Occurrence and molecular characterization of some zoonotic bacteria in bovine milk, milking equipments and humans in dairy farms, Sharkia, Egypt


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Abstract
One hundred bulk tank milk samples, 50 milking equipments’ swabs and 50 dairy workers’ hand swabs were collected from three dairy farms at Sharkia Province, Egypt, to determine the occurrence of some zoonotic bacteria as well as to investigate the presence of some virulence genes in all isolates by PCR. Staph. aureus, St. agalactiae, Salmonella species and M. bovis were isolated from milk samples with the percentages of 17%, 4%, 9% and 1%, respectively. In dairy workers hand swabs, the respective isolation rates of Staph. aureus, St. agalactia and Salmonellae were 10%, 2% and 8%, while only Staph. aureus and St. agalactia were recovered from milking equipments (6%, each). Of the examined Staph. aureus isolates, 11.8% and 20% of milk and hand swabs’ isolates, respectively, were carrying sea gene. In St. agalactiae isolates, cfb virulence gene was identified in all the obtained isolates. Moreover, spvC virulence gene was detected in 40% and 33.3% of S. Typhimurium isolates recovered from milk and hand swabs’ samples, respectively. While the respective percentages of spvC gene identification in S. Enteritidis isolates from the same sources were 75% and 100%. M. bovis was isolated from 1% of milk samples only. In conclusion, the isolation of potentially virulent Staph. aureus, St. agalactia and Salmonellae in dairy farms is of public health significance to milk consumers. Although the low isolation rate, the detection of M. bovis in milk samples should be of great concern due to its high public health hazards.

Introduction
Milk is considered one of the high nutritional quality foods; however, it is an excellent medium for growth and transmission of different bacterial pathogens to humans (Donkor et al., 2007). Microbial contamination of milk can occur from a variety of sources such as cows with mastitis, milk handlers, milking equipments or deficient hygiene (Bramley and Mckinnon, 1990; De Oliveira et al., 2011). There is an increasing concern about the presence of zoonotic pathogens in raw milk due to the spread of such pathogens between cows during the milking time by milking equipments and milkers’ hands (Fox and Gay, 1993). Therefore it is essential to assess the role of raw milk and other possible sources of contamination within the farm in transmitting some zoonotic bacteria to milk consumers.

Enterotoxigenic Staphylococcus aureus is considered the world’s third most important cause of foodborne intoxication (Tirado and Schmidt, 2001). Eleven antigenic types of Staph. aureus enterotoxins (SEs) have been recognized and they retain their biological activity even after pasteurization (Asao et al., 2003). Thus, because of the importance of SEs in the public health and food sector, efficient screening to detect their prevalence is required (Morandi et al., 2007). Streptococcus agalactiae or group B Streptococcus (GBS) is associated with early and late onset disease in infants, with asymptomatic colonization of the uro-genital and gastro-intestinal tract (Zadoks et al., 2011). It is also implicated in causing septicemia and other clinical manifestations in non-pregnant adults. Moreover, St. agalactiae has been widely reported as an important cause of mastitis in cattle (Amosun et al., 2010).

Non-typhoid Salmonella species are considered the most important bacterial etiology for enteric infections worldwide including Egypt (Bulgin et al., 1982). Different serotypes of S. enterica have been isolated from dairy animals and their environment, some of which are considered pathogenic to humans (Blau et al., 2005). Human infection is mostly associated with consumption of food of animal origin including milk (Gomez et al., 1997).

Bovine tuberculosis caused by Mycobacterium bovis is a relevant zoonosis that can spread to humans through inhalation and consumption of milk (Thoen and Barletta, 2005). M. bovis is classified as Risk 3 pathogen for public health (OIE, 2005) and it has

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been associated with extra-pulmonary tuberculosis in infants and children due to ingestion of milk (Thoen et al., 2006). The objective of the current study was to investigate bacterial contamination of milk, milking equipments and milker’s hands in dairy farms with some zoonotic bacteria and to molecularly characterize the potential of some isolates to harbor virulence genes.

