

# Activity of $\beta$ -lactam antibiotics against certain microorganisms which cause mastitis in cows

Artur Burmańczuk<sup>1</sup>, Cezary Kowalski<sup>1</sup>, Zbigniew Roliński<sup>1</sup>,  
Rafał Zań<sup>1</sup>, Dorota Krasucka<sup>2</sup>

<sup>1</sup>Sub-Department of Pharmacology, Department of Preclinical Veterinary Sciences, Faculty of Veterinary Medicine,  
University of Life Sciences in Lublin, 20-033 Lublin, Poland

<sup>2</sup>Department of Veterinary Pharmacy,  
National Veterinary Research Institute, 24-100 Pulawy, Poland  
artur.burmanczuk@up.lublin.pl

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## Abstract

**Introduction:** The objective of this study was to determine the current profile of bacteria responsible for the infection of the mammary gland and to assess their sensitivity to selected  $\beta$ -lactam antibiotics. **Material and Methods:** The study was conducted on 119 ( $n = 119$ ) dairy cows of the Polish Black-White breed aged 4 to 10 years with inflammation of the mammary gland. The cows came from different farms: smallholder farms and large dairy cattle farms in the Lublin and Białystok Provinces. Before the process of collection of milk samples, the teats were cleaned and immersed in a liquid disinfectant. The first streams were collected into containers which were subsequently utilised. Afterwards, 2–4 mL of milk or secretions was milked into sterile disposable tubes. The milk samples were collected into plastic bottles and kept in a cooler with ice until transportation to the laboratory. Tests of resistance to  $\beta$ -lactam antibiotics were performed by disc diffusion. **Results:** Contagious and environmental bacteria were isolated from all dairy barns. In the group of contagious bacteria, the presence of typical pathogens responsible for the mammary gland infections, *i.e.* *Staph. aureus*, *Str. agalactiae*, and *C. bovis*, was detected. A relatively broad group of the isolates was formed by environmental bacteria responsible for inflammation of the mammary gland: *Str. dysgalactiae*, *Str. uberis*, *Staph. chromogenes*, *Staph. hyicus*, *Staph. warneri*, and *E. coli*. Among the environmental organisms, streptococci constituted the largest percentage (23%), followed by staphylococci (13.2%), and *E. coli* (8.8%). The largest group of infectious pathogens comprised *Str. agalactiae* (29.6%) and *Staph. aureus* (20.8%). **Conclusion:** Our investigation of the current profile of the isolates responsible for mastitis in the Lublin and Białystok Provinces showed that environmental bacteria are the major cause of the disease. In view of the substantially varying degrees of sensitivity of the microorganisms isolated from cases of mastitis to  $\beta$ -lactam antibiotics, each therapeutic treatment should be preceded by susceptibility testing.

**Keywords:** cow, mastitis, microorganisms,  $\beta$ -lactam antibiotics.

## Introduction

The bacteria which cause mastitis in cows are classified as contagious or environmental pathogens depending on epidemiological link with the disease (20). Infectious pathogens, *e.g.* *Staph. aureus* and *Str. agalactiae*, as well as the less common *C. bovis* and *M. bovis*, have an ability to survive in the mammary gland. Their presence may lead to inflammation, which is usually manifested as an increase in the number of somatic cells in milk from infected quarters (1). Environmental pathogens causing

the mammary gland infection occur in the immediate cow's environment, *e.g.* in litter, manure, and soil. This group includes streptococci, such as *Str. dysgalactiae*, *Str. uberis*, *Enterococcus* spp., *E. coli*, *Klebsiella*, and *Enterobacter* (18). In addition to bacteria, pathogenic environment includes fungi and algae (2). Inflammation of the mammary gland in cows is an important sanitary-epidemiological and economical issue (4, 15). The utility value of a cow is based on milk yield and its variations, which are determined by a series of factors that decide about an intensive or extensive breeding system. A principle in the treatment

of mastitis is introduction of therapeutic agents into the mammary gland quarters devoid of inflammatory secretions, if not accompanied by inflammation of the overall reaction system. Antibiotics should only be used where justified and any abuse may cause unintended reactions, regardless of the general contraindications for their introduction to the environment (4, 11-14, 23-25).

The effectiveness of treatment depends on the aetiological factors causing the disease, but also the clinical symptoms, sensitivity of the microorganism to antibiotics, and efficiency of the immune system. Overuse or inappropriate use of antibiotics can lead to the development of resistance in different bacterial strains (19). In recent years, there has been a growing need to reduce the use of antibiotics in the treatment and prevention of diseases of animals (23). Therefore, to increase the effectiveness of the treatment, it is important to identify the strain and determine its sensitivity to antibiotics (4, 11, 12, 14, 23, 24).

