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Methicillin-resistant *Staphylococcus lentus* strains isolated from chicken carcasses and employees of a poultry abattoir

Methicillin-resistente Staphylococcus lentus-Stämme isoliert von Geflügelschlachttierkörpern und Mitarbeitern eines Geflügelschlachthofs

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Summary

Methicillin-resistant coagulase-negative staphylococci (MR-CNS) are increasingly reported in animals and humans as colonizing organisms and as opportunistic pathogens. Within a collection of MR-CNS isolates from livestock, chicken carcasses, bulk tank milk, minced meat, and contact persons, matrix-assisted laser desorption ionization-time of flight mass spectrometry identified 37 isolates as *S. lentus*. All 37 methicillin-resistant *S. lentus* strains originated from either chicken carcasses (30 strains) or employees working in a poultry abattoir (seven strains). To assess the phenotypic antibiotic resistance to selected antibiotics (ciprofloxacin, clindamycin, erythromycin, gentamicin, rifampin, sulfamethoxazole/trimethoprime, tetracycline, vancomycin), the disk diffusion method was used and none of the strains was resistant to gentamicin, rifampin, or vancomycin. Pulsed-field gel electrophoresis (*Sma*I) was applied to evaluate the genotypic relationship of the 37 methicillin-resistant *S. lentus* strains. With a cut-off level of 80 % similarity, 30 (81.1 %) strains were grouped into only two clusters. Overall, the seven human strains showed between 85 % and 100 % similarity to the closest related chicken isolates. Our results suggest potential transmission of methicillin-resistant *S. lentus* between slaughtered chickens and abattoir personnel.

Keywords: Methicillin-resistant *Staphylococcus lentus,* antibiotic resistance, genotyping, transmission, poultry abattoir employees

Zusammenfassung

Methicillin-resistente, Koagulase-negative Staphylokokken (MR-CNS) kommen bei Mensch und Tier auf Haut und Schleimhaut vor und sind auch als Pathogene von zunehmender Bedeutung. Bei von Nutztieren, Geflügelschlachttierkörpern, Rohmilch, Hackfleisch und Kontaktpersonen isolierten MR-CNS wurden mittels MALDI-TOF MS 37 Isolate als *S. lentus* identifiziert. Diese 37 Methicillin-resistenten *S. lentus*-Stämme stammten entweder von Geflügelschlachttierkörpern (30 Stämme) oder von Mitarbeitern eines Geflügelschlachthofs (sieben Stämme). Die Prüfung der Empfindlichkeit dieser Stämme gegenüber ausgewählten Antibiotika (Ciprofloxacin, Clindamycin, Erythromycin, Gentamicin, Rifampin, Sulfamethoxazol/Trimethoprim, Tetracyclin, Vancomycin) erfolgte unter Verwendung des Agardiffusionsverfahrens. Dabei wies keiner der Stämme Resistenzen gegen Gentamicin, Rifampin oder Vancomycin auf. Zur Genotypisierung wurde eine Pulsfeld-Gelelektrophorese (*Sma*I) durchgeführt. Dreissig (81.1 %) der 37 Methicillin-resistenten *S. lentus*-Stämme wurden dabei lediglich zwei verschiedenen Clustern zugeordnet. Insgesamt zeigten die sieben humanen Stämme eine Übereinstimmung von 85–100 % zu den am nächsten verwandten Geflügelisolaten. Diese Ergebnisse sprechen für die Übertragung von Methicillin-resistenten *S. lentus* zwischen geschlachtetem Geflügel und Schlachthofmitarbeitern.

Schlüsselwörter: Methicillin-resistente *Staphylococcus lentus,* Antibiotikaresistenz, Genotypisierung, Übertragung, Geflügelschlachthof-Mitarbeiter

