

Full Length Research Paper

Prevalence and antimicrobial resistance patterns of methicillin-resistant staphylococci (MRS) isolated in a Veterinary Teaching Hospital in Brazil

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We investigated the prevalence of methicillin-resistant staphylococci (MRS) in humans and dogs and evaluated the antimicrobial resistance patterns of these bacteria at a Veterinary Teaching Hospital. Specimens from 50 human subjects and 50 dogs were studied. Isolates were identified by Gram-staining, biochemical reactivity and resistance to antimicrobials. While no isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) or methicillin-resistant *Staphylococcus intermedius* (MRSI) were isolated, two (4%) methicillin-resistant coagulase-negative staphylococci (MRCoNS) were isolated from dogs and 18 (36%) were isolated from humans. The percentage of MRCoNS isolates resistant to penicillin (100%), ciprofloxacin (30%), gentamicin (40%), clindamycin (25%), erythromycin (70%), trimethoprim-sulfamethoxazole (20%) or vancomycin (0%) was evaluated. The absence of MRS isolates resistant to vancomycin is of interest because this antimicrobial may be used as an important therapeutic alternative in cases of MRSA infections. Surveillance programs aimed against MRS should therefore be stimulated in veterinary health units.

Key words: Dogs, MRS, nosocomial, veterinary staff.

INTRODUCTION

Methicillin-resistant staphylococci (MRS) are important nosocomial pathogens frequently found to be resistant to various antimicrobials. Resistance to methicillin is due to the presence of the gene *mecA*, which encodes a penicillin-binding protein (PBP2a) (NCCLS, 2003; Martins and Cunha, 2007). Isolates of *Staphylococcus aureus* resistant to methicillin (MRSA) have been studied for decades in human health units and over the last years have been isolated from veterinary health units in Canada (Weese et al., 2004, 2006), the USA (Seguin et al., 1999; Middleton et al., 2005; O'Mahony et al., 2005), England (Baptiste et al., 2005; Loeffler et al., 2005) and Austria (Cuny et al., 2006). MRSA has also been isolated from clinically normal cats in Brazil (Lilembaum et al., 1998, 1999). Methicillin-resistant coagulase-negative

staphylococci (MRCoNS) and methicillin-resistant *Staphylococcus intermedius* (MRSI) were isolated from healthy dogs and horses in a community in Slovenia (Vengust et al., 2006). MRCoNS strains were also isolated from dogs and horses in Denmark (Bagcigil et al., 2007).

The transmission of methicillin-resistant staphylococci among men and animals probably does occur (Seguin et al., 1999; Manian, 2003; van Duijkeren et al., 2004a; Baptiste et al., 2005; Weese et al., 2006). MRSA isolates have been found from veterinary staff members at a veterinarian conference in the USA (Hanselman et al., 2006). In the Netherlands, a study reported that veterinarians presented a higher risk of MRSA carriage (Wulf et al., 2006). Such observations denote that MRSA colonization could become an occupational risk factor to veterinary professionals, as previously suggested (Hanselman et al., 2006).

Although *S. intermedius* has been historically ranked as the major coagulase-positive species in dogs, recent

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studies refer to the presence of *S. aureus* isolates (particularly MRSA) in these animals (van Duijkeren et al., 2004b; Baptiste et al., 2005; O'Mahony et al., 2005; Weese et al., 2006).

In the last decade, the presence of MRSA has increased in human communities and it appears to reflect in animals, this fact could increase the chance of the occurrence of diseases affecting both animals and humans, making the control of this pathogen difficult (Weese et al., 2006). Dogs may act as MRSA reservoirs, leading to an animal and public health problem (Baptiste et al., 2005; Weese et al., 2006).

Cephalosporins have been widely used in humans and by veterinary professionals; however, MRS isolates are resistant to these drugs and are usually resistant to other antimicrobials, making the treatment of such infections due to such nosocomial originated microorganisms difficult (NCCLS, 2003). Vancomycin has often been a usual choice to combat these infections (Martins and Cunha, 2007).

