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Antimicrobial Susceptibility of Udder Pathogens Isolated from Dairy Herds in the West Littoral Region of Uruguay

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Gianneechini RE, Concha C, Franklin A: Antimicrobial susceptibility of udder pathogens isolated from dairy herds in the west littoral region of Uruguay. Acta vet. scand. 2002, 43, 31-41. – A total of 522 strains belonging to streptococci, enterococci and staphylococci isolated from sub-clinical and clinical cases of bovine mastitis from the west littoral region of Uruguay were analysed for their susceptibility to several antimicrobial agents. The susceptibility patterns were studied by agar disk diffusion methods (ADDM) and broth micro-dilution to determine the minimum inhibitory concentration (MIC). The concentration that inhibits 90% (MIC_{90}) of the analysed strains reported in micrograms per millilitre, for Staphylococcus aureus were >8, 8, ≤0.5, ≤4, ≤1, ≤0.5, >64, ≤0.25, 0.5, ≤1 and ≤1 to penicillin, ampicillin, oxacillin, cephalotin, gentamicin, erythromycin, oxitetracycline, enrofloxacin, trimethoprim/sulfamethoxazole, neomycin, and clindamycin, respectively. Coagulase-negative staphylococci (CNS) had different values for penicillin (4) and ampicillin (2), while the other antimicrobial agents had the same MIC_{90} values as reported for *S. aureus.* The MIC_{90} values for streptococci were 0.12, 0.25, ≤4, 16, ≤0.25, 0.5, 0.25 for penicillin, ampicillin, cephalotin, gentamicin, erythromycin, oxytetracycline and trimethoprim-sulfamethoxazole, whereas MIC_{90} for enterococci were 4, 4, 4, ≤0.5, 2, >8 for penicillin, ampicillin, gentamicin, erythromycin, oxytetracycline and trimethoprim-sulfamethoxazole, respectively. Of 336 strains of *S. aureus,* 160 (47.6%) were resistant to penicillin. For 41 CNS strains, 10 (27%) presented penicillin-resistance. All the streptococcal strains were susceptible to penicillin, while 3 (7%) of the 43 enteroccocal strains were resistant. Non significant statistical differences were found between the results obtained by ADDM and broth micro-dilution for classifying bacterial isolates as susceptible or resistant according to the National Committee of Clinical Laboratory Standards.

cow; mammary qland; bacteria; resistant; sensitive.

Introduction

Bovine mastitis is the major problem for milk producers throughout the world and responsible for substantial losses of revenue annually. Antibiotic therapy is an important tool in the scheme of mastitis control. The treatments are more effective when directed by veterinarians; for example correct drug selection can be enhanced using an appropriate antimicrobial susceptibility test. The misuse or intensive use of antibiotics can lead to the development of resistance among different bacterial strains and contamination of foodstuff, with animal and human health implications (*Lingaas* 1998). The antimicrobial resistance is the result of mutations or exchange of genetic material such as plasmids and transposons (*Neu* 1992). Such resis-

tance determinants most probably are acquired by pathogenic bacteria from a pool of resistance genes in other microbial genera present in different environments (*Davies* 1994). Increased resistance of *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) isolated from bovine mastitis cases to antimicrobial agents has been reported by *Gentilini et al.* (1995), *Aarestrup & Jensen* (1998) and *Myllys et al.* (1998).

Milk production in Uruguay (South America) is important with a total of 410.000 dairy cows, yielding 1462 millions litres in 1999 (*OPYPA* 2000). In spite of the importance of this sector, only 3 surveys to evaluate the resistance of udder pathogens to antibiotics have been performed in Uruguay using agar disk diffusion (ADDM, *Bauer & Kirby* 1966): 1) *Del Baglivi et al.* (1976) testing *S. aureus* and *Streptococcus agalactiae* isolated from subclinical cases obtained from 43 dairy farms in the southern dairy region of Uruguay showed that 53% of *S. aureus* and 100% of *Str. agalactiae* were sensitive to penicillin. 2) *Herrera et al.* (1982) found 78% of *S. aureus* strains susceptible to penicillin in the dairy area around Tacuarembó city (north of Uruguay). 3) *Bouman et al.* (1999) studied the resistance patterns of *S. aureus* and CNS isolated in the laboratory routine during 4 years from milk samples collected in the southwestern region of Uruguay for penicillin, cloxacillin, nafcillin, rifampin and tetracycline obtaining: 58%, 16%, 5%, 6%, 29% of resistance for S.aureus and 75%, 42%, 17%, 12%, 26% for CNS, respectively.

