



Original Research Article

Fairly used adult male and female pants: Contributors of methicillin-resistant *Staphylococcus aureus* (MRSA) spread in Abakaliki, Ebonyi State, Nigeria

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Abstract

Introduction: The importation and sales of second-hand clothing, commonly known as "Okirika," have become prevalent in Nigeria due to their affordability and accessibility. However, these garments often harbor a variety of microorganisms, including *Staphylococcus aureus*, which can lead to skin infections and other health issues.

Aim and Objectives: This study was designed to determine the occurrence of Methicillin-resistant *Staphylococcus aureus* (MRSA) from fairly used male and female pants vended in Abakaliki, Ebonyi State, Nigeria and to evaluate their susceptibility and resistance to different classes of antibiotics.

Materials and Methods: A total of 50 samples (25 each from female panties and male panties) of fairly used panties from freshly loosened bales were analyzed. *Staphylococcus aureus* were isolated and characterized using standard microbiological and biochemical methods. Methicillin resistant *S. aureus* was determined using Chromatic agar. Antibiogram of the MRSA were determined using disc diffusion methods.

Result: The results showed that 36 out of the 50 samples harboured *Staphylococcus aureus*. Of the 36 *S. aureus* isolates, 26 (representing 72.2%) were positive for methicillin-resistance while only 10 (representing 27.8%) were negative. Of the 26 that were MRSA positive, 14 (53.85%) were obtained from female panties and 12 (46.15%) from male pants. The antibiogram of the MRSA positive isolates showed that imipenem and doxycycline were most active against them while tobramycin and ciprofloxacin were the least in their activity. However, the isolates from male pants were 100% resistant to meropenem, cefepime, ceftioxin and vancomycin. Their resistance to tetracycline and amoxicillin ranged from 91.7% to 100%.

Conclusion: The occurrence of multidrug resistant Methicillin-resistant *Staphylococcus aureus* in fairly used clothes is of public health concern and suggests the need for proper disinfection and laundry prior to use.

Keywords: Male pants, Female pants, MRSA, Antibiotic resistance

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1. Introduction

The quest for cheap, unique and quality clothes by Nigerian citizens has made fairly used materials, especially clothes, a lucrative and sustainable business in the region. Many buyers test it for fitness, while some even use it without washing perhaps due to lack of knowledge of the possible health risks. Fairly used clothes, also known as second-hand clothes, are items of clothing that have been used by someone different

from the current owner.¹ They are generally called "Okirika", "Bend-down-select" or simply "Ok" in Nigeria. These clothes can include used items such as panties, socks, shirts, skirts, bras.² Most of these clothes are imported from different countries of the world.¹ During importation, used clothes are packed in bundles called bales, and the demand for foreign goods, including clothing, continues to rise in Nigeria. While these clothes are often cheaper, the main attraction is their perceived better quality compared to new local products.

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Often time, people buy and use them not being aware of the associated health dangers.³ Items like fairly used clothes that come in contact with the human body can play vital role in transmitting infections.³ Owing to the widespread use and health dangers associated with fairly used clothes, Rwanda Standards Board (RSB) banned the trade and use of fairly used underwear in Rwanda.⁴

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a Gram-positive, non-motile, spherical shaped bacterium genetically different from other strains of *Staphylococcus aureus*.^{5,6} It is known to cause several hard-to-treat infections (such as skin infections, osteoarthritis, and respiratory tract infections) in humans, occurring in both community and hospital settings.⁷ Infections caused by MRSA can result in longer hospital stays, increased healthcare costs, and higher mortality rates.⁸ Carriers of MRSA are also at risk of septicemia, wound infections, and toxic shock syndrome. The WHO in 2017 classified MRSA as a "Priority pathogen" because of the spread of the associated life-threatening diseases and its significant impact on public health globally, causing high rates of illness, death, and increased healthcare expenses.⁸

The practice of buying and selling fairly used clothes is common in many regions, including Abakaliki, Ebonyi State, Nigeria, where markets such as the International Market facilitate the trade of these items. According to literature, microbiological surveys of second-hand clothes in Nigeria have often recovered *S. aureus*, faecal indicators, and other potential pathogens including *Corynebacterium*, *Clostridiales*, and *Candida species*. Research studies from Makurdi, Abraka, and Anambra markets reported lots of bacterial species (including *S. aureus*, *E. coli*, *Klebsiella species*, *Pseudomonas spp.*) and fungi (e.g., *Candida*, *Aspergillus*) from pre-washed and unwashed clothing items, with some level of antibiotic resistance among the isolates. The existence of such pathogens and their profiles vary by market, treatment (handling), and season and the steady occurrence of skin/enteric flora proposes that second-hand underwear can act as microbiome reservoirs and conveyors of the associated pathogens if not properly cleaned. Meanwhile, the closeness of underwear to the urogenital/perineal region, to the pathogens may pose risks for skin/soft-tissue infections, vulvovaginitis, or UTIs in susceptible individuals.^{1,2,9-11}

