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Research Article

PREVALENCE OF *Staphylococcus aureus* IN SOME STREET FOOD SAMPLES AND THEIR ANTIBIOTIC RESISTANCE STATUS

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ABSTRACT

Street-vended foods are widely consumed because of their appealing taste, reasonable price, and convenience, but present a high risk of contamination with Staphylococcus aureus, one of the leading causes of food poisoning worldwide. This study aimed to investigate the occurrence of S. aureus in 40 street food samples, their antibiotic-resistant status, and their enterotoxigenic genes. The results showed that 12.5% of food samples were positive for S. aureus and 7.5% for Methicillin-resistant S. aureus. Antibiotic susceptibility test revealed that 31/31 isolates were resistant to at least one of the tested antibiotics, including penicillin, gentamicin, cefoxitin, clarithromycin, and tetracycline. Multidrug-resistant S. aureus accounted for 45.2% of all isolates. Enterotoxin genes, including sea and seb, were detected at low percentages. One strain (namely BTT6) derived from mixed rice paper was identified as Methicillin-resistant S. aureus with the seb gene. The results indicated that the tested street foods became a public health hazard because of a high prevalence of antibiotic-resistant S. aureus, especially multidrug-resistant S. aureus.

Keywords: antibiotic resistance; MRSA; multidrug resistant*; Staphylococcus aureus;* street foods

1. Introduction

S. aureus is a well-known food-borne pathogen, producing various enterotoxins; among the most popular enterotoxins are staphylococcal enterotoxin (SE) A and SEB (Mejía et al., 2021). The latter is a superantigen, which can activate T-cells and macrophages with massive cytokine production and escalation of endotoxin shock (Krakauer, 2019). *S. aureus* can grow and produce enterotoxin in many foods, including milk, meat, meat products, dairy products, and ready-to-eat food (Lim et al., 2023). Remarkably, *S. aureus* is resistant to many antibiotics, which threatens community health since antibiotic-resistant genes can be

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transferred among bacterial strains (Mejía et al., 2021).

Vietnamese street foods are diverse in terms of dishes, in which some street foods are attractive not only by taste but also by the diversity of nutritional groups, consisting of meat, vegetables, and starch. Such street foods are a favorable breakfast or snack for people of all ages since they provide people with affordable and convenient meals. Although Vietnam has published a number of regulations aimed at enhancing hygiene and safety practices for street food, there are still gaps between the government's regulatory requirements and the compliance of street food sellers. As a result, street foods are reported to be a significant source of food-borne pathogens, including *S. aureus,* because they are prepared, stored, and sold with poor hygiene (Huynh et al., 2022). When contaminated with various bacteria, the food becomes the environment that enables the horizontal transfer of antibiotic-resistant genes among pathogenic bacteria (Urban-Chmiel et al., 2022).

To our best knowledge, limited research has been done on *S. aureus,* their antibiotic resistance, and their enterotoxin genes in street foods in Ho Chi Minh City, Vietnam. Especially, a few studies have reported on the antibiotic-resistant status of *S. aureus* derived from food in Ho Chi Minh City. Besides, ready-to-eat food was highly consumed in Ho Chi Minh City. Therefore, this study was carried out to detect *S. aureus* in street food samples, their antibiotic-resistant status, and their enterotoxigenic genes.

2. Materials and methods

2.1. Materials

A total of 40 Vietnamese street food samples, including 10 samples of "bánh tráng trộn" (BTT), 10 samples of "bánh ướt" (BU), 10 samples of "bánh mì thịt" (BMT), and 10 samples of "xôi mặn" (XM), were randomly purchased from street vendors in District 5, Ho Chi Minh City, Vietnam in October 2021. Samples were collected in sterile containers, stored at 4°C until examined.

2.2. Methods

2.2.1. Bacterial isolation

S. aureus from food samples was isolated and identified as the reported method (Tallent et al., 2020) with slight modifications. A volume of 225 mL of buffered peptone water (Merck, Darmstadt, Germany) and 25 g of each sample were added to a sterile Stomacher bag (Seward, England). Next, the sample was homogenized in a stomacher (Seward, England) at 200 rpm for 5 min. Then, the sample was serially diluted 10 times by sterile buffered peptone water, and 1 mL of various dilutions was equitably distributed to 3 plates and spread on Baird-Parker agar (Merck, Darmstadt, Germany) with egg yolk tellurite (Merck, Darmstadt, Germany). The plates were incubated at 35 ± 2°C for 24 h. The presence of *S. aureus* was confirmed by colony morphology, Gram stain, and biochemical tests, including mannitol fermentation, catalase test, and coagulase test.

