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Molecular detection of vancomycin and methicillin resistance in *Staphylococcus aureus* isolated from food processing environments

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ABSTRACT

Staphylococcus aureus is a well-known foodborne pathogen. The aim of this study was to investigate the presence of *S. aureus* isolated from serving utensils in food processing environments in Mymensingh city, Bangladesh and to determine their antibiogram and resistance determinants. A total of 120 environmental samples were collected from different food settings. Isolation and identification were conducted using conventional biochemical tests. Molecular identification of isolates and detection of methicillin and vancomycin resistance were done using primer-specific polymerase chain reaction (PCR) targeting *Tuf*, *nuc*, *mecA*, and *mecC* genes. Antibiotic sensitivity tests were performed, and resistance genes were also detected by amplifying bla_{TEM}, *vanA, vanB, and vanC* genes. Among the 120 samples, 81 (67.5%) were positive for *Staphylococcus* spp. and 41 (50.62%) were positive for the *nuc*-gene. Among the 41 isolates, 5 (12.20%) were positive for *mecA*, but none were positive for the *mecC* gene. A total of 12.2% of the isolates were *vanC*-positive, of which 4 isolates (9.76%) were also positive for the *mecA* gene. Antibiotic sensitivity testing revealed that all *S. aureus* isolates (100%) from hotel samples were sensitive to ciprofloxacin and chloramphenicol, 90.32% were sensitive to doxycycline, and 80.65% were sensitive to streptomycin. Conversely, all isolates (100%) were resistant to ampicillin, and 29.03% were resistant to vancomycin. All *S. aureus* isolates obtained from non-hotel samples were susceptible to chloramphenicol, ceftriaxone, ciprofloxacin, doxycycline, meropenem, and vancomycin; however, 40% of isolates were resistant to novobiocin. Among the hotel isolates, 29 (93.55%) of the ampicillin-resistant isolates harbored the *blaTEM* gene while 5 (55.55%) of the vancomycin-resistant isolates harbored the *vanC* gene. Four of the five *vanC* positive isolates were also positive for the *mecA* gene. The presence of methicillin-resistant *S. aureus* (MRSA) which is also vancomycin-resistant in food processing environments is a threat to public health. This is the first report on the molecular detection of methicillin and vancomycin-resistant *S. aureus* isolated from food processing environments in Bangladesh.

1. Introduction

Foodborne diseases represent a significant public health problem worldwide [1,2]. Good personal hygiene and proper sanitation are essential measures for protecting against foodborne diseases. Plates, dishes, glasses, cups, spoons, cutlery, and other serving utensils used in food service establishments can potentially spread foodborne diseases [3]. It is crucial to make sure that serving utensils are kept clean [4]. The apparent cleanliness of surfaces and utensils in a food processing environment does not necessarily indicate the absence of bacterial contamination [5]. Previous studies also reported that cleaning utensils and food contact surfaces contributed to cross-contamination in food service establishments [6].

Staphylococcal foodborne diseases are known to be significant threats to public health worldwide [7]. Staphylococci remain viable on hands and surfaces for a long time following the initial contact [8]. A minimal dose of staphylococcal enterotoxins can cause food poisoning in humans [2]. Staphylococcal enterotoxins caused more than 300

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foodborne outbreaks in the European Union in 2012 [9]. Staphylococcal food poisoning is also a major cause of hospitalization in the U.S. every year [9,10]. Similarly, *Staphylococcus aureus* is responsible for a significant proportion of foodborne outbreaks in China [11].

Because of the acquisition of *mecA* gene, *S. aureus* can sometimes display methicillin-resistance, which may complicate therapy of infections in animals and humans [12]. Methicillin-resistant *S. aureus* (MRSA) strains have been detected in raw and cooked meat, milk, cheese, and other food products [13–17]. Food handlers in food service establishments are in close contact with food products, which can lead to contamination of the serving utensils.

Vancomycin is the recommended antibiotic for infections caused by MRSA. Vancomycin-resistant MRSA has been isolated for the first time more than two decades ago [18]. After this, vancomycin-resistant *S. aureus* (VRSA) has been isolated from more countries in North America, Europe, Asia, Africa, and South America [19–23].

There is very limited data on the microbiological safety of serving utensils in Bangladesh. Mymensingh is a densely populated city that has many educational institutions and historic places that attract local and international visitors. Due to its frequent food processing environments, it was selected as a suitable location for this study on the microbiological safety of serving utensils.