Fairly used clothes, often originating from diverse sources, can therefore potentially harbor MRSA raising alarms on their roles in the spread of MRSA and other multi-drug resistant pathogens.² This study was intended to assess the occurrence and drug-resistance pattern of MRSA in fairly used male and female under-garments (pants) vended in Abakaliki, Nigeria. It focused on assessing the contribution of fairly used adult male and female pants, a major source of clothing materials in Nigeria, to the spread of methicillin-

resistant *Staphylococcus aureus* (MRSA) in Abakaliki, Ebonyi State, Nigeria.

2. Materials and Methods

2.1. Study area

This study was carried out in Abakaliki metropolis, Ebonyi State, Nigeria. Sampling locations were distributed across the different market sections where second-hand clothing are sold to capture variability in vendors and storage. Ebonyi State is in Southeast Nigeria, where the research was carried out. The state is geographically located between roughly 6°20'N latitude and 8°06'E longitude. It shares borders with Benue, Cross River, and Enugu. It is made up of both rural and urban areas with a range of socioeconomic origins. The state has a tropical climate with two distinct seasons: the dry season (November to March) and the rainy season (April to October).

2.2. Study design

A market-based sampling of fairly used (second-hand) adult pants stratified by sex (male versus female pants) was employed. Pants were sampled at point-of-sale prior to any washing by the customers. Sample collection and laboratory analysis of the samples lasted for a period of 5-months (March to July, 2025).

2.3. Sample collection

Twenty five (25) each of female and male pants were purchased from freshly loosen bales vended in the International Market, Abakaliki, Ebonyi State. The samples were put into sterile polyethylene bags and were transported to Applied Microbiology laboratory of Ebonyi State University, Abakaliki for microbiological analyses.

2.4. Bacterial isolation

S. aureus was isolated from the samples in line with the method described by.¹¹ The surfaces of each sample collected from different vendors in the International Market, Abakaliki in Ebonyi State, were swabbed with a sterile swab stick moistened with sterile distilled water. Each of the swabs was used to inoculate sterile 5 ml nutrient broth in a test tube. The tubes were incubated at 37°C for 24 - 48 hours. The broth in each of the test tubes after incubation, was properly mixed and a loopful was inoculated into aseptically prepared mannitol salt agar plates. The plates were incubated at 37°C for 24 hours. Then suspected colonies of *Staphylococcus aureus* were transferred into a freshly prepared mannitol salt agar plates and incubated at 37°C for 24 - 48 hours to obtain the colonies in pure forms.¹⁰

2.5. Identification of bacterial isolates

Suspected colonies of the isolated *S. aureus* were identified based on their morphological characteristics on mannitol salt agar, microscopic features and other biochemical properties.

2.5.1. Morphological identification of methicillin-resistant *S. aureus* (MRSA)

A pure culture of each of the isolates obtained was inoculated onto an aseptically prepared MRSA chromogenic agar plate using sterile wire loop. The inoculated plates were incubated at 37°C for 18-24 hrs. The MRSA positive isolates were identified macroscopically based on their color differentiation in the plates after incubation. MRSA positive isolates were greenish in color on the MRSA Chromatic agar. Pure colonies of the MRSA positive isolates were transferred onto nutrient agar slants and incubated at 37°C for 24 – 48 hours. The isolates were then stored in the refrigerator for further use.

2.6. Antibiotics susceptibility testing

This was determined as described using Kirby-Bauer disk diffusion technique.¹² Sterile petri dishes of Mueller –Hinton agar plates were inoculated using a young culture (18-24 h old culture) standardized to 0.5 McFarland turbidity equivalent standard. The following antibiotics discs:

cefoxitin, doxycycline, tetracycline, tobramycin, cefepime, imipenem, meropenem, amoxicillin, vancomycin, and ciprofloxacin (India), were aseptically placed onto the surfaces of the inoculated plates using a sterile forceps. The plates were then allowed to stand for 10 -15 minutes to pre-diffuse before incubation at 37°C for 18 – 24 hours. Their clear zones of inhibition measured to the nearest millimeters (mm) using a meter rule were taken. The results were measured as sensitive or resistant in line with the clinical Laboratory Standard Institute interpretation guideline for antibiotics sensitivity.¹³

3. Results

3.1. Morphological and biochemical characteristics

The results of the morphological and biochemical characteristics of the isolates are shown in **Table 1**. *S. aureus* obtained had golden yellow coloration on mannitol salt agar. There were gram positive, coagulase positive, catalase positive, indole, oxidase and methyl red negatives.

