



The correlation between coagulase production and DNase, phosphatas, type of hemolysis produced by mastitis *Staphylococcus aureus* isolates and its susceptibility to some antibiotics

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Abstract

This study was performed to investigate the ability of *Staphylococcus aureus* isolated from bovine mastitis to produce free and bound coagulase and its relation with the production of phosphatase, DNase and type of haemolysis., also study the susceptibility pattern of *Staphylococcus aureus* isolates against some antibiotics. 210 milk samples were collected from cows suffering from acute and subclinical mastitis from Al-Nasser station of cow in Al Saweraa` city, and Zobaa` village and Al-Radwanian in Abu-Graib zone. Diagnosis of the isolates which were carried out according to cultural, microscopical, biochemical tests and Api staph system showed that 74 of these bacterial isolates belong in its characteristics to *Staphylococcus aureus* represented 35.23% from milk samples. The results showed that all 74 isolates which gave positive tube and slide coagulase test were gave also positive results for phosphatase and haemolysis, while 67 isolates (90.54%) gave positive results for DNase test. The susceptibility test showed that *Staphylococcus aureus* isolates were a height resistance for penicillin, lincomycin and cloxacillin, while highest sensitivity for ciprofloxacin, gentamycin and sulphamethoprime.

Keyword: *Staphylococcus aureus*, Coagulase test, Mastitis, DNase.

Introduction

In veterinary medicine, *Staphylococcus aureus* is one of the most frequently isolated contagious pathogen causing clinical or subclinical mastitis (Gruet *et al.*, 2001; Kerro Dego *et al.*, 2002). Bovine mastitis is an inflammation of mammary glands usually due to a microbial infection (Watts, 1988), and the most important cause of losses in the dairy industry. These losses are mainly due to low milk yield, low milk quality, and high production costs, Although several bacterial pathogens can cause, *Staphylococcus aureus* is the main agent responsible for contagious bovine mastitis, and is very difficult eradicate given the wide range of virulence factors that contribute to the ability of the bacteria to survive in the host (Nickerson *et al.*, 1995). *Staphylococcus aureus* produces coagulase, an extracellular enzyme that bind to protein to form a complex with thrombin-like activity which converts fibrinogen to fibrin (koneman *et al.*, 1992; McDevitt *et al.*, 1992). Coagulase production is one of the most reliable criteria for the identification of *Staphylococcus aureus* (Sperber and Tatini, 1975; koneman *et al.*, 1992), thought to have a role in cellular attachment. This is important given that attachment is one of the first steps in the

pathogenesis of infection (Archer, 1998).

DNase and coagulase test both use for detecting Pathogensity, notice that (90-95%) of coagulase positive strains are produced by DNase (Rayman *et al.*, 1975). In addition DNase test used for diagnosis of *Staphylococcus aureus* which is isolated from bovine mastitis (Bergh and Mealand, 1986). Additionally, *Staphylococcus aureus* produce a wide variety of exoproteins, including hemolysins that contribute to their ability to colonize and cause disease in mammalian hosts. Alpha hemolysin is the most studied of the *Staphylococcus aureus* cytotoxins (Dinges *et al.*, 2000). Beta toxin is a sphingomyelinase expressed by most strains isolated from bovine intramammary infections, but rarely by human isolates (Cifrian *et al.*, 1996). It has been reported that both toxins increase *Staphylococcus aureus* adherence to mammary epithelial cells (Caiazza and Toole, 2003). The continuous emergence of methicillin-resistant *Staphylococcus aureus* (MRSA), glycopeptide-insensitive *Staphylococcus aureus* (GISA), and vancomycin-resistant *Staphylococcus aureus* (VRSA) strains have made it difficult to treat *Staphylococcus aureus* infections (Kozielec *et al.*, 2009).

In human medicine, antimicrobial multi-resistance is frequently encountered and methicillin-resistant *Staphylococcus aureus* (MRSA) (de Neeling *et al.*, 1998; McBryde, 2004) and methicillin-resistant CNS (MR-CNS) (de Neeling *et al.*, 1998) strains are among the most threatening bacteria involved in nosocomial infections. In veterinary medicine, MRSA as well as multi-resistant *Staphylococcus aureus* strains are reported occasionally (Lee, 2003; Seguin *et al.*, 1999).

The aim of this study was to investigate the ability of *Staphylococcus aureus* which was isolated from bovine mastitis to produce free and bound Coagulase and its relation with DNase, phosphates and type of haemolysis.

