



## Phenotypic, Genotypic Resistance and Virulotyping of *Staphylococcus aureus* Isolated from Ready-to-Eat Food in Egypt

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**Abstract** | The present study aimed to determine phenotypic and genotypic characterization of antimicrobial resistance and some virulent genes in *Staphylococcus aureus* (*S. aureus*) isolated from ready-to-eat (RTE) food purchased from commercial restaurants at Sharkia, Egypt. One hundred RTE food including kofta, shawarma, sausage and fried shrimp were streaked on Baird-Parker agar plates followed by biochemical identification of isolates. Twenty seven *S. aureus* isolates were screened for antibiotic resistance by disk diffusion test. Uniplex PCR was used to detect the antimicrobial resistance genes for methicillin (*mecA*), beta-lactamase (*blaZ*), aminoglycoside [*aac* (6') *aph*(2'')] and macrolides (*mph C*) and virulence genes (*spa* and *clfA*) in four phenotypically multidrug resistant (MDR) *S. aureus* isolates. Furthermore, enterotoxin genes were screened in the four isolates of *S. aureus* using multiplex PCR. In this study, *S. aureus* strains showed highest resistance to methicillin and cefotaxime (100%) followed by amoxicillin (85.18%) and gentamicin (81.48%). The multiple antibiotic resistance (MAR) index of *S. aureus* had a range of 0.36 to 0.9 with an average 0.58. Three (25%) isolates from kofta were multi-resistant to 10 antibiotics and one strain (9.09%) of *S. aureus* from shawarma was resistant to 10 antimicrobials. The four isolates of phenotypically resistant *S. aureus* to methicillin, amoxicillin and gentamicin were positive (100%) for each of *mecA*, *blaZ* and *aac* (6') *aph* (2'') resistance genes; while, two isolates showed resistance to macrolides were positive for *mphC* gene. The four multidrug resistant (MDR) *S. aureus* were negative for any enterotoxin genes. Additionally, all examined MDR isolates of *S. aureus* from RTE food harboring *spa* and *clfA* genes (100%). The higher contamination of different RTE food with MDR *S. aureus* indicates improper hygienic measures. Also, the higher MAR index of *S. aureus* isolates has high risk potential for consumers.

**Keywords** | Phenotypic resistance, *Staphylococcus aureus*, Ready-to-Eat Food, Virulence genes, Antimicrobial resistance genes

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## INTRODUCTION

*Staphylococcus aureus* (*S. aureus*) is considered as one of the major food borne bacterial pathogens recovered from RTE meat and causing several food poisoning outbreaks (Diep *et al.*, 2006). This pathogen have the ability to grow within temperature range of 15–45 °C but requires higher concentration (10%) of NaCl (Behling *et al.*, 2010). *S. aureus* could multiply quickly at the room temperature and

release toxins that induce illness. It has been estimated that *S. aureus* responsible for 241,000 cases of foodborne illness each year in United States of America (Wu *et al.*, 2018). This bacterium could be detected in meat (Jackson *et al.*, 2013) and fish (Saito *et al.*, 2011). The contamination of food with *S. aureus* could be resulted from inadequate handling, improper conditions of storage, inadequate hygienic measures and post-production microbial contaminations (Ray, 2001). The presence of *S. aureus* as one of microbial

contaminants in RTE meats created public health hazard and food safety concerns (Mehndiratta and Bhalla, 2014), due to their pathogenicity and zoonotic importance (Razmyar et al., 2017). The antimicrobial susceptibility testing of *S. aureus* isolates recovered from RTE meat and retail chicken were studied in Egypt (Morshdy et al., 2018b) and India (Laxminarayan and Chaudhury, 2016).