Staphylococcus aureus is not only a threat to human health but also to animals. Given that samples were taken from the environment, different aspects of the *One Health* concept were investigated in the present study. In addition to the isolation and molecular identification of *S. aureus*, we aimed to determine the antimicrobial resistance pattern of the isolated bacteria from serving utensils in food service establishments in Mymensingh city. We also detected the resistance genes using PCR. Results of this study could be used to develop a food sanitation strategy in the city, which involves both prevention and treatment. Results could also be used to plan a strategy for the use of drugs that can minimize resistant bacteria in the future.

2. Materials and methods

2.1. Sample collection and transportation

For sample collection, ten food service establishments were selected in Mymensingh city. A total of 120 samples were collected, including 40 plate samples, 40 glass samples, and 40 spoon samples. The term “hotel isolates” refers to *S. aureus* isolates obtained from food establishments that are located within hotels. The term “non-hotel isolates” refers to *S. aureus* isolates obtained from food establishments elsewhere. All utensils were ready to be served to the customers and were clean to the best of the knowledge of the owners of the food service establishment. We took precautions to minimize self-contamination by using sterile cotton buds to wipe the upper surfaces of plates, spoons, and inner surfaces of glass followed by dipping the cotton buds into sterile nutrient broth. In order to maintain a temperature of 4°C, the samples were immediately transferred to the laboratory and were incubated overnight at 37°C.

2.2. Isolation and identification

Following overnight incubation, the preliminary cultures were streaked onto Mannitol Salt agar (HiMedia, India) plates, which is specific for *Staphylococcus* spp. followed by incubation at 37°C for 24 h. All isolates were sub-cultured on Mannitol Salt agar to obtain pure colonies. For morphological confirmation, Gram staining was performed. For biochemical confirmation, catalase and coagulase tests were performed.

2.3. Molecular detection using genus-specific and species-specific primers

Bacterial DNA was extracted by the conventional boiling method [24], and the extracted DNA was used as a template DNA for the molecular detection of *Staphylococcus* spp. and *S. aureus* using specific primers (Table 1). To detect MRSA, the extracted DNA was amplified with specific primers as shown in Table 1. In all cases, the amplified products were electrophoresed at 60 V for one hour in 1.5% agarose gel [25]. After staining with ethidium bromide, the gel was examined under a UV transilluminator using the 100-bp/1-kb DNA ladder (Promega, USA).

2.4. Antibiogram test

The following ten commonly-prescribed antibiotics (HiMedia, India) were used for antimicrobial susceptibility testing: ampicillin (AMP, 10 mg), chloramphenicol (C, 30 mg), ceftriaxone (CTR, 30 mg), ciprofloxacin (CIP, 30 mg), doxycycline (DO, 30 mg), meropenem (MEM, 10 mg), novobiocin (NV, 30 mg), streptomycin (S, 10 mg), tetracycline (TE, 30 mg), and vancomycin (VA, 30 mg). For each culture suspension of the bacterial isolates, a McFarland 0.5 standard was maintained [26]. Antibiogram phenotyping was performed using the disk diffusion method in Mueller Hinton agar media (HiMedia, India). Results were recorded as sensitive, intermediate, or resistant in accordance with the CLSI recommendations [27].

2.5. Molecular detection of resistance genes

Phenotypically-resistant *S. aureus* isolates were screened for antibiotic resistance genes using PCR using specific primers (Table 1). The amplified products were processed as in section 2.3.

3. Results

Out of the 120 samples, 81 (67.5%) were positive for *Staphylococcus* spp. by observing the color change on Mannitol Salt agar plates, microscopic examination, and PCR using genus-specific *Tuf* gene. Using PCR amplification of the *nuc* gene, 41 (50.62%) out of the 81 isolates were positive for the *nuc* gene (Table 2). The highest rate was obtained from the hotel glass samples (18.52%), and the lowest rate was obtained from the non-hotel spoons (1.23%)

The 41 isolates were further analyzed by PCR using the specific primers *mecA* and *mecC* for MRSA and five were found to be positive for the *mecA* gene (Table 3). None were positive for the *mecC* gene. Among the five isolates positive for *mecA*, four (9.76%) were obtained from hotel glass samples and one (2.44%) was obtained from spoon samples (Table 3). Among the non-hotel *nuc*-positive isolates, none was positive for *mecA* or *vanC*. Among the hotel *nuc*-positive isolates, four isolates were positive for both *mecA* and *vanC*, while one isolate was only positive for *mecA*, and another isolate was only positive for *vanC* (Table 3).

