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# CONTAMINATION OF THE ENVIRONMENT BY RESISTANT PATHOGENIC BACTERIA

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## ABSTRACT

Between 1999 and 2002 antibiotic resistance was studied in 896 strains of mastitis agents (*Streptococcus agalactiae* - 250; *S. uberis* - 105; "β-haemolytic" streptococci - 118; coagulase negative staphylococci - 215) isolated from dairy cow milk. Of 250 strains of *Streptococcus agalactiae* all were susceptible to penicillin. Resistance to cephalotin, amoxicillin combined with clavulanic acid, oxacillin, cloxacillin and ampicillin ranged from 1.2 % to 2.8%. Of the above strains 74.8% were resistant to streptomycin, 78.0% to neomycin, 5.6% to tetracycline and 4.4% of strains were not susceptible to erythromycin. Of 208 *Staphylococcus aureus* strains 30.8% were resistant to penicillin and 30.1% to ampicillin. Resistance to oxacillin, cloxacillin, cephalotin, tetracycline and erythromycin ranged from 1.9% to 4.3% of the strains whereas 4.8 % of the strains were resistant to neomycin and 5.7% to amoxicillin combined with clavulanic acid. Resistance to streptomycin was observed in 5.3% of strains. In 695 strains of bacterial agents of mastitis that have been isolated from the bodies of dairy cows as well as from the environment a lower prevalence resistance to antibiotics was observed. Of 96 strains of *Streptococcus agalactiae* all proved to be susceptible to penicillin. Resistance to oxacillin, cloxacillin, ampicillin, cephalotin and amoxicillin combined with clavulanic acid ranged from 1.0 % to 2.1%. Of these strains 83.3% were resistant to streptomycin, 78.1% to neomycin, 7.3% to tetracycline and 4.2% of strains were not susceptible to erythromycin.

In 136 strains of *Staphylococcus aureus* resistant to some of the antibiotics tested the number of resistancies to these antibiotics and the characteristics of the antibioticograms were examined. Four strains appeared to be resistant to 1 antibiotic, 44 strains simultaneously to two antibiotics and 80, 4 and 4 strains were simultaneously resistant to 3, 4 and 5 antibiotics, respectively. The results point at a high occurrence of polyresistant *Staphylococcus aureus* strains in the housing environment of dairy cows as well as at a high proportion of these strains in intramammary infections.

**Key words:** Pathogenic bacteria, antibiotics, antibiotic susceptibility

## INTRODUCTION

On a cattle farm maintenance of health appears to be one of the limiting factors of performance. Observing the zoohygienic rules is closely related to morbidity of the dairy cows and also to the prevention of damage to the environment (Plachá et al., 2001; Sasáková et al. 1999). It is the task of hygiene in primary milk production to decrease the contamination of the housing facilities and the living environment in general by pathogens showing a high degree of resistance to the antibiotics used in the field and thus to prevent also morbidity of the dairy cows, but mainly the occurrence of the most frequent disease - mastitis. The basic means to control this disease is antibiotic treatment both during the dry period and during lactation for which intramammary preparations are used that are available on the market at sufficient amounts. With regard to this offer it is of special importance to prepare a survey of the occurrence of mastitis-causing strains resistant to antibiotics. Such surveys of sensitivity to the most frequently used antibiotics and their evaluation present the fundamental presupposition for obtaining information on the

development of resistance to the antibiotics applied, or on polyresistance and its type in the individual causative agents on the given territory. By these measures the basic presuppositions are given for successful and rational treatment on the dairy farms, choice of the disinfectants at simultaneous application of zoohygienic principles on the farm as well as for decreased contamination of the outer environment with poly- and multiresistant pathogens.

It was the aim of this research to examine the resistance to antibiotics in pathogens isolated from the milk of cows with clinical mastitis, from the body of dairy cows and from the environment as well as to characterize the antibioticograms in *Staphylococcus aureus* strains isolated from milk samples and the body of dairy cows but also smears taken from the environment.

