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Original Research Article

Phenotypic and genotypic characterization of methicillin resistant *Staphylococcus aureus*.

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ABSTRACT

Food contaminated with multiple antibiotic-resistant *S.aureus* can be a major threat to the public health. The purpose of this study was to isolate *S.aureus* from different food sources, determine their antimicrobial susceptibility as well as detection of *mec*A gene among some resistant isolates. Out of 125 samples, 19 *S.aureus* isolates were isolated, and the antimicrobial susceptibility testing showed high resistance against kanamycin, penicillin G, oxacillin, erythromycin and tetracycline were the most resistant antimicrobials agents. All the tested isolates isolates were multiple drug resistant (MDR).Eight out of 19 isolates were phenotypically resistant to oxacillin as well as they were carriers for *mec*A gene.

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Introduction

Antibiotics were extensively used in animals and poultry production in prophylaxis, therapeutic and growth promoter purposes, the correlation between extensive use of antimicrobial agents and development of resistant bacteria is well authenticated for pathogenic bacteria **(Hawkey and Jones, 2009)**. Food of animal origin contaminated with antibiotic-resistant bacteria can be an important threat to public health, the antibiotic resistance determinants can be transferred from resistant bacteria to other bacteria of human public health significance. The prevalence of antimicrobial resistance among food-borne pathogens has elevated during recent decades **(Threlfall *et al.*, 2000** and **Chiu *et al.*, 2002)**.

Molecular analysis of antibiotic-resistant genes has shown that identical elements founded in bacteria that affect both animals and humans, which explain the role of raw foods in the dissemination of resistance genes and resistant bacteria to humans via the food chain (**O'Brien *et al.*, 1982 and Teuber, 2001**).

Many studies in recent years undertaken to assess the antibiotic resistance of bacteria in food of animal origin such as raw milk (**Munsch-Alatossava and Alatossava, 2007)** and meat products (**White *et al.*, 2001**).

These studies reported that a significant proportion of isolates from the food products demonstrated extensive resistance to antibiotics. The resistance genes can be transfer from resistant bacteria to the intestinal flora of humans through food products and the commensally flora can be a reservoir of resistance genes for pathogenic bacteria (**Aarestrup *et al*., 2008**).

Multiple studies have discovered the high prevalence of multidrug-resistant *S. aureus*, including methicillin-resistant *S.aureus* (MRSA) from food of animal origin in Europe, Canada, and United States **(Khanna *et al*., 2008; Smith *et al*., 2010)**, which represents a huge problem in public health **(Morosini *et al* 2006).** Most of methicillin-resistant *S. aureus* (MRSA) isolates carried *mecA* gene which is responsible for methicillin resistance (**El-Jakee *et al*., 2011**)

Therefor the aim of this study was to isolate *S.aureus* from different food sources, determine their antimicrobial susceptibility as well as detection of *mec*A gene among some resistant isolates.

Material and Methods

Samples

One hundred and twenty five samples were randomly collected from milk, meat and their products from Giza and Beni-Suef Governorates markets (Table 1). all samples were aseptically collected in sterile plastic bags separately and transferred immediately under hygienic measures in ice box to the laboratory to be examined for presence of *S.aureus.*

Table (1): Samples collected from sale markets.

Product	Milk	Yoghurt	Kareesh	Minced meat	Burger	luncheon
Numbers	28	18	19	20	20	20

Isolation and identification of *S.aureus.*

One loopfull from prepared incubated samples was plated onto (Difco) mannitol salt agar (Difco), incubated for 18-24 hours at 37°C and examined for bacterial growth. The suspected colonies were identified morphologically and biochemically according to **(Cruickshank *et al*., 1975 and Quinn *et al.*, 2002).**

Antimicrobial sensitivity test for the identified isolates:

S. aureus isolates were subjected to antimicrobial susceptibility testing against 10 antimicrobial agents representing different classes by the disk diffusion method and evaluated according to Clinical and Laboratory Standards Institute **(CLSI, 2013).**

The following antibacterial agents and their concentrations (µg) were used:

