SJIF Impact Factor: 6.711

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

ISSN: 2457-0400
Volume: 9.
Issue: 2
Page N. 149-153
Year: 2025

Original Article www.wjahr.com

PREVALENCE AND ANTIBIOGRAM OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) ISOLATED FROM FAIRLY USED TOWELS SOLD IN INTERNATIONAL MARKET ABAKALIKI, EBONYI STATE, NIGERIA

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Received date: 16 December 2024 **Revised date:** 05 January 2025 **Published date:** 25 January 2025

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ABSTRACT

A strong preference for imported clothes encourages the importation of second-hand clothing, often without consideration of potential health risks. This study aimed to assess the prevalence and antibiogram of Methicillin-resistant Staphylococcus aureus (MRSA) from second-hand towels sold in the International Market, Abakaliki, Ebonyi State. A total of 25 samples of towels were analyzed. Standard microbiological and biochemical methods were used to isolate and identify *Staphylococcus aureus*, while MRSA was detected using Chromatic agar. Antibiotic susceptibility and resistance were determined via the disc diffusion method. The study found that 23 of the 25 samples contained *S. aureus*, with 15 (65%) being positive for methicillin-resistant and 8 (35%) not resistant. The susceptibility testing revealed that all MRSA isolates were highly susceptible to imipenem and doxycycline, but showed the least susceptibility to tobramycin and ciprofloxacin. Resistance was complete (100%) to meropenem, cefepime, cefoxitin, vancomycin, tetracycline and amoxicillin. The detection of MRSA and other bacteria in second-hand clothing highlights a public health risk, emphasizing the importance of proper disinfection and laundering before use.

KEYWORDS: Antibiogram, Methicillin resistant, *Staphylococcus aureus*, fairly used towels**.**

I. INTRODUCTION

Second-hand clothes, also known as fairly used clothes, are items previously worn by someone else before being used by the current owner.[1] In Nigeria, they are referred to as "Okirika" or "bend down select" and include items like used panties, socks, shirts, skirts, bras, and household linens such as pillowcases, towels, bedding, curtains, and tablecloths.[6] These clothes are primarily imported from Western countries (such as Canada, the United States, Germany, and France) and Asian nations like Indonesia, Thailand, and China.[1] Upon arrival, they are bundled into large packages called bales. While these clothes are cheaper, their appeal often lies in their superior quality compared to new clothes. Despite their affordability and perceived quality, customers often buy and wear these clothes without considering the potential health risks. Like any object that comes into contact with humans, these clothes can contribute to the transmission of infections through everyday activities.[5] Wearing contaminated second-hand clothes can lead to various skin infections.[22] Due to their previous usage and widespread handling, second-hand clothes pose significant public health risks. This awareness led the Rwanda Bureau Standards to ban the sale of second-hand undergarments in Rwanda.[13]

Methicillin-resistant Staphylococcus aureus (MRSA) is one of the many microorganisms that can be found in second-hand clothes.[9] MRSA is a group of Gram-positive, non-motile, spherical bacteria that are genetically distinct from other strains of Staphylococcus aureus.[4] MRSA is responsible for numerous difficult-to-treat infections, including sepsis, bacteremia, pneumonia, meningitis and respiratory tract infections.[15] MRSA-related infections have been linked to longer hospital stays, increased healthcare costs, and higher mortality rates.[22] Individuals carrying MRSA are also at greater risk for septicemia, wound infections, and, occasionally, toxic shock syndrome. The World Health Organization (WHO) recognized MRSA as a "Priority pathogen" in 2017 due to its ability to cause life-threatening diseases and its increasing spread, representing a significant global public health issue.[25] The spread of MRSA and other infections via second-hand clothing, if not properly sanitized, raises concerns about the potential for this pathogen to spread in communities, including places like

the International Market in Abakaliki, Ebonyi State, Nigeria, where the trade of such clothes is common.

II. MATERIALS AND METHODS

Sample Collection

A total of 25 samples of fairly used clothes from freshly loosen bales were bought and transferred into sterile polythene bags and immediately transported to Applied Microbiology laboratory of Ebonyi State University for microbial analysis.

Bacterial Isolation

This was carried out according to the method described by[19] Surfaces of each sample collected from different selling point in the international market, Abakaliki in Ebonyi State, was swabbed with a sterile swab stick moistened with sterile distilled water. Each swab stick was inoculated into a test tube containing 5ml of nutrient broth and incubated at 37^{o}C for 24 - 48 hours. After incubation, the broth was properly agitated and a loopful from each tube was inoculated in aseptically prepared mannitol salt agar plate. The plates were incubated at 37^{o}C for 24 hours. Then suspected colonies of *Staphylococcus aureus* was transferred into another aseptically prepared mannitol salt agar and incubated at 37^{o}C for 24 - 48 hours to get colonies in pure form.

