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Characterization of enterotoxin, antibiotic resistance genes, and antimicrobial susceptibility profiling of *Staphylococcus aureus* isolated from table eggs: Implications for food safety and public health

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ABSTRACT

Background: *Staphylococcus aureus* is a significant pathogen in both clinical and food safety contexts, capable of contaminating table eggs, which are a common dietary staple worldwide.

Aim: This study aimed to assess the prevalence, molecular characteristics, and antibiotic resistance profiles of *S. aureus* isolated from table eggs. The focus was on identifying methicillin-resistant *S. aureus* (MRSA) strains that produce enterotoxin (*seb*), resistance to β-lactam antibiotics (*blaTEM*), tetracycline (*tet*A), and vancomycin-resistant *S. aureus*.

Methods: A total of 200 egg samples were collected from various retail sources in Mymensingh City Corporation, Bangladesh. Swab samples (n = 100) were collected from eggshells, and another 100 samples were collected from the inner membrane, egg white, and yolk. Samples were enriched in trypticase soy broth and cultured on mannitol salt agar. *Staphylococcus aureus* was isolated through conventional culture techniques, confirmed by polymerase chain reaction targeting the *nuc*, and further screened for the *mec*A, *seb*, *blaTEM*, *tet*A, *van*A, and *van*C genes. Antibiotic susceptibility testing was conducted using the disc diffusion method against 13 antibiotics. Bivariate analysis is used to assess the strong and significant correlations between virulence genes and the pairs of any of two antibiotic-resistant *S. aureus*.

Results: *Staphylococcus* spp. and *S. aureus* were detected in 53% and 21% of eggshell samples, respectively, and 41% and 13% of egg content samples. Among 34 coagulase-positive isolates, 12 (57.14%) from eggshells and 4 (30.78%) from egg contents were positive for the *nuc* gene. Resistance was observed in eggshell isolates for *mec*A (33.33%), *blaTEM* (85.71%), *tet*A (33.33%), *van*A (19.04%), *van*C (33.33%), and *seb* (20.50%), whereas egg content isolates showed resistance to *blaTEM* (46.15%) and *van*C (7.80%). All coagulase-positive isolates exhibited significant resistance to β-lactam antibiotics, cephalosporins, and glycopeptides, especially vancomycin. Notably, 19 (90.47%) and 12 (92.30%) eggshell and egg content isolates, respectively, were multidrug-resistant, with multiple antibiotic resistance indices ranging from 0.23 to 0.76.

Conclusion: The study revealed a high prevalence of multidrug-resistant *S. aureus* in table eggs, indicating a significant public health risk. The presence of MRSA and strains with enterotoxins and resistance genes underscores the need for enhanced monitoring, stricter biosecurity measures, and robust control strategies for egg production and distribution to ensure food safety.

Keywords: Antibiogram, Enterotoxin, MDR, MRSA, Table eggs, VRSA.

Introduction

Table eggs are a widely consumed and affordable food source that are rich in protein, fats, vitamins, and minerals, making them a popular choice for a healthy diet worldwide (Puglisi and Fernandez, 2022; Myers and Ruxton, 2023). Although eggs are shielded by a hard shell and semipermeable membrane, they remain susceptible to microbial contamination, posing a potential risk of transmitting infectious agents that can induce illness in humans (Verma *et al.,* 2023). Even apparently clean, unbroken, and freshly laid shell eggs may contain harmful bacteria, potentially resulting in egg-borne diseases (Damena *et al.,* 2022). Consuming raw or undercooked eggs carries a notable risk of contamination due to pathogens naturally found in hens (Solís *et al.,* 2023). These pathogens can migrate

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from the eggshell surface to its inner structures through pores, a process influenced by factors, such as humidity, temperature, and storage duration (Kulshreshtha *et al.,* 2021; Rachtanapun *et al.,* 2022).

Inadequate handling and storage of eggs in unhygienic conditions at poultry farms or retail settings can negatively impact egg quality and present a potential health hazard to consumers (Jones *et al.,* 2018; Damena *et al.,* 2022). Previous studies have identified various bacterial species known to cause foodborne illnesses, such as *Listeria monocytogenes*, *Escherichia coli*, *Salmonella enterica*, and *Campylobacter jejuni*, in table eggs (Fonseca *et al.,* 2014; Kubo *et al.,* 2020; Ricke *et al.,* 2023; Sharan *et al.,* 2023). *Staphylococcus* spp. have been implicated in causing several diseases in poultry, with approximately 50% of *Staphylococcus aureus* strains producing enterotoxins capable of causing food poisoning in consumers (Pondit *et al.,* 2018). Staphylococcal food poisoning, ranking third among all foodborne diseases worldwide, is caused by *S. aureus*, a Gram-positive, facultative anaerobic, nonmotile, nonspore-forming bacterium with a round shape, renowned for its toxic properties (Kadariya *et al.,* 2014; Şanlıbaba, 2022). *Staphylococcus aureus* is typically found on the skin and in the nasal cavities of humans and animals as a commensal resident, which can spread from its natural habitats to other parts of the body, causing a range of clinical infections and foodborne intoxication (Achek *et al.,* 2021; Raineri *et al.,* 2022).

Staphylococcus aureus is a Gram-positive bacterium known for causing a wide range of infections, from minor skin conditions to life-threatening systemic diseases (Lang *et al*., 2024). *Staphylococcus aureus*, a medically significant bacterium, causes various infections, ranging from skin and soft tissue issues to more severe conditions such as pneumonia, fasciitis, otitis media, and urinary tract infections (David and Daum, 2010; Tong *et al.,* 2015). *Staphylococcus aureus* is a leading cause of food poisoning due to its production of heat-stable enterotoxins, which can persist in the food environment and cause illness (Grispoldi *et al.,* 2021; Liang *et al.,* 2023). Enterotoxin-producing *S. aureus* requires a temperature range of 10°C to 46°C for toxin secretion, with optimal growth and toxin production observed between 30°C and 40°C; conditions outside this range significantly reduce or inhibit toxin production (Argudín *et al.*, 2010; Hennekinne *et al*., 2012).

The rise of multidrug-resistant *S. aureus*, including MRSA, has challenged healthcare globally, resisting most β-lactam antibiotics such as penicillin and cephalosporins (Alghamdi *et al.,* 2023). Table eggs may contain medically significant bacterial species, including antibiotic-resistant strains, raising concerns (Kapena *et al.,* 2020). Evidence suggests that antimicrobial resistance can be transferred from food-producing animals to humans through the food chain, direct handling, or contact with infected animals and contaminated animal products (Samtiya *et al.,* 2022). The World Organization for Animal Health categorizes antibiotics based on their necessity for animal treatment, impacting both animal and human health settings (Scott *et al.,* 2019; Van *et al.,* 2020). *Staphylococcus aureus* is a prevalent pathogen causing a range of human infections, from skin issues to severe conditions such as bacteremia and endocarditis (Tong *et al.,* 2015). Resistant strains, such as MRSA and vancomycin-resistant *S. aureus* (VRSA), present significant treatment challenges (Cong *et al.,* 2020). Enterotoxin-producing *S. aureus* (SESA) can contaminate food, including eggs, leading to food poisoning (Grispoldi *et al.,* 2021). *Staphylococcus aureus*, commonly found in food, produces heat-resistant enterotoxins causing food poisoning, while antibiotic-resistant strains pose significant health concerns, with methicillin and vancomycin vital for treatment (Naeim *et al.,* 2023).

Table eggs contaminated with antibiotic-resistant *Staphylococcus* strains pose a risk of foodborne infections in humans, especially MRSA, a significant bacterium that can develop multidrug resistance through horizontal gene transfer (Parvin *et al.,* 2021). The rise of multidrug-resistant (MDR) microorganisms is a global public health concern, making it essential to study the prevalence of *S. aureus*, its antimicrobial resistance, and clonal lineages across human, animal, and food chain contexts. This research is particularly important given the diverse lifestyles influenced by diverse ethnicities, cultures, and food habits (Lozano *et al.,* 2016; Rao *et al.,* 2022).