**Material and Methods**

**Sampling**

A total of 100 bulk tank milk samples of cattle origin, 50 swabs from milking equipments and 50 hand swabs from dairy workers were collected from three randomly chosen dairy farms in Sharkia, Egypt. The farms included two small (up to 25 animals) and one medium sized (up to 50 animals) farms at Azizia (Menia Elkamhi), Belbeis and Bani-Helal (Menia Elkamhi), respectively. The farms used automatic milking except the small sized farm in Belbeis used manual milking. The produced milk in Azizia and Bani-Helal farms is distributed to milk factories and dairy products outlets, while the milk produced in Belbeis farm is distributed to street vendors. The study was conducted during the period from February to June, 2013.

**Milk samples**

Bulk tank milk samples were collected aseptically into screw capped bottles and kept at 4°C until microbiological examination. Twenty five ml from each sample were homogenized with 225 ml of buffered peptone water (BPW) (Oxoid, CM509, Adelaide, Australia) for pre-enrichment and incubated at 37°C for 24 h (Addis et al., 2011a).

**Hand and milking equipments’ swabs**

Moistened sterile swabs were rolled over the palm of hands, area between fingers, finger tips and nails. Also, swabs from milking equipments were collected. Each swab was inserted in tubes containing BPW for pre-enrichment.

**Isolation and identification of Staph. aureus**

A loopful from the pre-enriched culture homogenate in BPW was inoculated onto Baird parker agar medium (Oxoid, CM0275, Adelaide, Australia) (Iso-6888-1, 1999) and incubated at 37°C for 24 hours. The suspected colonies were subjected to Gram staining and biochemical identification using catalase, coagulase test, test tube method and oxidase test (Cruickshank et al., 1975).

**Isolation and identification of St. agalactiae**

The pre-enriched culture homogenate in BPW was inoculated onto Edward’s media plates (Oxoid CM0027, Adelaide, Australia) and incubated at 37°C for 24-72 hours. Suspected colonies were subjected to Gram staining and biochemical identification using catalase, oxidase, esculin hydrolysis, CAMP test and carbohydrates utilization (Cruickshank et al., 1975). The biochemically identified St. agalactiae strains were serogrouped with a commercial latex agglutination kit (Oxoid, Adelaide, Australia) for the identification of streptococcal groups A, B, C, D, F and G.

**Isolation and identification of Salmonellae**

The isolation and identification of Salmonellae were done according to ISO 6579 method (Iso-6579, 2002; 2007). Pre-enriched culture homogenate in BPW were enriched in Rapapport Vassilidis (RV) broth (Oxoid, CM669, Adelaide, Australia) and incubated at 41°C, moreover, another portion of the enriched broth was enriched in Muller–Kauffmann tetrahionate/ novobiocin broth (Oxoid, CM1048, Adelaide, Australia) and incubated at 37°C for 24 hs ± 2 hs. A loopful from the enriched broth was then streaked onto the surface of Xylose Lysine Deoxycholate (XLD) agar plates (Oxoid, CM469, Adelaide, Australia) and Brilliant Green agar plates (Oxoid, CM0263, Adelaide, Australia) and incubated at 37°C for 24 hours. The suspected Salmonella colonies were subjected to Gram staining and biochemical identification using oxidase test, hydrolysis of urea, H₂S production and lysine decarboxylation (Cruickshank et al., 1975). The biochemically identified Salmonella isolates were then subjected to serotyping following Kauffman-White Scheme with commercial antisera (Difco Laboratories Deteroeit, Mitchigeu, USA) for cell wall (O) and flagellar (H) antigen identification (Kauffman, 1974). Serological identification was carried out at Animal Health Research Institute, Dokki, Giza.

**Isolation of M. bovis**

Milking equipments’ swabs and hand swabs pre-enriched in BPW were streaked directly onto Löwenstein-Jensen media (Oxoid CM0884, Adelaide, Australia). While, for each milk sample, 8 ml were centrifuged at 10,000 rpm for 20 minutes and the supernatant was discarded. Decontamination of the fat and protein sediment was then performed by Petroff decontamination method by 4% sodium hydroxide and 4% sulfuric acid (Balian et al., 2002) and then a loopful was streaked onto Löwenstein-Jensen media.
The samples were incubated at 37°C for 90 days with weekly examination. The isolation of *M. bovis* was carried out in a class II biological safety Type A2 cabinet (NewTechnology) that has been cleaned with 10% sodium hypochlorite and 70% alcohol, followed by exposure to ultraviolet light for 30 minutes with air circulation system turned on (Franco et al., 2013).

