Prevalence and antimicrobial susceptibility pattern of *Staphylococcus aureus* isolated from dairy cattle's subclinical mastitis in EL-Sharkia Governorate.

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ABSTRACT

Subclinical mastitis is a disease of major economic importance to dairy industry causing reduced milk quality and loss in its production. Therefore, the present study was carried out for isolation and identification the main pathogen responsible for this disease and detection the drug of choice. A total of 475 milk samples of lactating dairy cows were collected from different localities in El-Sharkia Governorate, and subjected to physical, chemical tests (California mastitis test (CMT) as well as Somatic cell count (SCC), bacteriological examination and antimicrobial susceptibility pattern. The prevalence of subclinical mastitis at cow level was 21% (100/475). Accurately 80 Staphylococcal isolates were identified with prevalence of 61% *S. aureus* through beta hemolysis and coagulase positive. The obtained *S. aureus* isolates were highly sensitive to vancomycin, gentamicin, ciprofloxacin and chloramphenicol with percentages of 93.4%, 83.6%, 82% and 80.3%, respectively. Meanwhile, high frequency of resistance was observed to oxacillin, erythromycin and tetracycline, with percentages of 59%, 55.7%, and 47.5%, respectively. Therefore, the main choice drugs for subclinical mastitis were vancomycin and gentamicin.

Keywords: Subclinical mastitis, California mastitis test, Antibiotic susceptibility.

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1. INTRODUCTION

Subclinical mastitis is a major problem affecting dairy animals all over the world. It causes enormous losses for dairy animals through reduced milk quality, poor product hygiene and undesirable changes in the milk's composition and consequently influences the national income of the country (Kasozi et al., 2014). Subclinical mastitis is characterized by absence visible changes in the milk appearance and is the most prevalent form in dairy herds, detecting by the CMT and increase in SCC (Oliveira et al., 2011). *Staphylococcus aureus* seems to be the predominant organism causing subclinical mastitis (Kader et al., 2002). More than 200 infectious causes of bovine mastitis are known to date and the commonest pathogens in large animals are *Staphylococcus aureus*, *Streptococcus agalactiae*, other *Streptococcus* and *coliforms* (Kader et al., 2002 and Sharma and Maiti, 2010). However, *S. aureus* has emerged as one of the most prevalent pathogens which, once established in the mammary gland is difficult to eradicate (Nickerson et al., 1995). Further *S. aureus* is Gram positive producing smooth, circular colonies, convex and lustrous; size of the colony may be 0.5-1.5 µm in diameter. Under microscope, it appears like irregular three dimensional bunches of grapes like cluster of cells. The colony pigmentation may vary from grey, grey white, grey white

with yellowish to orange shades and in blood agar typical β- hemolysis may be produced; depending on the growth condition (Sushma et al., 2012). *Staphylococcus aureus* is often responsible for intra-mammary infection in bovine and is the main etiological agent of contagious clinical and subclinical mastitis in dairy herds (Gilbert et al., 2006).

The aim of this work directed to isolate and identify the main causative agent from bovine subclinical mastitis in dairy farms at Sharkia Governorate beside detection antimicrobial susceptibility pattern for these isolates.

2. MATERIAL AND METHODS

2.1. Samples collection

Actually, 475 milk samples (360 cows were picked out from four varied dairy farms in addition to 115 individual cases of dairy cows admitted by holders to Clinic of Faculty of Veterinary Medicine, Zagazig University and Animal Health Research Institute, Zagazig) were collected during the period extended from January 2013 until May 2014. These collected samples were aseptically put into sterile screw capped tubes, kept in icebox and transferred to the laboratory.

2.2. Mastitis markers

a) The California Mastitis Test (CMT):

The milk samples were tested by CMT for subclinical mastitis according to Schalm et al., (1971). CMT scored from one to five corresponding to no reaction, trace, mild reaction, moderate reaction, strong reaction, respectively. Cows were considered positive for SM when at least one quarter turned out to be positive for CMT.

b) Somatic cell count (SCC):

The relevant portion of each milk sample was examined for SCC according to Zecconi et al., (2006), using automatic reader (Bently Soma count, U.S.A).

2.3. Bacteriological examination

A loopful of each sample is cultured onto nutrient agar (oxoid) then stained by Gram's stain (Murray et al., 2006); Subculturing onto mannitol salt agar, Baird Parker agar (Baired-Parker, 1997) and blood agar (Cruickshank et al., 1975). Each colony showed typical character of *S. aureus* and Gram positive reaction was subjected to biochemical identification (catalase and coagulase tests).

