# ISOLATION AND IDENTIFICATION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) FROM POULTRY MEAT AND POULTRY PRODUCTS.

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## ABSTRACT

*A total of 500 different samples were collected from poultry meat, poultry products and their human contacts in abattoirs from different Egyptian Governorates (Damietta,Dakahlia,Giza and Cairo) in 2012 and 2013. the Samples were tested for presence ofS. aureusby isolation and biochemical identification, the results revealed that coagulase positive S. aureuswas present in83 samples (61.03%).The isolated strains of coagulase positive S.aureus were tested for antimicrobial sensitivity. Most strains were resistant to penicillin and ampicillin, less resistance was appeared to amoxicillin, oxacillin, Methicillin, and ceftriaxone, while all strains were sensitive to, amoxicillin clavulinic acid and vancomycin. Using PCR technique, amplification of 310 bp fragment of mecA gene from the extracted DNA of all isolated coagulase positive S.aureus strains isolated from different chicken samples and human contacts resulted in 5 samples from muscles and skin were positive, Using sequencing technique tomec A gene in three positive strains, the Phylogenetic analysis of mecA gene of these isolates were clustered together and little away from other published isolates of MRSA, Amino acid identities were 99.5% among the analyzed isolates, The three isolates shared 87.2%-*

*89.2% identity with other Staphylococcus aureus isolates.*

## INTRODUCTION

Poultry meat is a common vehicle of food borne illness, *S.aureus* usually being one of the causes of outbreaks involving large number of peoples ***(Georgnaras and Von Holy, 2001)***.Methicillin-resistant *S. aureus* (MRSA) included those strains that had acquired a gene giving them resistance to methicillin and essentially all other beta-lactam antibiotics. Soon after methicillin was introduced into human medicine to treat penicillin resistant staphylococci, this group of organisms had since emerged as a serious concern in human medicine. MRSA was first reported as a nosocomial pathogen in human hospitals. Although these organisms caused the same types of infections as other *S. aureus*, hospital-associated strains have become resistant to most common antibiotics, and treatment can be challenged ***(Fitzgerald et al .,2001)***.The *mecA*gene which confered resistance to methicillin encoded a variant penicillin-binding protein, PBP2a . Native PBP2 catalysed a key step in the synthesis of the bacterial peptidoglycan cell wall, and was bound and inactivated by penicillin-type antibiotics including meticillin. PBP2a was not inhibited by penicillins and could function instead of PBP2 ***(Pinhoet al.,2001)*****.** *S.aureus*had developed resistance to most classes of antimicrobial agents. Penicillin was the first choice of antibiotics to treat staphylococcal infection. In 1944, by destroying the penicillin by penicillinase, *S.aureus*becomes resistant. More than 90% *S.aureus* strains were resistant to penicillin. However, methicillin, semi synthetic penicillin, was used to treat Penicillin Resistant *S. aureus* but resistance finally emerged. MRSA is mediated by the presence of PBP-2a which is expressed by an exogenous gene, *mecA* ***(Livermore, 2001)*****.** In Japan

however, an MRSA strain of human origin isolated from raw chicken samples appeared capable of producing enterotoxin C ***(Kitaiet al., 2005)*.**

## MATERIAL AND METHODS

### 1. Material:

#### 1.1. Samples:

A total of 500 different samples were collected from poultry meat, poultry products and its human contacts of poultry in abattoirs from different Egyptian Governorates (Damietta, Dakahlia, Giza and Cairo) in 2012 and 2013.All samples used were collected under aseptic condition and safety precautions. ***(Rodgerset al., 1999)***

#### 1.2. Preparation of samples:

Muscle and skin samples were collected from chicken's carcasses using sterile scissor and forceps and under aseptic condition.Each sample was collected then marked and placed in an ice box and transferred to the laboratory as soon as possible. And Cloacal swabs were collected from cloacae of living poultry by using sterile cotton swabs contain 2 ml saline.

### 2. Methods:

Isolation and identification of *Staphylococcus aureus*was done according to ***(Sneathet al., 1986)***.

