MICROBIOLOGICAL ASSESSMENT OF FEMALE SECOND-HAND UNDERGARMENTS SOLD IN ABRAKA, NIGERIA.

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ABSTRACT
Second-hand female undergarments sold in Abraka, Nigeria, were analyzed for bacteria and fungi using standard methods. Bacteria isolated were subjected to antibiotic susceptibility tests and analyzed for Multi-Antibiotic Resistance (MAR) using the Kirby-Bauer disc diffusion method. Microbial load of samples revealed lower counts in control (new undergarments (Pants and bras)) – 2 x 10^2, 5.5 x 10^3 and 0.5 x 10^2cfu//ml; counts for fairly used pants were 4.6 x 10^4, 3.7 x 10^4 and 3.2 x 10^2 cfu/ml while fairly used bras were 2.7 x 10^3, 4.8 x 10^4 and 1.5 x 10^2 cfu/ml for total aerobic counts(TAC), coliform counts (CC) and fungal counts (FC) respectively. Among the bacteria isolated were *Staphylococcus* spp, *Bacillus* spp, *Escherichia* spp, *Klebsiella* spp and *Pseudomonas* spp. Fungi isolated were *Trichoderma* spp, *Geotrichum* spp, *Rhizopus* spp, *Trichophyton* spp, *Fusarium* spp, *Penicillium* spp and *Aspergillus* spp. The highest frequencies of occurrence were recorded for *Klebsiella* spp and *Pseudomonas* spp with each accounting for 20.7% of the bacteria isolated. *Rhizopus* spp, on the other hand, had the highest prevalence (30.8%) among isolated fungi. In respect to sample types, second hand pants were found to be most colonized by microorganisms as they accounted for 43% and 53% of the isolated bacteria and fungi respectively. *Bacillus* spp isolated from fairly used bras generally presented resistance to antibiotics with MAR index values of 0.4-0.7. All gram positive bacteria isolated were resistant to amoxicillin. *Escherichia* spp isolates from second pants were resistant to septrin, chloramphenicol, gentamycin, streptomycin, cotrimoxazole and sparfloxacin. However, fluoroquinolone antibiotics (ciprofloxacin and ofloxacin) were most effective against isolated bacteria with 100% susceptibility recorded. The isolation of fungi and MAR bacteria are of public health concern and suggest the need for proper laundry of second hand clothes prior to use.

Keywords: Undergarments, Second Hand, Multi-Antibiotic Resistance, Bacteria, Fungi, Abraka.

INTRODUCTION
Second-hand clothes popularly called ‘Okirika’, ‘Bend-down-select’ and more recently ‘thrift wears’ in Nigeria have gained a lot of importance among the populace due to the present rise in poverty and inflation. Due to economic problems the cost of purchasing brand new clothing has become expensive and as a result, most people settle for second-hand clothes which are

seemingly affordable and relatively new depending on the grade (Rakhshanpour *et al.*, 2020). Second-hand clothes are generally defined as clothing items (shirts, garments, under-wears, beddings, socks) that have been previously used by someone other than the present user (Agbulu *et al.*, 2015).

According to Ogbonnaya *et al.* (2017), the use of second hand clothing in Nigeria antedates the Nigerian civil war; a market which thrived mainly among the "Igbos" of Eastern Nigeria. Though, several times after the civil-war, there have been attempts by government to ban the importation of fairly used clothes, the business has survived and provides an alternative to individuals who are unable to afford entirely new clothes. Also, second hand clothes are sought after due to a general but sentimental acceptance that they are usually of better quality than brand new clothes commonly found in markets within Nigeria (Olajubu *et al.*, 2017).

Microorganisms are known to be ubiquitous and as such are seen to inhabit microenvironments within fomites with minimum nutrients. Therefore, Second hand clothes have an inherent ability to retain and transmit microorganisms from the previous user(s) to the final user (Atubu *et al.,* 2016). These microorganisms may be commensals or pathogens from the epidermis of previous users and may also be microbes that are picked up during packaging and exportation/importation. Usually clothes and undergarments come in contact with bodily fluids and secretions such as sweat, saliva, vaginal discharge, semen and secretions from wounds and carbuncles. Some of these secretions carry microorganisms while others promote growth of microorganisms which may persist within the fabric for a long period of time (Agbbulu *et al.,* 2016). According to Abney *et al.* (2021), there is a likelihood that any pathogen associated with human illness may be found in clothing and most other textiles. Several studies indicate that microorganisms may survive on textiles for extended periods of time and infectious diseases can be transmitted directly by contact with contaminated textiles (Owen and Laird, 2020)

In an assessment 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. The isolates, however, only occurred in some of the sampled bras, briefs and perianal regions of long pants. Also, Awe and Abuh (2016) isolated seven different bacterial species including *Staphylococcus aureus*, *E. coli*, *Klebsiella* sp., *Pseudomonas aeruginosa* and *Proteus vulgaris* from second-hand garments purchased from International market Lokoja, Kogi State Nigeria.

