

Antimicrobial resistance and genotypes of staphylococci from bovine milk and the cowshed environment

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Abstract

Investigation of antimicrobial resistance and genetic relatedness of staphylococci from milk of cows with mastitis and cowshed environment was the aim of this study. Antimicrobial resistance against 14 antimicrobials were determined by using a disc diffusion method. Genetic similarity between the most frequently isolated species was analysed by PFGE (pulsed-field gel electrophoresis). Haemolytic activity, DNase, protease and esterase production was also investigated. Coagulase-negative *Staphylococcus* species were isolated from 30.8% of milk samples from cows with mastitis. The most frequently isolated species was *Staphylococcus xylosus* and yield of these organisms was significantly associated with milk of mastitis cows. *S. epidermidis* was a predominant penicillin-resistant species. High frequency of resistance to lincomycin was observed among isolates of *S. sciuri* (54.2%) and *S. xylosus* (25.9%) from cows with mastitis. PFGE (pulsed-field gel electrophoresis) analysis of 29 *Staphylococcus aureus* isolates showed the presence of 17 PFGE pulsotypes. Isolates of *S. sciuri* (n=36) had unique PFGE patterns. Some *S. xylosus* isolates from milk and milker's hands had the same PFGE pulsotypes, and this observation could indicate that dairyman may be a potential source of the infection. The pulsotype of each of the remaining isolates of *S. xylosus* suggested that they might have come from common environmental sources; however, these isolates differed in antibiotic resistance pattern or virulence traits. Therefore, knowledge about antibiotic sensitivity pattern and virulence factors of a CNS isolate, besides its genotype, may be informative in tracking the source of the infection.

Keywords: Coagulase-negative staphylococci, *Staphylococcus aureus*, antimicrobial resistance, PFGE, mastitis

Introduction

Staphylococci are the bacteria most commonly isolated from bovine mastitis cases (Taponen et al. 2006, Sampimon et al. 2009, Sawant et al. 2009). Currently, the coagulase-negative staphylococci (CNS) strains are the most prevalent organisms isolated from milk samples in many modern dairy herds where efficient mastitis control protocols are implemented (Gillespie et al. 2009, Sampimon et al. 2009). Mastitis is a serious disease of dairy cattle, which results in significant economical losses; therefore it is important to identify bacteria causing this disease, and their antibiotic resistance profile (Halasa et al. 2007). This could help in choosing the best strategy for the treatment of the infection and has a significant impact on the choice of the proper antimicrobial agent.

The study of epidemiological associations between mastitis pathogens may also help in the fulfilment of the tasks mentioned above. Moreover, the knowledge about the source of infection and transmission routes within herds and between herds may be of benefit in control strategies against *S. aureus* and CNS mastitis. To establish the epidemiological evidence for an association between isolates the various phenotypic and genotypic typing methods are used. Among them, macrorestriction profiling by pulsed-field gel electrophoresis analysis (RFLP-PFGE) is considered a "gold standard" for its excellent typeability, high discriminatory power, and easy interpretation of results, and may be useful for typing of staphylococci from human and cattle (Capurro et al. 2010). Until now, PFGE typing has not been performed for diversification of *S. aureus* and CNS species isolated from bovine mastitis in Poland.

The aim of this study was to investigate antimicrobial resistance and genetic relatedness of staphylococcal isolates from milk of cows with subclinical and clinical mastitis from different dairy herds in the central part of Poland. Genetic relatedness between isolates was investigated by *SmaI*-PFGE typing in order the study the transmission routes of staphylococci within herds and between herds. The samples from the cowshed environment were included in the study in order to determine whether a dairyman may be a potential source of infection of the mammary glands in dairy animals.

Materials and Methods

Quarter milk samples from cows with subclinical and clinical mastitis that belonged to twenty seven herds were collected in the eastern part of the Mazovia and the north Lublin region of Poland be-

tween February 2009 and March 2010. A quarter was identified as infected when somatic cell counts were increased above 2×10^5 /ml (Casadevall and Pirofski 2000). Before sample collection, teats of cows were dipped in chlorhexidine, cleaned thoroughly and dried. Then, teat ends were sanitized with swabs containing 70% isopropyl alcohol and allowed to dry. The first streams were discarded, and then 10 ml of milk was collected aseptically into sterile vials. Milk samples were cooled and immediately transported to the laboratory. A total of 500 milk samples from the affected quarters were examined for the presence of staphylococci. Ten microliters of each milk sample was plated on 5% sheep blood agar (Graso, Poland). Plates were incubated at 37°C for 48 hours.