Disk diffusion method applied according to ***(Konemanet al., 1979)*** Amplification of *mecA* gene from DNA of *Staphylococcus aureus* isolates was done according to ***(Spanuet al., 2003)***.

## RESULTS

**Table (1):** Isolation rate of *Staphylococcus aureus* from different samples of poultry and its products

| | | No. of tested samples | positive | | negative | |
|---|---|---|---|---|---|---|
| | | | No. | % | No. | % |
| Muscles & skin | | 100 | 46 | 46 | 54 | 54 |
| Cloacal swabs | | 100 | 32 | 32 | 68 | 68 |
| products | Burger | 23 | 1 | 4.35 | 22 | 95.65 |
| | Pane | 20 | 0 | 0 | 20 | 100 |
| | parts | 57 | 3 | 5.26 | 54 | 94.74 |
| Human samples | | 200 | 54 | 27 | 146 | 73 |
| **total** | | **500** | **136** | **27.20** | **364** | **72.80** |

**Table (2):** percentages of coagulase positive *Staphylococcus aureus* obtained from different samples of poultry and its products

| | | No. of tested samples | positive | | negative | |
|---|---|---|---|---|---|---|
| | | | No. | % | No. | % |
| Muscles & skin | | 46 | 29 | 63.04 | 17 | 36.96 |
| Cloacal swabs | | 32 | 14 | 43.75 | 18 | 56.25 |
| products | Burger | 1 | 1 | 100 | 0 | 0 |
| | Pane | 0 | 0 | 0 | 0 | 0 |
| | parts | 3 | 2 | 66.67 | 1 | 33.33 |
| Human samples | | 54 | 37 | 68.52 | 17 | 31.48 |
| **total** | | **136** | **83** | **61.03** | **53** | **38.97** |

**Table (3):** Interpretation of antimicrobial sensitivity testing for all coagulase positive *S. aureus* isolates.

| Antimicrobial disk | Antibiotic sensitivity of Coagulase positive *S. aureus* | | | | | |
|---|---|---|---|---|---|---|
| | Resistant | | Intermediate | | Sensitive | |
| | No. | % | No. | % | No. | % |
| **P** | 64 | 77.11 | - | - | 19 | 22.89 |
| **MET** | 16 | 19.28 | 8 | 9.64 | 59 | 71.08 |
| **OX** | 25 | 30.12 | 4 | 4.82 | 54 | 65.06 |
| **AMP** | 61 | 73.49 | - | - | 22 | 26.51 |
| **AMOXY** | 23 | 27.71 | 2 | 24.09 | 58 | 69.88 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **AM+CL** | - | - | 3 | 3.61 | 80 | 96.39 |
| **VA** | - | - | - | - | 83 | 100 |
| **KF** | 7 | 8.43 | 13 | 15.66 | 63 | 75.90 |
| **CRO** | 6 | 7.2 | 34 | 40.96 | 43 | 51.81 |

{P (penicillin), MET (methicillin), OX (oxacillin), AMP (ampicillin), AMOXY (amoxicillin), AM+CL (amoxicillin + clavulinic acid), VA (vancomycin), KF (cephalothin), and CRO (ceftriaxone),}

## Amplification of 310 bp fragment of mecA gene from the extracted DNA of all isolated coagulase positive *S.aureus* strains