The aim of this study was to determine the current profile of bacteria responsible for the infection of the mammary gland in cows and to assess their sensitivity to selected  $\beta$ -lactam antibiotics.

## Material and Methods

The study was conducted on 119 dairy cows of the Polish Black-White breed aged 4 to 10 years with inflammation of the mammary gland and weighing about 650 kg each. The animals were fed farming feed concentrates comprising wheat, rye, and fodder grain (oats, barley), alternating with raw corn and pasture grazing. Access to food and water was provided *ad libitum*. The analyses were carried out between March and September. Cows were qualified for the research on guidance from the owners. The animals were verified using the DRAMINSKI Mastitis Detector 4×4Q (Dramiński®, Poland), a 4-quarter device for detecting subclinical mastitis in its earliest, visually undetectable stage, in order to assess the somatic cell count. Cows with a somatic cell count  $\geq 400\ 000/\text{mL}$  were classified for the study. The cows came from different farms (Table 2): smallholder farms with under 20 heads (1, 4, 6, 7, 8, and 12) and large dairy cattle farms holding over 50 animals (2, 3, 5, 9, 10, and 11) in the Lublin and Białystok Provinces. Before the collection of milk samples, the teats were cleaned and immersed in a liquid disinfectant – Avitaderm (Agrovet – Sniadowo, Poland). Afterwards, their mouths were wiped with a swab soaked in 70% ethanol. The first streams were collected into containers which were subsequently utilised. Afterwards, 2-4 mL of milk or secretions was milked into sterile disposable tubes. The milk samples were collected into plastic bottles and kept in a cooler with ice until transportation to the laboratory. Tests of resistance to  $\beta$ -lactam antibiotics were performed by disc diffusion on Mueller-Hinton

media, in accordance with NCCLS guidelines (NCCLS, 1987:199). The following Oxoid™ rings were used: penicillin G (10 IU), amoxicillin (10 mg), ampicillin (10 mg), cefoperazone (30 mg), cefacetrile (30 mg), cloxacillin (5  $\mu\text{g}$ ), cefalexin (30 mg), cefazolin (30 mg), and cefalothin (30  $\mu\text{g}$ ). *Staph. aureus* ATCC25 923 and *E.coli* ATCC25 922 were used as reference strains. Evaluation of the results was carried out according to the NCCLS criteria (4, 14, 17, 21) differentiating the bacteria as sensitive, moderately susceptible, and resistant strains. Because of the explicitness of the results obtained, no statistical calculations were performed.

## Results

The bacteriological examination showed that in all analysed dairy barns infectious and environmental bacteria were isolated. In the group of contagious bacteria, the presence of typical pathogens responsible for the mammary gland infections, *i.e.* *Staph. aureus*, *Str. agalactiae*, and *C. bovis*, was detected (5, 6, 8-11, 13, 16, 19, 22). A relatively broad group of isolates was formed by environmental bacteria responsible for mastitis: *Str. dysgalactiae*, *Str. uberis*, *Staph. chromogenes*, *Staph. hyicus*, *Staph. warneri*, and *E. coli*. A detailed list of the isolates is presented in Tables 1 and 2.

**Table 1.** Bacteria isolated from cows with mastitis

Mastitis-causing bacteria	Number of isolated bacteria
<i>Str. uberis</i>	5
<i>Str. agalactiae</i>	27
<i>Streptococcus</i> spp.	5
<i>Str. dysgalactiae</i>	11
<i>C. bovis</i>	4
<i>Staph. aureus</i>	19
<i>Staph. hyicus</i>	3
<i>Staph. haemolyticus</i>	9
<i>E. coli</i>	8

Among the environmental organisms, streptococci accounted for the largest percentage (23%), followed by staphylococci (13.2%), and *E. coli* (8.8%). The largest group of infectious pathogens comprised *Str. agalactiae* (29.6%) and *Staph. aureus* (20.8%). At the same time, it cannot be inferred whether the number of cows in the farm can influence the type and quantity of the isolated pathogens (Table 3).

As shown in Table 4, the tested isolates exhibited different levels of resistance to cephalosporins. *C. bovis* isolates showed 100% resistance to cefalothin, similarly as *E. coli* to cefazolin. Then, in successive order, 80% of the tested strains of *C. bovis* were resistant to cefacetrile and 60% of the isolates were resistant to cefoperazone. The susceptibility of the tested pathogens to cephalosporins was generally low.

