JBS
Research Article

Journal of Biological Studies

eISSN: 2209-2560

V. 7, N. 4:214-226, 2024

https://doi.org/10.62400/jbs.v7i4.11539

Prevalence of multidrug resistance *Staphylococcus aureus* isolated from secondhand items in Dhaka, Bangladesh

Sumi Akter[1], M. Aminur Rahman[2*], Md. Tanvir Ahsan[1], Ratna Akter[3], Md. Abdus Salam[3], Sharmin Sultana Jinia[2], Kasi Marimuthu[5]

[1]Department of Botany, Jagannath University, Dhaka-1100, Bangladesh
[2]Department of Fisheries and Marine Bioscience, Faculty of Biological Science and Technology, Jashore University of Science and Technology, Jashore-7408, Bangladesh
[3]Department of Marketing, University of Development Alternative, Dhaka-1209, Bangladesh
[4]Directorate of Secondary and Higher Education, Ministry of Education, Dhaka-1000, Bangladesh
[5]Department of Environmental Science, Tezpur University, Tezpur -784028, Assam, India

*Correspondence author: amin2019@just.edu.bd / aminur1963@gmail.com

Received 10 September 2024|Accepted 18 October 2024|Published 15 December 2024

Abstract

Used clothing has been linked to the spread of disease from one buyer to another, posing a serious risk to the public health. In the current study, the used items were collected from three well-known marketplaces in Dhaka, Bangladesh. The objective was to isolate and identify *Staphylococcus aureus*, including Methicillin-resistant *Staphylococcus aureus* (MRSA) and Methicillin-sensitive *Staphylococcus aureus* (MSSA), using culture-based and molecular techniques. Ninety-nine used items of fifteen distinct kinds were purchased from vendors. All analyzed samples had a prevalence of *S. aureus*, 22/90 (24.4%), with the highest load in men's sweaters (9.3×10^3 cfu/ml) and the lowest load in women's jackets (5.0×10^1 cfu/ml), according to colonial appearance on mannitol salt agar and CHROMagar™ Staphylococcus culture plates. In all, 22 isolates were selected for further molecular analysis. Of the 22 presumptive *S. aureus* isolates that were recovered from the current investigation, 20 isolates with the *nuc* gene were identified as *S. aureus* species, among them 6 isolates with the *mecA* gene were identified as MRSA. Based on the data acquired on antimicrobial susceptibility, the majority of MSSA isolates (71.43%) and all MRSA isolates (100%) were determined to be multidrug resistant (MDR) with a MARI value between 0.2 and 0.5. In situations when utilizing secondhand products cannot be completely avoided, it has become clear that these items should be well cleaned before use since they may harbor bacteria that cause skin and other illnesses.

Key words: Secondhand items, MRSA, MDR, Bangladesh

1. Introduction

Items that are secondhand have been used by someone else before the current user (Jadoon et al., 2022). The most used items of apparel are toys, sandals, boots, shirts, jackets, undergarments, socks, and shoes (Baharuddin and Nurhatira, 2020). Western and Asian countries are the main importers of these clothing. The US is the world's top exporter of used clothes, followed by Germany, the UK, and the Netherlands, while Sub-Saharan Africa, Southeast Asia, and Eastern Europe are the top importers of used clothing (Baharuddin and Nurhatira, 2020). A renowned recycling company in china that exports used clothes, shoes and bags to countries of Africa, Southeast Asia, and the Middle East (https://hissenglobal.com/about-us/). Due to the uncertain nature of the economy and the high cost of branded apparel, many prefer imported secondhand clothing over new apparel (Rakhshanpour et al., 2021). Imported used apparel of high quality, famous brands, and affordable pricing attract buyers to the market (Rakhshanpour et al., 2021). Due to this, they are more susceptible to microbiological infections from bacteria, fungus, parasites, and viruses (Rakhshanpour et al., 2021).

As history of past users of used clothing is unknown, there is a possibility to put users the risk of developing a serious infectious disease from bacteria on the used items. The public are on the dangers of some diseases or infections that could be contacted from secondhand items which can either be gotten from the first user to the present user or was contaminated in the market place (Atubu et al., 2016). The way these items of clothing are handled in the markets place by traders and buyers also exposes them to contamination, as different people touch them with sweaty, dirty hands, and traders spread them most times on the bare floor, or on dirty sacks and papers for sale (Atubu et al., 2016). Second hand items are a potential reservoir of wide varieties of pathogenic microorganisms that can easily contaminate user or consumers. This is an important route to transmit different types of pathogens to user persons and is considered as a potential source of public health problem for a number of years (Jadoon et al., 2022). Microorganisms are known to be ubiquitous and such are seen to inhabit microenvironments within fomites with minimum nutrients (Odum and Idise, 2022). The features of the fomites and garments, as well as the microorganisms' characteristics, are intrinsic variables that affect the survival of the microbes on them. Extrinsic elements that affect this survival include humidity, temperature, and other environmental conditions (Al-Easawi and Emran, 2017; Muthiani et al., 2017; Bandyopadhyay et al., 2019).

Secondhand clothing has an inherent ability to hold onto and transfer germs from the original user or users to the next user (Atubu et al., 2016; Odum and Idise, 2022). Since its initial use, each item of fomites or fabrics a person has used has probably harbored some microbes, including fungi and pathogenic or odor-producing bacteria. These microbes can create an optimal environment for microbial growth when they come into contact with human body, as they provide warmth, moisture, and oxygen (Pedroso-Butanas and Butanas, 2018; D. Ocampo et al., 2023). Researchers reported that this produces circumstances in the body that are favorable to other microorganisms (Sanders et al., 2022; D. Ocampo et al., 2023). According to a number of studies, bacteria may live on fomites or textiles for extended lengths of time, and contact with contaminated fomites or textiles can directly spread infectious illnesses (Agbulu *et al.,* 2015; Atubu et al., 2016; Owen and Laird, 2020; Odum and Idise, 2022). Buying used clothing is thought to be a risky way for people to contract serious illnesses, particularly when worn or used without being washed (Al-Easawi and Emran, 2017; Bandyopadhyay et al., 2019; Putri et al., 2019; Jadoon et al., 2022). In previous many researchers identified pathogenic bacteria such as *Bacillus* spp., *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus* spp., *Pseudomonas aeruginosa, Escherichia coli, Klebsiella* spp., *Enterobacter* spp., *Proteus vulgaris, Acinetobacter baumanii, Shigella* spp.,

