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Occurrence of the blaZ Gene in Penicillin Resistant *Staphylococcus aureus* Isolated from Bovine Mastitis in Denmark

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Vesterholm-Nielsen M, Ølholm Larsen M, Elmerdahl Olsen J, Møller Aarestrup F: Occurrence of the blaZ gene in penicillin resistant *Staphylococcus aureus* isolated from bovine mastitis in Denmark. Acta vet. scand. 1999, 40, 279-286. – Fifty-eight penicillin resistant Staphylococcus aureus isolates of known phage types from bovine mastitis in Denmark from the 1950'ties (10 isolates) and the 1990'ties (48 isolates) were tested for beta-lactamase production. Furthermore, the presence of blaZ and blaR1 and the location of blaZ was determined by PCR and hybridisation. All isolates produced beta-lactamase and contained blaZ and blaR1. The blaZ gene was located on the chromosome in 54 isolates and on plasmids of different sizes in 4 isolates. Sequence analysis of an internal region of blaZ in 2 isolates of bovine origin showed a high degree of homology to already published sequences from human isolates. BlaZ could be transferred from the 4 isolates with plasmid location whereas it was not possible to transfer blaZ from 3 isolates with chromosomal location of the gene. The blaZ gene and the blaR1 gene were located closely to each other as previously published. In contrast to observations among isolates of human origin, no correlation between penicillin resistance and phage pattern was indicated for the bovine isolates. Furthermore, in contrast to the observed shift towards increased occurrence of plasmid location of blaZ among isolates of human origin in Denmark, blaZ appears to remain predominately chromosomal located among isolates of bovine origin.

Introduction

Since the introduction of penicillin for treatment more than 50 years ago, resistance to this antibiotic has been found in an increasing number of Staphylococcus aureus strains isolated from clinical infection in both humans and animals (*Renneberg & Rosdahl* 1992a, *Virani & Noble* 1992, *Schito et al.* 1996, *Aarestrup & Jensen* 1998). This has had an important impact on the effectiveness of penicillin for treatment of staphylococcal infections.

Bovine mastitis is the economically most important disease in dairy production (*Kossaibati & Esslemont* 1997) and *S. aureus* is the most frequently isolated pathogen from mastitis in Denmark (*Aarestrup et al.* 1995a). At present about 20% of *S. aureus* isolates from bovine mastitis in Denmark are resistant to penicillin (*Aarestrup & Jensen* 1998) and the most common mechanism of resistance seems to be production of beta-lactamase (*Aarestrup et al. 1995*).

The structural beta-lactamase gene (blaZ) has been identified in *S. aureus* of human origin and the DNA sequence has been published (*Chan*

1986). An upstream antirepressor gene (blaR1) flanks the blaZ gene (*Rowland & Dyke* 1990). The gene can be positioned on plasmids, on the chromosome (*Asheshov* 1969, *Skov et al.* 1995) or in a transposon (*Asheshov* 1969, *Virani & Noble* 1992). A Danish study of *S. aureus* strains of human origin showed a strong correlation between phage pattern and genetic location of blaZ. All strains of phage group II and the 94,96 complex had a chromosomal location. All strains of the 83A complex, most of the 52,52A,80,81 complex and phage type 95 carried a penicillinase encoding plasmid. In addition, 71% of phage group III strains carried a penicillinase encoding plasmid (*Rosdahl* 1985). Investigations on the genetic location in isolates of phage group II of human origin in Denmark showed that blaZ was mainly placed on the chromosome in isolates from the 1960'ties and 1970'ties, while isolates from the 1990'ties mainly had blaZ located on a plasmid (*Skov et al.* 1995).

The purpose of this study was to investigate the genetic basis for beta-lactamase production in *S. aureus* isolates from bovine mastitis in Denmark. The genes encoding beta-lactamase production in isolates of bovine origin were compared with the published blaZ sequences and the genetic position was determined. Finally, the transferability of beta-lactamase production from *S. aureus* isolates of bovine origin was investigated.