The sale of antibiotics is free in Uruguay, while the mastitis treatment is usually performed by the herd dairyman, and the antimicrobial agents most commonly used are tetracyclines, beta-lactams, macrolides, and aminoglycosides.

The methods for susceptibility testing used to choose the appropriate drug are ADDM qualitative test and quantitative determinations by means of microdilution to determine the minimum inhibitory concentration (MIC) (*Amsterdam* 1996, *Acar & Goldstain* 1996). These methods can be interpreted following the National Committee for Clinical Laboratory Standards criteria (*NCCLS* 1999) or guidelines proposed by other national antibiogram committees (*Acar & Goldstain* 1996*)*.

The purposes of this work were: To determine the phenotypic expression of in vitro susceptibility of antimicrobials for pathogens (staphylococci, streptococci, and enterococci) isolated from dairy herds in Uruguay, and to compare the results obtained by the ADDM vs. broth micro-dilution method according to the NCCLS criteria.

Materials and methods

Sample

A total of 522 strains including streptococci, enterococci and staphylococci were used in the study. The strains were isolated from sub-clinical and clinical cases of bovine mastitis from a survey carried out in the west littoral region of Uruguay (*Gianneechini* 2001), where quarter foremilk samples from 1077 milking cows and 40 milk samples from clinical cases detected in one month were collected in 29 randomly selected dairy farms. All strains were identified according to the procedures of the laboratory at the Department of Mastitis and Diagnostical Products, National Veterinary Institute (SVA), Uppsala, Sweden (*National Veterinary Institute* 1998). The isolates were maintained frozen at –20 °C in Trypticase soy broth (Difco Laboratories, Michigan, USA) containing 10% glycerol until testing.

Susceptibility testing

Prior to the susceptibility testing all isolates were sub-cultured on Blood-esculin agar and incubated for 24 h at 37 °C. Two different tests

were carried out to determine the drug susceptibility for all strains:

1 - The ADDM was conducted and interpreted according to the recommendations and criteria of the NCCLS for bacteria isolated from animals (*NCCLS* 1999). The following disks (Becton Dickinson Microbiology System, Cockeysville, Maryland, USA) were used: penicillin, 10 μg; ampicillin, 10 μg; oxacillin, 1 μg; amoxicillin – clavulanic acid, 20 μg + 10 μg; cephalotin, 30 μg; gentamicin, 10 μg; erythromycin, 15 μg; enrofloxacin, 5 μg; tetracycline, 30 μg; neomycin, 30 μg; trimethoprim-sulfamethoxazole, 1.25 μg + 23.75 μg.

The staphylococci were tested against all the drugs above, while the streptococci against only 6 of these antimicrobial agents (penicillin, ampicillin, cephalotin, gentamicin, erythromycin and tetracycline), and enterococci against penicillin, ampicillin, gentamicin, erythromycin and tetracycline. The medium used was Mueller-Hinton Agar (Difco Laboratories, Detroit, USA) for sthaphylococci and Mueller-Hinton agar supplemented with 5% sheep blood for streptococci. *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were included as quality control strains. The plates were read after 18 h incubation at 37 °C under aerobic conditions. The isolates were categorised as susceptible, intermediate and resistant by measuring the inhibition zone.

2 - The MIC was determined using a commercially available microdilution system (VetMIC ™ +/- panels, SVA, Uppsala, Sweden). The tests were performed by manufacturer's instruction and interpreted according to international standards (*NCCLS* 1999) using Mueller-Hinton broth (Oxoid Limited, Basingstoke Hants, England) and *S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *E. coli* ATCC 25922, as quality control strains.