Antimicrobial Agent	Code	Disk Concentration
Apramycin	APR	15 µg
Cefazoline	KZ	30µcg
Amoxicillin	AML	10 µg
Erythromycin	E	15µg
Trimethoprim + sulfamethoxazole	SXT	25 µg
Gentamicin	CN	10 µg

Kanamycin	**K**	**30 µg**
Vancomycin	VA	30 µg
Penicillin	P	10units
Oxacillin	OX	1 µg
Tetracycline	TE	30 µg

Phenotypic characterization of methicillin resistant *S. aureus*

Disc diffusion sensitivity testing of *S. aureus* isolates was performed with 1µg oxacillin discs. On Mueller Hinton Agar, according to CLSI recommendation, oxacillin complete inhibition zone diameter of ≤12 mm were considered resistant,

those with inhibition zone of ≥13 mm were susceptible.

Genotypic characterization of methicillin resistant *S. aureus*

S.aureus isolates were inoculated on Triptycase Soya Agar. After incubation period, fresh colonies were suspended in 500 µl sterile saline. DNA was extracted from the suspension using a QIAamp DNA Mini Kit according to the manufacturer's instructions (Qiagen).

Detection of *mec*A gene using PCR

The Polymerase chain reaction was performed for detection of *mec*A gene using primers previously described by **McClure *et al.* (2006)**. Briefly 310bp gene was amplified using primers *mec*A-F (GTA GAA ATG ACT GAA CGT CCG ATA A) and *mec*A-R (CCA ATT CCA CAT TGT TTC GGT CTA A).

Polymerase chain reaction contained 6 µl of DNA template, 12.5 µl Emerald Amp GT PCR master mix (2x premix), 1 µl from each primer (20pmol) and finally 4.5 µl PCR grade water.

The following temperature profile was used for DNA amplification: initial denaturation at 94°C for 5 min followed by 35 cycles of amplification (denaturation at 94°C for 30 sec, annealing at 50°C for 45 sec and extension at 72°C for 45 sec) and final extension at 72°C for 10 min. PCR amplifications were performed using T3 Thermal cycler (Biometra).The PCR products were visualized by 1 × TBE electrophoresis in ethidium-bromide-stained, 1% agarose gel.

Positive control

Confirmed positive sample in RLQP (Reference laboratory for veterinary quality control on poultry production, Dokki, Giza).

RESULTS

Results of recovery rate of *S.aureus* isolates:-

Seventy two Gram positive cocci were recovered out of 125 food samples.Out of them, 19 isolates (15.2%) were catalase and coagulase positive also fermented maltose, trehalose, mannitol and sucrose so; they were characterized biochemically as *S.aureus* (table 2).

Table (2) Prevalence of the isolated *S.aureus* among different food products:

Source of the samples	Total number of samples examined	Recovered *S.aureus* / total No. of original samples	
		Number	Percent
Milk	28	8	28.5
Yogurt	19	1	5.2
Kareish cheese	18	0	0
Total milk and milk products	**65**	**9**	**13.8**
Minced meat	20	5	25

Burger	20	2	10
Luncheon	20	3	15
Total meat and meat products	**60**	**10**	**16.6**
Total collected samples	**125**	**19**	**15.2**

Results of antimicrobial sensitivity:

The results revealed that all *S. aureus* isolates were sensitive (100%) to apramycin, trimethoprim-sulfamethoxazole, gentamicin and cefazolin. On the other hand they were resistant to amoxicillin, kanamycin erythromycin, tetracycline, penicillin G and oxacillin, with a percentage of 31.6%, 42.1%, 36.3%, 57.8% 73.6% and 84.2%, respectively (table 3).

All the tested isolates are multiple drug resistant (MDR).

Table (3): Results of antimicrobial sensitivity test on 19 isolates of *S.aureus* recovered from raw milk, meat and their products.