The proliferation of retail shops, super shops, farm outlets, and wholesale marketplaces selling table eggs in Bangladesh has led to an elevated risk of MRSA transmission to humans through egg intake. However, no investigation has been conducted on the prevalence of MRSA-resistant genes in table eggs within the country. This research hypothesizes that table eggs sold in retail markets within Mymensingh City Corporation, Bangladesh, may harbor MDR-SA, including MRSA, enterotoxin-producing strains (*seb*), and VRSA. Therefore, the purpose of this study is to isolate and identify *S. aureus* from eggshells and egg contents, assess their phenotypic and genotypic antibiotic resistance patterns, and detect the presence of MRSA, VRSA, and enterotoxin-producing *S. aureus* (*seb*) in both the eggshells and egg contents.

Materials and Methods

Sample collection

A total of 200 random eggs were collected from different places in Mymensingh City Corporation, including retail shops, super shops, farm outlets, and wholesale markets. Each egg sample was placed in a separate, sterile plastic bag and brought to the Bacteriology Laboratory, Department of Microbiology

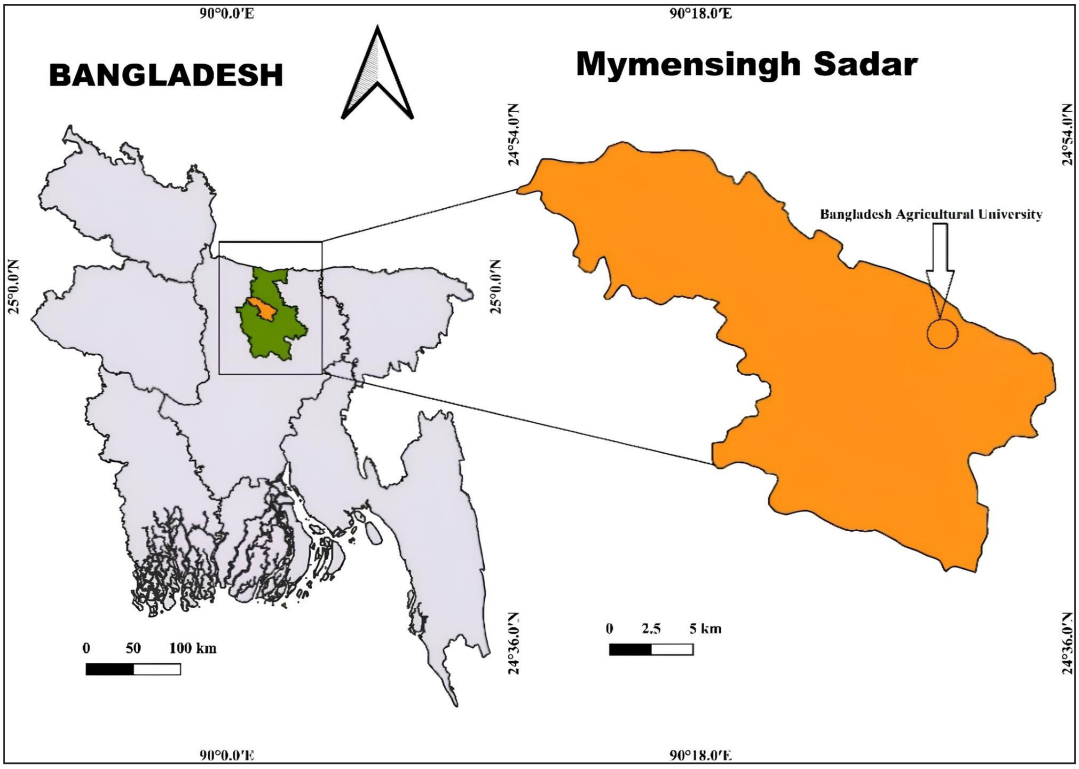

Fig. 1. Map of the site, where the study was conducted.

and Hygiene, Bangladesh Agricultural University, Mymensingh, Bangladesh, for testing (Fig. 1).

Bacteriological analysis

Sample preparation and isolation of *S. aureus*

The eggs were swabbed with sterile cotton buds and placed in trypticase soy broth for enrichment. The broth was then incubated in a bacteriological incubator overnight at 37°C. Following enrichment, samples were streaked onto mannitol salt agar (MSA) to isolate *S. aureus*. Pure colonies were obtained by subculturing all isolates on MSA plates, which were incubated for 24 hours at 37°C.

The surfaces of the eggs were disinfected by dipping them in 70% ethanol for 3–5 seconds, followed by air drying in a safety cabinet. Each egg was then cracked using a sterile rod, and the egg contents (the inner membrane, egg white, and yolk) were transferred into a sterile petri dish. Microbial analysis was performed on the mixer comprising three components of the egg: the inner membrane, egg white, and yolk. To isolate *S. aureus*, 1 mm of the egg mixture contents was diluted tenfold with sterile phosphate-buffered saline (1X PBS; HI Media, India) and homogenized for 1–2 minutes. Then, a 100-µl aliquot of the resulting mixture was spread onto an MSA plate (HI Media) and, after solidification, incubated for 24 hours at 37°C.

Identification of *S. aureus*

The identification of *S. aureus* was conducted by observing the cultural characteristics and colony morphology of MSA. Gram staining method and biochemical tests (catalase test and coagulase test) were performed to identify bacteria.

Molecular analysis

DNA extraction and polymerase chain reaction (PCR) detection of enterotoxin and antibiotic resistance genes

The boiling method was used to extract genomic DNA from isolated organisms (Ahmed and Dablool, 2017). Briefly, a 1 ml sample of the enriched culture was initially centrifuged at 5,000 rpm for 5 minutes. After discarding the supernatant, 200 µl of phosphate buffer solution was added to the pellet to create a suspension. The suspension was then boiled and allowed to cool for 10 minutes, followed by centrifugation at 10,000 rpm for 10 minutes. The resulting supernatant, which contained genomic DNA, was collected and stored at –20°C for future study.

A final reaction mixture of 20 µl was used for all PCR experiments, comprising 3 µl of nuclease-free water, 10 µl of master mix (Promega, Madison, WI), 1 µl each of forward and reverse primers, and 5 µl of DNA template. After amplification, the PCR results were analyzed by electrophoresis on a 1.5% agarose gel. The gel was then stained with ethidium bromide and documented using a Biometra UV trans illuminator (Göttingen, Germany). A 100-bp DNA ladder (Promega, Madison, WI) was used as a reference marker to confirm the anticipated size of the amplified PCR products.

A gene-specific PCR assay was performed to detect the specific gene associated with the detection of *S. aureus*. The assay successfully amplified the *nuc*, *mec*A, *seb*, *blaTEM*, *tet*A, *van*A, and *van*C genes using the PCR technique. The oligonucleotide primers used in this study for detecting these genes in *S. aureus* are listed in Table 1.

Antibiotic susceptibility testing

The antibiogram of *S. aureus* was performed using 13 commonly prescribed antibiotics: azithromycin (AZM, 15 µg), erythromycin (E, 5 µg), oxacillin (OX, 1 µg), penicillin (P, 10 µg), cefoxitin (CX, 30 µg), ciprofloxacin (CIP, 5 µg), levofloxacin (LEV, 5 µg), norfloxacin (NX, 10 µg), doxycycline (DO, 30 µg), tetracycline (TE, 30 µg), clindamycin (CD, 2 µg), gentamicin (GEN, 5 µg), and vancomycin (VAN, 30 µg). Prior to testing, each bacterial isolate was standardized to a McFarland 0.5 standard (Nix *et al.*, 2020; Haile *et al.*, 2022). The antibiotic disks were purchased from HI Media, India. The antibiotic sensitivity test was conducted using the disk diffusion method described by Bauer *et al*. (1966). The zones of inhibition produced by *S. aureus* were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2021).

Statistical analysis

The Statistical Package for Social Science (SPSS.v.25, IBM, Chicago, IL) and GraphPad Prism (Prism.v.8.4.2, San Diego, CA) were used to conduct analyses after the data from this study were integrated using Excel 365 (Microsoft/Office 365, Redmond, WA).