When the streptococcal strains were tested, 100 μl were inoculated in each well with erythromycin to obtain the following dilution: 0.25; 0.5; 1 and 2 μg/ml of the antimicrobial agent. These modifications were carried out to adapt to the breakpoints suggested by *NCCLS* (1999) for erythromycin. All panels were read on the same conditions as in the ADDM. The lowest dilution with no visible growth was considered as MIC for each strain. The concentration at which 50% and 90% of the isolates were inhibited, as well as the minimum and maximum range were determined.

The breakpoints suggested by the *NCCLS* (1999) for kanamycin were used for neomycin in both tests.

Oxacillin resistance testing

In order to confirm the presence of oxacillin resistance among staphylococci, VetMIC ™ GP_mo panels (SVA, Uppsala, Sweden) were used as recommended by *NCCLS* (1999). The procedures were conducted following the manufacturer's recommendations: the inoculum was prepared with colony material directly from the plate incubated 24 h before. A 1 μl loop with colony material was suspended in 4 ml of distilled water plus 0.02% Twin 80. From this suspension 100 μl were transferred to 10ml Mueller Hinton Broth + 2% NaCl (*Baker et al,* 1994), which achieved about 10^3 to 10^4 cfu/50 μl. Each oxacillin and control well of the panel was inoculated with 50 μl of this final bacterial suspension. The panel was incubated at 30 °C during 24 h under aerobic conditions. The strains *S. aureus* ATCC 29886 and *S. aureus* ATCC 29887 were included as negative and positive control strains, respectively.

ß-Lactamase Testing (Cloverleaf Method)

The assay to determine the production of ß-lactamase by staphylococci was described previously by *Franklin & Wierup* (1982). Briefly, the non-ß-lactamase-producing *S. aureus* Oxford

Table 1. In vitro susceptibility of 336 strains of *Staphylococcus aureus* obtained from clinical and sub-clinical bovine mastitis cases from the West Littoral Region of Uruguay.

Antimicrobial Agent	MIC[1] (µg/ml)				% Resistance	
	MIC_{50}[2]	MIC_{90}[3]	Range	Breakpoint	VETMIC	Agar disk diffusion test
Penicillin	0.5	>8	0.12->8	≥0.25	47.6	46.1 [5]
Ampicillin	0.5	8	≤0.12->16	≤0.5	46.7	46.6 [5]
Oxacillin	≤0.5	≤0.5	≤0.5-1	≥4	0.0	2.0 [5 6]
Cephalotin	≤4	≤4	≤4	≤32	0.0	0.0
Gentamicin	≤1	≤1	≤1-4	≥16	0.0	0.0
Erythromycin	≤0.5	≤0.5	≤0.5->4	≥8	3.0	2.6 [5]
Oxitetracycline	2	>64	≤0.25->64	≥16	13.4	14.0 [5 7]
Enrofloxacin	≤0.25	≤0.25	≤0.25-0.5	≥2	0.0	0.0
Neomycin	≤1	≤1	≤1-64	≥64	0.6	0.9 [5]
Clindamycin	≤1	≤1	≤1	≥4	0.0	….[4]….
Amoxicillin/ Clavulanic acid	….[4]….	….[4]….	….[4]….	….[4]….	….[4]….	0.0
Trimethoprim/ Sulfamethox.	0.25	0.5	< 0.06-8	≥4	0.3	0.0 [5]

[1] Minimum inhibitory concentration. [2] Lowest concentration inhibiting 50% of the isolated tested.
[3] Lowest concentration inhibiting 90% of the isolated tested. [4] Test not performed.
[5] No significant differences between methods. [6] All strains were sensitive at the confirmation test.
[7] Tetracycline disk were used to perform agar disks diffusion.

Table 2. In vitro susceptibility of 41 strains of *Coagulase Negative Staphylococcus* obtained from clinical and sub-clinical bovine mastitis cases from the West Littoral Region of Uruguay.