Poor information about MRS prevalence in veterinary health units in South America stimulated us to perform this work, which focused on the prevalence of methicillin-resistant staphylococci and the evaluation of their resistance to several antimicrobials, including vancomycin.

MATERIALS AND METHODS

Population study

Fifty dogs and 50 human subjects (students, staff members and veterinarians) from a small animal division of a veterinary teaching hospital in the city of Jaboticabal, São Paulo State, Brazil, were studied.

The dogs used in this study had been kenneled and belonged to the hospital; these animals were kept in 8 kennels (kennel G, kennel F, kennel E, kennel N, kennel PGO, kennel PM, kennel PMR and kennel PA). All animals remained in individual facilities and had no visible signs of staphylococcal infections, except one that exhibited nasal secretions. People involved in this research have worked or studied at the hospital and had been in frequent contact with these animals.

These samples represented approximately 95 and 90% of the population of dogs and human subjects of that hospital, respectively. The São Paulo State University Animal Experimentation Ethics Committee approved this study.

Sample collection

Samples were collected during one single day using a sterile cotton swab rubbed inside the nostrils and another sterile cotton swab rubbed on the nails, palms and between the fingers of both hands of each person. The same procedures were used on dogs, but instead of the paws, the perineum (hair and skin) was sampled (Vengust et al., 2006). Therefore, 100 samples from dogs and 100 samples from humans were collected. All samples were studied individually. Swabs were placed in tubes containing Brain Heart Infusion (BHI) (Oxoid, Cambridge, England) with 6% of NaCl (used as enrichment medium) and immediately processed.

Isolation and identification

Each tube containing enrichment media and swab was incubated aerobically at 35°C for 48 h and its contents were then placed on *Staphylococcus* 110 medium (Difco, Michigan, USA) and further incubated aerobically at 35°C for 48 h. Suspected colonies (three per plate) were collected and identified by their Gram-stained appearance, bacitracin and furazolidon resistance (Disk Diffusion) and their reaction to catalase (identification to genus level). The carbohydrate fermentation (maltose, mannitol, manose, raffinose, sucrose, trehalose and xylose), DNase presence, acetoin production (VP), tube coagulase using rabbit plasma and clumping factor tests lead to the identification to species level. Identification tests were performed as previously described (Mac Faddin, 1976; Koneman et al., 1997), and isolates were classified (Holt et al., 1994; Bannerman, 2003). Staphylococci isolated from the same human or canine carrier were differentiated by their biochemical characteristics and resistance patterns to various antimicrobials.

Antimicrobial susceptibility

All obtained isolates of staphylococci were submitted to a disk diffusion test against the following antimicrobials (Cefar, São Paulo, Brazil) using Muller-Hinton agar (Oxoid, Cambridge, England): penicillin (10 UI), oxacillin (1 µg), ciprofloxacin (5 µg), gentamicin (10 µg), clindamycin (2 µg), erythromycin (15 µg) sulfamethoxazole + trimethoprim (25 µg) and vancomycin (30 µg) (CLSI 2009). Additionally, a minimum inhibitory concentration (MIC) test using a broth macrodilution method was performed with all staphylococci isolates to assess resistance to vancomycin (NCCLS, 2003). The results from the disk diffusion and MIC tests were compared to standard guidelines (CLSI 2009). A PCR assay for the detection of a 533 bp fragment of the *mecA* gene was performed to assess methicillin-resistance of all staphylococci isolated (Gortel et al., 1999).

RESULTS

Ninety staphylococci isolates were found in 50 humans (59 from nostrils and 31 from skin) and 53 (34 from nostrils and 19 from skin) were found in 50 dogs. Table 1 demonstrates the percentages of different antimicrobial-resistant isolates. The only isolate of the only dog that had purulent secretion on its nostrils was classified as methicillin-susceptible *S. intermedius* (MSSI).

By PCR, 20 staphylococci isolates showing the presence of *mecA*, all coagulase-negative (MRCoNS) isolates, were found in 2/50 (4%) dogs and 18/50 (36%) in human beings. All MRS isolates were resistant to oxacillin by disk diffusion, except one, but this isolate was classified as MRS because *mecA* was detected by PCR. Using MIC, no MRS isolates were considered to be resistant to vancomycin. Table 2 shows the antimicrobial resistances of MRS isolates from dogs and humans.