Quarter milk samples for microbiological analysis were also collected in March 2009 and in March 2010 from cows without mastitis symptoms in a dairy herd No. 3 for comparison purposes. The herd No. 3 was chosen because there were no mastitis symptoms among cattle for at least two years before the start of samples collection. A total of 150 milk samples from the quarters were examined for the presence of staphylococci as described above.

The samples from the cowshed environment.

Swabs were taken from hands of farmers involved in milking herds No. 3 and 4. The palms of the hands were swabbed using sterile cotton swabs soaked in sterile physiological saline. The swabs were also taken from surface of teats of cows in dairy herds No 3. and 4 with sterile cotton swabs soaked in sterile physiological saline. The swabs were immediately directly streaked onto 5% sheep blood agar plates and incubated at 37°C for 48 hours.

From the cowshed of cattle belonging to herd No. 4, the samples of drinking water (100 ml) were collected into sterile containers, and centrifuged at 3000 g for 20 min. The pellets were suspended in 100 µl of sterile phosphate-buffered saline (PBS) (pH 7.2) and 10 µl was streaked onto 5% sheep blood agar. The samples of fodder and cow's bedding (10 g of each) were aseptically collected into sterile containers and after that transferred in 90 ml of PBS (pH 7.2). The suspensions were shaken for 10 min and 10 µl of each was streaked onto 5% sheep blood agar. Plates were incubated at 37°C for 48 hours and examined for the presence of staphylococci. A total of 84 environmental samples were examined.

Bacterial identification. Colonies grown on sheep blood agar plates were initially identified based on their morphology and hemolysis pattern. Gram-positive cocci were examined for catalase production. Isolates identified presumptively as staphylococci were streaked on 5% sheep blood agar to obtain pure cultures. *Staphylococcus* was differenti-

ated from *Micrococcus* species on the basis of susceptibility to furazolidone (100 µg) characterized by inhibition zones measuring ≥ 15 mm in diameter. Coagulase production was examined with a tube coagulase test and rabbit plasma (Downes and Ito 2001). Growth of coagulase-positive staphylococci on peptone agar (p-agar) (BBL, Becton Dickinson, Sparks, Md.) supplemented with 7 mg/l of acriflavin (Sigma-Aldrich, Steinheim, Germany) was considered a confirmation of the presence of *S. aureus* (Capurro et al. 1999). All staphylococci were also grown on DNase agar with methyl green (BBL, Becton Dickinson). The presumptive isolates were identified as CNS based on conventional microbiological procedures (Bannerman 2003) and with an API Staph ID 32 system (bioMérieux, Lyon, France). The Apiweb software (bioMérieux) was used to determine the probability of species identification. The identification with a probability $\geq 90\%$ was considered acceptable (Taponen et al. 2006). The isolates, for which the reliability of identification was below $< 90\%$ were then subjected to analysis with the VITEK GPI card system (VITEK 2 instrument, version 4.01, bioMérieux). Finally, the isolates with the probability of species identification $< 90\%$ by both systems were classified as *Staphylococcus* spp. The isolates were stored at -80°C in Brain Heart Infusion Broth (BHI; BBL, Becton Dickinson) with 15% glycerol.

Antibiotic susceptibility testing. The susceptibility of the isolates was tested with a disc diffusion method (CLSI 2011) using the following antibiotic discs (Oxoid, Basingstoke, UK): amoxicillin plus clavulanic acid (20 µg + 10 µg), ciprofloxacin (5 µg), clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), lincomycin (15 µg), norfloxacin (10 µg), ofloxacin (5 µg), penicillin (10 UI), teicoplanin (30 µg), tetracycline (30 µg), tobramycin (10 µg), and trimethoprim plus sulphamethoxazole (1.25 µg + 23.75 µg). The resistance to methicillin (MR) of isolates was assayed with ceftiofloxacin (30 µg). *S. aureus* ATCC 29213, *S. aureus* ATCC 25923, and a clinical *S. aureus* strain, which possess the *mecA* gene, were used as control strains.

Haemolytic activity and enzymes production. Haemolytic activity was performed on the plates with 5% sheep blood agar that were streaked with a fresh culture of staphylococci (24 h at 37°C in BHI broth) and incubated at 37°C for 48 hours (Konemam et al. 1997). Protease activity was determined on casein agar plates, according to the procedure described by Bjorklind and Arvidson (1977). For evaluation of the esterase production, the agar plates containing 0.1% Tween 80 and calcium chloride were streaked with a fresh culture of staphylococci (24 h at 37°C in BHI broth) and incubated at 37°C for 48 hours. The read-

ing was done after this period, according to the protocol proposed by Chapin and Murray (1999).

Pulsed-field gel electrophoresis (PFGE).