2.2.2. Biochemical tests

For the mannitol fermentation test, bacterial isolates were cultured in phenol red carbohydrate broth (peptone 10 g, sodium chloride 5 g, beef extract 1 g, phenol red 0.018 g, mannitol 10 g, distilled water 1,000 ml) for 18 to 24 h. Mannitol fermentation positive isolates were confirmed by the change of broth color from pink to yellow due to a decrease in pH to 6.8 or less.

The catalase test was carried out by the tube method. Bacteria were grown on brain heart infusion (BHI) agar plate for 18-24 h. Then, a small amount of a bacterial colony was collected using a sterile bamboo stick. Next, the stick was put in a test tube containing 2 mL of 3% hydrogen peroxide. After that, the tube was placed against a black background, and the immediate formation of bubbles at the end of the bamboo stick was observed. The bacterial strain that caused immediate bubble formation was considered a catalase-positive strain. No bubbles formed, showing that the strain was negative for catalase (Weldeselassie et al., 2020).

The coagulase test was detected by the tube method. Bacteria were cultured in test tubes containing 5 mL of BHI (brain heart infusion) broth for 18-24 h. Lyophilized rabbit plasma (BD BBL™, United States) was transferred into these test tubes. Next, they were incubated at 35 ± 2°C. Clot formation was observed every 4 h till 24 h. Any clot formation showed a coagulase-positive result. No clots showed a coagulase-negative reaction (Pervin et al., 2019). *S. aureus* ATCC 6538 was used as a positive control, and *Escherichia coli* ATCC 8739 was used as a negative control.

2.2.3. Antimicrobial susceptibility testing

The resistance of isolates to antibiotics was tested via disk-diffusion assay using Mueller-Hinton agar (MHA) as instructed by the guidelines of the Clinical and Laboratory Standards Institute 2021 (CLSI, 2021). Bacterial suspensions equivalent to 0.5 McFarlands units were spread on MHA (Merck, Darmstadt, Germany) plates, and antibiotic discs were placed on the surface of MHA medium. Next, the plates were incubated upside down at 35°C ± 2°C for 18-24 h. The inhibition zones were then measured and compared to the CLSI guidelines to report the result as susceptible (S), intermediate (I), or resistant (R). The antibiotic discs (Nam Khoa Biotek, Viet Nam), including penicillin (10 U), gentamicin (10 µg), ciprofloxacin (5 µg), cefoxitin (30 µg), clarithromycin (15 µg), and tetracycline (30 µg), were used in this study.

2.2.4. DNA extraction

A single colony on BHI agar was picked and transferred into an Eppendorf tube containing 200 µL of TE 1X buffer (Tris-HCl 1 mM, Na_2EDTA 0.2 mM). The tube was vortexed vigorously. Next, it was incubated at 95°C for 5 min, followed by centrifugation at 13,000 rpm for 5 min. Then, the supernatant was transferred into a fresh sterile Eppendorf tube and stored at -20°C until they were used for the PCR assay.

2.2.5. Detection of the mecA gene

A total of 13 cefoxitin-resistant isolates were tested for the *mecA* gene using specific primers (Table 1) in a conventional PCR assay, as previously mentioned (Wang et al., 2017). The primers were synthesized by PhuSa Genomics JSC (Can Tho province, Viet Nam).

2.2.6. Detection of enterotoxic gene sea and seb

S. aureus isolates were screened for *sea* and *seb* genes using PCR with specific primers (Table 1), as previously mentioned by Wang et al. (2017). The primers were synthesized by PhuSa Genomics JSC (Can Tho Province, Viet Nam). *S. aureus* ATCC 13565 was used as a positive control for *sea* gene, and *S. aureus* ATCC 14458 was used as a positive control for *seb* gene.

Table 1. *Primers used in this study*

Primer	Sequence (5'-3')	Product size (bp)	Reference
SEA_F	TTGGAAACGGTTAAAACGAA	120	(Nguyen et al., 2020)
SEA_R	GAACCTTCCCATCAAAAACA		
SEB_F	TCGCATCAAACTGACAAACG	478	(Nguyen et al., 2020)
SEB_R	GCAGGTACTCTATAAGTGCC		
MECA_F	AGCGACTTCACATCTATTAGG	509	(Yahya Ahmed et al., 2021)
MECA_R	TGTTATTTAACCCAATCATTGCTGTT		

2.2.7. PCR protocol to detect mecA, sea, and seb genes

The PCR reaction mixture (8X) was prepared with the following components: 12 µL of 10X Taq buffer, 12 µL of dNTPs (2 mM), 6 µL of $MgCl_2$, 0.4 µL of forward primer (100 pM), 0.4 µL of reverse primer (100 pM), 0.8 µL of Taq DNA polymerase (5 U), 1.5 µL of template DNA, and 86.9 µL of sterilized distilled water. The thermal cycling conditions were as follows: initial denaturation at 95°C for 5 min; followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 15 s; with a final extension at 72°C for 5 min.

