Occurrence, Virulence Factors, Antimicrobial Resistance, and Genotyping of *Staphylococcus aureus* Strains Isolated from Chicken Products and Humans

Rasha M. El Bayomi,[1] Heba A. Ahmed,[2] Maysa A. I. Awadallah,[2] Rasha A. Mohsen,[3] Abeer E. Abd El-Ghafar,[3] and Mahmoud A. Abdelrahman[3]

Abstract

Staphylococcus aureus in food is a consequence of inadequate hygienic handling and processing, posing a potential risk to public health. The current study aimed to characterize virulence factors, as well as antimicrobial resistance of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) isolated from retail chicken products and hand swabs from vendors in Egypt. In addition, genetic relatedness of the isolates from chicken and humans was evaluated by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) using protein A as a target. A total of 110 samples were collected from chicken products ($n = 80$) and vendors ($n = 30$). Overall, 30 (37.5%) chicken products samples were positive for *S. aureus*, whereas hand swabs from meat handlers revealed that 18 (60%) were positive. Ten MRSA strains were characterized by the presence of the *mec*A gene, comprising seven isolates from chicken and three from humans. Virulence-associated factors were evaluated by PCR, revealing that 31.3% of *S. aureus* isolates harbored the Panton–Valentine leukocidin (PVL) gene, whereas 10.4% were positive for the *sea* and *sed* genes each, and only two isolates were positive for γ-hemolysin–associated gene. Genotyping using *spa* PCR-RFLP showed identical restriction banding patterns of MRSA isolates of human and chicken meat origin, indicating the genetic relatedness of the isolates. To the best of our knowledge, this is the first study to characterize PVL-positive MRSA from chicken products and to utilize *spa*-RFLP for evaluating the genetic relatedness between MRSA of human and chicken origin in Egypt.

Key Words: *S. aureus*—MRSA—PVL—γ-Hemolysin—Enterotoxins—*spa*-PCR-RFLP.

Introduction

THE POULTRY INDUSTRY IN EGYPT has grown in the last years to keep up with the increased trend of consumption. *Staphylococcus aureus* is a widespread foodborne pathogen colonizing the nasal passages, skin, and intestinal tracts of different animals and humans (Hanson et al. 2011). The presence of *S. aureus* in food could be attributed to the contamination of raw meat during slaughtering, processing, and storage (Tassew et al. 2010, Hennekinne et al. 2012). Food poisoning due to *S. aureus* enterotoxins is ranked third worldwide; in addition, these toxins are heat stable, and as few as 20–100 ng of the toxin can produce illness (Asao et al. 2003, Schelin et al. 2011).

Resistance to antibiotics is an increasing concern worldwide and is associated mainly with uncontrolled usage of antimicrobials for treatment or as growth promoters in food animals (Barber et al. 2003). *S. aureus* resistant to penicillinase-resistant pencillins is referred to as methicillin-resistant *S. aureus* (MRSA). Resistance of methicillin is mediated by the *mec*A gene, which encodes penicillin-binding protein 2a, with a decreased binding affinity for β-lactams (Chambers 1997). Because of its multidrug resistance pattern, MRSA is considered a public health concern, especially for immunocompromised patients (Pesavento et al. 2007, Kraushaar and Fetsch 2014). Panton–Valentine leukocidin (PVL) is one of the important exotoxins causing severe skin and soft tissue infections and necrotizing pneumonia (Kraushaar and Fetsch

[1]Department of Food Control, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt.
[2]Department of Zoonoses, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt.
[3]Department of Microbiology, Animal Health Research Institute, Mansoura Branch, Mansoura, Egypt.

2014). The association of PVL with progressive, hemorrhagic, and necrotizing pneumonia has been reported in cases of community-acquired infection with MRSA (Gillet et al. 2002). γ-Hemolysin is also a toxin causing damage to erythrocytes (Prevost et al. 1995). The gene is encoded by the *hlg* locus, and previous reports have described the gene in more than 99% of *S. aureus* strains (Prevost et al. 1995). Production of enterotoxins by *S. aureus* is another virulence-associated factor that has an impact on the pathogenicity of the isolates. Eleven antigenic types of enterotoxins (staphylococcal enterotoxin type A [SEA] to SE type E [SEE]) are recognized; of them, SEA is considered the most commonly involved enterotoxin with human infection (Balaban and Rasooly 2000).

Typing of MRSA is essential for tracing the source of infection; therefore, typing methods should have the advantages of typeability, reproducibility, and good discriminatory power (Wichelhaus et al. 2001). Different molecular methods, *e.g.*, pulsed-field gel electrophoresis (PFGE), have been described as the gold standard typing method; however, PFGE is not widely used due to expense, time required, and sophisticated application (Wichelhaus et al. 2001). Molecular typing of *S. aureus* using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) has been shown to be of sufficiently high accuracy and corresponds to PFGE typing (Omar et al. 2014).

This study aimed to assess retail chicken products sold in Egypt as a potential vehicle for *S. aureus* and MRSA. Antimicrobial resistance profiling and molecular characterization of some virulence factors of the isolates obtained were carried out. Moreover, the genetic relatedness of MRSA isolates of human and chicken origin was determined by protein A (*spa*) PCR-RFLP.

