

## Prevalence of Methicillin Resistance *Staphylococcus aureus* in Cattle and She-camels Milk at Al-Qadisiya Province

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### Abstract

This study is conducted to investigate the methicillin resistant *Staphylococcus aureus* (MRSA) in the cattle and she-camels milk. In which the number of examined milk samples in cattle and she-camels was (400, 360) with percentage of *S. aureus* (19.4%, 13.4%) respectively. Actually the subclinical mastitis widely distributed (59%, 43.3%) with the methicillin resistant isolates (39.1%, 0%) respectively in the both species. Furthermore the antibiotics sensitivity test reveals high susceptibility of this pathogens to cefoxitin and streptomycin and to several antibiotic types in the she-camels isolates.

انتشار المكورات العنقودية المقاومة للمثيسيلين في حليب الابقار والنوق في محافظة القادسية

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### الخلاصة

هدفت الدراسة الحالية للكشف عن جراثيم المكورات العنقودية المقاومة للمثيسيلين في حليب الابقار والنوق، اذ تم فحص (400، 360) عينة لكلا النوعين قد شخصت نسبة التهاب الضرع فيهما (59، 43.3)% وعلى التوالي، وقد بلغت نسبة الاصابة بتلك الجراثيم (19.4، 13.4)%، بينما بلغت نسبة الجراثيم المقاومة للمثيسيلين (39.1، 0)% وعلى التوالي. لقد اظهر فحص حساسية المضادات الحيوية فعالية المضاد سيفوكستين وستربتومايسين في تثبيط العزلات الجرثومية لحليب الابقار بينما كانت العزلات الجرثومية لحليب النوق حساسة لجميع المضادات الحيوية.

### Introduction

Mastitis is considered one of the most important dairy cattle diseases and antibiotics use (1). The *S. aureus* is well known as causative agent of cattle and she-camels mastitis and characterized by acute, chronic, contagious and subclinical in nature (2, 3). Treatment of infections caused by *S. aureus* has become more difficult due to development of antimicrobial resistance, recently the MRSA widely distributed in all world with the risk of resistant to all beta-lactam antibiotics and other groups of antimicrobials therefore the therapeutic choices are limited significantly (4). The occurrence of MRSA in herds will develop a delay in the treatment of animal and human diseases with spreading in environment, therefore it has great zoonotic importance (5, 6, 7), therefore the aim of this study is to investigate the occurrence of MRSA in cattle and she-camels milk by using routine techniques, new chromogenic media and assess their antibiotics susceptibility to develop effective control strategies for this pathogens.

### Materials and Methods

This study includes collection of 400, 360 milk samples of cattle and she-camels taken from different farms in Al-Qadisiya province under aseptic condition, the California mastitis test composed of Alkyl Aryl sulfonate 3%, sodium hydroxide 1.5% and bromocresol purple as an indicator is done on milk samples to identify the subclinical cases from the health ones while the clinical cases had been excluded from this study, the positive

isolates are examined in laboratory of veterinary hospital in Al-Qadisiya by inoculation and culturing at 37° for 24h on the CHROMagar Staph medium (CAS) then *S. aureus* specific colony is streaked in quadrant manner on MRSA CHROMagar medium at same laboratory conditions, its composition in g/L (agar 15; peptone and yeast extract 40; salts 25; chromogenic mix 2.5 aren't described by the manufacturer; PH: 6.9) (CHROMagar, France), all these was done after sub culturing and identification by conventional tests which includes (oxidase, coagulase, catalase, mannitol, sucrose, D. trehalose, gelatinase, urease, phosphatase, nitrate reductase), the MRSA isolates found are evaluated with antibiotics sensitivity test according the Kirby-Bauer Method by using four antibiotics which ordinarily used for treatment of cattle, she-camels and human diseases, these antibiotics are: oxacillin (Ox-1), cefoxitin (Fox30), penicillin G (Gp-10) and streptomycin (S10), its potency are (1µg, 30µg, 10 unit and 10µcg) respectively and the standard zone of inhibition are (≥ 13, 22, 29, 15 mm) respectively (Difco, USA) (8).

