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Characterization of Resistance Patterns and Plasmid Profiles of Isolates from *Staphylococcus aureus* Animal Infections

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Abstract: Over 100 *Staphylococcus aureus* isolates from bovine and sheep infection were collected from farms of different regions of Slovakia. Nine of the collected isolates were multiresistant and harboured plasmids ranging in molecular sizes from 6.9 9.0 kb. Transformation experiment revealed that five from nine antibiotic resistant plasmids were able to replicate in *Escherichia coli* and *Corynebacterium glutamicum* RM3. Plasmid-mediated resistance to kanamycim, chloramphenicol, ampiciline and tetracycline was found.

Keywords: *Staphylococcus aureus*, Plasmids, Resistance, Animal infection, Antibiotics

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1. Introduction

Staphylococcus aureus is a pathogenic bacterium that causes a variety of diseases in humans and animals [1]. It causes skin lesions, serious infections including pneumonia, mastitis and meningitis, deep-seated infections and toxic shock syndrome by releasing exotoxins into the blood stream [2]. *Staphylococcus aureus* is, for dairy animals, one of the major reasons of intramammary infections (mastitis) of female's cows and it is frequently isolated from milk. The most common therapy of staphylococcal infections is the treatment with antibiotics. The problem of this cure was coupled with the raising resistance of staphylococci to antibiotics. *Staphylococcus aureus* resistant to semi-synthetic -lactamases was found in 1960s. These resistant bacteria became known as MRSA (methicillin resistant *Staphylococcus aureus*) [3]. In 1997 another *S. aureus* resistant to vancomycin was detected and at this time the resistance to antibiotics is widespread among staphylococci [4 6].

Clinical isolates of *Staphylococcus aureus* often harbor multiple plasmids, ranging from small rolling-circle replicating (RCR) plasmids that are cryptic or encode a single resistance determinant, to larger multi resistant and conjugative plasmids [7 9]. Increased attention has been focused to plasmid-encoded resistance to antiseptics and disinfectants detected on plasmid isolated from antibiotic-resistant staphylococci [10, 11].

The aim of the present study was to characterize the *Staphylococcus aureus* isolates collected from bovine mastitis for the presence of antibiotic resistance plasmids.

2. Experimental

Bacterial strains and media. *Staphylococci* isolates were collected from animals' bovine mastitis from different farms of Slovakia. The media used for grow were: Luria-Bertani (LB) broth [12] and blood agar (Imuna, Šarišské Michaľany, Slovakia). *S.aureus* cells and *E.coli* strains were grown at 37 C, *Corynebacterium glutamicum* were grown at 30 C.

Antibiotic susceptibility test: Staphylococci isolates were tested for antibiotic susceptibilities by the disc diffusion method in blood agar [13]. The following antibiotic discs (Biotika, Slovenská Ľupča, Slovakia) were used: ampicillin (10 μg), chloramphenicol (10 μg), ciprofloxacin (5 μg), clindamycin (10 μg), erythromycin (15μg), gentamycin (10 μg), kanamycin (10 μg), neomycin (30 μg), nitrofurantoin (10 μg), oxacillin (5 μg), penicillin (10 μg), sulphametoxazol (10 μg), streptomycin (25 μg) and tetracycline (30 μg). The minimum inhibitory concentrations (MIC) for staphylococci isolates were determined by microdilution method with LB medium, described by NNLS in document M7-A4 (NCCLS). The following antibiotics with concentration 10–600 μg/mL were used: ampicillin, chloramphenicol, kanamycin (ICN Biochemics) and tetracycline (Sigma). MIC was estimated as the lowest concentration of antibiotic that inhibited growth after 16–18 h of incubation at 37 C. MIC was also determined for strains *Escherichia coli* JM110 (Stratagene, USA) and *Corynebacterium glutamicum* RM3 [14], which were used as hosts for transformation of isolated staphylococci plasmids.

Plasmid isolation: Plasmid DNAs were isolated by alkaline lysis method [12]. Plasmid DNAs from corynebacterium were isolated by Santamaria et al. [15]. The same method with modification was used for isolation of staphylococci plasmid DNA. We used the combination of lysozyme (10 mg.mL ^{1}) and lysostaphin (2 mg.mL ^{1}) for lysis. Preparation and transformation of competent cells *Escherichia coli* JM110 were according to Hanahan [16]. Preparation and transformation of competent cells *Corynebacterium glutamicum* RM3 were performed according to van der Rest et al. [17].

3. Results and discussion

3.1 Antibiotic susceptibility test of staphylococci isolates

Staphylococcus aureus isolates Nos. 8, 32, 33, 60, 61, 90, 93, 96, 98 were tested for susceptibility to selected antibiotics by disc diffusion method on blood agar. Only *Staphylococcus aureus* isolate 33 was determined as resistant to six antibiotics with MIC (Minimum inhibitory concentrations) as follows: tetracycline 30 g, erythromycin 15 g, kanamycin, neomycin 30 g, penicillin 10 g, streptomycin 25 g. The other eight isolates were sensitive to the tested antibiotics (data not shown).

