkey Agar.

Table 1(a): Fungal and bacterial count from different categories of second-hand wears

Sample Used	Sample Number	Fungal Count CFU/mL	Bacterial Count CFU/mL
Shoes	1	8.00×10^4	5.50×10^3
	2	1.20×10^5	1.25×10^3
	3	4.10×10^5	2.50×10^3
	4	2.10×10^5	4.50×10^3
	5	2.50×10^5	2.50×10^3
	6	4.70×10^5	8.00×10^3
Socks	1	1.60×10^2	1.40×10^3
	2	5.00×10^2	1.85×10^3
	3	1.00×10^3	3.00×10^3
	4	3.50×10^3	2.50×10^3
	5	3.10×10^3	1.75×10^3
	6	1.40×10^3	1.55×10^3
Shirt	1	1.10×10^3	1.50×10^3
	2	1.90×10^3	2.30×10^3
	3	7.00×10^2	3.00×10^3
	4	7.00×10^2	3.10×10^3
	5	1.30×10^3	3.10×10^3
	6	9.00×10^2	5.30×10^3
Trouser	1	1.60×10^3	2.40×10^3
	2	7.00×10^2	1.50×10^3
	3	5.00×10^2	3.30×10^3
	4	1.10×10^3	1.85×10^3
	5	8.00×10^2	2.00×10^3
	6	5.00×10^2	1.10×10^4

Table 1(b): Bacterial count on vest Second-hand wears

Sample	Sample Number	Bacterial Count CFU/ml
Vest	1	2.10×10^3
	2	3.10×10^3
	3	3.40×10^3
	4	3.10×10^3
	5	3.20×10^3
	6	3.66×10^3

Table 2(a): Morphological and microscopic identification of fungal isolates

S/N	Cultural characteristics	Microscopy	Probable fungi
1	Bluish-green powdery colony with white filament,	Presence of conidiophores with hyphae	*Aspergillus fumigatus*
2	Darkly pigmented with white filament on the front and pale yellow on the reverse.	Conidiophores with phialides protrude from septate hyphae.	*Aspergillus niger*
3	Yellow-green powdery colony	Septate hyphae with conidiophores and phialides radiating from the vesicle.	*Aspergillus flavus*
4	White cottony appearance	Microconidia attached to hyphae.	*Trichophyton rubrum*

Table 2(b): Cultural characteristics, Gram-staining, and Biochemical Identification of Bacterial Isolates

Samples	**Cultural Characteristics**		**Microscopy**	**Biochemical Test**			**Probable bacteria**
	NA	**MA**	**GR**	**Mo**	**Cat**	**Coa**	
Vest	Round, smooth yellow colonies	-	+ve cocci	-ve	+ve	+ve	*Staphylococcus aureus*
Socks	Round, smooth yellow colonies	-	+ve cocci in clusters	-ve	+ve	+ve	*Staphylococcus aureus*
	White circular smooth colonies	Pink to red	-ve rod	+ve	+ve	-ve	*Escherichia coli*
	Large opaque mucoid	Brown lactose fermenters	-ve rod	+ve	+ve	-ve	*Pseudomonas spp*
Shoe	Round, smooth yellow colonies	-	+ve cocci in clusters	-ve	+ve	+ve	*Staphylococcus aureus*
	White circular smooth colonies	Pink to red	-ve rod	+ve	+ve	-ve	*Escherichia coli*
	Large opaque mucoid	Brown lactose fermenters	-ve rod	+ve	+ve	-ve	*Pseudomonas spp*
Shirts	Round, smooth yellow colonies	-	+ve cocci in clusters	-ve	+ve	+ve	*Staphylococcus aureus*
	Large opaque mucoid	Brown lactose fermenters	-ve rod	+ve	+ve	-ve	*Pseudomonas spp*
Trouser	Round, smooth yellow colonies	-	+ve cocci in clusters	-ve	+ve	+ve	*Staphylococcus aureus*
	Large opaque mucoid	Brown lactose fermenters	-ve rod	+ve	+ve	-ve	*Pseudomonas spp*

Note: +ve: Positive. -ve: Negative. Cat: Catalase, Coa: Coagulase, Mo: motility, NA: Nutrient agar, MCA: MacConkey agar, GR: Gram reaction.

Table 3(a): Distribution of Fungal Species isolated based on Categories of Second-hand clothes

Sample	*Aspergillus flavus*	*Aspergillus fumigatus*	*Aspergillus niger*	*Trichophyton rubrum*
Shoes	0	6	0	0
Socks	1	6	6	2
Shirts	1	5	6	0
Trousers	2	3	5	0

P= 0.158

Table 3(b): Distribution of Bacterial Species isolated based on Categories of Second-hand clothes

Samples	*S. aureus*	*P. aeruginosa*	*E.coli*
Vests	6	0	0
Socks	1	6	5
Shoes	4	6	3
Shirts	2	6	0
Trousers	6	5	0

P= 0.02.