Antimicrobial Agent	MIC[1] (µg/ml)				% Resistance	
	MIC_{50}[2]	MIC_{90}[3]	Range	Breakpoint	VETMIC	Agar disk diffusion test
Penicillin	0.5	>4	0.12->8	≥0.25	27.0	22.0 [5]
Ampicillin	0.5	2	≤0.12->16	≤0.5	24.4	22.0 [5]
Oxacillin	≤0.5	≤0.5	≤0.5-1	≥4	0.0	0.0
Cephalotin	≤4	≤4	≤4	≤32	0.0	0.0
Gentamicin	≤1	≤1	≤1-4	≥16	0.0	0.0
Erythromycin	≤0.5	≤0.5	≤0.5	≥8	0.0	0.0
Oxitetracycline	2	>64	≤0.25->64	≥16	13.9	13.9 [6]
Enrofloxacin	≤0.25	≤0.25	≤0.25-0.5	≥2	0.0	0.0
Neomycin	≤1	≤1	≤1-64	≥64	2.0	2.0
Clindamycin	≤1	≤1	≤1	≥4	0.0	….[4]….
Amoxicillin/ Clavulanic acid	….[4]….	….[4]….	….[4]….	….[4]….	….[4]….	0.0
Trimethoprim/ Sulfamethox.	0.25	0.5	< 0.06-8	≥4	7.3	0.0 [5]

[1] Minimum inhibitory concentration. [2] Lowest concentration inhibiting 50% of the isolated tested.
[3] Lowest concentration inhibiting 90% of the isolated tested. [4] Test not performed.
[5] No significant differences between methods. [7] Tetracycline disk were used to perform agar disks diffusion.

strain 209 is used as indicator. This strain is inoculated on PDM II agar plates (AB Biodisk, Solna, Sweden) to yield an almost confluent growth on the agar surface. In the centre of the agar plate a disk containing 10 μg of penicillin G (PDM Antibiotics Sensitive II, AB Biodisk) is placed in order to induce ß-lactamase production in the studied strain. The staphylococci to be tested were streaked in a line from the edge of the plate towards the centre of the penicillin disk. When the investigated strain was positive ß-lactamase producer, the indicator strain grew alongside this strain towards the penicillin disk, into the inhibited one. The *S. aureus* strains ATCC 29213 and ATCC 25923 were included as positive and negative control respectively.

Statistics analyses

The Z-test (*Milton* 1992) was performed to compare the proportions of resistant strains to each antimicrobial agents obtained by means of both test.

Results

All values obtained with control strains in both tests were within the expected ranges for all antimicrobial agents analysed. The ranges of MIC of each of the antimicrobial agents tested, MIC_{50} and MIC_{90} of the tested strains, and the percentage of resistance obtained by both micro-dilutions and ADDM are presented here for *S. aureus* (Table 1), CNS (Table 2), *Str. agalactiae* (Table 3), *Streptococcus dysgalactiae* (Table 4), *Streptococcus uberis* (Table 5) and *Enterococcus sp* (Table 6).

The differences found between both tests corresponding to each antimicrobial agent were not significant ($p > 0.05$). Of 336 strains of *S. aureus,* 215 (64%) were resistant to one or more antimicrobial agents in both tests. There was no resistance to oxacillin, cephalotin, gentamicin, enrofloxacin, clindamycin, and the combination of amoxicillin-clavulanic acid, whereas 160 (47.6%), 157 (46.7%), 45 (13.4%), 10 (3%), 2 (0.6%) and 1 strain (0.3%) were resistant to penicillin, ampicillin, tetracycline, erythromycin, neomycin and trimethoprim-sulphametoxazole, respectively. One hundred and fifty-six *S. aureus* isolates (46.4%) were ß-lactamase producers. While of 41 CNS strains, 10 (27%) presented resistance to penicillin and 9

Table 3. In vitro susceptibility of 60 strains of *Streptococcus agalactiae* obtained from clinical and sub-clinical bovine mastitis cases from the West Littoral Region of Uruguay.

