HUMAN AND BOVINE *STAPHYLOCOCCUS AUREUS* BIOTYPES ASSOCIATED WITH HAEMOLISIN PRODUCTION AND RESISTANCE TO OXACILLIN IN COWS WITH SUBCLINICAL MASTITIS IN FAMILY DAIRY FARMS

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Introduction

Staphylococcus aureus infection in dairy cows limits milk production due to mastitis development as well as increasing the herd's infection level (Gianneechini *et al.,*2002). The different clinical signs in *S. aureus* mastitis suggest differences in the phenotypical expression of virulence factors as well as strain genetic variability (Begoña and Iturralde, 2001). The phenotypical characterization of *S. aureus* biotypes establishes the presumptive origin of isolations (Magalhaes *et al*., 1990). α, β, and δ haemolysins are considered as primary factors in the development of infection (Buerke *et al.,* 2002). *S. aureus* resistance to β-lactamic antibiotics is associated to β-lactamase production, and the development of oxacillin (ORSA) and meticillin resistant strains (MRSA), which represents a high health risk in the milk herds as well as in public health, due to the possibility of transmission to man of ORSA/MRSA strains from animal origin increasing the infection pressure on human population (Gentilini *et al.,* 2000). The objective of this research work was to evaluate α ,β, γ, and δ haemolysin production, *in vitro* oxacillin resistance and β- lactamase production related to human and bovine biotypes in *Staphylococcus aureus* isolated from milking cows with subclinical mastitis in family dairy farms.

Material and methods

Sampling- A sectional study was performed in the Toluca Valley, Mexico in 26 family dairy farms in which Holstein and local racial types are found. By simple random sampling, 243 milk samples were collected from cows with different ages and production stage which showed a number 2 reaction in the California test. Teats were cleaned using 70% alcohol and 15 mL of milk were aseptically obtained in a combined sample from the four glandular

quarters. Milk was collected in sterile tubes and preserved at 4°C during transportation to the microbiology laboratory.

Bacteriology - Milk simples were incubated at 25°C for 15 minutes and vortexed (Fisher, USA). 10 µL of milk were aerobically cultured in Vogel Jonson agar plates with potassium telurite (Merck USA) at 37ºC/24 hrs. Colony forming units (CFU) were examined and morphologically described. Gram stain was performed as well as catalase and coagulase tests. *S. aureus* final identification was made using standarized procedures (National Mastitis Council, 1999) using the *API Staph* system (Biomeriux Vitek, Inc).

Virulence factors characterization - *S.aureus* virulence factors were phenotipically characterized *in vitro*. Biotypes A B, C and D were identified by culturing isolations in brain and heart agar with violet cristal (1:10000) at 37°C. Positive colonies were determined for presenting violet color. Different biotypes were considered according to color and growth. Biotype A growth and positive to violet crystal, biotype B white, biotype C yellowish and no growth for biotype D (Cohen, 1982) α, β, and δ haemolysin expression was observed in blood agar with 7% washed erythrocytes obtained from different species: human O^+, bovine, rabbit and horse. They were cultured under 10% CO_2 reduced atmosphere (Cottral, 1986). *In vitro* sensibility to antibiotics was performed by the Kirby-Bauer method modified by Barry and Thnsberry (1985). *S.aureus* isolations were cultured in Muller Hinton broth for 4 hrs. 10 µl were transferred to Muller Hinton liquified agar tubes, deposited over solid agar plates with penicillin 10U, ampicillin 30µg, cephfalotin 10µg and oxacillin 1 µg unidiscs (Beckton Dickinson USA) and cultured in anaerobiosis at 37°C/24 h. Inhibition halo around the unidiscs was measured in millimeters and compared to the values established by the test. *In vitro* β - lactamase production was made using the iodinemetric method in tubes.

Statistical analysis - *S. aureus* frequency isolation comparison, virulence factors expression and frequency of association, was determined by χ^2 test based on a cut point for positive and negative results. Statistical significance was established at a 5% level using the statistical package HandyStat 2 (1996).

Results

S. aureus infection rate in cows was 58 isolations (23.8%) which was different to non-coagulase *Staphylococcus* (10.5%) ($p<0.05$). Table 1 shows *S. aureus* haemolysin type distribution.

Table 1 . *Staphylococcus aureus* haemolysin type distribution

Haemolysin types	α	β	γ	δ	αβ	αδ	αγ	αβγ	αβγδ	γβδ	δγ	TOTAL
Number of strains	14	5	4	4	16	1	8	3	1	1	1	58
Strain %	24.2	8.6	6.9	6.9	27.6	1.7	13.8	5.2	1.7	1.7	1.7	100.0

($p<0.01$)

Table 2 shows *S.* aureus biotype distribution, β- lactamase production and *in vitro* oxacillin resistance. There was no difference in between the evaluated biotypes in the oxacillin resistant and β- lactamase production strains ($p>0.05$).

Table 2. *Staphylococus aureus* biotype distribution, β-lactamase production and *in vitro* oxacillin resistance.

S.aureus Biotypes	Presumptive Origin	*S.aureus* strains Num.	%	ORSA Strains Num.	%	β-lactamase Strains Num.	%
A	Human	28	48.4	8	13.79	27	46. 7
C	Bovine	26	44.8	4	6.89	25	43.0
B	Canine	4	6.8	0	0.0	2	3. 5
	TOTAL STRAINS	58	100.0	12	20.68	54	93.0

In vitro S.aureus resistance for β-lactamics penicillin and ampicillin was 93%. Oxacillin presented 19.0% and cephalotin showed low resistance 4% ($p<0.01$) (Table 3).

Table 3. *In vitro Staphylococcus aureus* sensibility pattern obtained from cows presenting subclinical mastitis in family dairy farms.

Antibiotics	Maximum mm	Minimum mm	Average mm	Standar Deviation mm	Number of R strains	β-lactamase Strains No.	%
Penicillin	30	8	18.47	6.79	54	54	93.0
Ampicillin	29	10	17.36	5.70	54	54	93.0
Cephalotin	30	0	14.10	10.25	4	2	4.0
Oxacillin	16	0	9.76	6.97	12	11	19.0

($P< 0.01$)

Discussion

The high *S.aureus* infection rate found in family dairy farms reflects high mammary intraglandular infection in the population, associated with high somatic cell counts, physicochemical alterations in milk and a drastic diminish in the nursing curve (Tollersrud *et al.,* 2000.) Biotypes A and C identified in human and bovine strains suggest a cross infection which can increase the infection pressure by animal origin *S. aureus* strains potentially pathogenic to man (Magalhaes *et al.,* 1990). *S. aureus* isolations obtained from dairy cows express a phonotypical regional variation associated to the genetic variability of the virulence factors in the strains (Younis *et al.,* 2000). The high resistance to β-lactamic antibiotics as well as β-lactamase production explains a metabolic resistance mechanism. Nevertheless,

oxacillin resistance (ORSA) indicates a phenotypical expression of *S.aureus* strains associated to meticillin resistance ones (MRSA) considered as a high risk in human health because of the genetic resistance of other antibiotic groups including vancomicin (Waage *et al*., 2002). This situation makes it necessary to develop an epidemiological surveillance in the family dairy farms to diminish the possibilities of transmission to man because of milk contamination related to α and β haemolysin production, carriers of oxacillin resistance, which establishes a potential health risk to animal and man health by the transmission of *S.aureus* ORSA strains from animal origin to man.

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