Introduction

Methicillin-resistant coagulase-negative staphylococci (MR-CNS) are increasingly reported in animals and humans as colonizing organisms and as opportunistic pathogens (Silva et al., 2001; Miragaia et al., 2002; Rajala-Schultz et al., 2004; van Duijkeren et al., 2004; Zhang et al., 2009). It is assumed that the *mec*A gene, which encodes methicillin resistance, had evolved in CNS and was then horizontally spread among staphylococci (Archer et al., 1994; Kloos et al., 1997; Barbier et al., 2010; Tsubakishita et al., 2010). Particularly *Staphylococcus (S.) sciuri* and *S. fleurettii* are thereby discussed as natural reservoir of the methicillin resistance gene *mec*A. Wielders et al. (2001) also showed evidence for in-vivo transfer of methicillin resistance encoding *mec*A between *S. aureus.* The *mec*A gene is located on a mobile genetic element called staphylococcal cassette chromosome and confers resistance to methicillin by encoding an altered penicillin-binding protein (PBP2α), which shows limited affinity to beta-lactam antibiotics.

During a previous study on the prevalence of MR-CNS in livestock (pigs, cattle, calves), chicken carcasses, bulk tank milk, minced pork and beef, and persons in contact with farm animals (veterinarians, pig farmers, employees of two abattoirs), 414 MR-CNS isolates (191 from livestock and chicken carcasses, 84 from bulk tank milk and minced meat, 139 from humans) were identified to species level (Huber et al., 2011). Overall, seven different species (*S. sciuri,* 32.6 %; *S. fleurettii,* 25.1 %; *S. haemolyticus,* 17.4 %; *S. epidermidis,* 14.5 %, *S. lentus,* 9.2 %; *S. warneri,* 0.7 %; *S. cohnii,* 0.5 %) were found. All 37 methicillin-resistant *S. lentus* strains were thereby isolated from either herd-wise pooled neck skin samples of chicken carcasses collected in a poultry slaughterhouse or nasal swabs of employees working in this poultry abattoir. The aim of the present study was (i) to assess phenotypic antibiotic resistance profiles of the methicillin-resistant *S. lentus* strains using the disk diffusion method and (ii) to genotype these strains using pulsed-field gel electrophoresis (PFGE) after macrorestriction with *Sma*I in order to evaluate relationship and to reveal potential transmission routes.

Materials and methods

Strain isolation and identification

The isolation of MR-CNS has previously been described in detail (Huber et al., 2011). Briefly, a two-step enrichment procedure in Mueller-Hinton broth supplemented with 6.5 % NaCl (24 h at 37 °C) and in phenol red mannitol broth supplemented with 75 µg/ml aztreonam and 5 µg/ml cefoxitin (24 h at 37 °C) was used. Samples were then plated onto an oxacillin-containing agar (Oxoid Brilliance MRSA agar; Oxoid Ltd., Hampshire, UK) and incubated for 24 h at 37 °C. Gram-positive, catalase-positive cocci grown beige or white on the agar were considered MR-CNS. A random selection of 414 isolates was confirmed as genetically methicillin-resistant by detection of the *mec*A gene (Mehrotra et al., 2000). MR-CNS species identification was performed using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) as described recently (Huber et al., 2011). MALDI-TOF MS was reported to be an effective tool for the reliable identification of staphylococci and CNS in particular (Carbonnelle et al., 2007; Dubois et al., 2010). The MR-CNS collection included 38 isolates from herd-wise pooled neck skin samples of chicken carcasses and 12 isolates from employees working in a poultry abattoir (Huber et al., 2011). Of the 38 isolates from chicken carcasses, 30 were identified as *S. lentus,* six as *S. sciuri,* and two as *S. epidermidis.* Of the 12 isolates from employees working in a poultry abattoir, seven were identified as *S. lentus* and five as *S. haemolyticus.* In the present study, the 37 methicillin-resistant *S. lentus* strains (30 from chicken carcasses and seven from employees of a poultry abattoir) were further characterized.

Antibiotic susceptibility testing

Phenotypic antibiotic resistance of the 37 methicillin-resistant *S. lentus* strains was tested using the disk diffusion method in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2008). The following disks were thereby used (BD BBL Sensi-Disc; Becton, Dickinson and Company, Sparks, MD, USA): ciprofloxacin (5 µg), clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), rifampin (5 µg), sulfamethoxazole/trimethoprime (23.75/1.25 µg), tetracycline (30 µg), and vancomycin (30 µg). Results were interpreted according to the CLSI guidelines, whereby intermediate results were considered resistant.