and *Salmonella* spp. *Micrococcus* spp., *Enhydrobacter* spp., and *Propionibacterium* spp. from second hand items which may survive for lengthy periods of time on secondhand items and put people in a risk to get a range of serious diseases (Atubu et al., 2016; Al-Easawi and Emran, 2017; Muthiani et al., 2017; Baharuddin and Nurhatira, 2020; Jadoon et al., 2022; Odum and Idise, 2022; D. Ocampo et al., 2023). Prior research on various fabrics and clothing showed that they were frequently colonized by *S. aureus*, including MRSA and MSSA, which are highly concerning for public health (Muthiani et al., 2017; Baharuddin and Nurhatira, 2020; Jadoon et al., 2022). Humans are more vulnerable to the spread of pathogenic germs and their detrimental effects when transient bacteria, such as *S. aureus*, are deposited on the skin's surface from environmental sources such used clothing (Al-Easawi and Emran, 2017; Jadoon et al., 2022).

A collection of different kinds of used items that are supplied from different regions of the world is referred to as used clothes or used apparel in Bangladesh. Bangladesh became the 92nd largest country in the world for buying of secondhand garments in 2021, putting in $7.28 million. Used apparel items were the 544th most imported product at the same year in Bangladesh. Bangladesh imports used clothing items from South Korea, Taipei, Kuwait, Japan and China (https://oec.world/en/profile/bilateral-product/used-clothing/reporter/bgd). Selling and buying second-hand items is a common practice in Bangladesh. Secondhand goods are offered for sale on hand carts or vans as well as in markets and open markets called roadside markets or footpath markets. Due to their excellent quality and affordable costs, second-hand goods have become more popular in Bangladesh among the country's lower and middle class population. Used items purchased from the market can be associated with pathogenic microorganisms, which should be reduced the microbial load and cured before used them. Microbiological quality of some of second-hand items in some parts of the world have been reported but so far as we know information is limited on microbial quality of used items sold at different markets in Dhaka city, Bangladesh. Therefore, the objectives of this investigation were to evaluate the microbial load of *S. aureus*, describe the *S. aureus* and MRSA bacterial isolates, and determine whether the isolates were sensitive to or resistant to the presence of specific antibiotics.

2. Materials and Methods

2.1. Sample Collection

In total, 90 samples were collected from 15 different types of secondhand items from the three renowned markets naming Begum Bazar, Gulistan street market and Dhaka new market in Dhaka city of Bangladesh. Two samples of each type of secondhand items were collected from each market, and thus, thirty samples were collected from each market. To prevent cross-contamination, each sample was collected independently in sterile polythene bags before being brought to the lab for bacterial isolation and identification.

2.2. Phenotypic identification of *S. aureus*

Bacterial sample was collected by a sterile cotton wool swab moistured in 5ml of phosphate buffer solution (PBS) (HIMEDIA, M1452, Pune) from different parts of used items, which were closely touched to skin. After that the swab was returned to it in sterile tube in PBS (HIMEDIA, M1452, Pune). To determine the overall *S. aureus* count, the serial dilution plate method (Akter et al., 2024) was applied. According to this technique, one ml of pre-enrichment sample from swab containing tube was transferred to nine ml of sterile normal saline for ten-fold (1:10) dilution and further diluted up to 10^5 dilutions based on requirement and spread onto mannitol salt agar (MSA; Himedia, Mumbai, India) using a sterile glass spreader. After that, the medium plates that had been inoculated were incubated for 48 hours at 35 ± 2 °C. The total number of colonies was determined after incubation using a digital colony counter (LT-37, Labtronics, Haryana) and expressed as cfu/ml. For patch inoculation,

individual yellow-colored colonies were selected and cultured for 24 hours at 37 °C on MSA (Himedia, Mumbai, India) and CHROMagar™ Staphylococcus (CHROMagar, Paris, France) medium plates. Following the incubation period, the presence of *S. aureus* was confirmed phenotypically by observation of yellow-colored colonies on MSA (Himedia, Mumbai, India) and mauve to pink-colored colonies on CHROMagar™ Staphylococcus (CHROMagar, Paris, France) medium (Merlino et al., 2000). Morphological, physiological and biochemical properties were considered to identify the isolated bacteria. Cell morphology was observed using an optical microscope (B-500TPH, Optika, Italy). Gram type of isolates was examined using a Gram staining kit (bioMerieux) according to the manufacturer's instructions. The test for motility was performed according to MacFaddin's method (MacFaddin, 1972). Standard method was followed to perform the physiological and biochemical characteristics (Cheesbrough, 2006; Holt, 1984). These were oxidase test, catalase test, methyl red test, Voges–Proskauer test, nitrate reduction, citrate utilization, urease activity, indole and H_2S production, hydrolysis of starch, gelatin and esculin. After confirmation, a stock culture for each isolate was prepared using Luria Bertani (LB) broth (Himedia, Mumbai, India) supplemented with 30% (v/v) glycerol (Nepa, Tangerang, Indonesia) and stored at −80 °C for further investigation.