This study, therefore, focused on a microbiological assessment of second-hand female underwear (pants and bras) sold in Abraka, Delta State, Nigeria. The major goal was to create awareness of the microbial quality of these goods and to drive home the need for proper hygienic measures after purchase prior to usage.

MATERIAL AND METHODS

Study Area

The study was conducted within Abraka, a University town, in Delta state, Nigeria. Abraka is marked by a uniform topography and humid weather. Due to the presence and influence of the Delta State University main campus, urbanization and population growth are constantly on the increase. However, the major populations in Abraka include students (majority of who are transient), farmers (mainly indigenes), civil servants and traders. According to Ugbomeh (2010), human population in Abraka is majorly constituted of females and people within the age brackets of 16 to 36 years.

Sample collection

Three categories of undergarment samples; fairly used panties, fairly used bras and brand new undergarments (a mixture of pants and bras) were purchased from Abraka main market. A total of 15 samples - five (5) for each category - were purchased from various stands within the market. Samples were collected distinctively in polythene bags previously sterilized by soaking in 95% ethanol and allowing to air-dry. The entire surface area of samples was swabbed with the use sterile cotton swabs immersed in 5ml sterile normal saline. The swabs were used to inoculate culture media plates.

Culture/Isolation

Nutrient agar and MacConkey agar were used for the cultivation of bacteria while Sabourad dextrose agar (SDA) was used for the isolation of fungi, Violet Red Bile (VRB) agar and Brilliant green lactose bile (BGLB) broth were used for confirmation of coliform bacteria. Upon inoculation, plates required for the growth of bacteria were incubated at 37^{o}C for 24hr while those for the isolation of fungi were incubated at room temperature (28 ± 2^{o}C) for a period of 72hr. Colonies were counted and recorded.

Identification of Isolates

Pure cultures of isolates were put in Agar slants and stored at 4^{o}C in a refrigerator until required. Bacterial isolates were identified using procedures outlined by Cheesbrough (2006) while pure fungal isolates were identified using the procedure reported by Harrigan and McCance (1976).

Antimicrobial Susceptibility Testing

The Kirby-Bauer disc diffusion method (Bauer *et al.,* 1996) was used to study the antibiotic susceptibility pattern of bacterial isolates obtained from undergarment samples. Overnight pure cultures inoculated into normal saline and standardized to 0.5 McFarland were used to inoculate sterile Mueller Hinton agar (MHA) plates and spread over the entire surface of the growth medium using sterile swabs. These were allowed to stand for 5 min to ensure drying. Commercially prepared antibiotic discs, purchased from Maxicare Medical Laboratories, Lagos Nigeria, were then placed onto the surface of the MHA and incubated at 35 ± 2^{o}C for 18-24 h. The antibiotic discs used consisted of pefloxacin (10µg), ceftriaxone (25µg), ciprofloxacin (10µg), erythromycin (10µg), gentamycin (10µg), streptomycin (30µg), cefuroxime (20µg), ampicillin/cloxacillin (30µg), cotrimoxazole (30µg) and amoxacillin (30µg) for gram positive isolates. Discs used for gram negative isolates were ciprofloxacin (10µg), ofloxacin (10µg), gentamycin (10µg), amoxacillin (30µg), streptomycin (30µg), pefloxacin (30µg), co-amoxiclav (30µg), chloramphenicol (30µg), cotrimoxazole (30µg) and sparfloxacin (10µg). After the incubation period, zones of inhibition were measured, recorded and interpreted following the Clinical Laboratory Standards Institute guidelines (CLSI, 2020).

Multi- Antibiotic Resistance (MAR) Index

The Multi-Antibiotic Resistance index of isolates obtained was calculated using the methods of Odum *et al.* (2020). Multiple Antibiotic Resistance (MAR) index was calculated as follows:

MAR index = a/b

Where, a = number of antibiotics resisted by an isolate; b = total number of antibiotics the isolate was exposed to.