Methicillin-resistant *S. aureus* (MRSA) occurs in humans and food of animal origin due to acquisition of genes that encode penicillin-binding protein-PBP2' or PBP2a (Chen et al., 2009). Besides, a divergent homologue of the *mecA* gene (*mecC*) verified 70% homology to the methicillin resistance (*mecA*) gene (Garcia-Alvarez et al., 2011). Also, frequent contamination of retail meat and poultry occur with MDR *S. aureus*; and 96% of *S. aureus* strains showed resistance to at least one antibiotic and 32% of susceptibility profiles were found in *S. aureus* isolates with several resistance to multiple antibiotic classes (Waters et al., 2011). Some studies highlighted the role of meat and poultry products in inducing human infection and colonization (de Jonge et al., 2010). The majority of virulence factors of *S. aureus* are cell surface proteins including matrix adhesion molecules which mediate bacterial binding to the host tissue and extracellular matrix including collagen and fibrinogen (Foster et al., 2014). The components of *S. aureus* surface include an elastin binding protein and clumping factors A and B (*clfA*, *clfB*) (Atshan et al., 2012). *S. aureus* surface proteins are critical for success of the bacteria as a commensal organism and also as a pathogen (Foster et al., 2014). Staphylococcal protein A (SPA), another important virulence factor, is a multifunctional surface protein located on FC fragment of IgG of mammalian species inducing immune evasion and could act as super antigen of B-cell (Foster et al., 2014; Sutra and Poutrel, 1994). Staphylococcal food poisoning is associated with presence of staphylococcal enterotoxins (SEs) (Le Loir et al., 2003). These enterotoxins are produced in the exponential phase of *S. aureus* growth. They cause food poisoning in human when the count of *S. aureus* reaches at least  $10^5$ – $10^8$  CFU/g in food (Seo and Bohach, 2003). Therefore, the aim of current study was to determine the phenotypic resistance and genotypic characterization of antimicrobial resistance genes, some virulence genes (*clfA* and *spa*) and enterotoxin genes in *S. aureus* strains recovered from some RTE food (kofta, shawarma, sausage and fried shrimp) purchased from restaurants in Sharkia Governorate, Egypt.

## MATERIALS AND METHODS

### COLLECTION OF FOOD SAMPLES

One hundred RTE food samples comprising of kofta, shawarma, sausage and fried shrimp (25, each) were purchased from commercial restaurants and supermarkets at Sharkia Governorate, Egypt during the period from

October, 2017 to April, 2018. The collected samples were packed into sterile lunch boxes. The samples of RTE food were directly placed in a cooler box; and then directly transferred to the Department of Food Control, Faculty of Veterinary Medicine, Zagazig University, and stored at 4 °C till analysis. All samples were microbiologically analyzed within 4 hrs of purchase.

### PROCESSING OF RTE FOOD SAMPLES

The food samples were prepared according to the previously described method (APHA, 1992). Twenty five gram of each RTE food samples were added to a volume of 225 ml sterile buffered peptone water (Oxoid, CM509, UK). The mixture was homogenized by a stomacher for 1 min, and then the homogenate was transferred in a sterile test tube. Afterwards, the homogenate (1 ml) was placed in sterile test tube containing 0.1% peptone water (9 ml), and then subjected to 10-fold serial dilution up to  $10^7$ .

### ISOLATION AND IDENTIFICATION

The isolation procedure of *S. aureus* was carried out according to the previously described method (Thapaliya et al., 2017). The prepared serial dilution (0.1 ml) was streaked on Baird–Parker agar plate (Oxoid, CM0275, UK) and incubated at 37 °C for 24 hrs. The suspected colonies are black-colored surrounded by a wide clear area with opacity. The presumptive colonies showing positive egg yolk reaction were subjected to biochemical identification. The motility test (ICMSF, 1996), catalase activity test (Macfaddin, 2000) and coagulase activity test (APHA, 1992) were carried out as described before. The colonies revealing coagulase positive, Gram positive cocci, catalase positive were biochemically distinguished as *S. aureus*.

