

Antimicrobial susceptibility of *Staphylococcus Aureus* and *Staphylococcus Coagulasa-negative* isolated from milk of cows with Mastitis from Téjaro, Michoacán

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Abstract

The aim of this study was to determine the antimicrobial susceptibility of *Staphylococcus aureus* and coagulase-negative staphylococci isolated from milk of cows with subclinical mastitis in Téjaro population, Michoacán. This work was conducted of September to January of 2013 in 17 dairy herds Téjaro, Michoacán, which have an average of 8 cows per stall. Subclinical mastitis was determined by testing according to California Wolter *et al.* (2004), 408 samples were obtained from 102 cows. Antibiotic susceptibility testing was performed on 102 strains of staphylococci (41 *S. aureus* and 61 coagulase-negative staphylococci) isolated from milk samples were obtained from cases of subclinical mastitis in dairy herds Téjaro Michoacán. We conclude that penicillin and ampicillin were the antibiotics that had higher resistance *Staphylococcus aureus*, but not coagulase-negative staphylococci, which showed increased resistance to erythromycin and tetracycline.

Antimicrobial susceptibility, *Staphylococcus aureus*, Coagulase-Negative Staphylococci, Subclinical mastitis

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Introducción

Staphylococcus aureus are the main object of study on antimicrobial resistance in subclinical mastitis due to its importance and the high frequency of isolation of strains resistant to methicillin (García, 2005). However, at present the incidence of mastitis caused by Coagulase Negative Staphylococci (NEC) has increased substantially (Torutoglu et al., 2006), due to poor handling, hygienic conditions and control of contagious pathogens in bovine farms. Staphylococci also develop resistance to certain antimicrobials such as Erythromycin, Ampicillin, Penicillin and Tetracyclines, as they participate in the most common infections in herds with subclinical mastitis.

The tests of susceptibility to these antimicrobials in Coagulase Negative Staphylococcus species, are due to the increase in resistance to Penicillin and Erythromycin when applied in the treatment of herds of cattle (Álvarez et al., 2008), whose purpose is to face the problem of the inadequate use of antimicrobials applied against *Staphylococcus aureus* and Coagulase Negative Staphylococci.

Objective

The objective of this study was to determine the antimicrobial susceptibility of *Staphylococcus aureus* and Coagulase-negative Staphylococci isolated from cow milk with subclinical mastitis in the population of Tejaro, Michoacán.

Material and Method

The present work was carried out during the period from September 2012 to January 2013 in 17 dairy farms in Tejaro, Michoacán, which had an average of 8 cows per stable. Subclinical mastitis was determined by the California test according to Wolter et al. (2004), 408 samples of 102 cows were obtained.

The antimicrobial susceptibility test was carried out in 102 staphylococcal strains (41 *S. aureus* and 61 Coagulase Negative Staphylococci) isolated from milk samples obtained from the cases of subclinical mastitis of the dairy herds of Tejaro Michoacán. The identification of staphylococci was carried out according to Sears and McCarthy (2003), that is, through their colonial morphology, catalase test, Gram stain, coagulase test, mannitol gelatin test and hemolysis.

The antimicrobial susceptibility test was carried out using the disk diffusion method in Agar Mueller-Hinton (Oxoid) according to Torutoglu et al. (2006). Ten colonies placed on blood agar medium, and incubated at 37 ° C for 18 hours, were suspended in 2 ml of sterile saline at a density approximately equal to the density of standard 0.5 of McFarland. A dry sterile cotton swab was placed in the suspension, then the excess broth was removed by pressing and turning the swab against the inside of the tube.

