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DETECTION OF *MECA* GENE IN METHICILLIN-RESISTANCE *STAPHYLOCOCCUS AUREUS* (MRSA) STRAINS ISOLATED FROM BEEF MEAT USING POLYMERASE CHAIN REACTION

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ABSTRACT

A total of 60 random samples of meat were collected from different butchers shops at Port- Said city and subjected to bacteriological examination. The prevalence of *Staph. aureus* coagulase positive was 10 (16. 6%). The coagulase positive *Staph. aureus* isolates were tested for their susceptibility to methicillin/ Oxaciillin. 6(60%) of the isolates were resistant to methicillin/ Oxaciillin. Multidrug resistance of MRSA strains was observed. The maximum resistance was observed against Ampicillin and Chloramphenicol (100%) while the least resistance was observed against Streptomycin (66.6%) followed by Gentamicin and Trimethoprim + Sulphamethoxazole (33.3%) and Erythromycin (16.6%). The isolates were analyzed for the presence of the genes encoding resistance to methicillin (mecA) by using Polymerase chain reaction (PCR) which is a powerful and a rapid procedure for the detection of *mecA* gene in methicllin-resistance (MRSA) strains. Only 3 out of 6 isolates were found to carry the *mecA* gene amplified at 310 bp. The *Staph. aureus* found in meat directly affect the human health and can cause a public illness if the meat is used in the food industry. Examination of meat using convetional PCR is a useful technique for detection of *mecA* gene in Methicillin-resistance *Staph. aureus* (MRSA).

Keywords*: Methicllin-resistance Staphylococcus aureus (MRSA) strains, meat, mecA gene, PCR.*

INTRODUCTION

Staph. aureus is the most commonly isolated human bacterial pathogen and is an important cause of skin and soft tissue infections, endovascular infections, pneumonia, septic arthritis, endocarditis, osteomyelitis, foreign-body infections, and sepsis (McDougal *et al*., 2003). Methicillin-resistant *Staph. aureus* (MRSA) is considered one of the most common pathogens causing nosocomial infection (Shore and Coleman, 2013). MRSA strains are categorized into two families. The first family is known as the community-acquired MRSA (CA-MRSA) strains and are associated with strains acquired in community settings and are expected to be sensitive for many antibiotics. The second family is the hospital-acquired MRSA strains which originate from hospital infections and are resistant to many antibiotics. Foodstuffs have been considered as sources of MRSA strains, therefore, surveying and surveillance activities for foodstuffs harboring MRSA strains is needed (Yamamoto *et al.,* 2013).

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Meat has an important role in human nutrition as it is desirable foodstuff. It is important sources for protein, fat, essential amino acids, minerals, vitamins and other nutrients (Biesalski, 2005). Meat is rich in nutrients required for microorganisms growth so they are considered an ideal culture medium for growth of many organisms because of the high moisture, high percentage of nitrogenous compounds of various degree of complexity, plentiful supply of minerals, accessory growth factors and some fermentable carbohydrates (glycogen) of a favorable pH for most of enteric microorganisms. It may become contaminated from different sources, these sources may be originated from human handling, manipulation and/or the animal itself or from environmental contamination which includes: Air and water which are the most important sources, dirty floors, tables, equipment and knives (Marritto and Gravani, 2006). During slaughtering processes MRSA can be contaminated on carcasses, contributing to the contamination rates of MRSA on meats in butchers shops, Therefore, meat acts as the vehicle in transmission of MRSA to the butchers and consumers (Boost *et al.,* 2012 and 2013). The surveillance of MRSA in meat was thought to be important. Several studies of MRSA in meat have been documented in various countries (Jones *et al.,*

2002; Kitai *et al.,* 2005; Van Loo *et al.,* 2007 and Weese *et al.,* 2010).