2.4. Antimicrobials sensitivity tests

Determination of the susceptibility of isolated Staphylococci to some antimicrobial agents was applied by disc diffusion method on Mueller Hinton agar (Barry and Berry, 1985). All isolates of *S. aureus* were tested with 10 different antimicrobial agents: vancomycin (VA), oxacillin (OX), clindamycin (DA), erythromycin (E), gentamycin (CN), ciprofloxacin (CIP), sulphamethaxazol-trimethoprim (SXT), ceftriaxone (CRO), chloroamphenicol (C), tetracycline (TE). Metric ruler was used to measure the diameter of the zone of inhibition according to NCCLs, 2007 and CLSI, 2014.

3. RESULTS

3.1. Incidence of subclinical mastitis in cows:

The current study revealed that out of 475 dairy cattle an overall prevalence of SM based on CMT and SCC was 21% (100/475) from different localities in EL-Sharkia Governorate (Table 1).

3.2. Cultural, morphological and biochemical characters of isolated S. aureus:

Eighty staphylococcus isolates were recovered from all examined subclinical mastitic samples and identified by conventional methods. All isolates grew on mannitol salt agar were Gram positive, non motile, non spore forming and arranged in grape like clusters but only 61/80 gave yellow colonies on mannitol salt agar (ferment salt agar). On Baird Parker medium, black small shiny, convex 1 mm

colonies after 24 hours and large 2.5 mm after 48 hours surrounded by an opalescent ring only in *S. aureus.* Additionally, all isolates were tube coagulase test positive, β-haemolytic, catalase positive and produced characteristic pigments. Moreover, it was noted that the pigmentation on milk agar must not be used as a sole mean for detection of *S. aureus* as10 coagulase positive isolates gave B-haemolysis on sheep blood agar but they gave white pigmentation on milk agar (Table2).

3.3. Antimicrobial sensitivity test:

Antimicrobial sensitivity pattern for 61 *S. aureus* isolates, which recovered from sub clinically mastitic milk samples, revealed that the majority were susceptible to vancomycin, gentamicin, ciprofloxacin, chloramphenicol, with percentages of 93.4%, 83.6%, 82% and 80.3%, respectively. Moreover, moderate sensitivity was observed to trimethoprim sulphathoxazole, clindamycin, tetracycline, with percentages of 65.6%, 60.7%, and 47.5%, respectively. Furthermore, frequent resistance was observed to oxacillin, erythromycin, and tetracycline, with percentages of 59%, 55.7%, and 47.5%, respectively (Table 3).

Table (1): Occurrence of *S. aureus* in milk samples from various localities in El- Sharkia Governorate.

Samples type	Origin of milk sample	Number of milk samples	Number of milk samples analyzed (CMT positive) (%)	Number of samples showing *S. aureus* (%)
Samples from apparently healthy cows	1-Abo Mandoor farm	75	16 (21.33%)	6 (37.50%)
	2-El salhia farm	95	26 (27.37%)	26 (100%)
	3-El Hosania farm	80	5 (6.25%)	0 (0%)
	4-Sami Assad farm	110	10 (9.09)	9 (90%)
	5-Animal Health Research Institute, Zagazig	50	25 (50%)	13 (52%)
	6-Clinic of Faculty of Veterinary Medicine, Zagazig University	65	18 (27.69%)	7 (38.8%)
Total		475	100 (21.05%)	61 (61%)

Table (2): Phenotypical characteristics of staphylococci isolated from subclinical mastitic milk.

Total milk samples	CMT positive	No. of staphylococci grown on to MSA	No. of MSA fermentor isolates	Bacteriological aspect (G+ve, cocci, grape like clusters)	% of pigment production			Baired parker (black colonies)		Catalase test (positive)	β-haemolytic and coagulase positive
					Golden Yellow	White	Lemon Yellow	With clear halo	No halo		
475	100	80 / 100 (80%)	61/80	61/80	51/80	29/80	0/80	61/80	19/80	61/80	61/80

MSA: Mannitol salt agar G+ve: Gram positive

Table (3): Result of antibacterial sensitivity test of 61 *S. aureus* isolates recovered from subclinical mastitic milk samples of cows against different antibacterial agents