Materials and Methods

Sampling: A total of 210 milk samples were collected from Al-Nasser station in Al-Sawera city, Al-Radwania and Zobia in Abu-Graib zone from cows suffering from clinical and subclinical mastitis. The udder was washed directly with tap water to remove dirt then dry with clean towel, the teat dip in Iodine solution 1:1000 and leave to dry then the teat was dip in 70% alcohol then dry, before sample taken one or two streams of milk discarded. Milk was collected in sterile vial (test tube 10ml) then the collected samples were transported to the laboratory in cooled container.

Identification of *Staphylococcus aureus*:

Cultural and microscopical characteristics: The shape and color of the colonies in addition to the type of haemolysis observed after plating the samples on blood and nutrient agar, the suspected isolates were cultured on mannitol salt agar by streaking method to differentiate between *Staphylococcus* species. Gram stain also done and examined by using light microscope.

Biochemical, sugar fermentation tests and RapID™ STAPH PLUS System tests were used to complete the diagnosis of *Staphylococcus aureus* isolates.

Study of some virulence factors: Slide coagulase test was done according to Coles (1986), while tube coagulase test was performed according to Sperber and Tatini (1975). The ability of these isolates to produce Phosphatase, DNase was performed according to Cruickshank *et al.* (1975)

Antibiotic susceptibility test: To study the susceptibility of the isolates against nine different antibiotics (ciprofloxacin, gentamycin, sulphamethoprim, chloramphenicol, ampicillin, cloxacillin, tetracycline, penicillin, lincomycin)

mueller-hinton agar according to Bauer-Kirby *et al.* (1966), the results were recorded as resistant or susceptible by the measurement of the inhibition zone diameter in milliliter.

Results and Discussion

Identification of the isolates: Identification of the isolates which were carried out according to cultural, microscopical, biochemical tests and Api staph system showed that 74 of these bacterial isolates belong to *Staphylococcus aureus* represented 35.23% from milk samples. According to Sutra and Poutrel (1994) *Staphylococcus aureus* is a ubiquitous micro-organism that is responsible for a wide range of both acute and chronic infections in humans and animals. In animals, *Staphylococcus aureus* is frequently associated with intramammary infections in lactating females, which cause substantial economic losses in dairy production.

The results of the virulence factors:

Coagulase test: Results showed that all isolates (100%) were positive for coagulase test. The slide coagulase test was revealed by the appearance of agglutination particles indicated that this bacteria had the ability to produce bound coagulase (clumping factor) enzyme which cause clotted of rabbit plasma, while the positive results of the tube coagulase test were obtained by the formation of clotted materials in the tube after four period of incubation 1, 3, 6 and 24hrs according to severity degree of pathogenic *Staphylococcus aureus* isolate (Table 1), the particles of agglutination indicated that the *Staphylococcus aureus* had the ability to produce free coagulase enzyme that clotted rabbit plasma (Figure 1). This test was used to detect free coagulase.



Figure (1): Tube coagulase test for *Staphylococcus aureus*

Table (1): Production of coagulase in tube method on different periods.

Total <i>Staphylococcus aureus</i> isolates	Time of incubation							
	1hr		3hrs		6hrs		24hrs	
	No.	%	No.	%	No.	%	No.	%
74	30	22.2%	10	13.5%	9	12.16%	25	33.8%

Although there are a few reports which indicated *Staphylococcus aureus* strains that give negative results in the tube coagulase test (TCT), this method is still generally considered as the gold standard for distinguishing between coagulase-positive and coagulase-negative *staphylococci* in the clinical microbiology laboratory (Selepak and Witebsky 1985).

The coagulase test correlates with pathogenicity. As some pathogenic *staphylococci* can be negative to the slide coagulase test but positive to tube coagulase test, while *Staphylococcus aureus* are usually haemolytic and often produce both the alpha-lysin and so exhibit double-haemolysis (Quinn, 2002).

Deoxyribonuclease test (DNase production): The result of the flooding of the plates with 1N HCl lead to the precipitate of the DNA and turns the plates cloudy. The appearance of clear zone around the growth spot indicated DNase production, result showed that 67 isolates (90.54%) were positive for DNase test while the remains (7 isolates) gave negative result for this test.

DNase test is not entirely reliable as an indicator to pathogenicity because it has been estimated that about 18 percent of coagulase-negative staphylococci have DNase activity (Quinn, 2002).