Antibacterial Agent	Milk and milk products (total No.=9)						Meat and meat products (total No.=10)					
	Sensitive		Inter-mediate		Resistant		Sensitive		Inter-mediate		Resistant	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Penicillin groups												
Penicillin	2	22.2	0	-	7	77.7	3	30	0	-	7	70
Penicillin	2	22.2	0	-	7	77.7	3	30	0	-	7	70
Amoxicillin	3	33.3	2	22.2	4	44.5	7	70	1	10	2	20
Glycopeptides group												
Vancomycin	8	88.8	0	-	1	11	9	90	0	-	1	10
Aminoglycoside group												
Gentamicin	9	100	0	-	0	0	10	100	0	-	0	-
Kanamycin	2	22.2	2	22.2	5	55.5	4	40	3	30	3	30
Apramycin	6	66.6	3	33.3	0	0	9	90	1	10	0	-
Macrolide group												
Erythromycin	6	66.6	1	11	2	22.2	5	50	2	20	3	30
Tetracycline group												
Tetracycline	2	22.2	0	-	7	77.7	3	30	3	30	4	40
Folate pathway inhibitor												
Sulfamethaxazole+trimethoprime	9	100	0	-	0	0	10	100	0	-	0	-
Cephalosporin group												
Cefazolin (first generation)	9	100	0	-	0	0	10	100	0	-	0	-

Phenotypic detection of Methicillin Resistant Staphylococcus aureus among the tested isolates

Out of 19 *S.aureus* isolates 16 isolates were phenotypically resistant to oxacillin disk so they were

characterized phenotypically as MRSA with a percentage of 84.2%.

Genotypic characterization Methicillin Resistant *Staphylococcus aureus* among the tested isolates

Eight oxacillin resistant isolates were selected for investigation using PCR to amplify *mec*A gene. They were all positive (100%) at an amplicon size of 310pb as shown in photo (1) so they were all confirmed as MRSA.

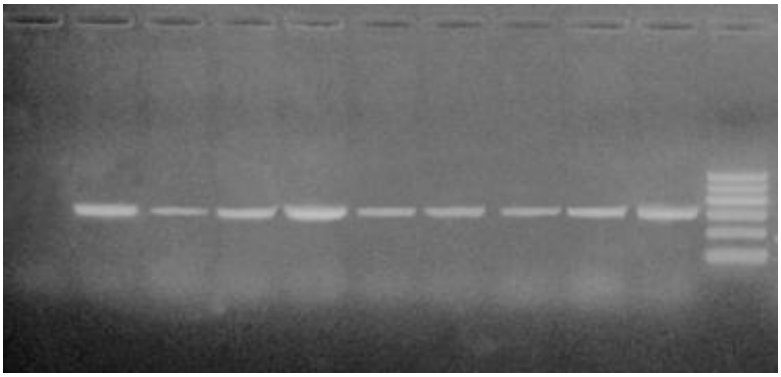

Photo (1): Showed amplification of *mec* A gene at amplicon size 310 pb

Lane 1: showed negative control.

Lane 2-9: positive amplification of *mec*A gene at 310 bp.

Lane M : DNA ladder (100-600 bp).

DISCUSSION

Food of animal origin is an ideal culture medium for growth of many organisms **(Hill, 1996)**. They are liable to harbor different types of microorganisms through processing, handling, distribution and storage as well as preparation. They are considered as serious sources of food borne diseases and have been linked to major outbreaks of food poisoning all over the world **(Hassanien, 2004).**

Result showed in table (2) revealed that *S.aureus* isolated from raw milk, milk products (Kareish cheese and yoghurt) and meat, meat products (burger and luncheon) with a percentage of 13.8% and 16.6% respectively. Nearer percentage was recorded by **(El-Jakee *et al.*, 2008; Song *et al.* 2014)**. Higher results for *S.aureus* contamination in raw milk was previously reported **(Gwida and El Ghary, 2013; El-jakee *et al.*, 2013)**. Occurrence of *S.aureus* in milk is variable in and this may be due to variation in season, geographical location, number of animals on the farm, farm size, hygienic measures, sampling, farm management practices, and differences in detection methods. **(El Sayed *et al.* 2011)** stated that the difference in white soft cheese in Egypt due to acidity as Domiati or Kareish acid coagulation, enzyme coagulation , different salt concentrations, keeping temperatures, ripening in brine solutions are factors affecting the microbiological quality of these varieties .

