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ORIGINAL ARTICLE

Characterization of *Staphylococcus aureus* isolated from chicken and quail eggshell

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ABSTRACT

Objectives: This study was conducted to assess the prevalence and characterization of *Staphylococcus aureus* from chicken and quail eggshells and to study the antibiogram of the isolates.

Materials and methods: A total of 300 eggs (220 chicken eggs and 80 quail eggs) were collected from different retail shops and farms in Mymensingh district. Swabs taken from the egg surfaces were cultured on Mannitol Salt Agar for the isolation of *S. aureus*. Polymerase chain reaction was conducted for confirmatory identification of the bacterial species targeting *nuc* gene, followed by confirmation of methicillin-resistant *S. aureus* by targeting the *mecA* gene. Antibiotic sensitivity test of the isolated bacteria was done against commonly used antibiotics by the disk diffusion method.

Results: The prevalence of *Staphylococcus* spp. and *S. aureus* in the chicken eggshell surface was 20.45% and 10.45%, respectively. Similarly, the prevalence of *Staphylococcus* spp. and *S. aureus* in quail eggshell surface was 16.25% and 5%, respectively. Overall, 27 isolates were identified as *S. aureus*, of which 23 were from the chicken eggshell surface and four from quail eggshell surface. Among the seven isolates tested, overall four (57.14%) were positive for the *nuc* gene. On the other hand, the *mecA* gene could be detected in three (50%) *S. aureus* out of six oxacillin resistant isolates. The antibiogram study indicated that most of the isolates were resistant to the antibiotics under β-lactam group.

Conclusion: The present study concludes that chicken and quail egg surface harbor multidrug-resistant bacteria which may cause public health hazards, if these antibiotic-resistant bacteria are transferred to a human.

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Introduction

Table eggs are devoured worldwide in varied forms and are viewed as a very nutritious and cheap source of protein. *Staphylococci* comprise an imperative part of the microflora which can be segregated from the table egg surface and its contents. They can possibly cause deterioration and infection in consumers through entering the food channel pathway [1]. The shell can be contaminated when going across the vent, but many researchers recommend that contamination mainly happens immediately after laying due to attachment with infected surfaces [2]. It has been estimated that after laying, bacteria deposited on egg surface can infiltrate the shell and subsequently infect egg contents [3].

Eggshell contains several microorganisms, including *Staphylococcus aureus*, *Salmonella* spp., *Streptococcus* spp., *Escherichia. coli*, *Bacillus* spp., and *Listeria monocytogenes* [4]. Several diseases occurred in poultry are caused by *Staphylococcus* spp. [5]. Nearly, 50% of *S. aureus* produce enterotoxins which create food poisoning in consumers [6]. Among all foodborne diseases in the world, Staphylococcal food poisoning is ranked as the third [7]. Animal originating *Staphylococcus* strains can potentially be harmful to humans. Most of the strains of *Staphylococcus* show resistance to antibiotics and cause zoonoses [8]. Eggs are the potential source of transmitting antibiotic-resistant *Staphylococcus* strains to human causing food-borne infection [9]. Methicillin-resistant *S. aureus* (MRSA) is considered as one of the important bacterium among the *Staphylococci*, which is genetically different from other strains. The MRSA is developed

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through horizontal gene transfer and natural selection. As a result, multidrug resistance (MDR) in the bacteria may develop [10].

Along with the chicken eggs, quail rearing and retail sale of its eggs are getting popular day by day in Bangladesh. Thus, there is a chance of transmitting the MRSA to human through egg consumption. However, to our knowledge, limited research has been conducted on MRSA bearing resistance gene in chicken and quail eggshell. Considering the above fact, the present experiment was conducted to isolate and characterize the *S. aureus* and/or MRSA from chicken and quail eggshell surface.

Materials and Methods

Sample collection

A total of 220 fresh chicken eggs and 80 quail eggs were collected for sampling from different farms and retail shops in Mymensingh during the period from January to June 2017. The eggs were transported to the Bacteriology Laboratory, Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh.

Isolation and identification of Staphylococcus aureus

The collected eggs were swabbed with sterile cotton buds and dispersed in nutrient broth for enrichment overnight at 37°C in bacteriological incubator (FIEM, Italy). After enrichment, the samples were streaked on to Mannitol Salt Agar (MSA). The colonies showing typical cultural characteristics of *S. aureus* were further inoculated on blood agar for further isolation and pure culture. Gram stain and sugar fermentation test, indole, coagulase, catalase, Methyl Red Voges Proskauer test, and motility test were performed for confirmation of the isolates [11].