### Results

The California mastitis test reveals in cattle and she-camels 236, 156 infected samples from total of 400, 360 milk samples respectively, these results show a significant differences ( $P < 0.01$ ) in the subclinical mastitis between the two types, but the infection percentage with *S. aureus* pathogens isn't significant ( $P > 0.05$ ) (Table 1). The *S. aureus* observed on (CAS, MRSA) culturing mauve and rose in color. The percentage of methicillin resistance *S. aureus* is 39.1% in mastitic cattle while there is no resistance compared with she-camels ( $P < 0.01$ ), furthermore the conventional microbiological tests confirmed these isolates and biochemically it was negative to oxidase but positive to coagulase, catalase, mannitol, sucrose, D. trehalose, gelatinase, urease, phosphatase and nitrate reductase. Additionally, it has characteristic growing colonies in blood agar ( $\beta$ -hemolysis), mannitol salt agar, DNase and SM110 media. The antibiotics sensitivity test detect high susceptibility to cefoxitin and streptomycin (80.4, 78.2)% in cattle respectively, while in she-camels there is high susceptibility to the all antibiotics without any resistance to oxacillin and penicillin G (Table 2).

**Table (1) Show the percentage rate of infection with *S. aureus***

No. of examined quarters		Subclinical mastitis			The infection					
					<i>S. aureus</i>			methicillin resistance		
		+	-	%	+	-	%	+	-	%
Cattle	400	236	164	59**	46	190	19.4	18	28	39.1**
		She-camels	360	156	204	43.3	21	135	13.4	0
Chi <sup>2</sup> values		X <sup>2</sup> <sub>tab.</sub>		X <sup>2</sup> <sub>cal.</sub>	X <sup>2</sup> <sub>tab.</sub>		X <sup>2</sup> <sub>cal.</sub>	X <sup>2</sup> <sub>tab.</sub>		X <sup>2</sup> <sub>cal.</sub>
		6.6		18.4	3.8		2.2	6.6		11.2

**Table (2) Show the susceptibility of *S. aureus* to antibiotics**

Antibiotics	cattle				She-camels			
	R	I	S	%	R	I	S	%
Ox-1	18	3	25	54.3	0	0	21	100
Fox30	6	3	37	80.4	0	0	21	100
Gp-10	24	0	22	47.8	2	0	19	90.4
S10	9	1	36	78.2	4	0	17	80.9

### Discussion

The isolation of *S. aureus* pathogens from raw cattle and she-camels milk has been described from different parts of the world namely Saudi Arabia (9), Jordan (10, 11), Egypt (12) and Morocco (13). In the present study the incidence of subclinical mastitis in cattle and she-camels is relatively comparative with those reported by (14, 15) (80, 36)% respectively these may be due to differences of geographical regions. The prevalence of

subclinical mastitis in cattle and she-camels differs significantly ( $P < 0.01$ ), these results may be due to feature of the anatomical structure of the she-camels udder teats, glands cisterns which are separated entirely with very narrow twin duct streak canals. Furthermore the udder didn't touch the earth even the recumbent of the animal this act as an important mechanical prevention to infection (16), However, many researchers demonstrated that she-camels milk contains inhibitors to pathogenic bacteria these includes: Lysozyme, immunoglobulins, lactoferrin and lactoperoxidase, these proteins involved with higher concentration in she-camels milk than in cattle milk (17, 18, 19, 20, 21), As well as there is another difference related to nature environments between cattle and camels which characterized by less contaminants than cattle environment (22). The percentage of MRSA isolated from cattle higher than 10.2% which reported by (23) these differences may be due to variation of the geographical regions. The causes of antibiotic resistance is explained by several authors reported numerous resistance methods such as: antibiotic-resistant genes, mutation, clonal evolution and plasmid transfer, target site alteration of ribosome, metabolic pathway alteration, efflux pumps and enzymatic cleavage of antibiotics (23, 24), recently (25) has discussed the causes of these resistance as a results of a protein (penicillin-binding protein 2a[PBP2a]). The results have also revealed high susceptibility and absence of antibiotics resistance in *S. aureus* isolated from she-camels milk, actually recent studies have showed absence of *mecA* gene responsible for MRSA and rare of antibiotics resistance related genes in the *S. aureus* isolated from milk of Arabian she-camels (26, 27), (28) demonstrate a small, soluble single-domain fragments derived from the unique variable region of dromedary heavy-chain antibodies which act as inhibitors to  $\beta$ -lactamase. this state arise from the camels rearing system in the desert regions of Iraq which leads to great decline in the therapeutic medication of the common bacterial infections. CHROMagar (CAS, MRSA) give best results for the detection of *S. aureus* and methicillin resistance pathogens comparatively with the conventional techniques, these results confirm data reported by (29). (30) has showed that primary streaking on (CAS) provides a convenient with less time consuming manner for the presumptive detection of *S. aureus* in routine clinical microbiology. (31) explained the very high accuracy of the use (CAS) medium and another author detected high sensitivity and specificity of the use (MRSA) medium (32). Conclusions, The high prevalence of antibiotic resistance suggests that subclinical mastitis in cattle plays a significant role in the spreading of multi-resistant staphylococci and represent a great public health concern in Iraq.

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