The use of antibiotics and other antimicrobial agents throughout the food chain contributes to the emergence of resistant bacteria that can be passed directly to humans after ingestion (Ruzauskas *et al.,* 2010). Antibiotic resistance bacteria can cause serious disease and is an important public health problem, antibiotic resistance bacteria can be prevented by minimizing unnecessary prescribing and overprescribing of antibiotics, correct use of prescribed antibiotics, good hygiene and infection control (Kumarasamy *et al.,* 2010).

Treatment of infections caused by ***Staph. aureus*** has been further complicated by antimicrobial resistance in the bacteria, particularly methicillin-resistant *Staph. aureus* (MRSA) Naimi *et al.* (2003). Methicillin-resistant *Staph. aureus* (MRSA) isolates are resistant to all available penicillins and other ß-lactam antimicrobial drugs. (Hennekinne *et al.*, 2012). Resistance to methicillin is mediated through the mec operon which is a part of the staphylococcal cassette chromosome mec (SCCmec) (El Karamany *et al.,* 2013). The *mecA* gene codes for an altered penicillin-binding protein, PBP2a, which has a lower affinity for binding β-lactam antibiotics (Mostafa, 2013). Thus, we aimed to investigate the prevalence of coagulase Positive *Staph. aureus* and detection of MRSA strains in meat collected from some Port- Said city butchers shops and detection of their antibiotic susceptibility profiles and presence of antibiotic resistant gene (*mecA*) by conventional PCR.

MATERIALS AND METHODS

1- Sampling: A total of 60 samples of meat were collected from different butchers shops at Port-Said city. Collected samples were double-bagged upon purchase to avoid cross-contamination and, transferred in an ice box and transported to the laboratory as soon as possible to be examined within 24 hours.

2- Bacteriological examination:

2-1 Isolation and Identification of *Staphylococcus aureus*: According to Singh and Prakash (2008) with slight modification. Enrichment was carried out in Peptone Water (PW) enrichment broth (Himedia, India), 10 gram of sample was homogenized with 90 ml sterile enrichment broth peptone water and enriched for 24 hrs at 37 °C. The selective medium used for isolation of *Staph. aureus* was Baird Parker Agar (BPA) (Himedia, India). A loopful of inoculum from enrichment broth was streaked onto BP agar and incubated aerobically for 48 hours at 37°C. Characteristic appearance of jet black colonies surrounded by a white halo was considered to be presumptive *Staph. aureus*. The pure colonies were streaked onto Nutrient agar slop (Himedia, India) and incubated aerobically for 24 hours at 37°C for further identification.

2-2 Morphological characteristics: Smear were prepared from the isolated colonies and stained with Gram's stain. The stained smear revealed Gram positive, spherical cells arranged in irregular clusters resembling to bunch of grapes according to Quinn *et al.* (2002).

2-3 Biochemical examination: The biochemical tests were performed to confirm *Staph. aureus* using Catalase test, Coagulase test, DNase test, Acetoin production, Oxidase test and D-mannitol fermentation according to Thaker *et al.* (2013).

3- Methicillin (oxacillin) susceptibility

Methicillin /Oxacillin sensitivity was determined by an agar screen test performed according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 2004) with Mueller-Hinton agar (Difco) containing 4% NaCl and 2µg of oxacillin (Sigma, St. Louis, Mo.) per ml. The strains were reported as sensitive, or resistant, to Methicillin /Oxacillin with inhibition zone diameter equal or more than 13mm and less than or equal to 10mm respectively. Disk diffusion testing was performed as recommended by the National Committee for Clinical Laboratory Standards NCCCL (2004); briefly, a broth culture suspension of the isolate to be tested was prepared in trypticase soy broth and turbidity adjusted to a 0.5 McFarland standard. The zone sizes were read after 24 hours of incubation in ambient air at 35°C. The MRSA isolates were tested for susceptibility to the following additional antibiotics: Ampicillin (10 µg), Chloramphenicol (30µg), Erythromycin (15µg), Gentamicin (10µg), Streptomycin (10µg), and Trimethoprim / Sulphamethoxazole (1.25+23.75).

