Compartaive Studies on Pathogenic Staphylococci Isolated From Human and Food of Animal Origin

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Abstract: This study was carried-out on a total of 480 random samples that, were collected in the study. From human 300 samples were collected that includes, 100 samples from anterior nares of patients with pneumonia and bronchitis and 100 pus samples from wounds and abscesses and 100 samples from throat. Also, 180 samples from the food of animal origin 120 samples were collected from milk, 30 samples from kareish cheese and 30 samples from roomy cheese. This study aimed to study whether the strains groups reflected a host- or tissue-adaptation and whether there is a predisposition of certain *cap* or *agr* types to colonise or infect certain ruminant hosts, and to evaluate the spread of resistance to methicillin and to the most commonly used antibiotics in the treatment of mastitis in ruminants. Our study concluded that, the *Staph. aureus* causes a great economic losses in human and milk industry, the the incidence of *staph aureus* in human samples (Pus, nasal swabs and throat swabs) of a higher incidences than that of the animal origin samples (milk, kareesh cheese and roomy cheese). Also, the best methods for detection of staph aureus enterotoxins genes in the samples of human or animal origin. Our results on antibiotic sensitivity test, cleared that, the staph. aureus is more sensitive to the oxacillin, chloramphenicol, Amoxcillin + Clavulnic acid, levofloxacin, gentamicin, streptomycin, Ciprofloxacin.
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1. Introduction

Staphylococcus aureus is an important agent of bacterial mastitis in milking animals and of foodborne intoxication in humans. ***(D'amico and Donnelly**, 2011 and Rehab Elbargisy, 2016).*

Microbial contamination of raw milk can occur from a variety of microorganisms from a variety of sources. Because of this, determining the cause of bacterial defects is not always straightforward. Although there is often one source of bacteria that cause high bulk tank counts, high bacteria counts can also result from a combination of factors (i.e., dirty equipment and marginal cooling). In some cases, selective plating procedures or bacterial culturing may be useful in identifying the source of high bacteria counts on the farm***. (Murphy and Boor, 2010 and Hu and Nakane, 2014).***

Worldwide bovine mastitis is the most common infectious disease affecting milk producing cows. Causing economic losses higher than any other disease of dairy cattle ***(Gillespie and Oliver, 2005).*** Also, posses food safety and any-microbial resistance threats, as it is the primary contamination source of milk and milk products especially in case of defective pasteurization ***(Zargar et al., 2014).***

S. aureus is one of the most frequent etiologic agents of mastitis in bovines, ovines and caprines, rendering livestock unable to adequately produce milk, which results in heavy economic losses for the dairy industry ***(Alves et al., 2008).***

Differences between host biotypes are also reflected at the genotypic level as determined by macro-restriction analysis of the chromosome ***(Hennekinne et al., 2003).*** Due to the specificity of host-pathogen interactions needed to produce mastitis, it has been postulated that the nature of the virulon and the regulation of its expression are determining factors when it comes to the ability of a strain to produce mastitis ***(Vautor et al., 2008).***

The recent release of the complete genome sequence of *S. aureus* ET-3, a bovine isolate, provides new insight into the genomic basis of a putative host adaptation and the existence of host specific genetic traits in *S. aureus* isolated from bovine hosts ***(Herron-Olson et al., 2007).***

We recently showed that diversification of the *S. aureus* core genome correlated with host origin in ruminants ***(Ben Zakour et al., 2008)***. However, in most studies, the panel of ruminant *S. aureus* strains comprised mostly strains isolated from cases of mastitis. The mammary gland tissues present characteristics such that some authors hypothesized that the specific traits found to be common in bovine strains were related to a tissue- rather than to host-specificity *(van **Leeuwen** et al., 2005).*

Outbreaks of staphylococcal food poisoning are most often associated with processed red meats, poultry products (especially chicken salad), sauces, milk products (especially cheeses), and custard or cream-filled bakery products. Ham and associated products are often involved in as many as 30% of outbreaks of staphylococcal food poisoning. Most outbreaks result from the combined effects of contamination of the food, often through unsanitary handling, with *Staphylococcus aureus*and holding the food at the wrong temp. Thus allowing growth and synthesis of enterotoxin by the pathogen***(Halpin et al., 1989a).***