The frequencies of the different variables were determined by descriptive analysis. By applying a prior technique (Brown, 2001) in GraphPad Prism, a binomial 95% confidence interval (CI) was computed to estimate the prevalence. To evaluate the possible relationship between the virulence genes of *S. aureus* isolates with a *p*-value of less than 0.05, a bivariate analysis was also carried out using SPSS. Additionally, SPSS bivariate analysis was used to determine any link between the two antibiotics that were shown to be resistant to the isolates. A *p*-value of less than 0.05 ($p < 0.05$) denoted statistical significance.

Ethical approval

No ethics approval was required for this study.

Results

Prevalence of Staphylococcus spp. and S. aureus

Based on cultural and biochemical assessments, the prevalence of *Staphylococcus* spp. was 53% (*n* =

Table 1. Oligonucleotide primers used in the PCR assays.

Target gene	Primer sequences (5′-3′)		Target size (bp)	References
nuc	F	5′-GCGATTGATGGTGATACGGTT -3′	279	Kalorey *et al.* (2007)
	R	3′-AGCCAAGCCTTGACGAACTAAAGC-5′		
*mec*A	F	5′-AGCCAAGCCTTGACGAACTAAAGC- 3′	533	Kalorey *et al.* (2007)
	R	3′-AAAATCGATGGTAAAGGTTGG -5′		
sea	F	5′-TTGGAAACGGTTAAAACGAA-3′	120	Lu *et al.* (2001)
	R	3′-GAACCTTCCCATCAAAAACA-5′		
seb	F	5′- TCGCATCAAACTGACAAACG -3′	478	Lee *et al.* (2007)
	R	3′- GCAGGTACTCTATAAGTGCC -5′		
sec	F	5′- AGATGAAGTAGTTGATGTGTATGG-3′	451	Wang *et al.* (2014)
	R	3′-CACACTTTTAGAATCAACCG-5′		
sed	F	5′-CTAGTTTGGTAATATCTCCTTTAAACG-3′	319	Omoe *et al.* (2002)
		3′-TTAATGCTATATCTTATAGGGTAAACATC-5′		
blaTEM	F	5′ -CATTTCCGTGTCGCCCTTAT-3′	793	Walker *et al.* (2001)
	R	5′ -TCCATAGTTGCCTGACTCCC-3′		
*tet*A	F	5′-GGTTCACTCGAACGACGTCA-3′	800	Randall (2004)
	R	3′-CTGTCCGACAAGTTGCATGA-5′		
*van*A	F	5′ -AATGTGCGAAAAACCTTGCG-3′	677	Lu *et al.* (2001)
	R	5′ -CCGTTTCCTGTATCCGTCC-3′		
*van*B	F	5′ -GCTCCGCAGCCTGCATGGA-3′	463	Lemcke and Bülte (2000)
	R	5′ -ACGATGCCGCCATCCTCCT-3′		
*van*C	F	5′- GAAAGACAACAGGAAGACCGC -3′	796	Lemcke and Bülte (2000)
	R	5′ - TCGCATCACAAGCACCAATC -3′		

Table 2. The overall prevalence of *Staphylococcus* spp. and *Staphylococcus aureus* in table eggshell and content samples.

Sample source	Type	No. of samples with *Staphylococcus* spp.	Prevalence of *Staphylococcus* spp. (%)	No. of samples with *S. aureus*[a,b]	Prevalence of *S. aureus* (%)	Total	
						No.	Prevalence (%)
Retail shop	ES	8 (25)	32	5 (25)	20	13	52
	EC	7 (25)	28	3 (25)	12	10	40
Super shop	ES	3 (25)	12	1 (25)	4	4	16
	EC	2 (25)	8	0 (25)	0	2	8
Farm outlet	ES	12 (25)	48	9 (25)	36	21	84
	EC	11 (25)	44	6 (25)	24	17	68
Wholesale market	ES	9 (25)	36	6 (25)	24	15	60
	EC	8 (25)	32	4 (25)	16	12	48
Total	ES	32 (100)	32	21 (100)	21	53	53
	EC	28 (100)	28	13 (100)	13	41	41

ES = egg shell, EC = egg content.
[a]Based on culture and biochemical characteristics.
[b]Based on coagulase testing.

Table 3. Molecular detection of *nuc* gene of *Staphylococcus aureus* from eggshells and contents.

Sample source	No. of *S. aureus* screened[a]	Prevalence of *nuc* gene positive *S. aureus* n (%)	95% CI	*p*-value
Egg shell	21	12 (57.14)	36.50–75.50	0.663
Egg content	13	4 (30.78)	12.70–57.60	0.266

[a]On the basis of the coagulase test.

100) in eggshell samples and 41% (n = 100) in egg content samples. Among these isolates, 21% of the eggshell samples and 13% of the egg content samples were confirmed to be *S. aureus* via coagulase test. The prevalence of *Staphylococcus* spp. and *S. aureus* is summarized in Table 2.

Molecular detection of enterotoxin and antibiotic resistance genes

All 34 coagulase-positive *S. aureus* isolates were analyzed using PCR to detect the *nuc* gene. The results showed that 12 out of 21 eggshell isolates (57.14%, CI: 36.50–75.50) and 4 out of 13 egg content isolates (30.78%, CI: 12.70–57.60) were positive for the *nuc* gene (Table 3 and Fig. 3A).

These isolates were also tested for several antibiotic resistance genes (*mec*A, *blaTEM*, *tet*A, *van*A, and *van*C) and the enterotoxigenic gene (*seb*). The results indicated that among the eggshell isolates, 7 (33.33%, CI: 17.20–54.60) carried the *mec*A gene, 18 (85.71%, CI: 65.40–95.00) carried *blaTEM*, 7 (33.33%, CI: 17.20–54.60) carried *tet*A, 4 (19.04%, CI: 7.70–40.00) carried *van*A, 7 (33.33%, CI: 17.20–54.60) carried *vanC*, and 6 (20.5%, CI: 13.80–50.00) carried the *seb* gene (Table 4 and Fig. 3B–G).

For the egg content isolates, 6 (46.15%, CI: 23.20–70.90) were positive for the *blaTEM* gene, and 1 (7.8%, CI: 00.40–31.50) was positive for the *van*C gene, respectively (Table 4 and Fig. 3E and F). However, none of the egg content isolates were positive for the *mec*A, *tet*A, *van*A antibiotic resistance genes and the *seb* enterotoxin gene.

Bivariate analysis revealed strong and significant positive correlations between several virulence genes. Specifically, strong correlations were observed between *mec*A and *tet*A (Pearson correlation coefficient, p = 1.000), *mec*A and *van*A (p = 0.686), *mec*A and *van*C (p = 1.000), *tet*A and *van*A (p = 0.686), and *van*A and *van*C (p = 0.686). A summary of the correlation results between the antibiotic resistance genes of the *S. aureus* isolates is provided in Table 5 and Figure 2.

Antibiotic susceptibility testing of S. aureus isolated from eggshells

All 34 coagulase-positive *S. aureus* isolates were tested for antibiotic susceptibility using eight groups of 13 commonly prescribed antibiotics. Among the 21 isolates from table eggshell samples, high resistance levels were observed to cephalosporins, specifically CX (100%), β-lactam antibiotics such as P (100%) and OX (95%), lincosamides such as CD (90%), and fluoroquinolones, particularly CIP (85%). Resistance to macrolides (E) and tetracyclines (TE) was observed in 57% of all eggshell isolates. The lowest resistance

