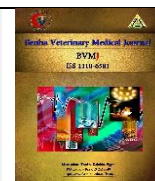




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Prevalence of methicillin-resistant *Staphylococcus aureus* in some ready-to-eat meat products

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ABSTRACT

Although *Staphylococcus aureus* (*S. aureus*) is a bacterium that remains widely studied because of its high pathogenic potential and its ability to develop resistance to antibiotics routinely used in clinical practice; this study investigated the occurrence of methicillin-resistant *Staphylococcus aureus* (MRSA) in some ready to eat (RTE) meat products collected from some public restaurants and street vendors in Benha city, Qalubiya governorate, Egypt; a total of 120 RTE beef products represented by kofta, burger, shawerma, and luncheon (30 of each) were examined for the prevalence of *S. aureus* and molecular detection of MRSA strains represented by the presence of *mecA* gene containing isolates; results revealed that kofta was the most contaminated samples with *S. aureus* where the mean count was 5.2×10^6 CFU/g; followed by burger, shawerma and luncheon samples. Molecular detection of MRSA isolates carrying *mecA* gene revealed that out of eight examined isolates, 2(25%) of examined isolates were MRSA strain. The presence of *S. aureus* especially MRSA strains in high prevalence among examined RTE meat products emphasizes the necessity of enforcing application of strict hygienic measures and GMP during preparation, handling, and serving; in addition, the health authorities have to exert more control over street vendors and fast food restaurants. .

1. INTRODUCTION

Nowadays, ready to eat (RTE) meat products-based sandwiches of shawerma, kofta, etc. are commonly prepared and sold by many restaurants which are widely distributed all over the country (Takeaway). *S. aureus* is one of the most important microorganisms which can contaminate or re-contaminate cooked foods by workers hands, equipment or utensils (Bryan, 1988). This microorganism is associated with nosocomial and community-acquired staphylococcal infections, primarily related to the emergence of drug-resistant organisms (DeLeo and Chambers, 2009). Methicillin-resistant *S. aureus* (MRSA) strains were firstly identified in 1961, immediately after the introduction of methicillin in clinical settings (Barber, 1961). Since then, increased resistance to methicillin among *S. aureus* isolates has been observed globally (Chambers, 1997). Because *S. aureus* is highly prevalent in food and food environments, MRSA may follow the same transmission pattern, and although MRSA infections have not been associated with the consumption of contaminated meats, the pathogen has entered the food chain. Methicillin-resistant *S. aureus* (MRSA) is mainly attributed to the presence of *mecA*

gene, located on one of Staphylococcal cassette chromosomes *mec* (SCC*mec*), that encodes penicillin-binding protein 2a (PBP2a) with a low affinity for essentially all beta-lactam antimicrobials resulting in difficult treatment of infections (Thaker et al., 2013). Methicillin-resistant *S. aureus* (MRSA) is known to be one of the most prevalent nosocomial pathogens throughout the world and is capable of causing a wide range of food poisoning, pneumonia, postoperative wound infections and nosocomial infections (Khosravi et al. (2017). In recent years, methicillin-resistant *S. aureus* (MRSA) has been identified in domestic animals and animal-derived food products worldwide (Hanson et al., 2011). Food products surveyed as meat and its products are widely known to be an important reservoir and main source of MRSA in humans (Contreras et al., 2015).

Therefore, the present study was conducted to investigate the incidence of coagulase-positive *S. aureus* and methicillin-resistant *S. aureus* (MRSA) strains in different popular ready-to-eat meat sandwiches (kofta, burger, shawerma, and luncheon) in Benha city.

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2. MATERIAL AND METHODS

2.1. Collection of samples:

A grand total of 120 samples of RTE meat products represented by “luncheon, burger, shawerma and kofta” (30 of each) were collected from different restaurants and street vendors in Benha city, Qalubiya governorate, Egypt; Samples were transferred to the laboratory under complete aseptic conditions in ice box within one hour and examined for bacteriological and molecular detection of the incidence of *S. aureus* and MRSA strains contamination.

2.2. Bacteriological examination:

- Preparation of sample according to APHA (2013).
- Determination of Staphylococci and *S. aureus* count according to (ISO 6881-1:1999, A1:2003).
- Identification of Staphylococcus aureus.
- Morphological examination (Cruickshank et al., 1975).
- Biochemical identification (MacFaddin, 2000).

2.3. Molecular detection of MRSA:

Two isolates of each confirmed coagulase positive *S. aureus* strains from each examined product were sent to the Central Laboratory for Food Analysis, Faculty of Veterinary Medicine, Benha University, Egypt; and molecularly examined for presence of *S. aureus* carrying *mecA* gene (MRSA) using PCR.