2.3. Molecular identification of *S. aureus* by using *nuc* and MRSA by *mecA* genes

Genomic DNA of isolated strains was extracted using the boiling lysis technique (Moniruzzaman et al., 2023). A single colony or two was collected from the pure culture CHROMagar™ Staphylococcus (CHROMagar, Paris, France) medium and inoculated into 3 ml of LB broth (Himedia, Mumbai, India). The mixture was then incubated overnight at 37°C in the 120 rpm Innova™ 4300 incubator shaker (Innova, NJ, USA). After that, 1.5 ml of the fresh culture was taken out and centrifuged for 5 minutes at 13,000 rpm. After discarding the supernatant, the pellet was again suspended in 600 µl of autoclaved distilled water and well mixed using a pipette. It was cooled in ice after boiling for 10 minutes at 110°C on a Stuart® scientific block heater (Stone, Staffordshire, UK), and then immediately centrifuged for 7–8 minutes at 13,000 rpm. At the end, 100 µl of the supernatant was kept for further PCR analysis at -20°C.

The *nuc* gene is a gold standard for *S. aureus* molecular confirmation (Brakstad et al., 1992). The *nuc* gene-positive isolates were further tested for the *mecA* gene by PCR method which confirmed the methicillin resistance property of MRSA (Omar et al., 2014). In both cases, PCR reactions were conducted on Bio-Rad T100™ Thermal Cycler (Bio-Rad, CA, USA). The extracted genomic DNA from bacterial cells was tested to detect the presence of species specific *nuc* gene of *S. aureus* and *mecA* gene for MRSA by simplex PCR method using specific primers (Nuc-F: GCG ATT GAT GGT GAT ACG GTT, Nuc-R: AGC CAA GCC TTG ACG AAC TAA AGC; MecA-F: TGG CTA TCG TGT CAC AAT CG, MecA-R: CTG GAA CTT GTT GAG CAG AG) (Brakstad et al., 1992; Omar et al., 2014). The GelDoc Go imaging system (Bio-Rad, USA) was used to view the bands generated through electrophoresis of the amplified PCR products on a 1% agarose gel under ultraviolet light.

2.4. Antimicrobial Susceptibility Testing

Using the disk diffusion technique, the antibiotic susceptibility patterns of the bacterial isolates were assessed (Jorgensen and Turnidge, 2015). After preparing a bacterial suspension from a 24-hour fresh culture, each isolate was cultured for two to three hours. After that, a little suspension was taken to make loan for each isolate in Mueller Hinton Agar medium plate using a cotton swab. After that, the medium containing the culture suspension was covered with antibiotic discs at different concentrations. Here, commercially available antibiotic discs (Thermo Scientific™ Oxoid™, Basingstoke, Hampshire, UK) e.g., azithromycin (AZM, 15 µg), chloramphenicol (C, 30 µg), ciprofloxacin (CIP, 5 µg),

clindamycin (DA, 2 µg), gentamicin (CN, 10 µg), linezolid (LZD, 30 µg), nitrofurantoin (F, 300 µg), tetracycline (TE, 30 µg), sulfamethoxazole-trimethoprim (SXT, 25 µg), and rifampicin (RD, 5 µg) were used (Huang et al., 2006). The MHA plates were then incubated for twenty-four hours at 37°C. After incubation, Clinical Laboratory Standards Institute (CLSI) (Humphries et al., 2018) guidelines were followed for measuring and interpreting the inhibition zones around each of the antibiotic discs. Multidrug resistance (MDR) capacity of the isolates was assessed according to Gurung et al. (2020). Any isolate that showed resistance to ≥3 different classes of antibiotics was considered a multidrug-resistant (MDR) strain (Gurung et al., 2020).

2.5. Multiple Antibiotic Resistance Index (MARI)

The number of antibiotics to which a microorganism is resistant was divided by the total number of antibiotics to which the organism was exposed for calculating the MARI (Ayandele et al., 2020).

2.6. Statistical analysis

Data collected from all the secondhand items were entered into a spreadsheet (Microsoft Excel 2010) and transferred into a statistical software program (STATA, version 18, Stata Corporation 2023, College Station, TX: StataCorp LLC.) for further analysis. Descriptive statistics were used to compute the prevalence of *S. aureus*, antibiotic resistance percentage, and frequency of MARI.

3. Results

3.1. Phenotypic confirmation of *S. aureus* isolates

The results from the current study indicated that different secondhand items were contaminated with *S. aureus*, which was phenotypically confirmed by MSA, CHROMagar™ Staphylococcus (Figure 1) and biochemical test (data are not shown).

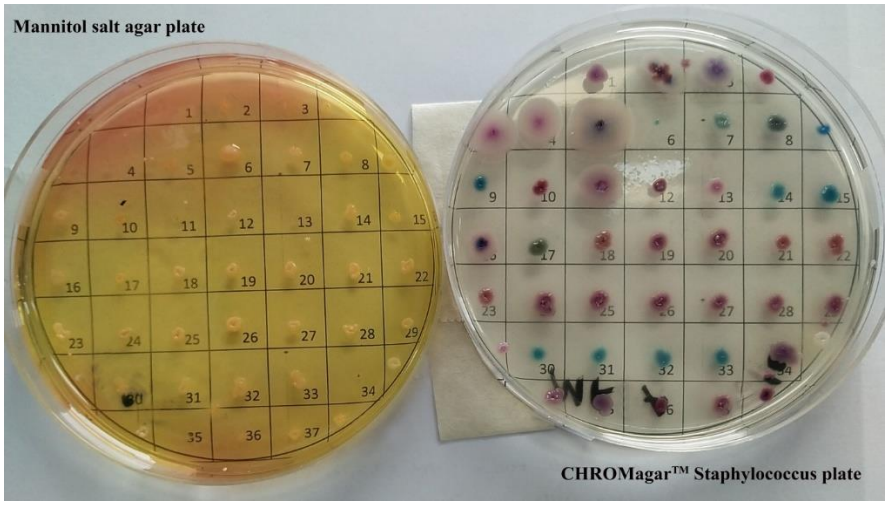

Figure 1. The appearance of *Staphylococcus aureus* colonies after patch inoculation on MSA and CHROMagar™ Staphylococcus plate. The colonies of *Staphylococcus aureus* were yellow colored on MSA plate and mauve to pink-colored colonies on CHROMagar™ Staphylococcus plate.