## MATERIALS AND METHODS

In the course of the years 1999 - 2002 the occurrence of resistance to antibiotics was examined in 896 strains of bacterial agents isolated from the secretion of the mammary gland in dairy cows and in 695 strains of mastitis agents isolated from the body of the animals and their environment. Sampling and bacteriological examination of the samples were carried out according to current methods (IDF, Bulletin No. 132, 1981). The agar-diffusion sensitivity test to 10 antibiotics was performed by the method of Bauer-Kirby (1966) on Müller-Hinten agar according to Urbášková et al. (1985) in Petri dishes of 90 - 100 mm diameter and 4 mm thickness of agar. The inoculum was prepared according to Urbášková et al. (1985) so that the resulting turbidity would correspond a 0.5 density of the McFarland scale. Inoculation was carried out by spreading with adapted sterile cotton tampons.

Table 1. Interpretation of the diameters of inhibition zones

Antibiotic <sup>1</sup>	Abbreviation <sup>2</sup>	Active substance contents of the disk <sup>3</sup> (µg; u)	Test evaluation (diameter of inhibition zone in mm) <sup>4</sup>	
			resistant <sup>5</sup>	susceptible <sup>6</sup>
Penicilín	PNC	10*	< 28	>29
Ampicilín	AMP	10	<28	>29
Oxacillin	OXA	1	<12	>13
Cloxacillin	CLOX	10	<10	>14
Amoxicillin + clavulan acid	AM+C	20/10	<19	>20
Cefalotín	CEF	30	<26	>35
Streptomycín	STR	10	<15	>20
Neomycín	NEO	30	<20	>26
Tetracyklín	TET	30	<14	>18
Erytromycín	ERY	15	<20	>26

◆= contents of active ingredient is given in int. units.

Reading of the test results was carried out after an 18-hour incubation of the plates in the thermostat at 37°C. Control of the quality of the media and the test disks used was carried out according to Urbášková et al. (1985) with the *Staphylococcus aureus* ATCC 25923 reference strain. The inhibition zones of the antibiotics examined in the single strains were evaluated according to Urbášková et al. (1985). Ten antibiotics were tested using Lachema (Brno CR) antibiotic test disks. Interpretation of the diameters of the inhibition zones was made according to Urbášková et al. (1985) and the NCCLS (1997).

## RESULTS AND DISCUSSION

The results of testing the susceptibility of bacterial strains isolated from the secretion of the udder of mastitis-infected dairy cows to 10 antibiotics are given in Table 2. All strains of *Streptococcus agalactiae* (250 strains) were sensitive to penicillin, showed excellent susceptibility to oxacillin, cloxacillin, ampicillin and the combination of amoxicilline with clavulanate and cephalosporin; good sensitivity was stated to tetracyclin and erythromycin. However, the number of strains resistant to streptomycin and neomycin was relatively high, i.e., 79.0% and 80.5%, respectively. Development of resistance to the antibiotics under examination in *Streptococcus uberis* (105 strains) and "beta-haemolytic streptococci" (118 strains) can, except the results found with streptomycin and neomycin, be considered as optimal. In *Staphylococcus aureus* (208 strains) a high occurrence rate of penicillin- and ampicillin-resistant strains was observed (30.8 and 30.1%, respectively); however, these strains revealed relatively good susceptibility to the other antibiotics. The highest numbers of penicillin- and ampicillin-resistant strains (36.3% and 11.2%, respectively) were recorded among the coagulase-negative staphylococci (215 strains). The occurrence of strains resistant to other antibiotics did not surpass the limit of 7.4%.

*Table 2: Susceptibility of 896 pathogenic bacteria to 10 antibiotics isolated from the milk of dairy cows with clinical mastitis*

Antibiotic tested	Streptococcus sp.			Staphylococcus sp.	
	<i>S. agalactiae</i> n = 250	<i>S. uberis</i> n = 105	„β-haemolytic“ n = 118	<i>S. aureus</i> n = 208	Coagul.- negative n = 215
	occurrence of resistant strains in %				
Penicilin	0.0	3.8	5.1	30.8	36.3
Oxacilin	2.8	3.8	3.4	2.4	6.5
Cloxacilin	2.0	4.8	3.4	2.4	3.7
Ampicilin	1.6	2.9	2.5	30.1	11.2
Amoxicilin + clavulan	1.2	1.0	4.2	5.7	5.1
Cefalotin	1.2	1.9	1.7	2.4	4.2
Streptomycin	74.8	24.8	19.5	5.3	7.4
Neomycin	78.0	39.1	22.9	4.8	6.5
Tetracyklin	5.6	7.6	5.9	4.3	5.6
Erythromycin	4.4	4.7	3.4	1.9	5.1