DISCUSSION

The absence of MRSA isolates in humans and dogs in this studied veterinary hospital is of epidemiological importance since MRSA are harmful to public health,

Table 1. Percentage of resistant staphylococci isolated in a veterinary teaching hospital^a.

Source	Isolates (n)	Percentage of resistance						
		ERI	CLI	GEN	CIP	SUT	PEN	VAN
		%	%	%	%	%	%	%
Dogs	53	21	28	2	2	8	68	0
MSSI ^b	6	17	0	0	0	0	17	0
MSCoNS ^c	45	20	31	0	2	7	73	0
MRCoNS ^d	2	50	50	50	0	50	100	0
Humans	90	66	18	16	10	18	90	0
MSSA ^e	1	0	0	0	0	0	100	0
MSCoNS	71	65	17	10	4	18	87	0
MRCoNS	18	72	22	39	33	17	100	0

a. Erythromycin (ERI), Clindamycin (CLI), Gentamicin (GEN), Ciprofloxacin (CIP), Sulfamethoxazole + trimethoprim (SUT), Penicillin (PEN), Vancomycin (VAN), b Methicillin-susceptible *Staphylococcus intermedius*, c Methicillin-susceptible coagulase-negative staphylococci, d Methicillin-resistant coagulase-negative staphylococci, e Methicillin-susceptible *Staphylococcus aureus*.

Table 2. Identification and antimicrobial resistance patterns of 20 isolates of methicillin-resistant staphylococci^{a, b}.

No	Species	Source	Site	ERI	CLI	GEN	CIP	SUT	PEN	VAN	OXA
1	<i>S. epidermidis</i>	Dog (Kennel PGO)	Skin	R	S	R	S	R	R	S	S
2	<i>S. simulans</i>	Dog (Kennel PA)	Skin	I	R	S	S	S	R	S	R
3	<i>S. epidermidis</i>	Human	Nostrils	R	R	R	R	R	R	S	R
4	<i>S. epidermidis</i>	Human	Nostrils	R	R	R	R	S	R	S	R
5	<i>S. epidermidis</i>	Human	Nostrils	R	R	R	I	S	R	S	R
6	<i>S. epidermidis</i>	Human	Nostrils	I	I	R	R	R	R	S	R
7	<i>S. epidermidis</i>	Human	Nostrils	R	R	I	R	S	R	S	R
8	<i>S. epidermidis</i>	Human	Nostrils	R	I	S	R	S	R	S	R
9	<i>S. epidermidis</i>	Human	Nostrils	R	I	R	S	S	R	S	R
10	<i>S. epidermidis</i>	Human	Nostrils	R	I	R	S	S	R	S	R
11	<i>S. epidermidis</i>	Human	Nostrils	R	I	R	I	S	R	S	R
12	<i>S. epidermidis</i>	Human	Nostrils	R	I	S	R	S	R	S	R
13	<i>S. epidermidis</i>	Human	Nostrils	R	S	S	S	S	R	S	R
14	<i>S. epidermidis</i>	Human	Nostrils	R	I	S	S	S	R	S	R
15	<i>S. epidermidis</i>	Human	Nostrils	I	I	S	S	S	R	S	R
16	<i>S. epidermidis</i>	Human	Nostrils	I	I	I	S	S	R	S	R
17	<i>S. epidermidis</i>	Human	Nostrils	I	I	S	S	S	R	S	R
18	<i>S. epidermidis</i>	Human	Skin	R	I	I	I	S	R	S	R
19	<i>S. epidermidis</i>	Human	Skin	R	I	S	S	R	R	S	R
20	<i>S. epidermidis</i>	Human	Skin	I	S	S	S	S	R	S	R
Resistant isolates		(%)		70	25	40	30	20	100	-	95
Intermediary isolates		(%)		30	60	15	15	-	-	-	-
Susceptible isolates		(%)		-	15	45	55	80	-	100	5

