ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIA ISOLATED FROM COWS WITH MASTITIS IN 2006-2007

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Abstract

The purpose of the study was determining the in vitro sensitivity of Streptococcus agalactiae, other Streptococcus sp., Staphylococcus aureus, coagulase-negative staphylococci, Escherichia coli, and Arcanobacterium pyogenes strains to antibiotics commonly used in veterinary praxis. These pathogens were isolated from cows with clinical or subclinical mastitis in 2006–2007. The activity of 10–14 antibiotics to each bacterial species was examined. The sensitivity was tested by disk diffusion method and performed according to CLSI guidelines in Mueller-Hinton agar. Streptococcus agalactiae strains were mostly sensitive to amoxicillin (93.6%), cefquinom (96%), cefapirin (94.1%), cefoperazone (88.9%), bacitracin (87.1%), and cephalexin (79%). Other streptococci were most susceptible to amoxicillin (91.4%), cefquinom (84.1%), bacitracin (89%), and cephalexin (79.7%). Amoxicillin/clavulanic acid (97.4%), cloxacinil (90%), cefquinom (92.9%), cephalaxin (90.3%), and bacitracin (88.6%) were the most active against Staphylococcus aureus. Coagulase-negative staphylococci were sensitive to amoxicillin/clavulanic acid (100%), cefquinom (89.2%), cephalaxin (89.3%), bacitracin (86.1%), rifaximin (86.8%), and cepafarin (86.5%). Norfloxacin (85.1%), enrofloxacin (83.9%), and marbofloxacin (84.4%) were active against Gram-negative bacilli. Only amoxicillin (87.1%) from ten analysed antibiotics exhibited very high activity against Arcanobacterium pyogenes.

Key words: cows, mastitis, aetiological agents, antibiotic sensitivity.

Udder inflammations are still the most frequent and costly diseases affecting dairy cows in the world (2, 15, 21, 40). Under default Dutch circumstances, mastitis costs € 140/cow per year (17). The average cost of the clinical mastitis case in the USA was calculated lastly as $179 (2). Staphylococcus aureus, coagulase negative staphylococci (CNS), Streptococcus agalactiae, and environmental streptococci are predominant aetiological agents in both subclinical and clinical forms of mastitis (4, 10, 26, 28, 32). Escherichia coli is the main aetiological agent of acute form of mastitis, though lastly chronic and even subclinical forms were noted (4, 26). Arcanobacterium pyogenes causes purulent inflammations that are called “summer mastitis” (26, 45). Cure rates especially in cases of Staph. aureus mastitis as well as Arc. pyogenes mastitis are rather poor (3, 25, 41, 42, 45). The resistance of the bacteria to antibiotics is proved to be, the main reason of low efficacy of the drugs the in treatment of mastitis (31), besides such factors as the low efficiency of the immunological system (5, 36), pathological lesions in the udder parenchyma (3), and improper pharmacokinetic properties of antimicrobial drugs (1, 12).
Committee for Clinical Laboratory Standards) guidelines in Mueller-Hinton agar (29). The following antibacterial agents (Oxoid) were used: penicillin (P; 10 i.u), amoxicillin (Aml; 25 μg), amoxicillin with clavulanic acid (Amc; 30 μg), ampicillin (Amp; 10 μg), cloxacillin (Ob; 5 μg), cefoperazone (Cfp; 30 μg), cefacetrol (Cef; 30 μg), ceftiquinom (Ceq; 10 μg), cephalaxin (Cl; 30 μg), tetracycline (Te; 30 μg), neomycin (N; 30 μg), streptomycin (S; 10 μg), gentamicin (Cn; 10 μg), bacitracin (B; 10 u), lincomycin (My; 15 μg), rifaximin (Rax; 40 μg), norfloxacin (Nor; 10 μg), enrofloxacin (Enr; 5 μg), and marbofloxacin (Mar; 5 μg). *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were the control strains.

Interpretation of the test results: sensitive (S), intermediate sensitive (I), and resistant (R) was based on CLSI criteria. Activity of 10–14 antibiotics to each bacterial species was analysed.

