ANTIBIOTIC SENSITIVITY OF STREPTOCOCCI AND ENTEROCOCCI FROM MASTITIS IN COWS IN 2010-2011

EDWARD MALINOWSKI, HENRYKA LASSA, AND ZDZISŁAW GAJEWSKI

Department of Pathophysiology of Reproduction and Mammary Gland, National Veterinary Research Institute, 85-090 Bydgoszcz, Poland
vetri@logonet.com.pl

Department and Clinic of Farm Animals, University of Life Sciences - SGGW, Faculty of Veterinary Medicine, 02-797 Warszawa, Poland

Received: November 2, 2011 Accepted: December 1, 2011

Abstract

The aim of the study was to establish the in vitro sensitivity of S. agalactiae (126 strains), S. dysgalactiae (82 strains), S. uberis (419 strains), and Enterococcus sp. (136 strains) isolated from mastitis in cows to 13 antibiotics. Bacteriological examinations of quarter milk samples were conducted with the recommendations of the IDF. Sensitivity to antibiotics was tested by disk diffusion method in Mueller-Hinton agar and performed according to CLSI guidelines. It was found that S. agalactiae strains were the most sensitive to bacitracin (97.6%), cefapirin (96.3%), and amoxicillin (93.7%), and resistant to neomycin (89.7%), tetracycline (49.2%), and clxocillin (38.9%). S. dysgalactiae showed the highest sensitivity to bacitracin (100%), cefapirin (100%), amoxicillin (97.6%), cephalaxin (97.6%), cefoperazone (93.9%), and resistance to neomycin (61.0%) and tetracycline (53.7%). S. uberis strains were mainly sensitive to cefapirin (98.9%), bacitracin (98.8%), cefquinome (97.6%), amoxicillin (97.4%) and resistant to neomycin (93.3%), tetracycline (35.3%) and lincomycin (22.4%). Enterococcus sp. showed the high sensitivity to amoxicillin (83.8%) and bacitracin (82.4%), and resistance to cloxaxill (76.5%), cephalaxine (58.1%), lincomycin (55.9%), neomycin (53.7%), penicillin (48.5%), and tetracycline (36.0%). In conclusion, strains of S. dysgalactiae were more sensitive to tested antibiotics than S. agalactiae, S. uberis, and especially Enterococcus sp. Only 8.8% of S. agalactiae, 4.3% of S. dysgalactiae, 4.3% of S. uberis, and 4.4% of Enterococcus sp. strains were sensitive to all tested antibiotics. The largest diversity in the resistance was found in Enterococcus sp.

Key words: cow, mastitis, streptococci, enterococci, antibiotic sensitivity.

Udder inflammations are still the most frequent and costly diseases affecting dairy cows all over the world. Streptococci along with staphylococci are the predominant aetiological agents of subclinical forms of mastitis (17, 21, 25, 31). Recent studies have reported that different species of Streptococcus were isolated from 5.8% of quarter milk samples (14), and from 13.1% (26), 18%-19% (21, 29) to 30% of samples (17). Prevalence of S. uberis and S. dysgalactiae intramammary infections in Holland was almost the same in the five samplings during the 30-year period, and was 1.1%-1.7% for S. uberis and 0.9%-1.5% for S. dysgalactiae but S. agalactiae was not found in the referred period (37).

Therapy with antimicrobials is the primary method of combating streptococcal udder infections. The cure rates of streptococcal mastitis treatment differ depending on aetiological agents. Very high efficacy was noted in cases of S. agalactiae inflammations following intramammary antibiotic infusions, as well as intramuscular injection of penicillin (18). Definitely worse therapeutic efficacy was noted in cases caused by other streptococci (24). Pyörälä and Pyörälä (32) reported 46.8% and 30% recoveries from mastitis caused by S. uberis and S. dysgalactiae after treatment with penicillin G and spiramycin, respectively. This was mainly caused by new infections, especially after treatment with spiramycin. Serieyes et al. (40) compared the effectiveness of intramuscular injections of penicillin G with the effectiveness of intramammary treatment with a combination of ampicillin and clxocillin. In their study, a 61% cure rate was observed following intramuscular therapy versus a 71% cure rate after intramammary treatment. The average bacteriological cure rate of streptococcal mastitis with three intramuscular injections of penethamate hydriodide was 70% (36). On the other hand, McDougall et al. (23) reported a very high cure rate in the treatment of clinical S. uberis mastitis with intramuscular injections of penethamate hydriodide or tylosin (almost 90% recoveries) and worse results for these antibiotics in the treatment of S. dysgalactiae clinical mastitis. Bradley and Green (3) carried out a survey on the effectiveness of cephalosporins in the intramammary treatment of clinical mastitis in the UK, France, and Germany. S. uberis (28.3%), Enterococcus sp. (1.2%), S. dysgalactiae (71.1%), and S. agalactiae (0.8%), among other bacterial species, were the main aetiological agents...
of the mastitis. The effectiveness was very low, and it was connected with new infections in quarters free from the primary infection, that was confirmed by Sandgren et al. (38) in cases caused by *S. uberis* and *S. dysgalactiae*.

