Schweiz. Arch. Tierheilk.

P. Decristophoris et al., Band 153, Heft 9, September 2011, 405–409
DOI 10.1024/0036-7281/a000233

Antibiotic treatments of a methicillin-resistant *Staphylococcus pseudintermedius* infection in a dog: A case presentation

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Summary

We report the antibiotic treatments administered to a female dog with mastitis and successive pyoderma. Microbiological investigations allowed the identification of *Staphylococcus pseudintermedius* after 54 days of various antibiotic treatments. The isolate carried the mecA gene and was resistant to 9 of 15 tested antibiotics. Consistent antibiotic treatment of the infection was possible only after accurate microbiological diagnosis.

Keywords: pyoderma, microbiological investigation, *Staphylococcus pseudintermedius*, antibiogram, antibiotic treatment

Antibiotische Behandlungen einer Methicillin-resistenten *Staphylococcus pseudintermedius* Infektion bei einem Hund: eine Fallvorstellung

Wir berichten über die antibiotischen Behandlungen einer Hündin mit Mastitis und folgender Pyodermie. Die mikrobiologische Untersuchung erlaubte die Identifizierung von *Staphylococcus pseudintermedius* nach 54 Tagen mit verschiedenen antibiotischen Behandlungen. Der isolierte Stamm beinhaltete das mecA-Gen und war gegen 9 der 15 getesteten Antibiotika resistent. Eine angemessene antibiotische Behandlung der Infektion war erst nach einer genauen mikrobiologischen Diagnose möglich.

Schlüsselwörter: Pyodermie, mikrobiologische Untersuchung, *Staphylococcus pseudintermedius*, Antibiogramm, antibiotische Behandlung

Introduction

Staphylococcus pseudintermedius is an opportunistic pathogen of various animal species, particularly dogs and cats, and causes skin and soft tissue infections (Bannoehr et al., 2007). This pathogen was first described in 2005 from four clinical specimens from a cat, a dog, a horse and a parrot (Devriese et al., 2005). Phylogenetic analyses showed that this was not a new emerging species among dogs, but rather a misidentified biotype of *Staphylococcus intermedius* (Sasaki et al., 2007). Phenotypic identification methods can lead to incorrect identification of *S. pseudintermedius*, whereas molecular methods (e.g. sequencing of the partial *hsp60* gene, MALDI-TOF MS) allow reliable identification of this species (Sasaki et al., 2007; Decristophoris et al., 2011). Methicillin resistance, encoded by the *mecA* gene, was described in *S. pseudintermedius* and multidrug resistance has been reported with increasing frequency in veterinary settings (Wettstein et al., 2008; Weese and van Duijkeren, 2010). This is of concern for the treatment of animal diseases and may carry potential public health consequences (Cohn and Middleton, 2010). To effectively manage antibiotic resistant bacteria and subsequent infections, it is mandatory to prevent inappropriate use of drugs and to improve the rapid prescription of appropriate antibiotics to a patient (Lloyd, 2010). We report the antibiotic treatments administered to a female dog before an infection by *S. pseudintermedius* had been diagnosed and a consistent antibiotic therapy was administered to the dog.

Case history

A 5-year-old, mixed breed, female dog with an ongoing mastitis was brought to an Italian veterinary practice. The dog had an ovariectomy 3 months before the visit

because of recurrent pseudopregnancies. For treatment of mastitis the antibiotic first chosen consisted of Synulox (amoxicillin-clavulanic acid, 14.3 mg/kg bw and 3.6 mg/kg bw respectively, bid for 7 days) together with Stomorgyl (spiramycin-metronidazole, 17.9 mg/kg bw, sid for 7 days). Due to the lack of efficacy, treatment with these antibiotics was stopped and the dog was given Baytril (enrofloxacin, 10.7 mg/kg bw, sid orally for 2 days) and Rocefin (ceftriaxone, sc, 35.7 mg/kg bw, sid for 31 days). At that stage the dog weighed 14 kg, was anaemic with a haematocrit of 31.9 % (37–55 %), had raised liver values (AP = 472 U/L; 23–212 U/L) and was febrile (40.8 °C). A complete blood cell count (CBC) revealed a leukocytosis of 23.47 K/µL (5.50–16.90 K/µL) characterized by a neutrophilia of 18.56 K/µL (2.00–12.00 K/µL) and a monocytosis of 3.55 K/µL (0.30–2.00 K/µL).