Table 1: Morphological and biochemical characteristic of *S. aureus* isolated from male and female pants

Colony colour on mannitol salt agar	Cell shape	Gram staining	Coagulase test	Catalase test	Indole test	Oxidase test	Methyl red test	Name of Organism
Golden Yellow	Cocci and clustered	+ve	+ve	+ve	-ve	-ve	-ve	<i>Staphylococcus aureus</i>

Keyword: +ve = positive result, -ve = negative result

Table 2: Prevalence of *S. aureus* isolated from fairly used male and female pants vended in International Market, Abakaliki

Samples source	No of samples analyzed	No. and percentage prevalence of <i>S. aureus</i> isolated
Female pants	25	20 (55.56)
Male pants	25	16 (44.44)
Total	50	36 (100)

Table 3: Frequency of occurrences of methicillin resistant *S. aureus* isolated from fairly used female and male pants vended in International Market, Abakaliki

Source of samples	Frequency of occurrence of MRSA (%)
Female panties	14 (53.85)
Male panties	12 (46.15)
Total	26 (100.0%)

Table 4: Antibiogram of methicillin resistant *Staphylococcus aureus* from male panties vended in International Market, Abakaliki

Antibiotics	Disk potency(µg)	% Resistance	% Susceptible
Meropenem	10	12 (100)	0 (0.0)
Cefepime	30	12 (100)	0 (0.0)
Ciprofloxacin	5	11 (91.7)	1 (8.3)
Tetracycline	30	12 (100)	0 (0.0)
Amoxicillin	20/10	11 (91.7)	1 (8.3)
Tobramycin	10	6 (50.0)	6 (50.0)
Doxycycline	30	3 (25.0)	9 (75.0)

Imipenem	10	1 (8.3)	11 (91.7)
Cefoxitin	30	12 (100)	0 (0.0)
Vancomycin	30	12 (100)	0 (0.0)

Table 5: Antibiogram of methicillin-resistant *S. aureus* isolated from female pants vended in International Market, Abakaliki

Antibiotics	Disk potency (µg)	% Resistance	% Susceptible
Meropenem	10	14 (100)	0 (0.0)
Cefepime	30	14 (100)	0 (0.0)
Ciprofloxacin	5	10 (71.4)	4 (28.6)
Tetracycline	30	13 (92.9)	1 (7.1)
Amoxicillin	20/10	14 (100)	0 (0.0)
Tobramycin	10	11 (78.6)	3 (21.4)
Doxycycline	30	7 (50)	7 (50)
Imipenem	10	0 (0.0)	14 (100)
Cefoxitin	30	14 (100)	0 (0.0)
Vancomycin	30	14 (100)	0 (0.0)

3.5 Antibiogram of methicillin-resistant *Staphylococcus aureus* isolated from female pants vended in International Market, Abakaliki

All the 14 MRSA positives from female pants were 100% susceptible to imipenem. Subsequently, 7(50%), 4(28.6%) and 3(21.4%) were susceptible to doxycycline, tobramycin and ciprofloxacin, respectively and only 1(7.1%) was susceptible to tetracycline (**Table 5**). Total resistance (100%) of the isolates to meropenem, cefepime, amoxicillin, cefoxitin, and vancomycin were also observed (**Table 5**).

3.2. Occurrences of *S. aureus* in female and male pants vended in International market, Abakaliki

In this study, out of the 50 samples (25 each of female panties and male panties) analyzed, a total of 36 isolates of *Staphylococcus aureus* were obtained. Of the 36 isolates, 20 representing 55.56% were obtained from female pants while 16 representing 44.44% were from male pants (**Table 2**).



Figure 1: *S. aureus* on mannitol salt agar

3.3 Prevalence of methicillin resistant *S. aureus* in fairly used female and male pants vended in International Market, Abakaliki

A total of 36 isolates of *Staphylococcus aureus* were screened for methicillin resistance. The result showed that only 26(72.22%) isolates were methicillin-resistant positive while

10(27.78%) were negative. Out of the 26 isolates that were methicillin-resistant positive the highest number 14 (53.85%) were obtained from female panties while 12 (46.15%) were from male pants (**Table 3**).