Antimicrobial Agent	MIC[1] (µg/ml)				% Resistance	
	MIC_{50}[2]	MIC_{90}[3]	Range	Breakpoint	VETMIC	Agar disk diffusion test
Penicillin	0.12	0.12	≤0.06-0.12	≥4	0.0	0.0
Ampicillin	0.25	0.25	≤0.12-0.25	≥8	0.0	0.0
Cephalotin	≤4	≤4	≤4	≥32	0.0	0.0
Gentamicin	8	16	4-16	≥16	85.0	85.0
Erythromycin	≤0.25	≤0.25	≤0.25-1	≥1	3.4	3.4
Oxitetracycline	≤0.25	0.5	≤0.25-2	≥8	0.0	0.0 [5]
Trimethoprim/ Sulfamethox.	0.25	0.25	≤0.06-2	≥4	0.0	….[4]….

[1] Minimum inhibitory concentration. [2] Lowest concentration inhibiting 50% of the isolated tested.
[3] Lowest concentration inhibiting 90% of the isolated tested. [4] Test not performed.
[5] Tetracycline disk were used to perform agar disks diffusion.

Table 4. In vitro susceptibility of 9 strains of *Streptococcus dysgalactiae* obtained from clinical and sub-clinical bovine mastitis cases from the West Littoral Region of Uruguay.

Antimicrobial Agent	MIC[1] (µg/ml)				% Resistance	
	MIC_{50}[2]	MIC_{90}[3]	Range	Breakpoint	VETMIC	Agar disk diffusion test
Penicillin	≤0.06	0.12	≤0.06-0.12	≥4	0.0	0.0
Ampicillin	≤0.12	0.25	≤0.12-0.25	≥8	0.0	0.0
Cephalotin	≤4	≤4	≤4	≥32	0.0	0.0
Gentamicin	8	8	4-8	≥16	0.0	0.0
Erythromycin	≤0.25	≤0.25	≤0.25	≥1	0.0	0.0
Oxitetracycline	8	>64	4->64	≥8	89.0	89.0 [5]
Trimethoprim/ Sulfamethox.	0.12	0.25	≤0.06-0	≥4	0.0	….[4]….

[1] Minimum inhibitory concentration. [2] Lowest concentration inhibiting 50% of the isolated tested.
[3] Lowest concentration inhibiting 90% of the isolated tested. [4] Test not performed.
[5] Tetracycline disk were used to perform agar disks diffusion.

strains (22.5%) were ß-lactamase producers.
Seven suspected oxacillin resistant strains of *S. aureus* on the ADDM were susceptible in the confirmatory test.
All isolates of *Str. agalactiae, Str. dysgalactiae* and *Str. uberis* were susceptible to penicillin and ampicillin, while 3 (7%) of 43 strains of *Enterococcus sp.* were resistant to penicillin.

Discussion

The *ß-lactams* (penicillins and cephalosporins) have become the first line of antimicrobial agents used for treatment of bovine mastitis in Uruguay. Within this class, penicillin, amoxicillin, cloxacillin and ampicillin are the mostly used agents. In the Nordic countries penicillin is used as the first-line antibiotic treatment of bovine mastitis, because of a low resistance rate

Table 5. In vitro susceptibility of 33 strains of *Streptococcus uberis* obtained from clinical and sub-clinical bovine mastitis cases from the West Littoral Region of Uruguay.

Antimicrobial Agent	MIC[1] (µg/ml)				% Resistance	
	MIC_{50}[2]	MIC_{90}[3]	Range	Breakpoint	VETMIC	Agar disk diffusion test
Penicillin	≤0.06	0.12	≤0.06-0.12	≥4	0.0	0.0
Ampicillin	≤0.12	0.25	≤0.12-0.25	≥8	0.0	0.0
Cephalotin	≤4	≤4	≤4	≥32	0.0	0.0
Gentamicin	16	>16	≤1->16	≥16	88.0	83.0 [5]
Erythromycin	≤0.25	≤0.25	≤0.25	≥1	0.0	0.0
Oxitetracycline	≤0.25	0.5	≤0.25-64	≥8	0.0	0.0 [6]
Trimethoprim/ Sulfamethox.	0.12	0.25	≤0.06-0.5	≥4	0.0	….[4]….