Results

Results of the examinations are presented on Figs 1-6. The most active antibiotics against *Streptococcus agalactiae* (Fig. 1), from 13 analysed, were amoxicillin (93.6%), ceftiquinom (96%), cefapirin (94.1%), cefoperazone (88.9%), bacitracin (87.1%), and cephalaxin (79%). Taking together sensitive (S) and intermediate sensitive (I) strains, penicillin, ampicillin, and cloxacillin were also effective. *Streptococcus agalactiae* strains were mostly resistant (R) to neomycin (80%) and tetracycline (40.2%).

From 12 examined antibiotics, the most active against other *Streptococcus* sp. (Fig. 2) were amoxicillin (91.4%), ceftiquinom (84.1%), bacitracin (89%), and cephalaxin (79.7%). Taking together S and I strains, penicillin, ampicillin, and cefoperazone were also effective. High percentage of the strains was resistant to tetracycline (62.3%), lincomycin (46.6%), and cloxacillin (31.1%).

From 11 analysed antibiotics, amoxicillin/clavulanic acid (97.4%), ceftiquinom (92.9%), cephalaxin (90.5%), cloxacillin (90%), and bacitracin (88.6%) were the most active against *Staph. aureus* (Fig. 3). Taking together S and I strains, cefoperazone (97.7%), amoxicillin (76.9%), and neomycin (77.3%) were also effective. High percentage of strains was resistant to penicillin (66.9%), tetracycline (41.7%), and lincomycin (39.4%).

From 13 analysed antibiotics, ceftiquinom (89.2%), cephalaxin (89.3%), bacitracin (86.1%), rifaximin (86.8%), and cefacetrol (86.5%) were the most active against coagulase-negative staphylococci (Fig. 4). Taking together S and I strains, amoxicillin (89.5%), cloxacillin (88.3%), cefoperazone (97.5%), and neomycin (86.8%) were also effective. In addition, all the strains (100%) from 200 tested were sensitive to amoxicillin/clavulanic acid. A high percentage of strains were resistant to penicillin (60.5%), ampicillin (69.1%), lincomycin (46.6%), and tetracycline (29.9%).

From 14 examined antibiotics, norfloxacin (85.1%), enrofloxacin (83.9%), and marbofloxacin (84.4%), were the most active against Gram-negative bacilli (Fig. 5). Only 9.7%, 9.6%, and 12% of the strains were resistant to these antibiotics, respectively. Taking together S and I strains, effective in vitro were also gentamicin (60.8% + 26.9%) and amoxicillin/clavulanic acid (62.7% + 20.9%). The highest percentage of the strains was resistant to rifaximin (76.1%), streptomycin (61.5%), ceftacetril (53.5%), tetracycline (47.1%), and neomycin (40.2%).

Only amoxicillin (87.1%) from 10 analysed antibiotics showed very high activity against *Arc. pyogenes* (Fig. 6). Taking together S and I strains, the most active in vitro was also cefoperazone (100%) and cephalaxin (85.9%). Penicillin (60%) and ampicillin (46.2%) in the highest percentage showed intermediate activity. The least active were tetracycline (83.9%), cloxacillin (46.6%), lincomycin (45.2%), and ceftiquinom (36.8%).

Discussion

The examined strains of bacteria are most frequently isolated as main aetiological agents of clinical and subclinical mastitis in cows (4, 7, 10, 14, 26, 28, 32, 45). Their in vitro susceptibility to antibiotics was similar or different comparing to data reported by authors, who applied the disc diffusion method (6, 7, 9, 22, 24, 27, 28, 37, 38, 44) or MIC determination (18, 20, 30, 32, 34, 43, 46). It should be emphasised that many scientists assume that comparison of results achieved by different methods in the sensitivity testing is not legitimate (19, 34). However, from literature data it can be also concluded that both above mentioned methods gave comparable results (8, 13, 33).

From this study, it is visible that *Streptococcus agalactiae* strains were the most sensitive to amoxicillin, ceftiquinom, cefoperazone, bacitracin, and cefalexin. Other authors also reported a high sensitivity of *Streptococcus agalactiae* to the mentioned antibiotics (14, 22, 38). Above 50% of these strains were only intermediate sensitive to penicillin, ampicillin, and cloxacillin. The analysed strains were less resistant to Pe, Amp, Ob, and Cfp and more resistant to Te comparing to data published five years earlier (24).