**Table 2.** Number of contagious and environmental microorganisms isolated in each farm

Farm (n = 12)	1 S	2 L	3 L	4 S	5 L	6 S	7 S	8 S	9 L	10 L	11 L	12 S	Total	%
Contagious microorganisms	4	2	3	5	1	4	2	3	6	1	1	2	34	37.3
Environmental microorganisms	4	5	3	3	6	5	7	3	3	7	6	5	57	62.7

S-small farm, L-large farm

**Table 3.** The amount of isolated microorganisms depending on the number of dairy cows on the farm

Isolated microorganisms	The number of cows below 20	The number of cows above 50
<i>Str. uberis</i>	4	1
<i>Str. agalactiae</i>	11	16
<i>Streptococcus</i> spp.	5	0
<i>Str. dysgalactiae</i>	9	2
<i>C. bovis</i>	4	0
<i>Staph. aureus</i>	9	10
<i>E. coli</i>	5	3
<i>Staph. hyicus</i>	2	1
<i>Staph. haemolyticus</i>	5	4
Envinronmental (%)	44.5	30
Contagious (%)	55.5	70

**Table 4.** Sensitivity of isolated mastitis-causing bacteria to selected  $\beta$ -lactam antibiotics

Pathogens Antibiotic	<i>Staph. Aureus</i> %	<i>Str. agalactiae</i> %	<i>C. bovis</i> %	<i>Str. dysgalactiae.</i> %	<i>Str. Uberis</i> %	<i>E.coli</i> %	<i>Staph. haemolyticus</i> %
benzylpenicillin G	S - 20 AS- 0 R - 80	S- 80 AS-20 R - 0	S- 100 AS- 0 R- 0	S- 80 AS- 20 R- 0	S-20 AS-80 R-0	S-ne AS-ne R-ne	S-40 AS-0 R-60
cloxacillin	S - 60 AS-20 R - 20	S- 40 AS- 0 R - 60	S- 0 AS- 100 R- 0	S- 0 AS- 20 R- 80	S-0 AS-20 R-80	S-ne AS-ne R-ne	S-40 AS-0 R-60
ampicillin	S - 60 AS- 0 R - 40	S- ne AS- ne R- ne	S- 100 AS- 0 R- 0	S- ne AS-ne R-ne	S-80 AS-20 R-0	S-0 AS-60 R-40	S-0 AS-20 R-80
amoxycillin	S - 80 AS- 20 R - 0	S- 0 AS- 100 R- 0	S- 0 AS- 100 R- 0	S-20 AS-80 R-0	S-20 AS-80 R-0	S-60 AS-40 R-0	S-20 AS-60 R-20
cefacetril	S - 0 AS- 100 R - 0	S- 0 AS- 100 R- 0	S- 0 AS-20 R- 80	S-30 AS-50 R-20	S-20 AS-80 R-0	S-0 AS-60 R-40	S-50 AS-0 R-50
cefazolin	S - 0 AS- 80 R - 20	S- 50 AS- 50 R- 0	S- ne AS- ne R- ne	S-80 AS-20 R-0	S-ne AS-ne R-ne	S-0 AS-0 R-100	S-20 AS-40 R-40
cefalothin	S - 0 AS- 80 R - 20	S- ne AS- ne R- ne	S-0 AS0 R-100	S-ne AS-ne R-ne	S-ne AS-ne R-ne	S-ne AS-ne R-ne	S-ne AS-ne R-ne
cefoperazone	S - 20 AS- 89 R - 0	S- 35 AS- 35 R- 30	S-40 AS-0 R-60	S-20 AS-80 R-0	S-ne AS-ne R-ne	S-ne AS-ne R-ne	S-20 AS-20 R-60

S – sensitive, AS – moderately sensitive, R - resistant, ne – not examined

The highest percentage (80%) of *Str. dysgalactiae* isolates was susceptible to cefazolin, while 50% of *Str. agalactiae* strains were sensitive to it. *Staph. haemolyticus* strains showed the same sensitivity to

cefacetrile. The sensitivity of all other isolates to four tested cephalosporins ranged between 20% and 35% (Table 4). Resistance to benzylpenicillin G ranged from 80% (*Staph. aureus*) to 60% (*Staph. haemolyticus*).

