Introduction

*Staphylococcus spp.* are significant bacteria in the etiology of avian diseases and may contaminate foods as a result of processed carcasses (Pepe et al., 2006). Although enterotoxins producing *S. aureus* is the most common cause of food-borne human illness throughout the world (Do Carmo et al., 2004), the other species such as *S. hyicus, S. sciuri, S. xylosus* or *S. cohnii* are also important, particularly because of carriage the genes encoding antimicrobial resistance (Aarestrup et al., 2000). *Staphylococcus* are one of the most predominant groups during the slaughtering and processing of poultry, and they have been recovered from air samples (Ellerbroek, 1997), neck skin of chicken carcasses (Olivier et al., 1996), and machinery surfaces (Huys et al., 2005). By this reason contaminated poultry products could be the source of possible transmission of different staphylococci species including resistant strains to humans, during food processing at home. Staphylococcal food poisoning is caused by ingestion of enterotoxins performed in the food contaminated essentially through human manipulation or raw material obtained from animals. Although coagulase-positive *Staphylococcus aureus* is the main agent responsible for food intoxication, some researches emphasise that coagulase-negative *Staphylococcus* (CNS) are able to produce staphylococcal enterotoxins and may be a potential cause of food poisoning (Da cunha et al., 2006). About 80%-90% of CNS isolates associated with hospital infections are methicillin-resistant coagulase negative *Staphylococcus* (MRCNS). So the aim of these study was to detect the occurrence of *Staphylococcus spp.* in chicken production cycle (Table eggs, unhatched eggs, baby chicks, broilers and chickens meat), As well as detection of mecA gene and entrotoxins production genes.

Materials and methods

**Sample Collection**

A total of 150 samples were collected from chickens and chicken byproducts from different farms and markets in Luxor city.
(30 samples were collected from each type (unhatched eggs, baby chicks, broilers, chicken meat and table eggs).

**Isolation of Staphylococci was done according to Sneath et al. (1986)**

The collected samples were inoculated in BPW (Difco), cultured onto Mannitol Salt agar (Difco) then incubated for 24-48 hours at 37°C. The resulted colonies were examined for identifying morphological characteristic appearance of Staphylococcus species.

**Identification and characterization of coagulase positive and negative Staphylococcus Species**

The isolates were identified according to MacFadin (2000) by using conventional techniques such as: catalase test, oxidase test, growth at 10% NaCl, Mannitol fermentation, coagulase test as well as using PCR by detection of 16rRNA gene specific for genus staphylococcus and clfA gene specific for S. aureus. (Mason et al., 2001).

**Serotyping of Staphylococcus isolates**

Coagulase negative staphylococci were selected and serotyped using INTEGRAL SYSTEM STAFILOCOCCHI kit based on biochemical tests (NCCLS, 2004).

**Detection of enterotoxins and mecA by Polymerase chain reaction (PCR)**

**Extraction**

All coagulase positive staphylococci CPS and CNS isolates were extracted according to QIAamp DNA mini kit (instructions (Qiagen, Germany)).

**Preparation of Master Mix**

According to Emerald Amp GT PCR master mix (Takara) Code No. RR310A kit, the primers used have specific sequence and amplify specific products as shown in Table 1.

**Cycling conditions of the primers during cPCR**

Temperature and time conditions of the primers during PCR are shown in Table 2.