The resistance of bacteria is proved to be the main reason for the low efficacy of antibiotics in the treatment of mastitis (28), among others factors, such as the low efficiency of immunological system (4) pathological changes in the udder parenchyma (1) and improper pharmacokinetic properties of antimicrobial drugs (12).

Many authors examined the *in vitro* susceptibility of streptococci, isolated from mastitic cows, using the disk diffusion method (5, 6, 14, 17, 25, 39) or MIC determination (19, 31, 33, 34). Reported results differed depending on the species tested, farm, and country.

Faibis et al. (10) concluded that resistance of streptococci to antibiotics increased and was noted in all species, and no family of antibiotics is unaffected by the resistance. Some authors (7, 34) suggest that only limited information is available on the antimicrobial susceptibility of these organisms although streptococci are frequently isolated from bovine mastitis. On the other hand, Guérin-Foublée et al (15) have reviewed the results of many authors on the sensitivity/resistance of *S. uberis*, *S. dysgalactiae*, and *S. agalactiae* to tetracyclines, macrolides, lincosamides, β-lactams, and aminoglycosides. All *S. dysgalactiae* and *S. agalactiae* isolated recently in Sweden were susceptible to penicillin. Bimodal MIC distributions for tetracycline in *S. dysgalactiae* and *S. uberis* indicated an acquired resistance in some isolates (2).

The surveillance of the antimicrobial resistance of aetiological agents of mammary gland inflammations is important to ensure optimal results of antimicrobial treatment, and minimise the risk for selection and spread of antimicrobial resistance (29, 30). Therefore, the aim of the study was to establish the *in vitro* sensitivity to antibiotics of *Streptococcus* sp. and *Enterococcus* sp. strains isolated from subclinical and clinical mastitis in cows in Poland in 2010-2011.

### Material and Methods

A total of 763 strains of bacteria including *S. agalactiae* (n=126), *S. dysgalactiae* (n=82), *S. uberis* (n=419), and *Enterococcus* sp. (n=136) were examined. These bacteria were isolated in 2010–2011 (to June 30) from subclinical and clinical cases of mastitis in cows belonging to herds in different regions of Poland. Quarter milk samples were collected aseptically by field veterinary surgeons. Before sampling, the first streams of milk were discarded, and teat ends were doubly disinfected and allowed to dry. In the laboratory, samples were streaked onto 5% blood agar and incubated at 35-37°C, and then were examined for growth after 24 and 48 h. Preliminary identification was based on colony morphology, haemolysis, and Gram staining. Identification of particular *Streptococcus* species was determined using API 20 Strep tests (bioMérieux).

Antimicrobial sensitivity of *Streptococcus* sp. and *Enterococcus* sp. was tested by the disk diffusion method and performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines in Mueller-Hinton agar. The following antibacterial agents (Oxoid) were used: penicillin (P; 10 i.u), amoxicillin (Aml; 25 µg), ampicillin (Amp; 10 µg), cloxacillin (Ob; 5 µg), cephalxin (Cl; 30 µg), cefoperazone (Cfp; 30 µg), cefapirin (Cpr; 30 µg), cefquinome (Ceq; 10 µg), erythromycin (E; 15 µg), tetracycline (Te; 30 µg), neomycin (N; 30 µg), lincomycin (My; 15 µg), and bacitracin (B; 10 u). *S. aureus* ATCC 25 923 and *Escherichia coli* ATCC 25 922 were the control strains. The isolates were categorized as susceptible, intermediate, and resistant by measuring the inhibition zone based on CLSI criteria.

### Results

Results of the examinations are presented in Figs 1–4. Figure 1 shows that *S. agalactiae* strains were mostly sensitive to bacitracin (97.6%), cefapirin (96.3%), amoxicillin (93.7%), and cefquinome (88.8%), and mostly resistant to neomycin (89.70%), tetracycline (49.2%), and cloxacillin (38.9%). Data presented in Fig. 2 demonstrate that *S. dysgalactiae* were mostly sensitive to bacitracin (100%), cefapirin (100%), amoxicillin (97.6%), cefapirin (97.6%), cefoperazone (93.9%), and cefquinome (92.5%), and resistant to neomycin (61.0%) and tetracycline (53.7%). As shown on Fig. 3, *S. uberis* strains were mostly sensitive to cefapirin (98.9%), bacitracin (98.8%), cefquinome (97.6%), amoxicillin (97.4%), and cefapirin (97.1%) and mostly resistant to neomycin (93.3%), tetracycline (35.3%), and lincomycin (22.4). Figure 4 demonstrates that *Enterococcus* sp. strains were mostly sensitive to amoxicillin (83.8%) and bacitracin (82.4%) and resistant to cloxacillin (76.5%), cefapirin (58.1%), lincomycin (55.9%), neomycin (53.7%), penicillin (48.5%), tetracycline (36.0%), and ampicillin (35.3%). It is also visible that *S. agalactiae* showed immediate sensitivity mainly to penicillin, ampicillin, and cloxacillin; *S. dysgalactiae* to penicillin and ampicillin; *S. uberis* to penicillin, ampicillin, and cloxacillin; and *Enterococcus* sp. to penicillin, ampicillin, and cefoperazone.