Materials and methods

Isolates and cultural methods

The isolates used in the present study are shown in Table 1. *S. aureus* isolates from cases of bovine mastitis in Denmark were selected on the basis of resistance to penicillin among culture collections at the Danish Veterinary Laboratory. Furthermore, 3 isolates (nos. 60-62) were included as negative controls. Isolate 59 was used in transferability studies. In total, 58 isolates resistant to penicillin were investigated. These isolates consisted of 10 *S. aureus* strains from 1952-1956, 8 from 1992, 17 from 1993 and 23 from 1996.

Table 1. Isolates of *Staphylococcus aureus* used in this study.

Isolate number	Year of isolation	Antibiotic resistance pattern
1-4	1952-1955	Pr
5-12	1992	Pr
13-29	1993	Pr
30-35	1954-1956	Pr
36-58	1996	Pr
59*		Ps, Rr, Fr
60	1952	Ps
61	1992	Ps
62	1993	Ps

Pr, penicillin resistant; Ps, penicillin sensitive; Rr, rifampicin resistant; Fr, fusidic acid resistant; Tr, tetracycline resistant; Ts, tetracycline sensitive.
All isolates were from a collection of *S. aureus* from cases of bovine mastitis in Denmark kept at the Danish Veterinary Laboratory (DVL), Copenhagen, Denmark.
*: ATCC 25923 made resistant to rifampicin and fusidic acid.

All isolates were subcultured on bovine blood agar (Columbia agar, CM 331, supplemented with 5% (vol/vol) sterile lysed bovine blood), and stored at -70 °C in Luria Bertani-medium with 15% glycerol until use.

Test of penicillin resistance

Resistance towards penicillin was investigated using tablet diffusion on bovine blood agar with 5 mg penicillin (Rosco Diagnostics, Taastrup, Denmark). Production of beta-lactamase after induction was detected using beta-lactamase diagnostic tablets according to the manufacturers guidelines (Rosco Diagnostics, Taastrup, Denmark).

Phage typing

Phage typing was performed at Statens Serum

Table 2. Primers used to characterize the blaZ gene and the blaR1/blaZ sequence from penicillin resistant *S. aureus*[a].

Primer	Sequence (5′-3′)	Position*
BlaZ A1	AAG AGA TTT GCC TAT GCT TC	169-188
BlaZ A2	GCT TGA CCA CTT TTA TCA GC	667-686
BlaR1 B1	GTT GCG AAC TCT TGA ATA GG	5018-5037
BlaZ B2	GAA GCA TAG GCA AAT CTC TT	5567-5586

[a] Primers blaZ A1 and blaZ A2 were used for PCR amplification of the internal region of the *blaZ* gene. Primers blaR1 B1 and blaZ B2 were used for PCR amplification of a sequence running from the *bla*R1 gene flanking the *blaZ* gene into the *blaZ* gene.

*Positions refer to sequences published in GenBank. For primers blaZ A1 and blaZ A2 positions are based on the published sequences of the *blaZ* gene, access numbers are M15526, M25252, M25253, M25254, M25257, U58139, X04121, X16471 and X25734. For primers blaR1 B1 and blaZ B2 positions are based on the published sequence of the transposon Tn552, access number is X52734.

Institut, Copenhagen as previously described (*Aarestrup et al.* 1995).

PCR amplification of blaZ

PCR amplification was performed as previously described (*Aarestrup et al.* 1996). Primers were designed according to the published sequences of the blaZ gene (*Chan* 1986, *Wang & Novick* 1987, *East & Dyke* 1989, *Gillespie & Skurray* 1989, *Rowland & Dyke* 1990, *Massaida & Varaldo* unpublished) and the blaR1/blaZ sequence from the transposon Tn552 (*Rowland & Dyke* 1990). All primers used are listed in Table 2.

All PCR amplifications were run with 1.5 mM $MgCl_2$ and an annealing temperature of 54 °C.