Enrofloxacin was stopped after 2 days and treatment was continued with ceftriaxone (sc, 35.7 mg/kg bw, sid) alone. Skin lesions with purulent and sanguineous exudates appeared on the back and around the occipital bone (Fig. 1). An Elizabethan collar was used to hinder scratching, because the dog was very pruritic. Diagnosis of deep pyoderma was made on the basis of the clinical appearance of the skin, but no skin tests were carried out. Due to the lack of efficacy of ceftriaxone alone, Antirobe (clindamycin; 10.7 mg/kg bw, bid for 5 days) was added to the ongoing treatment together with methylprednisolone tablets (0.3 mg/kg bw, bid for 30 days) to control the pruritus. The mastitis resolved but the pyoderma persisted and the owner decided to bring the dog to another veterinary clinic, where clindamycin treatment was stopped and the antifungal Sporanox (itraconazole, 7.1 mg/kg bw, sid for 5 days) was added to the ongoing ceftriaxone and corticosteroid treatment; also in this case no skin scraping or other cytological tests were performed. Pyoderma persisted and blood analysis revealed anaemia with a haematocrit of 24.5 % and Hb = 9.8 g/dL (12.0–18.0 g/dL) and eosinophilia (2.51 K/µL; 0.10–1.49 K/µL), together with elevated liver enzyme values (ALT = 187 U/L, 10–100 UL; AP value = 2400 U/L). Ceftriaxone treatment resulted in no skin improvement, therefore after 31 days of treatment the owner went to a third veterinary clinic where the drug was replaced by doxycycline (7.1 mg/kg bw, sid for 9 days). Progressive decreasing methylprednisolone doses and treatment with doxycycline were continued with no significant improvement of the skin, leading to the decision to carry out a microbiological analysis. A sterile swab (Amies agar gel 108C; Copan, Italy) was taken directly from the eyebrow arch lesion, plated out on blood agar with nalidixic acid within 24h after collection, and incubated during 24h at 35 °C.

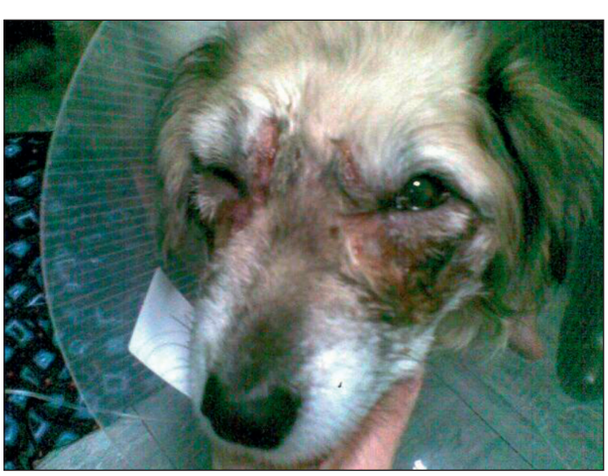

Figure 1: Pyoderma with exudates and blood appearing around the occipital bone.

Bacteriology

Heavy growth of Gram positive haemolytic, catalase positive cocci was observed. The strain was identified as *Staphylococcus aureus* by RapiDEC Staph biochemical tests (bioMérieux® SA, France), but this result was not confirmed by latex agglutination test (Pastorex®; Bio-Rad, France). The strain was eventually identified as *S. pseudintermedius* by MALDI-TOF MS analysis (matrix assisted laser desorption ionisation – time of flight mass spectrometry) with a confidence of 99.9 % (Decristophoris et al., 2011). MALDI-TOF MS produces a fingerprint spectrum of peptides and proteins of the analyzed microorganism. The diagnosis was later confirmed by sequencing of the partial *hsp60* gene (Kwok et al., 1999). According to the disk diffusion method (Clinical and Laboratory Standards Institute, previously NCCLS guidelines) (NCCLS, 2004), the isolated methicillin-resistant *S. pseudintermedius* (MRSP) was phenotypically resistant to 9 antibiotics of 15 tested, but sensitive to minocycline, a long acting tetracycline (minimal inhibitory concentration assessed by E-test method: 0.19 µg/mL) (Tab. 1). Oxacillin susceptibility test, used to predict *mecA*-mediated resistance in *S. pseudintermedius,* was evaluated according to Bemis and co-workers (Bemis et al., 2009). The *mecA* gene responsible for methicillin resistance was detected by PCR (Cotter et al., 1997). Minocycline (14.29 mg/kg bw, bid orally for 7 days) was administered based on dosage recommendation of CliniPharm/Clinitox-Datenbanken from the Institute of Veterinary Pharmacology and Toxicology, Zürich (www.vetpharm.uzh.ch). The dog's general clinical condition improved, the areas of pyoderma decreased in size and the sanguineous secretions diminished. Leukocytosis disappeared (11.18 k/µL). Topical treatment with a cream containing allicin, a garlic extract, was administered to help healing of the lesions. Despite of their improvement, two weeks later, the dog had a febrile attack which was clinically traced back to the *S. pseudintermedius* and the dog was therefore given rifampicin (21.4 mg/kg bw, sid orally for 7 days). After the administration of this antibiotic the pyoderma continued to improve, the liver values returned to the normal range (ALT = 33 U/L) and

Table 1: Phenotypic antibiotic test with the Kirby-Bauer method.