[1] Minimum inhibitory concentration. [2] Lowest concentration inhibiting 50% of the isolated tested.
[3] Lowest concentration inhibiting 90% of the isolated tested. [4] Test not performed.
[5] No significant differences between methods. [6] Tetracycline disk were used to perform agar disks diffusion.

Table 6. In vitro susceptibility of 43 strains of *Enterococcus sp.* obtained from clinical and sub-clinical bovine mastitis cases from the West Littoral Region of Uruguay.

Antimicrobial Agent	MIC[1] (µg/ml)				% Resistance	
	MIC_{50}[2]	MIC_{90}[3]	Range	Breakpoint	VETMIC	Agar disk diffusion test
Penicillin	0.5	4	0.12->8	≥16	7.0	7.0
Ampicillin	0.5	4	≤0.12-16	≥16	2.3	2.3
Gentamicin	≤1	4	≤1-4	≥16	0.0	0.0
Erythromycin	≤0.5	≤0.5	≤0.5-4	≥8	4.6	4.6
Oxitetracycline	2	2	≤0.25-2	≥16	0.0	2.0 [5 6]
Trimethoprim/ Sulfamethox.	>8	>8	1->8	≥4	97.7	….[4]….

[1] Minimum inhibitory concentration. [2] Lowest concentration inhibiting 50% of the isolated tested.
[3] Lowest concentration inhibiting 90% of the isolated tested. [4] Test not performed.
[5] No significant differences between methods. [6] Tetracycline disk were used to perform agar disks diffusion.

and narrow spectrum. This is an important tool to limit the development of antibiotic resistance as much as possible (*Aarestrup & Jensen* 1998). In our study 47.6% of *S. aureus* were classified as penicillin resistant, MIC ≥0.25 µg/ml (Table 2), 96% of which produced ß-lactamase. This was the same comparing the proportion of resistance (47%) as obtained by *Del Baglivi et al.* (1976) in the southern dairy area of Uruguay. The comparison between these results obtained in Uruguay over the years demonstrated that the situation in general has not changed during the last 25 years in relation to penicillin resistance. Whereas, the prevalence of resistance to penicillin was similar in Argentina (40%) (*Gentilini et al.* 2000) and Finland (50.7%) (*Myllys et al.* 1998). However, it was higher than in Norway, 4.2% from clinical cases and 18% from sub-clinical cases (*Hofshager et al.* 1999), and Sweden, 6% (*Franklin* 1998).

In relation to CNS, 27% of 41 isolates were penicillin resistant (Table 1). Results from Finland 37% (*Myllys et al.* 1998) and Norway 26% (*Hofshager et al.* 1999) agree with our findings. The MIC_{90} of penicillin was 4 µg/ml for our survey, and another study determined 0.5 µg/ml in New Zealand (*Salmon et al.* 1998).

The detection of ß-lactamase production in staphylococci is a useful and rapid method to detect penicillin resistance. At the National Veterinary Institute, Uppsala, ß-lactamase results are used as rapid screen to indicate penicillin resistance (*National Veterinary Institute* 1998). In this study 96% and 90% of penicillin resistant strains of S.aureus and CNS were positive as indicated by the cloverleaf method. Test for ß-lactamase producing should always be done to obtain the true picture of resistance to penicillin in staphylococci.

The streptococci and enterococci showed high susceptibility (streptococci 100%) to penicillin in our study (Tables 3, 4, 5 and 6). This agree with the results from monitoring studies done in the Scandinavian countries, where the streptococci populations isolated from mastitis were highly susceptible to penicillin (*Pyörälä & Myllys* 1995). Only 7% of the *Enterococcus sp.* strains were classified as resistant against penicillin (>8 µg/ml). The MIC_{90} of penicillin for *Str. agalactiae, Str. dysgalactiae,* and *Str. uberis* was 0.12 µg/ml in each case and for enterococci 4 µg/ml .

Oxacillin was included here as recommended

by the *NCCLS* (1999) to detect methicillin-resistant strains of *S. aureus* and CNS. In our study oxacillin resistance was not found in staphylococci. However, CNS strains with higher MIC than >0.5 *g/ml of oxacillin should be tested for possible carriage of the *mec*A gene, in order to verify the occurrence of this gene (*Hussain et al.* 2000).