Amoxicillin, ceftiquinom, bacitracin, cefalexin penicillin, ampicillin, and cefoperazone were the most active against other *Streptococcus* sp. Strains than *Streptococcus agalactiae*. It agrees with many reports (7, 32, 38) in which the same antibiotics were compared. Owens *et al.* (31) found very high in vitro susceptibility of *Streptococcus uberis, Str. dysgalactiae*, and other *Streptococcus* sp. to ampicillin, ceftiofur, cephalin, cloxacillin, enrofloxacin, penicillin, pirlimycin, and tetracycline. Except of enrofloxacin and pirlimycin (not examined), our strains were less susceptible to cloxacillin, penicillin, ampicillin, and tetracycline. The sensitivity of the presently examined isolates was almost identical comparing to earlier data (24).
Fig. 1. Sensitivity to antibiotics of *Str. agalactiae* strains isolated from cows with mastitis: S – sensitive, I – intermediate sensitive, R – resistant.

Fig. 2. Sensitivity to antibiotics of other *Streptococcus* sp. strains isolated from cows with mastitis: S – sensitive, I – intermediate sensitive, R – resistant.
**Fig. 3.** Sensitivity to antibiotics of *Staph. aureus* isolated from cows with mastitis: S – sensitive, I – intermediate sensitive, R – resistant.

**Fig. 4.** Sensitivity to antibiotics of CNS isolated from cows with mastitis: S - sensitive, I – intermediate sensitive, R – resistant.
Fig. 5. Sensitivity to antibiotics of Gram-negative bacilli isolated from cows with mastitis: S – sensitive, I – intermediate sensitive, R – resistant.

Fig. 6. Sensitivity to antibiotics of *A. pyogenes* isolated from cows with mastitis: S – sensitive, I – intermediate sensitive, R – resistant.
Amoxicillin/clavulanic acid, cefquinom, cefalexin, cloxacillin, bacitracin, and cefoperazone were the most active against *Staph. aureus*. The next group included amoxicillin and neomycin. The examined strains were resistant mostly to penicillin, tetracycline, and lincomycin. These strains were more susceptible to penicillin, bacitracin, and tetracycline than staphylococci isolated in the eastern part of Poland (37). Other authors found higher sensitivity to Cl, Cef, Ceq, Cn, Cfp, Amp, and P (38), or to ampicillin, cefotilin, cephradin, cloxacillin, enrofloxacin, penicillin, pirlimycin, and tetracycline (31). All the strains examined by Corti et al. (7) were sensitive to Amc, Ob, Cfp, Ceq, N, Cn, and My and only 9% were resistant to penicillin and 7% to ampicillin. More than 50% of strains examined in Finland were resistant to penicillin (32). Comparing to older data, the presently examined *Staph. aureus* strains exhibited lower degree of resistance to ampicillin, cloxacillin, cefoperazone, lincomycin, and neomycin (24).

Amoxicillin/clavulanic acid, cefquinom, cephalxin, bacitracin, rifaximin, cefacetril, amoxicillin, cloxacillin, cefoperazone, and neomycin were the most active against coagulase negative staphylococci. A high percentage of the strains were resistant to penicillin, ampicillin, lincomycin, and tetracycline.

Owens et al. (31) demonstrated very high susceptibility *in vitro* of *Staphylococcus* sp. to ampicillin, cefotilin, cefpharin, cloxacillin, enrofloxacin, penicillin, pirlimycin, and tetracycline, Schroder et al. (38) to cephalotilin, cefacetril, cefquinom, cefoperazone, tetracycline, ampicillin, and penicillin, and Pittaka (32) to cephalotilin, gentamicin or neomycin. Our strains were also susceptible to cloxacillin, as well as to cefalosporins. However, they were more resistant to ampicillin, penicillin, and tetracycline. Other authors noted lack of resistant strains to cefoperazone, cefquinom, neomycin, and gentamicin (7) or to cephalotilin and cefotilin (34). The examined strains were more resistant to penicillin and ampicillin than five years ago (24).