Pulsed-field gel electrophoresis

PFGE analysis of the 37 methicillin-resistant *S. lentus* strains was performed following the slightly modified protocol of MacKenzie et al. (2002). Briefly, after incubation of a colony in BHI broth (5 h, 37 °C, 350 rpm agitation) and centrifugation of 0.4 ml of the overnight culture, the pellet was washed in 0.9 ml of NET buffer (10 mM Tris, 1 mM EDTA, 10 mM NaCl), re-suspended in 0.25 ml of NET buffer, and mixed with 200 U of achromopeptidase (Sigma-Aldrich, Buchs, CH) and an equal volume of 2 % SeaKem™ Gold Agarose (Bio Concept, Allschwil, CH) at 50 °C. The cell/agarose suspension was loaded into plug moulds (Bio-Rad Laboratories, Reinach, CH) and allowed to solidify at 4 °C. Cells were lyzed by overnight incubation at 50 °C in lysis buffer (6 mM Trizma base, 100 mM EDTA, 1 M NaCl, 0.5 % Brij 58, 0.2 % sodium deoxycholate, 0.5 % lauroyl sarcosine) and 200 U of achromopeptidase. Plugs were washed three times for 30 min at 50 °C in TE buffer (10 mM Trizma base, 1 mM EDTA). One quarter of each agarose plug was digested with 30 U of *Sma*I (Roche Diagnostics, Rotkreuz, CH) according to the manufacturer's instructions and loaded into the wells of a 1 % PFGE certified agarose gel (Bio-Rad Laboratories). Electrophoresis was performed in 0.5 x TBE buffer (44.5 mM Trizma base, 44.5 mM boric acid, 1 mM EDTA) by the contour clamped homogeneous electric field method with a CHEF-DR III system (Bio-Rad Laboratories). Fragments were separated with a linear ramped pulse time of 3–33 s over a period of 22 h at 10 °C. Gels were stained with 1 µg/ml ethidium bromide (Sigma-Aldrich) solution for 30 min, visualized under UV, and photographed. *Salmonella* Branderup H9812 (ATCC BAA 664) cutted with *Xba*I was used as molecular weight standard and control for standardizing gel-to-gel comparison. PFGE patterns were compared using the GelCompar™ II version 6.0 software program (Applied Maths NV, Sint-Martens-Latem, B).

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TABLE 1: *Resistance patterns of the 37 methicillin-resistant Staphylococcus lentus strains (30 from chicken carcasses and 7 from employees of a poultry abattoir) to eight selected antibiotics.*

	No. of strains	Antibiotics tested[a]							
		Cip	Cli	Ery	Gen	Rif	SxT	Tet	Van
S. lentus strains isolated	19	s[b]	s	s	s	s	s	s	s
from chicken carcasses	4	s	s	s	s	s	s	r	s
(herd-wise pooled neck	3	s	r	r	s	s	s	s	s
skin samples)	2	s	r	s	s	s	s	s	s
	1	s	s	r	s	s	r	s	s
	1	s	s	r	s	s	s	s	s
S. lentus strains isolated	2	s	s	s	s	s	s	s	s
from employees of a poultry	1	s	r	s	s	s	s	s	s
abattoir (nasal swabs)	1	s	r	r	s	s	s	s	s
	1	r	r	s	s	s	s	r	s
	1	s	r	r	s	s	s	r	s
	1	s	r	s	s	s	r	s	s

[a] Cip, ciprofloxacin; Cli, clindamycin; Ery, erythromycin; Gen, gentamicin; Rif, rifampin; SxT, sulfamethoxazole/trimethoprime; Tet, tetracycline; Van, vancomycin.
[b] r, resistant; s, susceptible.

Results and Discussion

Amongst the 414 MR-CNS strains previously isolated from livestock, chicken carcasses, bulk tank milk, minced meat, and contact persons (Huber et al., 2011), methicillin-resistant *S. lentus* were detected in samples from chicken carcasses and employees of a poultry abattoir. *S. lentus* was thereby the predominant species in samples from chicken carcasses (79.0 %) and seven of the 12 strains isolated from employees of a poultry abattoir were also identified as *S. lentus.* On the other hand, no methicillin-resistant *S. lentus* were found in samples from employees of a pig/cattle abattoir, veterinarians or pig farmers, in samples from bulk tank milk and minced pork and beef, or in samples from farm animals other than chickens (pigs, cattle, calves).

