

Slime Production by *Staphylococcus aureus* Strains Isolated from Cases of Bovine Mastitis

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Abstract

The objective of the present research was to determine the frequency of slime production by *Staphylococcus aureus* strains recovered from bovine mastitis and comparison of slime formation frequency, depending on the determination procedure employed. The investigations embraced 59 *Staphylococcus aureus* strains obtained from the inflammatory secretion of mammary glands of 45 cows. Slime production was determined using Christensen method and Congo red agar (CRA) method. Out of the 59 *S. aureus* isolates, 47.45% produced slime as shown by Christensen method and 42.37% by the CRA method. However, 7 strains (11.86%) demonstrated slime production ability only when tested by the Christensen method, whereas 4 strains (6.77%) only using the CRA method.

Key words: *Staphylococcus aureus*, cow, mastitis, slime production

The mammary gland inflammation induced by *Staphylococcus aureus* is characterized by very high somatic cell count (SCC), decreased milk yield and low therapy success rate. There are estimates that 19–41% of all cows are infected by this pathogen (Nickerson, 1993b). Initially, *Staphylococcus aureus* damages the tissues lining the teat and gland cisterns to move up into the duct system and establishes deep-seated pockets of infection in the alveolar tissue (Nickerson, 1993a; 1993b). One of the most important factors accounting for *S. aureus* pathogenicity is its capacity for forming glycocalyx, that is slime. Glycocalyx is a polysaccharide covering (biofilm) on the surface of cells. The glycocalyx is associated with a number of important functions, such as protecting the bacterial surface against chemical and mechanical damages. Full synthesis of slime requires the expression of the *icaA* and *icaD* genes (Gad *et al.*, 2004; Szymankiewicz *et al.*, 2004).

The objective of the present research was to determine the ability of *Staphylococcus aureus* strains isolated from bovine mastitis cases to produce slime and to compare the frequency of slime formation ability detection depending on the protocol used.

The studies were done on 59 *S. aureus* strains obtained from the inflammatory secretion of the mam-

mary gland of 45 cows from 12 farms. All strains were subcultured on sheep blood agar to check their macro- and microscopic (by Gram-staining) morphology and their hemolytic activity. The isolates were identified as *S. aureus* using conventional biochemical tests: detection of catalase and coagulase, as well as latex agglutination assay, which detects clumping factor (Slidex Staph Kit, bioMerieux Poland Ltd).

Strains of *S. aureus* were tested for the ability to form slime by the modification of the standard macrotube method described by Christensen *et al.*, 1982; Christensen *et al.*, 1985; Freeman *et al.*, 1989 and Szymankiewicz *et al.*, 2004. Shortly, one colony from 20-hour incubation on the trypticase soy agar plate (bioMerieux Poland Ltd) was added to 5 ml trypticase soy broth (TSB, bioMerieux Poland Ltd) and incubated for 48 hours at 37°C without shaking. The content was then decanted and 1 ml volume of a 0.1% aqueous solution of safranin (POCH S.A.) was added. Each tube was then gently rotated to ensure uniform staining of any adherent material on the inner surface than the liquid was gently removed and the tubes were then placed upside down to drain. A positive result was indicated by the presence of an adherent layer of stained material on the inner surface

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of the tube. The presence of stained material at the liquid-air interface alone was not regarded as indicative of slime production. Slime formation intensity was not quantitatively evaluated.

Slime production ability assessment by Congo red agar (CRA) method was performed according to Freeman *et al.* (1989) and Szymankiewicz *et al.* (2004). The medium was composed of brain heart infusion broth (Oxoid Ltd) 37 g/l, sucrose 50 g/l, agar No 1 (Oxoid Ltd) 10 g/l and Congo red (POCH S.A.) 0.8 g/l. Congo red stain was prepared as a concentrated aqueous solution and autoclaved (121°C for 15 minutes) separately and added to the growth medium cooled to 55°C. One colony of a studied isolate from 18–20 h growth on the trypticase soy agar (TSA, bioMerieux Poland Ltd) was spread on Congo red agar plate and incubated aerobically for 24 hours at 37°C. A positive result was indicated by black colonies with a dry crystalline consistency. Non-slime producers usually remained pink.

The differences between both screening methods (macrotube vs CRA) were compared using the proportion test u_0 and the results are presented in Tables. A $p \leq 0.05$ was considered significant.