The only 28 strains (8.8%) of *S. agalactiae*, five (4.3%) of *S. dysgalactiae*, 18 (4.3%) of *S. uberis*, and six (4.4%) of *Enterococcus* sp. were sensitive to all tested antibiotics. The resistance of the examined species to the tested antibiotics is presented in Table 1. Strains of *S. agalactiae* were mostly resistant to one or two antibiotics, 13.2% of strains were resistant to three-five antibiotics, and 13.2% to seven antibiotics. The majority of *S. dysgalactiae* were resistant only to one-two, or sometimes to three antibiotics. Isolates of *S. uberis* showed resistance mostly to one or two, and sometimes to four-six antibiotics. The largest diversity in the resistance was exhibited by *Enterococcus* strains. More than 40% of the isolates were resistant to seven-ten antibiotics.
**Fig. 1.** Sensitivity of *S. agalactiae* to antibiotics: Aml-amoxicillin, P-penicillin, Amp-ampicillin, Ob-cloxacillin, Cl-cephalexin, Cfp-cefoperazone, Cpr-cefapirin, Ceq-cefquinome, E-erythromycin, Te-tetracycline, N-neomycin, My-lincosycin, B-bacitracin. R-resistant, I-intermediate, S-sensitive.

**Fig. 2.** Sensitivity of *S. dysgalactiae* to antibiotics. Symbols as in Fig. 1.

**Fig. 3.** Sensitivity of *S. uberis* to antibiotics. Symbols as in Fig. 1.
Fig. 4. Sensitivity to antibiotics of Enterococcus sp. 
Symbols as in Fig. 1.

Table 1
Strains resistant to antibiotics

<table>
<thead>
<tr>
<th>Species</th>
<th>Strains resistant to different numbers of antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>S. agalactiae</td>
<td>n</td>
</tr>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td>S. dysgalactiae</td>
<td>n</td>
</tr>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td>S. uberis</td>
<td>n</td>
</tr>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td>Enterococcus</td>
<td>n</td>
</tr>
<tr>
<td></td>
<td>%</td>
</tr>
</tbody>
</table>

Discussion

The examined streptococci, especially S. uberis, are frequently isolated as aetiological agents of clinical and subclinical mastitis in cows (6, 21). Their sensitivity to 13 antibiotics tested in this study distinctly differed among species. S. dysgalactiae strains were the most sensitive, compared to Enterococcus sp., which was the most resistant. Other authors also demonstrated substantial differences in the susceptibility patterns for the various organisms referred to as the environmental streptococci (7, 34).

Taking together the susceptible and intermediately susceptible isolates in regard to the examined antibiotics, amoxicillin, bacitracin, cefapirin, cefoperazone, and ceftiraxone were the most active against S. agalactiae. Penicillin and ampicillin were also effective in vitro. Other authors reported a high sensitivity of S. agalactiae to the above-mentioned antibiotics (15, 17, 39) as well. It is interesting that more than 50% of these strains were only intermediately sensitive to penicillin, ampicillin, and cloxacillin. All strains of S. agalactiae tested by Guerin-Faublée (15) were sensitive to β-lactams. The analysed strains were less resistant to penicillin, ampicillin, cloxacillin, and cefoperazone and more resistant to tetracycline compared to data published 8 years earlier (20).

Amoxicillin, ceftiraxone, bacitracin, cefalexin, penicillin, ampicillin, and cefoperazone were the most active against S. dysgalactiae. This agrees with many reports (6, 31, 39). All strains of S. dysgalactiae examined by Kalmus et al. (17) were sensitive to ampicillin, penicillin, and cephalotin, and about 90% of strains were sensitive to clindamycin, erythromycin, and gentamycin. Owens et al. (28) demonstrated very high in vitro susceptibility of S. dysgalactiae and other Streptococcus sp. to ampicillin, ceftiofur, cephapirin, cloxacillin, enrofloxacin, penicillin, pirlimycin, and tetracycline. Except for enrofloxacin and pirlimycin (not examined), our strains were less susceptible to cloxacillin, penicillin, ampicillin, and tetracycline.
S. uberis was less resistant compared to S. agalactiae and more resistant compared to S. dysgalactiae. However, some authors showed a small level of resistance of this species against many antibiotics (17, 28, 30). Taking together susceptible and intermediately susceptible strains, it can be stated that cefoperazone, amoxicillin, cefquinome, ampicillin, cephalxin, cefapirin, and bacitracin were highly active in vitro against S. uberis. The sensitivity of the presently examined isolates is almost identical compared to earlier data (20).