**DNA extraction**

DNA extraction from biochemically identified *Staph. aureus, St. agalactiae*, and *Salmonella* isolates was performed using Bacterial DNA extraction kit (Spin-Column) (BioTeke Corporation, Shanghai, China) according to the manufacturers guidelines. The DNA from suspected *M. bovis* cultures was extracted using GF-1 Tissue DNA Extraction Kit according to the manufacturer guides (Vivantis Technology, GF-TD-050).

**Multiplex PCR for identification of *Staph. aureus* enterotoxins**

Purified DNA of *Staph. aureus* isolates was subjected to a multiplex PCR for the identification of enterotoxins (Cremonesi et al., 2005). Oligonucleotide primers sequences (AlphaDNA, Montreal, Quebec, Canada) for the amplification of thermostable nuclease gene (*nuc*), enterotoxins *sea*, *sec* and *sed* genes are shown in Table 1. The reaction was performed in 25 µl reaction volume containing 12.5 µl of ready made 2x power Taq PCR mastermix (BioTeke Corporation, Shanghai, China), 0.6 µM each *cfa* primers and 2 µl of the purified DNA. A reaction mixture with no added DNA was run in the PCR as a negative control.

The reaction conditions consisted of one cycle of 94°C for 2 min followed by 30 cycles of 94°C for 1 min, 56°C for 1 min and 68°C for 1 min and a final cycle of 72°C for 7 min. The reaction was carried out in Primus Thermal Cycler (MWG-Biotech Thermal Cycler, Ebersberg, Germany). Amplification products were resolved in 1.2% (w/v) agarose gels along with 100bp molecular weight ladder (BioTeke Corporation, Shanghai, China). The agarose gel was prepared in 1x TBE (89 mM Tris- Borate, 2 mM EDTA, and pH 8.3) stained with 5 µM ethidium bromide. The gels were run in 1xTBE, 5 µM ethidium bromide for at least 45 minutes at 100 volts and then visualized under Ultra Violet light of ultraviolet transilluminator (Spectroline, Westbury, NY, USA).

**Molecular identification of *St. agalactiae* virulence gene**

Purified DNA of *St. agalactiae* isolates was subjected to PCR for the screening of CAMP virulence factor specific gene (*cfa*) (Chen et al., 2005). The oligonucleotide primers sequences (AlphaDNA, Montreal, Quebec, Canada) are listed in Table 1. The reaction was performed in 25 µl reaction volume containing 12.5 µl of ready made 2x power Taq PCR mastermix (BioTeke Corporation, Shanghai, China), 0.6 µM each *cfa* primers and 2 µl of the purified DNA. A reaction mixture with no added DNA was run in the PCR as a negative control. The reaction conditions consisted of one cycle of 94°C for 5 min followed by 30 cycles of 94°C for 30 sec, 60°C for 30 sec and 72°C for 45 sec and a final cycle of 72°C for 5 min. The gel preparation, electrophoresis and visualization were performed as previously mentioned in *Staph. aureus* section.

**Molecular identification of Salmonella species virulence gene**

Purified DNA of *S. Typhimurium* and *S. Enteritidis* isolates was subjected to PCR for the identification of *Salmonella* virulence plasmid (*spvC*) (Betancor et al., 2010). The oligonucleotide primers sequences (AlphaDNA, Montreal, Quebec, Canada) are listed in Table 1. The reaction was performed in 25 µl reaction volume containing 12.5 µl of ready made 2x power Taq PCR mastermix (BioTeke Corporation, Shanghai, China), 0.6 µM each *spvC* primers and 2 µl of the purified DNA. A reaction mixture with no added DNA was run in the PCR as a negative control. The reaction conditions consisted of one cycle of 95°C for 5 min followed by 30 cycles of 94°C for 1 min, 51°C for 45 sec and 72°C for 1 min, and a final extension at 72°C for 10 min. The gel preparation, electrophoresis and visualization were performed as previously mentioned in *Staph. aureus* section.