Table I
Slime production by *S. aureus* isolates

Strain	Number (n)	Slime-producing strains in			
		Christensen method		CRA method	
		n	%	n	%
<i>S. aureus</i>	59	28	47.45	25	42.37

The results of examinations of slime-forming ability of the *S. aureus* strains recovered from the cases of bovine mastitis are given in Table I and II. Out of the 59 *S. aureus* isolates, 47.45% produced slime as shown by the macrotube method and 42.37% by the plate test (CRA method). The comparative analysis performed with the test u_0 concerning frequency of slime-producing strain incidence subject to a procedure employed, revealed that both experimental methods demonstrate similar sensitivity. Still, 7 strains (11.86%) were found to produce slime only when the Christensen method was used (Christensen *et al.*, 1982; Christensen *et al.*, 1985) while 4 strains (6.77%) only with the Congo red agar method. In total, 32 strains showed the capacity to form slime.

The beginning of the biofilm science and engineering was associated with the industrial fluid handling system, e.g. water pipes. Sediments and biological pollutants that routinely foul the water pipeline system make a good example of naturally occurring biofilms (Górski and Palmer, 2007). Bacterial biofilm consists of organized slimy clusters of bacteria adhered to the inner surface area of the live organisms, like mucous membranes, blood vessel and lymphatic endothelium or the surface of medical and veterinary devices. Biofilm, also called a biological membrane, does not assume the form of amorphous sediment but resembles a highly structured complex. It contains slime and bacteria which surround themselves with the complex polymeric matrix they secrete. The slime helps the bacteria embedded in it to be protected from the attack of medicines as well as phagocytosis. It inhibits chemotaxis of granulocytes and their opsonic activity, thus affecting an inflammatory response (Dorocka-Bobkowska and Konopka, 2007; Fox *et al.*, 2005; Górski and Palmer, 2007; Szymankiewicz *et al.*, 2002; Szymankiewicz *et al.*, 2004). Numerous authors state that bacteria in a biofilm are 10 to 1000-fold more resistant to antibiotics than in their planktonic form (Górski and Palmer, 2007; Melchior *et al.*, 2006a; 2006b). For clinicians, a statement of a practical value is that the administration of antibiotics at therapeutic doses results in eradication of bacteria on the outside of the biofilm surface, while those on the inside do survive an antibiotic attack and provide material for further growth (Górski and Palmer, 2007).

S. aureus belongs to the major pathogen group of bovine mastitis and along with mycoplasmas and *S. agalactiae* is classified as contagious pathogens. This pathogen can severely damage the bovine mammary gland as it secretes toxins that destroy the secretory epithelial cells (Nickerson, 1993b). The study results presented by Melchior *et al.* (2006a) and Cucarella *et al.* (2001; 2004) indicate that *S. aureus* recovered from mastitis cases is highly resistant to the antimicrobial agents when it grows in a biofilm. Baselga *et al.* (1993) found that the slime-producing strains (SP) showed a higher colonization capacity, whereas the non-slime-producing (NSP) higher virulence. Vasudevan *et al.* (2003) noted that *ica* genes associated with bacterial biofilm formation were present in a total of 35 isolates of *S. aureus* obtained

Table II
Comparison of slime production by *S. aureus* by two methods

Strain	Number (n)	Slime-producing strains							
		Only in Christensen method [a]		Only in CRA method (b)		In both (c)		Total (a + b + c)	
		n	%	n	%	n	%	n	%
<i>S. aureus</i>	59	7	11.86	4	6.77	21	35.59	32	54.23

from bovine mammary gland inflammatory secretion. However it is noteworthy that, in vitro the biofilm was produced by only 24 strains, while the slime on Congo red agar (CRA) by 32 strains. Fox *et al.* (2005) decided to test their hypothesis that *S. aureus* isolated from bovine milk (chosen randomly) produces a biofilm more frequently than the *S. aureus* strains obtained from teats (chosen randomly, too) as well as from the milking devices. The research results have supported this hypothesis: 41.4% of *S. aureus* recovered from bovine milk formed a biofilm, while from the teats – 24.7% and the milking equipment – 14.7%.

The present results are comparable to those presented by other authors (Fox *et al.*, 2005; Oliveira *et al.*, 2006) as well as to the findings from the studies carried out on slime formation by *S. aureus* isolated from humans (Szymankiewicz *et al.*, 2004). Melchior *et al.* (2006b) in their review of biofilm function in recurrent mastitis reported that this disease pathogenesis often resembles the symptoms of human disorders induced by pathogens producing a biofilm. Therefore, the issue presents a new challenge that needs to be dealt with by veterinary medical scientists and microbiologists studying this disorder.

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