The examined enterococci were distinctly more resistant to tested antibiotics than streptococci. This finding agrees with other authors’ results (7, 34). Only amoxicillin and bacitracin were highly active in vitro. More than 70% of the strains were also sensitive (sensitive and intermediate sensitive together) to erythromycin, cefapirin, and cefoperazone. Owens et al. (27) reported that only penicillin and ampicillin demonstrated moderate activity against Enterococcus faecalis, but our examination gave an opposite result.

The performed study also shows that the tested bacteria were resistant against two-three (S. agalactiae, S. dysgalactiae) or even against six-ten antibiotics (S. uberis, Enterococcus sp.). The multi-resistance of these pathogens has been reported by other authors. Corti et al. (6) examined 100 strains of Streptococcus sp. isolated from clinical mastitis in Switzerland and found that 30% of the strains were sensitive to all the antibiotics tested. Four percent were resistant to penicillin G, 4% to amoxicillin/clavulanic acid, 1% to cefoperazone, 2% to cefquinome, 35% to neomycin, 22% to gentamicin, 61% to kanamycin, and 11% to lincomycin. Forty-two percent of the strains showed multiple resistance. The high level of resistance of S. agalactiae, S. dysgalactiae, S. uberis, and enterococci against streptomycin, penicillin, and cloxacillin, and low level of sensitivity to cephealin, clindamycin, gentamycin, amoxicillin, tetracycline, oxytetracycline, ampicillin, and chloramphenicol was observed in Iran (7). The in vitro resistance against two or more antibiotics has also been described (10).

It is possible that mastitigenic bacteria cannot lose their sensitivity to antibiotics over time but sometimes even regain this function. Erskine et al. (8) reported the increase in sensitivity to oxacillin, gentamicin, and pirlimycin of S. uberis, and to erythromycin, gentamicin, sulfa-trimetoprim, and tetracycline of S. dysgalactiae.

The in vitro testing is considered to be a predictor of therapy outcome for mastitis (1, 22, 28). The examination is especially useful in infections caused by Staphylococcus sp., newly acquired S. aureus, S. uberis, S. dysgalactiae, and S. agalactiae (28). Our earlier examination showed that recovery rates from acute mastitis equaled “zero” if pathogens were resistant to antibiotics used in intramammary treatment (22). However, the utility of the in vitro susceptibility testing of mastitigenic bacteria before treatment was sometimes useless (5, 16). It was raised that criteria used for categorising isolates as susceptible or resistant are based on human data, so they cannot be applied to predict clinical efficacy in bovine mastitis treatment (33). On the other hand, it is necessary to monitor mastitis pathogens to assess any changes in their antibiotic resistance patterns (15). First of all, prudent use of antibiotics can avoid the increase and dissemination of antimicrobial resistance arising from the use of antimicrobial drugs in animals (11).

The sensitivity of bacteria to antibiotics is tested using the disk diffusion method or MIC determination. Many scientists assume that comparison of results achieved by different methods of the sensitivity testing is not legitimate (19, 33). According to suggestions by Rossito et al. (34), the use of the agar disk diffusion (Kirby-Bauer) susceptibility test with human-based interpretative criteria is contraindicated, and these tests should only be performed with mastitis-specific interpretative criteria. However, from the data found in the literature it can be also concluded that both of the above-mentioned methods give comparable results (9, 13). These suggestions were recently confirmed by Saini et al. (35), who proved that the agar disk diffusion method, compared to the reference manual broth microdilution test, showed moderate to high diagnostic accuracy and very good essential and categorical agreement for most udder pathogens-antimicrobial combinations. Independently of the methods used, the examination of resistance to antibiotics of bacteria isolated from milk samples provides useful data for surveillance proposes and a baseline for identifying changes in antimicrobial sensitivity (30). Based on our study, amoxicillin, bacitracin, and cephalosporins can be advised for the treatment of mastitis caused by streptococci, and amoxicillin or bacitracin for the treatment of inflammation caused by Enterococcus sp. However, continuous monitoring of the sensitivity of these organisms, especially enterococci, should be carried out.

In conclusion, streptococci isolated from mastitic milk in 2010-2011 differ in their sensitivity to antibiotics that are frequently applied in mastitis treatment. Enterococcus sp. is significantly more resistant than Streptococcus sp. The large percentage of streptococci intermediately sensitive to penicillin, ampicillin, and cloxacillin indicates the spread of resistance to penicillin among both species.

References