3.2. Incidence and contamination level of *S. aureus*

The results obtained from the assessment of *S. aureus* of different secondhand items sold in three different markets of Dhaka, Bangladesh are presented in Table 1. The incidence of *S. aureus* in the total examined samples was 22/90 (24.4%), according to the phenotypic examination. *S. aureus* load in the different items of secondhand samples was ranged from 5.0×10^{1} to 9.3×10^{3} cfu/ml with the lowest count in women's jacket and highest count in men's sweater except no count found in any samples of adult shoes, men's T-shirt and men's trouser. Twenty two (22) isolates were chosen for further molecular analysis.

Table 1. Prevalence of *S. aureus* from secondhand items based upon cultural and PCR.

Sample types	Code	Total number of tested sample	Contaminated with *S. aureus*	Highest load of *S. ureus* (cfu/ml)	*nuc* (279bp) gene	*mecA* (310bp) gene
T-1 (Baby fabric toy)	BFT	6	1	8.7×10^{2}	1	1
T-2 (Baby shoes)	BS	6	2	1.7×10^{3}	2	1
T-3 (Baby romper)	BR	6	1	2.7×10^{2}	1	
T-4 (Baby sweater)	BS	6	3	6.7×10^{3}	3	1
T-5 (Baby dress)	BD	6	2	2.6×10^{2}	1	
T-6 (Kids Jeans)	KJ	6	2	9.0×10^{1}	2	
T-7 (Adult shoes)	AS	6	0	0		
T-8 (Shirt)	S	6	1	2.3×10^{2}	1	
T-9 (Men's T-shirt)	MTS	6	0	0		
T-10 (Men's sweater)	MS	6	2	9.3×10^{3}	2	1
T-11 (Men's jacket)	MJ	6	1	9.0×10^{1}	1	1
T-12 (Men's trouser)	MT	6	0	0		
T-13 (Men's Jeans)	MJ	6	3	1.6×10^{2}	2	
T-14 (Women's sweater)	WS	6	3	4.3×10^{2}	3	1
T-15 (Women's jacket)	WJ	6	1	5.0×10^{1}	1	
		90	22 (24.4%)		20 (22.2%)	6 (30%)

3.3. Molecular confirmation of *S. aureus* and MRSA isolates

The extracted DNA from the phenotypically confirmed *S. aureus* isolates was tested for the presence of species-specific *nuc* and *mecA* gene by simplex PCR with specific primers, which confirmed the molecular identity of *S. aureus* and MRSA. Amongst the 22 presumptive *S. aureus* isolates retrieved from the study, 20 were positive to the *nuc* gene detection confirming 91% (20/22) as *S. aureus* species (Figure 2). Among the 20 isolates of *S. aureus*, only 6 isolates were confirmed genetically as MRSA, having the *mecA* gene (Figure 3).

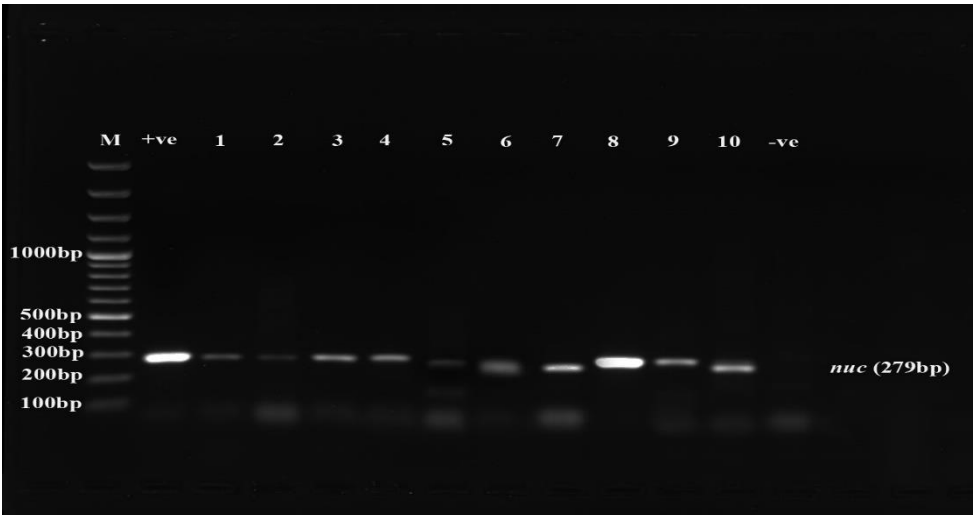

Figure 2. Photography of gel electrophoresis showing PCR product of nuc gene extracted from isolated *S. aureus* bacterial strain in secondhand items. Marker (M): 100 bp DNA ladder; + ve control: S. aureus ATCC29213; Lane 1-10: nuc (279 bp) gene amplification visualize band.

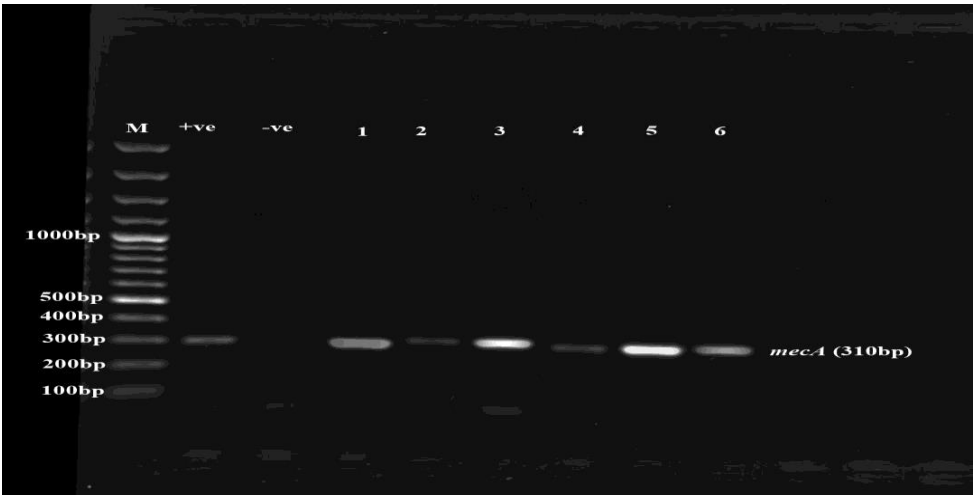

Figure 3. Photography of gel electrophoresis showing PCR product of *mecA* gene extracted from isolated *S. aureus* bacterial strain in secondhand items. Marker (M): 100 bp DNA ladder; + ve control: *S. aureus* ATCC33591; Lane 1-6: *mecA* (310 bp) gene amplification visualize band.