A total of 29 *S. aureus* isolates from the milk of dairy cows with clinical mastitis, as well as 91 of *S. xylosus* and 36 of *S. sciuri* isolates from all the sample sources examined in this study were analysed by pulsed-field gel electrophoresis. Macrorestriction analysis of chromosomal DNA using PFGE was performed according to the protocol described by Thorberg et al. (2006) with some modifications. For PFGE analysis, isolates were grown on blood agar for 24 h at 37°C and suspended in Tris-NaCl (10 mM Tris-HCl, 1 M NaCl, pH=7.6) to a cell density in the range of MacFarland 6-7. To 300 µl of cell suspensions 23 IU of lysostaphin was added and gently mixed with 300 µl of 1.5% Low Melt Agarose (BIO-RAD) dissolved in TE buffer (10 mM Tris, 1 mM EDTA; pH 8.0). The resulting mixture was poured into plug molds and allowed to solidify at room temperature for 10-15 min, followed by 5 min at 5°C . The plugs were then incubated in 2 ml of EC buffer (6 mM Tris-HCl, pH=7.6, 1 M NaCl, 100 mM EDTA, 1% N-lauryl sarcosine) with 100 µl lysostaphin (1 mg/ml), 23 IU lysostaphin, and incubated for 20 h at 37°C . After this time the plugs were incubated in 2 ml of EC buffer with 20 µl proteinase K (20 mg/ml) at 55°C for 4-5 h. Individual plugs were washed with sterile water and then three times with TE buffer at 54°C with agitation. For restriction endonuclease digestion, the plugs were incubated in a restriction buffer with 60 U of *SmaI* for 4 h at room temperature.

Electrophoresis was performed using CHEF-DRIII system (Bio-Rad). The gels were run at 5.5 V/cm with an angle of 120° and with pulse time of 5 to 40 s for 21 h. A lambda marker (New England BioLabs, Beverly, MA, USA) was used as size standard. The gels were stained with ethidium bromide and photographed under UV transillumination. The images of gels were acquired using Gel Doc 1000 Gel Documentation System (BioRad). The fingerprinting patterns were compared with use of the Molecular Analyst Software Fingerprinting (BioRad). The macrorestriction patterns (pulsotypes) were normalized by interpolation to the nearest reference line. Dice's similarity coefficients were calculated to generate dendrogram using the UPGMA method.

Statistical analyses. Chi-square statistics in Statistix 8.0 (Analytical Software, Tallahassee, FL) was used for calculations in order to determine the dependence between the number of isolates and the source of isolation. In cases when the numbers in subclasses had the value < 5 , Yates's correction was used and the adjusted value of statistics χ^2_{emp} was calculated.

Results

Staphylococcal isolates. A total of 184 staphylococcal isolates from milk of cows with clinical and subclinical mastitis, 48 isolates from milk samples from cows without mastitis symptoms, as well as 38 isolates from cowshed environment were obtained.

The most common species of the staphylococci isolated from the milk samples of cows with and without mastitis and from the cowsheds environment were shown in Table 1. *S. aureus* isolates were detected in 6% of the milk samples from cows with mastitis. In 154 of the milk samples from these cows (30.8%) CNS species were detected and within them 12 different species were identified. The predominant species were *S. xylosus*, *S. sciuri* and *S. epidermidis*. In the milk samples from mastitis cattle additionally *S. cohnii* (n=5), *S. simulans* (n=3), *S. lentus* (n=3), *S. capitis* (n=2), *S. caprae* (n=2), *S. haemolyticus* (n=2), *S. vitulinus* (n=2), *S. auricularis* (n=1) and *S. equorum* (n=1) were identified. CNS isolates were also found in the milk samples from cows without mastitis symptoms, but the most frequently CNS species isolated from these samples were *S. epidermidis* and *S. xylosus*. Besides them, the following CNS species were identified: *S. sciuri* (n=4), *S. simulans* (n=3), *S. equorum* (n=2), *S. lentus* (n=1), *S. capitis* (n=1) and *S. haemolyticus* (n=1). The samples from the cattle cowshed environment yielded 37 CNS isolates. *S. xylosus* was also isolated from teats skin (n=15), from milker's hands (n=6), from water (n=2) and from cow's beddings (n=1). *S. sciuri* was isolated from cow's beddings (n=6) and fodder (n=2). One isolate of *S. epidermidis* from teat skin was also identified. The occurrence of all identified *S. xylosus* isolates was significantly associated with their origin and the number of bacteria was highest in the milk samples from cows with mastitis.