**Real time PCR for identification of *M. bovis***

Extracted DNA from the suspected *M. bovis* colonies were identified by performing a real time PCR using MTPlex dtec-RT-qPCR Test (Edificio-Quorum3, Spain) that comprises a series of species-specific targeted reagents designed for detection of all species contained in the *Mycobacterium tuberculosis* complex.

The primers and TaqMan probe target a sequence conserved for all strains of each single species belonging to *Mycobacterium tuberculosis* complex. According to the source of *Mycobacteria* isolation, the species is determined, in the current study, the source of isolation was milk of bovine origin; therefore, the positive reaction using MTPlex reaction refers to contamination with *M. bovis*. Moreover, a screening test for tuberculosis was carried out for all dairy
Table 1. Primer sequences and expected product sizes

<table>
<thead>
<tr>
<th>Organism</th>
<th>GenBank acc.</th>
<th>Primer sequences (5’→3’)</th>
<th>Expected product size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>MW5583</td>
<td>TACCAAGTCTTGAGCGATT</td>
<td>409 bp</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SE-FL720</td>
<td>TACAGAAGTGGTGTATAGG</td>
<td>132 bp</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SE-FL1549</td>
<td>CATCTGACCGAGGAAATT</td>
<td>180 bp</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SE-FL467</td>
<td>TUCATATCCAAGGGTTTCT</td>
<td>371 bp</td>
<td></td>
</tr>
<tr>
<td>S. enteritidis</td>
<td>SE63078</td>
<td>TACATCCAGAAGAATGTC</td>
<td>359 bp</td>
<td></td>
</tr>
<tr>
<td>M. bovis</td>
<td>56396</td>
<td>TACATCCAGAAGAATGTC</td>
<td>339 bp</td>
<td></td>
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<tr>
<td>S. agalactiae</td>
<td>108263</td>
<td>GGTGAAGATGTTTTGAGTA</td>
<td>288 bp</td>
<td></td>
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<td>Salmonella typhi</td>
<td>207622</td>
<td>GGTGAAGATGTTTTGAGTA</td>
<td>200 bp</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Prevalence of some zoonotic bacteria in milk, milking equipments and milkers’ hands

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Staph. aureus</th>
<th>St. agalactiae</th>
<th>Salmonella spp.</th>
<th>Staph. aureus enterotoxins</th>
<th>S. enteritidis</th>
<th>S. typhimurium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk tank milk samples</td>
<td>2/17 (11.8%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Milking equipments</td>
<td>5/5 (100%)</td>
<td>1/1 (100%)</td>
<td>1/1 (100%)</td>
<td>1/1 (100%)</td>
<td>1/1 (100%)</td>
<td>1/1 (100%)</td>
</tr>
<tr>
<td>Milkers’ hands</td>
<td>1/3 (33.3%)</td>
<td>1/3 (33.3%)</td>
<td>1/3 (33.3%)</td>
<td>1/3 (33.3%)</td>
<td>1/3 (33.3%)</td>
<td>1/3 (33.3%)</td>
</tr>
</tbody>
</table>

The percentage of isolation is indicated between brackets.

Table 3. Prevalence of virulence genes in Staph. aureus, St. agalactiae and Salmonella isolates

<table>
<thead>
<tr>
<th>Source of sample</th>
<th>Staph. aureus</th>
<th>St. agalactiae</th>
<th>Salmonella spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk tank milk samples</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Milking equipments</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Milkers’ hands</td>
<td>-</td>
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</tr>
</tbody>
</table>

The percentage of isolation is indicated between brackets.

Results

Prevalence of some zoonotic bacteria in milk, milking equipments and milkers’ hands

One hundred bulk tank milk samples, 50 milking equipments’ swabs and 50 hand swabs from dairy workers were examined for the prevalence of Staph. aureus, S. agalactiae, and Salmonella spp. by cultural, biochemical and serological characterization. The results show that the prevalence rates of the aforementioned bacteria in the examined milk samples were 17%, 4% and 9%, respectively (Table 2). The respective prevalence rates from milking equipments’ swabs were 10%, 2% and 8%. Only Staph. aureus and Salmonella spp. were isolated from 6% of milk and hand swabs, each.