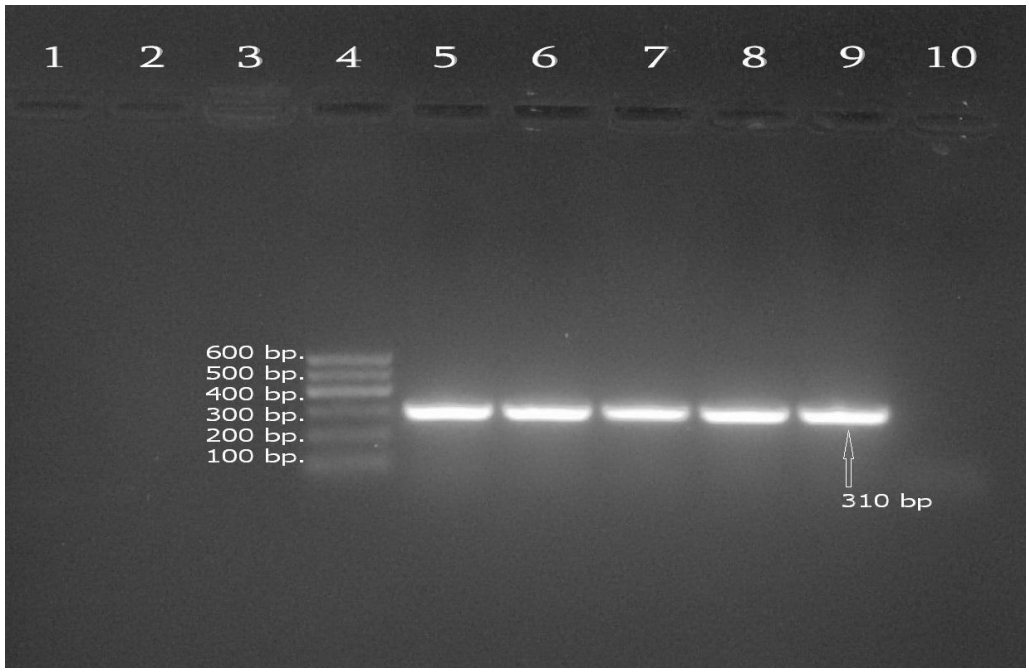


**Photo (1):** showed the agrose gel electrophoresis with positive PCR amplification of 310 bp fragment of mecA gene from DNA of coagulase positive *S. aureus* isolates from different samples. Lanes (5, 6, 7, 8).

**Lanes (1, 2, 3):** Negative samples

**Lane 4:** the DNA molecular weight marker (Gelpilot 100bp ladder).

**Lane 9:** positive control

**Lane 10:** negative control

Purified and sequenced *mecA* gene of three isolates were reveled 87.2%-89.2% identity with other *Staphylococcus aureus* isolates.

Percent Identity

Divergence

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | 99.6 | 99.6 | 99.6 | 99.6 | 99.6 | 99.6 | 99.6 | 99.6 | 99.6 | 99.6 | 99.6 | 99.6 | 99.6 | 99.6 | 99.6 | 98.4 | 99.6 | 99.6 | 99.6 | 89.2 | 88.7 | 88.7 | 1 |
| 2 | 0.4 | | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 98.8 | 100.0 | 100.0 | 100.0 | 89.2 | 88.7 | 88.7 | 2 |
| 3 | 0.4 | 0.0 | | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 98.8 | 100.0 | 100.0 | 100.0 | 89.2 | 88.7 | 88.7 | 3 |
| 4 | 0.4 | 0.0 | 0.0 | | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 98.8 | 100.0 | 100.0 | 100.0 | 89.2 | 88.7 | 88.7 | 4 |
| 5 | 0.4 | 0.0 | 0.0 | 0.0 | | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 98.8 | 100.0 | 100.0 | 100.0 | 89.2 | 88.7 | 88.7 | 5 |
| 6 | 0.4 | 0.0 | 0.0 | 0.0 | 0.0 | | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 98.8 | 100.0 | 100.0 | 100.0 | 89.2 | 88.7 | 88.7 | 6 |
| 7 | 0.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 98.8 | 100.0 | 100.0 | 100.0 | 89.2 | 88.7 | 88.7 | 7 |
| 8 | 0.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 98.8 | 100.0 | 100.0 | 100.0 | 89.2 | 88.7 | 88.7 | 8 |
| 9 | 0.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 98.8 | 100.0 | 100.0 | 100.0 | 89.2 | 88.7 | 88.7 | 9 |
| 10 | 0.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 98.8 | 100.0 | 100.0 | 100.0 | 89.2 | 88.7 | 88.7 | 10 |
| 11 | 0.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 98.8 | 100.0 | 100.0 | 100.0 | 89.2 | 88.7 | 88.7 | 11 |
| 12 | 0.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | 100.0 | 100.0 | 100.0 | 100.0 | 98.8 | 100.0 | 100.0 | 100.0 | 89.2 | 88.7 | 88.7 | 12 |
| 13 | 0.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | 100.0 | 100.0 | 100.0 | 98.8 | 100.0 | 100.0 | 100.0 | 89.2 | 88.7 | 88.7 | 13 |
| 14 | 0.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | 100.0 | 100.0 | 98.8 | 100.0 | 100.0 | 100.0 | 89.2 | 88.7 | 88.7 | 14 |
| 15 | 0.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | 100.0 | 98.8 | 100.0 | 100.0 | 100.0 | 89.2 | 88.7 | 88.7 | 15 |
| 16 | 0.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | 98.8 | 100.0 | 100.0 | 100.0 | 89.2 | 88.7 | 88.7 | 16 |
| 17 | 1.6 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | | 98.8 | 98.8 | 98.8 | 87.7 | 87.2 | 87.2 | 17 |
| 18 | 0.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.2 | | 100.0 | 100.0 | 89.2 | 88.7 | 88.7 | 18 |
| 19 | 0.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.2 | 0.0 | | 100.0 | 89.2 | 88.7 | 88.7 | 19 |
| 20 | 0.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.2 | 0.0 | 0.0 | | 89.2 | 88.7 | 88.7 | 20 |
| 21 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.8 | 0.0 | 0.0 | 0.0 | | 99.5 | 99.5 | 21 |
| 22 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 2.3 | 0.6 | 0.6 | 0.6 | 0.6 | | 99.5 | 22 |
| 23 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 2.4 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | | 23 |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | |

1 Staphaureus-R99-SCCmecIVa-andACM-geneti
2 Staphyaureus-CA-347.
3 Staphaureus-typeIX-staphylococcuscasset
4 Staphaureus M1 COMPLETE
5 Staphaureus-SCC4610-andtypeV-Staphyloco
6 Staphaureus-st MERSA P126
7 Staphaureus-Z172
8 Staphaureus-st-USA300-R114
9 Staphaureus SA40
10 StaphAUREUS SA957
11 Staphaureus-stATCC 4300
12 Staphaureus-Bmb9393
13 Staphaureus-SCCmec-isolate-CMFT535
14 Staphaureus-SCCmec-isolate-CMFT352
15 Staphaureus-SCCmec-isolate-CMFT33
16 Staphaureus-SCCmec-isolate-CMFT3119
17 Staphylococcus sp. X7602B
18 Staphylococcus aureus strAIN X196 MERSA
19 Staphylococcus aureus strain ATCC 43300
20 Staphylococcus SP CNS 45C
21 mersa42-f
22 MERSA-43
23 AHNEDHEGAZY-F

Mersa42-f: sample (1) mersa43: sample (2) Mersa-F: sample (3)

## DISCUSSION

Today, poultry meat industry has become the predominant source of protein from meat in the diet of the population of most developing countries ***(Robert, 1990).*** But during conventional slaughter procedures and further processing, microorganisms are introduced into and onto carcasses ***(Holder et al., 1997).***

During processing, contamination of carcasses with *S. aureus* increased to > $10^3$ CFU /g of skin. , Plucking and evisceration appeared to be the main stages at which contamination of carcasses with *S. aureus* occurred ***(Notermanset al., 1982)***.

In the present work, all *S.aureus* isolates showed yellow colonies surrounded by yellow halo on mannitol salt agar that agree with ***Howard and Kloss, 1993*** who mentioned that the selectivity of MSA is based on its high concentrations of salt. Also after 18 to 24 hrs incubation, Staphylococci appear as opaque, smooth, circular colonies with a butyrous consistency in blood agar.

