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Antibiotic Resistance Profile of Coagulase Positive Staphylococcal infection in Dairy Buffaloe

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ABSTRACT

For isolation and identification of the antibiotic resistance profile of Staphylococcal infection derived from buffalo (*Bubalus bubalis*) milk, a total of 100 milk samples have been collected from different villages in Qena governorate, Egypt, these animals were owned by small scale breeding farmers. *S. aureus* formed 23%, 58% other Staphylococci and other pathogens contained19% of the total number of samples. The *S. aureus* isolates have been tested for antibiotic susceptibility against 14 different widely used antibiotics, all *S. aureus* strains were resistant to Nalidixic acid by 100% and the lower percentage of resistance was to Ampicillin by 4.8%. The Multiple antibiotic resistances have been calculated for all *S. aureus* strains, the highest value was 1 and the least one was 0.143. The Buffaloe is considered one of the most important dairy animals in Egypt especially in the small scale breeding and the presence of *S. aureus* and other Staphylococci causing a great risk on the productive level of these animals due to mastitis as well as for the human beings, from here comes the importance of the detection of the antibiotic resistance profile.

Key words: *S. aureus*, Antibiotic susceptibility test, Mastitis

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INTRODUCTION

Subclinical mastitis has no gross pathological changes in the udder and the milk looks apparently normal. The main bacterial agents inducing mastitis are *Staphylococcus aureus, Escherichia coli, Streptococcus uberis, Streptococcus dysgalactiae and streptococcus agalactiae*, which account for around 80% of all diagnostic cases (Bradely, 2002).

The prevalence of *S. aureus* mastitis varies, depends on many factors from country to another, such as the top 17 dairy producing states, USA which accounted for 79.5 % of dairy operations and 82.5 % of U.S. milk cows were infected 43% at the herd level (Lombard et al., 2008), 30% in 112 Holstein herds in Mexico (Miranda-Morales et al., 2008), 52% in bulk tank milk on Prince Edward Island (Riekerink et al., 2006), in Finland decreased from 11.1% in 1995 to 10.1% in 2001 (Pitkala et al., 2001), mastitis and teat canal infections in South African dairy herds increased from 8.1% and 24.1% in 2002, to 15.4% and 30.0% in 2006, respectively (Petazer et al., 2009) and in a similar study was done in the department of animal medicine, South Valley University revealed that the *S. aureus* constitutes only 24 (14.7%) isolates out of 163 *staphylococci* isolates grown on Baird-Parker media and a percentage of (9.7%), which was similar to another study that showed a percentage of 24.8% (El-jakee et al., 2008) and 20.7% (Jakeen et al., 2010) and 21% (Malahat et al., 2010) respectively.

The penicillin significantly decreased the *S. aureus* produces by four major penicillin binding proteins (PBPs), PBP 1 to 4, which catalyze the transpeptidation reaction that cross-links the peptidoglycan of the bacterial cell wall which is essential for the cell wall synthesis, but beta lactam antimicrobials can bind to the original PBP active site and thereby inhibit the cell wall synthesis. However, PBP-2a, which has a reduced affinity for binding with beta-lactam antimicrobials (Hartman and Tomasz, 1984).

The aim of the present study was to estimate the antibiotic resistance profile of coagulase positive Staphylococci in dairy Buffaloe which is considered a very important step to compare the phenotypic and genotypic characterizations of *S. aureus* isolates in further investigations.

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MATERIAL AND METHODS

Sampling

A total of 100 raw milk samples were collected from dairy buffaloes in Qena Governorate, Egypt. The animals are apparently healthy with no local or systemic infection. Samples were immediately subjected to analysis within 24 hours, the milk samples were collected and kept in an ice in sterile single plastic tube (Pamela, 2005).

Isolation and culturing of *Staphylococcus aureus*

Samples were mixed thoroughly with vortex till the complete homogenization of the samples and the preparation of the Baired-Parker media according to Vanderzant and Splittstoesser (1992). 3-4 Typical and large colonies were harvested and picked up by a sterile metal bacteriological loop and then immersed in the glycerol stock in Eppendorf tube and kept immediately at -70 to -80 ºC for further studies (Jones et al.,1991).