Materials and Methods

Sampling and isolation of S. aureus

A total of 80 chicken products' samples (40 raw chicken breast fillet, 20 sliced luncheon meat, and 20 chicken nuggets) were collected randomly from local pluck-shop markets and outlets at Sharkia Governorate, Egypt. Moreover, 30 hand swabs from chicken handlers at the pluck-shop markets and outlets were collected. Twenty-five grams from each sample were homogenized with 225 mL of buffered peptone water (BPW; cat. no. CM509, Oxoid, Adelaide, Australia) for pre-enrichment and incubated at 37°C for 24 h. A loopful from the pre-enriched culture homogenate in BPW was inoculated onto Baird–Parker agar medium (cat. no. CM0275, Oxoid, Adelaide, Australia) and incubated at 37°C for 24 h (ISO-6888-1, 1999). In addition, serial dilutions were prepared for *S. aureus* count. One suspected *S. aureus* colony from each plate was subjected to Gram staining and biochemical identification (catalase, coagulase, oxidase, and DNase). *S. aureus* identification was then confirmed by amplification of the species-specific *nuc* gene (Gao et al. 2011).

Antimicrobial susceptibility test

A disc diffusion test was carried out to detect the minimum inhibitory concentration (MIC) of *S. aureus* isolates against 14 antimicrobials according to National Committee for Clinical Laboratory Standards (NCCLS 2013). The antibiotics that were used to evaluate resistance are listed in Table 2 (below).

Molecular characterization and spa-RFLP

S. aureus isolates were subjected to PCR for the detection of *mecA* (Zhang et al. 2004), PVL (Lina et al. 1999), γ-hemolysin (Kumar et al. 2009), and enterotoxin encoding genes (*sea*, *sec*, and *sed*) (Mehrotra et al. 2000). Moreover, MRSA strains isolated from different sources were subjected to *spa*-PCR-RFLP for genotyping (Omar et al. 2014). Extraction of the bacterial DNA was carried out using a QIAamp DNA Mini Kit according to the manufacturer's guidelines (Qiagen, Germany, GmbH). For restriction, HindIII enzyme was used according to the protocol described by Omar et al. (2014). The synthesized primers and the amplicon sizes are summarized in the Table S1 (Supplementary Data are available at www.liebertpub/vbz/). Typeability of *spa*-RFLP is defined as the number of strains that are located in a type by a typing system (Hallin et al. 2007).

Results

Biochemical identification revealed that 67 isolates were suspected *S. aureus* (data not shown). Only biochemically suspected *S. aureus* isolates confirmed by *nuc* gene PCR were included in the study ($n = 48$). A total of 110 samples comprising 40 chicken breast fillet, 20 sliced luncheon meat, 20 chicken nuggets, and 30 hand swabs from vendors were examined. Overall, 30 (37.5%) chicken samples were positive for *S. aureus*, with isolation rates of 42.5%, 40%, and 25% from chicken fillet, nuggets, and sliced luncheon meat, respectively (Table 1). The difference in the contamination rate of the investigated products with *S. aureus* was insignificant ($\chi_1^2 = 1.8$, $p = 0.4$). Examination of hand swabs collected from vendors revealed that 18 (60%) were positive for *S. aureus* (Table 1). The total *S. aureus* counts in chicken products were $3.4 \times 10^4 \pm 8.4 \times 10^3$ (chicken fillet), $1.1 \times 10^4 \pm 3.3 \times 10^3$ (nuggets), and $6.3 \times 10^4 \pm 1.5 \times 10^4$ (sliced luncheon meat) (Table 1). Out of the examined isolates from chicken ($n = 80$) and vendors ($n = 30$), the *mecA* gene specific for MRSA was detected in seven (8.8%) and three (10%) samples, respectively (Table 1).

The results of the antibiotic resistance of 48 *S. aureus* isolates of chicken and human origin showed that all of the isolates (100%) were sensitive to vancomycin, and only 7.8% resistance was observed against ofloxacin (Table 2). High resistance was detected against doxycycline (64.6%) and amoxicillin (60.4%). However, resistance rates ranging from 18.8% and 47.9% were reported to the other examined antimicrobials (Table 2).

Acquisition of virulence factor–associated genes was investigated by molecular amplification of PVL-, γ-hemolysin–, and enterotoxin-associated genes (*sea*, *sec*, and *sed*) Fig. S1. The results showed PVL, γ-hemolysin–, *sea*-, and *sed*-associated genes were detected, respectively, in 31.3%, 4.2%, 10.4%, and 10.4% of the *S. aureus* isolates obtained (Table 3). The comparable occurrence of PVL, γ-hemolysin–, *sea*-, and *sed*-associated genes in MRSA versus non-MRSA isolates was 90% vs. 15.8%, 0 vs. 5.3%, 40% vs. 2.6%, and 30% vs. 5.3%, respectively. None of the examined *S. aureus* isolates (MRSA or non-MRSA) was positive for the *sec*-associated gene (Table 3).