Antimicrobial agents	Sensitive	Intermediate	Resistant
	No.	No.	No.
Vancomycin (VA 30µg/ml)	57	0	4
	93.4%	0%	6.6%
Oxacillin (OX 30µg/ml)	16	9	36
	26.22%	14.7%	59%
Clindamycin (DA 2µg/ml)	37	8	16
	60.7%	13.1%	26.2%
Erythromycin (E 15µg/ml)	20	7	34
	32.8%	11.5%	55.7%
Gentamicin (CN 10µg/ml)	51	0	10
	83.6%	0%	16.4%
Ciprofloxacin (CIP 5µg/ml)	50	0	11
	82%	0%	18%
Sulfamethoxazole + trimethoprim (SXT 25µg/ml)	40	5	16
	65.6%	8.2%	26.2%
Ceftriaxone (CRO 30µg/ml)	15	30	16
	24.6%	49.2%	26.2%
Chloramphenicol (C 30µg/ml)	49	1	11
	80.3%	1.6%	18%
Tetracycline (TE 30µg/ml)	29	6	26
	47.5%	9.8%	47.5%

4. DISCUSSION

Mastitis, a complex disease, even at subclinical stage has a major economic impact on the dairy industry (Mir et al., 2014). Staphylococcal mastitis was the commonest and economically the greatest concern wherever dairy farming practiced the chief reservoir of bacterium was an infected udder (Tarfarosh and Purohit, 2008). In this study, the prevalence of subclinical mastitis was 21% (100/475) in El-Sharkia Governorate. This result was nearly near to Abou-Zaid and Bahout, (1993) who reported that the prevalence of subclinical mastitis per animal was 20.6% and 21.2%. This result was somewhat higher than that obtained by Abdel-Rady and Sayed (2009) (19.14%), El- Seedy et al., (2010) (14.5%) and lower than that obtained by EL-Khabaz et al., (2011) (31.6%) and Elhaig and Selim (2015) (71.6 %) in Egypt. Moreover, the incidence of subclinical mastitis differed among countries such as 8.3% in Tanzania (Karimuribo et al., 2005), 74.49% in Bangladesh (Islam et al., 2014) and 87.9% in United States (Kasozi et al., 2014). The difference in prevalence of subclinical mastitis observed between the reports from different parts and the present study could be attributable to differences in farm management practices and husbandry condition in the area and lack of awareness of farmers to the loss caused by mastitis (Radostitis et al., 2000). In this study, phenotypic identification revealed the incidence of 61% *Staphylococcus aureus* isolates 61% (61/100) was isolated from subclinical mastitis; this result was nearly consistent with Gianneechini et al., (2002) and Marija, et al., (2014) with ratio 62.8% and 56%, respectively. On the other hand, this prevalence rate is higher than that recorded in Egypt (Islam et al., 2014; Abdel-Kareem, 2015; Elhaig and Selim, 2015) (11.64%, 17.3%, 38.3 %, respectively); 8.0% in Colombia by Ramírez et al., (2014);

16.6% in Ugandan by Kasozi et al., (2014); 16.9% in China by Yang et al., (2015). Meanwhile this prevalence rate was somewhat lower than that recorded in Egypt by Abd El Tawab et al., (2015) (69%) and recorded in Brazil by Santos et al., (2014) (71.2%). The high incidence of *S. aureus* is indicative of poor hygienic measures during production, handling and distribution, stated in the findings of Zakary et al., (2011). In the current data of antibiogram revealed that vancomycin (toxic glycopeptides), gentamycin are the drugs of choice for isolates as reported previously (Belayneh et al., 2014 and Abdel-Kareem, 2015). Moreover, 59% of *S. aureus* isolates were resistant to methicillin (MRSA) (oxacillin) as reported previously (Tapleton and Tylor, 2002 and Waters et al., 2011). This ratio is highr than Brînda et al., 2010 (52%) while lower than El-Deen, *et. al.*, (2014) and Abdel-Kareem, (2015) with ratio 100%. The obtained methicillin resistance attributed to the inhibition of penicillin binding proteins (PBPs) that are involved in the synthesis of peptidoglycan, an essential mesh like polymer that surrounds the cell, through the expression of a foreign PBPs (PBP2). Allthough several authers recorded absolute susceptability to oxacillin by Wang et al., (2014) and Jahan et al., (2015). MRSA are often multidrug resistant, therefore, infection is either impossible to treat or requires prolonged duration of treatment. In this study, all isolates were moderately susceptible to sulphamethoxazole-trimethoprim, clindamycin (potent lincomycin) and ceftriaxone, which disagreed with Khakpoor et al., (2011) and Wang et al., (2014).

5. CONCLUSION

In light of the results, the present study has focused on the incidence of staphylococcal subclinical mastitis in El-Sharkia Governorate and mentioned that vancomycin, gentamycin ciprofloxacin and chloramphenicol are the drugs of choice for these cases in dairy herds.

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