Primer sequences of *S. aureus* used for PCR system following (Jukes et al., 2010) as tabulated in table (1). DNA Extraction using QIA amp kit (Shahet et al., 2009). Amplification of *S. aureus* enterotoxin genes (Jukes et al., 2010).

Table 3 Primer sequences of *S. aureus* used for PCR system.

Target gene	Oligonucleotide sequence (5' → 3')	Product size (bp)	Ref.
<i>mecA</i> (F)	5' TAGAATGACTGAAC GTCCG 3'	533	Jukes et al. (2010)
<i>mecA</i> (R)	5' TTGCATCA ATGTTACCGTAG 3'	533	Jukes et al. (2010)

2.4. Statistical Analysis:

The obtained results were statistically evaluated by application of Analysis of Variance (ANOVA) test according to Feldman et al. (2003).

3. RESULTS

Referring to the results demonstrated in table (2), *S. aureus* could be isolated from 61 (50.8%) samples, represented by 66.6, 43.3, 36.6, 56.6% with mean counts of 5.2x10, 3.2x10, 2.6x10, and 1.9x10CFU/g from kofta, burger, shawerma, and luncheon samples, respectively; statistical analysis of variance indicated a significant difference between kofta and the other samples when $p \leq 0.05$.

Table 2 Statistical analytical results of Staphylococcus aureus in examined ready to eat meat products (n=30)

Product	Positive samples		<i>S. aureus</i> count (CFU/g)		
	No.	%*	Min.	Max.	Mean ± SE
kofta	17	56.6	1.2x10	3.3x10	1.9x10 ^b ± 0.3x10
Burger	13	43.3	1.0x10	8.0x10	3.2x10 ^b ± 0.8x10
Shawerma	11	36.6	1.4x10	5.6x10	2.6x10 ^b ± 0.4x10
Luncheon	20	66.6	3.5x10	6.7x10	5.2x10 ^a ± 0.4x10
Total	61	50.8**			

Values within a column with different superscript letters were significantly different at ($P \leq 0.05$). *Percentage in relation to total number of each sample (30). **Percentage in relation to total number of samples (120).

In vitro antibiotic sensitivity test was conducted on 61 *S. aureus* isolates as demonstrated in table (3); although, isolates showed variable sensitivities against different antibiotics, in general, they showed multi-drug resistance for about 42.8% of tested antibiotics represented by Oxacillin (70.5%), Methicillin (70.5%), Nalidixic acid (60.6%), Amoxicillin (59.0%), Cefotaxime (55.7%), and Ampicillin (50.8%); while were most sensitive to Norfloxacin (95.1%).

Performing of PCR detection of MRSA strains revealed positive detection of *mecA* gene band at 533bp in two isolates out of examined eight isolates (25%) as presented in photo (1).

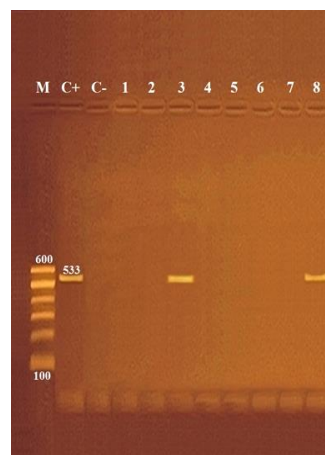


Fig. 1 Agarose gel electrophoresis of multiplex PCR of *sea* (120 bp), *seb* (478 bp), *sec* (257 bp) and *sed* (317 bp) enterotoxin genes for characterization of *S. aureus*. Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive for *sea*, *seb*, *sec* and *sed* genes. Lane C-: Control negative. Lane 2: Positive *S. aureus* strain for *sea*. Lane 3: Positive *S. aureus* strain for *seb* gene. Lane 7: Positive *S. aureus* strain for *sea* and *sed* genes. Lanes 1, 4, 5, 6 & 8: Negative *S. aureus* for enterotoxins

4. DISCUSSION

Since few decades ago, *S. aureus* was reported as incriminated pathogen in 25% of all foodborne illnesses in the United States of America; with continuously misuse of antibiotics and emerging multi-drug resistant bacteria, MRSA strains have been aroused as one of the most feared nosocomial germs that play important role in food poisoning; and however low prevalence of MRSA in food, the thread comes from difficulties of treating of infections due to multi-drug resistance of MRSA (Bean et al. (1997); Sciezynska et al. (2012); Cha et al. (2014)).