In Table 3 the occurrence of resistance to 10 antibiotics is given in 695 strains of bacterial agents isolated from the body of the dairy cows and the outer environment. Out of 96 strains of *Streptococcus agalactiae* all proved to be susceptible to penicillin; the occurrence rate of strains resistant to oxacillin, cloxacillin, ampicillin, cephalotin and to the combination amoxicillin plus clavulanic acid ranged from 1.0% to 2.1%. However, resistance to erythromycin was 4.2% which is 3.0% less than that to tetracyclin (7.3%) whereby the greatest proportio of resistant strains was found to occur with streptomycin (83.3%) and neomycin (78.1%). Out of 136 strains of *Staphylococcus aureus* the majority proved to be resistant to penicillin (49.3%) and ampicillin (47.1%) and surprisingly to the

combination of amoxicillin plus clavulanic acid (12.9%). Out of 150 strains of coagulase-negative staphylococci (12.0%) as many as 36.3% and 11.6% appeared to be resistant to penicillin and ampicillin, respectively. However, there was a high occurrence of strains resistant also to streptomycin (9.8%), tetracyclin (9.2%) and neomycin and oxacillin (8.2%).

Table 3: Susceptibility of 695 pathogenic bacteria isolated from the environment and bodies of dairy cows to 10 antibiotics

Antibiotic tested	Streptococcus sp.			Staphylococcus sp.	
	S. agalactiae n = 96	S. uberis n = 141	„β-haemolytic“ n = 115	S. aureus n = 136	Coagul. - negative n = 207
	Occurrence of resistant strains in %				
Penicilin	0.0	4.3	6.1	49.3	43.0
Oxacilin	1.0	2.8	4.3	3.7	8.2
Cloxacilin	1.0	2.8	1.7	3.7	4.8
Ampicilin	1.0	2.5	2.6	47.1	11.6
Amoxicilin + clavulan	2.1	4.2	2.6	5.9	4.4
Cefalotin	1.0	2.5	1.7	3.7	2.4
Streptomycin	83.3	23.3	29.6	5.9	9.8
Neomycin	78.1	35.0	28.7	5.1	8.2
Tetracyclin	7.3	7.5	6.1	5.1	9.2
Erytromycin	4.2	9.2	5.2	2.2	5.8

In table 4 the number of resistances to antibiotics and the characteristics of the antibiograms are given for 136 resistant *Staphylococcus aureus* strains isolated from milk samples and smears from the environment and the body of the dairy cows. Resistance to only 1 antibiotic was found in 4 strains of *Staphylococcus aureus*, resistance to 2 antibiotics was observed in 44 strains the majority of which (40) were resistant to penicillin and ampicillin. Simultaneous resistance to 3 antibiotics was determined in 80 resistant strains of *Staphylococcus aureus* of which 79 revealed resistance to penicillin, ampicillin and another tested antibiotic and only one strain was simultaneously resistant to penicillin, streptomycin and tetracyclin. Four strains were simultaneously resistant to 4 antibiotics (2 strains showed a combined resistance to penicillin, ampicillin, tetracyclin and amoxicillin + clavulanic acid, one strain was resistant to penicillin, neomycin and tetracyclin and another one to penicillin, ampicillin, oxacillin and tetracyclin). Similarly 4 strains were simultaneously resistant to 5 antibiotics (2 to penicillin, ampicillin, neomycin, tetracyclin and amoxicillin + clavulanic acid, one to penicillin, ampicillin, oxacillin, tetracyclin and streptomycin and one to penicillin, ampicillin, tetracyclin, amoxicillin + clavulanic acid and erythromycin. As can be seen from the results, as many as 88 strains proved to be simultaneously resistant to three and more antibiotics.