Prevalence of some virulence genes in Staph. aureus, St. agalactiae and Salmonella isolates

Staph. aureus isolates from 11.8% of milk and 20% of hand swabs were positive for sea gene responsible for the production of enterotoxin A (Table 3 and Figure 1). All St. agalactiae isolates from milk and hand swabs were positive for cfb gene (Table 3 and Figure 2). The obtained results showed that 40% and 75% of S. Enteritidis and 33.3% and 100% of S. Typhimurium isolates from milk and hand swabs were positive for spvC gene, respectively (Table 3 and Figure 3).

Discussion

Milk and milk products are implicated in 2-6% of bacterial food-borne outbreaks as reported by surveillance systems from several countries (De Buyser et al., 2001). The level of microbial contamination of raw milk is influenced by several factors such as: the health status of milking cows, hygiene of housing and milking environment and hygiene of milkers (Bramley and Mckinnon, 1990). Milk is considered a good substrate for Staph. aureus growth and enterotoxin production leading to food poisoning which is characterized by nausea, vomiting, abdominal cramps and diarrhea (Bramley and Mckinnon, 1990). Staph. aureus contamination of milk was detected in 17% of bulk tank milk samples (Table 2). Nearly similar results of 18.2% in Turkey (Ekici et al., 2004), 20.8% in Turkey (Aydin et al., 2011) and 21% in Iran (Ahmadi et al., 2009) were previously reported in milk samples collected from dairy farms. Also, Staph. aureus was detected in 10.8% of bulk tank milk samples in Brazil (Fagundes et al., 2010). Higher percentage of 68% in Brazil (De Oliveira et al., 2011) was also documented, this could be due to the examination of milk samples obtained from points of sales not from dairy farms, which in
In Egypt, Ibrahim (2010) isolated *Staph. aureus* from milk samples at Zagazig, Sharkia province with nearly similar isolation rate (16.5%). However, a higher prevalence of 25% was obtained from milk samples collected from buffalo attending the veterinary hospital in Zagazig, Sharkia (Suelam et al., 2012). Although the study of Suelam et al. (2012) was carried out at the same geographical area, the reported prevalence of *Staph. aureus* was higher than that obtained during the current study (17%). This could be attributed to the presence of a source of infection in the examined buffalos which is indicated by high prevalence of *Staph. aureus* in throat swabs (27.3%) and skin swabs (36.4%) collected from humans dealing with such animals at the veterinary hospital (Suelam et al., 2012). In Aswan, Egypt, a study reported the isolation of the organism from milk samples with the percentage of 34.1% (Abdel-All et al., 2010). Milking equipments are important fomites for transmission of *Staph. aureus* in dairy herds from bovine and human skin to milk (Fox et al., 1991). The spread of *Staph. aureus* throughout the farm especially in milk, farm environment and milking equipments highlights the potential role of such vehicles in transmitting the organism to humans (Jørgensen et al., 2005). Lower *Staph. aureus* isolation rate of 3.6% from milking equipments than the current study (6%) was previously reported (Lee et al., 2012).

About 95% of staphylococcal food poisoning are caused by SE types SEA to SEE (Tamarapu et al., 2001), of which, SEA is considered the most commonly involved enterotoxin (Balaban and Rasooly, 2000). Such toxins are thermoresistant and the ingestion of at least 1 gram of enterotoxin per 100 grams of food is enough to induce food poisoning (Tranter, 1996; Cremonsei et al., 2005). Therefore, the determination of SEs producing strains in food is important with respect to assessing public health risks (Aydin et al., 2011). In the present study, 11.8% of the milk isolates were found encoding *sea* gene (Table 3 and Figure 1). These results are in accordance with 11.7% and 11.6% isolation rates of enterotoxigenic *Staph. aureus* isolates detected in bulk tank milk reported by Neder et al. (2011) in Argentina and Morandi et al. (2007) in Italy, respectively, while nearly similar to 9.7% reported by Aydin et al. (2011) in Turkey. These findings are also in agreement with the frequently reported SEA in dairy products (Normanno et al., 2005; Rall et al., 2008). Higher SEA isolation rate of 21.4% was reported in milk samples collected from Aswan, Egypt (Abdel-All et al., 2010). The detection of the *nuc* gene in the present study in all the positive samples encoding *sea* gene is strongly correlated with enterotoxin production and it is considered as a marker of food contamination with enterotoxigenic *Staph. aureus* (Barski et al., 1996; Tamarapu et al., 1996; Cremonesi et al., 2005).

