**Bacterological and molecular studies of S.aureus isolated from foods and human contact**

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**Abstract**

A total of 50 samples of human ,foods were collected from different food samples and human contact from different foods shopes . Samples were examined microbiologically for the presence of Staphylococcus aureus was isolated from the samples in a ratio present 8 and identified by biochemical identification. S.aureus strains were tested for antimicrobial sensitivity and all strains showed a 100% resistant to ampicillin. The resistance to oxacillin, amoxicillin, trimethoprim, gentamicin and tetracycline was in a different ratio. However, All the strains were sensitive to levofloxacin and ciprofloxacin.

Using PCR technique, amplification of some virulence gene as(tst,icaD, sea )and antibiotic resistance genes (mecA , blaz , vanA) was performed from the extracted DNA of S.aureus strains .All extracted DNA samples of S.aureus showed a positive results for mecA ,tst , blaz, icaD and sea genes . However, all the samples did not give any PCR product on

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agrasoe gel. Using sequencing technique gene in two 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% which had accession number MF774211 corresponding GenBank sequence.

**Key work** :S.aureus ,mecA,blaZ ,vanA,tst ,icoD and sea genes ,antibiotic sensitive ,sequences mecA gene ,human ,foods

###### Introduction

The mast important bacterial pathogens in human foods that are

responsible for food –human infection include E.coli ,salmonella and

coagulase positive S.aureus (,nassif 2015)

Meat and meat products are the mast palatable of highly material value

foods for human being as source for protein ,fat,mineral,vitamin and

other nutrient (Biesalski 2005)

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

(Geornaras and Von Holy, 2001).

In the twenty first century, the bacterium S. aureus continues to be a

global threat to human and animal health. There is currently no vaccine

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for preventing S.aureus infections and this bacteria have developed

resistance to many if not most antibiotics. Hence, the therapeutic options

are rapidly disappearing. The genetic and physiological flexibility allows

this commensal bacteria to become a powerful pathogen and elucidating

the myriad of mechanisms its employ to avoid the host defense and/or

antimicrobial agents . Theretofore, it presents an important area of

research Greg A.somerville(2016)

S. aureus possesses many virulence factors and the most notable are the

five major classical types of staphylococcal enterotoxins (SEs: SEA to

SEE), the non-classical SE-like toxins (SEl: SEG to SEU), and other

virulence genes such as toxic shock syndrome toxin 1 (TSST-1),

exfoliative toxins and cytolytic toxins (leukocidin and hemolysins).

Staphylococcal enterotoxins (SEs) are heat stable proteins that are mainly

associated with food poisoning outbreaks [. Hennekinne J.A 2012,

Argudin M.A. 2012], while TSST-1 is a superantigenic exotoxin that

causes toxic shock syndrome [. Fueyo J.M. 2005]. The exfoliative toxins

are responsible for staphylococcal scalded skin syndrome that typically

affects infants and young children [. Ladhani S 2003], lukPV cytotoxin

causes leukocytosis with necrotic lesions in the skin or mucosa .[Lina G.1999]

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S.aureus has 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.aureusbecomes 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).

The aim of this work bacteriological and molecular studies of S.aureaus isolated from foods and humane

**2-Material and Methods**

2-1:Sample cullection

A total 50 random samples collection from human foods and human

were examined for bacterialogical samples. Samples were collected from

different shops at Sixth of October City, Aussem, Boulak, Dokki, Giza

,Cairo during the period from 2016 and 2o17. and transferred with

minimum delay to the laboratory for studying its bacteriological

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examination .Each sample was subjected to bacteriological status taken

alone in sterile plastic bags, kept in icebox samples used were collected

under aseptic condition and safety precautions. (Rodgerset al., 1999)

2-2 :Bacterialogical examination .

Pre-enrichid non selective media ( Buffer peptone water) inoculated with the collected samples and then inculated at 37c for 24h inder aerobic condition . Aloopful from incubated nutrient broth was streaked into mannitol salt agar and Barid parker agar and incubated for 24-48h at 37c .the developed colonies were picked up and subculture for purification .the purified colonies were morphological identified by gram stain and biochemical test (Swayne 1998)

2-3: antibotical sensitivity test .

The disk diffusion test technique was applied according as(Konemanet al,1979)Eight types of antibiotic from different groups (oxacillin, ampicillin ,amoxicillin , trimethoprim, levofloxacin , entamicin ,ciprofloxacin ,tetracycline) .The interpretation of inhibition zone of tested culture was according to (Nccls2002)

2-4: Detection of some virulence and resistancegenes of isolates of S.aureus:

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By using QIAamp(R) DNA minimum kitset reaction (catalogue no M50IDIoo )(Sambrook and Russall Davids 1989) It was applied on: random isolates S.aureus for detect virulence genes as (icoD ,tst , sea ) and resistance gene as (mecA ,blaz ,vanA)

2.5:Sequencing and phylogenetic analysis of (Sangel .et.al.1977)