RESULTS

The results obtained from microbial load assessment of fairly used undergarment sold in Abraka, Nigeria are presented in Table 1. It indicated that fairly used undergarments were pleated with higher populations of microorganisms as compared with controls (new

undergarments). Fairly used pants had the highest total aerobic and fungal counts while second hand bras presented the highest coliform counts.

Table 1: Microbial load of samples (cfu/ml)

Samples	TAC	CC	FC
Fairly used Pants	4.6 x 104	3.7×10^4	3.2×10^3
Fairly used Bras	2.7×10^3	4.8×10^4	1.5×10^3
Control (new pants and bras)	2×10^2	5.5×10^3	0.5×10^2

Key: TAC = Total aerobic counts, CC = Coliform counts, Fc = Fungal counts

The prevalence of bacterial species on samples are presented in Tables 2. *Bacillus* spp and *Staphylococcus* spp were the only gram positive isolates obtained. *Bacillus* spp was most predominant (17.24%) while *Staphylococcus* spp was 3.45% prevalent. However, *Staphylococcus* spp was only isolated from controls as against *Bacillus* spp which was present in both controls and fairly used bras. Among the gram negative isolates, *Pseudomonas* spp and *Klebsiella* spp presented the highest prevalence, with both organisms having an occurrence of 20.69% each. *Escherichia* sp. was isolated from fairly used pants and controls. Of all sample types, fairly used pants presented the highest number of bacterial isolates which were only gram negative organisms.

Table 2: Prevalence of bacterial isolates

Isolates	Prevalence N (%)			
	Fairly used Pants	Fairly used Bras	Control	Total (%)
Gram +ve				
Staphylococcus spp	0(0)	0(0)	1(3.45)	1(3.45)
Bacillus spp	0(0)	3(10.34)	2(6.90)	15(17.24)
Gram –ve				
Escherichia spp	3(10.34)	0(0)	2(69)	5(17.24)
Klebsiella spp	5(17.24)	0(0)	1(3.45)	6(20.60)
Pseudomonas spp	3(10.34)	3(10.34)	0(0)	6(20.69)
Salmonella spp	0(0)	2(6.90)	1(3.45)	3(10.35)
Shigella spp	3(10.34)	0(0)	0(0)	3(10.34)
Total (%)	14(48.28)	8(27.59)	7(24.14)	29(100)

Fungi isolated from fairly used pants were *Trichoderma* spp, *Geotrichum* spp, *Rhizopus* spp and *Penicillium* spp (Table 3). It was observed that controls carried less fungal load than fairly used

garments with only *Rhizopus* spp and *Aspergillus* spp isolated. Highest prevalence of fungi was observed with fairly used pants (53.85%).

Table 3: Prevalence of fungi

Isolates	Prevalence N (%)			
	Fairly used (%) Pants	Fairly used Bras	Control	Total
Trichoderma spp	1(7.69)	1(7.69)	0	2(15.39)
Geotrichium spp	1(7.69)	0	0	1(7.69)
Fusarium spp	1(7.69)	0	0	1(7.69)
Rhizopus spp	2(15.39)	1(7.69)	1(7.69)	4(30.77)
Penicillium spp	1(7.69)	0	0	1(7.69)
Aspergillus spp	0	1(7.69)	1(7.69)	2(15.39)
Trichophyton spp	1(7.69)	1(7.69)	0	2(15.39)
Total (%)	7(53.85)	4(30.77)	2(15.39)	13(100)

The antibiotic resistance profiles of bacterial isolates are presented in Tables 4 and 5 for gram positive and gram negative isolates respectively. *Bacillus* spp isolated from fairly used bras generally presented high resistance to antibiotics with MDR index of 0.7 while *Staphylococcus* sp. isolated from the controls was found to be resistant to six (6) out of ten (10) antibiotics. Gram positive bacteria were all resistant to amoxicillin. Among the gram negative isolates, *Pseudomonas* spp had minimum resistance to the various antibiotics tested with MDR index range of 0.1 to 0.3. On the other hand, *Klebsiella* spp and *Escherichia* spp isolated from fairly used pants demonstrated high resistance to antibiotics. *Escherichia* spp isolates from second hand pants generally exhibited resistance to six antibiotics tested- septrin, sparfloxacin, chloramphenicol, gentamycin, perfloxacin and streptomycin. Ciprofloxacin and ofloxacin (fluoroquinolones) however, appeared to be the most effective antibiotics against bacteria isolated from fairly used pants and bras as 100% susceptibility was recorded.