The bacterial suspension was inoculated onto the Mueller-Hinton agar with the sterile swab in such a way that the entire surface of the agar was covered. Subsequently discs containing the following antimicrobials: ampicillin (Bio-Rad, 10 µg), cephalothin (Bio-Rad, 30 µg), cefotaxime (Bio-Rad, 30 µg), ceftazidime (Bio-Rad, 30 µg), cefuroxime (Bio-Rad, 30 µg), Dicloxacillin (Bio-Rad, 1 µg), erythromycin (Bio-Rad, 15 µg), gentamicin (Bio-Rad, 10 µg), pefloxacin (Bio-Rad, 5 µg), penicillin (Bio-Rad, 10 U), tetracycline (Bio-Rad, 30 µg), and trimethoprim / sulfamethoxazole (Bio-Rad, 25 µg) were placed on the surface of the medium and incubated aerobically at 37 ° C for 18 hours.

The results were recorded as sensitive or resistant by the diameter of the inhibition halo according to the interpretative standards of the NCCLS. The reference strain used for the antimicrobial susceptibility assays was ATCC 25923 of *S. aureus*.

Results and Discussion

Next, information on the in vitro activity of 12 antimicrobials against strains of staphylococci isolated from subclinical bovine mastitis is presented. The antibiotic resistance rates of the strains isolated from *S. aureus* and ECN from bovine mastitis are detailed in table 1, in which it is observed that *S. aureus* and NEC isolates present a high rate of resistance to penicillin and ampicillin, and slightly to erythromycin.

The sensitivity of staphylococci isolated from milk of cows with mastitis to select antimicrobial agents has been previously reported (Gooraninejad et al., 2007). Sensitivity to penicillin predicts sensitivity to other β -lactam antimicrobial agents such as ampicillin. In this study 29.2% (12) of the strains isolated from *S. aureus* were resistant to penicillin, 21.9% (9) to ampicillin, 9.7% (4) to erythromycin and only 2.4% (1) of the strains they were resistant to tetracyclines. Regarding the sensitivity of strains of *S. aureus*, it was found that 100% (41) of these were sensitive to cephalothin, cefotaxim, cefepime, cefuroxime, dicloxacillin, gentamicin and trimethoprim / sulfamethoxazole.

This result of resistance to penicillin is below that reported by Bezek, (1998) in a study conducted with cows with mastitis in the United States (44%), by Gentilini et al. (2002) in Argentina (40%), by Malinowski et al. (2002) in Poland (66.7%) and by Mylus et al. (1998) in Finland (50%).

Regarding ECN strains, 21.3% (13) of these were resistant to erythromycin, 13.1% (8) to tetracyclines, 11.4% (7) to penicillin, 8.1% (5) to ampicillin, 8.1% (5) to gentamicin, 11.27% (7) were resistant to trimetropin / sulfamethoxazole, dicloxacillin, cefotaxim, cefepime and cefuroxime respectively. However, 100% (61) of strains isolated from ECN were sensitive to cefalotin and levofloxacin.

In other studies conducted on penicillin resistance by NEC, it was found that these results are also below what was found in Argentina (30%) by Gentilini et al. (2002), Finland (37.2%) by Myllus et al. (1998) and Denmark (36.1%) by Aarestrup et al. (nineteen ninety five).

This resistance to penicillin observed in this study as pointed out by Torutoglu et al. (2006) may be because this antibiotic represents the main antibiotic recommended for the treatment of mastitis in cows. Therefore, it is concluded that *Staphylococcus aureus* were the ones that showed greater resistance to Penicillin and Ampicillin, but not Coagulase Negative Staphylococci, which showed greater resistance to Erythromycin and Tetracyclines.

These differences observed in the activity of antibiotics used against staphylococci show the importance of antibiotic susceptibility tests for the identification of bacterial agents. So in the treatment of animals infected by these bacteria, it is important to determine through an antibiogram the resistance they present to antibiotics and thus apply the appropriate treatment.

Conclusions

It is concluded that Penicillin and Ampicillin were the antibiotics to which *Staphylococcus aureus* showed greater resistance, but not Coagulase Negative Staphylococci, which showed greater resistance to Erythromycin and Tetracyclines.

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