Identification of Bacterial Isolates

Isolated bacteria were identified by using Bergey's Manual of Determinative Bacteriology, on the basis of morphological, Microscopic examination and biochemical properties.[16]

Morphological characterization

Colonies developed on mannitol salt agar, then classified according to their shape, color and odor.

Microscopic examination

Microscopic examination was done for the gram-stained slides in order to characterize gram-positive bacteria from others.

Biochemical characteristics

The biochemical test was performed to identify the bacterial isolated. This test includes catalase test and indole production test.

Identification of Methicillin-Resistant *S. aureus* (MRSA)

The pure culture of each *S. aureus* obtained was inoculated onto an aseptically prepared MRSA chromogenic agar plate using sterile wire loop. The plates were incubated at 37^{o}C for 18-24 hrs. After incubation, MRSA positive isolates were identified macroscopically based on their color differentiation in the plates. Pure colonies of the species were transferred into nutrient agar slants and incubated at 37^{o}C for 24 – 48 hours. The isolates were then stored in the refrigerator for further use.

Antibiotics Susceptibility testing

The susceptibility and resistance pattern of the MRSA to different antibiotic agents was determined using Kirby-Bauer disk diffusion technique.[8] Sterile Petri dishes of Mueller –Hinton agar plates were inoculated using a young culture (1 day old) of a 0.5 McFarland turbidity standard of the test organism. Commercially available antibiotics disc including cefoxitin, doxycycline, tetracycline, tobramycin, cefepime, dorpenem, meropenem, amoxicillin, vancomycin, and ciprofloxacin (India), were aseptically placed onto the surfaces of the inoculated plates using a sterile forceps. Subsequently, the plates were allowed to stand for 15 minutes to enable the inoculated organism to pre-diffuse. They were inoculated at 37^{o}C for 18 – 24 hours and their clear zones of inhibition measured to the nearest millimeters (mm) using a meter rule. The interpretation of the measurement as sensitive or resistant was made according to the clinical Laboratory Standard Institute interpretation guideline for antibiotics sensitivity.[12]

III. RESULTS

Table 1: Morphological characteristic of the bacteria isolate.

Organism isolated	Chromatic MRSA agar	Mannitol salt agar	Colony Shape	Gram staining	Catalase test	Indole test
Staphylococcus aureus	Green	Golden Yellow	Clustered	+ve	+ve	-ve

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

Plate 1: Pictorial representation of S. aureus on mannitol salt agar.

Table 2: Prevalence of *Staphylococcus aureus* isolated from fairly used clothes sold in International Market, Abakaliki.

Samples sources	No of samples	*S. aureus* (%)
Towels	25	23 (92.0)

A total of 25 samples were analyzed from towels. The result showed that 23 (92.0) isolates of *Staphylococcus aureus* were obtained.

Table 3: Prevalence of methicillin resistant *Staphylococcus aureus* isolated from fairly used clothes sold in International Market, Abakaliki.

Samples sources *S. aureus*	MRSA distribution (%)
Towels 23 (92.0)	15 (65.0)

Twenty three (23) isolates of *Staphylococcus aureus* were screened for methicillin resistance and the result showed only 15 representing 65 % were positive for methicillin resistant while 8 representing 35% were negative.

Table 4: Antibiotics susceptibility and resistance profile of methicillin resistant *Staphylococcus aureus* from Towels sold in International Market, Abakaliki.

Sample code	Resistant	Susceptible
T3	MEM, FEP, TE, AML, TOB, FOX, VA	CIP, IPM, DO
T4	MEM, FEP, CIP, TE, AML, FOX, VA	TOB, DO, IPM
T5	MEM, FEP, TE, AML, TOB, DO, FOX, VA	CIP, IPM, DO
T8	MEM, FEP, CIP, TE, AML, FOX, VA	TOB, DO, IPM
T12	MEM, FEP, CIP, TE, AML, TOB, DO, IMP, FOX, VA	
T13	MEM, FEP, CIP, TE, AML, DO IMP, FOX, VA	TOB
T15	MEM, FEP, TE, AML, TOB, FOX, VA	CIP, DO, IPM
T16	MEM, FEP, CIP, TE, AML, TOB, DO, FOX, VA	IPM
T17	MEM, FEP, CIP, TE, AML, FOX, VA	TOB, DO, IPM
T18	MEM, FEP, CIP, TE, AML, FOX, VA	TOB, DO, IPM
T19	MEM, FEP, CIP, TE, AML, TOB, DO, FOX, VA	IPM
T22	MEM, FEP, CIP, TE, AML, FOX, VA	TOB, DO, IPM
T23	MEM, FEP, CIP, TE, AML, TOB, DO, IMP, FOX, VA	
T24	MEM, FEP, CIP, TE, AML, TOB, DO, FOX, VA	IPM
T25	MEM, FEP, CIP, TE,AML, TOB, DO, FOX, VA	IPM