Biochemical tests

The coagulase test was performed by two different methods; the slide coagulase test and tube coagulase test (Wichelhaus et al., 1999) and thermostable nuclease test "deoxyribonuclease activity" (Lachia et al., 1971)

Antibiotic susceptibility test

Antimicrobial susceptibility was tested by the single diffusion method according to (Deresse et al., 2012) for S. aureus. The antibiotic discs used with variable concentrations were Nalidixic acid (30 mg), Kanamycin (30 mg), Neomycin (30 mg), Sulfamethoxazole (25 mg), Gentamicin (10 mg), Oxytetracycline (30 mg), Penicillin (10 IU), Chloramphenicol (30mg), Norfloxacin (10mg), Streptomycin (10mg), Amoxicillin (30mg), Ciprofloxacin (5mg), Erythromycin (15mg) and Ampicillin (10mg). They were used to determine the susceptibility of the isolated S. aureus strains (Oxoid Limited, Basingstoke, and Hampshire, UK). Therefore, the antimicrobial susceptibility testing was applied according to the guidelines stipulated by National Committee for Clinical Laboratory Standards "NCCLS" (2001). The tested strains were evaluated as susceptible, intermediate and resistant. MAR index for each strain was determined according to the formula stipulated by Singh et al. (2010) as following:

MAR index= Number of resistance (Isolates classified as intermediate were considered sensitive for MAR index) / Total Number of tested antibiotics

RESULTS

Prevalence of staphylococci

The total prevalence of different staphylococci *and S. aureus* which isolated from a total of 100 raw milk samples collected from dairy buffaloes at farmers' houses which isolated on Baird–Parker agar media were *S. aureus* 23 %, other Staphylococci 58% and other pathogens 19% (Table1).

Biochemical examination

All *S.aureus* isolated from examined raw milk samples showed coagulase positive with a percentage of 100% and D-Nase positive with a percentage of 86.9 % and D-Nase negative with a percentage of 13.04% (Table 2).

Antibiotic sensitivity tests

The frequency of different levels of resistance to various antibiotics among *S. aureus* isolates are shown in Table 3, the highest resistance level was recorded for the Nalidixic acid (100%) and the lower one was for Ampicillin (4.8%) and the antimicrobial resistance profile of different *S. aureus* isolates are shown in Table 4.

Table1. Incidence of *S. aureus* and other Staphylococci isolated from Buffaloe's milk in Qena, Egypt

Number of milk samples	S. aureus	Other Staphylococci	Other Pathogens
100	23(23%)	58(58%)	19(19%)

Table 2. Biochemical reactions of *S.aureus* isolated from Buffaloe'smilk in Qena, Egypt

Number of S. aureus	Coagulase Test		D-Nase Test	
Twenty three	Positive	Negative	Positive	Negative
	23 (100%)	0 (0%)	20 (86.9%)	3 (13.4%)

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Table 3. Antimicrobial susceptibility of *S. aureus* isolated from Buffaloes' milk in Qena, Egypt

Antimicrobial agent	S	I	R
Nalidixic acid (30 mg)	-	-	21(100%)
Kanamycin (30mg)	-	1(4.8%)	20(95.2%)
Neomycin (30mg)	1(4.8%)	-	20(95.2%)
Sulfamethoxazole (25mg)	1(4.8%)	2(9.5%)	18(85.7%)
Gentamicin (10mg)	2(9.5%)	2(9.5%)	17(80.9%)
Oxytetracycline (30mg)	3(14.3%)	1(4.8%)	17(80.9%)
Penicillin (10 IU)	3(14.3%)	4(19.0%)	14(66.7%)
Chloramphenicol (30mg)	5(23.8%)	3(14.3%)	13(61.9%)
Norfloxacin (10mg)	6(28.6%)	4(19.0%)	11(52.4%)
Streptomycin (10mg)	8(38.1%)	2(9.5%)	10(47.6%)
Amoxicillin(30mg)	9(42.9%)	6(28.6%)	6(28.6%)
Ciprofloxacin (5mg)	14(66.7%)	2(9.5%)	5(23.8%)
Erythromycin (15mg)	16(76.2%)	2(9.5%)	3(14.3%)
Ampicillin (10mg)	19(90.5%)	1(4.8%)	1(4.8%)