A relatively high sensitivity to benzylpenicillin G in the range of 80-100% was noted in *Str. agalactiae* and *C. bovis*. A high percentage of the tested isolates was resistant to cloxacillin: *Str. agalactiae* - 60%, *Staph. haemolyticus* - 60%, and *Str. dysgalactiae* - 80%. While estimating the results for aminopenicillins, it was assumed that the resistance to ampicillin in the case of *E. coli* and *Staph. haemolyticus* strains was higher in comparison to amoxycillin, while the highest percentage of the strains sensitive to both aminopenicillins was found in the case of *Staph. aureus* and *Str. uberis* (Table 4).

## Discussion

The average percentage of cows with mastitis caused by infectious bacteria was 37.3 and mastitis caused by environmental organisms accounted for 62.7 of the analysed cases. A variation between the farms in the occurrence of contagious and environmental bacteria among the examined isolates was observed. For example, in farms 4 (small farm) and 9 (large farm), the dominant pathogens responsible for mastitis were infectious bacteria (*Staph. aureus* and *Str. agalactiae*). However, in farms 5 (large farm), 7 (small farm), 10 (large farm), and 11 (large farm), environmental bacteria dominated. In other farms, both infectious and environmental microorganisms were isolated in similar quantities. While analysing the diversity in the occurrence of pathogens responsible for mastitis in particular farms, no rules can be deduced from the distribution of contagious and environmental microorganisms. It should be stressed that in the analysed regions of our country, the environmental bacteria were the aetiological factors of mastitis in a large percentage of cases.

In a study conducted in 2001-2006 (24), it was reported that the average percentage of staphylococcal isolates resistant to benzylpenicillin G was relatively low (28.9%), especially in comparison to the resistance of these pathogens (60%-80%) isolated in the examined farms. A higher level of resistance of staphylococci (55.5% *Staph. aureus* and coagulase-negative - 49.4%) was noted by other authors in the national research (14). The existing data confirm that staphylococci resistant to penicillin G and cloxacillin are still a major problem in the treatment of mastitis. According to many authors (14, 23, 24) as well as our data, the high resistance to ampicillin in staphylococci and *E. coli* strains should be a prerequisite for not using this antibiotic in cases of mastitis without verifying its effectiveness *in vitro*. Moreover, during the treatment of so-called acute cases of mastitis, ampicillin should not be the medicine of choice when it is necessary to introduce an antibiotic at random. The relatively high percentage of cephalosporin-resistant isolates among all the evaluated microorganisms against the tested cephalosporins prompts more prudent use if the

susceptibility of aetiological factor has not been determined.

Assessment of sensitivity of mastitis isolates to antibiotics allows for proper selection of medicines, reduces the probability of lack of treatment efficiency, and contributes to decreasing the scale of antibiotics use. The *in vitro* tests of the isolated bacteria's sensitivity to chemotherapeutics also provide early assessment of the scale of increase of resistant strains in farms or in medical practice. The results of the bacteriological examinations presented here confirm the need for continuous monitoring of the sensitivity to antibiotics of both contagious and environmental microorganisms.

In conclusion, our investigation of the current profile of the isolates responsible for mastitis in the Lublin and Białystok Provinces showed that environmental bacteria are the major cause of the disease. It was also shown that *Streptococci* were the predominant pathogen in this group of microorganisms. The results concerning the resistance of the analysed pathogens to  $\beta$ -lactam antibiotics showed a different level of their sensitivity to these chemotherapeutics. For example, 100% resistance was detected in the *C. bovis* strains to cefalothin and up to 80% susceptibility of *Str. dysgalactiae* to cefazolin was found. A particularly high proportion of resistance in all isolates tested was recorded in the case of cloxacillin. In the group of aminopenicillins, there was a higher resistance of *E. coli* and *Staph. haemolyticus* strains to ampicillin compared to amoxycillin. In view of the substantially varying degrees of sensitivity of microorganisms isolated from cases of mastitis to  $\beta$ -lactam antibiotics, each therapeutic treatment should be preceded by susceptibility testing.

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## References

- Bradley A.J.: Bovine mastitis: an evolving disease. *Vet J* 2002, 163, 1-13.
- Costa E.O., Ribeiro A.R., Watanabe E.T., Melville P.A.: Infectious bovine mastitis caused by environmental organisms. *J Vet Med B* 1998, 45, 65-71.
- Eenennaam A.L., Van Gardner A.L., Holmes J., Perani L., Anderson R.J., Cullor J.S., Guterbock W.W.: Financial analysis of alternative treatments for clinical mastitis associated with environmental pathogens. *J Dairy Sci* 1995, 78, 2086-2090.