4- Molecular detection of *mecA* gene in Methicillin-resistance *Staph. aureus* (MRSA) strains:

4.1- DNA extraction: DNA extraction from the isolated strains was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56^{O}C for 10 min. Then 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

4.2- Oligonucleotide Primer *Staph. aureus*: Primers used were supplied from Metabion (Germany) are listed in table (1).

Table 1: Primer sequence, target gene, amplicon size and cycling conditions of *Staph. aureus* MRSA strain.

Target gene	Primers sequences	Amplified segment (bp)	Primary Denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
mecA F	GTA GAA ATG ACT GAA CGT CCG ATA A	310	94˚C 5 min.	94˚C 45 sec.	50˚C 45 sec.	72˚C 45 sec.	72˚C 10 min.	McClure *et al.*, (2006)
R	CCA ATT CCA CAT TGT TTC GGT CTA A							

4.3- PCR amplification: Primers were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentrations, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an applied biosystem 2720 thermal cycler.

4.4- Analysis of the PCR Products: The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the uniplex PCR products were loaded in each gel slot. A 100 bp DNA ladder and a 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

RESULTS

Table 2: Incidence of coagulase positive *Staph. aureus* in examined meat samples.

sample	No.	Coagulase Positive *Staph. aureus*	
		No.	%
Meat	60	10	16.6%

Table 3: Methicillin (oxacillin) susceptibility of coagulase positive *Staph. aureus* (n=10).

Sensitive		Resistant	
No.	%	No.	%
4	40%	6	60%

Table 4: Susiptability of MRSA to other antimicrobials (n=6).

Antimicrobial	Disc potency	No.	%
Ampicillin (AMP)	(30mg)	6	100%
Chloramphenicol (C)	(30µg)	6	100%
Erythromycin (E)	(15mg)	1	16.6%
Gentamicin (CN)	(10mg)	2	33.3%
Streptomycin (S)	(10µg)	4	66.6%
Trimethoprim + Sulphamethoxazole (SXT)	(1.25 + 23.75)	2	33.3%

Table 5: illustrating the results of PCR for the detection of *mecA* genes.

Staph. aureus strain	1	2	3	4	5	6	Total	%
mecA gene	+	+	+	-	-	-	3/6	50

+: Positive for *mecA* gene.
-: Negative for *mecA* gene

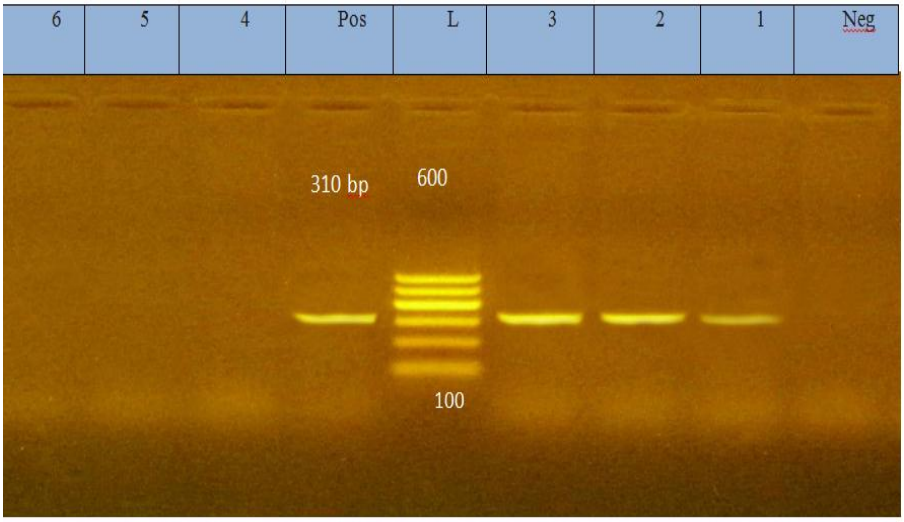

Fig. (1): Showing Agarose gel electrophoresis of PCR products after amplification of: ***mecA*** gene. MWM-molecular weight marker (100 – 600 bp DNA ladder), + control (Positive, Negative) + different strains of ***Staph. aureus*** (*mecA* gene products at 310 bp).

