Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Document heading doi:

A comparative evaluation of methicillin-resistant staphylococci isolated from harness racing-horses, breeding mares and riding-horses in Italy

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PEER REVIEW

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Comments

This is a good study in which the authors evaluated the distribution of staphylococcal species and levels of antibiotic resistance that were found to be different between isolates. Antibiotic pressure may lead to these differences. The results are interesting and suggested that MRS are present especially in harness racing-horses.
(Details on Page 172)

ABSTRACT

Objective: To investigate the prevalence of methicillin-resistant staphylococci (MRS) which is a potencial risk factor of transmission between animals and humans in different types of horses (harness racing-horses, breeding mares and riding-horses) and to compare the antimicrobial resistance of the isolates. **Methods:** A total of 191 healthy horses, housed at different locations of the Campania Region (Italy), were included in the study. Nasal swab samples were collected from each nostril of the horses. The *mecA* gene was detected by a nested PCR technique. Antibiotic susceptibility was tested for each isolate. **Results:** MRS was isolated from nasal samples of 68/191 (35.6%; 95% *CI*: 28.9%-42.9%) healthy horses. All isolates were coagulase-negative with the exception of two coagulase-positive MRS strains, identified as *Staphylococcus aureus* and *Staphylococcus pseudintermedius*, 2/83 (2.4%; 95% *CI*: 0.4%-9.2%). Interestingly, both coagulase-positive MRS isolates were from harness racing-horses. These horses also presented a significantly higher positivity for MRS (53.3%; 95% *CI*: 40.1%-66.1%) than the breeding mares and riding-horses groups. Antibiotic susceptibility testing showed difference between isolates due to different origins except for an almost common high resistance to aminopenicillins, such as ampicillin and amoxicillin. **Conclusions:** It can be concluded that harness racing-horses may act as a significant reservoir of MRS as compared to breeding mares and riding-horses.

KEYWORDS

Methicillin-resistant staphylococci, Mares, Harness racing-horse, Riding-horse, Nasal swabs

1. Introduction

Methicillin resistance in *Staphylococcus aureus* (*S. aureus*) and coagulase-negative species is primarily mediated by the overproduction of PBP2a, an additional altered penicillin-binding protein with extremely low affinities for β-lactam antibiotics[1]. PBP2a is encoded by the gene *mecA*, residing on a large mobile genetic element designated staphylococcal chromosome cassette mec. Until now, seven major variants of staphylococcal chromosome cassette mec, type 1 to 7, have been described[2].

Methicillin-resistant *S. aureus* (MRSA) have been reported in almost all domesticated species, including dogs, cats, horses, cattle and sheep[3-6]. Skin and soft tissue MRSA infections[7], bacteraemia, septic arthritis and osteomyelitis[8,9], implant-related infections, metritis[10], omphalitis[11], catheter-related infections and pneumonia[12] have all been reported in horses.

In addition, *mecA*-harboring coagulase-negative staphylococci (CoNS) have been isolated from several domesticated and healthy animals[13,14]. In Japan, *mecA* positive CoNS were cultured from the skin and nares of healthy horses[15]. Recently, high prevalence of the CoNS was found in healthy horses in the Netherlands[16] and Slovenia[17] and in Italy[18].

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Foundation Project: Supported by Faculty of Veterinary Medicine, University of Naples "Federico II", Naples, Italy.

Article history:
Received 1 Dec 2012
Received in revised form 5 Dec, 2nd revised form 13 Dec, 3rd revised form 25 Dec 2012
Accepted on 10 Feb 2013
Available online 28 Mar 2013

The increasing resistance of staphylococci to β-lactam antibiotics has become a major clinical problem and the strains also generally exhibited multiple resistance to tetracyclines, aminoglycosides, macrolides, lincosamides, and some other antimicrobial drugs[19-22]. Furthermore, since horses are often in close contact with their owners and farm staff members, the risk of transmission of these bacteria between animals and humans (or *vice versa*) must be taken into consideration. However, it is not well known the distribution of *mecA*-harboring staphylococci and the antimicrobial resistance with reference to the different habitats in which horses live. The aim of this study was to investigate the correlation between the prevalence of methicillin-resistant staphylococci (MRS), both coagulase-negative and coagulase-positive, from different standing of healthy horses in Italy, particularly in harness racing-horses, breeding mares and riding-horses.

2. Materials and methods

2.1. Sample collection

A total of 191 healthy horses, housed at different locations of the Campania Region (Italy) were sampled. The following are three different locations: (1) 60 harness racing-horses aged between 3-6 years randomly selected at the Agnano racetrack; (2) 64 breeding mares aged between 4-15 years from an Equine Reproduction Centre; (3) 67 horses aged more than 15 years from a large Riding Centre.