Genotyping of the *spa* region in 10 MRSA isolates (seven of chicken and three of human origin) showed that 80% of the identified MRSA isolates produced a single band of 1200 bp (type S1), whereas only 20% produced no bands

TABLE 1. OCCURRENCE OF *S. AUREUS* AND MRSA[a] AND *S. AUREUS* COUNT ($\log_{10}$ CFU/GRAM) IN THE SAMPLES EXAMINED

Samples	Number examined	S. aureus	MRSA	Mean ± SE
Chicken fillet	40	17 (42.5, 27–59.1)	1 (2.5, 0.1–13.2)	$3.4 \times 10^4 \pm 8.4 \times 10^3$
Nugget	20	8 (40, 19.1–63.9)	2 (10, 1.2–31.7)	$1.1 \times 10^4 \pm 3.3 \times 10^3$
Sliced Luncheon	20	5 (25, 8.7–49.1)	4 (25, 5.7–43.7)	$6.3 \times 10^4 \pm 1.5 \times 10^4$
Total	80	30 (37.5, 29.9–49)	7 (8.8, 3.6–17.2)	—
Vendors hand swabs	30	18 (60, 40.6–77.3)	3 (10, 2.1–26.5)	—
Total	110	48 (43.7, 34.2–53.4)	10 (9.1, 4.4–16.1)	—

[a]Number (proportion, 95% confidence interval).
MRSA, methicillin-resistant *S. aureus*; CFU, colony-forming units; SE, standard error.

(Fig. 1). The absence of PCR product is considered a separate type, namely S2. Restriction of the produced amplicon by the HindIII enzyme produced two banding patterns of 240 bp and 696 bp (Fig. 1). The typeability of the method was calculated to be 80%.

Discussion

Contaminated chicken meat is considered an important source of foodborne pathogens posing risk for zoonotic transmission (Osman et al. 2015). In the current study, the proportion positive for *S. aureus* in chicken products' samples was 37.5% (Table 1). In Egypt, *S. aureus* isolation rates of 6% (EI-Jakee et al. 2013, Osman et al. 2015) and 77.6% (Karmi 2013) from chicken meat samples have been reported. The difference between the isolation rate obtained and those previously reported in Egypt could be attributed to the identification of *S. aureus* isolates using only biochemical identification in the aforementioned three studies. Phenotypic identification of staphylococci has been reported to be unsatisfactory, irreproducible, and unreliable (Heikens et al. 2005). Moreover, the variation could be attributed to the sample size, location, and hygienic measures posed by chicken meat handlers (Bhargava et al. 2011). Comparable

isolation rates were reported in Japan (34.2%; Ogata et al. 2012), the United States (42.1%; Abdalrahman et al. 2015), and South Africa (44%; Oguttu et al. 2014).

As illustrated in Table 1, *S. aureus* contamination was found to be higher in chicken breast fillet (42.5%) and nuggets (40%) than in sliced luncheon meat (25%); however, the difference between the three products was insignificant. *S. aureus* was recorded in 46.2% of chicken fillets in Croatia (Kozačinski et al. 2006), 75% of nuggets in Italy (Pepe et al. 2006), and 40% of luncheon meat samples in Egypt (Karmi 2013). The lower contamination rate of sliced luncheon meat than chicken fillet and nuggets might be attributed to the complete heat treatment of luncheon meat during processing. Although there was a low contamination rate of sliced luncheon meat, the potential of handling after cooking is suspected, which, in turn, poses a risk for human infection (Oguttu et al. 2014).

MRSA is a potential cause of hospital-acquired infection; however, community-acquired infections are also of growing concern (Tiemersma et al. 2004, Witte et al. 2004). The isolation rate of MRSA from chicken products in the present study was 8.8% (Table 1). Similarly, 7.1% of chicken meat samples were positive for MRSA in Korea (Cho et al. 2014). Variable isolation rates of MRSA from chicken meat have

TABLE 2. ANTIMICROBIAL RESISTANCE OF *S. AUREUS* AND MRSA ISOLATED FROM CHICKEN PRODUCTS AND HUMANS

Antimicrobial class	Antimicrobial agent	Concentration (μg)	*Number of resistant* S. aureus *isolates (%)*		
			Chicken products (n = 30)	Humans (n = 18)	Total n = (48)
Cephalosporins	Cefoperazone	75	10 (33.3%)	13 (72.2%)	23 (47.9%)
Lincosamides	Clindamycin	2	13 (43.3%)	9 (50%)	21 (43.8%)
Glycopeptides	Vancomycin	30	0	0	0
Aminoglycosides	Gentamicin	10	16 (53.3%)	4 (22.2%)	20 (41.7%)
	Apramycin	15	10 (33.3%)	6 (33.3%)	16 (33.3%)
Penicillins	Amoxicillin	30	16 (53.3%)	13 (72.2%)	29 (60.4%)
Tetracyclines	Doxycycline	10	17 (56.7%)	14 (77.8%)	31 (64.6%)
Macrolides	Erythromycin	15	10 (33.3%)	11 (61.1%)	21 (43.8%)
Sulfonamides	Sulfatrimethoprim	15	16 (53.3%)	7 (38.9%)	23 (47.9%)
Quinolones	Levofloxacin	5	10 (33.3%)	8 (44.4%)	18 (37.5%)
	Nalidixic acid	30	13 (43.3%)	7 (38.9%)	20 (41.7%)
	Ofloxacin	5	7 (23.3%)	3 (16.7%)	10 (7.8%)
	Enrofloxacin	5	7 (23.3%)	2 (11.1%)	9 (18.8%)
	Ciprofloxacin	5	10 (33.3%)	6 (33.3%)	16 (33.3%)

MRSA, methicillin-resistant *S. aureus*.