Figure 2: MRSA +ve and -ve *S. aureus*

3.4. Antibiogram of methicillin-resistant *Staphylococcus aureus* from male pants vended in International Market, Abakaliki

The results of the 12 MRSA positive isolates from male pants screened using different classes of antibiotics showed that 11(91.7%) were susceptible to imipenem. This was respectively followed by 9(75%) and 6(50%) to doxycycline and tobramycin; 8.3% each to amoxicillin and ciprofloxacin. Meanwhile, a 100% resistance each to meropenem, cefepime, tetracycline, cefoxitin, and vancomycin was also observed (**Table 4**).

3.6 Multiple antibiotic resistance indices (MARI) of the MRSA isolated from fairly used male and female pants

Multiple Antibiotic Resistance Indices (MARI) of the MRSA isolated from male and female pants were determined and the result showed that the MRSA female pants had an average MARI of 0.8 while those from the male pants had 0.7 (**Table 6**).

Table 6: Multiple antibiotic resistance indices (MARI) of MRSA isolated from fairly used male and female pants

Sample sources	Average MARI
Female pants	0.8
Male pants	0.7

4. Discussion

This study was initiated to investigate the possible involvement of fairly used under-garments in the spread of MRSA, which has been classified by WHO as one of the global public health concerns. The study found that second-hand clothes vended in the studied area were contaminated with *Staphylococcus aureus*, including the highly pathogenic MRSA. All 50 samples of used clothing examined in the study exhibited high levels of staphylococcal contamination (55.56% in female pants and 44.44% in male pants). The source of these often exotic products is the most likely contributing factor. Because the buyers of the fairly used clothing are not aware of the health situation of the past users, there is high tendency of contracting a highly infectious disease from these clothing, as some pathogens are exceedingly resilient and can persist in hard environs for some periods.¹⁴

It was observed in this study that *S. aureus* is more prevalent in female pants 20 (55.56%) than male pants 16 (44.44%). This suggests that female pants might harbor a higher number of *S. aureus* compared to male undergarments. The difference in the occurrence of *S. aureus* in male and female fairly used pants could be attributed to several factors including the health conditions of the previous users and related extrinsic factors, including environmental temperature and humidity.¹⁵ Not like some bacteria species that find it difficult to survive in the absence of water, *S. aureus* according to literature can persist for days to weeks on textiles/fabrics and other dry surfaces.¹⁶ Their survival could be linked to the presence of thick peptidoglycan cell wall, biofilm formation, toxin, enzyme production.^{17,18} It could also be associated with the storage conditions of the used clothes where *S. aureus* can persist on textile or on dry surfaces for 7 days or more, with some studies reporting persistence up to weeks or months at room temperature.¹⁶ Our finding agreed with the report of Olajubu *et al.*¹⁹ who recorded up to 60.7% of *Staphylococcus aureus* among other bacteria species isolated from fairly used clothes. Also, according to the report by Kong *et al.*, Odum and Idise, Bauer *et al.*, Isa *et al.*, Al-Easawi and Emran,^{2,9-11,20} there is likelihood that women harbor *S. aureus* in the vaginal and perineal areas, because of their close contact with undergarments, thereby increasing the possibility of bacterial transfer to clothing which pose serious risks of skin/soft-tissue infections, vulvovaginitis, or UTIs to susceptible individuals. This aligns with the higher prevalence of *S. aureus* in female pants in our study. Another report by Mellor *et al.*²¹ noted that females have a slightly higher density of *S. aureus* in their skin flora, which might have also contributed to the observed higher

isolation rate from female clothing. Therefore, the finding that 55.56% of *S. aureus* isolates came from female pants when compared with their male counterpart (44.44%) aligns with other researchers findings suggesting that women may have higher skin colonization rates of this bacterium. This organism is associated with pneumonia, urinary tract infections, bacteremia, endocarditis, osteomyelitis, meningitis, bacteremia and lungs abscess.²²

Methicillin resistant *Staphylococcus aureus* (MRSA) recognized by WHO as one of the most serious global public health threat was observed in this study. Out of the 36 isolates of *Staphylococcus aureus* screened, as many as 26 (representing 72.22%) were positive for methicillin resistance. Meanwhile, of the 26 that were positive for methicillin resistant, higher proportion, 14(53.85%) were from female pants while 12(46.15%) from male pants. These findings propose a relatively higher prevalence of methicillin-resistant *S. aureus* (MRSA) in female compared to male pants. The occurrence of MRSA on undergarments may be linked to factors such as personal hygiene, frequency of washing and exposure to MRSA prevalent environments. Individuals who frequently use shared gym facilities or healthcare settings are more at risk of harboring MRSA, which could subsequently be transferred to their clothing.²³ Also, the type of fabric, sweat production, and duration of contact with the skin may all play a role in its prevalence. Our findings agrees with Benedict *et al.* and Lauder *et al.*^{24,25} who also reported the presence of MRSA in clothing. Nevertheless, the varied distribution of MRSA in female and male pants suggests that gender-based factors may influence MRSA prevalence. The outcome of this research also supported the work of Ocampo *et al.*²⁶ who reported the survival of MRSA for long times on used clothes thereby spreading life threatening diseases that can cause a substantial public health problem.