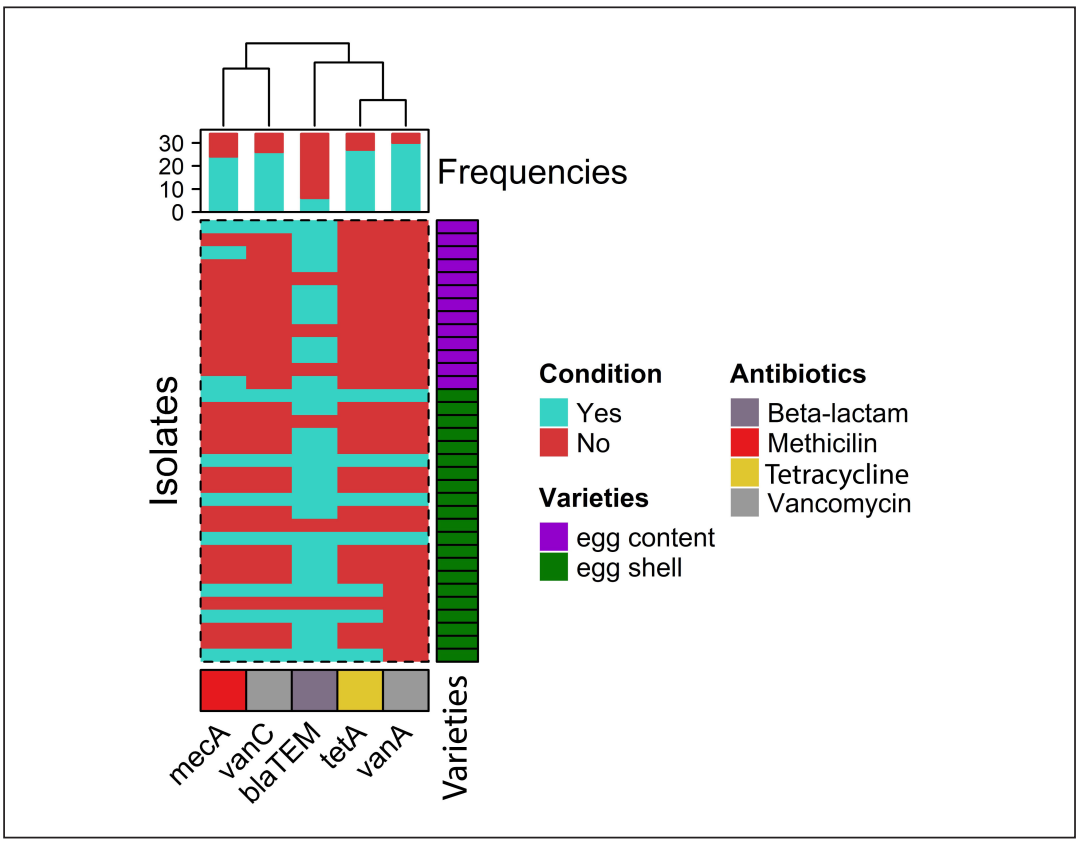

Fig. 2. The heatmap represents the distribution of the different types of antibiotic-resistant genes of *Staphylococcus aureus* isolated from eggshells and contents. Here, the top of the dendrogram represents the frequency of present genes and the neighborhood relationship among the genes.

levels were noted for aminoglycosides, such as GEN (23%), and glycopeptides, such as VAN (33%).

Conversely, fluoroquinolones, including LEV, NX, and DO, demonstrated the highest sensitivity (100%) across all isolates. Moderate sensitivity was observed for aminoglycosides (GEN [61%]) and glycopeptides (VAN [42%]). Lower sensitivity rates were observed for macrolides (E [14%]) and fluoroquinolones (CIP [14%]). Additionally, 19% of the isolates were sensitive to tetracyclines (TE) (Table 6 and Fig. 4).

These resistance and sensitivity patterns were further supported by strong and statistically significant positive associations observed between several antibiotics in the correlation analysis. Notably, AZM showed strong correlations with E (Pearson correlation coefficient, p = 0.826), TE (p = 0.826), GEN (p = 0.586), and VAN (p = 0.539). This finding aligns with the observed resistance trends, in which E and TE displayed notable resistance levels, whereas GEN and VAN showed lower resistance. Similarly, E was strongly correlated with CIP (p = 0.471), a fluoroquinolone that exhibited both high resistance and low sensitivity, as well as CD (p = 0.795), reflecting the resistance trends in lincosamide.

Further strong associations were noted between E and TE (p = 1.000), CIP and TE (p = 0.471), E and GEN (p = 0.484), TE and GEN (p = 0.484), E and VAN (p = 0.612), TE and VAN (p = 0.612), and GEN and VAN (p = 0.791). These correlations underscore the complex relationship between resistance patterns and antibiotic effectiveness, particularly among macrolides, aminoglycosides, and glycopeptides (Table 7).

Antibiotic susceptibility testing of S. aureus isolated from egg contents

Thirteen coagulase-positive *S. aureus* isolates from table egg contents were tested for antibiotic susceptibility. The higher resistance levels were observed to β-lactam antibiotics, including P (100%) and OX (100%), cephalosporins such as CX (92%), lincosamides such as CD (84%), fluoroquinolones such as CIP (69%), and tetracyclines, specifically TE (61%). Additionally, 30% of the isolates showed resistance to macrolides (E) and aminoglycosides (GEN), whereas 46% were resistant to VAN. Among the egg content isolates, all (100%) were sensitive to LEV and DO, with NX and GEN showing sensitivity in 92% and 69% of the isolates, respectively. Sensitivity to E and VAN was observed in 38% of the isolates. The lowest sensitivity rates were