TABLE 3. VIRULENCE-ASSOCIATED GENES IN MRSA AND NON-MRSA *S. AUREUS* ISOLATES

Isolates	Virulence-associated genes				
	PVL	γ-Hemolysin	sea	sed	sec
MRSA ($n=10$)	9 (90%)	0	4 (40%)	3 (30%)	0
Non-MRSA ($n=38$)	6 (15.8%)	2 (5.3%)	1 (2.6%)	2 (5.3%)	0
Total ($n=48$)	15 (31.3%)	2 (4.2%)	5 (10.4%)	5 (10.4%)	0

MRSA, methicillin-resistant *S. aureus*.

been reported in different studies (de Boer et al. 2009, Fessler et al. 2011, Hanson et al. 2011, Agerso et al. 2012, Chairat et al. 2015). The results obtained revealed that contamination of poultry meat during slaughter is assumed to be the most important source for infection of human consumers. Such an assumption was supported by the reported observation in The Netherlands that 8% of poultry were MRSA positive before slaughtering; however, after slaughtering, 35% of poultry meat was contaminated (Mulders et al. 2010).

Infected food handlers are responsible for foodborne illnesses due to bacterial pathogens (Centers for Disease Control and Prevention 2000). It has been estimated that more than 50% of healthy individuals have a high prevalence of *S. aureus* on the hands and fingertips and that food handlers have been involved in outbreaks (Jones et al. 2002, Le Loir et al. 2003, Lues et al. 2006). The results showed that 60% of hand swabs collected from food handlers at retail markets were positive for *S. aureus*, with MRSA comprising 10% (Table 1). *S. aureus* was isolated from 57.5% of food handlers in Botswana (Loeto et al. 2007) and 38.3% in Nigeria (Okareh and Erhahon 2015). Moreover, in Nigeria, 48.8% of hand swabs from meat handlers were positive for MRSA, and the authors attributed such a high isolation rate to the observed unhygienic handling of meat (Nnachi et al. 2014). On the contrary, MRSA was not recovered from hand swabs of meat handlers in The Netherlands as a consequence of the continuous active surveillance against antibiotic-resistant microorganisms (de Jonge et al. 2010).

According to the legal requirements of the Egyptian Organization for Standardization and Quality Control (EOSQC 2005), the permissible level of *S. aureus* in poultry meat varies from one product to another. In chicken fillets, *S. aureus* should be less than 10^2 colony-forming units (CFU)/gram,

whereas partially and completely heat-treated chicken products (nuggets and luncheon meat, respectively) should be *S. aureus* free. The results obtained showed that the mean *S. aureus* count in chicken breast fillet, nuggets, and sliced luncheon meat are beyond the permissible limits of EOSQC (Table 1), thus indicating that chicken products in retail at the study area pose risk to human consumers. Lower *S. aureus* counts of 2.74 ± 0.56 log CFU/gram were reported from breast fillet in Croatia (Kozačinski et al. 2006) and $>10^3$ in ready-to-eat chicken meat in South Africa (Oguttu et al. 2014), while, in The Netherlands, colony counts in chicken meat products were found below 10 CFU/gram (de Boer et al. 2009).

The emergence of antimicrobial-resistant *S. aureus* has been associated with the uncontrolled use of drugs such as growth promoters in animals; hence, there should be a restriction of usage at the farm level (Huber et al. 2010, Buyukcangaz et al. 2013). In the present study, 64.6% and 60.4% of the isolates were resistant to doxycycline and amoxicillin (Table 2). In accordance with these results, *S. aureus* isolates have shown increased resistance to tetracyclines and penicillins in different studies (Aydin et al. 2011, Pu et al. 2011, Buyukcangaz et al. 2013). The spread of penicillin resistance has been found to be rapid due to plasmid transfer and is most reported among isolates of food origin (Aydin et al. 2011, Buyukcangaz et al. 2013). *S. aureus* isolates of human origin were significantly more resistant to cefoperazone than isolates of chicken origin ($\chi_1^2 = 5.34$, $p = 0.02$). The higher resistance of human isolates could be attributed to the common use of cefoperazone in treatment of dairy cattle infected with mastitis, which consequently results in more exposure of human consumers to resistant *S. aureus* from dairy sources (Fessler et al. 2014). Interestingly, all *S. aureus* isolates in the present study were sensitive to
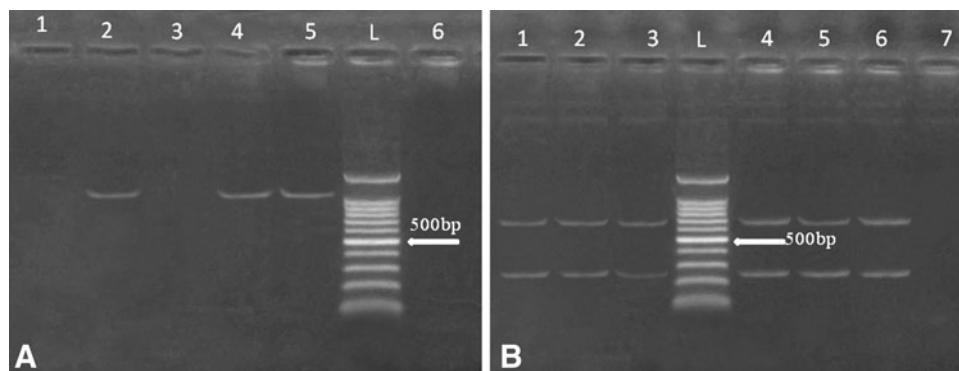

FIG. 1. Exemplar for 1% agarose gel of spa-RFLP. Lane L, 100-bp ladder. (**A**) Amplification of 1200-bp fragment. (**B**) Restriction bands of 240 bp and 696 bp).

vancomycin, thus concurring with previously reported results (Buyukcangaz et al. 2013, Cho et al. 2014, Kim et al. 2015).

