Antimicrobial activity of an acetic and boric acid solution against *Staphylococcus pseudintermedius*

Gevoeligheid van Staphylococcus pseudintermedius *voor de combinatie azijnzuur-boorzuur*

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ABSTRACT

Incubation of 10^7 colony forming units/ml of *Staphylococcus pseudintermedius* in an undiluted, a 1:2 and a 1:4 diluted aqueous 2% acetic acid and 2% boric acid solution resulted in inactivation of the bacteria within 30, 60 and 120 minutes, respectively. This indicates that a combination of these acids might be useful for local treatment of *S. pseudintermedius* infections. Further clinical studies are necessary, however, to confirm these *in vitro* results.

SAMENVATTING

Respectievelijk 30, 60 en 120 minuten na het suspenderen van $5x10^7$ kolonie vormende eenheden van *Staphylococcus pseudintermedius* isolaten in 5 ml van een onverdunde, 1/2 en 1/4 verdunde waterige oplossing van 2% azijnzuur en 2% boorzuur, werden geen levende bacteriën meer gevonden. Dit duidt erop dat een combinatie van deze zuren nuttig zou kunnen zijn voor de lokale behandeling van *S. pseudintermedius* infecties. Bijkomende klinische studies zijn evenwel noodzakelijk om de hierbeschreven *in vitro* bevindingen te bevestigen.

INTRODUCTION

In dogs, coagulase positive staphylococci have been associated with several suppurative conditions, including pyoderma, otitis externa, endometritis, urinary tract infections, conjunctivitis and wound infections (Hermans *et al.*, 2004; Quinn *et al.*, 1999). *Staphylococcus aureus* has only occasionally been isolated from these lesions and in the literature it is commonly accepted that *Staphylococcus intermedius* is the main major pathogenic *Staphylococcus* species associated with dogs (Hermans *et al.*, 2004). However, recent studies have shown that canine staphylococcal isolates previously identified as *S. intermedius,* in fact belong to the newly described species *Staphylococcus pseudintermedius* (Bannoehr *et al.,* 2007; Devriese *et al.*, 2006; Sasaki *et al.*, 2007a).

Infections caused by *S. pseudintermedius* are often treated with antibiotics. However, acquired antimicrobial resistance is frequent in canine isolates (Donné *et al.*, 2000). Although these isolates are usually susceptible to cephalosporins and the combination amoxicillin-clavulanic acid, methicillin resistant *S. pseudintermedius* strains showing acquired resistance to all beta-lactam antibiotics have been described (Bannoehr *et al.,* 2007; Sasaki *et al.*, 2007b).

As an alternative to antimicrobial therapy, acetic and boric acid have been suggested for local treatment of bacterial infections. Both acids have been shown to exert antibacterial effects on different bacterial species, including staphylococci (Houlsby *et al.*, 1986; Russel and Diez-Gonzalez, 1998).

A 2% acetic acid and 2% boric acid aqueous solution is commercially available as an ear and skin cleaner for use in dogs. In the present study, the bactericidal effect of different dilutions of this product on canine *S. pseudintermedius* isolates was determined.

MATERIALS AND METHODS

Two isolates (81 and 336), phenotypically identified as *S. pseudintermedius* (Devriese *et al.*, 2006), were used in this study. Isolate 81 was obtained in 2005 from the uterus of a dog with pyometra. It showed acquired resistance to beta-lactamase susceptible beta-lactam antibiotics, macrolides, lincosamides, tetracyclines and neomycin. Isolate 336 was obtained in 2006 from the umbilicus of a pup that died shortly after birth. Acquired antimicrobial resistance was not detected in this isolate. Both isolates were cultured overnight at 35°C on Columbia agar with 5% sheep blood (Oxoid, Basingstoke, Hampshire, UK) in a 5% CO_2 atmosphere.