DISCUSSION

Meat is an important vector for the transfer of antibiotic resistances from animals to humans, and antimicrobial resistance has always been a major concern for nosocomial infections in hospital environments. Such transfer can occur in three ways: by means of antibiotic residues in food, through the transfer of resistant food borne pathogens, or through the ingestion of resistant parts of the original food micro flora and resistance transfer to pathogenic microorganisms (Mayrhofer *et al*., 2004).

Staph. aureus is considered to be one of causes of food borne illnesses. Meat is often contaminated with strains of this bacterium, that may occur directly from infected food–producing animals or may result from poor hygiene during transportation, and hygienic level during slaughter also, during evisceration contamination may come from intestinal contents as well as from water during rinsing and washing of carcasses (Marritto and Gravani, 2006). In the present study 10(16.6%) isolates of coagulase positive *Staph. aureus* could be detected from the 60 examined meat samples (Table 2). Nearly to findings were obtained by (Goja *et al.*, 2013), who isolated *Staph. aureus* 7 (12%) from meat, while various studies done by Aseel *et al.* (2010) and Ezzat *et al.* (2014) showed lower prevalence of *Staph. aureus* (5.55%) and (10%), respectively. Another studies showed higher prevalence of *Staph. aureus* was obtained by (Mansour and Basha 2009; Andrew *et al.*, 2011 and Sajith *et al.*, 2012) in a percentage of (20%),(37%) and (40.2%), respectively from meat. Meat can be contaminated at several points throughout the processing operations. Moreover, retail cuts could result in greater microbial load owing to large amount of exposed surface area, more readily available water, nutrient and greater oxygen (Nørrung *et al.*, 2009). The higher incidence of *Staph. aureus* may be due to the unsanitary condition of the butcher, absence of the health services in butcheries shops, unhygienic manner, processing, transportation and storage.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major human pathogen, causing severe morbidity and mortality worldwide both in hospitals and the community. In our study the susiptability of coagulase positive *Staph. aureus* strains isolated from examined meat to Methicillin / Oxaciillin was shown in Table (3), out of 10 coagulase positive *Staph. aureus* strains 6 isolates were found to be resistant (60%). Similar findings were obtained by (Jackson *et al.*, 2013). While higher incidences were observed by (de Boer *et al.*, 2009; Ogata *et al*., 2012; Nnachi *et al.*, 2014 and Food Microbiology and Safety, 2015).

Multi-drug resistance (MDR) refers to a condition enabling pathogenic organisms to resist distinct antibiotics of a wide variety of structure and function targeted at eradicating the organism. Multidrug resistance, defined as intermediate or complete resistance to 3 or more antimicrobial classes. In our study the confirmed MRSA isolates were examined for its multidrug resistance ability to different antimicrobials and the study revealed that 6(100%) of the isolates were resistant to Ampicillin and Chloramphenicol, 4(66.6%) were resistant to Streptomycin, 3(33.3%) were resistant to Gentamicin and trimethoprim- sulphamethoxazole and 1(16.6%) was resistant to Erythromycin (table 4). Frequently, MRSA isolates are also resistant to other antimicrobial classes (Weese, 2010 and Borah *et al.*, 2016). Strains of *Staph. aureus* have been observed to

show resistance against multiple antimicrobials (Kumar *et al.,* 2010 and Khalifa *et al.,* 2014). A comparison of the antibiotic resistance pattern with that reported by Abass, (2014), revealed much similarity, who reported that *Staph. aureus* from meat were resistant to Chloramphenicol, on the other hand they were sensitive to trimethoprim-sulphamethoxazole, erythromycin, while she disagree in susceptibility of strains Streptomycin.