Characterization of some virulence-associated genes was essential to investigate the potential of the isolates to pose risk to human consumers. Of 48 *S. aureus* isolates, the PVL-associated gene was found in 31.3%, whereas 90% of MRSA isolates were PVL positive (Table 3). In agreement with the current study, all MRSA isolates from retail meat were PVL positive in the United States (Pu et al. 2009, Bhargava et al. 2011), whereas 37.2% of *S. aureus* isolates from clinical cases were positive for PVL (Lina et al. 1999). In Egypt, 19.04% PVL positive MRSA isolates were characterized from patients at Zagazig City, Egypt (Enany et al. 2010). Other studies reported that MRSA isolates from retail meat were negative for the PVL-associated gene (Fessler et al. 2011, Hanson et al. 2011, Ogata et al. 2012). The significance of PVL-positive MRSA isolates was associated with their ability to produce skin infections and pneumonia (Liassine et al. 2004, Labandeira-Rey et al. 2007).

Although γ-hemolysin previously was reported to be produced by more than 99% of *S. aureus* (Prevost et al. 1995), only two isolates were positive in the present study (Table 3). Likewise, Li et al. (2015) reported that none of the *S. aureus* isolates from retail foods in China were positive for the *hlg* gene. Contrary to our results, γ-hemolysin–associated gene was detected in all MRSA isolates from human cases (Lina et al. 1999).

S. aureus isolates of poultry origin mainly produce toxins C and D; however, production of toxins A and B was reported recently in isolates from raw chicken meat (Kitai et al. 2005). In the present study, 10.4% of *S. aureus* isolates were positive for both *sea*- and *sed*-associated genes (Table 3). In Japan, Kitai et al. (2005) reported that 78 *S. aureus* isolates were enterotoxigenic, of which, 17.9% and 2.6% were found to produce SEA and SED, respectively. Enterotoxins were reported also in 29.5% of *S. aureus* isolated from meat and meat products in China (Jiang et al. 2001).

In the present study, 40% and 30% of MRSA isolates were found to harbor *sea* and *sed* genes, respectively (Table 3). Similarly, Corrente et al. (2005) reported that 71 MRSA isolates of human origin in Italy were able to synthesize SEA, whereas, in contrast, SED was the most frequently synthesized toxin among MRSA isolates from foods of animal origin in Italy (Normanno et al. 2007). Most of MRSA isolates (60%) were shown to harbor more than one of the investigated virulence factors, whereas only two non-MRSA isolates showed the simultaneous presence of two virulence determinants. These results indicate that MRSA isolates pose more risk to human consumers compared to non-MRSA isolates (Kim et al. 2015).

The *spa* gene encoding for protein A was chosen for typing the isolates in the current study on the basis of the previously reported heterogeneous nature indicating variability among the examined strains (Wichelhaus et al. 2001). The results obtained showed that a single band of 1200 bp was produced by 80% of MRSA isolates, whereas 20% of the strains had no bands (Fig. 1). These results were consistent with Omar et al. (2014), who reported that PCR-RFLP of the *spa* gene produced a single band (type S1) in 84% of MRSA isolates; 5.2% were of type S4 that had no PCR products. Moreover, the authors reported the same banding patterns after restriction and 92% typeability of the method. The absence of the *spa*

gene was also reported in 3.8% (Shakeri et al. 2010) and 5% (Adesida et al. 2006) of *S. aureus* isolates. These observations were supported by the speculation that the *spa* gene is not consistently expressed in all *S. aureus* isolates (Frénay et al. 1994).

The isolation of *S. aureus* with long *spa* bands (1200–1392 bp) has been reported to have a role in adherence to respiratory epithelium (Omar et al. 2014). The current study reports that *spa* bands from MRSA isolates of human and chicken meat origin are long enough (1200 bp) to adhere to respiratory epithelium in case of infection. In accordance, different studies have also reported the amplification of the *spa* gene in *S. aureus* producing long amplicons (Mehndiratta et al. 2009, Shakeri et al. 2010, Soltan Dallal et al. 2010). Two bands of 240 bp and 696 bp were produced by all MRSA isolates after restriction (Fig. 1). The identical restriction banding pattern of MRSA isolates of human and chicken meat origin indicated the genetic relatedness of the isolates. Interestingly, the current results showed that the seven MRSA isolates were from the shops where MRSA-positive vendors were identified. Such findings highlighted the possibility of MRSA transmission between meat handlers and chicken meat. The potential of contaminated meat consumption to produce risk for *S. aureus* infection to consumers was suggested by Ogata et al. (2012), who identified identical genetic fingerprint between *S. aureus* isolates of human and meat. Also, MRSA isolates of chicken origin were closely related to animal and human isolates in Korea (Lee 2003).

