



RESEARCH ARTICLE

Methicillin resistant *Staphylococcus aureus* (MRSA) colonization at clinics of a Faculty of Veterinary Medicine

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Öz

Öztürk D, Türütoğlu H, Şahan Ö. Bir veteriner fakültesinin kliniklerinde metisiline dirençli *Staphylococcus aureus* (MRSA) kolonizasyonu.

Abstract

Ozturk D, Turutoglu H, Sahan O. Methicillin resistant *Staphylococcus aureus* (MRSA) colonization at clinics of a faculty of veterinary medicine.

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Amaç: Metisiline dirençli *S. aureus* (MRSA), birçok antibiyotiğe direnç nedeniyle, insan ve veteriner hekimliğinde enfeksiyonların tedavisinde güçlüklereden neden olan önemli bir zoonotik ajandır. Bu çalışmada, bir veteriner fakültesinin kliniklerinde çalışan veteriner hekimler, personel ve öğrencilerin el ve burun mukozaları ile klinik ortamından alınan sıvap örneklerinde MRSA kolonizasyonu araştırıldı.

Gereç ve Yöntem: Bu amaçla, 16 veteriner hekim, 2 klinik personeli ve 17 öğrencinin herbirinden alınan dört (iki el ve burun mukozası) ve klinikte el ile temasının sık olduğu düşünülen 41 farklı çevresel yüzeyden sıvap örnekleri toplandı.

Bulgular: Yüz on sekiz *S. aureus* izolatının 75'i (%63.6; 34 veteriner hekim, 8 personel, 24 öğrenci, 9 çevresel yüzey) disk difüzyon testi ile metisiline fenotipik olarak dirençli bulundu. On dört veteriner hekim ve 7 öğrenciden alınan örneklerin en az birinde metisiline fenotipik dirençli *S. aureus* taşıyıcılığı belirlenirken, iki personelden alınan örneklerin tümünde metisiline fenotipik dirençli *S. aureus* tespit edildi. Veteriner hekimler (13 izolat), personel (1 izolat) ve öğrencilerden (10 izolat) izole edilen 24 (%20.3) *S. aureus* izolatında, metisiline direnci kodlayan *mecA* geni pozitif bulunurken, çevresel yüzeylerden izole edilemedi.

Öneri: MRSA kolonizasyonunun klinikte çalışan insanlar ve öğrencilerde yüksek olduğu, insanlar ve hayvanlar arasındaki geçiş riski nedeniyle sanitasyon önlemlerinin, özellikle de personel hijyeninin gerekli olduğu kanısına varıldı.

Anahtar kelimeler: Kolonizasyon, MRSA, *Staphylococcus aureus*, veteriner klinikleri

Aim: Methicillin resistant *Staphylococcus aureus* (MRSA) is an important zoonotic agent causing difficulties in treatment of the infections in human and veterinary medicine, due to multiple antibiotic resistances. In this study, the colonization of MRSA was investigated by the swabbing samples from the skin and nasal mucosa of veterinarians, personnel and students and from the environment at clinics of a Faculty of Veterinary Medicine.

Materials and Methods: For this purpose, samples were obtained from 16 veterinarians, 2 clinic personnel and 17 students that each was sampled 4 times and from 41 different environmental surfaces thought to be common sites of hand contact.

Results: Out of 118 *S. aureus* isolates, 75 (63.6%; 34 veterinarians, 8 personnel, 24 students and 9 environmental surfaces) were found phenotypically resistant to methicillin by a disk diffusion test. All samples taking from two personnel were colonized with phenotypic resistant *S. aureus*, while the 14 veterinarians and 7 students sampled were carried the phenotypic resistant *S. aureus* at least to one of samples. Although 24 (20.3%) *S. aureus* isolated from veterinarians (13 isolates), personnel (1 isolate) and students (10 isolates) were determined to be positive for *mecA* gene encoded resistance to methicillin, *mecA*-positive isolates can not be isolated from environmental surfaces at clinics.

Conclusions: It has been determined that MRSA colonization has high in working people and students at the clinic, thus standard sanitation measures, particularly personnel hygiene, are required because of the risk of transmission between humans and animals.