Table 4: Antibiogram of g+ve bacterial isolates

Isolate	Sample	MARI	Antibiotics resistant to
Staphylococcus spp	Control (new pants and bras)	0.6	Streptomycin, cotrimoxazole, erythromycin, ampicilin/cloxacilin, amoxacilin,ceftriaxone
Bacillus spp	Control (new pants and bras)	0.4	Gentamycin, cotrimoxazole, amoxacilin, ampicilin/cloxacilin
Bacillus spp	Fairly used bras	0.7	Streptomycin, cotrimoxazole, erythromycin, Pefloxacin, amoxacilin, ampicilin/cloxacilin, ceftriaxone

Table 5: Antibiogram of g-ve bacterial isolates

Isolate	Sample	MARI	Antibiotics resistant to
Escherichia spp	Control (new pants and bras)	0.4	Cotrimoxazole, chloramphenicol, Pefloxacin, Streptomycin
Escherichia spp	Fairly used pants	0.7	Cotrimoxazole, chloramphenicol, sparfloxacin, co-amoxiclav, Gentamycin, Pefloxacin, Streptomycin
Klebsiella spp	Control (new pants and bras)	0.4	Cotrimoxazole, amoxacilin, Pefloxacin, Streptomycin
Klebsiella spp	Fairly used pants	0.7	Cotrimoxazole, sparfloxacin, amoxacilin, co-amoxiclav, Gentamycin, Pefloxacin, Streptomycin
Pseudomonas spp	Fairly used pants	0.2	co-amoxiclav, Gentamycin
Pseudomonas spp	Fairly used bras	0.3	Cotrimoxazole, co-amoxiclav, , Pefloxacin
Salmonella spp	Control (new pants and bras)	0.3	Cotrimoxazole, sparfloxacin, Streptomycin
Salmonella spp	Fairly used bras	0.2	Cotrimoxazole, Streptomycin
Shigella spp	Fairly used pants	0.5	Cotrimoxazole, , sparfloxacin, , co-amoxiclav, Gentamycin, Streptomycin

The frequency of MAR indexes among isolated bacteria is presented in Table 6. Bacterial isolates with MAR indexes above 0.2 are assumed to be from high risk contamination sources. A total of 79.31% of the bacteria isolated from fairly used clothes and controls presented MAR indexes above 0.2. However, 17.24% resistance was observed for 6 and 7 antibiotics respectively.

Table 6: Frequency of MAR index among isolates from samples

Number of antibiotics tested	Number of antibiotics resisted	MAR Index	Frequency of MAR Index in isolates n (%)	Risk Source (%)
10	1	0.1	1(3.45)	20.69
	2	0.2	5(17.24)	
	3	0.3	4(13.79)	79.31
	4	0.4	6(20.69)	
	5	0.5	3(10.35)	
	6	0.6	5(17.24)	
	7	0.7	5(17.24)	
	Total		29	

DISCUSSION

The microbial loads obtained from fairly used underwear and controls in this study is not surprising as it is well known that textiles support the growth of microorganisms. They inherently possess the ability to degrade natural fibres and to a lesser extent, synthetic fibres (Sanders *et al,* 2021). Textile-degrading taxa include the fungal genera Aspergillus and Penicillium and bacterial genera Bacillus and Pseudomonas which are among the microbes isolated from fairly used undergarments in this study. The difference in microbial loads of fairly used undergarments and new ones which served as controls can be attributable to several factors such as previous usage of fairly used clothing and packaging of new undergarments which helps reduce exposure to microbes or spores carried by air and hand (Briones *et al.,* 2016). However, the findings of this study suggest that clothes, whether new or fairly used, carry microorganisms and should be subjected to processes that improve hygiene (washing and ironing) prior to use. The findings of this study are in line with those of Olajubu *et al.* (2017) who reported lower microbial counts in new garments as against fairly used clothes. The researchers further suggested that the source of microorganisms on new clothes could be traced to sellers' carrier status and frequent touching by buyers amongst other factors Olajubu *et al.* (2017).