In this study, Table (1) showed that the total prevalence of coagulase positive *S.aureus* from chickens and human contacts samples were 61.03 % of the samples (83/136), while, 38.97 % were coagulase negative Staphylococci (53/136).

out of 46 muscles and skin samples, 29 samples were coagulase positive with the percentage of 63.04%, that nearly agree with the results of ***Kawano et al. (1996)*** who isolated *S.aureus* strains from the skin of 103 chickens with percentage of 79.2% of the 130 chickens.

Regarding to the current study 32 cloacal swabs dubjected for isolation of *S.aureus*, The overall isolated coagulase positive *S.aureus* was 14 with the percentage of 43.75 %,On the other hands ***Kinsman et al. (1981)*** concluded that the natural populations of*S.aureus* found on the skin and respiratory tract of healthy birds were probably contributing to the source of infection when the clinical disease develops.

Studying of 83 strains of coagulase positive *S.aureus* against 9antimicrobial discs revealed different dgree of sensitivity. those results

coincide with many authors as ***Gardiniet al. (2003)*** who found that *Staphylococci* were generally susceptible to beta -lactams, but 12 strains were resistant to methicillin, 8 were resistant to oxacillin, and 9 were resistant to penicillin G.

***Archer and Niemeyer (1994)*** determined that The *S. aureus* had acquired a gene (*mec*A) coding for the altered penicillin-binding protein 2A, allowing the organism to grow in the presence not only of methicillin but also all new β-lactams. While ***Strommengeret al. (2006)*** confirmed that all isolated *S.aureus* that carrying the *mecA* gene mediated resistance to β-lactam antibiotics.

Few studies were planned for detection of *mecA* among chickens ***(Perez-Roth et al., 2001)***. In present study 5 from 83 samples were containing *mecA* gene which are lower than that recorded by ***Lee (2003)*** who found only three (10%) from chickens (6%).

There are also concerns about MRSA as a possible zoonosis. Both human-to-animal and animal-to-human transmission are known to be possible; however, it has not yet been determined whether animals are an important primary source of MRSA infections for humans, or if most animals are colonized after contact with human carriers ***(Baptisteet al.,2005; Duquette and Nuttall, 2004;Weeseet al., 2006).*** In contrary, some authors conclude that, currently the risk to human health from zoonotic MRSA seems to be very small ***(Duquette and Nuttall, 2004).***

the Phylogenetic analysis of mecA gene of three *Staphylococcus aureus* isolates with other *staph* isolates based on amino acid sequence showed that all isolates under study clustered together and little away from other published isolates of MRSA, Amino acid identities is 99.5% among the analyzed isolates, The three isolates shared 87.2%-89.2%

identity with other *Staphylococcusaureus* isolates. Nucleotide similarity report of 195 base and amino acid similarity report of 66 amino acids of three isolates with other reference staph isolates showed that Sequenced part of the mecA gene showing partial homology to other *Staphylococcus aureus* strains.

## REFERENCES


- ***Archer, G.L. and Niemeyer, D.M. (1994):*** Origin and evolution of DNA associated with resistance to methicillin in *Staphylococci*. Trends.Microbiol., 2: 389–392.

- ***Baptiste, K. E.; Williams, K.; Willams, N. J.; Wattret, A.; and Clegg, P. D.; Dawson, S.; Corkill, J. E.; O'Neill, T. and Hart, C. A. (2005):*** Methicillin-resistant *Staphylococci* in companion animals. Emerg. Infect. Dis., 11(12):1942-1944**.**

- ***Duquette, R. A. and Nuttall, T. J. (2004):*** Methicillin-resistant *Staphylococcus aureus* in dogs and cats: an emerging problem? J. Small Ani.Pract., 45(12): 591-597.

- ***Fitzgerald J.R., Sturdevant D.E., Mackie S.M., Gill S.R. and Musser J.M. (2001):*** Evolutionary genomics of *Staphylococcusaureus*: insights into the origin of methicillin-resistantstrains and the toxic shock syndrome epidemic. ProcNatlAcadSci U S A. ,98(15):8821-6.

- ***Gardini, F.; Tofalo, R. and Suzzi, G. (2003):*** A survey of antibiotic resistance in Micrococcaceae isolated from Italian dry fermented sausages. J. Food Prot., 66(6): 937-945.