The *nuc*-gene positive isolates were tested for antibiotic susceptibility using ten commonly prescribed antibiotics. Among 31 *S. aureus* isolates from hotel samples, 100% were resistant to ampicillin, 45.16% were resistant to novobiocin, and 29.03% were resistant to vancomycin. All isolates were sensitive to chloramphenicol and ciprofloxacin while 90.32% and 80.65% of the isolates were sensitive to doxycycline and streptomycin, respectively. Among the 10 *nuc*-gene positive non-hotel isolates, four (40%) were resistant to novobiocin, while six (60%) were sensitive to novobiocin. All other isolates exhibited sensitive or intermediate resistance to chloramphenicol, ceftriaxone, ciprofloxacin, doxycycline, meropenem, and vancomycin (Table 4, Fig. 1, Fig. 2). A total of 29 out of the 31 (93.55%) ampicillin-resistant isolates (as detected phenotypically) were positive for the bla_{TEM} gene. Vancomycin resistance genes were detected by PCR using *vanA*, *vanB*, and *vanC* primers. Results showed that none of the isolates harbored *vanA* or *vanB* genes. Five (55.55%) isolates harbored the *vanC*-resistance gene. Four isolates which were methicillin resistant (as detected phenotypically) were found to harbor the *vanC* gene. To the best of our knowledge, this is the first report to detect the *vanC* resistance gene in *S. aureus* isolated from food service settings in Bangladesh.

Table 1
Oligonucleotide primers used for the amplification of *Staphylococcus aureus* genes.

Primer	Target gene	Primer sequence (5′-3′)	Amplicon size (bp)	Reference
Tseq271	*Tuf*	5′-AAYATGATIACIGGIGCIGCICARATGGA-3′	884	[28]
Tseq1138		5′-CCIACIGTICKICCRCCYTCRCG-3′		
nuc-F	*nuc*	5′-GCGATTGATGGTGATACGGT-3′	279	[29]
nuc-R		5′-AGCCAAGCCTTGACGAACTAAAGC-3′		
mecA-F	*mec*A	5′-AAAATCGATGGTAAAGGTTGG-3′	533	[30]
mecA-R		5′-AGTTCTGGCACTACCGGATTTTGC-3′		
mecC-P1	*mecC*	5′-GAAAAAAAGGCTTAGAACGCCTC-3′	138	[31]
mecC-P2		5′-GAAGATCTTTTCCGTTTTCAGC-3′		
$bla_{TEM\text{-}1}$-F	bla_{TEM}	5′-CATTTCCGTGTCGCCCTTAT-3′	793	[32]
$bla_{TEM\text{-}1}$-R		5′-TCCATAGTTGCCTGACTCCC-3′		
vanA-F2	*vanA*	5′-AATGTGCGAAAAACCTTGCG-3′	677	[33]
vanA-R2		5′-CCGTTTCCTGTATCCGTCC-3′		
vanB-F2	*vanB*	5′-GCTCCGCAGCCTGCATGGA-3′	463	[34]
vanB-R2		5′-ACGATGCCGCCATCCTCCT-3′		
vanC1-F	*vanC*	5′-GAAAGACAACAGGAAGACCGC-3′	796	[34]
vanC1-R		5′-TCGCATCACAAGCACCAATC-3′		

Table 2
The percentage of *nuc*-gene positive samples in *Staphylococcus aureus* isolates in the current study.

Study area	*Staphylococcus*-positive	Number (%)	*S. aureus*-positive (*nuc*-positive)	Number (%)
Hotel	15 (Spoons)	15/120 (12.5)	10	10/81 (12.35)
Food Establishments	20 (Glass)	20/120 (16.67)	15	15/81 (18.52)
($N = 60$)	18 (Plates)	18/120 (15.0)	6	6/81 (7.41)
Non-hotel Food	6 (Spoons)	6/120 (5.0)	1	1/81 (1.23)
Establishments	12 (Glass)	12/120 (10.0)	4	4/81 (4.94)
(N = 60)	10 (Plates)	10/120 (12.0)	5	5/81 (6.17)
Total samples = 120	81	81/120 (67.5)	41	41/81 (50.62)

Table 3
The percentage of *mecA*-positive *Staphylococcus aureus* isolates in the current study.