Identification of bacterial pathogens in milk from cows with mastitis is the definitive diagnosis of mastitis infctions. It also, provides information important for prevention and control of the disease. In most clinical laboratories identification methods are based on microbiological culture of milk and biochemical identification of bacterial isolates recovered. However, these microbiological culture limited by the dynamic nature of infections. Subclinically infected cows are intermittent shedders of organisms through low and high shedding patterns during lactation that leading to negative cultures ***(Phuektes et al., 2001).***

Because S. aureus is a common contaminant of cheese, an understanding of the ecology of this pathogen and of the antimicrobial susceptibility and toxigenicity of various strains will ultimately contribute to the development of control practices needed to enhance the safety of artisan and farmstead cheese production. ***(D'amico and Donnelly,*** *2011).*

This study aimed to study whether the strains groups reflected a host- or tissue-adaptation and whether there is a predisposition of certain *cap* or *agr* types to colonise or infect certain ruminant hosts, and to evaluate the spread of resistance to methicillin and to the most commonly used antibiotics in the treatment of mastitis in ruminants.

2. Materials and Methods

1-Sampling: A total of 480 random samples were collected in the study. From human 300 samples were collected that includes, 100 samples from anterior nares of patients with pneumonia and bronchitis and 100 pus samples from wounds and abscesses and 100 samples from throat. Also, 180 samples from the food of animal origin 120 samples were collected from milk, 30 samples from kareish cheese and 30 samples from roomy cheese.

2. Examination of the samples:

1. Bacteriological examination of milk samples ***(Quinn et al., 2002)***

2. Purification:-The suspected colonies (Gram-positive arranged in clusters) were picked-up and subcultured for purification. The pure isolates was preserved into semisolid agar till further identification.

3. Identification of the obtained isolates:

Isolates were identified according to colonial morphological characteristics (Pigmentation and haemolysis), microscopic appearance and through biochemical tests according to ***Collee et al., (1996), Quimn et al., (2002), and Boerlin et al., (2003).***

a- Morphological identification:- *Staphylcocci* was preliminary identified morphologically by dried stained film according to Gram's stain. Positivity revealed as irregular clusters arrangement or "bunches of grapes".

b. Cultural characters.

1. Biochemical characters:- Catalase test, oxidase test, urease test, coagulase test, DNase test, haemolysis, pigment production, aerobic fermentation of mannitol, maltose and acetone production, were carried as further identification according to ***Quinn et al., (2002) and Boerlin et al., (2003).***

2. Direct detection of *S. aureus* in milk by a tube coagulase test. ***(Yazdankhah and Olsen1998).***

3. Detection of *S. aureus* DNA by PCR:

c-Data analysis and statistics: The statistical analysis were made for detection of the significance of the incidence of isolated microorganisms in milk of cows and buffaloes using Chi^2-test according to ***(SAS, 2004).***

3. Results and Discussion

Staph aureus causes a great economic losses to milk industry through discarding of the milk that contaminated with *staphylococcus* bacteria as well as this bacteria reduced the amount of milk produced, as the milk is very essential to human as we can synthesis from it cheese, butter, ice-cream and a lot of other products***. (Cohen et al., 2005 and Bessen., 2009).***

Our results on the cultural characters of Staphylococci isolates cleared that, isolates of Staphylococcus aureus gave the results which includes Gram +ve stain cocci of grabe like appearance, -ve results for indole test, MR, VP, citrate, triple sugar iron test and –ve for motility staining. Also, the isolated cocci showed +ve results for catalase test, oxidase test, urease test, oxidation fermentation test, +ve aerobic fermentation of mannitol, coagulase test and hemolysis on blood. (Table, 1). These results agreed with those of ***Seo and Bohach (2007) and FDA (2012)*** they reported that the characters of *staph. aureus* are Gram +ve, and it gave +ve results for catalase test, oxidase test, urease test, oxidation fermentation test, +ve aerobic fermentation of mannitol, coagulase test and hemolysis on blood.

While, our results on the incidence of *staphylococcus aureus* among examined samples

collected from workers and owners related to examined animals, the results cleared that, the incidences of *Staph. aureus* among all examined samples collected from the owners and workers of related animals reached to (63.34 %). (Table, 2).