Gram-negative isolates, mostly coliforms, were sensitive to quinolones (Nor, Enr, and Mar) and gentamicin. Other authors reported higher sensitivity to tetracycline, cephalxin, ampicillin, gentamicin, and dihydrostreptomycin (20) than in this study. Corti et al. (7) found that all examined strains were sensitive to Amc, Cfp, Ceq, Cn, and only 9% were resistant to neomycin and 20% to ampicillin. On the other hand, some authors reported high percentage of resistance to ampicillin and tetracycline (34, 43) or to streptomycin (43). These authors found that the examined strains were genotypically different, multidrug resistant, and carried multiple resistance genes. Comparing to earlier examinations (24), the analysed strains were less resistant to amoxicillin but more resistant to neomycin. Sensitivity to streptomycin and cefoperazone did not change.

*Arcanobacterium pyogenes* strains were highly sensitive only to ampicillin and cefoperazone. Characteristic feature was the intermediate sensitivity of more than 50% of the analysed strains to penicillin and ampicillin. Schröder et al. (38), found that all examined strains were sensitive to penicillin, ampicillin, cefoperazone, cefacetril, and cephalonium. Other authors on the basis of MIC determination reported that strains of bovine origin were susceptible to penicillins, cepham, gentamicin, erythromycin, tilmicosin and lincomycin, and highly resistant to tetracycline (46) as well as to tylosin (18). The sensitivity to some penicillins and cephalosporins and resistance to tetracycline of our isolates was similar to above-mentioned data. However, the sensitivity of *Arc. pyogenes* isolated from bovine mastitis to antibiotics was not frequently tested.

The majority of authors have noted the increase in the resistance to antibiotics of bacteria, mostly staphylococci, isolated from mastitis (28, 32, 35, 44). In Finland, the proportion of *Staph. aureus* strains resistant to at least one antimicrobial drug increased from 36.9% in 1988 to 63.6% in 1995, and that of CNS from 26.6% to 49.7% and multiresistance also increased (28). Other authors found that only 35 of *Staphylococcus* sp. isolates were susceptible to all 15 tested antibiotics, while the remaining 204 isolates were resistant at least to one of the antibiotics (44). On the other hand, Oliveira et al. (30) have determined MIC concentration for *Staph. aureus* strains from 11 countries, and they demonstrated that overall level of resistance, tested regardless of country, was generally low for all antimicrobial agents that were currently available commercially to treat bovine mastitis.

It is possible that mastitogenic bacteria can lose the sensitivity to antibiotics over the time or even acquire sometimes this feature. Erskine et al. (9) reported an increase in the sensitivity to ampicillin, penicillin, and erythromycin of *Staph. aureus*, to oxacillin, gentamicin, and pirlimycin of *Str. uberis*, to erythromycin, gentamicin, sulfadiazine, and tetracycline of *Str. dysgalactiae*, and to ampicillin and cephalotilin of *E. coli* strains during seven years. The *in vitro* resistance to antibiotics of bacteria isolated in the same farm can change from one year to the next one (22).

The *in vitro* testing is considered to be a predictor of therapy outcome for mastitis (3, 23, 27, 31, 44). The examination is especially useful in cases caused by *Staphylococcus* sp., newly acquired *Staph. aureus*, *Str. uberis*, *Str. dysgalactiae*, and *Str. agalactiae* (31). Our earlier investigations showed that recovery rates from acute mastitis equals “zero” if the pathogens were resistant to antibiotics used in intrammary treatment (27). However, the usefulness of the *in vitro* susceptibility testing of mastitogenic bacteria before treatment was sometimes useless (6, 16). It was raised that the interpretative criteria used for categorising of isolates as susceptible or resistant are based on human data, so they cannot be used to predict clinical efficacy in bovine mastitis treatment (34). On the other hand, it is necessary to monitor mastitis pathogens to assess any changes in their antibiotic resistance patterns (14). Prudent use of antibiotics first of all can avoid the increase and dissemination in antimicrobial resistance.
arising from the use of antimicrobial drugs in animals (11, 39).

References