Study area	*nuc*-positive	*mecA*-positive (%)	*vanC*-positive (%)
Hotel Food	10 (Spoons)	1 (2.44)	0 (0)
Establishments	15 (Glass)	4 (9.76)	4 (9.76)
	6 (Plates)	0 (0)	1 (2.44)
Non-hotel Food	1 (Spoons)	0 (0)	0 (0)
Establishments	4 (Glass)	0 (0)	0 (0)
	5 (Plates)	0 (0)	0 (0)
Total	41	5 (12.20)	5 (12.20)

Multidrug-resistance (MDR) to two, three, four, or five antibiotic classes was detected in 19 out of 31 (61.3%) *nuc*-gene positive isolates. Only one isolate (3.2%) was resistant to five classes of antibiotics, three (9.67%) isolates were resistant to four classes of antibiotics, seven (22.58%) isolates were resistant to three classes of antibiotics, and eight (25.81%) isolates were resistant to two classes of antibiotics.

4. Discussion

Methicillin-resistant *S. aureus* (MRSA) causes zoonotic infections that have both public health and veterinary importance, especially with the MDR patterns that can be associated with MRSA. MRSA causes food poisoning, suppurative pneumonia, pyogenic endocarditis, and otitis media infections in humans [35]. It causes a variety of infections in animals including botryomycosis and localized purulent infection in horses, localized pyogenic infection and severe acute mastitis in cattle, pustular dermatitis and food poisoning in dogs and cats [35]. In addition, it causes greasy pig disease in swine and bumble-foot disease in birds [35]. Through skin infections and other routes, there can be human-to-animal and animal-to-human transmission of MRSA. The excessive use of antibiotics in the veterinary sector can result in MRSA-infected animal reservoirs that can cause zoonotic MRSA infections in humans [35]. To fully control MRSA infections, a *One Health* approach that considers animals and humans in addition to the environment needs to be implemented.

Multidrug-resistant MRSA has been detected in animals and animal-derived food products (such as frozen chicken meat and processed raw meat) in Bangladesh. [36, 37]. In addition, *S. aureus* has been isolated from raw milk samples in Bangladesh, in which mastitis is known to be a major problem [38]. Contamination of milk can happen while milking and is thus dependent on the cleanliness of the environment and the utensils that are used for the milking process. This highlights the impact of the environment, which is the third component of the *One Health* triangle.

MRSA can also be transmitted between humans and their companion animals or even by stray dogs and cats. MRSA was isolated from dogs and cats [39,40] and from ornamental birds [41] in Bangladesh. Some of the isolates from ornamental birds were also found to be resistant to vancomycin [41].

Cross-contamination of food due to improper handling and non-hygienic utensils can cause foodborne outbreaks and infections [42]. Sources of contamination include human hands, breath, hair, raw food products, and food processing environments. People suffering from respiratory tract infections, or gastrointestinal disorders should not

Table 4
Antibiotic resistance profile of *Staphylococcus aureus* isolates in the current study

Antimicrobial agent	Number of isolates and their antibiotic susceptibility patterns (%)					
	Resistant		Intermediate		Sensitive	
	Hotel (n = 31)	Non-hotel (n = 10)	Hotel (n = 31)	Non-hotel (n = 10)	Hotel (*n* = 31)	Non-hotel (*n* = 10)
Ampicillin	31 (100%)	–	–	6 (60%)	–	4 (40%)
Chloramphenicol	–	–	–	–	31 (100%)	10 (100%)
Ceftriaxone	12 (38.71%)	–	–	–	19 (61.29%)	10 (100%)
Ciprofloxacin	–	–	–	–	31 (100%)	10 (100%)
Doxycycline	1 (3.23%)	–	2 (6.45%)	–	28 (90.32%)	10 (100%)
Meropenem	–	–	10 (32.26%)	–	21 (67.74%)	10 (100%)
Novobiocin	14 (45.16%)	4 (40%)	3 (9.67%)	–	14 (45.16%)	6 (60%)
Streptomycin	–	–	6 (19.35%)	2 (20%)	25 (80.65%)	8 (80%)
Tetracycline	–	–	14 (45.16%)	5 (50%)	17 (54.84%)	5 (50%)
Vancomycin	9 (29.03%)	–	–	–	22 (70.97%)	10 (100%)

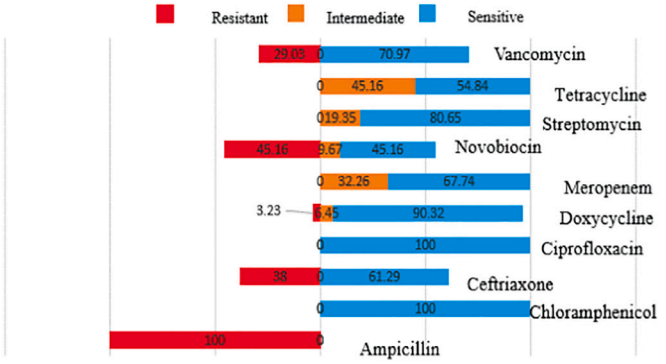

Fig. 1. Antibiotic Susceptibility patterns of hotel *Staphylococcus aureus* isolates.