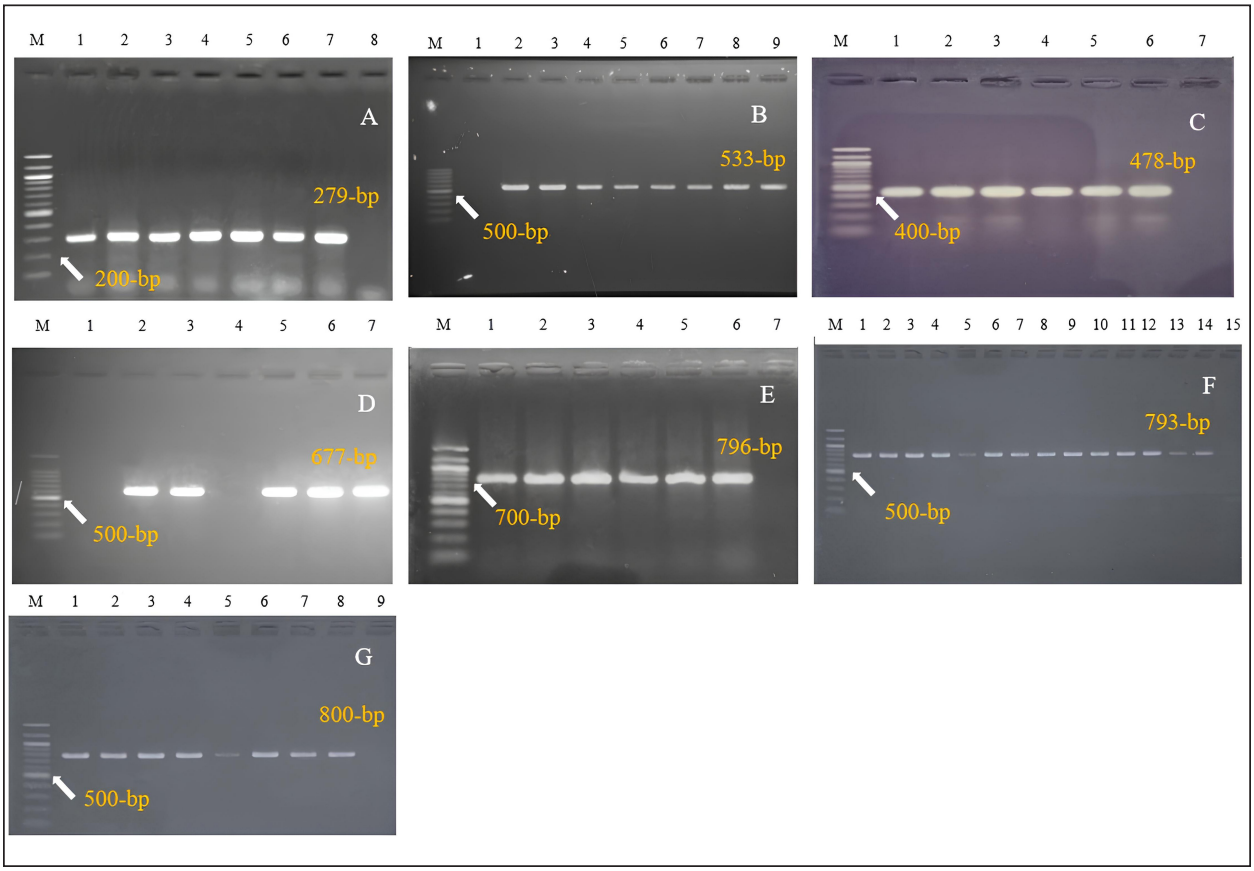

Fig. 3. PCR detection of *Staphylococcus aureus*. (A) Amplification of 279-bp fragment of *nuc* gene (Lane 1–6: DNA samples, Lane M: 100-bp size DNA marker, Lane 7: positive control, and Lane 8: negative control) of *S. aureus*. (B) Amplification of 533-bp fragment of *mec*A gene (Lane 2–8: DNA samples, Lane M: 100-bp size DNA marker, Lane 9: positive control, and Lane 1: negative control) of *S. aureus.* (C) Amplification of 478-bp fragment of *seb* gene. (Lane 1–5: DNA samples, Lane M: 100-bp size DNA marker, Lane 6: positive control, and Lane 7: negative control) of *S. aureus* (D) Amplification of 677-bp fragment of *van*A gene (Lane 2, 3, 5, and 6: DNA samples, Lane M: 100-bp size DNA marker, Lane 7: positive control, and Lane 4: negative control) of *S. aureus.* (E) Amplification of 796-bp fragment of *van*C gene (Lane 1–5: DNA samples, Lane M: 100-bp size DNA marker, Lane 6: positive control, and Lane 7: negative control) of *S. aureus.* (F) Amplification of 793-bp fragment of *blaTEM* gene (Lane 1–13: DNA samples, Lane M: 100-bp size DNA marker, Lane 14: positive control, and Lane 15: negative control) of *S. aureus.* (G) Amplification of 800-bp fragment of *tet*A gene (Lane 1–7: DNA samples, Lane M: 100-bp size DNA marker, Lane 8: positive control, and Lane 9: negative control) of *S. aureus.*

observed for CX and TE, with only 7% of the isolates responding to these antibiotics (Table 6 and Fig. 4).

These resistance and sensitivity patterns align with the statistical findings of the bivariate analysis, in which strong and positive significant correlations were observed between several antibiotics. In this study, AZM was strongly correlated with E ($p = 0.033$, coefficient = 0.592), GEN ($p = 0.001$, coefficient = 0.822), and VAN ($p = 0.033$, coefficient = 0.592). These correlations reflect the observed resistance trends, particularly in the case of E, GEN, and VAN, in which resistance was noted in 30% and 46% of the isolates, respectively.

Additionally, E exhibited strong correlations with CIP ($p = 0.025$, coefficient = 0.617), TE ($p = 0.004$, coefficient = 0.732), and VAN ($p = 1.000$, coefficient = 1.000), supporting the resistance trends observed for these antibiotics. The correlation between E and CIP, for instance, is consistent with the moderate resistance levels observed for CIP (69%) and E (30%).

Moreover, significant correlations between CIP and VAN ($p = 0.617$, coefficient = 0.617), TE and VAN ($p = 0.732$, coefficient = 0.732), and GEN and VAN ($p = 0.720$, coefficient = 0.720) underscore the observed patterns of resistance and sensitivity. The resistance observed in VAN (46%) is particularly notable in light of these correlations, which highlight the complex interplay between these antibiotics in egg content isolates (Table 8).

Table 4. Molecular detection of enterotoxin and antibiotic resistance genes of *Staphylococcus aureus* isolated from eggshells and contents.

Sample Source	Name of gene	Number of detected genes	Prevalence of detected genes of *S. aureus* (ES, *N* = 21; EC, *N* = 13)	95% CI	*p*-value
ES	*mec*A	7	33.33	17.20–54.60	0.189
EC	*mec*A	0	0	00.00–22.80	NA
ES	*blaTEM*	18	85.71	65.40–95.00	0.001
EC	*blaTEM*	6	46.15	23.20–70.90	1
ES	*tet*A	7	33.33	17.20–54.60	0.189
EC	*tet*A	0	0	00.00–22.80	NA
ES	*van*A	4	19.04	7.70–40.00	0.007
EC	*van*A	0	0	00.00–22.80	NA
ES	*van*C	7	33.33	17.20–54.60	0.189
EC	*van*C	1	7.8	00.40–31.50	0.003
ES	*seb*	6	20.5	13.80–50.00	0.078
EC	*seb*	0	0	00.00–22.80	NA

Table 5. Pearson correlation with different types of antibiotic resistance genes of eggshell isolates.

Name of gene		*mec*A	*blaTEM*	*tet*A	*van*A	*van*C
*mec*A	PCC	1				
	p-value	-				
bla_{TEM}	PCC	0.289	1			
	p-value	0.204	-			
*tet*A	PCC	1.000**	0.289	1		
	p-value	0	0.204	-		
*van*A	PCC	0.686**	0.198	0.686**	1	
	p-value	0.001	0.39	0.001	-	
*van*C	PCC	1.000**	0.289	1.000**	0.686**	1
	p-value	0	0.204	0	0.001	

PCC = Pearson correlation coefficient.
**Significant correlation at the 0.01 level (*p*-value).

Phenotypic MDR of S. aureus isolated from eggshells and egg contents

Out of 21 eggshell coagulase-positive *S. aureus* isolates, 19 (95% CI: 71.1%–98.3%) were identified as MDR based on their phenotype. A total of nine resistance patterns were observed, of which eight were classified as MDR patterns. The overall prevalence of MDR was 90.47%. Among the MDR patterns, pattern number 3 (CX-P-CD-CIP) and pattern number 9 (CX-P-OX-CD-CIP-E-TE-AZM-VAN-GEN) were the most frequently observed, each found in 5 out of 21 isolates (23.80%, 95% CI: 10.6%–45.1%). Another notable MDR pattern, number 6 (CX-P-OX-CD-CIP-E-TE-AZM), was found in 4 out of 21 isolates (19.04%, 95% CI: 0.77%–40.0%). In contrast, resistance pattern number 1 (CX-P-OX) was not considered MDR in terms of phenotype, although it was present in two isolates (95% CI: 0.17%–28.9%). Additionally, five isolates exhibited resistance to four different classes of antibiotics, corresponding to patterns 2, 4, 5, 7, and 8 (Table 9).

Similarly, of 13 coagulase-positive *S. aureus* isolates from the egg content, 12 (95% CI: 66.7%–99.6%) were identified as MDR based on their phenotype. Eight resistance patterns were observed, of which seven were classified as MDR patterns, resulting in an overall MDR prevalence of 92.30%. The most frequently observed MDR patterns were pattern number 2 (OX-

Table 6. Antibiotic susceptibility profiles of *Staphylococcus aureus* isolated from eggshells and egg contents.

Name of antibiotics classes	Name of antibiotics	Antimicrobial susceptibility *n* (%)					
		Egg shells			Egg contents		
		R	I	S	R	I	S
Macrolides	Azithromycin	10 (47.62)	3 (14.28)	8 (38.09)	4 (30.77)	3 (23.07)	6 (46.15)
	Erythromycin	12 (57.14)	6 (28.58)	3 (14.28)	6 (46.15)	2 (15.38)	5 (38.46)
β-lactam	Oxacillin	20 (95.23)	0 (0.0)	1 (4.76)	13 (100)	0 (0.0)	0 (0.0)
	Penicillin	21 (100)	0 (0.0)	0 (0.0)	13 (100)	0 (0.0)	0 (0.0)
Cephalosporins	Cefoxitin	21 (100)	0 (0.0)	0 (0.0)	12 (92.30)	0 (0.0)	1 (7.70)
Fluoroquinolones	Ciprofloxacin	18 (85.72)	0 (0.0)	3 (14.28)	9 (69.23)	1 (7.70)	3 (23.07)
	Levofloxacin	0 (0.0)	0 (0.0)	21 (100)	0 (0.0)	0 (0.0)	13 (100)
	Norfloxacin	0 (0.0)	0 (0.0)	21 (100)	0 (0.0)	1 (7.70)	12 (92.30)
	Doxycycline	0 (0.0)	0 (0.0)	21 (100)	0 (0.0)	0 (0.0)	13 (100)
Tetracyclines	Tetracycline	12 (57.14)	5 (23.82)	4 (19.04)	8 (61.53)	4 (30.78)	1 (7.69)
Lincosamides	Clindamycin	19 (90.47)	2 (9.53)	0 (0.0)	11 (84.62)	0 (0.0)	2 (15.38)
Aminoglycosides	Gentamycin	5 (23.81)	3 (14.28)	13 (61.91)	4 (30.77)	0 (0.0)	9 (69.23)
Glycopeptides	Vancomycin	7 (33.33)	5 (23.81)	9 (42.86)	6 (46.15)	2 (15.38)	5 (38.47)

I = intermediate; R = resistant; S = sensitive.