**Results**

A total of 50 (33.3%) isolates were identified as Staphylo-

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Primer sequence (5'-3')</th>
<th>Length of amplified product</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sec</td>
<td>GSEA-F</td>
<td>GOTTATCAATGTGCCGCTGG</td>
<td>102 bp</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GSEA-R</td>
<td>CCAGCACTCTTCCCTTGCG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sed</td>
<td>GSEBF-1</td>
<td>GTATGGGTGTAGTAACTGAGC</td>
<td>164 bp</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GSEBR-2</td>
<td>CCAATACGGAGAAGATTGGAGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sec</td>
<td>GSEC-F</td>
<td>AGATGAAGTGGATGATGATGAGA</td>
<td>451 bp</td>
<td>Mehrotra et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>GSEC-R</td>
<td>CAGACTCTTGAATTCAACCG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sed</td>
<td>GSEDF-1</td>
<td>CCAATAAAGGAGAAATAAAG</td>
<td>278 bp</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GSEDR-2</td>
<td>ATIGGTAATTTCCTGACA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sec</td>
<td>GSEEF-1</td>
<td>AGGGTTTTTCAGCGATATGC</td>
<td>209 bp</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GSEER-2</td>
<td>CTTTTTTTCTTGCGTCAATCT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mecA</td>
<td>MecA-F</td>
<td>GTAGAAATGACTCAGCTCGATTA</td>
<td>310 bp</td>
<td>McClure et al. (2006)</td>
</tr>
<tr>
<td></td>
<td>MecA-R</td>
<td>CCATTCCATGGTGTAGCTGTA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16S rRNA</td>
<td>16S rRNA-F</td>
<td>CTTACCAACCTGGGATGCTTTCTCGGG</td>
<td>791 bp</td>
<td>Mason et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>16S rRNA-R</td>
<td>CTTAGGCTTTCAACCTTCCGCTGCG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>clfA</td>
<td>clfA-F</td>
<td>GCAGAAATGCAGCAACAGGAACAAC</td>
<td>638 bp</td>
<td></td>
</tr>
<tr>
<td></td>
<td>clfA-R</td>
<td>CTTGATCTTCCAGCCATATAATGGG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primary denaturation</th>
<th>Secondary denaturation</th>
<th>Annealing</th>
<th>Extension</th>
<th>No. of cycles</th>
<th>Final extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>mecA</td>
<td>94°C 5 min.</td>
<td>94°C 30 sec.</td>
<td>50°C 30 sec.</td>
<td>72°C 45 sec.</td>
<td>35</td>
<td>72°C 7 min.</td>
</tr>
<tr>
<td>Enterotoxins genes</td>
<td>94°C 5 min.</td>
<td>94°C 30 sec.</td>
<td>50°C 45 sec.</td>
<td>72°C 45 sec.</td>
<td>35</td>
<td>72°C 10 min.</td>
</tr>
<tr>
<td>16S rRNA and clfA</td>
<td>94°C 5 min.</td>
<td>94°C 30 sec.</td>
<td>55°C 45 sec.</td>
<td>72°C 45 sec.</td>
<td>35</td>
<td>72°C 10 min.</td>
</tr>
</tbody>
</table>
coccus species, 14% of the samples were coagulase positive S. aureus (21/150), while, 19.33% were CNS (29/150). Coagu-
lase positive S. aureus revealed from 16.7% (5/30) of table
eggs, 20% (6/30) of unhatched eggs, 13.3(4/30) of baby chicks
and 10% (3/30) of broilers and chicken meat, while CNS re-
vealed from from10% (3/30) of table eggs, 26.7% (8/30) of un-
hatched eggs, 13.3% (4/30) of baby chicks, 33.3% (10/30) of
broilers and 13.3% (4/30) of chicken meat as shown in Table 3
and Fig. 1.

The INTEGRAL SYSTEM STAFILOCOCCI kit was used for
identification of CNS isolates. The results were as follow, out
of 29 CNS isolates, 10 isolates were S. xylosus (34.49%), 5 S.
warneri (17.25%), 3 isolates of each of S. epidermidis, S. sapro-
phyticus, S. simulans and S. hominis (10.34%) and 2 isolate of
S. capitis (6.9%).

mecA gene was detected as 66.7% and 51.7% among S.
aureus and CNS respectively and it was found in CNS isolate
as follow S. xylosus, S. warneri, S. epidermidis and S. capitis
with the percentages 50%, 60%, 33.3%, 100% respectively
and S. simulans and S. hominis with 66.7%.

Enterotoxins were found in 5 (23.8%) of S. aureus as fol-
lowing: seb and sec (9.5%) and see (4.8%) and found in 6
(20.6%) of CNS as following: sec and see (10.3%). sec was de-
tected in 2 isolates of S. xylosus and 1 isolate of S. simulans.
see was detected in S. xylosus, S. warneri, S. simulans as shown
in Table 4 and Figs 2, 3.

Table 3. Occurrence of S. aureus and coagulase negative staphylococci from examined samples

<table>
<thead>
<tr>
<th>Source of samples</th>
<th>No. of examined samples</th>
<th>Coagulase positive S. aureus</th>
<th>CNS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Table eggs</td>
<td>30</td>
<td>5</td>
<td>16.7</td>
<td>3</td>
</tr>
<tr>
<td>Unhatched eggs</td>
<td>30</td>
<td>6</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>Baby chicks</td>
<td>30</td>
<td>4</td>
<td>13.3</td>
<td>4</td>
</tr>
<tr>
<td>Broilers</td>
<td>30</td>
<td>3</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Chickens meat</td>
<td>30</td>
<td>3</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>21</td>
<td>14</td>
<td>29</td>
</tr>
</tbody>
</table>

Table 4. Occurrence of enterotoxin genes and mecA gene among the staphylococcus isolates.