Conclusion

Although there was a low isolation rate of MRSA from the chicken products examined, this rate could present a risk to consumers. To minimize the spread of MRSA by contaminated raw foods, handlers should follow appropriate hygienic measures during the handling and preparation of food. Moreover, knowledge about the sources, mode of transmission, and prevention tools is required. To the best of our knowledge, this is the first study to characterize PVL-positive MRSA from chicken products and to use *spa*-RFLP for evaluating the genetic relatedness between MRSA of human and chicken origin in Egypt.

Author Disclosure Statement

No competing financial interests exist.

References

Abdalrahman LS, Stanley A, Wells H, Fakhr MK. Isolation, virulence, and antimicrobial resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin sensitive *Staphylococcus aureus* (MSSA) strains from Oklahoma retail poultry meats. Int J Environ Res Public Health 2015; 12: 6148–6161.

Adesida SA, Likhoshvay Y, Eisner W, Coker AO, et al. Repeats in the 3′ region of the protein A gene is unique in a strain of *Staphylococcus aureus* recovered from wound infections in Lagos, Nigeria. African J Biotech 2006; 5:1858–1863.

Agerso Y, Hasman H, Cavaco LM, Pedersen K, et al. Study of methicillin resistant *Staphylococcus aureus* (MRSA) in Danish pigs at slaughter and in imported retail meat reveals a novel MRSA type in slaughter pigs. Vet Microbiol 2012; 157: 246–250.

Asao T, Kumeda Y, Kawai T, Shibata T, et al. An extensive outbreak of staphylococcal food poisoning due to low-fat milk in Japan: Estimation of enterotoxin A in the incriminated milk and powdered skim milk. Epidemiol Infect 2003; 130:33–40.

Aydin A, Muratoglu K, Sudagidan M, Bostan K, et al. Prevalence and antibiotic resistance of foodborne *Staphylococcus aureus* isolates in Turkey. Foodborne Pathog Dis 2011; 8:63–69.

Balaban N, Rasooly A. Staphylococcal enterotoxins. Int J Food Microbiol 2000; 61:1–10.

Barber DA, Miller GY, McNamara PE. Models of antimicrobial resistance and foodborne illness: Examining assumptions and practical applications. J Food Prot 2003; 66:700–709.

Bhargava K, Wang X, Donabedian S, Zervos M, et al. Methicillin-resistant *Staphylococcus aureus* in retail meat, Detroit, Michigan, USA. Emerg Infect Dis 2011; 17:1135–1137.

Buyukcangaz E, Velasco V, Sherwood JS, Stepan RM, et al. Molecular typing of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) isolated from animals and retail meat in North Dakota, United States. Foodborne Pathog Dis 2013; 10:608–617.

Centers for Disease Control and Prevention (CDC). Report of the FDA retail food program database of foodborne illness risk factors. (Washington, DC: US Food and Drug Administration, 2000.

Chairat S, Gharsa H, Lozano C, Gomez-Sanz E, et al. Characterization of *Staphylococcus aureus* from raw meat samples in Tunisia: Detection of clonal lineage ST398 from the African continent. Foodborne Pathog Dis 2015; 12:686–692.

Chambers HF. Methicillin resistance in staphylococci: Molecular and biochemical basis and clinical implications. Clin Microbiol Rev 1997; 10:781–791.

Cho J, Joo I, Choi J, Jung K, et al. Distribution of Methicillin-resistant *Staphylococcus aureus* (MRSA) in raw meat and fish samples in Korea. Food Sci Biotech 2014; 23:999–1003.

Corrente M, Monno R, Totaro M, Martella V, et al. Characterization of methicillin resistant *Staphylococcus aureus* (MRSA) isolated at the Policlinico Hospital of Bari (Italy). New Microbiol 2005; 28:57–65.

de Boer E, Zwartkruis-Nahuis JT, Wit B, Huijsdens XW, et al. Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. Int J Food Microbiol 2009; 134:52–56.

de Jonge R, Verdier JE, Havelaar AH. Prevalence of meticillin-resistant *Staphylococcus aureus* amongst professional meat handlers in the Netherlands, March–July 2008. Euro Surveill 2010; 15.

El-Jakee J, Marouf SA, Ata NS, Abdel-Rahman EH. Rapid method for detection of *Staphylococcus aureus* enterotoxins in food. Global Veterinaria 2013; 11:335–341.

Enany S, Yaoita E, Yoshida Y, Enany M, et al. Molecular characterization of Panton-Valentine leukocidin-positive community-acquired methicillin-resistant *Staphylococcus aureus* isolates in Egypt. Microbiol Res 2010; 165:152–162.

Egyptian Organization for Standardization and Quality Control (EOSQC). Standard specification no. 3493 for heat treated chicken meat. 2005.

Fessler AT, Kadlec K, Hassel M, Hauschild T, et al. Characterization of methicillin-resistant *Staphylococcus aureus* isolates from food and food products of poultry origin in Germany. Appl Environ Microbiol 2011; 77:7151–7157.

Fessler AT, Turnidge J, Schwarz S. Quality control ranges for cefoperazone 30 μg disks for *Staphylococcus aureus* ATCC® 25923 and *Escherichia coli* ATCC® 25922. Vet Microbiol 2014; 171:284–289.

Frénay HM, Theelen JP, Schouls LM, Vandenbroucke-Grauls CM, et al. Discrimination of epidemic and nonepidemic methicillin-resistant *Staphylococcus aureus* strains on the basis of protein A gene polymorphism. J Clin Microbiol 1994; 32:846–847.