The antibiogram of the methicillin-resistant *Staphylococcus aureus* (MRSA) obtained in this study showed that isolates from females and male pants were most susceptible to imipenem 100% and 91.7%, respectively. The susceptibility of the isolates from female pants to tetracycline, ciprofloxacin, tobramycin and doxycycline ranged from 7.1% to 50%. There were 100% resistant to meropenem, cefepime, and amoxicillin. Isolates from male pants were 75% and 50% susceptible to doxycycline and tobramycin, respectively. However, there were 91.7% each resistant to amoxicillin and ciprofloxacin. The low susceptibility to ciprofloxacin (21.4%) and tobramycin (28.6%) suggests the presence of resistance mechanisms such as efflux pumps or enzymatic modification of target sites, which are commonly associated with fluoroquinolone and aminoglycoside resistance.²⁵ This study supported the report by Gajdács *et al.*²⁷ who observed that doxycycline and ciprofloxacin are progressively ineffective against MRSA, especially in healthcare settings where MRSA has been severely exposed to antibiotics. The isolates were 100%

resistant by meropenem, cefepime, tetracycline, ceftazidime and vancomycin. A study by Dautzenberg *et al.*²⁸ reported extensive resistance of MRSA isolates to beta-lactam antibiotics, such as amoxicillin and cefepime from healthcare settings which aligned with our findings. In this study, only 7.1% of the MRSA isolates were susceptible to tetracycline, which is consistent with other reports which indicated tetracycline resistance to be prevalent in MRSA populations. Abdulrahman *et al.*²⁹ documented high rates of tetracycline resistance in community-acquired MRSA strains, which are known to carry the tetracycline resistance gene (tetK), further in support of the low susceptibility found in this study. This antimicrobial susceptibility pattern is of great concern, especially with the high resistance rates of the MRSA to commonly used antibiotics like vancomycin, which is traditionally used as a last-line treatment for MRSA and ceftazidime. Their high susceptibility to imipenem is also notable as carbapenems are usually reserved for multi-drug resistant organisms. However, the 100% susceptibility to imipenem might suggest that these isolates have not yet developed resistance to imipenem.

Furthermore, the Multiple Antibiotic Resistance Indices (MARI) of the MRSA isolated from male and female pants were 0.7 and 0.8, respectively and this indicates that the isolates were highly multi-drug resistant. Our finding corroborates with the study by Ikeh *et al.* and Isa *et al.*^{9,10} who also reported high multidrug resistance index values of >0.6 among the methicillin resistant *S. aureus* (MRSA) screened from hospital fomites and community samples in South-Eastern Nigeria. This therefore suggests that fairly used clothes including bed linens and items in that category could serve as reservoirs of resistant strains of pathogenic organisms including MRSA.

5. Conclusion

In conclusion, this study revealed that fairly used clothes harbor life threatening microorganisms including MRSA. Presence of MRSA in the fairly used cloth screened climaxes the need for adequate hygienic practices before wearing newly purchased clothes, particularly undergarments. Additionally, while fairly used clothes are cheap alternative to new clothes, they can serve as carriers of dangerous infectious agents between human populations. Given that MRSA is of a significant concern due to its high resistance to beta-lactam antibiotics, including methicillin, this result therefore highlights the need for better hygienic practices and monitoring of antimicrobial resistance in everyday items such as undergarments. We recommend that Nigeria's public health policy makers should ensure effective surveillance and monitoring protocols for fairly-used clothes and related products against foreign pathogen transmission, or consider the option of banning importation of fairly-used clothes to save the nations citizens from the associated health risks.

6. Study limitation

Because of limited resources, the limitation of this study is the relatively small sample size used, as restricted number of fairly used adult male and female pants investigated may not be a good representative of the complete range of second-hand clothing items circulating in Abakaliki or beyond. Also, the study focused exclusively on methicillin-resistant *Staphylococcus aureus* (MRSA). Other pathogens that might also pose other serious health challenges were not investigated.

7. Source of Funding

The study was funded collaboratively by the authors.

8. Conflict of Interest

None.

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