S. lentus along with *S. sciuri* and *S. vitulinus* form the *S. sciuri* group (Stepanovic et al., 2005). Members of this group are widely spread in nature and they are also found in the mucosal flora of healthy humans and various animal species. Data on the occurrence (and characteristics) of methicillin-resistant *S. lentus* in healthy humans were so far lacking, but *S. lentus* have been detected in samples from patients with bacteremia and various infections (Stepanovic et al., 2005; Karachalios et al., 2006; Koksal et al., 2009). On the other hand, methicillin-resistant *S. lentus* were described in several species of healthy animals including horses and ruminants (Busscher et al., 2006; Zhang et al., 2009; De Martino et al., 2010). But to the authors' knowledge this is the first report on methicillin-resistant *S. lentus* isolated from chickens. Besides, *S. lentus* were isolated from healthy chickens (Hinton and Ingram, 2000; Simjee et al., 2007; Zhang et al., 2009), from chickens showing clinical illness (Awan and Matsumoto, 1998), and also from the surface of table eggs (Stepien-Pysniak et al., 2009).

The results of the phenotypic antibiotic resistance testing of the 37 methicillin-resistant *S. lentus* strains are summarized in Table 1. Overall, resistance to clindamycin, erythromycin, tetracycline, sulfamethoxazole/trimethoprime, and ciprofloxacin were found in 27.0 %, 18.9 %, 16.2 %, 5.4 %, and 2.7 % of the strains, respectively. On the other hand, none of the strains was resistant to gentamicin, rifampin, or vancomycin. Amongst the *S. lentus* strains isolated from chicken carcasses, 19 (63.3 %) showed no resistance to the tested antibiotics. Of the seven human strains, only two showed this phenotype. Compared to the resistance rate in human isolates, the proportion of clindamycin-resistant strains was clearly lower in chicken strains. This may be due to the fact that clindamycin is not used in the Swiss poultry production (personal communication Prof. Richard Hoop, Vetsuisse Faculty University of Zurich). However, further data on the antibiotic resistance of methicillin-resistant *S. lentus* from humans or chickens are lakking in literature.

PFGE analysis of the 37 methicillin-resistant *S. lentus* strains yielded 28 different band patterns. Based on a cut-off level of 80 % similarity, as suggested by McDougal et al. (2003) for methicillin-resistant *S. aureus,* 34 isolates were grouped into four clusters, whereas the remaining three isolates clustered separately (Fig. 1). Thirty (81.1 %) of the 37 strains were thereby grouped in only two clusters. With regard to the sample origin, PFGE patterns were randomly distributed among the isolates. The largest cluster comprised 20 strains (54.1 %), four of human and 16 of chicken origin. Within this cluster, two human strains showed 100 % similarity and likewise did seven chicken strains. These seven chicken strains originated from seven different farms and were taken on six different days of sampling. This suggests that this genotype is prevalent on chicken farms of different areas in Switzerland. The second largest cluster comprised ten strains (27.0 %) and only one of them was of human origin. As the only human isolate in the present study, this strain showed 100 % similarity to a chicken isolate. The other six human methicillin-resistant *S. lentus* strains showed a similarity between 85 % and 96 % to adjacent chikken isolates (Fig. 1).

In conclusion, since similarities of 85 % and more were found between PFGE patterns of methicillin-resistant *S. lentus* from chicken carcasses and humans, our findings support the hypothesis of transmission between slaughtered chickens and abattoir personnel. Both methicillin-resistant and methicillin-susceptible staphylococci have been suggested to be transmitted between humans and animals (Lee, 2003; Voss et al., 2005; Juhász-Kaszanyitzky et al., 2007; van Duijkeren et al., 2008; Graveland et al., 2010; Jaglic et al., 2010). The potential direction of spread could not be verified since the results illustrate only punctual time events. However, the fact that *S. lentus* was only found in employees of a poultry abattoir but not in other human samples may indicate that the direction of transmission was from chickens to humans.