In the current study the incidence of *S.aureus* in meat products is in agreement with **[Pesavento *et al.* 2007; Abdaslam *et al.* 2014; Hanson *et al.* 2011].**while **[Ali *et al.* 2010]** isolated *S.aureus* from meat sample with a percentage of 7% lower than our results. But **[Li *et al.* 2015**; **Song *et al.* 2014; Hassanin 2007]** found *S.aureus* with a percentage higher than our results

Antibiotic resistant *S. aureus* strains can be transmitted by contaminated foods with resistant bacteria and spread between animals and humans (**Gundogan *et al.*, 2006)**. There is a relationship between the prevalence of antibiotic resistance and the consumption of antibiotic agents. Some researchers reported a primary factor for antibiotic resistant bacteria in food is related to using of antibiotics for therapeutic purposes in animals **(Al-Zu'Bi *et al.*, 2004).**

The studied *Staphylococcus aureus* isolates were sensitive (100%) to apramycin, trimethoprim-sulfamethoxazole, gentamicin and cefazolin. On the other hand they were resistant to amoxicillin, erythromycin, kanamycin, tetracycline, penicillinG, oxacillin with a percentage of 31.6%, 36.3%, 42.1%, 57.8%, 73.6% and 84.2% respectively **(table 3)** as

well as high prevalence of multidrug resistance. **Jamali *et al.* (2015)** stated that *S. aureus* resistant to tetracycline with a percentage of (56.1%) and gentamicin (2.1%) but low incidence in case of penicillin G, erythromycin, streptomycin, kanamycin and oxacillin. The high prevalence of multidrug resistance (MDR) *S. aureus* isolates in our study is agreement with [**Haran *et al*., 2012**; **Albuquerque *et al*, 2007; Tan *et al.* 2014**]. The same results obtained by **Argudín *et al* (2011)** they found *S. aureus* resistant to trimethoprim- sulfamethoxazole (4%) and oxacillin (95%) but differ in erythromycin (70%), tetracycline (100%), gentamicin (14%) and kanamycin (29%). **Gundogan *et al.* 2011** reported that 10.5% of the *S.aureus* isolates were resistant to vancomycin. **[Hanson *et al.* 2011]** reported the same results but with low incidence in oxaciliin resistant in *S.aureus* isolated from meat.

The accurate and rapid detection of antibiotic resistance genes is extremely important in preventing the spread of infections. PCR-based molecular methods are preferred for determination of antibiotic resistance genes. During the last 10 years, many studies have demonstrated the extremely high capacity of molecular methods such as PCR and Pulsed-field gel electrophoresis (PFGE), this methods were increasingly used for their rapid, specific, reliable and accurate detection of bacteria and genes of interest **(Millan Laplana *et al*., 2007)**. Recently, the detection of antibiotic resistance genes was accomplished by PCR methods directed to *mec*A gene [**Al-Zu'Bi *et al*., 2004**].

Methicillin-resistant S. aureus (MRSA) as one of the most common pathogens that cause nosocomial infection worldwide **(Al-Zu'Bi, Bdour, & Shehabi, 2004**). Since the first identification of an MRSA isolate in 1960 in UK **(Donnio, Preney, Gautier-Lerestif, Avril, & Lafforgue, 2004)**. MRSA described the first foodborne outbreak of that caused death of five out of twenty-one patients **(Kluytmans *et al*., 1995)**. Methicillin-resistant Staphylococcus aureus (MRSA) has recently emerged as a health concern and currently causes approximately 94,000 invasive infections yearly in the United States of America, leading to an estimated 18,650 deaths **(Klevens *et al* .2007).**

The present results revealed that all isolates of *S.aureus* which were resistant to oxacillin were carrier for *mec*A gene that agree to that of **Shahraz *et al.* (2012)** and **Jamali *et al.* (2015)** . Lower prevalence of *mec*A gene in food of animal origin was detected by **[Normanno *et al.*, 2007].**

Lee (2003) stated that contaminated foods of animal origin may represent a source of MRSA infection for humans.

It can be concluded that *S. aureus* contaminated milk and meat under study as well as their products in noticeable percentage. Worrisome most of the recovered isolates are MDR and MRSA and they could be transmitted to human being representing public health hazard.

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