3. Results and discussion

3.1. Occurrence of *S. aureus* in street food samples

Isolation results showed that all 40 street food samples tested were contaminated with bacteria. The food samples in this study were sold by street vendors with poor food hygiene and safety practices, with very few vendors wearing gloves when preparing food. All ingredients were previously prepared and stored at ambient temperature until they were mixed and distributed to the consumer. Improper preparation and storage of ingredients can be the reason for bacterial contamination and growth.

A total of 619 isolates, with grey to black convex colonies with an opaque zone around the colonies on Baird Parker agar with egg yolk tellurite (Figure 1A), were detected in 17/40 food samples (42.5%). All isolates appeared as staphylococci, Gram-positive (Figure 1B) under the microscope, and were catalase positive (Figure 1C) and mannitol positive. However, only 31/619 isolates were coagulase positive (Figure 1B), indicating that they were *S. aureus* isolates.

The percentage of coagulase-positive *S. aureus* among the staphylococci isolates was quite low, at 5%, suggesting that the investigated food samples were contaminated with other *Staphylococcus* species in much larger numbers than *S. aureus*. Coagulase-negative staphylococci were reported to predominate in food, including ready-to-eat foods (Chajęcka-Wierzchowska et al., 2020). Although they were commonly regarded as non-pathogenic bacteria, their safety remains uncertain (Heo et al., 2020). Some studies showed that they could produce enterotoxins (Chajęcka-Wierzchowska et al., 2020).

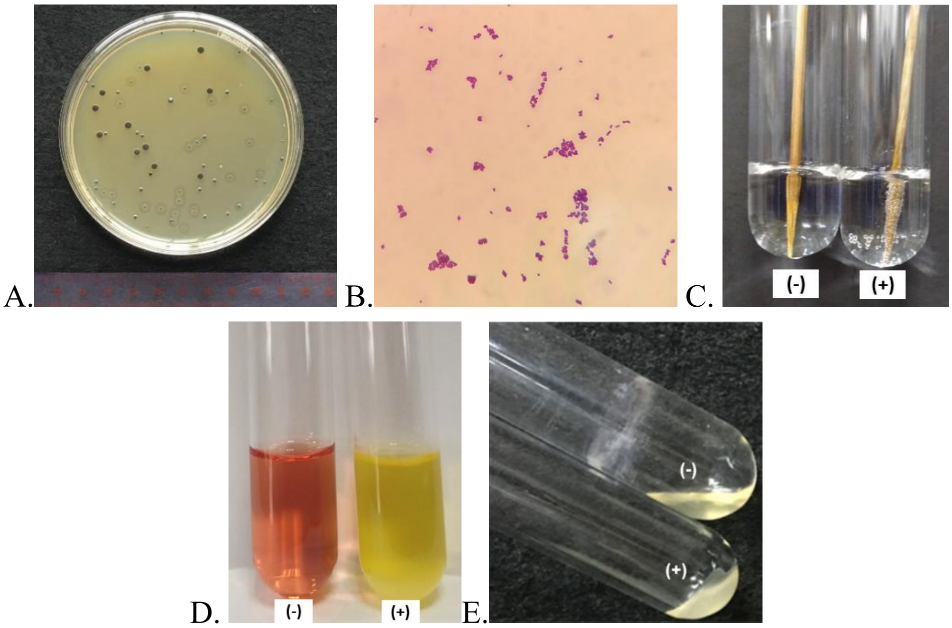

Figure 1. *Colony morphology on Baird-Parker (A), Gram stain (B), catalase (C) of mannitol fermentation (D), and coagulase reaction (E) of S. aureus isolates*