<table>
<thead>
<tr>
<th>Source of samples</th>
<th>No. of examined samples</th>
<th>seb</th>
<th>%</th>
<th>sec</th>
<th>%</th>
<th>see</th>
<th>%</th>
<th>mecA</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPS (S. aureus)</td>
<td>21</td>
<td>2</td>
<td>9.5</td>
<td>2</td>
<td>9.5</td>
<td>1</td>
<td>4.8</td>
<td>14</td>
<td>66.7</td>
</tr>
<tr>
<td>S. xylosus</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>20</td>
<td>1</td>
<td>11.1</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>S. warneri</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>20</td>
<td>3</td>
<td>60</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>33.3</td>
</tr>
<tr>
<td>S. saprophyticus</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. simulans</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>33.3</td>
<td>1</td>
<td>33.3</td>
<td>2</td>
<td>66.7</td>
</tr>
<tr>
<td>S. hominis</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>66.7</td>
</tr>
<tr>
<td>S. capitis</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>2</td>
<td>4</td>
<td>5</td>
<td>10</td>
<td>4</td>
<td>8</td>
<td>29</td>
<td>58</td>
</tr>
</tbody>
</table>
In the environment (Addis et al. and mucosa of humans and animals while some are free living. CNS (60%) was higher than that of CPS (40%). The high percentage of CNS was high (58%) compared to CPS (42%) and Pranab (2016), the latter found that the percentage of CNS was high (58%) compared to CPS (42%) and disagreed with Al-khalaf et al. (2013); Yurdakul (2013) who isolated S. aureus from newly hatched at a prevalence of 14.7% and 15% respectively and disagreed with Al-khalaf et al. (2013). Also, Tugba (2011), who found CNS in 25.2% chicken. and disagreed with Youssef and Hamed (2012), who observed that 67.5% of CNS isolates were Methicillin-resistant, and 10% for S. aureus and 10% for S. hyicus and S. xylosus were isolated from whites, yolks and shells of eggs.

In the present study, staphylococci were isolated from 30 unhatched eggs (18 and 21 day) dead and live embryo in 14(46.67%). S. aureus were 6 (20%) and coagulase negative staphylococcus isolates were 8 (26.7%). (37.5%) S. xylosus, 12.5% S. hominis, 12.5% S. simulans, 12.5% S. saprophyticus and 25% S. warneri but (Babaca, 2014) isolated staphylococcus species from dead-in-shell chicken in 21.6%.

S. aureus contamination is very important cause of arthritis in chicks and early chick mortalities (Abd El-Latif, 1995). Examination of baby chicks revealed that 8 (26.6%) staphylococcus species were isolated from 30 baby chicks (1-7days). 4 (13.3%) from each S. aureus and CNS. Coagulase negative staphylococcus isolates were 2 (50%) S. xylosus. 1 (25%) S. saprophyticus and 1 (25%) S. epidermidis. and this result agreed with Abd El-Gali et al. (1984) and Azmy (1996) who isolated S. aureus from newly hatched at a prevalence of 14.7% and 15% respectively and disagreed with Al-khalaf et al. (2013), who isolated S. echinatus and 4 (13.3%) from each S. aureus and CNS. CNS can contaminate foods because of the major problems in broiler chickens as reported by Skeeles (1997).

A total 50 staphylococcal isolates were isolated from 150 samples of chicken production cycle in present study. 21(14%) isolates were identified as coagulase positive staphylococcus aureus and 29 (19.33%) isolates were identified as CNS by using staphylococcus specific genus primer (165 rRNA) and S. aureus primer (clfA) (Mason et al., 2001). It is clear that percentage of CNS was high (58%) compared to CPS (42%) and this agreed with Goja et al. (2013); Yurdakul et al. (2013); Piyali and Pranab (2016), the latter found that the percentage of CNS (60%) was higher than that of CPS (40%). The high number of CNS isolated in this study could be justified by the fact that CNS are found abundantly in the normal teat skin flora and mucosa of humans and animals while some are free living in the environment (Addis et al., 2011).