PCR amplification of the internal region of the *blaZ* generates an expected PCR product of 518 bp.

PCR amplification of the sequence running from blaR1 into blaZ generates an expected PCR product of 569 bp.

DNA sequencing

The nucleotide sequences of the amplification products from 2 isolates were determined by cycle sequencing (*Sears et al.* 1992) using the Amplitaq FS dye determinator kit and a 373A automatic sequencer (Applied Biosystems, Perkin-Elmer, Foster City, Calif.). DNAsis software (Hitachi Software Engineering Co., Ltd.) was used for sequence analysis.

Plasmid DNA

Plasmid DNA was prepared from 40 isolates (1-29, 35, 36, 43, 46-48, 50, 54, 55, 57 and 58) as described by *Wegener & Schwartz* (1993) with the following modifications. Ten mL of trypticase soy broth (TSB) supplemented with 10% yeast extract was inoculated with one colony from bovine blood agar. Benzylpenicillin (Løvens Kemiske Fabrik (LEO), Ballerup, Denmark) was added to a final concentration of 8 μg/mL and incubated at 37 °C until the optical density (OD) at 650 nm was between 0.5 and 0.8. For each plasmid preparation 2 mL/OD_{650} was used.

After centrifugation the pelleted chromosomal DNA and proteins were removed and the supernatant was extracted once with an equal volume of phenol:chloroform:iso-amylalcohol (25:24:1) and once with an equal volume of chloroform:iso-amylalcohol (24:1). Plasmid DNA was sedimented by addition of 35 μL ammonium acetate (3 M) and 350 μL iso-propanole and incubated at -20 °C for one h. The plasmid DNA was sedimented by centrifugation at 13,000 rpm for 10 min. at 4 °C. The supernatant

was discarded and the DNA pellet dried and resuspended in 20 μL TE-buffer (10 mM/mL Tris, one mM/mL EDTA).

Chromosomal DNA

Bacterial chromosomal DNA was obtained from isolates 1-58 as previously described (*Aarestrup et al.* 1995) with the following modifications. Bacterial isolates were grown overnight in 10 mL TSB, supplemented with 10% yeast extract. Benzylpenicillin was added to a final concentration of 8 μg/mL. The overnight culture was centrifuged and the precipitate resuspended in 567 μL TE-buffer. After lysation of the cells, proteins were removed by addition of 15 μL of proteinase K (10 mg/mL) (Merck, Darmstadt, Germany). After extraction with phenol chloroform, the DNA was precipitated with 500 μL iso-propanol and centrifuged at 13,000 rpm for 10 min. at 4 °C. The supernatant was discarded and pellet was washed with one mL 70% ethanol followed by centrifugation. The precipitate was dried and resuspended in 100 μL TE-buffer.

Enzymatic digestion of chromosomal DNA

The amount of DNA in each sample was measured using Genequant RNA/DNA calculator (Pharmacia, Copenhagen, Denmark) following the manufacturers recommendations.

Two μg of DNA was digested overnight with a restriction enzyme. EcoRI, *Hin*dIII, *Pst*I and EcoRV (Amersham International, Arlington Heights, IL, the United States) were used. The 3 first enzymes have no restriction sites in the blaZ gene, while the last enzyme has one restriction site in the gene according to the published sequences for the blaZ gene (*Chan* 1986, *Wang & Novick* 1987, *East & Dyke* 1989, *Gillespie & Skurray* 1989, *Rowland & Dyke* 1990, *Massaida & Varaldo* unpublished).

Gel electrophoresis

DNA fragments were separated in a 0.8% agarose gel. Plasmids in *Escherichia coli* V517 and *E. coli* 39R861 served as molecular weight markers for size determination of plasmids. *Hin*dIII digested lambda-DNA served to determine the size of the digested chromosomal fragments.

DNA blotting

DNA fragments were transferred onto a Hybond-N membrane (Amersham, Arlington Hights, IL, the United States) by standard Southern blotting using 20xSSC as transfer buffer.