Phosphatase test: After incubation the plate of phosphatase agar, filter paper saturated with ammonia were added to the medium, all isolates were gave positive result for this test which indicated by the color of the colonies and around its which turn to the pink or red.

According to Baird-Parker,(1963); Barber and Kuper (1951); Barnerb and Morris, (1957);

phosphatase activity has often been evaluated as a test for identifying staphylococci. Originally, the assay of this enzyme was used for separating *Staphylococcus aureus* from other staphylococci, since it was thought that only the former possessed phosphatase activity (Barner and Morris, 1957; White and Pickett, 1953). Later, it was shown that a significant percentage of non-*Staphylococcus aureus* staphylococci also carry this enzyme (Baird-Parker, 1963, Barber and Kuper, 1951; Pennock and Huddy, 1967).

Haemolysis activity: The haemolysis activity was found in all 74 isolates (100%) (Table 2), the haemolysis activity was investigated by culture the bacteria on blood agar base supplied with sheep blood agar for alpha (α), beta (β) haemolysis.

Beta-toxin is one of the haemolysin produced by *Staphylococcus aureus*, and works as a sphingomyelinase C, degrading sphingomyelin in the outer layer of eukaryotic cell membranes (Bayles *et al.*, 1998). According to Aarestrup *et al.*, (1999) and Larsen *et al.* (2002) a high percentage (75–97%) of *Staphylococcus aureus* strains isolated from bovine mastitis produce beta-toxin, whereas only 10–15% of human isolates express this factor. However, some studies have suggested that this toxin is important in intra-mammary infection.

The results of antibiotic susceptibility test: All *Staphylococcus aureus* isolates were sensitive for ciprofloxacin, cloromphencol, (trimethoprin + sulphamthozole), gentamycin, while the isolates was resistant to tetracycline, ampicillin, lincomycin, cloxacillin, pencillin (Table 3 and Figure 2).

Table (2): The haemolytic activity of *Staphylococcus aureus*

β haemolysis		Type of haemolysis				Total <i>Staphylococcus aureus</i> isolates
		α and β haemolysis		α haemolysis		
No.	%	No.	%	No.	%	
20	27%	44	59.45%	10	13.5%	74

Table (3): The results of antibiotic susceptibility test of *Staphylococcus aureus* against nine of antibiotics.

Antibiotics	Sensitive		Resistance	
	No.	%	No.	%
Ciprofloxacin	74	100%	0	0%
Gentamycin	67	90.5%	7	9.45%
Sulphamethoprime	64	86.48%	10	13.5%
Chloromphincol	35	47.29%	39	52.7%
Ampicillin	28	37.8%	46	62.16%
Cloxacillin	0	0%	74	100%
Tetracycline	8	10.8%	66	89.18%
Pencillin	0	0%	74	100%
Lincomycin	0	0%	74	100%

Figure (2): Antibiotic susceptibility test for *Staphylococcus aureus*

There seem to be regional differences in the resistance patterns of *Staphylococcus aureus* (Myllys *et al.*, 1998; Salmon *et al.*, 1998; de Oliveira *et al.*, 2000; Orchoa-Zarzosa *et al.*, 2008), but in most countries penicillin resistance is the commonest form of antimicrobial resistance. Based on the results of previous studies from Argentina, Belgium, Brazil, Finland, Germany, Great Britain (England), Ireland, Portugal, Switzerland, the United States and Uruguay (Devriese *et al.*, 1997; Gentilini *et al.*, 2000; de Oliveira *et al.*, 2000; Erskine *et al.*, 2002; Giannechini *et al.*, 2002; Makovec and Ruegg, 2003; Tenhagen *et al.*, 2006; Nunes *et al.*, 2007; Rabello *et al.*, 2007), approximately one-third to two-thirds of bovine *Staphylococcus aureus* isolates were resistant to penicillin and these agreed with our results which showed that all *Staphylococcus aureus* isolates were also resistant to penicillin.

Staphylococcal resistance to penicillin is mediated by penicillinase or a form of β -lactamase production. Penicillinase-resistant penicillin such as

methicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin and flucloxacillin are able to resist degradation by staphylococcal penicillinase found that important finding MRSA isolates reveals that resistant to nearly all antibiotics, sensitive to oxacillin and vancomycin. This is due to the fact that MRSA is often multidrug resistant (Mehta *et al.*, 1998; Udaya *et al.*, 1997).

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