These results agreed with those of ***Sourek*** *(1980)* who found that the incidences of *Staph. aureus* in samples collected from human related to animals reached to 271 strains produced staphylococcal enterotoxins from 870 strains of *staph. aureus* isolated. Also, ***Wang et al. (2012)*** found that, the higher incidences of *Staph. aureus* observed in pus samples (80 %), throat samples (60 %) and the lower incidences level observed in nasal samples (50 %).

Our results agreed with those of ***Ghose et al. (2003)*** where they reported that, the main bacteria isolated from pus samples were were *Staphylococci* (58.85%), *Streptococci* (32.53%), *Micrococci* (5.74%), *Corynebacteria* (1.91%) and *E.coli* (0.96%) and the pus of a higher bacterial count than that of milk samples.

Our results on the incidences of staphylococcus aureus among examined samples of milk, kareesh cheese and roomy cheese, cleared that, the incidences of Staph. aureus among all examined samples of animal origin reached to (12.78 %). The higher incidences of Staph. aureus observed in roomy cheese samples (20 %), Kareesh cheese samples (16.66 %) and the lower incidences level observed in milk samples (10 %). (Table, 3).

Table (1): The microbiological identification depend upon the following tests:

Tests	Isolate4
Gram stain	G+ve
Indole test	-
MR	-
VP	-
Citrate	-
Triple sugar iron test	-
Catalase test	+ve
Oxidase test	+ve
Urease test	+ve
Motality staining	-
Oxidation-Fermentation	+ve
Aerobic fermentation of monnital	+ve
Coagulase test	+ve
Hemolysis on blood agar	+ve
Suspected bacteris	Staph. Aureus*

* Pigmented colony (Cream buff, golden yellow, orange)

* Results of oxidation fermentation tests showed the ability of the colonies to produce acid under aerobic and anaerobic condition.

Table (2): Results of examined samples collected from workers and owners related to examined animals.

Source of samples	Types of samples	Number of samples	+ve samples	%
Human	Nasal swab	100	50	50
	Pus	100	80	80
	Throat	100	60	60
	Total	300	190	63.34

$Chi^2 = 6.25$** ** = Significant at ($P < 0.01$)

Table (3): Results of examined samples of animal origin.

Source of samples	Types of samples	Number of samples	+ve samples	%
Food of animal origin	Raw milk	120	12	10
	Kareish cheese	30	5	16.66
	Romy cheese	30	6	20
Total		180	23	12.78

$Chi^2 = 5.44$** ** = Significant at ($P < 0.01$)

Our results agreed with those of *(Sara El-nomrousey, 2014)* where it found that, In this study, bacteriological examination of 80 bovine milk samples and 200 milk product samples revealed that 20 isolates of *S.aureus* were recovered from 80 milk samples with a percentage 25% and 83 S.aureus isolates were recovered from 200 milk product samples with a percentage 41.5%. All isolates were identified as *S.aureus* by using Specific culture media and biochemical testsAs regards to milk samples, 22 out of 50 examined street milk samples were *Staphylococcus.* 16 isolates were S.aureus with apercent 32%. While 20% of examined farm milk were positive for S.aureus. As regards to milk product samples, 28 isolates of S.aureus were recovered from 50 street Kareish cheese samples. While 23 isolates were recovered from 50 street ice cream samples.

Our results on the incidence of *staphylococcus aureus* among examined samples collected from workers and owners related to animals and collected

samples of milk, kareesh cheese and roomy cheese, the results cleared that, there is a significant differences of the incidences of staphylococcus aureus among different examined samples of workers and owners related to animals (nasal swabs, throat and pus) as well as samples of milk and kareesh cheese and roomy cheese. The incidences of Staph aureus incidences in human collected samples (nasal swabs, throat and pus) of a higher level (63.34 %), than that observed in (kareesh cheese, roomy cheese and milk samples), (12.87 %). While, in human collected samples, the higher incidences of *Staph. aureus* observed in pus samples (80 %), throat samples (60 %) and the lower incidences level observed in nasal samples (50 %). The incidences of *Staph. aureus* in samples of animal origin (kareesh cheese, roomy cheese and milk samples) cleared that, the higher incidences of Staph. aureus observed in roomy cheese samples (20 %), Kareesh cheese samples (16.66 %) and the lower incidences level observed in milk samples (10 %). (Table, 4).