Hybridisation

Digoxigenin labelled probe (518 bp) for the blaZ gene was prepared by PCR amplification using the primers described in Table 2. Probe DNA was purified using Qiagen spin columns (Hilden, Germany). The obtained PCR product was labelled with digoxigenin-II-dNTP using a DNA labelling and detection kit (Boehringer, Mannheim, Germany). The filters were hybridised at 60 °C using a digoxigenin staining kit according to the manufacturer's recommendations (Boehringer, Mannheim, Germany).

Transfer studies

The 4 isolates (21, 35, 47 and 55) containing a beta-lactamase encoding plasmid and isolates 4, 8 and 9 with blaZ positioned on the chromosome were investigated for their ability to transfer beta-lactamase production to isolate 59 using surface (plate) mating technique. Selective Mueller Hinton plates containing (i) 5 μg/mL penicillin; (ii) 100 μg/mL rifampicin (Sigma Chemical Co., St. Louis, MO, the United States) and 20 μg/mL fusidic acid (Løvens Kemiske Fabrik (LEO), Ballerup, Denmark); (iii) 5 μg/mL penicillin with 100 μg/mL rifampicin and 20 μg/mL fusidic acid were used for selection.

Table 3. Size of chromosomal fragments containing the blaZ gene after digestion with the restriction enzyme EcoRI in relation to decade of isolation.

Location of *blaZ*	Source (decade)	Fragment size(s) (kbp)	Number of isolates
Chromosomal	1950'ties	9.5	5
		14	4
	1990'ties	8	7
		9.5	27
		13	3
		17	1
		20	4
		6 and 8	3
Total number of isolates			54

Results

Beta-lactamase test

All 58 penicillin resistant *S. aureus* isolates produced beta-lactamase. The 3 penicillin sensitive *S. aureus* isolates (60-62) did not produce beta-lactamase.

blaZ and blaR1/blaZ coding regions

Amplification products of the expected size were obtained from all beta-lactamase producing isolates (1-58), whereas no products were obtained from beta-lactamase negative isolates (60-62). Sequence determination of the PCR products from 2 of these isolates (1 and 2) showed 99.6% homology to each other and 95.3% homology to the published sequences (*Chan* 1986). Similar, from the PCR amplification of the fragment spanning the region from blaR1 into blaZ using primers blaR1 B1 and blaZ B2 no product was obtained from isolate 60, whereas products of the expected sizes were obtained from isolates 1-58.

Localisation of the S. aureus blaZ gene

The blaZ gene probe hybridised to the chromosome in 54 isolates. The distribution of chromosomal fragment sizes obtained from digestion with EcoRI is shown in Table 3.

In 4 isolates (21, 35, 47 and 55) blaZ was positioned on a plasmid. Plasmid screening of 36 isolates with blaZ located on the chromosome showed no beta-lactamase encoding plasmids. The hybridisation positive plasmids were of different sizes ranging between 36 kbp and 147 kbp. One of the 4 isolates harbouring a beta-lactamase plasmid was from the 1950'ties (phage group III), whereas the other 3 were from the 1990'ties (2 from phage group III and 1 non typable (NT)).

The blaZ probe hybridised to a single band in 55 isolates and to 2 bands in 3 isolates (15, 16 and 48). These were all from the 1990'ties. These isolates were further digested with restriction enzymes *Eco*RV, *Hin*dIII and *Pst*I to investigate the possibility of existence of the blaZ gene in more than 1 copy. The hybridisation patterns for these isolates, when digested, appeared exactly identical. When digested with *Pst*I 2 bands occurred. When digested with *Eco*RV and *Hin*dIII only one band appeared.

Phage pattern and localisation of the blaZ gene in beta-lactamase producing *S. aureus* during the 1950'ties and 1990'ties are shown in Table 4. Bovine isolates were predominantly of phage group I and III, irrespectively of the period of isolation. No significant changes in the location of blaZ were observed between isolates from the 1950'ties and the 1990'ties.