Gao J, Ferreri M, Liu XQ, Chen LB, et al. Development of multiplex polymerase chain reaction assay for rapid detection of *Staphylococcus aureus* and selected antibiotic resistance genes in bovine mastitic milk samples. J Vet Diagn Invest 2011; 23:894–901.

Gillet Y, Issartel B, Vanhems P, Fournet JC, et al. Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. Lancet 2002; 359:753–759.

Hallin M, Deplano A, Denis O, De Mendonça R, et al. Validation of pulsed-field gel electrophoresis and *spa* typing for long-term, nationwide epidemiological surveillance studies of *Staphylococcus aureus* infections. J Clin Microbiol 2007; 45:127–133.

Hanson BM, Dressler AE, Harper AL, Scheibel RP, et al. Prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) on retail meat in Iowa. J Infect Public Health 2011; 4:169–174.

Heikens E, Fleer A, Paauw A, Florijn A, et al. Comparison of genotypic and phenotypic methods for species-level identification of clinical isolates of coagulase-negative staphylococci. J Clin Microbiol 2005; 43:2286–2290.

Hennekinne JA, De Buyser ML, Dragacci S. *Staphylococcus aureus* and its food poisoning toxins: Characterization and outbreak investigation. FEMS Microbiol Rev 2012; 36:815–836.

Huber H, Koller S, Giezendanner N, Stephan R, et al. Prevalence and characteristics of methicillin-resistant *Staphylococcus aureus* in humans in contact with farm animals, in livestock, and in food of animal origin, Switzerland, 2009. Euro Surveill 2010; 15.

ISO-6888-1. Horizontal method for the enumeration of coagulase positive *Staphylococcus* (*S. aureus* and other species). Part 1.Technique using Baird-Parker agar medium. Ed I. 1999.

Jiang C-m, Liu P-h, Ding J-p, Liu X-g, et al. Incidence and pollution of enterotoxigenic *Staphylococcus aureus* in milk, meat and fish in China. Jpn J Food Microbiol 2001; 18:43–47.

Jones TF, Kellum ME, Porter SS, Bell M, et al. An outbreak of community-acquired foodborne illness caused by methicillin-resistant *Staphylococcus aureus*. Emerg Infect Dis 2002; 8:82–84.

Karmi M. Prevalence of methicillin-resistant *Staphylococcus aureus* in poultry meat in Qena, Egypt. Veterinary World 2013; 6:711–715.

Kim YJ, Oh DH, Song BR, Heo EJ, et al. Molecular characterization, antibiotic resistance, and virulence factors of

methicillin-resistant *Staphylococcus aureus* strains isolated from imported and domestic meat in Korea. Foodborne Pathog Dis 2015; 12:390–398.

Kitai S, Shimizu A, Kawano J, Sato E, et al. Prevalence and characterization of *Staphylococcus aureus* and enterotoxigenic *Staphylococcus aureus* in retail raw chicken meat throughout Japan. J Vet Med Sci 2005; 67:269–274.

Kozačinski L, Hadžiosmanović M, Zdolec N. Microbiological quality of poultry meat on the Croatian market. Veterinarski Arhiv 2006; 76:305–313.

Kraushaar B, Fetsch A. First description of PVL-positive methicillin-resistant *Staphylococcus aureus* (MRSA) in wild boar meat. Int J Food Microbiol 2014; 186:68–73.

Kumar JD, Negi YK, Gaur A, Khanna D. Detection of virulence genes in *Staphylococcus aureus* isolated from paper currency. Int J Infect Dis 2009; 13:e450–e455.

Labandeira-Rey M, Couzon F, Boisset S, Brown EL, et al. *Staphylococcus aureus* Panton-Valentine leukocidin causes necrotizing pneumonia. Science 2007; 315:1130–1133.

Le Loir Y, Baron F, Gautier M. *Staphylococcus aureus* and food poisoning. Genet Mol Res 2003; 2:63–76.

Lee JH. Methicillin (Oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. Appl Environ Microbiol 2003; 69: 6489–6494.

Li G, Wu C, Wang X, Meng J. Prevalence and characterization of methicillin susceptible *Staphylococcus aureus* ST398 isolates from retail foods. Int J Food Microbiol 2015; 196: 94–97.

Liassine N, Auckenthaler R, Descombes MC, Bes M, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* isolated in Switzerland contains the Panton-Valentine leukocidin or exfoliative toxin genes. J Clin Microbiol 2004; 42:825–828.

Lina G, Piemont Y, Godail-Gamot F, Bes M, et al. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis 1999; 29:1128–1132.

Loeto D, Matsheka MI, Gashe BA. Enterotoxigenic and antibiotic resistance determination of *Staphylococcus aureus* strains isolated from food handlers in Gaborone, Botswana. J Food Prot 2007; 70:2764–2768.

Lues JF, Rasephei MR, Venter P, Theron MM. Assessing food safety and associated food handling practices in street food vending. Int J Environ Health Res 2006; 16:319–328.

Mehndiratta PL, Bhalla P, Ahmed A, Sharma YD. Molecular typing of methicillin-resistant *Staphylococcus aureus* strains by PCR-RFLP of *spa* gene: a reference laboratory perspective. Indian J Med Microbiol 2009; 27:116–122.

Mehrotra M, Wang G, Johnson WM. Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. J Clin Microbiol 2000; 38:1032–1035.