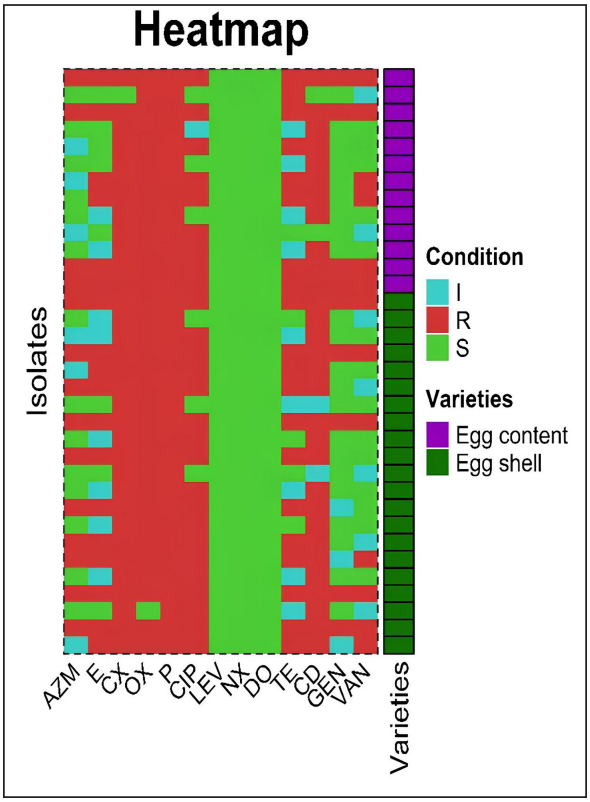

Fig. 4. Antibiotic susceptibility profiles of *S. aureus* isolated from eggshells and contents isolate. I = Intermediate; R = Resistant; S = Sensitive; AZM = Azithromycin; E = Erythromycin; CX = Cefoxitin; OX = Oxacillin; P = Penicillin; CIP = Ciprofloxacin; TE = Tetracycline; CD = Clindamycin; GEN = Gentamycin; VAN = Vancomycin.

P-CX-CIP) and pattern number 7 (OX-P-CX-CD-CIP-TE-E-VAN-AZM-GEN), which were present in 3 out of 13 isolates (23.07%, 95% CI: 0.82%–50.3%) and 4 out of 13 isolates (30.76%, 95% CI: 12.7%–57.6%), respectively. Resistance pattern number 1 (OX-P-TE) was detected in 2 isolates (95% CI: 0.17%–28.9%) but was not classified as MDR phenotypically. Antibiotic resistance was detected in seven isolates against four different classes of antibiotics, corresponding to patterns 2, 4, 5, 7, and 8 (Table 10). The antibiotic resistance profiling of each *S. aureus* isolates varied, with multiple antibiotic resistance indices ranging from 0.23 to 0.76 (Tables 9 and 10).

Discussion

The issue of antibiotic-resistant bacteria, particularly those caused by resistant strains of *S. aureus*, poses a significant challenge to public health. This challenge is especially pronounced in individuals undergoing prolonged antibiotic treatment, where controlling infections becomes increasingly difficult (Nwobodo *et al*., 2022; Tălăpan *et al*., 2023). The spread of resistant bacteria to animals, the environment, and food is a growing concern, especially because the antimicrobial resistance profiles of *S. aureus* strains found in egg samples are alarmingly similar to those identified in humans. This resemblance, particularly in certain regions of the world, is a cause for concern and underscores the need for vigilant monitoring and control measures (Manyi-Loh *et al*., 2018; Samreen *et al*., 2021).

When food contaminated with *S. aureus* is ingested, it can lead to a severe health condition known as

Table 7. Pearson correlation coefficient to assess the pairs of any of the two antibiotic-resistant *Staphylococcus aureus* isolated from table eggshell samples.

Antibiotics		AZM	E	CX	OX	P	CIP	TE	CD	GEN	VAN
AZM	PCC	1									
	p-value	-									
E	PCC	0.826**	1								
	p-value	0	-								
CX	PCC	.b	.b	.b							
	p-value	-	-	-							
OX	PCC	0.213	0.258	.b	1						
	p-value	0.353	0.258	-	-						
P	PCC	.b	.b	.b	.b	.b					
	p-value	-	-	-	-	-					
CIP	PCC	0.389	0.471*	.b	–0.091	.b	1				
	p-value	0.081	0.031	.	0.694	-	-				
TE	PCC	0.826**	1.000**	.b	0.258	.b	0.471*	1			
	p-value	0	0	-	0.258	-	0.031	-			
CD	PCC	0.309	0.375	.b	–0.073	.b	0.795**	0.375	1		
	p-value	0.172	0.094	-	0.755	-	0	0.094	-		
GEN	PCC	0.586**	0.484*	.b	0.125	.b	0.228	0.484*	0.181	1	
	p-value	0.005	0.026	-	0.589	-	0.32	0.026	0.431	-	
VAN	PCC	0.539*	0.612**	.b	0.158	.b	0.289	0.612**	0.229	0.791**	1
	p-value	0.012	0.003	-	0.494	-	0.204	0.003	0.317	0	-

AZM = azithromycin; b = not calculable, as at least one of the input variables is fixed; CD = clindamycin; CIP = ciprofloxacin; CX = cefoxitin; E = erythromycin; GEN = gentamycin; OX = oxacillin; P = penicillin; PCC = Pearson correlation coefficient; TE = tetracycline; VAN = vancomycin.
*Significant correlation at the 0.05 level (*p*-value).
**Significant correlation at the 0.01 level (*p*-value).

staphylococcal food poisoning (Romano *et al*., 2023). Eggs, which are easily contaminated, can harbor *S. aureus*, and even cooking may not completely eliminate the heat-stable enterotoxins produced by this bacterium (Grispoldi *et al*., 2021; Khoothiam *et al*., 2023). Limited studies on *S. aureus* in eggs have demonstrated that the bacteria can be present on the eggshell, yolk, and egg white, with some isolates also exhibiting resistance to antimicrobials (Zhang *et al*., 2023).

Previous studies have shown that bacteria can penetrate eggshells (Gole *et al*., 2014; Kulshreshtha *et al*., 2021). The widespread use of antibiotics in treating farm animals and in poultry feed may contribute to the presence of MRSA in eggs, facilitating the infection of eggs by resistant bacteria (Abreu *et al*., 2023). The current study aimed to detect *S. aureus* in egg samples collected from various retail sources to understand its prevalence, molecular characteristics, antimicrobial sensitivity, and MDR profile in Bangladesh. This research sheds light on how bacteria enter eggs, potentially through human or animal sources.

The study found that *Staphylococcus* spp. were present in 53% of eggshell samples and 41% of egg contents. Among these, 21% of the eggshell isolates and 13% of the egg content isolates were confirmed as *S. aureus*. These findings emphasize the presence of potentially harmful bacteria in both the outer and inner parts of table eggs, underscoring the importance of good hygiene practices in egg production and consumption to ensure food safety and protect public health. Previous studies have indicated that bacteria, especially *Staphylococcus* spp., can be found on the outer shell of eggs (Verma *et al.,* 2023). Punom *et al.* (2020) investigated unhatched leftover duck eggs from selected mini hatcheries in Kishoreganj, Bangladesh, and found a high prevalence of *Salmonella* spp., *E. coli*, *Staphylococcus* spp., and *Clostridium* spp. in unhatched leftover duck eggs. These bacterial species potentially contribute to decreased hatchability and increased embryo mortality. Pondit *et al.* (2018) also reported a high prevalence rate of *S. aureus* in chicken and quail eggshells in Mymensingh, Bangladesh.