Antimicrobial resistance. The frequency of the resistant *S. aureus* and CNS isolates from bovine milk samples and the cowshed environment to antimicrobial agents were shown in Fig. 1. More than half of *S. aureus* (52.9%) and CNS (53.8%) isolates exhibited phenotypic resistance to penicillin. Numerous isolates of both *S. aureus* and CNS species were resistant to clindamycin. About fifteen percent of *S. aureus* isolates were also resistant to trimethoprim with sulphamethoxazole. More than ten percent of CNS isolates showed resistance to erythromycin and tetracycline. Over 27% of CNS isolates were resistant to lincomycin, and the results of χ^2 test revealed that resistance to this antibiotic was significantly associated with CNS isolates.

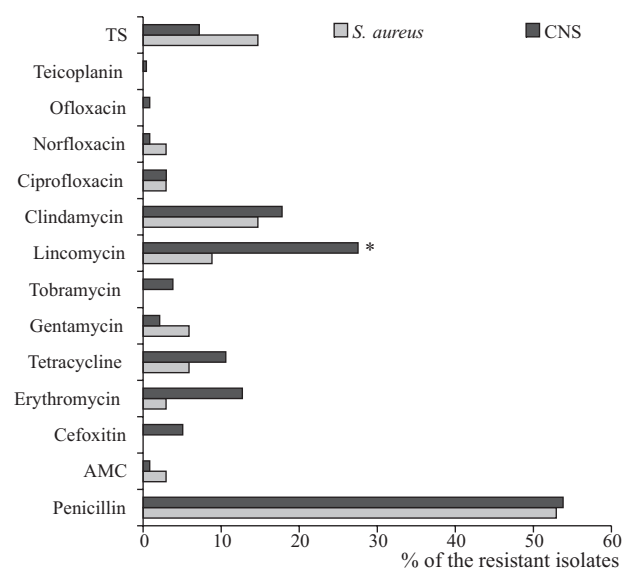


Fig. 1. Antibacterial resistance of *S. aureus* and CNS isolates from bovine milk samples and the cowshed environment.

AMC, amoxicillin plus clavulanic acid; TS, Trimethoprim plus sulphamethoxazole.

*, The resistance to lincomycin was significantly associated with CNS isolates (χ^2 test).

Table 1. Prevalence of *S. aureus* and CNS species in the milk samples collected from cows and the cowsheds environment.

| Species | No. of isolates (% of samples) | | | Value of χ^2_{emp} comparison of: | | |
|----------------------------|---|--|--|--|---------|---------|
| | milk from cows with mastitis A (n = 500) | milk from cows without mastitis B (n = 150) | cowshed samples ^a C (n = 84) | A and B | B and C | A and C |
| | <i>S. aureus</i> | 30 (6.0) | 3 (2.0) | 1 (1.2) | 3.83 | 0.37 |
| <i>S. xylosus</i> | 58 (11.6) | 9 (6.0) | 24 (28.6) | 3.91* | 96.68** | 17.16** |
| <i>S. sciuri</i> | 24 (4.8) | 4 (2.7) | 8 (9.5) | 1.27 | 27.19** | 3.09 |
| <i>S. epidermidis</i> | 20 (4.0) | 10 (6.7) | 1 (1.2) | 1.86 | 0.25 | 1.64 |
| Other CNS species | 21 (4.2) | 8 (5.3) | 0 | – | – | – |
| <i>Staphylococcus</i> spp. | 31 (6.2) | 14 (9.3) | 4 (4.8) | – | – | – |
| Total | 184 | 48 | 38 | | | |

a – The swabs from teats skin, milker's hands, samples of cow's beddings, fodder and water.

*, The significant dependence between the number of isolates and the source of isolation.