Milkers’ hands are considered as an initial point of contamination with *Staph. aureus* in dairy farms (Olivindo et al., 2009). Approximately, 30-50% of humans carry *Staph. aureus* and one third to one half of the organisms have been shown to be enterotoxigenic (Bergdoll, 1989). *Staph. aureus* was isolated from 10% of milkers’ hand swabs and 20% of the isolates were enterotoxigenic encoding for *sea* gene (Table 3 and Figure 1), these results are consistent with those
reported by Ruzickova (1994). The production of SEA is mostly correlated to human strains, thus the contamination of milk during the milking process and handling by milkers could be assumed (Akineden et al., 2008). The isolation of Staph. aureus from milkers’ hands with the percentages of 3.3% in Brazil (Lee et al., 2012) and 45.9% (Adesiyun et al., 1998) in Trinidad were previously reported. Moreover, an isolation rate of 44.1% was reported in skin swabs of dairy workers in Aswan, Egypt, of which 43.5% were found to encode sea gene (Abdel-All et al., 2010). Also, 60% prevalence of Staph. aureus was reported from dairy workers in Ismailia, Egypt (Fadel and Ismail, 2009). Several reports of human Staph. aureus isolation from milk have been reported suggesting that humans are the source of milk contamination in dairy farms (Roberson et al., 1998; Zadoks et al., 2002; Matyi et al., 2013).

The obtained 4% prevalence rate of St. agalactiae from bulk tank milk in the current study (Table 2) was consistent with 3.9% obtained in Portugal (Bexiga et al., 2005) and 3% in USA (Makovec and Ruegg, 2003). Higher prevalence rates of St. agalactiae in milk were previously reported in Kenya (Mosabi et al., 1997), Egypt (Amin et al., 2011) and Brazil (Elias et al., 2012). The higher prevalence could be attributed to type of examined milk (mastitic or non-mastitic) and inadequate hygienic and control measures within the examined dairy farms. Although St. agalactiae was isolated with low percentage from milkers’ hands (Table 2), this result highlights the role of milkers’ hands as a source of infection with the bacterium especially when good hygienic measures have not been maintained (Amosun et al., 2010).

The potential for human St. agalactiae isolates to be a zoonosis has previously been investigated and results revealed that although human and bovine St. agalactiae isolates represent distinct populations, human host associated St. agalactiae subtypes may occasionally be transmitted to bovines (Dogan et al., 2005). Moreover, some bovine isolates have been shown to have a genetic relationship with human isolates (Evans et al., 2008). In a previously conducted study, it has been shown that St. agalactiae group B serotype III has been incriminated as the major colonizing strain in pregnant women (Ekin and Gurturk, 2006). These findings showed that milk could be a risk factor playing an important role in transmitting some St. agalactiae strains with potential zoonoses to humans. Moreover, identical St. agalactiae isolates from milk and dairy workers have been reported (Jensen and Aarestrup, 1996).

The pathogenicity of St. agalactiae in humans is attributed to several virulence factors; therefore, in order to investigate the potential of the strains isolated during the current study to pose risk to milk consumers, the presence of CAMP factor specific gene (cfb) was investigated. The presence of cfb gene in all St. agalactiae isolates (Table 3 and Figure 2) indicated the potential of such strains to be virulent causing risk to milk consumers, these results coincide with those reported by Jiusheng et al. (2008). It was suggested that the release of CAMP factor during systemic infections could impair the host immune response indicating its role as a putative virulence factor (Gase et al., 1999).

Salmonella spp. were isolated from 9% of bulk tank milk (Table 2), nearly similar isolation rates of Salmonella spp. from milk were previously reported by Van Kessel et al. (2003) and Kars et al. (2005) in USA. Other studies reported the isolation of Salmonella spp with the prevalence of 6.1% in USA (Jayarao and Henning, 2001), 6% in Pennsylvania (Jayarao et al., 2006) and 2.6% in USA (Van Kessel et al., 2004). Higher isolation rates of 28.1% (Van Kessel et al., 2011) and 28.6% (Addis et al., 2011b) were documented in USA and Ethiopia, respectively.