### ANTIMICROBIAL SUSCEPTIBILITY TESTING

The biochemically identified isolates of *S. aureus* were tested for their antimicrobial susceptibility against eleven antibiotics on Mueller–Hinton agar plates (Oxoid, England) using the Kirby Bauer disk diffusion test (Bauer et al., 1966). The antimicrobial panels and their disc concentrations including methicillin (ME; 10 µg), cefotaxime (CTX, 30 µg); Amoxicillin–clavulanic acid (AMC, 30 µg), gentamicin (CN, 10 µg), erythromycin (E, 15 µg), clindamycin (DA, 2 µg), doxycycline (DO, 5 µg), ciprofloxacin (CIP, 5 µg), sulfamethoxazole–trimethoprim (SXT, 25 µg), chloramphenicol (C, 30 µg) and vancomycin (VA, 30 µg) were obtained from Thermo scientific, Oxoid, USA. Interpretation of the results was carried out according to the standards of Clinical Laboratory Standard Institute guidelines (CLSI, 2016). The multiple antibiotic resistance index of *S. aureus* isolate was calculated according to the formula stipulated by (Singh et al., 2010) as the following equation:

$$MAR \text{ index} = \frac{\text{Number of antibiotics with resistance profile}}{\text{the number of used antibiotics}}$$

## MOLECULAR DETECTION OF ANTIMICROBIAL RESISTANCE AND VIRULENCE GENES

The genomic DNA was extracted from four biochemically identified *S. aureus* isolates with phenotypic resistance and isolated from shawarma, sausage, fried shrimp and kofta using QIAamp DNA mini kit (Takara Kit, Catalogue no.51304, Japan). The selected primer sequences and cycling conditions were carried out to amplify the resistance genes to methicillin (*mecA*) (McClure et al., 2006), beta-lactamase (*bla<sub>Z</sub>*) and aminoglycoside [*aac(6')*aph(2'')] (Duran et al., 2012) and macrolide (*mphC*) (Schlegelova et al., 2008) in *S. aureus* isolates using uniplex PCR (Table 1).

The two virulence factors including staphylococcal protein A (*spa* gene) (Wada et al., 2010) and clumping factor A (*clf A* gene) (Mason et al., 2001) were molecularly detected in *S. aureus* isolates using uniplex PCR. Also, the oligonucleotide sequences and PCR cycling conditions to identify enterotoxin genes (*sea*, *seb*, *sec*, *sed* and *see*) in *S. aureus* were performed by multiplex PCR according to (Mehrotra et al., 2000) (Table 1). The electrophoresis of PCR products was performed on 1.5% agarose gel having ethidium bromide (0.5 µg/ml) with gel pilot 100 bp ladder (QIAGEN, USA). Positive control isolate of *S. aureus* was included in each reaction, while *Escherichia coli* isolate was used as negative one.

## RESULTS AND DISCUSSION

*S. aureus* is one of the most important food borne pathogens in RTE food products and is incriminated in different infections all over the world (Diep et al., 2006). Additionally, this bacterium is responsible for 12.5% of food borne bacterial outbreaks in China and is considered the third most frequently pathogen after *Vibrio parahaemolyticus* outbreaks (27.8%) and *Salmonella* food poisoning (23.1%) (Wei-Wei et al., 2018). *S. aureus* could adapt to different environmental conditions and quickly acquire resistance to virtually all antimicrobials (McCallum et al., 2010). Recently, MDR *S. aureus* were recorded in outbreaks of food poisoning that were related with consumption of food products (Papadopoulou et al., 2018). Our study showed that the incidence of *S. aureus* in RTE food was 27%. However, *S. aureus* was recovered with lower percentage (17.9%) from RTE food in Taiwan (Fang et al., 2003). In the present study, the highest isolation percentage of *S. aureus* (48%, 12/25) was detected in kofta sandwiches, followed by shawarma sandwiches (36%, 9/25); while the lowest incidence (12%, 3/25) was recovered from sandwiches of sausage and fried shrimp. On the contrary, lower isolation percentages of *S. aureus* was recovered in minced meat (10%), sausage (8%) and beef burger (10%) in Egypt (Morshdy et al., 2018a). In Egypt, higher incidence of *S. aureus* was detected in examined

kofta (90%), luncheon (50%), burger (75%), shawarma (80%), hawawshi (65%), and sausage sandwiches (60%) (Morshdy et al., 2018b). These variations could be attributed to difference in the system of cooking (frying, roasting and grilling), the microbiological quality of raw ingredients, size and shape of meat pieces, cooking utensils (oven, grill, stew pot) and type of seasoning ingredients (sauces, spices, vegetables) (Daelman et al., 2013).