Most of the bacterial genera isolated from fairly used undergarments as well as controls which are deemed brand new have reportedly been isolated by other researchers. In line with the findings of this study, Agbulu *et al.* (2015), Awe and Abuh (2016), Olajubu *et al.* (2017) and Baharuddin and Nurhatira (2020) all reported the isolation of *Bacillus* sp from fairly used undergarments. Contrary to the findings of Olajubu *et al.* (2017) and Briones *et al.* (2016), *Staphylococcus* sp was not among organisms isolated from fairly used undergarments. Although, the presence of the organisms in new undergarments, which served as controls, may be traced to the process of touching during bargaining. It is evident that the organism is rarely found in fairly used undergarments due to the conditions of storage and transportation which may not be friendly for its survival. Similar to this finding are those of Agbulu *et al.* (2015) as well as Baharuddin and Nurhatira (2020) who reported that the bacteria isolated from fairly used female undergarment belong to the Bacillus genera. Also, gram negative bacterial isolates such as *Pseudomonas* sp. and *Escherichia coli* have been previously reported as inherent in second hand female undergarments (Awe and Abuh, 2016; Olajubu *et al.,* 2017). Abney *et al.* (2021) stated that enteric disease causing bacteria such as *Salmonella* sp. have been associated

with textiles. The presence of these microorganisms in second hand clothes is an indication of their poor hygienic conditions.

The isolation of molds from second hand undergarments is attributable to their ability to degrade fabric and their ability to survive under harsh conditions. Although some fungi such as *Geotrichum* sp. are considered members of the human normal flora (sputum and feces), majority of the fungal isolates can be traced to environmental sources. Similar to the findings of this study, Agbulu *et al* (2015) isolated *Penicillium* sp and *Tricophyton* sp. from female second hand undergarments sold within Makurdi metropolis, Nigeria. Also, Olajubu *et al* (2017) among other fungal isolates recorded the presence of *Penicillium* sp. and *Aspergillus* spp. in female undergarments sold in a Lagos market, Nigeria. Similarly, Awe and Abuh (2016) isolated *Penicillium, Aspergillus, Trichoderma and Fusarium* from second hand clothes sold in an International market in Lokoja, Nigeria. *Rhizopus* and *Aspergillus* have long been known as capable of causing deadly infections especially in immunocompromised individuals. *Trichophyton* is known to be a dermatophyte capable of infecting the skin, hair and nails. Also, *Fusarium* sp. is capable of inducing fusarial infections on skin and wound sites even with immunocompetent individuals. It is, therefore, suggestible that individuals who indulge in the purchase of fairly used undergarments are predisposed to various forms of skin related infections both from fungi and bacteria.

Antibiotic resistance is a growing challenge within the health care sector. The isolation of antibiotic resistant bacteria from fairly used undergarments indicates a pending public health risk for those who may eventually purchase and wear them. *Escherichia* sp., *Klebsiella* sp. and *Staphylococcus* sp., which are among bacteria isolated from undergarments in this study, have been implicated in vaginitis (Kaambo *et al.*, 2018). Apart from predisposition to infectious organisms, difficult-to-treat infections as a result of high antibiotic resistance among isolates may set in. The high rates of resistance to cotrimoxazole is similar to the reports of Mulu *et al.* (2015) who reported high resistance of vagina bacterial isolates to amoxicillin (82.2%) and cotrimoxazole (62.2%). Same researchers, in line with the findings of this study, reported high levels of sensitivity to ciprofloxacin (79.6%) and gentamycin (77.6%).

According to Ayandele *et al.* (2020), MAR index is an effective method in source tracking of antibiotic resistant organisms. They suggested that an MAR value greater than 0.2 indicates a high risk source of contamination where antibiotics are frequently used or abused. With 79.31% of bacteria isolated in this study presenting MAR indexes above 0.2, it is safe to suggest that

most of the second hand clothes sampled were from high risk contamination sources where previous users or sellers are either frequent users or abusers of antibiotics. Despite the high rates of MAR recorded, fluroquinolone antibiotics (ciprofloxacin and ofloxacin) appeared to be the most efficient among antibiotics used in this study, with 100% susceptibility rates recorded. These drugs may, however, serve as a last resort in cases of recalcitrant infections.

CONCLUSION

The study has demonstrated that fairly used clothes are heavily colonized by microorganisms including bacteria and fungi which may be pathogenic. Also, the isolation of microorganisms from controls indicates the necessity for proper laundry and hygienic practices prior to use of recently purchased clothing. It can also be concluded that fairly used clothes, though a cheaper alternative to brand new clothes, may serve as agents of infection dispersion/transmission between populations. Furthermore, the high resistance to antibiotics among isolated bacteria calls for special care and awareness. Marketers of fairly used clothes can also do well to track the sources of their goods as the findings of this study suggests that most of the sampled second hand undergarments may have originated from high risk contamination sources.

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