Antibiotic	Phenotypic behaviour	Measured diameters (mm)	Clinical breakpoints (mm)		Possible therapeutic options after
			S	R	(Weese, 2006; Lloyd, 2010)
Oxacillin	R [1]	6	≥ 18	≤ 17	No
Tobramycin	R	11	≥ 15	≤ 12	No
Doxycycline	R	8.5	≥ 16	≤ 12	No
Chloramphenicol	R	6	≥ 18	≤ 12	No
Ciprofloxacin	R	6	≥ 21	≤ 15	No
Clindamycin	R	6	≥ 21	≤ 14	No
Erythromycin	R	6	≥ 23	≤ 13	No
Fusidic acid	R	18	≥ 20	≤ 19	No
Trimethoprim-sulfamethoxazole	R	6	≥ 16	≤ 10	No
Minocycline	S	0.19 *	≤ 4 **	≥ 16 **	First line
Rifampicin	S	31.5	≥ 20	≤ 16	restricted
Linezolid	S	26.5	≥ 21	–	restricted
Quinupristin-dalfopristin	S	21	≥ 19	≤ 15	restricted
Mupirocin	S	22	≥ 14	≤ 13	restricted
Vancomycin	S	16	≥ 15	≤ 14	restricted

[1] when resistant to oxacillin and presenting the mecA gene, the strain is considered resistant also to all the other beta-lactams (i.e. penicillins, cephalosporins and carbapenems). Cefoxitin breakpoints are not predictive of mecA-mediated resistance to methicillin/oxacillin in Staphylcococcus pseudintermedius (Papich, 2010). R = resistant, S = susceptible.

* in µg/mL

** human interpretative criteria of the E-test for the assessment of the minimal inhibitory concentration (MIC) measured in µg/mL

the fever disappeared. Since the treatment, the animal has been suffering from an undiagnosed articular pain.

Discussion

Infections due to *S. pseudintermedius* and MRSP have been reported with increased frequency in dogs since the species was described in 2005. *S. pseudintermedius* was isolated as colonising agent from the ear, perineum and nasal mucosae of both healthy dogs and dogs with atopic dermatitis (Fazakerley et al., 2009). Moreover, MRSP was isolated as urinary tract infection agent also in cats (Wettstein et al., 2008) and *S. pseudintermedius* infections in humans were documented (Van Hoovels et al., 2006; Chuang et al., 2010). Lack of the use of molecular identification methods might lead to incorrect characterisation of *S. pseudintermedius*. The RapiDEC Staph is a test based on biochemical reactions for the identification of the main staphylococci isolated from human specimens (*S. aureus, S. epidermidis* and *S. saprophyticus*) but also for the presumptive identification of *S. intermedius*, a staphylococcal species of animal origin. The use of RapiDEC Staph for the diagnosis of the dog isolate led to incorrect identification of the recovered microorganism. This observation suggest that the use of this phenotypic test can entail misidentification of *S. pseudintermedius*, because this species shares many phenotypic characteristics and mechanisms of antibiotic resistance with *S. aureus* (Devriese et al., 2005; Weese and van Duijkeren, 2010).

The described clinical manifestations due to *S. pseudintermedius* in pets mainly consist of pyoderma and skin infections (Vincze et al., 2010). Here we report a MRSP strain isolated from a pyoderma lesion in a female dog previously affected by mastitis. The origin of the infection is unclear. The dog might have contracted the MRSP infection during the ovariectomy. Correspondingly, cases of nosocomial wound infections are reported for methicillin-resistant *S. aureus* (MRSA) in humans after surgery (Sisirak et al., 2010). On the other hand, it is also possible that the dog carried the *S. pseudintermedius* strain as part of its normal skin bacterial community, so that the multi-drug resistant strain was selected by the antibiotics. Humans and other in-contact pets could also have been a source of MRSP transmission to the dog, but this possibility was not further explored.

