SHORT COMMUNICATION

Prevalence of Methicillin-Resistant *Staphylococci* on a Farm: Staff can Harbour MRS When Animals Do Not

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Impacts

- Methicillin-resistant *Staphylococci* (MRS) are multi-resistant bacteria which have been a severe public health problem in the entire world, mainly causing nosocomial infections.
- In this study MRS have been detected in humans but not in animals.
- The results show that staff can harbour MRS and not necessarily transmit it to the animals.

Keywords:
Farm animals; MRSA; MRS; *mec*A; resistance to methicillin; *Staphylococcus* spp.

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Summary

The aim of this work was to establish the prevalence of methicillin-resistant *Staphylococci* (MRS) in the animals and staff of a teaching and research farm. Samples of dairy cattle (36), beef cattle (26), sheep (19), horses (21), pigs (23), goats (23) and humans (13) were collected and screened for the presence of MRS. The detection of *mec*A gene was performed by PCR to determine the resistance of the samples to methicillin. Antimicrobial-resistance testing to penicillin, meropenem, ceftriaxone, cephalothin, oxacillin, levofloxacin, enrofloxacin, chloramphenicol, ciprofloxacin, gentamicin, clindamycin, erytromycin, linezolid, sulfamethoxazole/trimethoprim, tetracycline, doxycycline and vancomycin was performed on the *mec*A+ isolates. From the 161 samples, four methicillin-resistant coagulase-negative *Staphylococci* (MRCoNS) were isolated from human beings (31%), whereas none was isolated from animals (0%). No methicillin-resistant *Staphylococcus aureus* (MRSA) were isolated. All of the MRCoNS isolates from this work presented different antimicrobial resistance patterns. MRCoNS may be present in humans associated with animals while not present in the animals. Selective pressure outside of the farm and a lack of MRCoNS transmission between humans and animals may be responsible for this lack of correlation.

Introduction

Methicillin-resistant *Staphylococci* have been an important public health problem in the entire world, mainly through nosocomial infections. MRS are often multi-resistant bacteria and the species most representative of this group is *Staphylococcus aureus* (MRSA). The detection of MRS in veterinary units within different countries (Lilenbaum et al., 1998; Gortel et al., 1999; van Duijkeren et al., 2004a; Weese et al., 2004), as well as the likelihood of MRSA transmission between animals and man (van Duijkeren et al., 2004b; Juhász-Kaszanyitzky et al., 2007), have demonstrated the importance of monitoring the presence of MRS and the level of antimicrobial resistance in animals. Prevalence studies focused on different environments are necessary to enhance the big picture of MRS epidemiology; thus, this study aimed to determine the prevalence of MRS in healthy animals and the people associated with them (researchers and farm workers) at a farm that is used for teaching and researching activities (located in the Faculty of Agrarian Sciences and Veterinary Medicine of the São Paulo State University, in Jaboticabal-SP, Brazil) and further characterize the antimicrobial resistance patterns in the isolates.

Materials and Methods

In total, 161 samples were collected from June 2008 to March 2009 (36 from dairy cattle, 26 from beef cattle, 19 from sheep, 21 from horses, 23 from pigs, 23 from goats and 13 from human beings). This study was approved by the São Paulo State University Ethics committee. All samples were collected from the nostrils of clinically healthy animals and people using sterile cotton swabs. The swabs were immediately placed in tubes containing an enrichment broth (brain heart infusion supplemented with NaCl – 6%) and incubated for 48 h at 35°C. After incubation, 10 μl of each tube was inoculated and streaked onto Manitol salt agar with oxacyllin (2 μg/ml). The agar plates were then incubated for 48 h at 35°C. Two visually typical *Staphylococci* colonies were picked from each plate and inoculated in BHI agar slants. To identify samples that were of the *Staphylococcus* genus, the isolates were submitted to the following tests: gram staining, catalase and modified oxidase. Isolates of the genus *Staphylococcus* were indentified either as coagulase-positive *Staphylococci* (CoPS) or coagulase-negative *Staphylococci* (CNS) through the tube coagulase test with rabbit plasma. All *Staphylococci* isolated were submitted to a thermal lyses procedure in order to obtain the DNA template. Detection of gene *mec*A was done by PCR (Gortel et al., 1999). In cases in which both isolates from the same carrier were *mec*A positive, only one isolate was used in further procedures.

MRS isolates were submitted to antimicrobial resistance testing by the disk-diffusion method (Kirky-Bauer). The following antimicrobials were tested: penicillin (10 UI), meropenem (10 μg), ceftriaxone (30 μg), cephalothin (30 μg), oxacillin (1 μg), levofloxacin (5 μg), enrofloxacin (5 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), gentamicin (10 μg), clindamycin (2 μg), erytromycin (15 μg), linezolid (30 μg), sulfamethoxazole/trimethoprim (25 μg), tetracycline (30 μg), doxycycline (30 μg) and vancomycin (30 μg), and the corresponding breakpoints were obtained using human and veterinary guidelines (Clinical and Laboratory Standards Institute, 2005, 2008). Inducible resistance to clindamycin was tested by the 'D zone' test (CLSI, 2008).