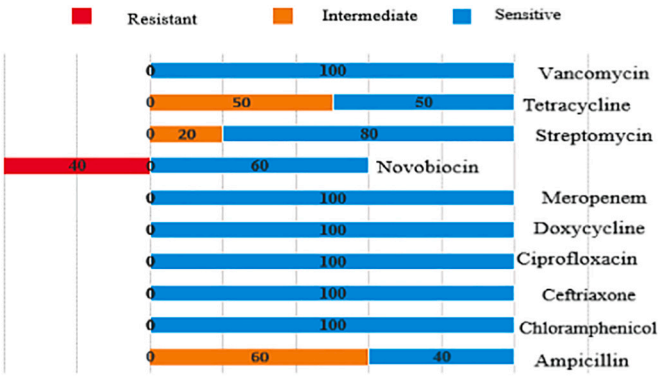

Fig. 2. Antibiotic susceptibility patterns of non-hotel *Staphylococcus aureus* isolates.

handle food. As *S. aureus* can survive for a long period in the respiratory tract of infected patients, on cloths, and in other environments, there is a possibility of contaminating food processing environments as well as the serving utensils. The pathogenic *S. aureus* can also adhere to gloves of employees in food processing establishments and can be transmitted to other humans or to the environment if gloves are not changed frequently [43]. It is important to ensure that the nasopharyngeal and oropharyngeal tracts of food handlers are free of *S. aureus* in order to prevent any foodborne outbreaks [44–46]. Utensils can also become contaminated with *S. aureus* from food products including raw meat and vegetables. Dairy cows, chickens, and pigs are known to have the ability to transmit MRSA to humans [47]. Improper food handling by infected personnel is responsible for 20% of bacterial foodborne illnesses [48]. An earlier study reported a carriage rate of 79% in *S. aureus* among food handlers [49].

In order to prevent infections, there needs to be daily cleaning and disinfection of tables and environments in which food is served A major concern with *S. aureus* is its biofilm production ability, which increases its tolerance to dryness and dehydration, which helps it to persist and multiply [50,51]. A study reported that 34.3% of kitchen sponges from food establishments were positive for *S. aureus*. Moreover, *S. aureus* was isolated from 30% of the kitchen sponges of restaurants, 36.4% of the kitchen sponges of hotels, 33.3% of the kitchen sponges of pastry shops, and 34% of the kitchen sponges of cafeterias [52].

Other potential sources of cross-contamination include the use of paper bills and coins. The older the paper bill or coin is, the more the microbial contamination [53]. A study conducted in Bangladesh reported that the majority of paper bills were contaminated with *Staphylococcus* spp. [54] Another report indicated that bacterial contamination of paper bills in Bangladesh was as high as 93.7% [55]. In Nigeria, the rate of contamination of paper bills with *S. aureus* was reproted to be 22.5% [56]. Contaminated paper bills can lead to cross-contamination of food items or serving utensils. Flies can act as mechanical vectors for food contamination, and microorganisms such as *E. coli*, *Vibrio cholera*, and *S. aureus* have been isolated from house flies. It is important to note that dishwashing detergents were found to have different capacities to eliminate bacteria in kitchen sponges [57]. Bacteria can remain on hands and utensils for up to several days after the initial contact.

The emergence of antibiotic-resistant *S. aureus* such as MRSA and VRSA is a worldwide public health threat [58,59]. Our data showed that *S. aureus* is prevalent in serving utensils in food processing environments. Many isolates in our study were found to be antibiotic-resistant and can be a potential source of transmission of resistance to humans. Serving utensils played a significant role in transmission of multidrug-resistant staphylococci detected in the present study. Continuous monitoring and surveillance for antibiotic resistance are key for the control and treatment of MRSA infections. A multifaceted *One-Health* approach involving public health and veterinary consultants in addition to environmental microbiologists needs to be implemented for the effective control of MRSA infections.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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