

# In-Vitro Study

## Susceptibility of Selected Otitis External Pathogens to Individual and Mixture of Acetic and Boric Acids

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### BACKGROUND:

The anatomical nature of the ear canal of feline and canine species may predispose to otitis externa by normal bacterial flora, yeast and fungi<sup>1</sup>. The

challenge to the veterinarian treating such diseases is the prompt initiation of a treatment regime which does not lead to the development of drug resistance in the infecting pathogens. Thus the use of mild acids would alleviate such a concern for the patient and reduce exposure of the individual administering the treatment. A recent report describes the effective use of a mixture of acetic and boric acids as effective in the treatment of *Malassezia*<sup>2</sup>. A standardized mixture of 2% boric acid plus 2% acetic acid used in the treatment of the *Malassezia* otitis externa successfully resolved the infection in the ears of dogs. The mechanism of action of these acids was not determined although possible explanations were discussed.

### OBJECTIVE:

The objective of this study was to determine the in vitro susceptibility and mechanism of action of individual solutions and mixtures of acetic and boric acid against isolates of *Staphylococcus intermedius*, *Pseudomonas aeruginosa* and *Candida albicans* obtained from canine cases of otitis externa.

### MATERIALS AND METHODS:

Fresh isolates of *S. Intermedius*, *Ps. aeruginosa* and *C. albicans* were obtained from the Clinical Microbiology Laboratory of the Veterinary Hospital of The University of Pennsylvania. These isolates were maintained on Mueller-Hinton (MH) agar slants (for bacteria) and Sabouraud's glucose (SG) agars (for the *Candida*). The bacterial cultures were grown overnight at 37C and stored at refrigeration temperature until used. The *Candida* was grown on SG agar at room temperature for three days prior to storage in the refrigerator. The storage time was less than three days prior to the performance of the experiment. Acetic acid and boric acids were prepared in 1X MH Broth (MHB) or Sabouraud's broth (SB) at twice the use concentration and

filtered sterilized (0.22 u Millipore )MHB) or Sabouraud's broth (SB) at twice the use concentration and filtered sterilized (0,22 u Millipore filter) and stored in sterile bottles. These solutions were dispensed into a 96 well microtiter plate (50 ul/well) to achieve individual and all possible combinations of the acids on the same plate. Individual solutions were raised to volume (100 ul) with the appropriate broth, duplicates were placed on the same plate. Two plates were used per organism. Four wells on each plate contained only the growth medium as a control of the growth response. All plates were incubated at 37 overnight as sterility control. The microbes were grown overnight in the appropriate broth and diluted with fresh growth medium to yield 10 colony forming units per ml (cfu). The plates were inoculated with 10 ul of diluted suspension and the plates were incubated at 37 C (bacteria) for 24 hours or at room temperature (*Candida*) for 3 days. At that time, 1 ul was transferred from the microtiter plates to MH agar (or SA) plates using a replicator (six rows of eight stainless steel rods per row; the diameter of each rod was 2.0 mm and the top of the rod will transfer 1 ul of fluid). The replicator was surface sterilized by dipping the device into 95% ethanol and passing through a flame. The agar plates were incubated at the appropriate temperature and visually examined daily for five days. In this fashion, the cidal activity of the solution can be determined. All microtiter plates were then reincubated for an additional period as previously described and aliquots again transferred to agar plates. The microtiter plates were visually examined using a back-lighted magnifying mirror and growth was scored as a plus (+). The data was collected on individual sheets and the results compared at the completion of the experiment.

A mixture of the two acids was required to achieve uniform killing effect of all three microbes. This is interpreted as indicating that the action of the single acid has a 'static' mechanism and that the combination of the acids act in synergy to kill the organism (i.e., the 'cidal' activity).

### RESULTS:

The results are listed as the lowest concentration inhibiting or killing most

microbes. The fraction indicates the number of inhibited, or killed compared to the total number of different assays for a particular concentration. Boric acid effectively inhibited *Staphylococcus intermedius*, *Pseudomonas aeruginosa* and *Candida albicans*. The bacteria were considerably more sensitive to boric acid concentrations than was *Candida albicans*. A mixture of the two acids was required to achieve uniform killing effect of all three microbes. This is interpreted as indicating that the action of the single acid has a 'static' mechanism and that the combination of the acids act in synergy to kill the organism (i.e., the 'cidal' activity).

*Pseudomonas* was the most sensitive of the two microbes with growth being inhibited at 0.5% boric acid but bactericidal activity was not observed until the mixture of 0.5% boric acid/0.5% acetic acid was used. Growth of *S. intermedius* was completely inhibited at 2.0% boric acid but the killing activity was not observed until the mixture of 5.0% boric acid/0.5% acetic acid was monitored. Growth of *Candida* was completely inhibited at 5.0% boric acid but killing was not achieved until the boric acid was raised to 3.5% and acetic acid was present at 0.5%.

### DISCUSSION:

These studies indicate that the individual solution of boric acid is effective to inhibit or retard the growth of all three microbes. This inhibition is interpreted as a minimal inhibitory concentration. The transfer of small specimens to agar plates after the microbe has been exposed for a specific period clearly indicates the killing activity of the mixtures for each microbe. Thus, while boric acid was an effective inhibitor of bacterial and *Candida* growth, the most effective cidal activity is achieved only when both boric and acetic acids are used together.

### REFERENCES:

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