All samples were collected in the summer-autumn period of 2007 and these centres documented no history of MRSA. Owner's consent was obtained prior to enrolment of each horse. A cotton-tipped swab was collected from each nostril and kept at 4 °C (not longer than 24 h) in Amies medium until processing. Demographic information (age, breed, gender and use) was recorded for each horse at the time of sample collection.

2.2. Bacteriological assays

Nasal swab samples were plated on mannitol-salt agar and incubated aerobically at 37 °C for 24-48 h. Staphylococcal isolates were identified by colonial morphology, gram-stain, catalase and staphylo-coagulase (tube coagulase) reactions. Oxacillin (OX, methicillin) susceptibility test of all isolates was performed by OX disk diffusion method in accordance with Clinical and Laboratory Standards Institute-National Committee for Clinical Laboratory Standards (M31-A2, 2002). The OX susceptible *S. aureus* (ATCC 29213) and OX resistant *S. aureus* (ATCC 43300) strains were used as controls. Identification was confirmed with API-ID 32 Staph system (bioMérieux, Marcy L'Etoile, France) and methicillin-resistance was also confirmed by a positive PBP2a latex agglutination test (PBP2′ Test kit, Oxoid, Basingstoke, Hampshire, England). Stock cultures were stored at −70 °C in microbank vials (PRO-LAB Diagnostics, Richmond Hill, ON, Canada) for further analysis.

2.3. Extraction of DNA

Cells cultured in 1.5 mL of trypticase soy broth at 37 °C for 24 h were harvested and centrifuged at 16 000 r/min for 3 min. The pellet was washed with 1.0 mL of sterile distilled water, resuspended in 50 μL of Triton X-100 lysis buffer [100 mmol/L NaCl, 10 mmol/L Tris-HCl (pH 8.0), 1 mmol/L EDTA (pH 9.0), 10 g/L Triton X-100], boiled for 10 min, and then centrifuged at 16 000 r/min for 3 min. The suspension was cooled at room temperature for 5 min and centrifuged at 16 000 r/min for 3 min. The supernatant was used as template.

2.4. Detection of mecA gene by PCR

The *mecA* gene was detected by a nested PCR technique as described previously[15]. For the first amplification, the following primers were used (Invitrogen Ltd., Glasgow, UK): 5′-GTT GTT GTA GTT GTC GGG TTT GG-3′ (position 37 to 56, sense) and 5′-CCA CCC AAT TTG TCT GCC AGT TTC TCC-3′ (position 1 828 to 1 854, antisense); size of the amplified fragment was 1 818 bp. For the second (nested) amplification, the following internal primers were used: 5′-AGA TTG GGA TCA TAG CGT CA-3′ (position 375 to 394, sense) and 5′-GAA GGT ATC ATC TTG TAC CC-3′ (position 613 to 632, antisense); size of the amplified fragment was 258 bp. All PCRs were performed in duplicate. All PCR reagents were purchased from Invitrogen.

Briefly, PCR was performed in a 50 μL mixture containing template DNA, PCR buffer [10 mmol/L Tris-HCL (pH 8.4), 50 mmol/L KCl, 1.0 mmol/L $MgCl_2$], a 100 nmol/L concentration of each PCR primer, a 200 μmol/L concentration of each deoxyribonucleoside triphosphate, and 1.25 IU of *Taq* polymerase. Both reactions were performed in a DNA thermal cycler without mineral oil (Mastercycler Gradient Eppendorf, Eppendorf, Hamburg, Germany). The PCR and the nested PCR consisted of a preheating at 94 °C for 2 min, 25 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min, and incubation at 72 °C for 5 min. The final PCR products were loaded on a 1% agarose gel with 2 μg/mL of ethidium bromide to determine the size of the amplified products. As positive control, 100 ng of genomic DNA separated from a suspension of OX resistant *S. aureus* (ATCC 43300) was included in each experiment. Negative controls contained all reagents except DNA template.

2.5. Antibiotic susceptibility testing

Laboratory trials were performed in accordance with the principles described in the standard method of the National Committee for Clinical Laboratory Standards (2002), using the following antimicrobial agents: OX 10 μg, amoxicillin/clavulonic acid (AMC) 30 μg, ampicillin 10 μg, imipenem 10 μg, meropenem 10 μg, colistin sulfate 10 μg, cefaclor 30 μg, cefuroxime 30 μg, cefprozil 30 μg, ceftriaxone 30 μg, cefoxitin 30 μg, erythromycin 15 μg, lincomycin (MY) 2 μg, tetracycline 30 μg, doxycycline 30 μg, amikacin 30 μg, trimethoprim/sulphametoxazole 25 μg and ciprofloxacin 5 μg. The susceptibility of each isolate to the panel of antibiotics was revealed by the diameter size of the clear zones around the dish as directed by the manufacturer.

2.6. Statistical analysis

Statistical analysis was performed by the Student-Newman-Keuls Multiple Comparison test using GraphPad InStat Version 3.00 for Windows 95 (GraphPad Software, San Diego, CA, USA).