Table (4): Types and number of collected samples and percent of positive samples for staphylococcus aureus.:-

Source of samples	Types of samples	Number of samples	+ve samples	%
Human	Nasal swab	100	50	50
	Pus	100	80	80
	Throat	100	60	60
	Total	300	190	63.34
Food of animal origin	Raw milk	120	12	10
	Kareish cheese	30	5	16.66
	Roomy cheese	30	6	20
Total		180	11	6.12

$Chi^2 = 9.22$** ** = Significant at ($P < 0.01$)

Our results on the multiplex PCR for detection of *S. aureus* enterotoxins genes cleared that, the total number of 19 isolates of *S. aureus* (5 isolates from Roomy cheese, 4 isolates from nose and throat human lesion, 6 isolates from human pus, 2 isolates from milk and 2 isolates from Kareesh cheese) tested by using multiplex PCR by using sets of primers for enterotoxins (A,B,C,D). The results obtained by multiplex PCR showed that the enterotoxin A produced by 9 isolates (2 from human pus, 4 from human lesion, 2 from Roomy cheese and 1 from kareesh cheese) which gave characteristic band at 102 bp. No isolates produce enterotoxin B which gave characteristic band at 164 bp. Enterotoxin C produced by 4 isolates (1 from milk, 1 from roomy cheese and 2 from Kareesh cheese) which gave characteristic band at 451 bp. Enterotoxin D produced by 6 isolates (2 from human pus, 1 from human lesion and 3 from roomy cheese) gave characteristic band at 278 bp. (Fig., 1 and 2).

Our results agreed with those of ***(Omoe et al., 2002 and Sara El-nomrousey, 2014)*** investigated the distribution of *staphylococcal* enterotoxin (SE) A to I (SEA to SEI), genes (sea to sei) in *S.aureus*, 146 isolates obtained from Japan, from humans involved in, and samples from food poisoning outbreaks, healthy humans, cows with mastitis, and bovine raw milk were analyzed by mulitplex PCR. 77.4 % *S.aureus* isolates were found to be positive for one or more se genes, most of the *S.aureus* isolates harboring seg and about 60 % of the isolates harboring sei did not produce a detectable level of SEG or SEI. These results suggest the importance of quantitative assessment of SEG and SEI production in foods in order to classify the relationship between these new SEs and Food poisoning.

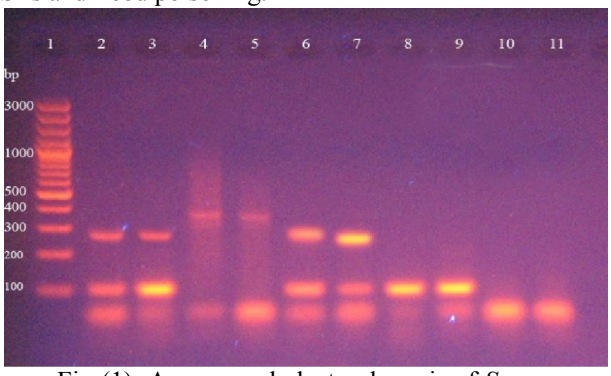

Fig (1): Agarose gel electrophoresis of *S. aureus* PCR products using enterotoxins Staphylococcus primer

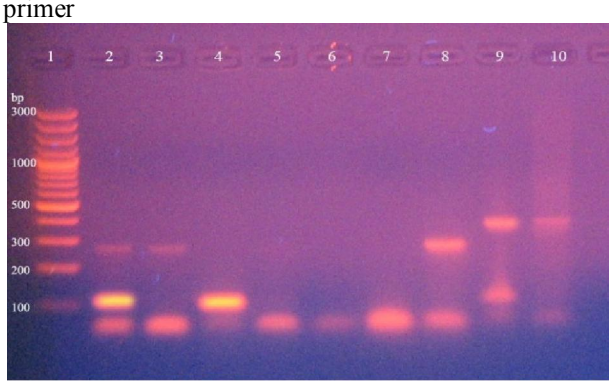

Fig (2): Agarose gel electrophoresis of *S. aureus* PCR products using enterotoxins Staphylococcus primer.