Key words: Colonization, MRSA, *Staphylococcus aureus*, veterinary clinics





Introduction

It is considered that *Staphylococcus aureus* is a party of normal flora on the skin andosa of human and can commonly be founded in animals (Kaszanyitzky et al 2003). Up to 30% of human are colonized with *S. aureus* on the skin and in the nasal mucosa, but only minorities of these *S. aureus* are methicillin resistant (Eiff et al 2001, Weigelt 2008). Methicillin resistant *S. aureus* (MRSA) is one of the important pathogens of nosocomial infections of humans in the world and leads to difficulty in treatment of the infections due to multiple antibiotic resistances (Moodley et al 2006, Weese et al 2006). For many years, although MRSA was considered only a human pathogen, it was also described in domestic animals such as cat, dog, cattle, horse and pigs (Seguin et al 1999, Manian 2003, Duijkeren et al 2004, Duquette and Nuttall 2004, Loeffler et al 2005, Kaszanyitzky et al 2007).

MRSA carriage in veterinarians, veterinary staff, environmental surface, pet animals and their owners were reported by researchers (Manian 2003, Duijkeren et al 2004, Weese et al 2004, O'Mahony et al 2005, Moodley et al 2006, Kaszanyitzky et al 2007). The isolation of the same MRSA strains from domestic animals and their owners has indicated the possibility of transmission of the agent from humans to animals or vice versa (Manian 2003, Duquette and Nuttall 2004, Loeffler et al 2005, Moodley et al 2006). Also, it has been stated that veterinarians, veterinary staff and students contacted to infected or colonized animals with MRSA may be high risk groups for MRSA carriage (Weese et al 2004, Anderson et al 2008, Wulf et al 2008). The transmission of MRSA between animals and humans is not only via contact with people or animals infected or colonized with MRSA, but also possible when an animal or human comes into contact with objects including door handles, floors, gloves, gowns, the male clients, toilets, marker pens on the ultrasound booking, computer terminals, intravenous catheters, bloodstreams, muzzles and overbed tables (Weese et al 2004, Loeffler et al 2005, Moodley et al 2006).

The aim of this study was to evaluate the skin and nasal mucosa colonization of MRSA in veterinarians, personnel and students and also environmental surfaces of clinics in a Faculty of Veterinary Medicine.

Materials and Methods

Samples

Swabbing samples were collected from both the nostrils and hands of 16 veterinarians, 2 clinic personnel, 17 veterinary students and 41 different environmental surfaces of clinics such as door handles, operation tables, microscop switches, thermometers, lamb switches, boxes, rontgen apparatus, PC, dressing boxes, tables, soap dispensers, taps and muzzles

presumed to be common sites of hand contact at clinics of a Faculty of Veterinary Medicine (Burdur, Turkey). The sampling was randomly performed without informing the veterinarians, clinic personnel and students, and samples were collected with sterile gloves by individuals who work in microbiology laboratory. Four samples were taken from each individual. The samples were taken from the median septum mucosa of both nostrils and from the skin in the interval of fingers of both hands of the person. A dry cotton wool sterile swap was wetted with saline water and then was touched the places mentioned above. Swabs in sterile tubes were cooled and immediately transported to the laboratory.

Isolation of *S. aureus*

All of the swab samples were streaked on blood agar containing 5% sheep blood (Oxoid Ltd, Hampshire, England) and incubated at 37°C for 24 hours. The colonies were identified according to conventional methods such as Gram staining, catalase, coagulase, haemolysis, clumping factor, DNase, anaerobic fermentation of mannitol and Voges Proskauer reaction (acetoin production) (Winn et al 2006).