** , The highly significant dependence between the number of isolates and the source of isolation.

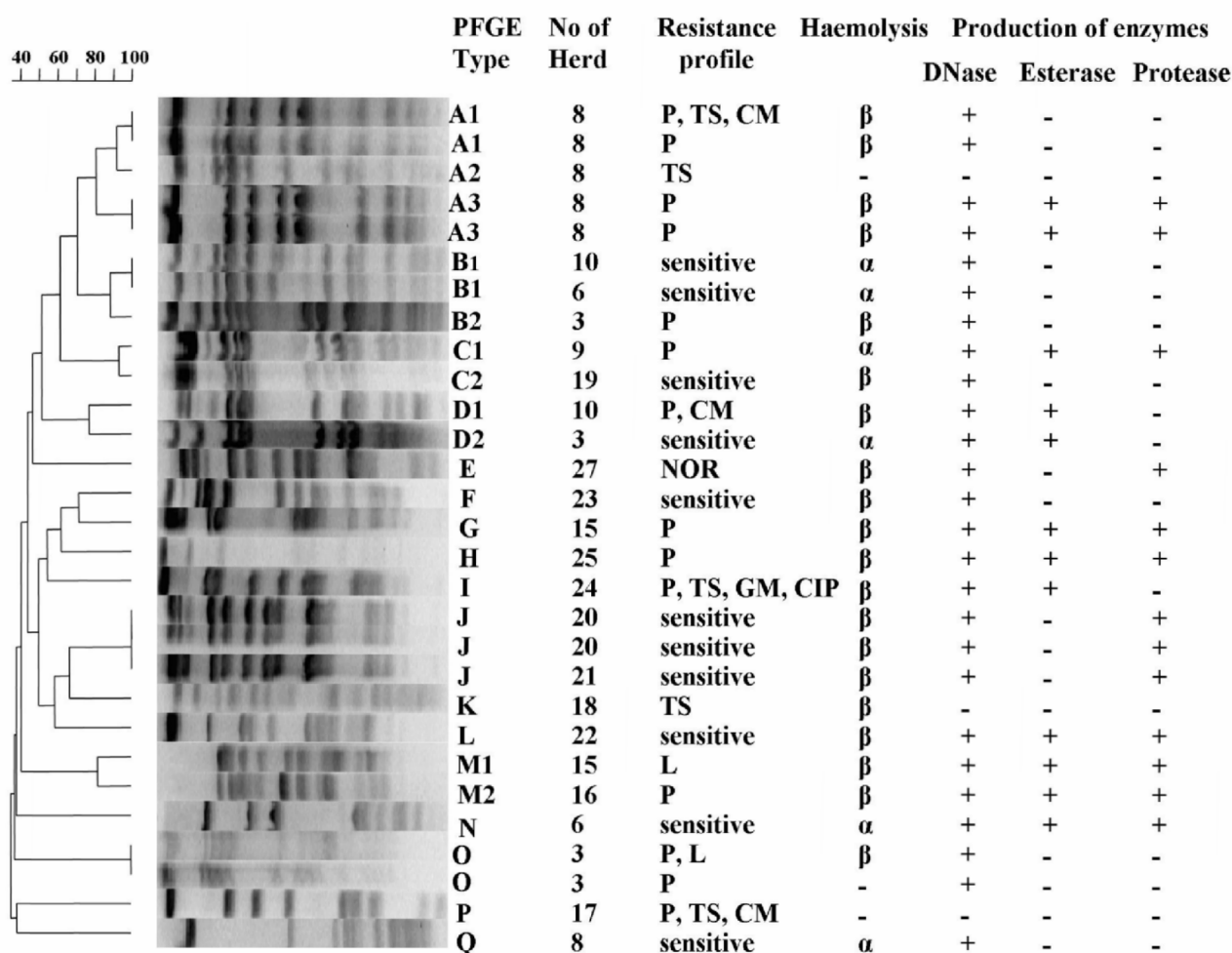


Fig. 2. PFGE patterns, resistance profiles and phenotypically expressed virulence factors of 29 *S. aureus* isolates from dairy cows with clinical mastitis from 18 herds in the eastern part of the Mazovia and the north Lublin region of Poland.

The isolates were assigned to 17 pulsotypes, designated by letter (A-Q) and subtypes, indicated by number (1-3). The phenotypic resistance profile (P – Penicillin, GM – Gentamicin, L – Lincomycin, CM – Clindamycin, CIP – Ciprofloxacin, NOR – Norfloxacin, TS – Trimethoprim with sulphamethoxazole), haemolysis pattern (α , β , -) and production of enzymes (-, +) are presented for each isolate.

The highest number of penicillin-resistant isolates was found among staphylococci from the milk samples of cows with mastitis and *S. epidermidis* was a dominant species (75%). An increased level of resistance to lincomycin was observed among isolates of *S. sciuri* (54.2%) and *S. xylosus* (25.9%). The isolates of *S. xylosus* displayed also an elevated level of resistance to erythromycin (19%). Over sixteen percent of the *S. sciuri* isolates were resistant to tetracycline. Similar pattern of resistance was observed for *S. xylosus* and *S. epidermidis* isolates from the milk samples of cows without symptoms of mastitis, except for lack of resistance to erythromycin among *S. xylosus* isolates.