Our results on antibiotic sensitivity test, cleared that, the higher inhibition zone observed in levofloxacin, Ciprofloxacin, Amoxicilline + Clavulinic acid and choloramphenicol as the inhibition zone of them reached to 31.63, 29.63, 24.26 and 20.21 mm respectively, while, the lower inhibition zone observed in Ceftizoxime, tetracycline and Mecillinam as the inhibition zone of zem reached to 10.32, 10.79 and 3.68 mm. While, the intermediate inhibition zone size observed in streptomycin, gentamycin, ampicillin and sulphatrimethoprime as its inhibition zone level reached to 19.32, 17.11, 13.11 and 11.95 mm.

Also, the sensitivity tests of antibiotics to Staph. Aureus isolates:-

The results cleared in Table (5 and 6) indicated that, the staph. Aureus is more sensitive to the oxacillin, chloramphenicol, Amoxcillin + Clavulnic acid, levofloxacin, gentamicin, streptomycin, Ciprofloxacin. While, the intermediate sensitivity observed in sulphatrimethoprime and Ampicillin. While, the staph. aureus showed a high resistant for Cefizoxime, Mecillinam and tetracycline.

Our results agreed with those of (***D'amico and Donnelly, 2011)*** observed, limited antimicrobial resistance was among the staphylococcus isolates, with resistance to ampicillin (12.51%) or penicillin (17.04%) most common.

Our study concluded that, the *Staph. aureus* causes a great economic losses in human and milk industry, the incidences of *staph aureus* in human samples (Pus, nasal swabs and throat swabs) of a higher incidences than that of the animal origin samples (milk, kareesh cheese and roomy cheese). Also, the best methods for detection of staph aureus enterotoxins genes in the samples of human or animal origin. Our results on antibiotic sensitivity test, cleared that, the staph. aureus is more sensitive to the oxacillin, chloramphenicol, Amoxcillin + clavulnic acid, levofloxacin, gentamicin, streptomycin, Ciprofloxacin.

Table (5): Average diameter of Zone inhibition of different antibiotics.

	Code	Drug	Mcg	Mean, Std. Error (Cm)	Minimum	Maximum
1	Sxt	Sulphatrimethoprim	23.75 µg	11.95±2.39	.00	26.00
2	CRO	Ceftizoxime	30 µg	10.32±1.13	.00	25.00
3	OX	Oxacillin	5 µg	15.11±1.78	.00	25.00
4	C	Chloramphenico	30 µg	20.21±1.85	10.00	30.00
5	AMC	Amoxicillin+clavulinic acid	300 µg	24.26±1.26	6.00	31.00
6	AM	Ampicillin	10 µg	13.11±1.33	.00	20.00
7	LEV	Levofl oxacin	10 µg	31.63±0.68	26.00	36.00
8	CN	Gentamicin	23.75 µg	17.11±1.27	8.00	25.00
9	MEC	Mecillinam	30 µg	3.68±0.77	.00	10.00
10	S	Streptomycin	10 µg	19.32±0.96	6.00	25.00
11	CIP	ciprofloxacin		29.63±0.90	22.00	40.00
12	TE	Tetracyclin		10.79±2.19	.00	30.00
				17.26±0.67	.00	40.00

Means within the same column of different litters are significantly different at ($P < 0.01$).

Table (6): Sensitivity and resistance of *staphylococcus aureus* isolates to different antibiotic used.

	Code	Drug	Mean, Std. Error (Cm)	Susceptability	Intermediate	Resistance
1.00	Sxt	Sulphatrimethoprim	11.95		I	
2.00	CRO	Ceftizoxime	10.32			R
3.00	OX	Oxacillin	15.11	S	-	
4.00	C	ChloramphenicoL	20.21	S	-	
5.00	AMC	Amoxicillin+clavulinic acid	24.26	S	-	
6.00	AM	Ampicillin	13.11		I	
7.00	LEV	Levofl oxacin	31.63	S		
8.00	CN	Gentamicin	17.11	S		
9.00	MEC	Mecillinam	3.68		-	R
10.00	S	Streptomycin	19.32	S	-	
Total	CIP	Ciprofloxacin	29.63	S		
	TE	Tetracyclin	10.79			R
			17.26	S		

S = Susceptable I-Intermediate R= Resistant

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