The prevalence of antimicrobial drug resistance among food-borne pathogens is increased due to its use in human therapy and animal farming for therapeutic and prophylactic purposes. Multidrug resistant *Staph. aureus* are frequently isolated from food sources. Such strain are more dangerous and of great food safety concern (Wise, 2007 and Van *et al.,* 2012).

The threat of antibiotic-resistant bacteria has initiated studies on the nature of genes encoding resistance and the mechanism by which these genes spread and evolve. The isolated MRSA strains were examined by conventional PCR for the presence of resistant gene (*mecA*). The PCR assay confirmed the presence of *mecA* gene in 3strains (50%) at 310 bp Table (5) and Figures (1), which is agree with (Riffon *et al.,* 2001; John, 2003; Sajith Khan *et al.,* 2012 and Kamal *et al.,* 2013) who found that PCR assay was rapid and accurate procedure for the detection of MRSA strains as compared to the conventional methods since the reporting time is less and can help efficiently in infection management. In a study performed by Podkowik *et al.* (2012) the authors observed prevalence *mecA* genes conferring resistance to Oxacillin, Penicillin, belonging to twelve staphylococcal isolates obtained from ready-to-eat porcine, bovine, and chicken products.

Conclusion and Recommendations

We can conclude from the obtained results that meat could be a source of resistant bacteria, which may create a health risk for consumers. Some bacteria that are capable of causing serious disease are becoming resistant to most commonly available antibiotics. Moreover, the microbiological safety of food has to be guaranteed in order to prevent the transmission of pathogen or opportunist microorganisms to the consumer. PCR assay was found to be a rapid and accurate procedure for the confirmation identification and determination of the *mecA* genes of *Staph. aureus* by conventional methods which requires a minimum of two-day period, since the time taken for PCR assay is much less a few hours, prompt treatment can be initiated in view of medical and less economic costs. Therefore, The conventional PCR assay can be used as an accurate, safe, and fast technique for the confirmation of *Staph. aureus* and its antibiotic resistance genes in meat samples.

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الكشف عن جين *mecA* فى عترات ميكروب المكور العنقودي الذهبي المقاومة للميثيسيللين (MRSA) المعزولة من اللحوم البقرية باستخدام تفاعل البلمرة المتسلسل

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تمت هذه الدراسة لإلقاء الضوء على تواجد الميكروب المكور العنقودي الذهبي في ٦٠ عينة عشوائية من اللحوم و قد أظهرت نتائج الفحص البكتريولوجي والتعريف البيوكيميائي للعينات المستخدمة ايجابية ١٠عترة (١٦.٦%) من اجمالي العينات كانت ايجابية الميكروب المكور العنقودي الذهبي الايجابي لاختبار التجلط. ولقد تم دراسة حساسية ١٠ عترات من الميكروب المكور العنقودي الذهبي المعزولة من عينات اللحوم وجد منها ٦ عترات مقاومة للميثيسيللين. وتم عمل اختبار الحساسية للعترات المقاومه للميثيسيللين لبعض المضادات الحيوية الأخرى واظهرت النتائج ان العترات أعلي مقاومة للامبيسيللين والكلورامفينيكول بنسبة (١٠٠%) ومقومة أقل للاستربتوميسين (٦٦.٦%) و يليها كل من الجنتاميسين والسلفا ترايميثوبريم بنسبة (٣٣.٣%) والأريثروميسين (١٦.٦%). وللتأكد من وجود (*mecA*) جين في ٦ معزولات المقاومة للميثيسيللين تم اجراء تفاعل البلمرة المتسلسل (PCR) حيث اظهرت النتائج تواجد هذا الجين في (٣) عترات مما يعني ان استخدام اختبار البلمرة المتسلسل اكد فعاليته في الكشف عن جين المسؤل عن مقاومه للميثيسيللين فى العترات المعزوله من الميكروب المكور العنقودي الذهبي.