3.4. Antibiotic susceptibility and MARI profiles of *S. aureus*

S. aureus strains containing *nuc* gene were subjected to antibiotic sensitivity assay. The susceptibility pattern of *S. aureus* isolates are presented in Table 2. Disk diffusion testing demonstrated that all isolates of *S. aureus* including MRSA and MSSA were highly resistant (100%) to azithromycin, whereas all were sensitive to gentamicin (100%) and tetracycline (100%).

Table 2. Antibiotic resistance patterns of *S. aureus* isolates.

			MRSA			MSSA		
Antibiotics	**Disc Code**	**Concentration**	**R (%)**	**I (%)**	**S (%)**	**R (%)**	**I (%)**	**S (%)**
Azithromycin	AZM	15 µg	6 (100)	0 (0.0)	0 (0.0)	14 (100)	0 (0.0)	0 (0.0)
Clindamycin	DA	2 µg	3 (50)	1 (16.6)	2 (33.3)	14 (100)	0 (0.0)	0 (0.0)
Gentamicin	CN	10 µg	0 (0.0)	0 (0.0)	6 (100)	0 (0.0)	0 (0.0)	14 (100)
Tetracycline	TE	30 µg	0 (0.0)	0 (0.0)	6 (100)	0 (0.0)	0 (0.0)	14 (100)
Ciprofloxacin	CIP	5 µg	6 (100)	0 (0.0)	0 (0.0)	0 (0.0)	8 (57.1)	6 (42.8)
Trimethoprim-sulfamethoxazole	SXT	25 µg	2 (33.3)	2 (33.3)	2 (33.3)	8 (57.1)	3 (21.4)	3 (21.4)
Cholramphenicol	C	30 µg	0 (0.0)	0 (0.0)	6 (100)	0 (0.0)	4 (28.5)	10 (71.4)
Nitrofurantoin	F	300 µg	2 (33.3)	0 (0.0)	4 (66.6)	11 (78.5)	3 (21.4)	0 (0.0)
Linezolid	LZD	30 µg	6 (100)	0 (0.0)	0 (0.0)	6 (42.8)	0 (0.0)	8 (57.1)
Rifampicin	RD	5 µg	0 (0.0)	1 (16.6)	5 (83.3)	0 (0.0)	4 (28.5)	10 (71.4)

Table 3. Frequency of MARI of *S. aureus* isolates from secondhand items samples.

	Isolates	No. of antibiotics tested	Total antibiotic resistance	No. of isolates	MAR Index	Frequency of MARI in isolates n (%)	Antibiotic-resistant pattern	MDR isolates
MRSA	S6, S7	10	5	2	0.5	2 (33.3)	AZM/DA/CIF/F/LZD	MDR
	S55, S63	10	4	2	0.4	2 (33.3)	AZM/CIP/SXT/LZD	MDR
	S21	10	4	1	0.4	1 (16.6)	AZM/DA/CIF/LZD	MDR
	S80	10	3	1	0.3	1 (16.6)	AZM/CIP/LZD	MDR
MSSA	S11, S20	10	5	2	0.5	2 (14.28)	AZM/DA/SXT/F/LZD	MDR
	S14, S31, S35, S60, S77, S83	10	4	6	0.4	6 (42.8)	AZM/DA/SXT/F	MDR
	S82, S88	10	4	2	0.4	2 (14.28)	AZM/DA/F/LZD	MDR
	S28, S73	10	3	2	0.3	2 (14.28)	AZM/DA/LZD	
	S45	10	3	1	0.3	1 (7.14)	AZM/DA/F	
	S23	10	2	1	0.2	1 (7.14)	AZM/DA	

4. Discussion

Used goods provide a risk to human health because they may act as a vehicle for the spread of infectious organisms (Baharuddin & Nurhatira, 2020; Jadoon et al., 2022; Odum and Idise, 2022). Several previous studies reported that *S. aureus* was the most frequently isolated

bacterium from secondhand items (Atubu et al., 2016; Muthiani et al., 2017; Olajubu et al., 2017; Garba et al., 2019; Jadoon et al., 2022; D. Ocampo et al., 2023). The prevalence rate of *S. aureus* (22.2%) detected from used items in this study was lower than 60.7% in Nigeria (Olajubu et al., 2017), 75% in Nigeria (Atubu et al., 2016), and 80% in Pakistan (Jadoon et al., 2022). *S. aureus* occur commonly in our environment and thus, it is possible to cultivate this species from clothing as well as almost any surface in our surroundings (Al-Easawi and Emran, 2017; D. Ocampo et al., 2023). There are various ways to spread it, including coming into close touch with infected materials. *S. aureus* is present in the nose, back of the throat, and skin of around 30% of healthy people (Jadoon et al., 2022). Since clothing and fomites regularly come into close touch with the skin of their former owners, it is logical to assume that these objects, as the study has shown, are contaminated with potentially harmful bacteria like *S. aureus* (Al-Easawi and Emran, 2017; D. Ocampo et al., 2023). This particular species of bacteria may live for years in clothes, fabric, and solid particles. It may last in used fabrics and clothing for an extended period of time because to its resistance to dryness and tolerance of high temperatures (Al-Easawi and Emran, 2017; D. Ocampo et al., 2023). These microorganisms might only need to be present in trace amounts to infect the following host if they are able to survive on surfaces long enough to come into touch with a host (Al-Easawi and Emran, 2017; D. Ocampo et al., 2023).