Genetic similarities between isolates. Genetic similarity between the most frequently isolated species was analysed by PFGE. Each isolate of *S. sciuri*

had a unique DNA fingerprint pattern, even though some of the isolates were taken from the same herd (data not shown). As the result of PFGE analysis of *S. aureus* isolates from 18 different herds, the presence of 17 PFGE types, designed by letters from A through Q was shown. The subtypes were designed by numbers (Fig. 2). PFGE type A was observed within the isolates from the samples taken in herd No. 8. Within PFGE type A, three subtypes were detected that shared 80% similarity of their restriction patterns. The isolates belonging to type A differed in their antibiotic resistance and production of enzymes. The isolates of subtype A2 did not express haemolysins. Subtype A3 consisted of two isolates with identical band patterns and showed the same resistance profile and biochemical activity. Two subtypes B1 and B2 con-

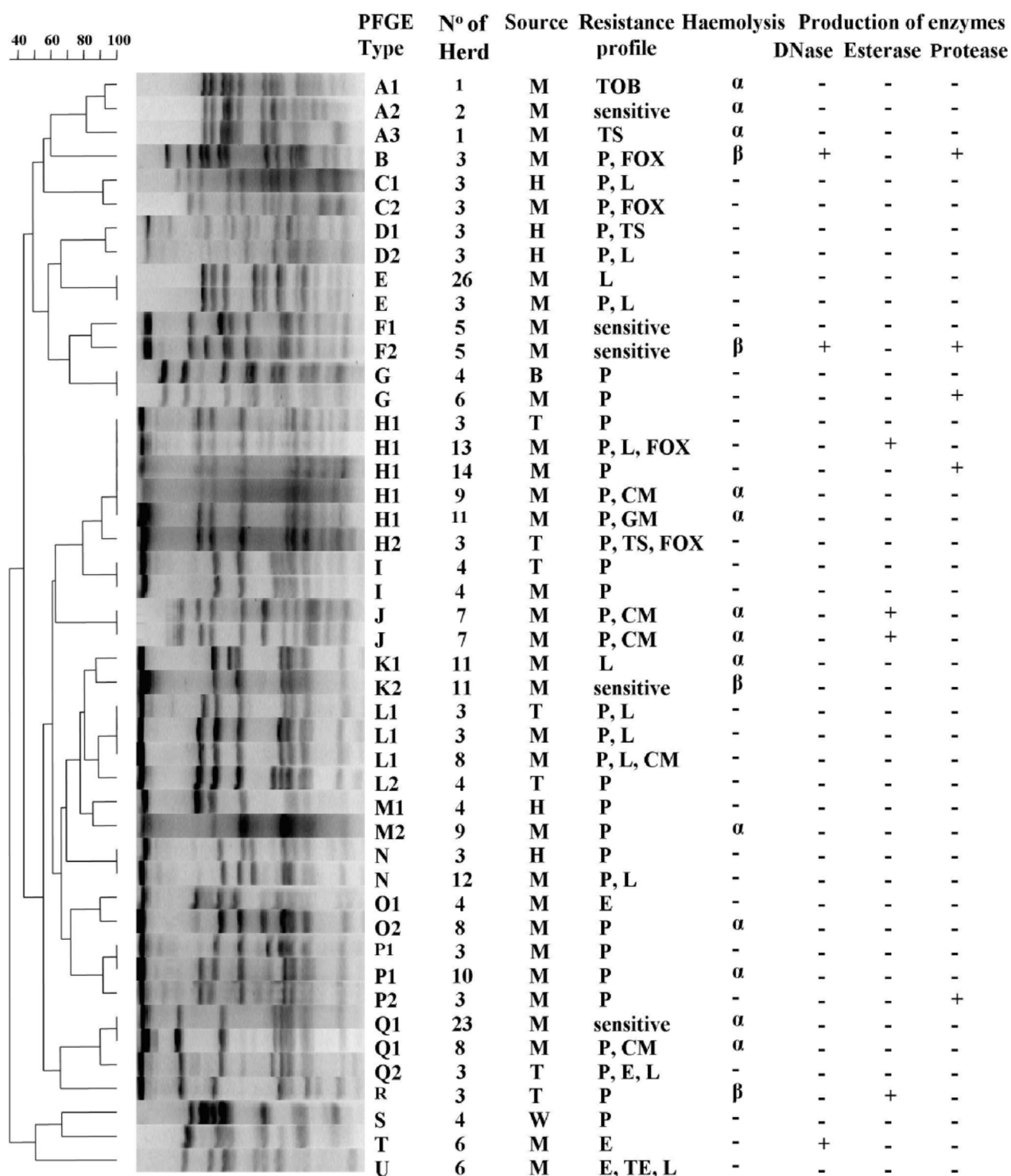


Fig. 3. The representative 46 PFGE patterns, resistance profiles and phenotypically expressed virulence factors of *S. xylosus* isolates.