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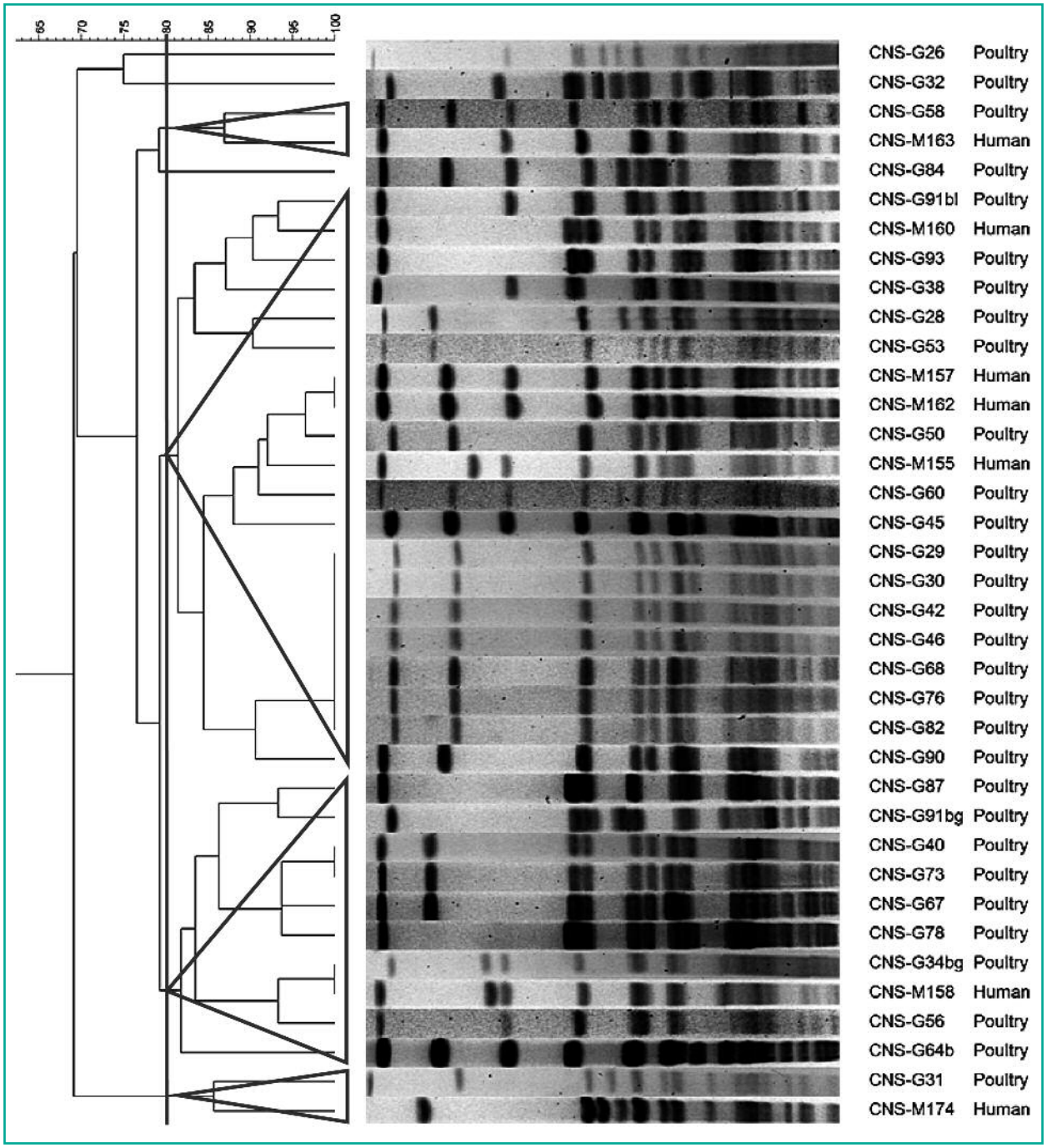

FIGURE 1: *PFGE patterns and dendrogramm of the 37 methicillin-resistant Staphylococcus lentus strains (30 from chicken carcasses and 7 from employees of a poultry abattoir). The delineation level for clusters (indicated by bold vertical line) was set at 80 % similarity.*

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