The examined secondhand items were found to be contaminated with MRSA. According to earlier research, pathogenic bacteria MRSA and MSSA, which pose a serious danger to public health, were identified from SHI in Kenya (Muthiani et al., 2017), Pakistan (Jadoon et al., 2022), and the Philippines (D. Ocampo et al., 2023). According to data from community-associated MRSA infection outbreaks, skin-to-skin and skin-to-fomite contact are significant and frequent alternate modes of acquisition for the skin-infecting strain (Muthiani et al., 2017; D. Ocampo et al., 2023). Both sporadic infections and outbreaks of differing degrees are caused by staphylococci. As mentioned by several researchers group, it could result in a variety of diseases, including deep-seated illnesses like osteomyelitis and endocarditis, as well as more dangerous conditions like pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections (Muthiani et al., 2017; Jadoon et al., 2022).

An estimated 5 million fatalities are attributed to antimicrobial resistance (AMR), directly causing 1.27 million deaths globally in 2019 (An et al., 2024). AMR is a serious danger to public health worldwide (An et al., 2024). According to An et al. (2024), *S. aureus* is one of the Gram-positive bacteria that is most responsible for antibiotic resistance-related deaths. Among the investigated isolates in this study, high susceptibilities to antibiotics including gentamicin (100%), tetracycline (100%), chloramphenicol (80%), and rifampicin (75%) were found. The majority of *S. aureus* were reported to be sensitive to gentamicin, tetracycline, and chloramphenicol in previous investigations (Olajubu et al., 2017; Bandyopadhyay et al., 2019; Garba et al., 2019; Jadoon et al., 2022; D. Ocampo et al., 2023). According to the results of the current study, which were consistent with earlier research conducted in Bangladesh, the United States, and Vietnam (Islam et al. 2018; Vicetti Miguel et al. 2019; Khatun et al. 2023; An et al., 2024), isolated *S. aureus* strains, including MRSA and MSSA, exhibited the maximum resistance to azithromycin (100%) and clindamycin (85%). Of all the risks associated with antibiotic resistance, MRSA infections are one of the most common and can be quite dangerous (Ventola, 2015). Nearly all antibiotics used in therapy have evolved resistance mechanisms in *S. aureus* strains (Mlynarczyk-Bonikowska et al., 2022). The majority of issues were brought on by MRSA strains, which resulted in infections that were challenging to treat (Mlynarczyk-Bonikowska et al., 2022). The MRSA isolates in this investigation exhibited the highest resistant to azithromycin (100%), ciprofloxacin (100%) and linezolid (100%) with 100% MDR phenotypes. Many previous studies showed high resistance

of MRSA strains to azithromycin, ciprofloxacin and linezolid (Wilson et al., 2003; Elshabrawy et al., 2017; Rafique et al., 2019; Gurung et al., 2020; Parvin et al., 2021; An et al., 2024).

Present study conducted on secondhand items in Bangladesh has reported a relatively high prevalence of MRSA (30%) and high rates of MDR (100%) amongst the isolates. An important finding of concern in this study is that all the isolates of MRSA detected from secondhand items were MDR, previous report in Bangladesh also documented that 100% MRSA strains isolated from food, environmental and clinical samples were MDR (Islam et al., 2018; Islam et al., 2019; Parvin et al., 2021). During the last ten years, rates of community-acquired MRSA infections have sharply increased among the general population (Ventola, 2015). MDR microbes have the potential to infect humans (Sobur et al., 2021; Haque et al., 2023). Because MDR strains are expanding due to AMR, pathogens represent a significant danger to healthcare systems worldwide (Sobur et al., 2021; Haque et al., 2023). In this study, six MRSA (100%) and ten MSSA (71.43%) isolates were phenotypically MDR in nature. MARI analysis is now widely used to identify bacteria isolated from settings where traditional antibiotics are both safe and effective when used to treat humans. Generally, plasmids containing one or more resistance genes are associated with bacterial MARI (Sobur et al., 2021; Mlynarczyk-Bonikowska et al., 2022; Haque et al., 2023). More than 0.20 MARI value means that the source of these bacterial strains is considered high-risk, meaning that many antibiotics are either overused or misused. This means that a significant percentage of the bacterial isolates have been exposed to multiple antibiotics and have consequently developed resistance to them (Odum & Idise, 2022). The majority of the used clothing samples came from high-risk contamination sources where previous users or sellers were either frequent antibiotic users or abusers, as indicated by the fact that 95% of the *S. aureus* isolates in this investigation had MARI values larger than 0.2 (Odum and Idise, 2022). Previously, Odum and Idise (2022) reported that 79.31% of various types of bacterial strains isolated from secondhand items showed a MARI of more than 0.2, whereas, 95% of *S. aureus* strains in our present study showed a MARI of more than 0.2 and Odum & Idise also presented that isolated *S. aureus* strains had MARI value of 0.6, which is more than in our current investigation.

5. Conclusion

The research findings indicate that Methicillin-resistant *Staphylococcus aureus* (MRSA) was detected in used items obtained from markets of Dhaka, Bangladesh. The detection of MRSA in used clothing revealed that although secondhand clothing is less expensive than new, it can act as an agent for the spread of infections and their transmission among different groups. Kirby-Bauer disk diffusion susceptibility testing revealed that all isolated MRSA bacterial isolates were MDR. Due to the high level of antibiotic resistance seen in isolated MRSA and MSSA strains, extra caution and awareness are necessary. The results of this investigation also raised the possibility that some of the analyzed used goods came from sources of high risk contamination. Although individuals utilize these clothes because of their living standards, it is strongly advised that they be properly laundered and cleaned before wearing secondhand clothing that has been acquired. Future research is essential to isolate and identify other pathogenic bacteria and assess their potential for producing diseases or creating problems in human life, moreover exploring their emergence and spread of resistance in those bacteria.

Acknowledgments

This research was funded under the “University Grants Commission 2023-24” of the Jagannath University, Dhaka, Bangladesh. We would like to thank the reviewers for their valuable comments and suggestions to improve paper quality.