Detection of nuc *and* mecA *genes in the* S. aureus

The genomic DNA was extracted from the isolated organisms by boiling method [12]. Polymerase chain reaction (PCR) was conducted to amplify the *nuc* and *mecA* genes following the methods described by Kalorey et al. [13] and Hussain et al. [14]. Oligonucleotide primers targeting *nuc* and *mecA* genes of *S. aureus* are mentioned in Table 1. For the amplification of both the genes, a final reaction volume of 25 µl for PCR was used consisting of 2 µl of each primer (10 pmol/µl), 12.5 µl 2X PCR master mixture, 3 µl of template DNA (about 10 ng), and 5.5 µl of nuclease-free water. Thermocycler machine (Applied Biosystem, Singapore) was used for amplification of the genes, and the thermal profile used for both *nuc* and *mecA* genes consisted of an initial denaturation for 5 min at 95°C, followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 45 sec, and extension at 72°C for 1 min. The final extension was set at 72°C for 10 min. An amount of 5 µl PCR products were separated in 1.5% agarose gel and was visualized using UV trans-illuminator (Biometra, Germany) after staining with ethidium bromide.

Antibiotic sensitivity test

Antibiogram of *S. aureus* was done against nine commonly used antibiotics, including amoxicillin (30 µg), ciprofloxacin (5 µg), erythromycin (5 µg), gentamycin (5 µg), nalidixic acid (µg), oxacillin (1 µg), penicillin (10 µg), tetracycline (30 µg), and vancomycin (30 µg). The antibiotics disks were purchased from Himedia, India. The antibiotic sensitivity test was performed by the disk diffusion method [15]. The zone of inhibition produced by *S. aureus* was interpreted according to the standards of the Clinical and Laboratory Standards Institute [16].

Results

Overall prevalence of Staphylococcus *spp. and* Staphylococcus aureus

On the basis of cultural and biochemical characteristics, 20.45% (n = 45/220) chicken egg samples were found to be associated with *Staphylococcus* spp. Among these 45 isolates, 23 (10.45%) were identified as *S. aureus* on the basis of the coagulase test (Table 2). Similarly, 13 (16.25%) out of 80 quail eggs were associated with *Staphylococcus* spp., of which four (5%) were *S. aureus* (Table 3).

Molecular detection of nuc and mecA

From the coagulase positive isolates, seven (randomly selected) were used for PCR amplification targeting the *nuc* gene, of which four (one from quail and three from chicken eggshell) were positive (Table 4, Fig. 1a). Among the coagulase positive isolates (n = 27), six isolates originated from

Table 1. Oligonucleotide primers used in this study.

Target gene	Primer	Primer Sequence (5′–3′)	Target size (bp)	Reference
nuc	*nuc*F	CGCGATTGATGGTGATACGGTT	279	[14]
	*nuc*R	ACGCAAGCCTTGACGAACTAAAGC		
mecA	*mecA* F	AAAATCGATGGTAAAGGTTGG	533	[14]
	mecA R	AGTTCTGGCACTACCGGATTTTGC		

Table 2. Overall prevalence of *Staphylococcus* spp. and *S. aureus* in chicken egg sample.

Sample source	Sample(*N*)	*Staphylococcus* spp. positive[#]	Prevalence of *Staphylococcus* spp. (%)	*S. aureus* positive[*]	*S. aureus*(%)
BAU poultry farm	50	15	30	10	20
Janota poultry Farm	50	2	4	0	0
KR market	20	4	20	2	10
Shesh more	20	6	30	3	15
Wapda more	20	5	25	2	10
Kewatkhali Bazar	20	3	15	1	5
Poultry more	20	4	20	1	5
Mesua bazar	20	6	30	4	20
Total	220	45	20.45	23	10.45

[#]On the basis of cultural and biochemical properties, [*]On the basis of coagulase test.

Table 3. Overall prevalence of *Staphylococcus* spp. and *S. aureus* in quail egg sample.

Sample source	Sample(*N*)	*Staphylococcus* spp. positive[#]	Prevalence of *Staphylococcus* spp. (%)	*S. aureus* positive[*]	*S. aureus*(%)
BAU poultry farm	20	4	20	2	10
KR market	20	2	10	1	5
Shesh more	20	3	15	0	0
Mesua bazar	20	4	20	1	5
Total	80	13	16.25	4	5

[#]On the basis of cultural and biochemical properties, [*]On the basis of coagulase test.