- ***Georgnaras, I. and Von Holy, A. (2001):*** Antimicrobial susceptibilities of isolates of *Staphylococcus aureus,* Listeria species and Salmonella serotypes associated with poultry processing. Int. J. Food Microbiol., 70(1/2): 29-35.

- ***Holder, J. S.; Corry, J. E. L. and Hinton, M. H. (1997):*** Microbial status of chicken portions and portioning equipment, British Poultry Sci., 38: 505-511.

- ***Howard, B. J. and Kloss, W. E. (1993):*** *Staphylococci.* Chapter 12. 243-256. In: Clinical and pathogenic microbiology, 2nd edition.

- ***Kawano, J.; Shimizu, A.; Saitoh, Y.; Yagi, M.; Saito, T. and Okamoto, R. (1996):*** Isolation of methicillin resistant coagulase negative *Staphylococci* from chickens. J. Clin. Microbiol., 34(9): 2072-2077.Patho., 62:142-145.

- ***Kinsman, O. S.; White, M. I. and Noble, W. C. (1981):*** Inflammatory reactions to *Staphylococcal* protein A in mice. Br. J. Exp.

- ***Kitai, S.; Shimizu, A.; Kawano, J.; Sato, E.; Nakano, C.; Uji, T. and Kitagawa, H. (2005):*** Characterization of methicillin-resistant *Staphylococcus aureus* isolated from retail raw chicken meat in Japan. J. Vet. Med. Sci., 67(1): 107-110.

- ***Koneman, E. W.; Allen, S. D.; Sowell, V. R. and Sommers, H. M. (1979):*** Color atlas and textbook of diagnostic microbiology. J.B. Lippincott company, Philadelphia. Toronto. J. Infect. Dis., 123: 97-98.

- ***Kreiswirth, B.; Kornblum, J.; Arbeit, R. D.; Eisner, W.; Maslow, J. N.; McGeer, A.; Low, D. E. and Novick, R. P. (1993):*** Evidence of a clonal origin of methicillin resistance in *Staphylococcus aureus*. Sci., 259:227-230.

- ***Lee, J. H. (2003):*** Methicillin (Oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. Appl. Environ. Microbiol., 69(11): 6489-6494.

- ***Livermore D.M. (2001):*** Antibiotic resistance in staphylococci. Int J Antimicrob Agents, 16: 3–10.

- ***Notermans, S.; Dufrenne, J. and van Leeuwen W. J. (1982):*** Contamination of broiler chickens by *Staphylococcus aureus* during processing; incidence and origin. J. APPl.Microbiol., 52 (2): 275-280.

- ***Perez-Roth, E.; Claverie-Martin, F.; Villar, J. and Mendez-Alvarez, S. (2001):*** Multiplex PCR for simultaneous identification of *Staphylococcus aureus* and detection of methicillin and mupirocin resistance. J. Clin. Microbiol., 39(11): 4037-4041.

- ***Pinho M.G., Filipe S.R., de Lencastre H. and Tomasz A. (2001):*** Complementation of the essential peptidoglycan transpeptidase function of penicillin-binding protein 2 (PBP2) by the drug resistance

protein PBP2A in *Staphylococcus aureus*. *J Bacteriol* ,183 (22): 6525-6531.

- ***Robert, E. C. (1990):*** Poultry science in the year 2000. Poultry Sci., 69: 2102-2105.

- ***Rodgers, J. D.; McCullagh, J. J.; McNamee, P. T.; Smyth, J. A. and Ball, H. J. (1999):*** Comparison of *Staphylococcus aureus* recovered from personnel in a poultry hatchery and in broiler parent farms with those isolated from skeletal disease in broilers. Vet. Microbiol., 69: 189-198.

- ***Sneath, P. H. A.; Mair, N. S.; Elisabeth Sharpe, M.; Holt, J.G.; Murray, R. G. E.; Chairman; Brenner, D.J.; Bryant, M.P.; Krieg, N.R.; Moulder, J.W.; Pfennig, N.and Staley, J. T.(1986):*** Section 12 gram-positive cocci, Bergey's Manual of Systemic Bacteriology, Vol. 2, Williams& wikins.USA.