Conflict of interests

The authors declare that they have no competing interests.

References

Agbulu, C. O., Gberikon, G. M., & Ajine, B. O. (2015). Isolation and characterization of microorganisms associated with Secondhand female undergarments and children wear sold in Makurdi metropolis. International Journal of Current Microbiology and Applied Science, 4(1):716-724.

Akter S., Zuhura F. T., Ferdous Z., Hossain M. N., Ahsan M. T., & Salam M. A. (2024). Detection of multi-antibiotic resistant *Vibrio parahaemolyticus* isolated from fresh produce in Dhaka, Bangladesh. Journal of advanced biotechnology and experimental therapeutics, 7(2), 303-313.

Al-Easawi, N. A. F., & Emran, F. K. (2017). A microbial survey of second hand clothe samples collected from Baghdad market. Journal of Al-Nahrain University, 20(3), 127-136.

An, N. V., Hai, L. H. L., Luong, V. H., Vinh, N. T. H., Hoa, P. Q., Hung, L. V., Son, N. T., Hong, L. T., Hung, D. V., Kien, H. T., Le, M. N., Viet, N. H., Nguyen, D. H., Pham, N. V., Thang, T. B., Tien, T. V., & Hoang, L. H. (2024). Antimicrobial resistance patterns of *Staphylococcus aureus* isolated at a general hospital in Vietnam between 2014 and 2021. Infection and drug resistance, 17, 259–273.

Atubu, J.O., Gberikon, G.M., & Agbulu C.O. (2016). Microbial analysis of secondhand children socks sold in some selected markets in Makurdi Metropolis, Nigera. Journal of Applied Life Sciences International, 6(4), 1-8.

Ayandele, A. A., Oladipo, E. K., Oyebisi, O., & Kaka, M. O. (). Prevalence of multi-antibiotic resistant *Escherichia coli* and *Klebsiella* species obtained from a tertiary medical institution in Oyo State, Nigeria, Qatar Medical Journal, 2020,20209

Baharuddin, A. & Nurhatira J. (2020). The existence of *Staphylococcus aureus* at the imported clothing in Makassar city. International Journal of Science and Healthcare Research, 5(1), 191-197.

Bandyopadhyay, R., Ghosh, S., Chatterjee, S., & Mandal, S. (2019). Bacteriological profiling of toys and clothes of children from children's day care centre of Tarakeswar, Hooghly West Bengal, India. Bioscience Biotechnology Research Communications, 12(1).

Brakstad, O. G., Aasbakk, K., & Maeland, J. A. (1992). Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. Journal of clinical microbiology, 30(7), 1654-1660.

Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries. Part 2, 2nd Edition, Cambridge University Press Publication, South Africa, 1-434.

D. Ocampo, M. Z., D. Aquino, L. A., R. Aquino, M. A., P. Buenavente, R., & M. Tropel, N. A. (2023). Isolation, characterization, and identification of the most common bacterial isolates from second-hand clothes in selected ukay-ukay stores in Dasmarinas, Cavite. *Food, Nutrition, and Health. Reaffirming the Critical Role of Transformative Research and Knowledge Production in the Age of Post-Truth,* Proceedings of the 5th De La Salle University Senior High School Research Congress, De La Salle University, Philippines, 27-29 June.

Elshabrawy, W. O., Zaki, M. E., & Kamel, M. F. (2017). Genetic and phenotypic study of methicillin-resistant *Staphylococcus aureus* among patients and health care workers in Mansoura University Hospital, Egypt. Iranian journal of microbiology, 9(2), 82–88.

Garba, L., Barambu, H., Adamu, M. T., Yarma, A. A., & Aisami, A. (2019). Bacterial contaminants associated with female handbags and their antibiotic susceptibility profile. Saudi Journal of Pathology and Microbiology, 4(4): 338-342

Gurung, R. R., Maharjan, P., & Chhetri, G. G. (2020). Antibiotic resistance pattern of Staphylococcus aureus with reference to MRSA isolates from pediatric patients. Future science OA, 6(4), FSO464.

Haque, Z. F., Islam, M. S., Sabuj, A. A. M., Pondit, A., Sarkar, A. K., Hossain, M. G., & Saha, S. (2023). Molecular detection and antibiotic resistance of *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Vibrio alginolyticus* from shrimp (*Penaeus monodon*) and shrimp environments in Bangladesh, Aquaculture Research, *2023*, 5436552, 1-11.

Holt, J.G. (1984). Bergey's manual of determinative bacteriology. 9th Edition, Lippincott Williams and Wilkins, Baltimore.

Huang, H., Flynn, N. M., King, J. H., Monchaud, C., Morita, M., & Cohen, S. H. (2006). Comparisons of community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) and hospital-associated MSRA infections in Sacramento, California. Journal of Clinical Microbiology, *44*(7), 2423-2427.

Humphries, R. M., Ambler, J., Mitchell, S. L., Castanheira, M., Dingle, T., Hindler, J. A., & Sei, K. (2018). CLSI methods development and standardization working group best practices for evaluation of antimicrobial susceptibility tests. Journal of clinical microbiology, *56*(4), e01934-17.

Islam, M. A., Parveen, S., Rahman, M., Huq, M., Nabi, A., Khan, Z. U. M., Ahmed, N., & Wagenaar, J. A. (2019). Occurrence and characterization of methicillin resistant *Staphylococcus aureus* in processed raw foods and ready-to-eat foods in an urban setting of a developing country. Frontiers in microbiology, 10, 503. https://doi.org/10.3389/fmicb.2019.00503

Islam, T., Kubra, K., & Hassan Chowdhury, M. M. (2018). Prevalence of methicillin-resistant *Staphylococcus aureus* in hospitals in chittagong, Bangladesh: A threat of nosocomial infection. Journal of microscopy and ultrastructure, 6(4), 188–191.