Table 4. Molecular detection of *nuc* and *mecA* genes in *S. aureus*.

Gene	Total sample	Coagulase positive	Coagulase positive used for PCR	PCR positive
nuc	300	27	7	4
mecA	300	27	6	3

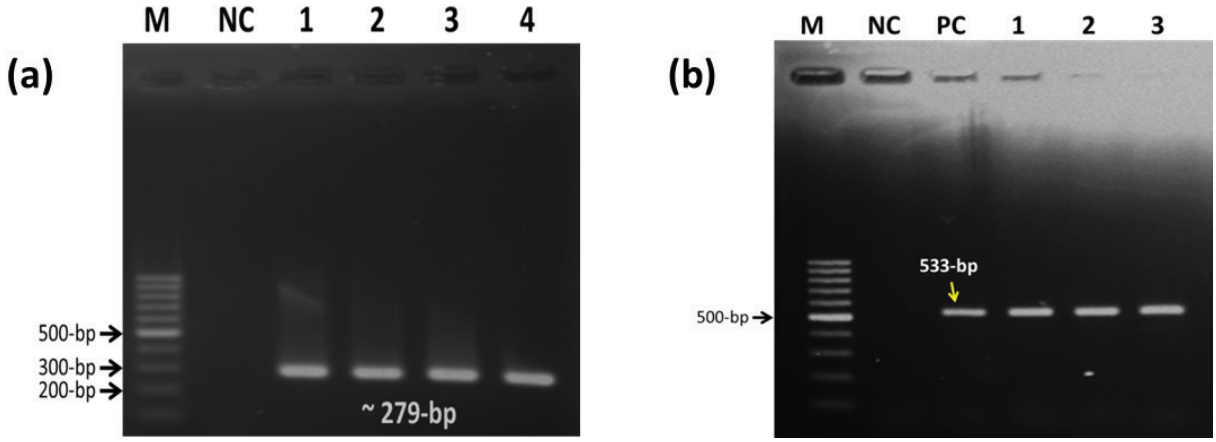

Figure 1. PCR amplification of *nuc* (a) and *mecA* (b) genes of *S. aureus*. (a) M = marker, NC = negative control, Lane 1–4 = test samples, (b) M = marker, NC = negative control, PC = positive control, Lane 1–3 = test samples.

chicken eggs were resistance to oxacillin, of which three were found positive for the *mecA* gene (Table 4, Fig. 1b).

Antibiotic sensitivity test

All the 23 isolates of *S. aureus* from chicken egg sample were subjected to antibiotic sensitivity test against nine commonly used antibiotics (Table 5). The results showed that vancomycin was sensitive to 73.91% isolates. Besides, 91.30% isolates were found to be resistant to amoxicillin (Table 5). On the other hand, all the four *S. aureus* isolates from quail egg samples were highly susceptible (75%) to vancomycin, oxacillin, and tetracycline. In contrast, 75% isolates were resistant to amoxicillin, nalidixic acid, and penicillin (Table 5).

Discussion

Out of 300 eggs, 58 (19.33%) eggshells yielded growth of *Staphylococcus* spp., which was supported by Syed et al. [17] in Pakistan, who reported 21.3% prevalence of *Staphylococcus* spp. However, the prevalence found in our study was comparatively higher than the findings of Parveen et al. [18], Eid et al. [19], Chaemsanit et al. [20], Pyzik et al. [21], and Pyzik and Marek [22] who observed the prevalence was 5.5%, 14.5%, 18%, 7.61%, and 15.6% in Dinajpur (Bangladesh), Sharkia (Egypt), Thailand, and Lubin city, respectively. The prevalence of *Staphylococcus* spp. (25%) in table eggs collected from different markets of Dhaka city, as reported by Fardows et al. [23], was higher as compared with our study. In developing countries like Bangladesh, an increased percentage of bacterial contamination have been found on egg surface due to inappropriate refrigeration and even no refrigeration during the market storing. Thus, variation in the prevalence of *Staphylococcus* spp. in eggshell might be due to inappropriate storage condition at market level.