The cows belonged to 16 dairy herds in the eastern part of the Mazovia and the north Lublin region of Poland. The isolates were assigned to 21 pulsotypes, designated by letter (A-U) and subtypes, designed by number (1-3). The phenotypic resistance profile (TOB – Tobramycin, TS – Trimethoprim with sulphamethoxazole, P – Penicillin, FOX – Cefoxitin, L – Lincomycin, CM – Clindamycin, GM – Gentamicin, E – Erythromycin, TE – Tetracycline), haemolysis pattern (α , β , -) and production of enzymes (-, +) are presented for each isolate. M, milk samples from cows with sub- and clinical mastitis (only the isolates from herd No. 3 were from cows without mastitis symptoms); T, teats skin; H, milker's hands; B, the sample of cow's beddings; W, water.

tained the isolates from different herds with various resistance profiles and types of haemolysis. Two subtypes within types C and D were observed, which differed in their antimicrobial-resistance patterns, the type of haemolysis, and the production of esterase and protease for subtypes C1 and C2. PFGE type J consisted of the isolates from herd No. 20 and 21, for which the identical pulsotypes were noted. *S. aureus* from herds No. 15 and 16 (subtype M1 and M2, respectively) shared 80% similarity and had different resistance profile. The isolates from herd No. 3 belonged to type O and each of them had different resistance profile and haemolytic activity. The PFGE patterns of the remaining *S. aureus* isolates yielded various pulsotypes.

The representative 46 PFGE patterns of *S. xylosus* isolates were shown in Fig. 3. The isolates from herds No. 1 and 2 shared PFGE banding pattern, although they had different resistance profiles. Pulsotypes C and D grouped the isolates collected in the herd No. 3. They were isolated from milker's hands or milk (subtype C1 and C2), and from milker's hands (subtype D1 and D2) with different antibiotic susceptibilities. Moreover, *S. xylosus* isolates from herd No. 3 had common pulsotypes with isolates from other herds (pulsotype E, H, L, P, Q). Pulsotype F (subtype F1 and F2) shared 80% of their restriction fragments. PFGE type G contained the isolates from cow's beddings (herd No. 4) and from milk (herd No. 6), which differed in production of protease. PFGE type H with 2 subtypes (H1 and H2) was common for the isolates from milk of cows with or without mastitis symptoms and from teats skin. These isolates had different resistance to antibiotics and some of them showed α -haemolysis and production of esterase or protease.

Two of seven isolates from herd No. 4 that belonged to the same clonal group (type I) originated from teat skin and a milk sample of the same cow. Two isolates from milk of cows from herd No. 7 yielded the same pulsotype J. Subtypes K1 and K2 from herd No. 11 showed different haemolytic activity and susceptibility to lincomycin.

The other common type (L) was observed among isolates from herds No. 3, 4 and 8. The isolates from milk and teat skin (herd No. 3) within the same subtype L1 were identical. Pulsotypes M and N grouped isolates that came from the milker's hands or milk and displayed different haemolytic activity (subtype M1 and M2) or susceptibility patterns (type N). The PFGE type O and Q consisted of isolates from various herds with different resistance profile and haemolytic activity for subtypes O1 and O2.

Discussion

The prevalence, antimicrobial resistance and PFGE profiles of staphylococci isolated from bovine

milk and the cowshed environment in the central region of Poland were investigated.

CNS species were detected in 30.8% of the milk samples from cows affected with bovine mastitis and it has been twice more than the proportion of the contaminated milk samples (14.6%) reported by Malinowski et al. (2006). The species most often isolated in our survey from the milk of cows with mastitis symptoms was *S. xylosus*. This observation confirms the previous report by Malinowski et al. (2006) as well as by Bochniarz and Wawron (2011) who showed that *S. xylosus* was the species most frequently isolated from the milk samples from mastitis cattle in different regions of Poland.

In this study, *S. sciuri* and *S. epidermidis* were also isolated from milk samples what is in agreement with the results of others (Gillespie et al. 2009, Sampimon et al. 2009). Moreover, *S. aureus* was detected in 6.0% of milk samples from cows with mastitis. Malinowski et al. (2006) showed that among the etiological agents of bovine mastitis in western part of Poland *S. aureus* was isolated in 8.6% of cases.

Since antimicrobials play a major role in the control of bovine mastitis, monitoring of antimicrobial susceptibility of mastitis pathogens seems to be critical, especially in the light of reduced susceptibility to β -lactams, macrolides and lincosamides of *Staphylococcus* species isolated from milk (Gentilini et al. 2002). In the present study, resistance to penicillin was most often observed among CNS isolates from cows with mastitis (49.3%). These results suggest that penicillin might not be a proper choice for treatment of mastitis caused by CNS in Poland. The highest percentage of CNS isolates resistant to penicillin was among *S. epidermidis*. The above observation is in accordance with results of Sampimon et al. (2011), who showed that penicillin resistance in *S. epidermidis* was significantly more common than in other species.

