IDENTIFICATION IN COWS PRESENTING SUBCLINICAL MASTITIS

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Introduction

S. aureus β-lactamic resistance represents a worldwide epidemiological alert because of the presence of meticillin resistant strains (Madigan *et al*., 1999) that can produce severe intrahospital infections caused by MRSA strains characterized for the presence of the *mecA* gene (Seguin *et al*., 1999). In the *nuc* gene, the desoxirribonuclease is specifically identified in *S. aureus* (Brakstad *et al*., 1992). There is a possibility that dairy herds with a high level of infection produced by *S. aureus* penicillin resistant strains may represent a potential risk in the MRSA strains dissemination to human population, due to the possibility of crossed transmission infections and product contamination with the possibility of epidemics in animal and human population. That is why it is necessary to know if the *S. aureus* endemic strains in dairy cows can express resistance to oxacillin (ORSA), by carrying the *mec A* gene. This study was made to identify using PCR the presence of the *mecA* gene in ORSA strains as a resistance indicator to meticillin, in *S. aureus* isolations as a resistance indicator in isolations obtained from dairy cows presenting subclinical mastitis.

Material and methods

22 *S. aureus* field strains obtained from cows with subclinical mastitis in a dairy herd with history of β-lactamic antibiotic resistance in the Toluca Valley were tested for *in vitro* sensibility tests using unidiscs containing the following antibiotics: penicillin (10 UI /ml), ampicilline (10 µg/ml), oxacillin (30 µg/ml) and cephafalotin (1 µg/ml) following the modified method of Barry and Thnsberry (1985). The molecular strain characterization was made by DNA extraction of each culture according to Welsh, *et al*. (1990), using as controls *S. aureus* ATCC 25293, *S. epidermidis* ATCC 12228 and the 305 *S. aureus* strains.

PCR test protocol was followed to detect specific *S. aureus* nuc gene obtaining a final amplification of 270 base pairs (bp) (Brakstad *et al*., 1992), and for *mecA* gene related to

meticillin resistance obtaining a final 310 bp product (Vanuffel e*t al*., 1995). Reaction cycles were adjusted to each of the employed methods. The amplified reactions were run over a 3% agarose gel at 90V for 60 minutes, using a low range molecular weight marker (622 to 15 bp). The amplified products corresponding to *nuc* and *mec*A genes were visualized in the 270 and 310 bp under a UV transluminator.

Results

The resistance pattern to antibiotics in the 22 isolations studied was the following: 11 for penicillin (50%), ampicillin 12 (54.54%), oxacillin 6 (27.27%) and cephalotin 3 (13.63%). *S. aureus* ATCC 25293 control strain *in vitro* sensibility was: cephalotin sensible resistant to all the antibiotics used. *S. epidermidis* ATCC 12228 strain was resistant to penicillin and ampicillin. Field strain 305 was resistant to all the antibiotics.

PCR test in the *S. aureus* control strains corresponds to *nuc* and *mecA* genes. In the *S. epidermidis* control strain *nuc* gene was not identified. All the *S. aureus* isolations studied were confirmed by PCR, showing *nuc* gene. In the control strains *mec A* gene was only confirmed in the field *S. aureus* 305 strain*,* presenting the 310 bp band. Of the 6 oxacillin resistant *S. aureus* strains (ORSA) just one showed a band corresponding to the *mec A* gene, characterized as meticillin resistant (MRSA).

Discussion and cnclusions

PCR is confirmed useful for differentiating *S. aureus* from other *Staphylococcus spp.* strains, by the *nuc* gene identification (Brakstad, *et al*., 1992). The high frequency of penicillin and ampicillin resistance observed in the *S. aureus* evaluated isolations is frequently related to β-lactamase producing strains and oxacillin resistance is explained by the low *in vitro* sensibility to β-lactamic antibiotics (Corrente *et al.,* 2003; Kaszanyitzky *et al.,* 2004).

Multiple β-lactamic and cephalosporin resistance in the studied strains suggest other mediator mechanisms for antibiotic resistance. ORSA strain presence shows cross resistance with MRSA strains (National Committee for Clinical Laboratory Standards, 1993). These need the confirmation of the *mec A* gene, indicating the capacity of expressing genetic resistance to meticillin, which constitutes a risk to animals and man (Vannuffel, *et al*., 1995; Corrente *et al.,* 2003) by establishing an important risk factor in public health from the MRSA animal origin strains increasing the risk of infection amplification to humans (Vanwamel *et al.,* 1995; Olsen *et al.,* 1998). It is concluded that within the *S. aureus* penicillin resistant

isolations obtained from cows with subclinical mastitis only one oxacillin resistant strain carrying the *mecA* gene was identified, related with meticillin resistance.

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