Recent results revealed that, luncheon samples (consumed immediately without pre heat-treatment) recorded lower *S. aureus* counts than examined pre-consumption heated treated samples (kofta, burger, and shawerma); it may be referred to under cooking or improper heat treatment, handling, and/or added chemical preservatives to luncheon during processing that play a direct powerful antimicrobial action against *S. aureus*.

Tabulated results of table (1) were somewhat agreed with those reported by Rawash (2015), Laban (2018)

who recorded that the incidence of *S. aureus* in their examined RTE kofta, burger, shawerma, and luncheon

was 60, 46.6, 40, and 60%, respectively.

Table 3 *In Vitro* anti-microbial Sensitivity test for isolated *S. aureus* strains

Antimicrobial agents	Disk concentrations	Sensitive		Intermediate		Resistant		AA
		No.	%	No.	%	No.	%	
Oxacillin	1 µg	3	4.9	15	24.6	43	70.5	R
Methicillin	5 µg	6	9.8	12	19.6	43	70.5	R
Nalidixic acid	30 µg	6	9.8	18	29.5	37	60.6	R
Amoxicillin	25 µg	9	14.7	24	39.3	36	59.0	R
Ampicillin	20 µg	20	32.8	10	16.3	31	50.8	R
Cefotaxime	30 µg	6	9.8	21	34.4	34	55.7	R
Streptomycin	S/10	10	16.4	33	54.1	18	29.5	IS
Trimethoprim/ Sulphamethoxazol	SXT/25 (1.25/23.75) mcg	21	34.4	32	52.4	8	13.1	IS
Neomycin	30 µg	19	31.1	32	52.5	10	16.4	IS
Ciprofloxacin	5 µg	48	78.6	6	9.8	7	11.4	S
Gentamicin	10 µg	47	77.0	10	16.4	4	6.5	S
Lomefloxacin	10 µg	54	88.5	4	6.5	3	4.9	S
Norfloxacin	10 µg	58	95.1	0	0.0	3	4.9	S
Erythromycin	15 µg	24	39.3	35	57.3	2	3.4	IS

No.: Number of isolates. %: Percentage in relation to total number of isolates (61). AA: Antibiogram activity. R: Resistant. S: Sensitive. IS: Intermediate

The obtained results have been lower than those recorded by Abd Allah-Mona (2017), Laban (2018), and Morshdy et al. (2018) who recorded that the mean *S. aureus* counts in examined shawerma, kofta, luncheon, and burger samples was 3.9×10^3 , 6.4×10^3 , 2.5×10^3 , and 1.9×10^3 CFU/g, respectively; while they were higher than those recorded by Abd Allah-Enas (2011), Ali and Abd-El-Aziz (2011), and Heweidy (2016) who detected *S. aureus* in 35, 25, 25, 8.6% of examined kofta, burger, shawerma, and luncheon samples, respectively.

Variations between authors may be attributed to the differences in manufacturing, processing and handling procedures. Presence of *S. aureus* in such RTE foods highlighted preparation, handling, storage or service faults which may come through cross-contamination from raw food, food handlers and the surrounding environment; in addition, spices, equipment, dressings, knives, and other additives are considered as the source of contamination.

Results of antimicrobial sensitivity test summarized in table (2) were somewhat agreed with the results recorded by Bahbah (2019), Hosny (2016) who recorded a multidrug resistance of their *S. aureus* isolated from meat and meat products. Most of *S. aureus* isolates were resistant to all β -lactams antibiotics, which is conferred by the *mecA* gene, which codes for an altered penicillin-binding protein (PBP2a or PBP20) that has a lower affinity for binding β -lactams (penicillins, cephalosporins, and carbapenems). This allows resistance to all β -lactam antibiotics and obviates their clinical use during MRSA infections as mentioned by Chambers (2001).

From the other hand, results of molecular detection of the presence of MRSA in examined RTE samples were in agreement with Laban (2018); Morshdy et al. (2018) who could detect *mecA* gene containing *S. aureus* isolates from examined RTE samples.

5. CONCLUSION

The high prevalence of *S. aureus* among the tested samples, mainly in kofta and luncheon samples, and the presence of the MRSA in prepared foods

highlighted the necessity of enforcing hygienic practices within fast food and street vended foods kitchens. In the future, the molecular and ecological characterization of isolated MRSA strains must be performed to determine the origin of contamination. Better knowledge of strict hygienic practices during collection of raw materials, preparation of food, holding, storage and serving must be educated to food handlers.

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