In the current study, the phenotypic resistance of 27 isolates of *S. aureus* from RTE food comprising kofta (12 isolates), shawarma (9), sausage (3) and fried shrimp (3) were investigated against 11 antimicrobials using the disc diffusion method (Table 2). In our study, isolates of *S. aureus* clarified the peak resistance to methicillin and cefotaxime (100%) followed by amoxicillin (85.18%) and gentamicin (81.48%). Similarly, the resistance percentages of *S. aureus* from RTE meat products to methicillin and amoxicillin were 100% and 57.1%, respectively in Egypt (Morshdy et al., 2018a). The moderate resistance of *S. aureus* isolates was detected for erythromycin and clindamycin (62.96%), doxycycline (59.25%) and ciprofloxacin (48.14%); while the lower resistance of *S. aureus* isolates was found to sulfamethoxazole-trimethoprim (29.62%) and chloramphenicol (25.92%). Another study in Egypt investigating MDR *S. aureus* isolates derived from RTE meat sandwiches, and the results demonstrated that 84% of *S. aureus* isolates were resistant to erythromycin and 64% for oxytetracycline and sulphamethoxazole (Morshdy et al., 2018b). However, erythromycin showed moderate resistance (52.7%) for *S. aureus* isolates recovered from retail meat and meat products in China followed by clindamycin (29.6%), gentamicin (19.4%), ciprofloxacin (16.9%) and amoxicillin/clavulanic acid (11%) (Wu et al., 2018). Our finding showed that 27 *S. aureus* isolates from RTE food were sensitive (100%) to vancomycin as listed in Table 2. However, resistance of *S. aureus* to vancomycin was found in 27.6% of camel meat and 54.5% of human hand swabs in Egypt (Al-Amery et al., 2019). In the current study, maximum resistance (100%) of *S. aureus* isolates derived from RTE food were found to methicillin as MRSA could be transmitted to cooked food products owing to temperature abuse during the storage and thus resulting in multiplication of MRSA strains. Moreover, the higher MRSA contamination of different RTE meat and chicken products may be possible associated with utilization of antibiotics in animal husbandry (de Boer et al., 2009). Also, the major reason for high resistant *S. aureus* strains could be related with supplements and growth promoters for animal feed and for human treatments. The difference in resistance percentage of *S. aureus* strains to antimicrobials could be accounted for size of sample, geographic area and period of sample collection (Ge et al., 2017).



**Table 1:** Primer sequences and PCR conditions for antimicrobial resistance genes, enterotoxin genes and virulence determinants in *S. aureus* strains derived from ready to eat food.