Table 4 Number of resistancies to antibiotics and characteristics of the antibiograms in 136 strains of *Staphylococcus aureus* isolated from milk samples and smears of the outer environment and the bodies of the animals

Not susceptible to	ANTIBIOGRAM	Number of strains
<b>one antibiotic</b>	STM	1
	NEO	1
	TET	1
	ERY	1
<b>two antibiotics</b>	PNC AMP	40
	PNC CLOX	1
	PNC STM	1
	PNC NEO	1
	PNC CEF	1
<b>three antibiotics</b>	PNC AMP TET	6
	PNC ANP OXAC	8
	PNC AMP CLOX	9
	PNC AMP AM+C	15
	PNC AMP CEF	9
	PNC AMP NEO	12
	PNC AMP STM	15
	PNC STM TET	1
	PNC AMP ERY	5
<b>four antibiotics</b>	PNC AMP TET AM+C	2
	PNC AMP NEO TET	1
	PNC AMP OXA TET	1
<b>five antibiotics</b>	PNC AMP/ TET AM+C ERY	1
	PNC AMP OXA TET STM	1
	PNC AMP NEO TET AM+C	2
<b>Total</b>		<b>136</b>

From the results it is obvious that *Streptococcus agalactiae* maintains its extreme susceptibility to conventional penicillin which coincides with the results of ŠKARDA et al. (1990) and OWENS et al. (1997). *Streptococcus agalactiae* showed excellent susceptibility to oxacillin, cloxacillin, ampicillin, amoxicillin + clavulanic acid which is in accordance with the data reported by WATTS (1995). The high percentage of occurrence of *Streptococcus agalactiae* strains resistant to streptomycin and neomycin (80.8% and 83.6%, respectively) is mainly caused by the naturally increased MIC values which for this bacteria are as follows: streptomycin - 16.0 - 128.0 µg/ml, neomycin 8.0 - 128.0 µg/ml (OWENS et al. , 1990; VASIL, 1998).

Evaluating the occurrence of resistance in *Staphylococcus aureus* strains it is important to go back to history. Already in 1974 CHRISTIE et al. pointed at the fact that in England, introduction of the broad use of antibiotics in the treatment of mastitis in dairy cows caused an increase in the occurrence of resistant strains of *Staphylococcus aureus* from 6.0% in 1969 to 45.4% in 1972. In the field of human medicine in Slovakia KRČMÉRY et al. (1975) noted that in examinations carried out in the years 1973 - 1975 the occurrence of *S. aureus* strains resistant to penicillin, ampicillin, streptomycin, tetracyclin and erythromycin presented 82.4%, 40.6%, 27.6%, 35.6% and 25.7% and this situation has persisted since.

Malinowski et al. (1992) draw attention to the unfavourable situation regarding the susceptibility of *S. aureus* strains to antibiotics in Poland (penicillin resistance 48.6%). In *Staphylococcus aureus* strains obtained from clinically and latent mastitis of sheep Šimko and Bartko (1996) reported an occurrence of 60.0% of penicillin-resistant strains.

Vasil' (1998) described the occurrence of resistance to antibiotics in bacterial agents isolated from mastitic dairy cows in East Slovakia during the period from 1995 - 1997 and reported good susceptibility to oxacillin, erythromycin and oxytetracyclin but high resistance to penicillin (31.3%) in *Staphylococcus aureus* strains. Myllys et al. (1998) compared the development of both prevalence of mastitis in dairy cows and resistance to antibiotics in *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) based on the examinations of two representative sets in Finland. They found prevalence of mastitis in dairy cows to decrease from 47.8% in 1988 (4495 animals examined) to 37.8% in 1995 (2648 animals examined). *Staphylococcus* sp. presented the largest group of isolated pathogens, however, the occurrence of *S. aureus* decreased proportionally to the increased isolation of CNS. The occurrence of *Staphylococcus aureus* strains resistant to at least one of the antibiotics examined increased from 36.9% (1988) to 63.6% (1995) and the situation was similar for CNS (increase from 26.6% to 49.7%). This increase was most frequently caused by the beta-lactamase producing strains of *Staphylococcus aureus*. The authors reported also an increased number of multiresistant strains of *Staphylococcus aureus* and CNS; however, this increase was proportionate to that of the antibiotic-resistant strains.

From the results presented in this paper it follows that the fact must be kept in mind that qualitatively new epizootiological - zoohygienic relations are arising under the new conditions of dairy cow breeding (Vasil', 1992; Pačajová and Venglovský, 1997). Therefore regular observation of the occurrence of resistance to antibiotics in the mastitis-causing agents gain increasing importance both because of the morbidity of the animals and the possible contamination of the environment by multiresistant pathogenic bacteria that present a danger for humans as well.

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