**3-Results**

**Table (1):** Incidence of *Staphylococcus aureus* from different samples of foods and human samples

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **origin** | **Types of samples** | **Total number** **of samples** |  |  |
| **Positive samples**  | **Negative samples** |
| **Foods** | Product meat | 8 | 1 | 7 |
| Chickens | 15 | 0 | 15 |
| Sandwiches(aubergine,bean,sausage) | 3 | 0 | 3 |
| Pasta | 7 | 2 | 5 |
|  Broth | 3 | 0 | 3 |
| Fish | 1 | 0 | 1 |
|  Appetizer | 4 | 1 | 3 |
| **Human**  | Sample from hand | 9 | 4 | 5 |

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**Table (2):** 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.** | **%** |
| **AX** | 7 | 87.5 | - | - | 1 | 12.5 |
| SXT | 4 | 50 | - | - | 4 | 50 |
| **OX** | 5 | 62.5 | - | - | 3 | 37.5 |
| **AMP** | 8 | 100 | -- | - | - |  |
| **LEV** | - | - | - | - | 8 | 100 |
| **GM** | 1 | 12.5 | 2 | 25 | 5 | 62.5 |
| **CIP** | - |  | - | - | 8 | 100 |
| **TE** | 6 | 75 | 1 | 12.5 | 1 | 12.5 |

{ OX (oxacillin), AMP (ampicillin), AX (amoxicillin), SXT (trimethoprim) , LEV (levofloxacin), GM (gentamicin) ,CIP (ciprofloxacin), TE (tetracycline) }

( (8 In relation to total number of isolates of S.aureus ( % )

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**Table (3) Oligonucleotide primers sequences source**

They have specific sequence and amplify a specific product

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Target** | **Gene** | **Primer Sequence** | **Amplified product** | **Reference** |
| Staph.Aureus |  mecA | F GTA GAA ATG ACT GAA CGT CCG ATA A | 310 bp | McClure *et al*., 2006 |
| R CCA ATT CCA CAT TGT TTC GGT CTA A |
| icaD | F AAA CGT AAG AGA GGT GG | 381 bp | Ciftci *et al*., 2009 |
| R GGC AAT ATG ATC AAG ATA |
| *blaZ* | F ACTTCAACACCTGCTGCTTTC | 173 bp |  Duran *et al*., 2012 |
| R TGACCACTTTTATCAGCAACC |
| *Sea* | F GGTTATCAATGTGCGGGTGG | 102 bp | Mehrotra *et al*., 2000 |
| R CGGCACTTTTTTCTCTTCGG |
| *Tst* | F ACCCCTGTTCCCTTATCATC | 326 bp |
| R TTTTCAGTATTTGTAACGCC |
| *vanA* | F CATGACGTATCGGTAAAATC | 885 bp | Patel *et al*., 1997 |
| R ACCGGGCAGRGTATTGAC |

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**Table (4)Amplification of fragment of** resistance genes. **(mecA , blaz , vanA) genes from the extracted DNA of all isolated positive *S.aureus* strains**

|  |  |  |
| --- | --- | --- |
| **Staph.aureus**  | **Sample ID** | **Results** |
| ***mecA*** | ***blaZ*** | ***vanA*** |
| 1 | 38 | + | + | - |
| 2 | 8 | + | + | - |
| 3 | H4 | + | + | - |
| 4 | A5 | + | + | - |

Sample number (1,2) isolated from food and (3,4) isolated from humans

**Table (5) Amplification of fragment of** virulence genes ( **tst ,icaD, sea) genes from the extracted DNA of all isolated positive *S.aureus* strains**

|  |  |  |
| --- | --- | --- |
| **Staph. aureus** | **Sample ID** | **Results** |
| ***Tst*** | ***icaD*** | ***Sea*** |
| 1 | 38 | + | + | + |
| 2 | 8 | + | + | + |
| 3 | H4 | + | + | + |
| 4 | A5 | + | + | + |

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**photo (1):** showed the agrose gel electrophoresis with positive PCR amplification of (**102bp**) fragment of virulance **sea** gene from DNA of positive *S. aureus* isolates from 4 samples (2 food ,2 human)

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**photo (2):** showed the agrose gel electrophoresis with positive PCR amplification of (**381bp**) fragment of virulance **icaD** gene from DNA of positive *S. aureus* isolates from 4 samples (2 food ,2 human)

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**photo (3):** showed the agrose gel electrophoresis with positive PCR amplification of (**326bp**) fragment of virulance **tst** gene from DNA of positive *S. aureus* isolates from 4 samples (2 food ,2 human)

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**photo (4):** showed the agrose gel electrophoresis with positive PCR amplification of (**310bp**) fragment of resistance **mecA** gene from DNA of positive *S. aureus* isolates from 4 samples (2 food ,2 human)

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**photo (5):** showed the agrose gel electrophoresis with negative PCR amplification of (**885 bp**) fragment of resistance **vanA** gene from DNA of positive *S. aureus* isolates from 4 samples (2 food ,2 human)

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**photo (6):** showed the agrose gel electrophoresis with positive PCR amplification of (**173 bp**) fragment of resistance **blaZ** gene from DNA of positive *S. aureus* isolates from 4 samples (2 food ,2 human)

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-Purified and sequenced *mecA* gene of two identity *Staphylococcus aureus* isolated from two strains one of them isolated from food and other isolated from humen **.**The result of sequence of mecA gene of S.aureus .We have provided a GenBank accession number is MF 774211 .The sequence altaned were 99% identical to the corresponding GenBank sequence .