| Reference                          | PCR cycling conditions   | Length of amplified product (bp) | Primer sequence (5'-3')  | Gene            |
|------------------------------------|--|----------------------------------|--|-----------------|
| (McClure <i>et al.</i> , 2006)     | 94°C-5min;35cycles<br>(94°C-30s, 50°C- 30s,<br>72°C-30s);72°C-7 min      | 310                              | GTA GAA ATG ACT GAA CGT CCG ATA A<br>CCA ATT CCA CAT TGT TTC GGT CTA A | mecA            |
| (Duran <i>et al.</i> , 2012)       | 94°C-5min; 35cycles<br>(94°C-30s, 54°C- 30<br>s,72°C-30s); 72°C-7 min    | 173                              | ACTTCAACACCTGCTGCTTTC<br>TGACCACTTTTATCAGCAACC                         | blaZ            |
|                                    | 94°C-5min;35cycles<br>(94°C-30s, 50°C- 40 s,<br>72°C-45s); 72°C-10 min   | 491                              | GAAGTACGCAGAAGAGA<br>ACATGGCAAGCTCTAGGA                                | aac(6')aph(2'') |
|                                    |  | 102                              | GGTTATCAATGTGCGGGTGG<br>CGGCACTTTTTTCTCTTCGG                           | sea             |
|                                    |  | 164                              | GTATGGTGGTGAAGTACTGAGC<br>CCAAATAGTGACGAGTTAGG                         | seb             |
| (Mehrotra <i>et al.</i> , 2000)    | 94°C-5min;35cycles<br>(94°C-30s,50°C- 40<br>s,72°C-45s);72°C-10 min      | 451                              | AGATGAAGTAGTTGATGTGTATGG<br>CACACTTTTAGAATCAACCG                       | sec             |
|                                    |  | 278                              | CCAATAATAGGAGAAAATAAAAG<br>ATTGGTATTTTTTTTCGTTTC                       | sed             |
|                                    |  | 209                              | AGTTTTTTTCACAGGTCATCC<br>CTTTTTTTTCTTCGGTCAATC                         | see             |
| (Schlegelova <i>et al.</i> , 2008) | 94°C-5min;35cycles<br>(94°C-30s,55°C- 40<br>s,72°C-45s); 72°C-10 min     | 722                              | GAGACTACCAAGAAGACCTGACG<br>CATACGCCGATTCTCCTGAT                        | mphC            |
| (Wada <i>et al.</i> , 2010)        | 94°C-5min; 35cycles<br>(94°C-30s, 55 °C- 30 s,<br>72°C-30s); 72 °C-7 min | 226                              | TCA AGCA AAG AAC AAC AAA ATG C<br>GCT TTC GGT GCT TGA GAT TC           | spa             |
| (Mason <i>et al.</i> , 2001)       | 94°C-5min; 35cycles<br>(94°C-30s, 55°C- 40 s,<br>72°C-45s); 72°C-10 min  | 638                              | GCAAAATCCAGCACAAACAGGAAACGA<br>CTTGATCTCCAGCCATAATTGGTGG               | clfA            |

**Table 2:** Antimicrobial susceptibility of 27 *S. aureus* isolates derived from ready to eat food by disc diffusion method.

| Antimicrobials (disc concentration/µg) | R          | S          | I          |
|--|------------|------------|------------|
| ME (10)                                | 27 (100)   | 0 (0.00)   | 0 (0.00)   |
| CTX (30)                               | 27 (100)   | 0 (0.00)   | 0 (0.00)   |
| AMC (30)                               | (85.18)    | 4(14.81)   | 0 (0.00)   |
| CN (10)                                | 22 (81.48) | 2 (7.40)   | 3(11.11)   |
| E (15)                                 | 17 (62.96) | 5 (18.51)  | 5(18.51)   |
| DA (2)                                 | 17(62.96)  | 9 (33.33)  | 1(3.70)    |
| DO (5)                                 | 16(59.25)  | 10(37.03)  | 1(3.70)    |
| CIP (5)                                | 13(48.14)  | 10 (37.03) | 4(14.81)   |
| SXT (25)                               | 8(29.62)   | 9 (33.33)  | 10 (37.03) |
| C (30)                                 | 7(25.92)   | 10(37.03)  | 10(37.03)  |
| VA (30)                                | 0 (0.00)   | 27(100)    | 0 (0.00)   |

R: resistant; I: intermediate; S: sensitive; Data were represented by No (%); ME: methicillin; CTX: cefotaxime; AMC: Amoxicillin- clavulanic acid; CN: gentamicin; E: erythromycin; DA: clindamycin; DO: doxycycline; CIP: ciprofloxacin; SXT: sulfamethoxazole-trimethoprim; C: chloramphenicol; VA: vancomycin.