Phenotypic methicillin resistance of *S. aureus* isolates

Phenotypic methicillin resistance of *S. aureus* isolates were determined by disk diffusion methods according to NCCLS (2003). Ten colonies were suspended in sterile saline water in a density equal to McFarland Opacity Standart No. 0.5. The bacterial suspension was inoculated on Muller Hinton agar (Oxoid) containing 2% NaCl. The oxacillin disk (1 µg, Oxoid) was placed on the agar and plate was incubated aerobically at 35°C for 24 h. The inhibition zone diameter was recorded as susceptible (≥ 13 mm), intermediate susceptible (11-12 mm) and resistant (≤ 10 mm) according to NCCLS (2003). *MecA*-positive *S. aureus* 27R (Department of Microbiology and Clinical Microbiology, Faculty of Medicine, Hacettepe University, Ankara, Turkey) and *mecA*-negative *S. aureus* ATCC 25923 (Department of Microbiology, Faculty of Veterinary Medicine, Mehmet Akif Ersoy University, Burdur, Turkey) were used as control strains for antimicrobial susceptibility tests.

Detection of *mecA* gene

S. aureus isolates were investigated for the presence of *mecA* gene encoded methicillin resistance by PCR. Primers (F-CCTAGTAAAGCTCCGGAA and R-CTAGTCCATTCGGTCCA) for *mecA* were selected from published sequences (Choi et al 2003). PCR was performed in a 25 µL reaction mixture containing 5 µL DNA, 12.5 µL 2XPCR mastermix (Applied Biosystem, Roche, USA), 1 µL primerF (100 pmol), 1 µL primerR (100 pmol) and 5.5 µL ddH₂O. The amplification was performed on a thermal cycler (CLP, ATC401, USA) and PCR products (10 µL) were electrophoresed in a 1.5% agarose gel



at 100V for 45 min. *S. aureus* 27R and ATCC 25923 strains were used as control in PCR assay.

Results

Isolation rate of *S. aureus* isolates

S. aureus was isolated from 118 (65.2%) of 181 samples (64 samples from 16 veterinarians, 8 samples from 2 personnel, 68 samples from 17 veterinary students and 41 samples from 8 different environmental surfaces of clinics). These isolates were obtained from veterinarians (45 isolates), personnel (8 isolates), students (54 isolates) and environmental surfaces (11 isolates). *S. aureus* was isolated from both nostrils of 2 personnel, 13 veterinarians and 14 students. For *S. aureus*

carriage on both hands, 6 veterinarians, 2 personnel and 10 students were positive. Of samples from 41 environmental surfaces, 11 were contaminated with *S. aureus*. The high rate of *S. aureus* isolation among the environmental surfaces was found in soap dispensers (66.7%), consultation tables (60%) and taps (50%) of the clinic (Table 1).

Phenotypic and genotypic methicillin resistance of *S. aureus* isolates

Out of 118 *S. aureus* isolates, 75 (63.6%; 34 veterinarians, 8 persons, 24 students and 9 environmental surfaces) were found phenotypically resistant to methicillin by a disk diffusion test. While all samples taking from two personnel were colonized with phenotypic resistant *S. aureus*, 14 veterina-

Table 1. The presence of *mecA* gene and phenotypic methicillin resistance in *S. aureus* isolates from veterinarians, personnels, students and environmental surfaces at clinics of a Faculty of Veterinary Medicine.