This study, CNS isolates were Staphylococcus xylosus (34.49%), Staphylococcus warneri (17.25%), Staphylococcus epidermidis (10.34%), Staphylococcus saprophyticus (10.34%), Staphylococcus simulans (10.34%), Staphylococcus hominis (10.34%), Staphylococcus capsici (6.9%) that had been identified by INTEGRAL SYSTEM STAFILOCOCCI KIT (NCCLS 2004) and biochemically according to MacFadden (2000). This similar to Da cunha et al. (2006), who detected CNS strains from food samples, S. epidermidis (40%), S. xylosus (20%), S. warneri (20%), S. saccharolyticus (15%), and S. hominis (5%).

Results revealed that staphylococci were detected in 26.7% of table eggs; 16.7% for Staphylococcus aureus and 10% for CNS (6.66%) S. saprophyticus and 3.34% S. epidermidis of CNS isolates and this agreed with Stepien et al. (2009) who found both coagulase-positive strains (S. aureus and S. hyicus) and coagulase-negative strains, particularly S. lentus, S. warneri, S. epidermidis and S. xylosus were isolated from whites, yolks and shells of eggs.

In the present study, staphylococci were isolated from 30 unhatched eggs (18 and 21 day) dead and live embryo in 14(46.67%). S. aureus were 6 (20%) and coagulase negative staphylococcus isolates were 8 (26.7%). (37.5%) S. xylosus, 12.5% S. hominis, 12.5% S. simulans, 12.5% S. saprophyticus and 25% S. warneri but (Babaca, 2014) isolated staphylococcus species from dead-in-shell chicken in 21.6%.

S. aureus contamination is very important cause of arthritis in chicks and early chick mortalities (Abd El-Latif, 1995). Examination of baby chicks revealed that 8 (26.6%) staphylococcus species were isolated from 30 baby chicks (1-7days). 4 (13.3%) from each S. aureus and CNS. Coagulase negative staphylococcus isolates were 2 (50%) S. xylosus. 1 (25%) S. saprophyticus and 1 (25%) S. epidermidis, and this result agreed with Abd El-Gali et al. (1984) and Azmy (1996) who isolated S. aureus from newly hatched at a prevalence of 14.7% and 15% respectively and disagreed with Al-khalaf et al. (2013), who isolated S. echinatus and 4 (13.3%) from each S. aureus and CNS. CNS can contaminate foods because of the major problems in broiler chickens as reported by Skeeles (1997). In these study staphylococcus species were detected in 13 out of 30 (43.3%) from broiler, 3 (10%) were S. aureus and 10 (33.3%) strains were CNS (S. xylosus (5), S. simulans (2), S. capitis (2), S. warneri (1) and this agreed with Sobhy et al. (2014), who detected S. capitis, S. simulans, S. sciuri, S. haemolyticus, S. xylosus and S. saprophyticus from broilers chicken and agreed with Youssef and Hamed (2012), who isolated S. aureus (11.7%) from apparently healthy broilers in Ismailia governorate. On the other hand, they were inconsistent with the results of Rasheed (2011) that isolated S.aureus at the percentage of 50.98% from different broiler chickens farm, this may be due to the samples obtained from healthy and diseased bird.

The result of this study revealed that staphylococcus species found in 7 (23.3%) from 30 raw chicken samples (breast, neck and thigh). 2 (6.67%) strains were S. aureus and 4 (13.3%) were CNS (S. hominis (50%) and S. warneri (50%), the result agreed with Mohammad et al. (2014) that isolated S. aureus from15.7% raw chicken meat. also agreed with Sumru and Tugba (2011), who found CNS in 25.2% chicken. and disagreed with Yurdakul et al. (2013) who isolated 22 coagulase negative staphylococci from 50 chicken meat samples and this may be due to difference of the sample collection site.

It was observed that 66.7% (14/21) and 51.7% (15/29) of S. aureus and coagulase negative staphylococcus isolates (CNS) were positive for detection of mecA gene. The results were agreed with Feber et al. (2012), who isolated MRSA (Methicillin-resistant Staphylococcus aureus) from (50.0%) of staphylococcus isolates, Helen et al. (2011) detected MR-CNS in 48.3% of examined samples and Koksal et al. (2009), who observed that 67.5% of CNS isolates were Methicillin-resistant, while the result disagreed with Akbar and Anai (2013), who detected mecA gene in 18.18% and EL-Shareek and Ali (2012) that found the gene in 29.6%.