In this study, the range of MAR index for *S. aureus* was 0.36 to 0.9 with an average 0.58 as listed in Table 3. Moreover, 3 (25%) isolates of kofta origin were multi-resistant to 10 antibiotics, while one isolate (9.09) derived from shawrma was multi drug resistant to 10 antimicrobials and other two *S. aureus* strains (22.2%) originated from shawrma were resistant to 9 antibiotics (Table 3). Similarly, the average MAR index of *S. aureus* isolates was 0.59 from RTE sandwiches in Egypt (Morshdy *et al.*, 2018b). In another study, 95.65% of *S. aureus* from pork meat in India have MAR index above 0.3 (Savariraj *et al.*, 2019). The difference in the MAR index could be attributed to variations in the sources of food samples and geographic distributions, which had differential selective pressures for levels of antibiotic resistance and the methodologies of test (Robert-Pillot *et al.*, 2014). In the current study, *S. aureus* strains with higher value of MAR index (>0.2) reflected high risk sources and poses high risk hazard for consumers; where they eat those RTE foods contaminated with such strains as previously supported (Robert-Pillot *et al.*, 2014). The highest MAR index

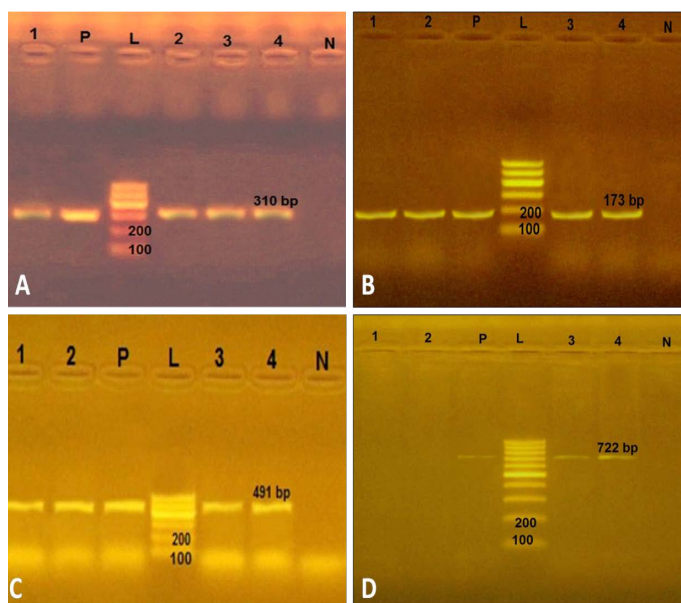
**Table 3:** Antimicrobial resistance profiles and MAR index of *S. aureus* isolates derived from ready to eat food.

| MAR  | Resistance profile                       | No. of iso-<br>lates (%) | No. of resistant<br>antibiotics | Resistance<br>Pattern | Ready to eat<br>food         |
|------|--|--------------------------|---------------------------------|-----------------------|------------------------------|
| 0.9  | ME, CTX, AMC, CN, E, DA, DO, CIP, SXT, C | 3(25)                    | 10                              | R1                    | Kofta<br>(12 isolates)       |
| 0.54 | ME, CTX, AMC, CN, E, DA                  | 6 (50)                   | 6                               | R2                    |                              |
| 0.45 | ME, CTX, CN, DA, DO                      | 2(16.7)                  | 5                               | R3                    |                              |
| 0.36 | ME, CTX, AMC, DO                         | 1(8.3)                   | 4                               | R4                    |                              |
| 0.9  | ME, CTX, AMC, CN, E, DA, DO, CIP, SXT, C | 1 (9.09)                 | 10                              | R5                    | Shawrma<br>(9 isolates)      |
| 0.81 | ME, CTX, AMC, E, DA, DO, CIP, SXT, C     | 2(22.2)                  | 9                               | R6                    |                              |
| 0.72 | ME, CTX, AMC, DA, DO, CIP, SXT, C        | 1(9.09)                  | 8                               | R7                    |                              |
| 0.63 | ME, CTX, CN, DA, CIP, SXT, DO            | 1(9.09)                  | 7                               | R8                    |                              |
| 0.54 | ME, CTX, CN, E, DO, AMC                  | 1(9.09)                  | 6                               | R9                    |                              |
| 0.36 | ME, CTX, AMC, CN                         | 3(33.3)                  | 4                               | R10                   | Sausage<br>(3 isolates)      |
| 0.54 | ME, CTX, AMC, CN, DO, CIP                | 2(66.7)                  | 6                               | R11                   |                              |
| 0.45 | ME, CTX, AMC, CN, E                      | 1(33.3)                  | 5                               | R12                   | Fried shrimp<br>(3 isolates) |
| 0.63 | ME, CTX, AMC, CN, E, DA, CIP             | 1(33.3)                  | 7                               | R13                   |                              |
| 0.54 | ME, CTX, AMC, E, DO, CIP                 | 2(66.7)                  | 6                               | R14                   |                              |
| 0.58 |  |                          |                                 |                       | Average                      |