Table 4. Genetic location of the *S. aureus* blaZ gene in relation to phage pattern and decade of isolation.

Phage pattern	1950'ties		1990'ties	
	Plas-mid	Chromo-somal	Plas-mid	Chromo-somal
Group I		2		17
Group II				7
Group III	1	6	2	10
Group V				
Type 95				
NI		1		10
NT			1	1
Total	1	9	3	45

NI: Sensitive to at least 2 types of phages belonging to different patterns. NT: Non typable.

Transferability

Beta-lactamase production was transferred to isolate 59 from isolates 21, 35, 47 and 55, but not from isolates 4, 8 and 9.

Discussion

Production of beta-lactamase is the most important mechanism of penicillin resistance in *S. aureus* isolates of both human and bovine origin (*Lacey* 1984, *Rosdahl* 1985, *Owens & Watts* 1988, *Aarestrup et al.* 1995). In the present study, the gene blaZ was detected by PCR in all bovine, penicillin resistant *S. aureus* isolates tested. By use of a second PCR, constructed to amplify the region between blaR1 and blaZ, the location of these 2 genes was further confirmed. The sequences of the internal region of the blaZ gene showed 95.3% homology with the originally published sequences (*Chan* 1986). Thus, blaZ seems to be a highly conserved gene, which has also previously been indicated from sequence studies of genes obtained from human isolates of *S. aureus* (*Wang & Novick* 1987, *East & Dyke* 1989, *Gillespie & Skurray* 1989, *Roland & Dyke* 1990). The full sequence of the blaZ gene from a bovine isolate still remains to be determined before the exact degree of homology between isolates of bovine and human origins can be determined. The present study suggests that human and bovine isolates share the same beta-lactamase encoding gene.

The blaZ gene was found mainly to be located on the chromosome as only 4 isolates had blaZ located on a plasmid. The chromosomal fragments that contained blaZ after digestion with EcoRI were all above 6 kb in size. Only 1 hybridisation positive band was observed in all but 3 strains. As blaZ is 846 bp long (*Chan* 1986), and the published sequences do not contain EcoRI restriction sites, it was concluded that the majority of isolates only contain 1 copy of blaZ. The distribution of fragment sizes, however, showed that the gene did not seem to be in the same genetic surroundings in all isolates investigated.

The hybridisation patterns indicated the presence of 4 different penicillinase plasmids in the investigated *S. aureus* strains with sizes ranging from 36 kbp to 147 kbp. Three out of 4 isolates that carried blaZ gene on a plasmid were of phage group III. *Rosdahl* (1985) found that 71% of human penicillin resistant isolates that belonged to phage group III, contained a beta-lactamase encoding plasmid. In the present material of bovine origin this proportion was 16%. Taking into account that only 58 isolates of bovine origin were investigated, this might not be the true proportion.

Investigations of human isolates of phage group II showed that a greater proportion of recent isolates carried the blaZ gene on a plasmid compared to older strains (*Skov et al.* 1995). Our material of bovine origin contained 7 isolates belonging to phage group II. These were all from the 1990'ties and all had a chromosomal location of the blaZ gene. Comparison of the isolates from the 1950'ties and the 1990'ties showed no tendency towards an increasing proportion of isolates with the blaZ gene on a plasmid. One out of 10 isolates had blaZ located on a plasmid in the 1950'ties and 3 out of 48 isolates in the 1990'ties.

Three isolates had an EcoRI hybridisation pattern, which could be indicative of the presence of more than 1 copy of the blaZ gene. The restriction patterns obtained after digestion with *Eco*RV and *Hin*dIII were, however, as expected from the published sequences of blaZ (*Chan* 1986, *Wang & Novick* 1987, *East & Dyke* 1989, *Gillespie & Skurray* 1989, *Rowland & Dyke* 1990). Thus, the presence of 2 bands after digest with EcoRI is probably caused by the presence of a restriction site within blaZ in these 3 isolates.