Our results revealed that the resistance of CNS from milk of cows with mastitis to erythromycin (14.3%) was higher than in Germany (7.4%) (Luthje and Schwarz 2006). We also observed the highest percentage of the isolates resistant to erythromycin among *S. xylosus* from milk of cows with mastitis symptoms. Sampimon et al. (2011) have shown that erythromycin resistance was more common in *S. equorum* than in other species.

The highest percentage of the isolates resistant to lincomycin was noted among *S. sciuri*. Lincomycin resistance was more common in *S. sciuri* isolates from milk samples of cows without mastitis symptoms and from the cowshed environment than in the isolates from the mastitis cases. The high percentage of the strains resistant to lincomycin has also been observed among isolates of *S. succinus* (80%) and *S. xylosus*

(53%) by Resch et al. (2008) who investigated CNS contaminating food. Resistance to lincomycin has clinical relevance, since this antibiotic is widely used for mastitis therapy in dairy cows (Sawant et al. 2009). Lincomycin was used in Poland as intramammary antimicrobial solution for clinical bovine mastitis therapy since 2001. Similar phenomenon of an increasing resistance to this antibiotic, commonly used for treatment of infections in animals, has been reported by Lüthje and Schwarz (2006) who observed an increase in resistance to pirlimycin (lincosamide antibiotic) up to 6.4% among CNS isolates from mastitis after five years of its veterinary therapeutic use in Germany.

In the present study, the analysis of genomic similarities between *S. aureus* isolates from the milk samples of mastitis cattle from 18 different dairy herds was performed with RFLP-PFGE, following the approach of the other epidemiological studies of bovine mastitis (Thorberg et al. 2006, Gillespie et al. 2009, Sawant et al. 2009). The isolates have been compared based on their pulsotypes, as well as their specific antimicrobial resistance patterns, haemolytic activities and production of DNase, esterase and proteases. It has been found that these isolates belonged to 17 different PFGE types, though some of them originated from the same herd. Thus, it may indicate that *S. aureus* causing mastitis is very diverse in this region of Poland. Capurro et al. (2010) have investigated genotypic diversity of 82 *S. aureus* isolates from cases of acute clinical mastitis in cattle and showed that some pulsotypes have spread widely among herds within the country and were found in all regions of Sweden. Similarly to our results, most pulsotypes were found only once or twice. Moreover, we have shown that haemolysis type, resistance profile or productions of enzymes are different within some pulsotypes. Variations in haemolysis type or β -lactamase production have also been found within some pulsotypes by Cappurro et al. (2010).

CNS isolates from mastitis and other sources have been compared with ribotyping and PFGE in study conducted by Thorberg et al. (2006). They compared *S. epidermidis* isolates from milker's hands and from bovine mastitis and showed that *S. epidermidis* strains causing mastitis could have originated from humans. In our study, the analysis of genomic similarities between *S. xylosus* isolates from milk of cows with- and without mastitis and from milker's hands, teats skin, cow's beddings and water samples have been performed with PFGE. The most of *S. xylosus* isolates had their own pulsotypes. A large diversity of PFGE pulsotypes in *S. xylosus* has also been found by Dordet-Frisoni et al. (2007). They have not observed any relationship between the origin of *S. xylosus* strains and their *SmaI* PFGE results. In our study, the com-

mon PFGE patterns of *S. xylosus* from the milk samples have been found. The isolates with the same PFGE patterns from various herds have different resistance profile, haemolysis type or the level of enzymes production. We have also observed common PFGE pulsotypes for isolates from milker's hands and from milk samples. *S. xylosus* is a commensal of the skin of humans and animals; however, it may be also related to animal and human opportunistic infections (Siqueira and Lima 2002, Sampimon et al. 2009). The same PFGE types of the isolates from milk and milker's hands could indicate humans as the source of these infections. We have also noted similar PFGE patterns among the isolates from teats skin and the milk samples originating from the same individual cows as well as for the isolates from milk and cow's beddings.

In our study, coagulase-negative *Staphylococcus* species were the most frequently isolated from the milk samples from cows with mastitis. The predominant species were *S. xylosus*, *S. sciuri* and *S. epidermidis*, which showed various patterns of resistance to antibiotics. Some *S. xylosus* isolated from milk and milker's hands had the same PFGE pulsotypes and this observation could indicate that dairyman may be a potential source of the infection. We have also observed the identical PFGE pulsotypes of isolates taken from the samples without evident epidemiological links. These isolates differed in antibiotic resistance and virulence phenotypes. This may indicate that phenotypic characteristics are also important and might be helpful in determining the sources of the mastitis etiological factors.

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