- ***Spanu , T.; Sanguinetti, M.; D'Inzeo,T.; Ciccaglione, D.; Romano, L.; Leone, F.; Mazzella, P. and Fadda, G.(2003):*** Identification of methicillin-resistance isolates of *staphylococcus aureus* and coagulase negative staphylococci responsible for blood stream infections with the phoenix $^{TM}$ system. Diag. Microbiol. Infect. Dis., 48.221-227.

- ***Strommenger, B.; Kehremberg, C.; Kettlitz, C.; Cuny, C.; Verspohl, L.; Witt, W. and Schearz, S. (2006):*** Molecular characterization of Methicillin-resistant *Staphylococcus aureus* strains from pet animals and their relationship to human isolates. J. of antimicrob.Chemother., 57:461-465.

- ***Weese, J. S.; Caldwell, F.; Willey, B. M.; Kreiswirth, B. N.; McGeer, A.; Rousseau, J. and Low, D. E. (2006):*** An outbreak of methicillin-resistant *Staphylococcus aureus* skin infections resulting from horse to human transmission in a veterinary hospital. Vet. Microbiol., 114 (1-2):160-164.

عزل وتصنيف الميكروب المكور العنقودي الذهبي المقاوم للميزيسلن من لحوم ومنتجات الدواجن

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**[**]المعمل المرجعي للرقابة البيطرية على الإنتاج الداجني ، معهد بحوث صحة الحيوان ، دقي ، جيزة ، مصر**

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**[****]معهد بحوث صحة الحيوان ، المنصورة ، الدقهلية ، مصر**

من الفحص البكتيري لعدد 500 عينة لحوم ومنتجات الدواجن وعمال المجازر لها من أجل عزل الميكروب العنقودي الذهبي كانت 83 عينة ايجابية التخثر للميكروب العنقودي الذهبي بنسبة 61.03 % وهذه النسبة الايجابية كانت ممثلة فى55.4% من الدجاج ( 59.2% ، 30.4%، 6.5 % من الجلد والعضلات، مسحات المجمع و منتجات الدواجن بالترتيب) وسلبية بنسبة 44.6 % من العاملين المخالطين للحوم الدواجن في المجازر. وقد تم عمل اختبار الحساسية للعينات المعزولة ايجابية التخثر للميكروب العنقودي الذهبي. ووجد أن معظم المعزولات كانت مقاومة للبنيسيللين و الامبيسيللين وأقل مقاومة كانت ظاهرة للميزيسلن واللاموكساسيللين، اوكساسيللين، ، بينما كل المعزولات كانت حساسة الى الفانكوميسين. على الوجه الاخر اظهرت المعزولات مقاومة متوسطة لكلا من سيفاتركسون، و سيفالوسينواموكساسيللين + كلافيولينك اسيد وباستخدام تفاعل البلمرة المتسلسل تم زيادة جزء من جين mecA وزنه الجزيئى 310 bp بعد استخلاص الحامض النووي من العينات الايجابية التجلط للميكروب العنقودي الذهبي المعزولة من عينات الدجاج المختلفة والعاملين المخالطين لها. نتج عن

تفاعل البلمرة المتسلسل 10.9. % (46/5) عينة ايجابية من عينات الجلد والعضلات من عينات الدجاج.بينما العينات ايجابية التخثر للميكروب العنقودي الذهبي المعزولة من مسحات المجمع ومنتجات الدواجن ومن العاملين المخالطين كانت لا تحتوى على جين mecA. وبعمل تحليل جيني لثلاثة من هذه المعزولات وإسناد النتائج لعترات المكورات العنقودية الذهبية المرجعية الموجودة في بنك الجينات وجد انها تتشابه فيما بينها بنسه 99.5 % ونسبة التماثل علي مستوي الأحماض الامينية بين المعزولات الاخري كانت 87.2 %.