Comparison of phage patterns among penicillin resistant isolates from the 1990'ties with phage patterns in a collection of penicillin sensitive isolates of bovine origin (*Aarestrup et al.* 1995) showed no significant difference between the 2 populations indicating that there is no correlation between phage pattern and penicillin resistance in bovine isolates. This is in contrast to findings among human isolates where a correlation between changes in phage pattern and development of penicillin resistance has been shown (*Rosdahl* 1988, *Renneberg & Rosdahl* 1992). Comparison to phage patterns among naturally occurring penicillin resistant strains of human origin (*Rosdahl* 1985) showed some differences in the distribution of phage patterns. The major difference was that no phage group V or phage type 95 was found among the bovine isolates.

In conclusion, this study demonstrated blaZ in all *S. aureus* isolates from bovine mastitis resistant to penicillin. The sequences had a high degree of homology with the sequences of the blaZ gene published from human isolates, and the blaZ gene and the blaR1 gene were located close to each other. The blaZ gene was mainly located in the chromosome among bovine isolates from the 1950'ties and 1990'ties. There was no indication of the existence of a common plasmid containing blaZ. No correlation between penicillin resistance and phage pattern was observed in isolates of bovine origin. Transferability of the blaZ gene was demonstrated from isolates containing plasmids but not from the chromosome.

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References

Aarestrup FM, Wegener HC, Rosdahl VT: Evaluation of phenotypic and genotypic methods for epidemiological typing of *Staphylococcus aureus* isolates from bovine mastitis in Denmark. Vet. Microbiol. 1995, *45,* 139-150.

Aarestrup FM, Wegener HC, Rosdahl VT, Jensen NE: Staphylococcal and other bacterial species associated with intramammary infections in Danish dairy herds. Acta Vet. Scand. 1995a, *36,* 475-487.

Aarestrup FM, Ahrens P, Madsen M, Pallesen LV, Poulsen RL, Westh H: Glycopeptide susceptibility among Danish *Enterococcus faecium* and *Enterococcus faecalis* isolates of animal and human origin and PCR identification of genes within the VanA cluster. Antimicrob. Agents Chemother. 1996, *40,* 1938-1940.

Aarestrup FM, Jensen NE: Development of penicillin resistance among *Staphylococcus aureus* isolated from bovine mastitis in Denmark and other countries. Microb. Drug Resist. 1998, *4,* 247-256.

Asheshov EH: The genetics of penicillinase production in *Staphylococcus aureus* strain PS80. J. Gen. Microbiol. 1969, *59,* 289-301.

Chan PT: Nucleotide sequence of the *Staphylococcus aureus* PC1 beta-lactamase gene. Nucleic Acids Res. 1986, *14,* 5940.

East AK, Dyke KGH: Cloning and sequence determination of six *Staphylococcus aureus* beta-lactamases and their expression in *Escherichia coli* and *Staphylococcus aureus.* J. Gen. Microbiol. 1989, *135,* 1001-1015.

Gillespie MT, Skurray RA: Nucleotide sequence of the *blaZ* gene of the *Staphylococcus aureus* beta-lactamase transposon Tn4002. Nucleic Acids Res. 1989, *17,* 8854.

Kossaibati MA, Esslemont RJ: The costs of production diseases in dairy herds in England. Vet. J. 1997, *154,* 41-51.

Lacey RW: Antibiotic resistance in *Staphylococcus aureus* and streptococci. Brit. Med. Bull. 1984, *40,* 77-83.

Owens WE, Watts JL: Antimicrobial susceptibility and beta-lactamase testing of staphylococci isolated from dairy herds. J. Dairy Sci. 1988, *71,* 1934-1939.

Renneberg J, Rosdahl VT: A nationwide 30 years study of changes in antibiotic resistance in *Staphylococcus aureus* with special emphasis on penicillin-resistance. Zbl. Bakt. Suppl. 1992, *26,* 27-30.