In Egypt, Salmonella spp. were isolated from milk samples in Sharkia dairy farms with a higher isolation rate of 28% (Awad, 2002), while another study reported that all of the examined milk samples were free from Salmonella in Sharkia dairy farms (Ahmed, 2005). The inconsistency between the results of the present study and those aforementioned studies carried out in Sharkia, could be attributed to the difference of the hygienic measures applied in each study area and the use of pooled milk in bulk tanks during the current study while other studies used individual milk samples.

Salmonella species were isolated from 8% of hand swabs of dairy workers (Table 2), this result constitutes public health hazards due to the possibility of transferring the organism to milk and consequently increasing the risk of infection for milk consumers. In Ismailia, Egypt, Fadel and Ismail (2009) isolated Salmonella spp. from 3.7% of dairy workers’ hands swabs. The probability of fecal contamination of milk, and thus the risk of pathogens transfer appears to be modulated more by farm management and hygiene of dairy workers (Ricci et al., 2013).

Virulence plasmids are one of several Salmonella virulence determinants involved in survival and growth in host cells (Finlay and Falkow, 1989). Previous studies have shown that a 7.8-kb region of virulence plasmids consists of five genes described as spvRABCD (Gulig et al., 1992). The carriage of spvC gene in Salmonella isolates seems to increase the growth rate of Salmonella spp. in host cells.
and affect their interaction with the host immune system (Gulig et al., 1993). SpvC gene was found in 75% of S. Typhimurium isolates and 40% of S. Enteritidis isolates from bulk tank milk (Table 3 and Figure 3). Moreover, the gene was detected in 100% of S. Typhimurium isolates and 33.3% of S. Enteritidis isolates from hand swabs (Table 3 and Figure 3). SpvC gene was previously identified in S. Typhimurium isolates of human origin (Swamy et al., 1996; Abouzeed et al., 2000). Moreover, the gene was detected in 100% and 90% of S. Enteritidis isolates of bovine and human origin, respectively, in Iran (Amini et al., 2010). The detection of spvC in the isolated strains from milk and human sources is of great concern because strains carrying virulence plasmids can cause systemic disease, while those lacking such plasmids can cause local or asymptomatic disease (Heithoff et al., 2008).

Raw milk derived from infected cows with M. bovis was regarded as a principle vehicle of infection for humans (Dankner et al., 1993). In the current study, M. bovis was isolated from bulk tank milk with the percentage of 1% (Table 2). Similar isolation rate (1%) was reported in bulk tank milk in Brazil (Franco et al., 2013), while nearly similar prevalence rates of 0.7%, 0.8% and 1.6% were also reported in Turkey (Aydin et al., 2012), Brazil (Fujimura Leite et al., 2003) and Tunisia (Ben Kahla et al., 2011), respectively. Although the low isolation rate of M. bovis from bulk tank milk samples, the presence of M. bovis in milk poses a public health risk. The low prevalence of M. bovis could be attributed to the dilution of milk in the bulk tank (Passchyn et al., 2012) and also due to the low number of excreting the organism from infected cows (Gonzalez et al., 1986). Combinations of preventive measures are recommended in dairy farms in order to minimize the risk of milk contamination with potential pathogens. For instance; routine screening of dairy animals and milk, eradication of rats, cleaning and disinfection of farm environment and milking equipments and personal hygienic practices for dairy workers.

**Conclusion**

In conclusion, the current study reported the detection of potentially virulent bacteria of zoonotic importance in bulk tank milk, milking equipments and dairy workers from some dairy farms in Sharkia Province, Egypt. The presence of such bacteria in raw milk underlines the importance of preventive measures during milking to ensure the quality of milk and to prevent the dissemination of foodborne zoonotic bacteria to consumers. Contaminated milk does not only pose a risk due to its consumption but also it is used in the production of cheese and other dairy products which constitute another potential public health risk. Although the isolation rate of M. bovis is low, it is highly recommended to use PCR for confirmation of the bacteria due to high public health risk.

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