S: susceptible, I: intermediate, R: resistant

Table 4. Antimicrobial resistance profile of *S. aureus* strains isolated from Buffaloes' milk in Qena, Egypt

No.	Id. Strain	Antimicrobial resistance profile	MAR index
1	*S. aureus*	NA,K,N, SXT, G, T, P, C, NOR,S, AMX, CP, E, AM	1
2	*S. aureus*	NA,K,N, SXT, G, T, P, C, NOR, S, AMX, CP, E	1
3	*S. aureus*	NA,K,N, SXT, G, T, P, C, NOR, S, AMX, CP, E	0.928
4	*S. aureus*	NA,K,N, SXT, G, T, P, C, NOR, S, AMX, CP	0.928
5	*S. aureus*	NA,K,N, SXT, G, T, P, C, NOR, S, AMX, CP	0.857
6	*S. aureus*	NA,K,N, SXT, G, T, P, C, NOR, S, AMX,	0.857
7	*S. aureus*	NA,K,N, SXT, G, T, P, C, NOR, S	0.786
8	*S. aureus*	NA,K,N, SXT, G, T, P, C, NOR, S	0.714
9	*S. aureus*	NA,K,N, SXT, G, T, P, C, NOR, S	0.714
10	*S. aureus*	NA,K,N, SXT, G, T, P, C, NOR, S	0.714
11	*S. aureus*	NA,K,N, SXT, G, T, P, C, NOR	0.643
12	*S. aureus*	NA,K,N, SXT, G, T, P, C	0.571
13	*S. aureus*	NA,K,N, SXT, G, T, P, C	0.571
14	*S. aureus*	NA,K,N, SXT, G, T, P	0.500
15	*S. aureus*	NA,K,N, SXT, G, T	0.500
16	*S. aureus*	NA,K,N, SXT, G, T	0.500
17	*S. aureus*	NA,K,N, SXT, G, T	0.500
18	*S. aureus*	NA,K,N, SXT	0.428
19	*S. aureus*	NA,K,N	0.286
20	*S. aureus*	NA,K,N	0.286
21	*S. aureus*	NA	0.143
Average			**0.598**

DISCUSSION

Using culture method identified about 23% of examined samples infected with *S. aureus*. These results were higher than 14% (Abdel et al., 2004), while were in accordance with 22.9% (Türkyilmaz et al., 2010) and 24.8% (El-Jakee et al., 2008) but were lower than 44.4% (Mahmoud et al., 2008).

Identification of these isolates was performed using biochemical (phenotypic) methods, 100% of these isolates showed positive for coagulase test and 86.9% for D-Nase test. Present results were higher than the report of Abdel et al. (2010), that recorded 33.55% of isolates were positive for coagulase test and 32.06 % were positive for D-Nase test while the present study results were some extent close to Mohamed (2012) as recorded 98.5% of isolates were positive for coagulase test. El-Jakee et al. (2008) reported that the production of coagulases and thermonuclease were not unique features of *S. aureus* but were shared by *S. intermedius* and *S. hyicus.* The prevalence of subclinical mastitis caused by *S. aureus* were studied by many investigators, the obtained result came in coincides with the results of Fox and Gay (1993) as they stated that *S. aureus* caused mastitis in cattle ranged from 7-40 % of total number of animals, while it was lower

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for high extent than that recorded by Janosi and Balty (2004) which was 80%. The antibiotic sensitivity testing of the isolated strains (n= 21) to various antibiotics shown in Table 3 and 4. The results of penicillin (66.7%) were similar to Moronie et al. (2006) 69.1% and higher than Gentilini et al. (2002) 40% but less than Abera et al. (2010) 94.4%. The results of erythromycin 14.3% were similar to 15.7% (Mohamed, 2012), but lower than 40.9% (Ghaleb, 2006). The results of amoxicillin 28.6% were similar to 34.2% (Mohamed, 2012) but less than 81.3% (Klimien et al., 2011).

CONCLUSION

Subclinical mastitis of *S. aureus* was common in dairy buffaloes in Qena Governorate affecting milk yield (quality and quantity) and consumer safety. Culturing method and biochemical tests were not enough for detection of *S. aureus* subclinical mastitis but it is considered an important preliminary step but it needs further confirmation for detection of MRSA strain which acquire the mecA gene that responsible for the beta-lactam resistance.

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