Staphylococcal enterotoxins (SE) constitute a family of biologically and structurally related toxins and the ingestion of these toxins results in gastrointestinal effects such as nausea, vomiting, diarrhea and abdominal pain. The SEs are the main cause of many outbreaks of food borne diseases (Lamaia et al, 2005).

Although enterotoxins are produced mainly by coagulase positive staphylococci, some coagulase-negative staphylococci (CNS), involved in a variety of human and animal infections (Kloos et al., 1995), CNS can contaminate foods because of...
humans are common carriers of these microorganisms and some may be related to specific human infections (Bergdoll, 1995). The data illustrated that 5/21 S. aureus isolates (23.8%) and 6/29 CNS strains (20.7%) showed positive results for presence of enterotoxin genes. Three classical enterotoxin genes (seb, sec and see) were detected in the present work. This was agreed with Kitai et al. (2005) (21.7%); Naffa et al. (2006) (23%) ; Holmberg and Blake (1984), (26.5%) of S. aureus isolates produced SE and Çepoğlu et al. (2010), who found that 20% of 20 CPS was produced staphylococcal enterotoxin.

In this study seb enterotoxin was produced by 9.5% of S. aureus and was not produced by CNS. The result agreed with Polledo et al. (1985), who found that the distribution of enterotoxins seb were 8 (9.6%) of CPS strains and disagreed with Kitai et al. (2005), who detected seb in 64.1% of S. aureus and Rasoul et al. (2015) in 4.1%. Staphylococcal enterotoxin B (SEB) is the toxin most commonly associated with classic food poisoning. CDC (2014) reported that SEB has been studied as a potential biological warfare agent because it can easily be aerosolized; it is very stable; and can cause widespread systemic damage, multi-organ system failure, and even shock and death when inhaled at very high dosages. However, SEB is classified as an incapacitating agent because in most cases aerosol exposure does not result in death but in a temporary, though profoundly incapacitating, illness lasting as long as 2 weeks (Ulrich et al., 1997).

Studies have shown that sec is the most thermostable enterotoxin, followed by seb and sea (Notermans et al., 1988), while sec enterotoxin was found in this study by 9.5% and 10.3% of S. aureus and CNS respectively. This agreed with Polledo et al. (1985), who found sec in 8.4% from CPS strains and Kitai et al. (2005), who found sec in 10.3% of S. aureus and disagree with Enas et al. (2016) that detected sec gene in 23% of S. aureus isolates.

Staphylococcal enterotoxin poisoning outbreak where see has been confirmed as the causative agent (Ostyn et al., 2010). In this study see enterotoxin was detected in 4.8% and 10.3% from S. aureus and CNS respectively, this was agreed with Holmberg and Blake (1984), who detected see in 4.3% of S. aureus. However, Asadollahi et al. (2014) who reported a high level of see gene (31%). Other studies reported low level of see gene distribution (2.4%) (Polledo et al., 1985).

The se genes seb and sec were found in similar percentages (Rall et al., 2010b), which agreed with results from this study, which also demonstrated the presence of sec and see genes in 30% of S. xylosus, which agreed with Da cunha et al. (2006), who detected sec gene in one S. xylosus isolate. S. xylosus, are used as a starter culture in fermented meat products (Montel et al., 2000)

In present study S. warneri carried see enterotoxin gene in 20%. S. simulans produced sec and see enterotoxin in 33.3%. However, S. epidermidis, S. saprophyticus, S. hominis and S. capitis not produced enterotoxins.

Valle et al. (1991) found a toxigenic capacity in 45 (16.5%) CNS isolates, including S. epidermidis, S. haemolyticus, S. warneri, and S. xylosus, Stepien et al. (2016) suggested a strong association between coagulase-negative S. simulans and endocarditis in broiler chicken.

Conclusion

From above mentioned data, it was observed that coagulase positive Staphylococci and coagulase negative staphylococci contain MecA gene and different types of enterotoxin genes. So, attention should be given to CNS because there is no more studies on it.

Acknowledgement

Many thanks to Dr. Waleed Younis, Assistant lecturer of Microbiology, Dept. of Microbiology, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt. For his technical support.

References