The eosinophilia present in the blood could have been an indicator for an allergic or parasitic origin particularly because of the pruritus observed. Skin scraping for parasites can be easily carried out in any practice together with a Fungassay to rule out any fungal infections, as well as an impression smears to confirm diagnosis by showing intracytoplasmic bacteria on cytological examination. If underling systemic disease is suspected, a full blood workup has to be done taking care to include also differential count of the WBC and eventually also a skin biopsy. These clinical diagnostic methods should have been applied also in the described case before the administration of different antibiotics. In any case bacterial culture and sensitivity test should be carried out if antibiotic treatment does not yield the expected results.

Treatment of MRSP is critical because of its resistance to all beta-lactams and, often, other classes of antibiotics. As for the antibiotic therapy of other bacterial infections, the administration of antibiotics until completion of full treatment is of primary importance. An effective antimicrobial therapy depends on several parameters such as bacterial susceptibility, pharmacokinetic characteristics of the drug, and dosage regime. Lack of treatment completion or prolonged antibiotics use may result in selection for resistant strains (Prescott, 2000). Delayed or inadequate prescriptions can reduce the efficacy of treatment and favour the spread of the infection, both in human and in veterinary medicine (Buckley, 2009). As in human medicine, treatment of animal infections should rely on a stepwise approach that includes successive use of first, second and third line antibiotics (Lloyd, 2010). In this case, the first therapy consisted of amoxicillin-clavulanic acid and spiramycin-metronidazole. Due to lack of efficacy of this empiric approach, enrofloxacin was administered together with ceftriaxone. These drugs, however, belong to antibiotic classes that are usually employed as third line treatment in human medicine: thus, they should be used in animals only for a limited number of cases, i.e. where all other antibiotics fail (Ungemach et al., 2006). Minocycline is a semi-synthetic, long acting tetracycline which has been suggested to be effective against staphylococci resistant to semisynthetic penicillins and cephalosporins (Minuth et al., 1974). In Japan, minocycline is commonly used in veterinary dermatological practices (Kawakami et al., 2010), whereas in other countries, such as Switzerland, drugs containing this antimicrobial principle are not approved for use in animals (www.vetpharm.uzh.ch).

If the infection persists after a first line empiric treatment, the choice of a more specific, active drug to be administered should rely on an accurate microbiological analysis and antibiogram, which allow to evaluate the susceptibility of the pathogen against different drugs (Lloyd, 2010). In the described case, the antibiotic treatment of pyoderma included the administration of six different antibiotics, one corticosteroid and one antifungal agent, during a total of 54 days, before a microbiological investigation was eventually carried out. The microbiological analysis allowed determination of the antibiotic susceptibility profile of the MRSP strain within 48 h.

Possible therapeutic options after failure of the treatment with minocycline and successive relapse were limited (Tab. 1). These consisted of antibiotic agents for which the use in veterinary medicine should be restricted to life-threatening infections, when culture and susceptibility testing indicate no other options (Lloyd, 2010). The dog was treated with rifampicin, even if this drug is rarely used in dogs, at the recommended doses of 10–15 mg/kg orally sid. This is a bactericidal staphylococcal agent which inhibits the bacterial RNA polymerase activity resulting in a block of the protein synthesis. Normally, with deep pyoderma antibiotic treatment is given for at least six weeks, extended to no less than two weeks after clinical resolution. However as rifampicin is very hepatotoxic and plasma liver enzymes should be monitored weekly, the veterinary surgeon preferred to give a higher dose for only seven days. Topical treatment was hardly used in this case; only an ointment containing allicin was applied toward to the end of the treatment, although it is very useful in the treatment of pyoderma. It helps removing debris and bacteria and favours drainage of exudative and deep lesions. There are many products on the market, such as soaks with chlorhexidine or iodine and special shampoos containing benzoyl peroxide or ethyl lactate. Creams tend to be used for localised lesions. In this case the frequent change of veterinary practices did not permit a continuity of the dog follow up and this had a deleterious effect on the outcome.

Conclusion

This clinical case emphasizes the importance of a rapid and accurate microbiological diagnosis, based on the identification of the pathogen and an antibiogram for an effective treatment of severe cutaneous infections in dogs, especially when an empiric first line treatment is not successful. This is particularly important in the case of infections that might result from microorganisms such as *S. pseudintermedius*, known to develop multidrug resistance (Cohn and Middleton, 2010).

Acknowledgements

We are grateful to Nadia Ruggeri for the technical support. Thanks go to Dr. med. vet. Ulli Scheu, PD Dr. Orlando Petrini, Dr. Barbara Wicht and also to anonymous reviewers for helpful comments and criticisms.

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Received: 4 January 2011
Accepted: 31 March 2011