The percentage of food samples positive for *S. aureus* was 12.5% (5/40). The prevalence of *S. aureus* in food samples varies between food products. Of these, 3/10 samples of BMT, 1/10 sample of BU, and 1/10 sample of BTT were contaminated with *S. aureus*. All of the XM samples tested were negative for *S. aureus* (Table 2). BTT consists of rice paper, chili powder, dried shrimp, seasoned fried onion, laksa leaves, some shredded mango, dried beef, and boiled quail eggs. BU, made from a thin, wide sheet of steamed rice flour, usually consists of Vietnamese pork ham, fried shallots, lettuce, fresh mint, pork pie, and bean sprouts. BMTs are made with cucumber, onion, cilantro, chili, pate, and pork. XM consists of sticky rice, pork pie, pork, pate, ham, and sausage. In Ho Chi Minh City, steamed thin rice pancake, steamed sticky rice, and grilled pork bread are widely eaten for breakfast, while mixed rice paper is often used as a snack. When sold in public places, sidewalks, streets, or around schools and universities, they were at a very high risk of bacterial contamination because of poor sanitary practices in preparation, storage, and sale. The prevalence of *S. aureus* detected in food samples in this study was comparable to that of another survey on street vended food, at 12.1% (Sivakumar et al., 2019) while *S. aureus* was found at higher percentage in study on food samples of animal origin, at 34.3% (Yahya Ahmed et al., 2021), 39.1% (Weldeselassie et al., 2020).

Table 2. *Number of street food samples contaminated with S. aureus and other bacteria*

Street food	No. of contaminated samples (no. of isolates)		
	Staphylococci	***S. aureus***	**Other bacteria**
Mixed rice paper	2 (63)	1 (10)	10
Steamed thin rice pancake	5 (114)	1 (5)	10
Roasted pork bread	8 (396)	3 (16)	10
Steamed sticky rice	2 (46)	0 (0)	10
Total	17 (619)	5 (31)	40

3.2. *Antibiotic resistance*

All 31 coagulase-positive *S. aureus* isolates were tested for antibiotic susceptibility by the Kirby-Bauer disk-diffusion method as directed by CLSI 2021. The results showed that a high rate of isolates was resistant to penicillin (80.6%), cefoxitin (41.9%), and clarithromycin (71.0%). Besides, *S. aureus* isolates resistant to tetracycline reached the rate of 32.3%. Whereas the percentage of isolates resistant to gentamicin was quite low, only 6.5%, and 4/31 isolates were intermediately resistant to ciprofloxacin, but no ciprofloxacin-resistant isolates were detected (Table 3).

Table 3. *Number of antibiotic-resistant S. aureus isolates from street food*

Antibiotic class	Antibiotic used in this study	No. of resistance isolates			
		Mixed rice paper (n = 10)	**Steamed thin rice pancake (n = 5)**	**Grilled pork bread (n = 16)**	**Total (n = 31)**
Beta-lactams	Penicillin	9	3	10	22
Cephalosporins	Cefoxitin	8	3	2	13
Flouroquinolones	Ciprofloxacin	0 (4 I)	0	0	0
Aminoglycosides	Gentamicin	1	0	1	2
Macrolides	Clarithromycin	7	2	13	22
Tetracyclines	Tetracycline	3	4	3	10

The results also revealed that 31/31 isolates were resistant to at least one tested antibiotic, accounting for 100% (Table 4). Isolates resistant to one antibiotic accounted for 29.0%, and isolates resistant to two antibiotics accounted for 29.0%. While 45.2% of isolates (14/31) were regarded as multi-resistant strains, including 32.2% (11/31) isolates resistant to three antibiotics, and 9.7% (3/31) isolates resistant to four antibiotics (Table 4). Remarkably, an isolate from mixed rice paper (namely BTT6) was resistant to penicillin, gentamicin, cefoxitin, clarithromycin, and intermediate resistant to ciprofloxacin, and susceptible to only one antibiotic tested, tetracycline. Contaminated food is considered an important vehicle for resistant *S. aureus* strains (Castro et al., 2017). Antibiotic-resistant *S. aureus* contamination in foods could be derived from food animals in husbandry and food vendors. These antibiotic-resistant bacteria could be transferred to humans through the food chain, and their antibiotic resistance could make treatments of human potential infections low effective and even ineffective (Jia et al., 2020).