4. Gruet P., Maincent P., Berthelot X., Kaltsatos V.: Bovine mastitis and intramammary drug delivery: review and perspectives. *Adv Drug Deliver Rev* 2001, 50, 245–259.
5. Guérin-Faubleé V., Carret G., Houffschmitt P.: *In vitro* activity of 10 antimicrobial agents against bacteria isolated from cows with clinical mastitis. *Vet Rec* 2003, 152, 466–471.
6. Heringstad B., Klemetsdal G., Ruane J.: Selection for mastitis resistance in dairy cattle: a review with focus on the situation in the Nordic countries. *Livest Prod Sci* 2000, 64, 95–106.
7. Hornish R.E., Katarski S.F.: Cephalosporins in veterinary medicine. Ceftiofur use in food animals. *Curr Top Med Chem* 2002, 2, 717–720.
8. Kłosowska A., Malinowski E., Kuźma K.: Relationship between somatic cell counts in cow quarter foremilk samples and etiological agents of mastitis. *Med Weter* 2005, 61, 53–57.
9. Krukowski H.: Intramammary infections caused by environmental pathogens in cows. *Med Weter* 2006, 62, 189–192.
10. Kurek C., Niemczuk K.: Patterns of phages of staphylococci isolated from udders of cows and their antibiotic resistance. *Med Weter* 1987, 43, 270–273.
11. Lassa H., Kubiak J., Małkińska-Horodyska M.: Antibiotic susceptibility of the bacteria most often isolated from clinical mastitis in cows. *Życie Wet* 2013, 88, 651–653.
12. Malinowski E., Kłosowska A., Kuźma K., Krakowski H.: Antibiotic sensitivity of bacteria isolated from bovine mastitis. *Med Weter* 1992, 48, 366–400.
13. Malinowski E., Kłosowska A., Lassa H.: Variability among etiological agents of clinical mastitis in cows. *Pol J Vet Sci* 2001, 4, 41–45.
14. Malinowski M., Kłosowska A.: Cow mastitis pathogen resistance to antibiotics. *Med Weter* 2003, 59, 230–235.
15. Malinowski M., Kłosowska A.: Diagnostyka zakażeń i zapaleń gruczołu mlekowego krów. *PIWet*, Puławy, 2002.
16. Muhammad G., Hoblet K., Jackwood D., Bech-Nielsen S., Smith K.: Interspecific conjugal transfer of antibiotic resistance among staphylococci isolated from the bovine mammary gland. *Am J Vet Res* 1993, 54, 1432–1440.
17. NCCLS, (1987–1998) Performance Standards for Antimicrobial Susceptibility Testing. Eleventh Informational Supplement, NCCLS document M 100–911 (ISBN 1–56238–426–0). NCCLS, 2001, Pennsylvania, USA.
18. Quinn, P.J., Carter, M.E., Markey, B., Carter, G.R.: *Corynebacterium* species and *Rhodococcus equi*. In: *Clinical Veterinary Microbiology*. Wolf Publishing, London 1994, pp. 327–344.
19. Rabello R.F., Souza C.R.V.M., Duarte R.S., Lopes R.M.M., Teixeira L.M., Castro A.C.D.: Characterization of *Staphylococcus aureus* isolates recovered from bovine mastitis in Rio de Janeiro, Brazil. *J Dairy Sci* 2005, 88, 3211–3219.
20. Rossitto P.V., Ruiz L., Kikuchi Y., Glenn K., Luiz K., Watts J.L., Cullor J.S.: Antibiotic susceptibility patterns for environmental streptococci isolated from mastitis in central California dairies. *J Dairy Sci* 2002, 85, 132–138.
21. Saran A., Leitner G.: Interactions between bacteria, immunity and therapy in mammary gland. *Proc. INF Inter. Symp. Immunology of ruminant mammary gland*, Stresa, Italy, 2000.
22. Watts J.L., Salmon J.A.: Activity of selected antimicrobial agents against strains of *Staphylococcus aureus* isolated from bovine intramammary infections that produce beta-lactamase. *J Dairy Sci* 1997, 80, 788–791.
23. Wawron W., Piech T., Bochniarz M.: Antibiotic sensitivity of pathogens isolated from cows with mastitis. *Med Weter* 2008, 64, 1132–1135.
24. Wawron W., Szczubiał M., Piech., Krzyżanowski J., Wrona Z., Krakowski L.: Antibiotic-sensitivity of bacteria isolated from cases of bovine mastitis. *Med Weter* 2000, 56, 675–680.
25. Weller J.L., Saran A., Zeligier Y.: Genetic and environmental relationships among somatic cell count, bacterial infection, and clinical mastitis. *J Dairy Sci* 1992, 75, 2532–2540.