The second method used for testing of nine staphylococci isolates for susceptibility to selected antibiotics in liquid medium was by microdilution. The antibiotic resistance and MIC were determined for the selected antibiotics: chloramphenicol (Cm), ampicillin (Ap), tetracycline (Tc) and kanamycin (Kn). As is shown in Table 1 from *Staphylococcus aureus* isolates, 33 and 96 were resistant to all four tested antibiotics.

Table 1. The MIC to antibiotics of staphylococci isolates 8, 32, 33, 60, 61, 90, 93, 96, 98.

Isolate *S.a.* / *antibiotic* (*g/ml)*	**8**	**32**	**33**	**60**	**61**	**90**	**93**	**96**	**98**
Cm	150	90	600	60	60	60	60	60	30
Ap	0	0	100	0	0	50	0	50	0
Tc	75	75	60	0	45	45	45	45	0
Kn	60	60	600	30	>600	0	>600	>600	>600

3.2 Plasmids profiling

Plasmid DNA from nine *Staphylococcus aureus* isolates was isolated and characterized by agarose gel electrophoresis (Fig. 1).

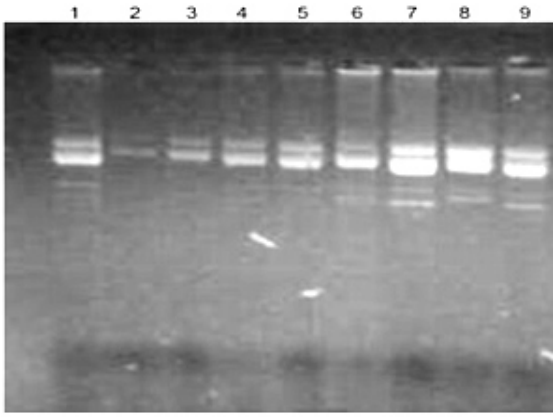

Fig. 1. Agarose gel electrophoresis of isolated plasmids from *Staphylococcus aureus* isolates. 1. pSA 8, 2. pSA 32, 3. pSA 33, 4. pSA 60, 5. pSA 61, 7. pSA 63, 8. pSA 66, 9. pSA 98.

Nine isolated plasmid DNAs were transformed into *E. coli* and *C. glutamicum* cells. The transformants were selected on agar plates with appropriate antibiotic addition according to previously determined MIC for each isolate (Table 1). Only five out of nine isolated plasmids were able to replicate in host cells: pSA32, pSA60 and pSA93 in *E. coli* and pSA 33 and pSA 33 in *E. coli* and *C. glutamicum*. The sizes of plasmids were estimated according to the restriction analysis (Table 2). The resistance to antibiotics linked with plasmid DNA was also determined (Table 2). We concluded that pSA33 and pSA90 carry resistance to antibiotics Cm, Ap, Tc, pSA32, pSA60 to Cm and Kn and pSA93 to Cm and Tc antibiotics (Table 2).

Table 2. Plasmid pSA32, pSA 33, pSA60, pSA90 and pSA 93 characterization.

Plasmid	Size (kbp)	Replication in	Phenotype Antibiotic resistance
pSA 32	8,2	*E.coli*	Cm^r, Kn^r
pSA 33	6,9	*E.coli*, *C. glutamicum*	Cm^r, Ap^r Tc^r
pSA 60	8,2	*E.coli*	Cm^r, Kn^r
pSA 90	6,9	*E.coli*, *C. glutamicum*	Cm^r, Ap^r,Tc^r
pSA 93	9,0	*E.coli*	Cm^r, Tc^r

4. Conclusions

The detail phenotypic and genotypic characterization of staphylococci isolates from mastitis is very comprehensive and helpful in specification of the infecting pathogens. The determination of antibiotic resistance patterns might provide important information about *Staphylococcus aureus* isolates originated from unrelated geographical origins [18]. Resistance typing may also be a useful primary typing control measure [19]. Antibiotics are used in animals for both prevention and treatment of infections. We found out that all the isolates were resistant to at least two antibiotics. *S. aureus* isolate 33 and 96 are resistant to all four tested antibiotics, so we can consider them as multiresistant. Despite the fact that plasmid originated from *Staphylococcus aureus* (Gram-positive), most of them were able to replicate in bacteria *E.coli* (Gram-negative) and only plasmids pSA33 and pSA93 in *Corynebacterium glutamicu*m (Gram-positive). The prevalence of multiple antibiotic resistant *Staphylococcus aureus* isolates present in the samples from bovine mastitis raises the important issues for infection control in environment.

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