Jadoon, A., Farooq, H., Ambreen, A., Rafi, N., Akram , M.H., Salam, M. A., Memon, F. S., Mirani, S.A., Gul, M., Ali, S., Kamran, M., Zafar, I., Riaz, M.U., & Mehmood, A. (2022). Isolation and identification of potentially pathogenic bacteria from second hand items of flea market of district Haripur, Pakistan. Natural Volatiles & Essential Oils, 9(1), 1067-1076.

Jorgensen, J. H., & Turnidge, J. D. (2015). Susceptibility test methods: dilution and disk diffusion methods. Manual of clinical microbiology, 1253-1273.

Khatun, R., Hasan, S., Khatun, N., & Akter, S. (2023). Prevalence of methicillin-resistant *Staphylococcus aureus* with their susceptibility pattern and their association of panton-valentine leukocidin gene in a tertiary care hospital in Bangladesh. Fortune Journal of Health Sciences, 6, 160-166.

MacFaddin, J. F. Biochemical tests for the identification of medical bacteria. Baltimore, Williams and Wilkins Company, 1972.

Merlino, J., Leroi, M., Bradbury, R., Veal, D., & Harbour, C. (2000). New chromogenic identification and detection of *Staphylococcus aureus* and methicillin-resistant *S. aureus*. Journal of Clinical Microbiology, 38(6), 2378-2380.

Mlynarczyk-Bonikowska, B., Kowalewski, C., Krolak-Ulinska, A., & Marusza, W. (2022). Molecular mechanisms of drug resistance in *Staphylococcus aureus*. International journal of molecular sciences, 23(15), 8088.

Moniruzzaman, M., Hussain, M. T., Ali, S., Hossain, M., Hossain, M. S., Alam, M. A. U., Galib, F. C., Islam, M. T., Paul, P., Islam, M. S., Siddiqee, M. H., Mondal, D., Parveen, S., & Mahmud, Z. H. (2023). Multidrug-resistant *Escherichia coli* isolated from patients and surrounding hospital environments in Bangladesh: A molecular approach for the determination of pathogenicity and resistance. Heliyon, 9(11), e22109.

Muthiani, Y. M., Matiru, V. N., & Bii, C. (2017). Potential skin pathogens on second hand clothes and the effectiveness of disinfection methods. *JKUAT Annual Scientific*

Conference, Jomo Kenyatta University of Agriculture and Technology, Kenya, 21 June, 144-162.

Odum, E.I., & Idise, O.E. (2022). Microbiological assessment of female second-hand undergarments sold In Abraka, Nigeria. Rivers State Univeristy Journal of Biology & Applied Sciences, 2(1).

Olajubu, F. A., Folorunso, V. T., & Olojede, O. (2017). The microbial diversity of fairly used wears sold in a Lagos market, Nigeria. IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPRS), 12(2), 63-68.

Omar, N. Y., Ali, H. A. S., Harfoush, R. A. H., & El Khayat, E. H. (2014). Molecular typing of methicillin-resistant *Staphylococcus aureus* clinical isolates on the basis of protein A and coagulase gene polymorphisms. International journal of microbiology, *2014*, 650328.

Owen, L. & Laird, K. (2020). The role of textiles as fomites in the healthcare environment: a review of the infection control risk. PeerJ. 8: e9790

Parvin, M. S., Ali, M. Y., Talukder, S., Nahar, A., Chowdhury, E. H., Rahman, M. T., & Islam, M. T. (2021). Prevalence and multidrug resistance pattern of methicillin resistant *S. aureus* isolated from frozen chicken meat in Bangladesh. Microorganisms, 9(3), 636. https://doi.org/10.3390/microorganisms9030636

Pedroso-Butanas, M. A., & Butanas Jr., B. M. (2018). Elemental and infrared characterization of contaminants in second-hand cotton garments from flea markets in Valencia city, Bukidnon, Philippines. Jurnal Penelitian Fisika dan Aplikasinya, 8(1), 10-16.

Putri, M. M., Selomo, M., Ruslan, & Amqam, H. (2019). Bacterial contamination analysis on children toys in the early childhood education program of Makassar. *Proceedings of the 3rd International Conference on Environmental Risks and Public Health*, ICER-PH 2018, 26-27, October, Makassar, Indonesia.

Rafique, H., Hussain, N., Saeed, M. U., Iqbal, H. M. N., Azim, G., & Bilal, M. (2022). Linezolid-resistance *Staphylococcus aureus* — Prevalence, Emerging Resistance Mechanisms, Challenges and Perspectives. Journal of Pure Applied Microbiology,16(3), 1492-1505. doi: 10.22207/JPAM.16.3.44

Rakhshanpour, A., Aghahossein-Shirazy, A., Shafiei, R. & Rahimi, M.T. (2021). Second hand clothes, a new threat for acquiring parasitic infections. Iran Journal of Public Health, 50(1), 211-212.

Sanders, D., Grunden, A. M., & Dunn, R. R. (2022). A review of clothing microbiology: the history of clothing and the role of microbes in textiles. Biology Letters, 17(1).

Sobur, M. A., Islam, M. S., Haque, Z. F., Orubu, E. S. F., Toniolo, A., Choudhury, M. A., & Rahman, M. T. (2021). Higher seasonal temperature enhances the occurrence of methicillin resistance of *Staphylococcus aureus* in house flies (*Musca domestica*) under hospital and environmental settings. Folia microbiologica, 67(1), 109–119. https://doi.org/10.1007/s12223-021-00922-9

Ventola, C. L. (2015). The antibiotic resistance crisis: causes and threats. P & T: a peer-reviewed journal for formulary management, 40(4), 277-283.

Vicetti Miguel, C. P., Mejias, A., Leber, A., & Sanchez, P. J. (2019). A decade of antimicrobial resistance in *Staphylococcus aureus*: A single center experience. PloS one, 14(2), e0212029.

Wilson, P., Andrews, J. A., Charlesworth, R., Walesby, R., Singer, M., Farrell, D. J., & Robbins, M. (2003). Linezolid resistance in clinical isolates of *Staphylococcus aureus*. Journal of Antimicrobial Chemotherapy, 51(1), 186-188.