^aErythromycin (ERI), Clindamycin (CLI), Gentamicin (GEN), Ciprofloxacin (CIP), Sulfamethoxazole + trimethoprim (SUT), Penicillin (PEN), Vancomycin (VAN) and Oxacillin (OXA), ^b R - resistant, I - intermediary, S - susceptible.

however, further studies on this subject should be performed at other veterinary units in Brazil. Furthermore, this work was performed in a single day, so it is possible that a MRSA carrier (dog or human) had left the hospital before that day or arrived after that day. Other animals

(cats) not studied but that were present at the hospital that day could be MRSA carriers, so it is not impossible that this studied veterinary hospital could have a MRSA prevalence different than zero. MRSA have been isolated from the skin of cats in Brazil (Lilembaum, 1998),

therefore, pets can act as MRSA carriers in this country in the same way it that has been demonstrated in several other countries.

The absence of MRSA in the dogs could be observed in animals without apparent staphylococcal infections, compared with results that have isolated MRSA from clinical specimens in other studies (Gortel et al., 1999; van Duijkeren et al., 2004b). MRSA were not found in healthy community dogs in Slovenia (Vengust et al., 2006), in two other studies, MRSA were not found in dogs that had visited veterinary hospitals (but were not clinical specimens) (Baptiste et al., 2005; Bagcigil et al., 2007). These results demonstrate that it is probably easier to isolate MRSA from clinical specimens than from colonizable sites (skin and nostrils) of dogs.

In this study, the MRCoNS prevalence in dogs (4%) was similar to that of MRS in clinical specimens from several animals (3.2%) (van Duijkeren et al., 2004b) and was also similar to the prevalence of MRCoNS found in dogs in a veterinary hospital in England (6%) (Baptiste et al., 2005), but differed from that of MRCoNS isolated from healthy dogs in Slovenia (11.5%) (Vengust et al., 2006) and in dogs at a veterinary hospital in Denmark (13%) (Bagcigil et al., 2007). It seems that this prevalence may vary among dogs from different countries.

Vancomycin-resistant isolates were not found in this study or in other studies of clinical specimens from dogs (Gortel et al., 1999) and many other animals (van Duijkeren et al., 2004b). This is valuable information because vancomycin has been a usual choice for the treatment of infections due to MRSA.

Except vancomycin, the drug that expressed the lowest resistance among all MRS was sulfamethoxazole-trimethoprim (20%), which is lower than that found in another study performed only with isolates from clinical specimens of dogs (39%) (Gortel et al., 1999). This drug has been an alternative for the treatment of infections due to MRSA. Thus, this result enhances the importance of surveillance of the level of resistance of sulfamethoxazole-trimethoprim. The intermediary susceptibility level to ciprofloxacin among MRS in this study (45%) was similar to that from another study performed with clinical specimens from dogs (57%) (Gortel et al., 1999).

Multiresistant MRCoNS are not unexpected findings since they have been reported in animals elsewhere (van Duijkeren et al., 2004b). This occurrence suggests that we need to consider that use of several antimicrobials might facilitate multiresistant MRCoNS selection. Since coagulase-negative staphylococci have been the most prevalent etiologic agents of central venous, catheter-related bloodstream infections in human hospitals (Casey et al., 2006), possible transmission of multiresistant MRCoNS from dogs to humans could be harmful. Moreover *S. epidermidis* may be associated with pathogenicity factors (biofilms, hemolysins, lipases and proteases) (Michelim et al., 2005).

MRCoNS could originate in the veterinary hospital itself by selective pressure or be brought to it from the community or human hospitals. Several studies have shown that some MRSA isolates from dogs and humans in veterinary health units are genetically similar to the most prevalent epidemic isolates from humans in the UK (EMRSA-15) (Baptiste et al., 2005; O'Mahony et al., 2005), suggesting that they have been brought from human hospitals or are genetically different from them, indicating that they have been acquired from the community (Middleton et al., 2005). However, it is not known whether this could be confirmed about MRCoNS.

Surveillance programs focused on nosocomial infectious agents have existed for many years in human hospitals, they should also be performed in veterinary hospital units because the emergence of multiresistant bacteria in this environment may become a risk to public and animal health.

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