MAR: Multiple antibiotic resistance; ME: methicillin; CTX: cefotaxime; AMC: Amoxicillin-clavulanic acid, CN: gentamicin; E: erythromycin; DA: clindamycin; DO: doxycycline; CIP: ciprofloxacin; SXT: sulfamethoxazole-trimethoprim; C: chloramphenicol; VA: vancomycin.

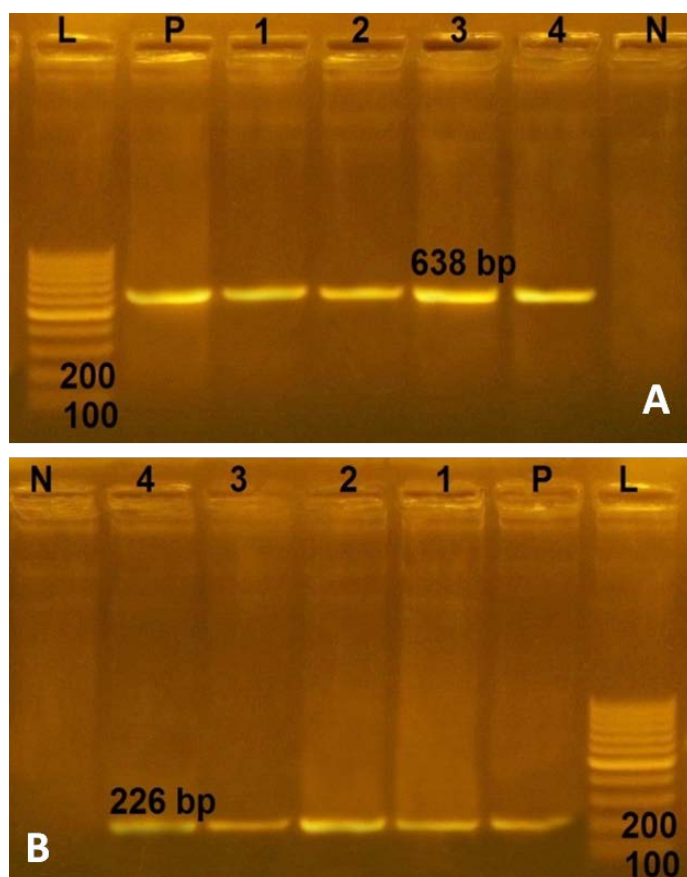
in our study could be accounted for frequent exposure of human and farm animals to antibiotics in animals raised for food and for various purposes such as growth promotion and therapeutics and these resistant pathogens could be transmitted to humans through food (Nygard et al., 2008). This study indicated that MDR *S. aureus* should be included in the list of antimicrobial resistant pathogens which cause contamination of the food supply. Therefore, monitoring of antimicrobial resistance is necessary to determine the effectiveness of new generations of antibiotics and *S. aureus* isolates revealed resistance to antimicrobials owing to their capability to produce an exopolysaccharide barrier (Gündoğan et al., 2006) and carrying broad variety of MDR genes on plasmids which could be exchanged and spread to other species of *Staphylococci* (Neihart et al., 1988).