**Analysis required: Sequence for staph.aureus**

**Number and Type of samples: 2 amplified DNA**

**Sample No 1**

**Results and Comments:**

TGGCCGGTTAAAGATATAAACATTCAGGATCGTAAAATAAAAAAAGTATCTAAAAATAAAAAACGAGTAGATGCTCAATATAAAATTAAAACAAACTACGGTAACATTGATCGCAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAGTTAGATTGGGATCATAGCGTCATTATTCCAGGAATGCAGAAAGACCAAAGCATACATATTGAAAATTTAAAATCAGAACGTGGTAAAATTTTAGACCGAAACAATGTGGTATCA.

**Sample is genetically characterized as**

[Staphylococcus aureus subsp. Aureus strain LA-MRSA ST398 isolate E154](https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_1108635204)

Staphylococcus aureus strain NZ15MR0322 genome assembly, chromosome.

Staphylococcus aureus strain ST93 SCCmec-Ivn genomic island , with99%identity

**Sample No3**

TGGCCGGTTAAAGATATAAACATTCAGGATCGTAAAATAAAAAAAGTATCTAAAAATAAAAAACGAGTAGATGCTCAATATAAAATTAAAACAAACTACGGTAACATTGATCGCAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAGTTAGATTGGGATCATAGCGTCATTATTCCAGGAATGCAGAAAGACCAAAGCATACATATTGAAAATTTAAAATCAGAACGTGGTAAAATTTTAGACCGAAACAATGTGGTATCA

**Sample is genetically characterized as**

Staphylococcus aureus subsp. Aureus strain LA-MRSA ST398 isolate E154,

Staphylococcus aureus strain ST93 SCCmec-Ivn genomic island

Staphylococcus aureus strain NZ15MR0322 genome assembly. With99%identity

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**4-Discussion**

Staphylococcus aureus is an important food borne pathogen , a major cause of food poisoning cases and out breaks worldwide **(Wang et** **al.,2012)** 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).

In this study, Table (1) showed that the total prevalence of positive S.aureus from food and human contacts samples were (8/50) of the samples, while, (42/50). were negative Staphylococci

Out of 15 chickens samples, 15 samples were negative with the percentage of 100%, that near agree with the results of (**Diaz-lopez et.al2011**)the 70 samples, 27 were from retail outlets and 43 from street vendors. All specimens were negative by both microbiological and molecular methods for staph.aureas bacteria

Regarding to the current study 9 cloacal swabs dubjected for isolation of

S.aureus, The overall isolated positive S.aureus was 4 with On the

other(**Wang X.et.al.2016)** which isolation and identification of

staphylococcus aureus were performed totally 67 s.aureus strains were

isolated .32 s.aureus strains were isolated from patient samples

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Studying of 8 strains of coagulase positive S.aureus against 8

antimicrobial 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, 8 were

resistant to oxacillin, while( **Aarestrup et al. 2000)** showed

antimicrobial susceptibility to chosen ntimicrobial agents among 118

Staphylococcal isolates in Denmark . High frequencies of S.aureus

(47%) were resistant to tetracycline ,30% were resistant to

ciprofloxacin

**Abd El-Salam (2014)** reported that all S.aureus isolates tested were susceptible to ciprofloxacin which could be a good choice for treatment . 100% of S.aureus isolates were resistant to methicillin and,more than half of isolates resistant to amoxicillin while the isolates showed a variable presentage of resistanes to trimethoprim and gentamicin.

**(Archer and Niemeyer .1994**) determined that The S. aureus had

acquired a gene (mecA) 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( **Strommenger.et al. 2006)** confirmed

that all isolated S.aureus that carrying the mecA gene mediated resistance

to β-lactam antibiotics.

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when used PCR technique of positive s.aureus strains to detection

of some genes the result was positive for resistance genes

(mecA,blaZ) and negative for (vanA) gene this result agree with

(**Anna c.shore.et.al.2011)** and Few studies were planned for

detection of mecA among chickens (**Perez-Roth et al., 2001**). In

present study 4 from 4 samples were containing mecA gene which

are more than that recorded by( **Lee .et.al 2003)** who found only

three (10%) from chickens (6%).

And positive for virulence genes (tst,icaD,sea) in 4 positive

s.aureus isolated which it agree with (**Klotz.M.et.al.2003)**who

detection of sea gene as well as the mecA gene ecoding methicillin

resistance and (**Manfredi.EA.et.al.2010**) who detection sea gene

from the food while (**piechowicz L.et.al 2008**)detect( tst )gene were

in most of the strains and (**Mottola C.et.al.2016**) which of (icaD)gene in more strain and one strain positive for (tst) gene

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).

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Amino acids of two isolates with other reference staph isolates

showed that Sequenced part of the mecA gene showing partial

homology to other Staphylococcus aureus strains in 99% .this reselt

is identical to the results obtained (Salwa M. Helmy .el.al2015)

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