| Samples | <i>S. aureus</i> positive % (Positive samples/samples) | Disk diffusion test for <i>S. aureus</i> (Oxacillin, 1 µg) | | | PCR for <i>S. aureus</i> | |
|---------------------------------------|---|---|---|---|-----------------------------|---------------|
| | | R | I | S | <i>mecA</i> + | <i>mecA</i> - |
| Veterinarians (n:16) | | | | | | |
| Right nostrils | 93.8 (15/16) | 14 | 0 | 1 | 5 | 10 |
| Left nostrils | 81.2 (13/16) | 10 | 1 | 2 | 5 | 8 |
| Right hands | 62.5 (10/16) | 5 | 0 | 5 | 2 | 8 |
| Left hands | 43.8 (7/16) | 5 | 0 | 2 | 1 | 6 |
| Veterinary clinic staff (n: 2) | | | | | | |
| Right nostrils | 100 (2/2) | 2 | 0 | 0 | 0 | 2 |
| Left nostrils | 100 (2/2) | 2 | 0 | 0 | 1 | 1 |
| Right hands | 100 (2/2) | 2 | 0 | 0 | 0 | 2 |
| Left hands | 100 (2/2) | 2 | 0 | 0 | 0 | 2 |
| Veterinary students (n:17) | | | | | | |
| Right nostrils | 88.2 (15/17) | 6 | 1 | 8 | 3 | 12 |
| Left nostrils | 88.2 (15/17) | 7 | 0 | 8 | 4 | 11 |
| Right hands | 64.7 (11/17) | 4 | 0 | 7 | 1 | 10 |
| Left hands | 76.5 (13/17) | 7 | 0 | 6 | 2 | 11 |
| Environmental surfaces (n:41) | | | | | | |
| Door handles | 14.3 (1/7) | 1 | 0 | 0 | 0 | 1 |
| Consultation tables | 60.0 (3/5) | 3 | 0 | 0 | 0 | 3 |
| Cabinet handles | 0 (0/5) | 0 | 0 | 0 | 0 | 0 |
| Soap dispensers | 66.7 (2/3) | 1 | 1 | 0 | 0 | 2 |
| Operation equipment | 25 (1/4) | 1 | 0 | 0 | 0 | 1 |
| Computer terminals | 100 (1/1) | 1 | 0 | 0 | 0 | 1 |
| Clinic equipment | 14.3 (2/14) | 2 | 0 | 0 | 0 | 2 |
| Taps | 50 (1/2) | 0 | 0 | 1 | 0 | 1 |



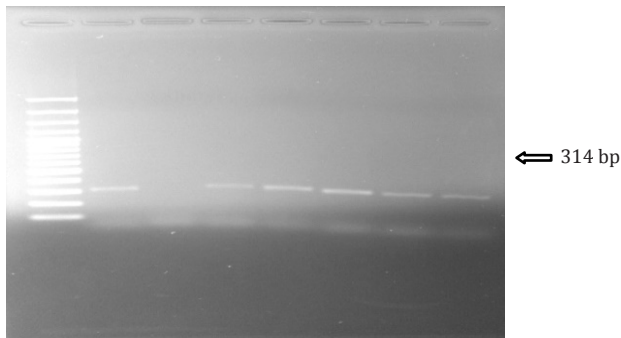


Figure 1. PCR results (1-Marker (100bp), 2- *S. aureus* 27R (*mecA*+), 3- *S. aureus* 25923 (*mecA*-), 4, 5, 6, 7, 8-*S. aureus* isolates (from veterinarians).

rians and 7 students sampled were carried the phenotypic resistant *S. aureus* at least to one of samples.

Although 24 (20.3%) *S. aureus* isolated from veterinarians (13 isolates), personnel (1 isolate) and students (10 isolates) were determined to be positive for *mecA* gene encoded resistance to methicillin (Figure 1), *mecA*-positive isolates can not be isolated from environmental surfaces of clinics. While *mecA*-positive *S. aureus* isolates were found on the only one hand of 3 veterinarians and 3 students, they were detected in both nostrils of 3 veterinarians and 2 students, in only one nostril of the others (Table 1).

Discussion

Approximately, 20-60% of the human may carry *S. aureus* in their anterior nares and the nasal *S. aureus* carriers have a risk higher than non carriers (Weigelt 2008, Loeffler et al 2010). In this study, *S. aureus* was isolated more from nostrils than from hands of the veterinarians, personnel and students. Colonization of *S. aureus* on the hands of personnel and students was determined to be higher than veterinarians. We thought that this may be originated from the poor hand hygiene and hand to face contact and careless contact with animals. Veterinarians generally handle animals using gloves and with attention to hand hygiene. But, the personnel and students may be rather careless about using gloves and hand hygiene in their interaction with animals.

