PHENOTYPICAL EXPRESSION OF *Staphylococcus aureus* VIRULENCE FACTORS ISOLATED FROM DAIRY COWS WITH SUBCLINICAL MASTITIS

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Introduction

Staphylococcus aureus is an important economic and clinical pathogen of dairy cows (Lammers *et al*.,1999; Joo *et al.*, 2001). The prevalence for subclinical and clinical mastitis in cows is related to the level of infection in the herd and the management and hygiene conditions of the animals (Kaphur *et al*., 1995), associated to the genetic variability as well as the agent's pathogenecity (Lammers *et al*.,2001; Schuberth *et al*.,2001; Begoña e Iturralde, 2001). The objective of this study was to identify the phenotypical expression of *S. aureus* virulence factors in dairy production cows with subclinical mastitis in family production units.

Material and methods

288 milk samples were taken from 11 family dairy production units in the Toluca Valley by a randomly stratified sampling method from cows in different milk production stages (Farver *et al.*,1985; Magalhaes *et al*., 1990). Milk was cultured on Vogel Jonson and Baird Parker with potassium telurite (3.5%) agar plates for 24 hrs at 37ºC. *API Staph* (Biomeriux Vitek, Inc.) system was used for final identification of the isolations. The phenotypical characterization of *S.aureus* virulence was made by using protein A, latex agglutination, Staphylase test (Oxoid, USA); and coagulase tubes with rabbit, bovine and human plasma incubated at 37ºC. The human, bovine and canine biotypes were characterized on brain and heart agar with crystal violet (1:10000) (Cottral, 1986) The type of haemolysin (α, β, γ, δ) was obtained from blood agar containing erythrocytes from different species at 10% CO_2. Capsule was observed in soft agar culture tubes (0.035% brain and heart infusion supplemented with milk sera 4%). Pigment production was determined on dextrose manitol (10%) agar (Velázquez *et al*., 1994). *In vitro* sensitivity to antibiotics was made by Kirby-Bauer's method, using penicillin 10U, ampicillin 30*m*g, cephalotin 10*m*g and oxacyllin 1*m*g unidiscs (Beckton Dickinson USA). β- lactamase test was confirmed by the iodinemetric assay (Dike, 1979). The results were evaluated by using the estimation of proportion test and ANOVA to evaluate the inhibition ring for antibiotics (Thrusfield, 1990).

Results

38 isolations were obtained, with an infection rate of 13.2% ($p<0.01$). The biotypes observed were; 9% human, 2.5% bovine and 2.5% canine ($p<0.01$). The coagulotypes were; 77.5% rabbit, 10% rabbit-bovine and 12.5% rabbit-human ($p<0.01$). The pigment production was predominantly creamy yellow 87.5% and golden yellow 12.5% ($p<0.05$). The haemolyin types were 50% α, 25% β, 10% γ, 7.5% δ with common associations α-β, y α-β-γ ($p<0.01$). 87.5% isolations presented capsule ($p<0.01$). Antibiotic resistance was 94.7% penicillin, 97.3% ampicillin , 42% cephfalotin and 44.7% oxacyllin ($p<0.01$). β-lactamase production type I was present in 68.4% isolations ($p<0.01$).The relation between β-lactamase production and resistant strains was observed in penicillin, ampicilln, cephalotin and oxacillin ($p<0.01$).

Discussion

Virulence factors suggest a multifactorial participation in the infection to animal and humans. In the dairy population it is important the findings about multiresistant and resistant *S. aureus* strains (Bartlett and Miller ,1993). The significant variation between the virulence factors associated to *S.aureus* strains, is a risk factor for the presentation of mastitis in the herd (Leitner *et al*.,2003). The presentation of haemolysins types α, β, γ and δ was considered as an important virulence factor related to the resistance and capsule production as observed in other countries (Younis t **al.**,2000). It is possible to reduce the glandular infection by *S. aureus* as well as the somatic cell count by developing prevention, control and surveillance practices to diminish health risks.(Foster, 1986;Watts, et al., 1986; Watts, 1988; Daley, et al., 1991; Blood, 1986; Mc Donald, 1987; Velázquez, 1994). (Schukken et al., 1990; Nickerson, 1988, Ziv, 1995).

Conclusion

The economic loss in dairy cows by mastitis has motivated intensive research to increase the cow's resistance to intramammay infections and the evaluation of phagocytosis activity in mammary gland (Capuco *et al.*,1986; Dekker *et al.*,1994; Marcus *et al.*,1994; Schutz *et al*.,1994 Marcus and Shuster ,1994), with the epiemiological surveillance related to the phenotypical expression of virulence factors associated with the potenial pathogenicity in animal and human population.

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