Table 8. Pearson correlation coefficient to assess the pairs of any of the two antibiotic-resistant *Staphylococcus aureus* isolated from table egg content samples.

Antibiotics		AZM	E	CX	OX	P	CIP	TE	CD	GEN	VAN
AZM	PCC	1									
	p-value	-									
E	PCC	0.592*	1								
	p-value	0.033	-								
CX	PCC	0.158	0.267	1							
	p-value	0.606	0.377	-							
OX	PCC	.b	.b	.b							
	p-value	-	-	-							
P	PCC	.b	.b	.b	.b						
	p-value	-	-	-	-						
CIP	PCC	0.365	0.617*	0.433	.b	.b	1				
	p-value	0.22	0.025	0.139	-	-	-				
TE	PCC	0.433	0.732**	–0.228	.b	.b	0.501	1			
	p-value	0.139	0.004	0.453	-	-	0.081	-			
CD	PCC	0.234	0.395	0.677*	.b	.b	0.178	0.101	1		
	p-value	0.443	0.182	0.011	-	-	0.561	0.742	-		
GEN	PCC	0.822**	0.720**	0.192	.b	.b	0.444	0.527	0.284	1	
	p-value	0.001	0.006	0.529	-	-	0.128	0.064	0.347	-	
VAN	PCC	0.592*	1.000**	0.267	.b	.b	0.617*	0.732**	0.395	0.720**	1
	p-value	0.033	0	0.377	-	-	0.025	0.004	0.182	0.006	-

AZM = azithromycin; .b = not calculable, as at least one of the input variables is fixed; CD = clindamycin; CIP = ciprofloxacin; CX = cefoxitin; E = erythromycin; GEN = gentamycin; OX = oxacillin; P = penicillin; PCC = Pearson correlation coefficient; TE = tetracycline; VAN = vancomycin.
*Significant correlation at the 0.05 level (*p*-value).
**Significant correlation at the 0.01 level (*p*-value).

Table 9. Multidrug resistance profiles of *Staphylococcus aureus* isolated from table eggshells.

No. of Pattern	No. of antibiotic classes	Multidrug resistance profiles of *S. aureus*	No. of isolates showed resistant *n* (%)	Overall MDR prevalence *n* (%)	MAR Index
1	2	CX-P-OX	2 (9.52)		0.23
2	3	CX-P-OX-CD	1 (4.76)		0.30
3	4	CX-P-CD-CIP	5 (23.80)		0.30
4		CX-P-OX-CD-CIP	1 (4.76)		0.38
5	6	CX-P-OX-CD-CIP-E-TE	1 (4.76)	19 (90.47)	0.53
6		CX-P-OX-CD-CIP-E-TE-AZM	4 (19.04)		0.61
7	7	CX-P-OX-CD-CIP-E-TE-VAN	1 (4.76)		0.61
8		CX-P-OX-CD-CIP-E-TE-AZM-VAN	1 (4.76)		0.69
9	8	CX-P-OX-CD-CIP-E-TE-AZM-VAN-GEN	5 (23.80)		0.76

AZM = azithromycin; CD = clindamycin; CIP = ciprofloxacin; CX = cefoxitin; E = erythromycin; GEN = gentamycin; OX = oxacillin; MAR = multiple antibiotic resistance; MDR = multidrug resistance; P = penicillin; TE = tetracycline; VAN = vancomycin.

Table 10. Multidrug resistance profiles of *Staphylococcus aureus* isolated from table egg contents.

No. of Pattern	No. of antibiotics classes	Multidrug resistance profiles of *S. aureus*	No. of isolates showed resistant *n* (%)	Overall MDR prevalence *n* (%)	MAR Index
1	2	OX-P-TE	1 (7.69)	12 (92.30)	0.23
2	3	OX-P-CX-CD	1 (7.69)		0.30
3	3	OX-P-CX-CIP	3 (23.07)		0.30
4	4	OX-P-CX-CD-CIP	1 (7.69)		0.38
5	5	OX-P-CX-CD-CIP-TE	1 (7.69)		0.46
6	7	OX-P-CX-CD-CIP-TE-E-VAN	2 (15.38)		0.61
7	8	OX-P-CX-CD-CIP-TE-E-VAN-AZM-GEN	4 (30.76)		0.76

AZM = azithromycin; CD = clindamycin; CIP = ciprofloxacin; CX = cefoxitin; E = erythromycin; GEN = gentamycin; MAR = multiple antibiotic resistance; MDR = multidrug resistance; OX = oxacillin; P = penicillin; TE = tetracycline; VAN = vancomycin.

The MDR profile analysis of coagulase-positive *S. aureus* isolates from egg content samples revealed a high prevalence of MDR, with 92.30% of isolates exhibiting resistance to multiple antibiotic classes. Previous studies have reported similarly high levels of MDR, with 95.10% of isolates from Shanghai and 12.5%–100% of isolates from *S. aureus* from Bangladesh showing similar resistance patterns (Ou *et al*., 2020; Ain *et al*., 2022). The most common resistance patterns observed in this study were OX, P, CX, CD, CIP, TE, E, VAN, AZM, and gentamycin (GEN). Similar resistance patterns have been reported in studies conducted in South Africa, northeast Ethiopia, and Panama (Shittu and Lin, 2006; Kibret and Abera, 2011; Mejía *et al*., 2021). Notably, certain resistance patterns were frequently observed, indicating the presence of potentially dominant strains with broad antibiotic resistance profiles (Russo *et al*., 2020; Bashir *et al*., 2023).

The present study also revealed significant differences in the prevalence of virulence and antibiotic resistance genes between coagulase-positive *S. aureus* isolates from eggshell and egg content samples. The eggshell, which is the primary barrier between the external environment and the egg interior, is more susceptible to contamination from various sources, such as handling, storage conditions, and exposure to contaminated surfaces (De Reu *et al*., 2006; Kulshreshtha *et al*., 2021). This external exposure increases the likelihood of acquiring diverse antibiotic resistance genes (Kunhikannan *et al*., 2021). In contrast, the egg content is relatively protected and is less exposed to environmental contaminants, which may explain the lower prevalence and diversity of resistance genes. Differences in the microenvironments of the eggshell and egg content could also influence bacterial adaptation and gene expression, contributing to the observed variations in gene prevalence (Grizard *et al*., 2015; Chen *et al*., 2019). Specifically, 57.14% of eggshell isolates were positive for the *nuc* gene, compared with 30.78% of egg content isolates. Antibiotic resistance genes were more prevalent in eggshell isolates, with *blaTEM* detected in 85.71% and *mec*A detected in 33.33% of these isolates. In egg content isolates, *blaTEM* was found in 46.15% of isolates and *van*C in 7.8%, while none tested positive for *mec*A, *tet*A, *van*A, or the enterotoxigenic gene *seb*. Similar patterns of antibiotic resistance and distribution of resistance genes have been detected in various bacteria, including *Salmonella* and *S. aureus*, across different bird species, such as chickens and quails (Pondit *et al*., 2018; Ganjeer *et al*., 2023). These findings underscore the higher prevalence and diversity of antibiotic resistance genes in eggshell isolates than in egg content isolates, highlighting potential differences in contamination sources or bacterial adaptation mechanisms.

The strong and statistically significant positive correlations observed between certain antibiotic resistance genes in the *S. aureus* isolates suggest a consistent co-occurrence of these genes, likely due to co-selection or co-inheritance mechanisms (Tasneem *et al*., 2022; Murray *et al*., 2024). The high correlation between *mec*A and *tet*A, mecA and vanC, and vanA and vanC (with Pearson correlation coefficients of 1.000) indicates that these genes often appear together within the same bacterial isolates (Ballah *et al*., 2022; Nepal *et al*., 2023). This co-occurrence may result from genetic linkage, where resistance genes are located close to each other on mobile genetic elements such as plasmids or transposons, facilitating their simultaneous transfer during bacterial reproduction or horizontal gene transfer (Stalder *et al*., 2012; Partridge *et al*., 2018). Additionally, exposure to multiple antibiotics can create selective pressure, encouraging the survival of bacteria harboring multiple resistance genes and further promoting the co-selection of these genes (Davies and Davies, 2010).