In the present study, the four strains of phenotypically resistant *S. aureus* to methicillin, amoxicillin and gentamicin were positive (100%) for *mec A*, *bla Z* and *aac (6') aph (2'')* genes with PCR products of 310, 173 and 491 bp, respectively (Figure 1). However, 2 out of 4 phenotypically resistant isolates to macrolide antibiotics (erythromycin) were positive for *mphC* gene with a PCR product of 722 bp (Figure 1). This finding agreed with previous reports of multidrug resistant *S. aureus* bearing *mec A*, *bla Z* and *aac(6') aph (2'')* from retail meat products (Morshdy et al., 2018a), chicken meat and giblets (Darwish et al., 2018) in Egypt and RTE meat products (Xing et al., 2014) in China. Our study indicated that RTE meat, chicken and shrimp could be considered as important transmission route for antibiotic resistant *S. aureus* bearing multiple antibiotic resistance genes.



**Figure 1:** Uniplex PCR to amplify some antimicrobial resistance genes in 4 *S. aureus* strains from ready to eat food. A) PCR products of *mec A* gene. P: positive control isolate; N: negative control isolate of *Escherichia coli*; L: Ladder 100 bp; Lanes 1, 2, 3 and 4: positive strains for *mec A* gene (310 bp) isolated from kofta, shawrma, sausage and fried shrimp, respectively; B) PCR products of *bla Z* gene. The four *S. aureus* isolates (lanes 1-4) were positive PCR product of 173 bp; C) PCR products of *aac(6')aph (2'')* gene. The lanes 1-4 were with amplicon of 491 bp; D) PCR products of *mph C* gene. The two *S. aureus* isolates from kofta and shawarma (Lanes 1 and 2) were negative, while other two *S. aureus* strains from sausage and fried shrimp (lanes 3 and 4) were positive with PCR product of 722 bp.

In the current study, the 4 multidrug resistant *S. aureus* were negative for any enterotoxin genes. However, more than 74% of *S. aureus* strains derived from RTE food, processed meat and fish products in Bangladesh harbored enterotoxin genes with a distribution percentage of 26% for *sea*, 11% for *seb*, 49% for *sec* and 13% for *sed* genes (Islam et al., 2019). Furthermore all 4 multidrug resistant *S. aureus* from RTE food harboring the two virulence genes (*spa* and *clfA* genes) with PCR products of 226 and 638 bp, respectively (Figure 2). Conversely, lower distribution (76.92%) of *clfA* gene in *S. aureus* strains recovered from chicken meat in Iran (Momtaz et al., 2013). This study revealed that multidrug resistant *S. aureus* isolated from RTE food were MRSA isolates, possessed two virulence genes (*clfA* and *spa* genes), represent public health hazard for consumers.



**Figure 2:** Agarose gel electrophoresies of two virulence genes (*clfA* and *spa*) in 4 strains of *S. aureus* isolated from ready to eat food. A) PCR products of *clfA* gene. P: positive control isolate; N: negative control; L: Ladder 100 bp; Lanes 1- 4: positive strains for *clfA* gene (638 bp) isolated from kofta, shawarma, sausage and fried shrimp, respectively; B) PCR products of *spa* gene. The four *S. aureus* isolates (lanes 1-4) were positive with amplicons of 226 bp.

## CONCLUSION

The higher contamination of different kofta, shawarma, sausage and fried shrimp as RTE food with multidrug resistant *S. aureus* indicates improper hygienic measures.

Also, higher MRSA contamination of such RTE food could be associated with the wide use of antimicrobials in animal husbandry. Moreover, higher MAR index of *S. aureus* isolates poses high risk potential for consumers to eat such contaminated RTE food. Therefore, RTE food could be considered as important transmission route for phenotypic resistant *S. aureus* that carry resistance genes to methicillin, beta-lactamase, macrolide and aminoglycosides. Strict hygienic measures should be taken during the preparation of RTE food, besides strong legislations should be followed to produce food of high keeping quality.

## AUTHORS CONTRIBUTION

All authors had contributed equally.

## CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare.

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