The bactericidal effect of a 2% acetic acid and 2% boric acid aqueous solution (Malacetic Otic, Dermapet, U.S.A.) against these isolates was determined. Approximately $5x10^7$ colony forming units (cfu) of each *S. pseudintermedius* isolate were suspended in 5 ml undiluted, 1:2 and 1:4 diluted (in distilled water) Malacetic Otic. As a control, approximately $5x10^7$ cfu of each *S. pseudintermedius* isolate were suspended in 5 ml distilled water. All suspensions were incubated at 30°C in a linear shaking bath (GLS400, Grant Instruments, Shepreth, UK). At

Table 1. Logarithmic mean and standard deviation of the number of colony forming units (log10 cfu) per ml for *Staphylococcus pseudintermedius* isolates 81 and 336 after incubation at 30°C in distilled water, and in an undiluted, a 1:2 and a 1:4 diluted aqueous 2% acetic acid and 2% boric acid solution (AA-BA sol.)

Suspension	Incubation time (minutes) 0	30	60	120	180	240	300	360
Isolate 81 – distilled water	6.74 ± 0.05	6.84 ± 0.04	6.87 ± 0.03	6.77 ± 0.17	6.79 ± 0.08	6.72 ± 0.08	6.82 ± 0.05	6.69 ± 0.17
Isolate 81 – 1:1 AA-BA sol.	6.00 ± 0.07	neg.*	neg.	neg.	neg.	neg.	neg.	neg.
Isolate 81 – 1:2 AA-BA sol.	6.53 ± 0.08	2.54 ± 0.28	neg.	neg.	neg.	neg.	neg.	neg.
Isolate 81 – 1:4 AA-BA sol.	6.68 ± 0.13	4.70 ± 0.13	2.66 ± 0,38	neg.	neg.	neg.	neg.	neg.
Isolate 336 - distilled water	6.91 ± 0.07	6.89 ± 0.03	6.88 ± 0.03	6.82 ± 0.02	6.71 ± 0.17	6.73 ± 0.07	6.79 ± 0.07	6.81 ± 0.05
Isolate 336 – 1:1 AA-BA sol.	5.22 ± 0.00	neg.	neg.	neg.	neg.	neg.	neg.	neg.
Isolate 336 – 1:2 AA-BA sol.	6.49 ± 0.09	2.91 ± 0.09	neg.	neg.	neg.	neg.	neg.	neg.
Isolate 336 – 1:4 AA-BA sol.	6.67 ± 0.04	4.10 ± 0.01	2.80 ± 0.02	neg.	neg.	neg.	neg.	neg.

*neg.: <1.22

0, 30, 60, 120, 180, 240, 300 and 360 minutes, three 60 µl samples were collected, and the bacterial titer (number of cfu per ml) in these samples was determined by plating tenfold dilutions on Columbia agar with 5% sheep blood. For each bacterial suspension and each time point, the logarithmic mean titer and the standard deviation were calculated.

RESULTS

The results are summarized in Table 1.

No differences were observed between the two isolates tested. In the control samples without Malacetic Otic, the bacterial titer did not change throughout the experiment. In the undiluted Malacetic Otic solution, the bacteria were inactivated within 30 minutes. Incubation of *S. pseudintermedius* in a 1:2 and a 1:4 diluted Malacetic Otic solution resulted in inactivation of the bacteria within 60 and 120 minutes, respectively.

DISCUSSION

It can be concluded that, under the conditions tested here, an aqueous solution containing at least 0.5% acetic acid and 0.5% boric acid exerts a bactericidal effect against *S. pseudintermedius.* This indicates that a combination of these acids might be useful for local treatment of *S. pseudintermedius* infections.

In the present study, pure cultures of *S. pseudintermedius* were incubated in an aqueous environment. The *in vivo* situation is far more complex. Several factors, including the presence of organic material, mixed infections of *S. pseudintermedius* with other bacteria or fungi, and the presence of bacteria in biofilms (Haesebrouck *et al.*, 2007) may influence the effects of acetic and boric acid. Therefore, further clinical studies are necessary to confirm the usefulness of these acids in the field.

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