The notable patterns of antibiotic resistance and sensitivity observed among coagulase-positive *S. aureus* isolates from eggshell and egg content samples can be attributed to several factors. The high levels of resistance to cephalosporins (CX), β-lactam antibiotics

(P and OX), lincosamides (CD), and fluoroquinolones (CIP) suggest widespread and possibly prolonged exposure to these antibiotics, either in veterinary medicine or through environmental contamination, leading to selective pressure favoring resistant strains (Ali *et al*., 2022; Urban-Chmiel *et al*., 2022). Moderate resistance to macrolides (E) and tetracyclines (TE) indicates some level of exposure, but not as intense or frequent as with previously mentioned antibiotics (Chopra and Roberts, 2001; LaPlante *et al*., 2022). The lower levels of resistance to aminoglycosides (GEN) and glycopeptides (VAN) could be due to their less frequent use or effective regulatory measures limiting their application in agriculture and veterinary practices (Jain *et al*., 2011; Miller *et al*., 2020). On the other hand, the highest sensitivity observed with fluoroquinolones (LEV, NX, and DO) suggests that these antibiotics may not be commonly used or that they remain effective against these *S. aureus* isolates, maintaining their efficacy (Clay *et al*., 2021). The moderate sensitivity to aminoglycosides (GEN) and glycopeptides (VAN) further supports this, indicating that although resistance exists, these antibiotics retain substantial activity against the isolates (Adhikari, 2010). The lower sensitivity to macrolides (E) and fluoroquinolones (CIP) points to emerging resistance, but not yet at a critical level, while the moderate sensitivity to tetracyclines (TE) suggests they remain effective to a certain extent but may be under increasing pressure (Wieczorek and Osek, 2013; Urban-Chmiel *et al.*, 2022). The high levels of antibiotic resistance found in *S. aureus* isolates from table eggs pose serious concerns about public health and the food industry. These findings underscore the crucial need for stronger regulatory mechanisms to monitor and limit antibiotic usage in chicken agriculture. Antibiotic stewardship initiatives combined with stricter limits on the use of important antibiotics may help reduce the emergence and spread of resistance strains. By aligning with established guidelines, such as those set by the World Health Organization and regulatory bodies such as the FDA, the food industry can contribute significantly to the global fight against antimicrobial resistance.

The bivariate analysis demonstrated strong and statistically significant positive correlations between certain pairs of antibiotics used against eggshell isolates, suggesting potential mechanisms of co-resistance or co-selection (Pouwels *et al.*, 2019; Robas *et al.*, 2021). The strong correlations between AZM and E, AZM and TE, AZM, and GEN indicate that resistance to these antibiotics may be linked, possibly due to the presence of multi-resistance plasmids or genes that confer resistance to multiple drugs simultaneously (Chen *et al.*, 2013; Abotsi *et al.*, 2022; Heidary *et al.*, 2022). Moderate correlations observed between AZM and VAN, E and CIP, and CIP and CD suggest a less direct but still significant association between resistance mechanisms for these antibiotics (Arabestani *et al.*, 2017; Yamanaka *et al.*, 2019). These relationships may be influenced by similar selective pressures or genetic elements that carry resistance determinants for multiple antibiotics (Munita and Arias, 2016). Strong correlations between E and TE, as well as between GEN and VAN, further support the idea of shared resistance pathways or genetic linkages (Chopra and Roberts, 2001; Conwell *et al*., 2017). Additionally, moderate correlations between E and GEN, TE and GEN, E and VAN, TE and VAN, and GEN and VAN highlight a network of interconnected resistance traits, reinforcing the complexity of antibiotic resistance patterns (Baquero *et al*., 2021).

The bivariate analysis of egg content isolates also revealed strong and statistically significant positive correlations between several pairs of antibiotics, indicating potential patterns of co-resistance or co-selection. Specifically, AZM exhibited strong correlations with E, GEN, and VAN, suggesting that resistance mechanisms affecting AZM may also confer resistance to E, GEN, and VAN, possibly due to shared resistance genes or the presence of multi-resistance plasmids that carry these genes together (Vrancianu *et al.*, 2020; Nusrin *et al.*, 2022). Additionally, E showed strong correlations with CIP and TE, indicating potential cross-resistance or co-selection mechanisms (Shariati *et al.*, 2022). This suggests that the use of E might be selected for bacteria that are also resistant to CIP and TE, likely because of linked genetic elements or overlapping resistance pathways (Shariati *et al.*, 2022; Hasan *et al.*, 2023). The strong correlation between CX and CD suggests a similar association, where resistance to one could imply resistance to the other, potentially due to co-located resistance genes (Duche *et al.*, 2023). Furthermore, the strong correlations between CIP, TE, GEN, and VAN with each other emphasize a network of interconnected resistance traits, indicating that resistance to one of these antibiotics often coincides with resistance to others. This interconnected resistance complicates treatment options and highlights the necessity for careful antibiotic use and comprehensive resistance management strategies (Gajdács *et al.*, 2021; Muteeb *et al.*, 2023).

The high prevalence of MDR among coagulase-positive *S. aureus* isolates from both eggshell and egg content samples, with prevalence of 90.47% and 92.30%, respectively, can be attributed to several factors. The frequent observation of resistance patterns involving CX, P, OX, CD, CIP, E, TE, AZM, VAN, and GEN suggests the widespread use of these antibiotics in agricultural settings (Parvin *et al.*, 2021; Ballah *et al.*, 2022). This widespread use likely exerts selective pressure on bacterial populations, favoring the survival and proliferation of resistant strains (Tello *et al.*, 2012). The frequent occurrence of certain resistance patterns indicates the presence of dominant strains with broad antibiotic resistance profiles (Russo *et al.*, 2020; Sultan *et al.*, 2020). These strains could have acquired multiple

resistance genes through horizontal gene transfer mechanisms, such as conjugation, transformation, or transduction, often facilitated by mobile genetic elements such as plasmids, transposons, and integrons (Bello-López *et al.*, 2019; Michaelis and Grohmann, 2023). Additionally, the shared resistance patterns between eggshell and egg content isolates suggest that these dominant strains are capable of colonizing both the external and internal environments of the eggs, possibly due to contamination during handling and processing (De Reu *et al.*, 2006; Gantois *et al*., 2009). The study's limitations include geographic bias and a narrow sampling of egg sources, potentially restricting its broad applicability. Future research should expand regionally, include diverse egg types and supply chain stages, and explore contamination trends through longitudinal studies. These findings underscore the importance of stringent antibiotic stewardship practices and improving biosecurity measures in poultry farming to reduce the selection pressure for resistant strains and prevent the spread of MDR bacteria.

Conclusion

The findings of this study indicate the significant presence of MDR *S. aureus* in eggs collected from the market of Mymensingh City Corporation, Bangladesh. This high prevalence poses a public health risk for consumers purchasing eggs from retail shops, supermarkets, local markets, and farm outlets. The presence of MDR *S. aureus* in eggs also highlights the potential for the spread of resistant strains to humans through the food chain. Implementing proper sanitation and biosafety measures is essential to mitigate this public health threat.

The antibiotic resistance profiles identified in *S. aureus* strains from table egg samples in this study provide a valuable reference for future research. Monitoring antibiotic use in livestock and farm animals is crucial for reducing the risk of MDR *S. aureus* infection. Expanding similar studies to other regions of Bangladesh and other countries would help determine the prevalence and distribution of MDR strains on a broader scale.

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Conflict of interest

The authors declare no conflict of interest.

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Author's contributions

PB designed and conducted the experiment, collected data, analyzed data, and wrote the manuscript. KAS, MBL, and MZR wrote the manuscript, collected samples, conducted the experiment, helped in various molecular research and analysis, and interpreted the results. TA, PS, and MM conducted laboratory work, gathered data, and performed a review. MMK participated in the methodology, wrote the first draft, reviewed it, revised the final version, and edited the manuscript. MAI conceptualized and designed the experiment, developed the draft, arranged funds, critically analyzed the data, and wrote and revised the final version of the manuscript. All authors contributed to the review of the manuscript and approved the final manuscript.

Data availability

The data generated from this study might be available on a valid request from the corresponding author.

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