Keyword: meropenem (MEM), cefepime (FEP), ciprofloxacin(CIP), tetracycline (TE), amoxicillin (AMC), tobramycin (TOB), doxycycline (DO), imipenem (IPM),cefoxitin (FOX), vancomycin (VA).

Result obtained from this study showed that all the 15 MRSA positives isolates obtained from towels were 12 (80%) susceptible to imipenem followed by 8 (53.3%) doxycycline, 6 (40%) Tobramycin and 3 (20%) ciprofloxacin while 100% resistant was observed in meropenem, cefepime, tetracycline, amoxicllin, cefoxitin, and vancomycin.

IV. DISCUSSION

This study found that second-hand clothes were frequently colonized by bacteria, including Methicillin-resistant *Staphylococcus aureus* (MRSA), which pose significant public health risks. All the 25 samples of second-hand clothes analyzed in this study showed varying levels of bacterial contamination. The level of patronage given to these clothes by all in the society makes it a matter of public health importance. The way they're handled at the source is critical; if they're not properly stored and bundled together, they could become more polluted.[20] The majority of consumers are unaware of the dangers of wearing secondhand clothing without following necessary disinfection procedures, putting them at risk of illness. Because used clothing does not know the history of past users, there is a risk of developing a highly serious infectious disease from bacteria on the clothing, as most germs are extremely resistant and can survive hard environments for long periods of time on clothing.[24] The bacteria displayed characteristics such as clustered, green colonies on Chromatic MRSA agar, golden yellow colonies on mannitol salt agar, and were Gram-positive during gram staining, catalase-positive and indole-negative under microscopic examination.

The prevalence of *Staphylococcus aureus* in the second-hand clothes samples showed that 23 (92.0) isolates were obtained from the 25 samples analyzed. The microbial loads of this fairly used clothes could be attributed to factors like prior usage of the clothes and environmental conditions such as temperature and humidity. This brings to bear, the need to know the source of these fairly used clothes.[3] reported that used clothing is not good to use because the present user don't know the history of the previous user as a result of this, there is possibility of contracting a very large infectious disease from bacteria on clothing especially when used without washing. These findings align with the study by[20] which reported *Staphylococcus aureus* (60.7%) as the most commonly

isolated bacterium from second-hand clothes. *Staphylococcus aureus* is linked to several infections, including nosocomial infections, skin infections, pneumonia, urinary tract infections, bacteremia, and more, highlighting the public health concerns of its presence on second-hand clothing.

The study also revealed a high prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) among the isolates. Of the 23 *S. aureus* isolates, 15 (65%) were positive for methicillin-resistant while 8 (35%) were negative. The presence of MRSA may be due to high rate of usage and lack of personal hygiene from previous user as shown in the study and also how the clothes are handled at sources during importation to prevent the growth of spores or other forms of contamination. MRSA infections are associated with significant morbidity and mortality, and this finding is consistent with[18] who noted that MRSA can survive for extended periods on second-hand clothes, contributing to the spread of life-threatening diseases.

In antibiotic susceptibility testing, the MRSA isolates were tested against ten conventional antibiotics. The results showed that MRSA exhibited multiple resistances, in line with findings from[9] The MRSA isolates in this study were most susceptible to imipenem and doxycycline, and least susceptible to tobramycin and ciprofloxacin. Additionally, the MRSA isolates were 100% resistant to meropenem, cefepime, cefoxitin, vancomycin tetracycline and amoxicillin, aligning with the study of[20], which also reported high resistance to ampicillin and amoxicillin.

V. CONCLUSION

The study has demonstrated that second-hand clothes are often heavily contaminated with microorganisms which are pathogenic. The isolation of these microorganisms from the samples highlights the need for proper washing and hygiene practices before using them. It can also be concluded that, while second-hand clothes are a more affordable option than new ones, they can act as vehicles for the transmission of infections. Additionally, the high level of antibiotic resistance found in the isolated bacteria underscores the importance of caution and awareness before using these clothes. Furthermore, vendors of second-hand clothes would benefit from tracing the origins of their products, as the study suggests that many of the sampled undergarments may have come from high-risk contamination sources.

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