Table 4: Antibiotic susceptibility profile of bacteria isolated from second-hand wears

Isolate	CPX (%)	E (%)	S (%)	OFX (%)	PEF (%)	SP (%)	SXT (%)	AM (%)	CH (%)	CN (%)	R (%)
S. aureus											
S	100	92.8	14.3	-	21.4	-	0	35.7	-	71.4	78.6
I	0	0	85.7	-	71.4	-	35.7	7.1	-	28.6	21.4
R	0	7.1	0	-	7.1	-	64.3	57.1	-	0	0
E. coli											
S	100	-	86.6	100	80	100	13.4	53.4	85	26.4	-
I	0	-	13.4	0	13.4	0	20	26.6	15	13.6	-
R	0	-	0	0	6.6	0	66.6	20	0	60	-
P. spp											
S	100	-	81.8	63.3	82	100	45.5	36.4	81.8	72.4	-
I	0	-	18.2	27.6	18.0	0	0	0	18.2	18.5	-
R	0	-	0	9.1	0	0	54.5	63.6	0	9.1	-

KEY: SXT: sulfamethoxazole-trimethoprim, CN: Gentamycin, AP: Ampiclox, CPX: Ciprofloxacin, SP: Sparfloxacin, OFX: Ofloxacin, AM: Amoxicillin, CH: Chloramphenicol, S: Streptomycin PEF: Pefloxacin, E: Erythromycin, (-) Not tested

E. coli, which are coliform bacteria, were also isolated in this study and are accountable for most uncomplicated cystitis cases in women, particularly young women. *E. coli* is generally a harmless microorganism originating from the intestines. If it spreads to the vaginal opening, it may invade and colonize the bladder, causing an infection.

Aspergillus species, which include *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, and *Trichophyton rubrum*, which were isolated in this study, can cause skin infections and respiratory problems, among other serious medical consequences. These fungi can cause respiratory issues like asthma exacerbations and skin infections such as athlete's foot and ringworm (Kwon-Chung & Sugui, 2013). They are commonly present in indoor and outdoor environments and can contaminate various items such as fabrics and shoes. When individuals come into contact with these contaminated items, fungal spores are likely to adhere to their skin, which can pose a health risk (Shelton *et al.*, 2002).

Fungi survive longer than bacteria on fabrics; hence, they are constantly on cloth materials. *Aspergillus spp*, the most commonly isolated fungi in this study, can potentially cause pulmonary and corneal infections (Olajubu *et al.,* 2017). *Aspergillus flavus,* also isolated in this study, has long been known to cause a potentially deadly threat of infections for hospital patients with injured or impaired immune systems (Neely & Orloff, 2001). The isolation of *A. niger* and *A. Flavus* in this study is in line with the work of Al-Easawi and Emran (2017), who also isolated these organisms from Second-hand cloths.

All bacteria isolated in this study were 100% susceptible to only one antibiotic, ciprofloxacin. Some are susceptible to more than one antibiotic but also highly resistant to many antibiotics. *Escherichia coli* were 100% sensitive to three antibiotics and highly resistant to more than one antibiotic, gentamycin and sulfamethoxazole-trimethoprim (60% and 66.6% respectively). The gram-positive bacteria Staphylococcus aureus were resistant to sulfamethoxazole-trimethoprim and amoxicillin but showed varying susceptibility patterns to other antibiotics: ciprofloxacin, erythromycin, and streptomycin. The pattern of antimicrobial resistance varies in different regions. However, the antibiotic sensitivity patterns of organisms change fast over a short period. This is especially true for developing countries where only medical practitioners do not sell antibiotics but are purchased from pharmacists without a prescription (Ibeawuchi and Mbata 2012). Therefore, periodic evaluation of sensitivity patterns is indispensable for the coherent and proper use of antibiotics (El-Astal *et al.,* 2005).

CONCLUSION

Assorted potential pathogens were isolated from the second-hand clothes, with trousers and shoes having the highest microbial count for bacteria and fungi. These isolated pathogens are also incriminated in life-threatening diseases. Though there was no significant association between the category of wear studied and fungi species isolated, there was a significant association between the category of wear studied and bacterial species examined. All bacteria isolated in this study were 100% susceptible to only one antibiotic, ciprofloxacin. Some are susceptible to more than one antibiotic but also highly resistant to many antibiotics.

RECOMMENDATION

Proper hygiene practices, such as washing and disinfecting second-hand clothing and footwear before use, are important to minimize the potential health implications of the isolated bacteria and fungi. The urge for fairly used clothes because of the assumption that they are cheaper and of better quality than new ones will have to be weighed carefully given the likely attending health implications. Bacterial and fungal contamination of this type of clothing can significantly impact everyday life, affecting not only personal health but also the cleanliness and hygiene of the living environment. Understanding the potential health risks associated with this type of clothing can help emphasize the importance of proactive measures to prevent and control these bacterial and fungal infections.

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