Cephalotin was included to determine the resistance against the first-generation cephalosporin class for all bacterial species except *Enterococcus sp.* All tested microorganisms were 100% sensitive to cephalotin and MIC_{90} was ≤4 μg/ml.

Despite their structural differences macrolide and lincosamides antimicrobials have similar biological properties, including their mechanism of action against the 50S subunit of the bacterial ribosome. These common properties easily allow the development of cross-resistance (*Prescott* 2000).

Erythromycin and clindamycin were included here to evaluate the resistance against these groups. Clindamycin was used in our survey to test resistance againts lincosamides in staphylococci and no strains were resistant. For our strains the MIC_{90} value was <1 μg/ml, a result remarkably different as compared with 8 μg/ml obtained by *De Oliveira et al.* (2000) for *S. aureus* strains isolated in the United States.

For erythromycin our findings (Tables 1 and 2) showed scarce resistance in *S. aureus* (3%) and in CNS (0%), similar to the result (2.4%) reported by *Del Baglivi et al.* (1976). The results were lower than reported by *Gentilini et al.* (2000) for *S. aureus* in Argentina (11.6%). In Finland, *Myllys et al.* (1998) found 2.6% and 11.5% resistance among *S. aureus* and CNS, respectively, while in Sweden, *Franklin* (1998) reported 1% resistance in S. aureus. The MIC_{90} of erythromycin in our study was ≤0.5 μg/ml to staphylococci.

Streptococci showed high erythromycin susceptibility, only 3.4% of *Str. agalactiae* and 4.6% of *Enterococcus sp.* were resistant in our study. Substantial differences were found in relation to results obtained in Finland (17%) for enterococci (*Myllys et al.* 1998), but no differences with respect to the erythromycin susceptibility result in streptococci (2,8%) obtained by *Del Baglivi et al.* (1976). Our MIC_{90} value of erythromycin for streptococci was ≤0.25 μg/ml except for *Str. uberis* (0.5 μg/ml), while for enterococci was ≤0.5 μg/ml.

Aminoglycosides are used with precaution in dairy animals in order to avoid the risk of prolonged residues in milk. However, products for direct infusion into mammary gland containing neomycin are used because of the limited systemic effect caused by this way of administration (*Prescott* 2000). The MIC_{90} (2 μg/ml) of neomycin (Table 1) in our survey for *S. aureus* was slightly different compared to the results obtained with *S. aureus* from different countries (*De Oliveira et al.* 2000).

The *S. aureus* and CNS bacteria were not gentamicin-resistant and the MIC_{90} values were ≤1 μg/ml for both. This was similar to the results obtained for *S. aureus* in Argentina (*Gentilini et al.* 2000). As expected we found high MICs of gentamicin in *Str. agalactiae* and *Str. uberis* (Tables 3 and 5), while *Str. uberis* and *Enterococcus sp.* had lower MICs (Tables 4 and 6). Aminoglycosides are not the antimicrobials agents of choice for streptococcal mastitis because streptococci have inherited resistance to this class (*Pyörälä & Myllys* 1995).

Our results regarding tetracycline-resistance for *S. aureus* (13.4%) and CNS (13.9%) were similar to those in Finland (*Myllys et al.* 1998), but higher than the results obtained in Norway for *S. aureus* (0.2 %) and CNS (3%) (*Hofshager et al.* 1999). The results were twofold higher than the 6% reported by *Del Baglivi et al.* (1976) in Uruguay. A possible explanation for this phenomenon could be that for many years

tetracyclines have been the most widely antimicrobial class used by the farmers to treat any infection.

In general the streptococci and enterococci were susceptible to oxytetracycline, with the exception of *Str. dysgalactiae* (Table 4). *Pyörälä & Myllys* (1995) stated that *Str. dysgalactiae* strains are less susceptible to tetracyclyne than *Str. uberis* strains, as also reported by *Brown & Roberts* (1991).

Staphylococci and streptococci were susceptible to trimethoprim-sulfamethoxazole, whereas enterococci were resistant (Table 6).