Although MRSA was considered one of the important pathogens of humans, there were a lot of studies that MRSA carriage and infection in animals was notified (Seguin et al 1999, Manian 2003, Duquette and Nuttall 2004, Duijkeren et al 2004, Loeffler et al 2005, Kaszanyitzky et al 2007). It was stated that MRSA may be transmitted to animals by the hands of colonized or infected persons (Manian 2003, Duijkeren et al 2004, Loeffler et al 2010). Moodley et al (2006) reported that MRSA isolates from veterinary staff were found similar to those isolated from infected animals. Baptiste et al (2005) stated that MRSA isolated from a dog was found the same strain as MRSA isolated from 2 veterinary staff and a student who has contact with this dog; and a few months

later, the same MRSA strain was isolated from two different dogs. In this study, the majority of people working in clinic were colonized with phenotypically or genotypically resistant *S. aureus*. Therefore, we thought that veterinarians, staff and students colonized with MRSA may be high risk groups in transmission of the agent to animals (Weese et al 2004, Anderson et al 2008, Wulf et al 2008).

It has been reported that MRSA carriage is less than 1% in population, up to 5-10% in healthcare workers and higher than 10% in veterinary staff (Loeffler et al 2005, Moodley et al 2006, Weese et al 2006, Wulf et al 2008, Loeffler et al 2010, Martino et al 2010). Wulf et al (2008) reported that MRSA carriage is 12.5% in the nostrils of veterinarians who have contact with pig farmers in the Netherlands. Similarly, MRSA carriage has been found in 17% of veterinarians and 18% of technicians attending a veterinary surgery conference in California (Burstiner et al 2010); 4.4% small animal personnel and 15.6% of equine personnel attending a veterinary internal medicine conference in the USA (Hanselmann et al 2007); and 10.1% of veterinary personnel attending an equine veterinary conference (Anderson et al 2008). In UK, MRSA carriage was determined in 17.9% of 78 veterinary staff (Loeffler et al 2005). In this study, 24 of the 75 phenotypic methicillin resistant *S. aureus* isolates were found *mecA*-positive and all of these were isolated from humans working in clinic. The *mecA*-positive *S. aureus* carriage of veterinarians was higher than veterinary students. These results are also in parallel with results from several studies (Loeffler et al 2005, Moodley et al 2006, Weese et al 2006, Hanselmann et al 2007, Anderson et al 2008, Wulf et al 2008, Loeffler et al 2010, Martino et al 2010), all of which reported high colonization rates in humans who have close contact with animals. These findings can also be associated with physical conditions of clinics, such as working a common service in the same area of the three different departments (surgery, internal medicine and reproduction) and increasing the human population in the presence of students. On the other hand, small and large animal were also treated in the same area of the clinic from where this study was also conducted. Therefore, MRSA to veterinarians, personnel and students may be transmitted from other persons and animals, especially healthy but colonized by MRSA.

Several researchers stated that environmental contamination could play an important role in MRSA transmission within veterinary and medicine hospitals (Boyce et al 1997, Weese et al 2004, Loeffler et al 2005, Moodley et al 2006, Oie et al 2007, Kilic et al 2010). In this study, 9 from 11 *S. aureus* isolates from environmental surfaces of veterinary clinic (such as door handles, operation tables, microscope switches, thermometers, lamp switches, boxes, x-ray apparatus, PC, dressing boxes, tables, soap dispensers, taps and muzzles) thought to be common sites of hand contact were phenotypically resistant to methicillin. None of these isolates were



determined as a true MRSA (*mecA*-positive) isolate. Ozturk et al (2010) were previously reported that phenotypically methicillin resistant *S. aureus* was isolated from dogs with otitis externa, skin wounds and pyoderma brought to the same faculty clinics. But, these isolates were not carrying *mecA* gene, which encoded a penicilin binding protein 2a (PBP2a). The *mecA* gene is an important molecular marker for methicillin resistance (Weese et al 2006) and methicillin resistance may appear in staphylococci which lack of *mecA* gene due to the overproduction of β -lactamase (Unal 1996, Kaszanyitzky et al 2003). In the presented study, *mecA*-negative isolates were susceptible to amoxicillin/clavulanic acid after repeated disk diffusion testing. Therefore, we thought that the overproduction of β -lactamase in isolates may cause a decrease in susceptibility to methicillin (Unal 1996, Kaszanyitzky et al 2003).

Conclusions

This study was demonstrated that MRSA colonization was high in people laboring and students at the veterinary clinic, so standard sanitation measures, particularly personnel hygiene, are required because of a possible risk of transmission between humans and animals.

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