Table 4. *Antibiotic resistance patterns of S. aureus isolates*

Resistance pattern	No. of isolates	Percentage (%)
Ch	6	19.4
Te	2	6.5
Pen	1	3.2
Pen-Te	3	9.7
Pen-Ch	5	16.1
Pen-Ceft-Ch	8	25.8
Pen-Ceft-Te	2	6.5
Pen-Gen-Te	1	3.2
Pen-Ceft-Ch-Te	2	6.5
Pen-Ceft-Gen-Ch	1	3.2
No resistance observed	0	0

Ch: clarithromycin, Te: tetracycline, Pen: penicillin, Ceft: cefoxitin, Gen: gentamicin

3.3. *mecA confirmation*

Cefoxitin resistance phenotype was regarded as Methicillin-resistant *S. aureus* (MRSA). Therefore, 13 cefoxitin-resistant isolates were screened for the *mecA* gene by PCR with specific primers (Table 1). The results showed that all cefoxitin-resistant isolates were *mecA*-positive (Figure 2), so MRSA accounted for 41.9% (13/31) of all *S. aureus* isolates. Among 13 MRSA isolates, 8 isolates were derived from BTT, 2 isolates from BMT, and 3 isolates from BU. MRSA was detected in 3/40 food samples (7.5%), including 1 BTT, 1 BU, and 1 BMT sample.

MRSA has already been isolated from food, indicating that the food production chain is a channel of transmission between resistant microorganisms and humans (da Silva et al., 2019). The percentage of MRSA in this study was rather higher than the percentage of strains isolated from food in the USA and in European countries (0.6-37.5%) (da Silva et al., 2019). The above results suggested that street foods became a public health hazard because of the occurrence of multidrug-resistant *S. aureus* at a high rate.

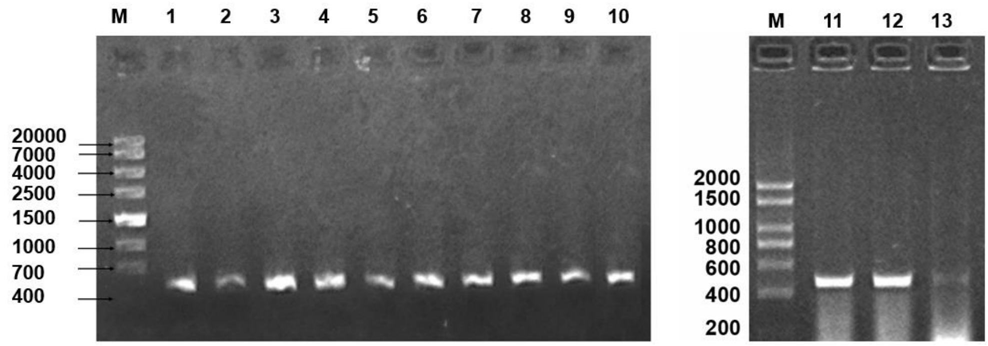

Figure 2. *Gel electrophoresis of the PCR product for the detection of the mecA gene Lane M shows DNA ladder, MW 200-20,000 bp fragments (ZipRuler Express DNA Ladder 2, ThermoFisher Scientific, Lithuania) and MW 200-2000 bp fragments (HTBiotech, Vietnam). Lanes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 13 show a band size of 509 bp of the mecA gene corresponding to positive isolates*

3.4. Enterotoxin gene

Staphylococcal enterotoxins are classified into 23 different SEs and SE-like toxins. Of them, SEA is one of the major serological types (Rodríguez-Lázaro et al., 2017). Therefore, *S. aureus* isolates in this study were screened for *sea* gene. The gene *sea* was detected in 2/31 isolates (6.5%), including a multidrug-resistant strain from BMT (namely BMT6), being resistant to penicillin, gentamicin, and tetracycline; another strain from BU (namely BU4), being resistant to penicillin, clarithromycin, and intermediate resistant to gentamicin. Besides, an isolate derived from BTT (namely BTT6) was detected carrying *seb* (Figure 3), which was also a multidrug-resistant *S. aureus,* being resistant to penicillin, cefoxitin, and clarithromycin. Staphylococcal food poisoning is due to the consumption of enterotoxin pre-formed in the food (Weldeselassie et al., 2020). Although *sea* gene was usually detected at a major part of *S. aureus* such as 56.3% (Sina et al., 2011), 45.2% (Arfatahery et al., 2016), it was detected at quite low rate in this study, at 6.5%, which is comparable with the prevalence of *sea* in another study on street vended food, at 7.7% (Sivakumar et al., 2019). Besides, the rate of strains carrying the gene encoding for superantigenic SEB was 3.2%, lower than other studies at 37.7% (Sina et al., 2011) or at 18.5% (Arfatahery et al., 2016).