In this study, *Staphylococcus* spp. showed golden-yellow colonies on MSA due to fermentation of mannitol, as reported by Konuku et al. [24] and Kwoji et al. [25]. Microscopically, *Staphylococcus* spp. was Gram-positive cocci arranged in a grape-like cluster [11]. The isolation of coagulase positive *Staphylococcus* spp. in this study warns that this organism may cause human infection elicited by toxins produced by them. In this study, 58 isolates were catalase positive, of which 27 were coagulase positive indicating that the isolates were *S. aureus*, as described by Kumar et al. [26].

Out of seven coagulase-positive *S. aureus*, *nuc* gene was confirmed to be present in four (57.14%) isolates. Six isolates were oxacillin resistant, of which three (50%) contained the *mecA* gene. In another study, Sadeghi and Mansouri [27] reported that 162 *S. aureus* isolates were confirmed to be present with the *nuc* gene, of which 56.8% were MRSA. Similar report was also reported by Pyzik et al. [21].

Nowadays, MDR is an emerging issue worldwide in treating infectious diseases. Here, *Staphylococcus* spp. originated from chicken eggs showed varying degrees of resistance to amoxicillin (91.30%) and oxacillin (26.08%). Similar results reported by Eid et al. [24] indicated that 87% isolates of *Staphylococcus* spp. were resistant to amoxicillin. However, slightly lower resistant to amoxicillin (73.3%) was recorded by Lee [28]. In another study, Nam et al. [29] reported that only 6.2% *Staphylococcus* spp. were resistant to oxacillin. The eggs collected directly from the farms and the grocery stores were not washed before being sold. Though isolation of *S. aureus* was performed, enumeration was not conducted from these samples. This study also limits on the characterization of bacteria on the eggshell surface rather than the inner content. So, it would be difficult to interpret the public health significance in its present form. However, this study described the presence

Table 5. Antibiotic sensitivity profile of *S. aureus* isolated from chicken and quail eggs.

Antimicrobial agents	Group	No. of isolates (%)					
		Chicken egg isolates			Quail egg isolates		
		R	I	S	R	I	S
Amoxicillin	β-lactam	21 (91.30)	2 (8.69)	0 (0.0)	3 (75.0)	1 (25.0)	0 (0.0)
Oxacillin	β-lactam	6 (26.08)	2 (8.69)	15 (65.21)	1 (25.0)	0 (0.0)	3 (75.0)
Penicillin	β-lactam	19 (82.60)	4 (34.78)	0 (0.0)	3 (75.0)	1 (25.0)	0 (0.0)
Ciprofloxacin	Quinolone	6 (26.08)	4 (17.39)	13 (56.52)	2 (50.0)	1 (25.0)	1 (25.0)
Nalidixic acid	Quinolone	19 (82.60)	2 (8.69)	2 (8.69)	3 (75.0)	1 (25.0)	0 (0.0)
Erythromycin	Macrolide	9 (39.13)	6 (26.08)	8 (34.78)	1 (25.0)	1 (25.0)	2 (50.0)
Gentamycin	Aminoglycoside	8 (34.78)	5 (21.73)	10 (43.47)	1 (25.0)	2 (50.0)	1 (25)
Tetracycline	Tetracycline	9 (39.23)	2 (8.69)	12 (52.17)	1 (25.0)	0 (0.0)	3 (75.0)
Vancomycin	Glycopeptide	4 (17.39)	2 (8.69)	17 (73.91)	0 (0.0)	1 (25.0)	3 (75.0)

R = Resistant, I = Intermediate, S = Sensitive.

of MRSA on eggshell in Bangladesh, and it would be interesting to continue working on the same track and develop the knowledge of the hygienic status in the egg production.

Conclusion

Our results reveal that eggshells are contaminated with *Staphylococcus* spp. at a higher proportion, and MDR *S. aureus* are recorded. This research confers a risk of being affected by MDR *S. aureus* from eggs of retail shops and farms unless they are properly washed and stored at the collection to marketing stage. So, it is important to establish proper hygienic practice and awareness among the people regarding the risk of MDR bacteria in consumers.

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Conflict of Interest

The authors declare that there is no conflict of interest towards the publication of this article.

Authors' Contribution

Amrita Pondit, Zobayda Farzana Haque, and Abdullah Al Momen Sabuj carried out the experiments, analyzed the data, and wrote the initial draft of the manuscript. Sukumar Saha and Md. Shahidur Rahman Khan designed and supervised research work, revised, and finalized the manuscript. All authors read and approved the manuscript before submission.

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