Enrofloxacin is approved for systemic administration to treat bovine mastitis in some Scandinavian countries. We found a high susceptibility in staphylococci (Tables 1 and 2) and a similar situation was found by *Myllys et al.* (1998).

Both antimicrobial susceptibility tests, ADDM and broth microdilution, used in this survey were performed according to the approved standard for bacteria isolated from animals and the interpretative criteria for veterinary use according to *NCCLS* (1999). The ADDM is most commonly used in the veterinary laboratories in Uruguay and many other countries. There were no significant differences between the methods when classifying bacterial isolates as susceptible or resistant according to NLCCS (Tables 1, 2, 3, 4, 5 and 6). The results from ADDM could be influenced by several factors, such as: compositions of agar medium, pH, inoculum density, agar depth, timing of drug applications, incubation time, etc (*Acar & Goldstain* 1996). However, *Myllys et al.* (1992) have obtained high correlation coefficient (0.875 to 0.975) between both methods in agreement with our results. *Kielbauch et al.* (2000) considered ADDM as a useful tool when the level of compliance with NCCLS guidelines was evaluated periodically.

Conclusion

This study did not show changes with respect to the penicillin and erythromycin resistance level of udder pathogens (staphylococci and streptococci) during the last 25 years in Uruguay, while a clear increase in tetracycline resistance was found for *S. aureus.*

The Agar Disk Diffusion Method was a good tool, inexpensive, and readily available for regional veterinary laboratories. However, considering the necessity to maintain the surveillance over antimicrobial resistance in a country, it is important to periodically evaluate the compliance with guidelines such as National Committee for Clinical Laboratory Standards guidelines. It is also important to monitor regularly the minimum inhibitory concentrations for the isolated strains from different regions of the country. A responsible antibiotic policy would be highly relevant in a future programme for mastitis control and udder health in Uruguay.

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Sammandrag

Känslighet för antimikrobiella ämnen hos juverpatogener isolerade från mjölkbesättningar i Uruguays västra Litoralregion.

Totalt 522 stammar av streptokocker, enterokocker och stafylokocker, isolerade från subkliniska och kliniska fall av bovin mastit från Uruguays västra Litoralregion analyserades med avseende på känslighet för olika antimikrobiella ämnen. Känslighetsmönstret studerades med hjälp av agardiskdiffusions metod (ADDM) och buljongspädningsteknik i mikroskala för att bestämma minsta hämmande koncentration (MIC). Känsligheten hos stafylokocker för olika antimikrobiella ämnen testades mot penicillin, ampicillin, oxacillin, cefalotin, gentamycin, erytromycin, oxitetracyklin, enrofloxacin, trimetoprim-sulfametazol, neomycin, klindamycin och amoxicillin-klavulansyra. Känsligheten hos streptokocker och enterokocker testades mot penicillin, ampicillin, cefalotin, gentamicin, erytromycin, oxitetracyklin och trimetoprim-sulfametazol. De MIC-värden som hämmar 90% av de analyserade stammarna, rapporterat i μg/ml, var för *Stafylokockus aureus (S.aureus)* >8, 8, ≤0.5, ≤4, ≤1, ≤0.5, >64, ≤0.25, 0.5, 2 respektive ≤1. Koagulasnegativa stafylokocker (KNS) visade andra värden för penicillin, ampicillin och oxitetracyklin: 4, 2 repektive >128. MIC_{90}-värdet för streptokocker var respective 0.12, 0.25, ≤4, 16, ≤0.25, 0.5, 0.25 och för enterokocker 4, 4, ≤4, 5, ≤0.5, 2, >8. Av 336 stammar av S. aureus var 160 (47,6%) resistenta mot penicillin. Av 41 KNS stammar var 10 (27%) pc-resistenta. Alla streptokockstammar var känsliga mot penicillin, medan 3 (7%) av de 43 enterokockstammarna var resistenta. Inga statistiska signifikanta skillnader erhölls i resultaten från ADDM och buljongspädningsteknik vid klassificering av bakterieisolaten som känsliga eller resistenta enligt National Committee of Clinical Laboratory Standards.

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