Results

Four *Staphylococci* were isolated that contained the gene *mec*A by PCR. All four were coagulase negative and thus methicillin-resistant coagulase-negative *Staphylococci* (MRCoNS). These isolates were obtained exclusively from the 13 human beings tested, demonstrating a prevalence of MRCoNS of 31% in the humans and 0% in the animals. Table 1 shows the resistance patterns of the MRCoNS isolates. None of the MRCoNS showed inducible resistance to clindamycin by the 'D zone' test. No MRSA were isolated.

Discussion

The lack of MRSA isolation from the animals and human beings tested in this study should be evaluated carefully and does not prove the absence of MRSA in the entire population at this site. However, the lack of MRSA in this study and in another performed in the veterinary teaching hospital of the same university (Maluta et al., 2010) supports a general trend of MRSA absence at this site.

The prevalence of MRCoNS in humans at this farm (31%) was similar to the human prevalence in the veterinary hospital cited above (36%). We hypothesize whether this similar prevalence in both cases indicates that the humans were contaminated in the community. These prevalences were lower than those found in three equine facilities in Denmark (63%) (Moodley and Guardabassi, 2009), indicating that different degrees of selective

Table 1. Resistance patterns of four methicillin-resistant coagulase-negative *Staphylococci* isolated from human beings and local of sampling*

Isolate ID	Site of sampling	OXA*,†	ERI†	CLI†	GEN†	LEV‡	ENR§	CIP‡	CLO†	LNZ‡	SUT†	TT†	DOX‡	VAN†
S107	Pig sector	R	R	R	S	S	I	S	R	S	R	R	R	S
S133	Goat sector	R	I	S	R	S	S	S	S	S	S	S	S	S
S160	Beef cattle sector	R	S	S	R	S	S	S	S	S	R	S	S	S
S161	Beef cattle sector	R	R	S	R	S	S	S	R	S	R	I	S	S

PEN, Penicillin; OXA, oxacillin; ERI, erytromycin; CLI, clindamycin; GEN, gentamicin; LEV, levofloxacin; ENR, enrofloxacin; CIP, ciprofloxacin; CLO, chloramphenicol; LNZ, linezolid; SUT, sulfamethoxazole/trimethoprim; TT, tetracyclin; DOX, doxycycline; VAN, vancomycin; R, resistant; I, intermediary; S, susceptible.

* Isolates resistant to oxacillin (methicillin) are to be reported as resistant to all beta-lactams antibiotics (Clinical and Laboratory Standards Institute, 2008) therefore the four isolates were considered resistant to penicillin, meropenem, ceftriaxone and cephalothin

†Breakpoints were attributed according to CLSI (2005, 2008). The results were the same using both guidelines.

‡Breakpoints were attributed according to CLSI (2005).

§Breakpoints were attributed according to CLSI (2008).

pressure in the community may have contributed to the differing prevalence at each site.

Absence of MRCoNS found in the animals in this study is corroborated by the absence of MRCoNS in food animals reported by Bagcigil et al., 2007; but is contradicted by their prevalence in horses found in other works, ranging from 42% to 82% (Vengust et al., 2006; Bagcigil et al., 2007; Moodley and Guardabassi, 2009). This discrepancy may be explained by the low (or even absent) selective pressure from antimicrobials on the *Staphylococci* population of animals in this work because they were healthy and thus not routinely exposed to antimicrobials.

The presence of MRCoNS in humans and their absence in animals (it is important to note that the number of humans beings tested was 13 while the number of animals tested was 148, and thus one would expect to find more MRCoNS in animals, since more animals were tested) raises an interesting question because cross-transmission could be expected. Because only two MRCoNS species (*S. vitulinus* and *S. haemolyticus*) were shown to be shared between animals and humans in a previous work (Moodley and Guardabassi, 2009), the host specificity of CNS species could hamper human to animal transmission of MRCoNS, explaining the zero prevalence in animals in this work.

Although, in general, MRS are resistant to several antimicrobials, the isolates of this work were not, except for the strain S107 (resistant to eight antimicrobials). All isolates presented different antimicrobial resistance patterns, to a lesser or greater degree (Table 1). The different resistance patterns indicate that the MRCoNS were not shared between humans.

In conclusion, MRCoNS may be present in humans associated with animals but not present in the animals. MRCoNS colonization may not correlate between humans and the animals associated with them. Selective pressure outside of the farm and a lack of MRCoNS transmission between humans and animals may be responsible for this lack of correlation.

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