Renneberg J, Rosdahl VT: Epidemiological studies of penicillin resistance in Danish *Staphylococcus aureus* strains in the period 1977-1990. Scand. J. Infect. Dis. 1992a, *24,* 401-409.

Rosdahl VT: Localisation of the penicillinase gene in naturally occurring *Staphylococcus aureus* strains. Acta Path. Microbiol. Immunol. Scand. B 1985, *93,* 383-388.

Rosdahl VT: Epidemiology of resistance among gram-positive bacteria: With special reference to staphylococcal infections. J. Hosp. Infect. Suppl. A 1988, *11,* 123-129.

Rowland SJ, Dyke KGH: Tn552 a novel transposable element from *Staphylococcus aureus.* Mol. Microbiol. 1990, *4,* 961-975.

Schito GC, Debbia EA, Pesce A: Susceptibility of respiratory strains of *Staphylococcus aureus* to fifteen antibiotics: Results of a collaborative surveillance study (1992-1993). The Alexander Project Collaborative Group. J. Antimicrob. Chemother. Suppl. A 1996, *38,* 97-106.

Sears LE, Moran LS, Kissinger C, Creasey T, Perry-O'Keefe H, Roskey M, Sutherland E, Slatko BE: CircumVent thermal cycle sequencing and alternative manual and automated DNA sequencing protocols using the highly thermostable VentR (exo-) DNA polymerase. Biotech. 1992, *13,* 626-633.

Skov RL, Williams TJ, Pallesen L, Rosdahl VT, Espersen F: Beta-lactamase production and genetic location in *Staphylococcus aureus:* Introduction af a beta-lactamase plasmid in strains of phage group II. J. Hosp. Infect. 1995, *30,* 111-124.

Virani Z, Noble WC: Antibiotic resistance and plasmids in *Staphylococcus aureus* from normal populations. J. Antimicrob. Chemother. 1992, *29,* 35-39.

Wang P-Z, Novick RP: Nucleotide sequence and expression of the beta-lactamase gene from *Staphylococcus aureus* plasmid pI258 in *Escherichia coli, Bacillus subtilis,* and *Staphylococcus aureus.* J. Bacteriol. 1987, *169,* 1763-1766.

Wegener HC, Schwartz S: Antibiotic resistance and plasmids in *Staphylococcus hyicus* isolates from pigs with exudative epidermitis and from healthy pigs. Vet. Microbiol. 1993, *34,* 363-372.

Sammendrag

Forekomst af blaZ genet i penicillinresistente Staphylococcus aureus isoleret fra bovin mastitis i Danmark.

Otteoghalvtreds penicillinresistente *Staphylococcus aureus* isolater med kendte fagtyper fra mastitis hos kvæg, som var isoleret i 1950erne (10 isolater) og 1990erne (48 isolater), blev undersøgt for beta-laktamaseproduktion, forekomst af *blaZ* og *bla*R1, samt lokalisering af *blaZ.* Alle isolater producerede betalaktamase og indeholdt *blaZ* og *bla*R1. *BlaZ* genet var placeret på kromosomet i 54 isolater og på plasmider af forskellig størrelse i 4 isolater. Sekventering af en del af *blaZ* i 2 isolater viste en høj grad af homologi til de publicerede sekvenser fra humane stammer. *BlaZ* kunne overføres til en følsom *S. aureus* stamme fra de 4 isolater, hvor *blaZ* var placeret på plasmider, mens det ikke var muligt at overføre *blaZ* fra 3 isolater med kromosomal placering. *BlaZ* og *bla*R1 generne var placeret nær hinanden som tidligere beskrevet. Det var ikke muligt at observere nogen korrelation mellem fagtype og penicillinresistens, hvilket er i modsætning til det, der tidligere er blevet rapporteret for humane stammer. *BlaZ* er tilsyneladende stadig primært kromosomalt placeret blandt danske bovine stammer, hvilket er i modsætning til situationen blandt humane isolater, hvor der er sket et skift mod en øget forekomst af plasmidmedieret betalaktamase produktion.

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