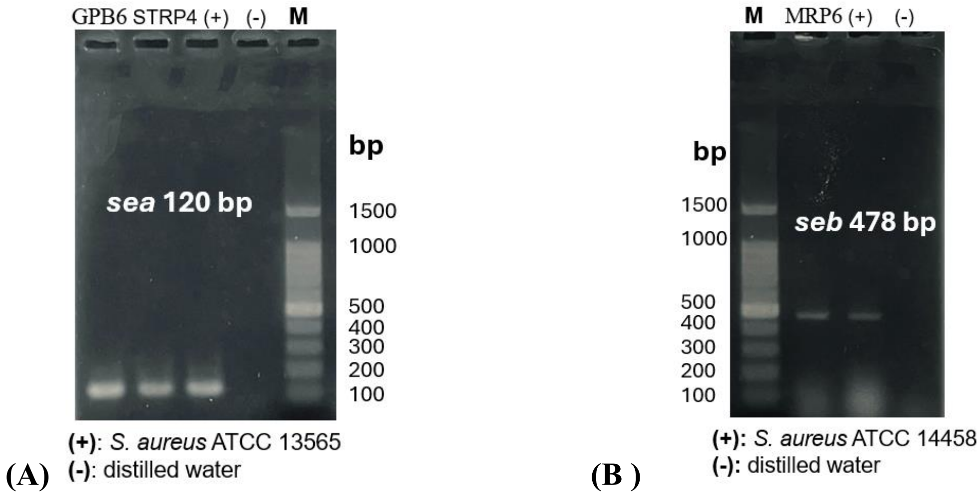

Fig 3. *Gel electrophoresis of the PCR product for the detection of sea and seb gene*

4. Conclusion

This study investigated the presence of *S. aureus* in 40 street food samples, their antibiotic resistance, and enterotoxigenic genes. The results showed that 12.5% of the samples were positive for *S. aureus*, with 7.5% positive for MRSA. All 31 isolates were resistant to at least one tested antibiotic, including penicillin, gentamicin, cefoxitin, clarithromycin, and tetracycline, with 45.2% being multidrug-resistant. Enterotoxin genes *sea* and *seb* were detected at low frequencies, including one *S. aureus* strain (BMT6) from grilled pork bread and one strain (BU4) from steamed thin rice pancake carrying the *sea* gene, and one MRSA strain (BTT6) from mixed rice paper carrying the *seb* gene. These findings underscore the potential public health risks posed by street foods due to the high prevalence of antibiotic-resistant *S. aureus*, particularly multidrug-resistant strains, emphasizing the urgent need for stricter food safety regulations. Further research is essential to investigate *S. aureus* contamination in a wider range of street foods, along with other bacterial pathogens, to better understand the overall bacterial contamination risks in street foods.

❖ ***Conflict of Interest:*** *Authors have no conflict of interest to declare.*

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SỰ HIỆN DIỆN VÀ SỰ ĐỀ KHÁNG KHÁNG SINH CỦA *Staphylococcus aureus* TRONG MỘT SỐ MẪU THỨC ĂN ĐƯỜNG PHỐ

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TÓM TẮT

Thực phẩm đường phố được tiêu thụ rộng rãi do hương vị hấp dẫn, giá cả phải chăng và sự tiện lợi, nhưng có nguy cơ cao nhiễm Staphylococcus aureus, một trong những nguyên nhân hàng đầu gây ngộ độc thực phẩm. Nghiên cứu này nhằm điều tra sự xuất hiện của S. aureus, tình trạng kháng kháng sinh và các gene độc tố của chúng trong 40 mẫu thực phẩm đường phố. Kết quả cho thấy 12,5% mẫu thực phẩm dương tính với S. aureus và 7,5% dương tính với S. aureus kháng methicillin. Tất cả 31/31 chủng phân lập được đều kháng ít nhất một trong các kháng sinh được thử nghiệm, gồm penicillin, gentamicin, cefoxitin, clarithromycin và tetracycline. S. aureus đa kháng thuốc chiếm 45,2% tổng số chủng. Các gene độc tố sea và seb, được phát hiện ở tỉ lệ thấp. Một chủng kí hiệu BTT6, có nguồn gốc từ bánh tráng trộn, được xác định là S. aureus kháng methicillin có mang gene seb. Kết quả cho thấy thực phẩm đường phố tiềm ẩn mối nguy hiểm đối với sức khỏe cộng đồng do tỉ lệ nhiễm S. aureus kháng kháng sinh cao, đặc biệt là S. aureus đa kháng thuốc.

Từ khóa: đa kháng thuốc; đề kháng kháng sinh; MRSA; *Staphylococcus aureus*; thức ăn đường phố