Mulders MN, Haenen AP, Geenen PL, Vesseur PC, et al. Prevalence of livestock-associated MRSA in broiler flocks and risk factors for slaughterhouse personnel in The Netherlands. Epidemiol Infect 2010; 138:743–755.

National Committee on Clinical Laboratory Standards (NCCLS). Performance standards for antimicrobial susceptibility Testing. Twenty-Third Informational Supplement. CLSI document M100-S23. 2013. (ISBN 1-56238-865-7 [Print]; ISBN 1-56238-866-5 [Electronic]).

Nnachi AU, Emele FE, Ukaegbu CO, Agah MV, et al. Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in raw meat and meat handlers in Onitsha, Nigeria. EJPM 2014; 2:9–15.

Normanno G, Corrente M, La Salandra G, Dambrosio A, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) in foods of animal origin product in Italy. Int J Food Microbiol 2007; 117:219–222.

Ogata K, Narimatsu H, Suzuki M, Higuchi W, et al. Commercially distributed meat as a potential vehicle for community-acquired methicillin-resistant *Staphylococcus aureus*. Appl Environ Microbiol 2012; 78:2797–2802.

Oguttu JW, McCrindle CME, Makita K, Grace D. Investigation of the food value chain of ready-to-eat chicken and the associated risk for staphylococcal food poisoning in Tshwane Metropole, South Africa. Food Control 2014; 45: 87–94.

Okareh OT, Erhahon OO. Microbiological assessment of food and hand-swabs samples of school food vendors in Benin City, Nigeria. Food and Public Health 2015; 5:23–28.

Omar NY, Ali HAS, Harfoush RAH, El Khayat EH. Molecular typing of methicillin resistant *Staphylococcus aureus* clinical isolates on the basis of protein A and coagulase gene polymorphisms. Int J Microbiol 2014; 2014:11.

Osman KM, Amer AM, Badr JM, Saad AS. Prevalence and antimicrobial resistance profile of *Staphylococcus* species in chicken and beef raw meat in Egypt. Foodborne Pathog Dis 2015; 12:406–413.

Pepe O, Blaiotta G, Bucci F, Anastasio M, et al. *Staphylococcus aureus* and staphylococcal enterotoxin A in breaded chicken products: Detection and behavior during the cooking process. Appl Environ Microbiol 2006; 72:7057–7062.

Pesavento G, Ducci B, Comodo N, Nostro AL. Antimicrobial resistance profile of *Staphylococcus aureus* isolated from raw meat: A research for methicillin resistant *Staphylococcus aureus* (MRSA). Food Control 2007; 18:196–200.

Prevost G, Couppie P, Prevost P, Gayet S, et al. Epidemiological data on *Staphylococcus aureus* strains producing synergohymenotropic toxins. J Med Microbiol 1995; 42:237–245.

Pu S, Han F, Ge B. Isolation and characterization of methicillin-resistant *Staphylococcus aureus* strains from Louisiana retail meats. Appl Environ Microbiol 2009; 75:265–267.

Pu S, Wang F, Ge B. Characterization of toxin genes and antimicrobial susceptibility of *Staphylococcus aureus* isolates from Louisiana retail meats. Foodborne Pathog Dis 2011; 8:299–306.

Schelin J, Wallin-Carlquist N, Cohn MT, Lindqvist R, Barker GC, Radstrom P. The formation of *Staphylococcus aureus* enterotoxin in food environments and advances in risk assessment. Virulence 2011; 2: 580–592.

Shakeri F, Shojai A, Golalipour M, Rahimi Alang S, et al. Spa diversity among MRSA and MSSA strains of *Staphylococcus aureus* in north of Iran. Int J Microbiol 2010; 2010:351397.

Soltan Dallal M, Salehipour Z, Eshraghi S, Fallah Mehrabadi J, et al. Occurrence and molecular characterization of *Staphylococcus aureus* strains isolated from meat and dairy products by PCR-RFLP. Annals Microbiol 2010; 60:189–196.

Tassew H, Abdissa A, Beyene G, Gebre-Selassie S. Microbial flora and food borne pathogens on minced meat and their susceptibility to antimicrobial agents. Ethiopian J Health Sci 2010; 20:137–143.

Tiemersma EW, Bronzwaer SL, Lyytikainen O, Degener JE, et al. Methicillin-resistant *Staphylococcus aureus* in Europe, 1999–2002. Emerg Infect Dis 2004; 10:1627–1634.

Wichelhaus TA, Hunfeld KP, Boddinghaus B, Kraiczy P, et al. Rapid molecular typing of methicillin-resistant

Staphylococcus aureus by PCR-RFLP. Infect Control Hosp Epidemiol 2001; 22:294–298.

Witte W, Cuny C, Strommenger B, Braulke C, et al. Emergence of a new community acquired MRSA strain in Germany. Euro Surveill 2004; 9:16–18.

Zhang K, Sparling J, Chow BL, Elsayed S, et al. New quadriplex PCR assay for detection of methicillin and mupirocin resistance and simultaneous discrimination of *Staphylococcus aureus* from coagulase-negative staphylococci. J Clin Microbiol 2004; 42:4947–4955.

Address correspondence to:
Dr. Heba A. Ahmed
Faculty of Veterinary Medicine
Zagazig University
Alzeraa Street
Zagazig 44511
Egypt

E-mail: heba_ahmed@zu.edu.eg