Evaluation of In Vitro Activity of Two Topical Products Against Three Organisms Isolated from Canine Referral Patients with Otitis Externa and Cutaneous Pyoderma*

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ABSTRACT

Canine otitis externa and cutaneous pyoderma are common problems that are often associated with Staphylococcus intermedius, Pseudomonas aeruginosa, and Malassezia pachydermatis. In vitro activity of two topical products against these organisms isolated from canine referral patients were evaluated. Organisms were grown and diluted to a concentration equivalent to $10^7$ colony-forming units (CFU) per mL and exposed to either a 0 or 1/5 dilution of Hexadene Flush with Spherulites (Virbac Animal Health Inc, Fort Worth, TX) or a 1/5 or 1/25 dilution of ResiCHLOR Lotion with Spherulites (Virbac Animal Health Inc, Fort Worth, TX) at time intervals from 1 to 30 minutes. Results showed that all three organisms were killed within 1 minute of contact time at 0 and 1/5 dilution of the flush. The lotion diluted to 1/5 also killed all three organisms. At 1/25 dilution, this lotion killed S. intermedius and P. aeruginosa within 1 minute of contact time, whereas M. pachydermatis was killed after 1 minute. The findings suggest that the two topical products exhibit efficacy against these common skin pathogens in vitro and can be useful in their clinical management.

INTRODUCTION

The microorganisms most frequently isolated from canine otitis externa and cutaneous pyoderma are the yeast, Malassezia pachydermatis, and the bacteria, Pseudomonas aeruginosa and Staphylococcus intermedius. Otitis externa and cutaneous pyoderma in dogs usually is treated by small animal practitioners without the benefit of microbiologic culturing. Mixed infections often occur making it necessary to employ treatments that are effective against more than one of these organisms. Several reports also
have revealed the emergence of a greater proportion of strains with resistance to antibacterial agents commonly used to treat these two diseases. Recently, Lloyd and Lamport reported the importance of the specific formulation of chlorhexidine rather than its concentration for efficacy against these organisms.

The objective of the study reported here was to compare the in vitro rate of killing of three organisms isolated from canine referral patients with otitis externa and cutaneous pyoderma by two topical products at different dilutions. Hexadene Flush is an antiseptic shampoo that contains 0.25% chlorhexidine gluconate, propylene glycol, triclosan, FD&C blue #1, and fragrance. ResiCHLOR Lotion contains 2% chlorhexidine gluconate, cetyl alcohol, stearyl alcohol, stearammonium chloride, dimethylstearamine, lactic acid, tetrasodium EDTA, methylchloroisothiazolinone, methylisothiazolinone, fragrance, FD&C blue #1, and FD&C yellow #5. Both products contain Spherulites, a product that carries chlorhexidine acetate designed by microencapsulation technology.

### MATERIALS AND METHODS
#### Bacteria, Sources, and Growth Conditions
The bacteria used in this study included ten isolates each of *S. intermedius*, *P. aeruginosa*, and *M. pachydermatis* obtained from canine patients with otitis externa or cutaneous pyoderma. The isolates were obtained from Tuskegee University, School of Veterinary Medicine, Tuskegee, AL; Auburn University, School of Veterinary Medicine, Auburn, AL; the State of Alabama Veterinary Diagnostic Laboratory, Auburn, AL; College of Veterinary Medicine, University of Georgia, Athens, GA; and College of Veterinary Medicine, Kansas State University, Manhattan, KS. The identity of the organisms was verified following standard procedures. *S. intermedius* and *P. aeruginosa* were grown in brain–heart infusion broth (Oxoid, Ogdensburg, NY), overnight at 37°C, while *M. pachydermatis* was grown on Sabouraud’s dextrose agar (10 g neopeptone, 20 g dextrose, 20 g agar) overnight at 32°C.

#### Preparation of Cultures
Each overnight bacterial culture was standardized in Mueller Hinton Broth (Oxoid) to approximately $1 \times 10^7$ CFU/mL ($A_{440} = 0.3$) using a “Spectronic 20” spectrophotometer (Bausch and Lomb, Rochester, NY).

#### In Vitro Activity of the Two Topical Products
In vitro activity determination was carried out essentially as described by Lloyd and Lamport. Twenty µL of the standardized test organism were added to 2 mL of either a 0 or 1/5 dilution in phosphate buffered saline (PBS; pH = 7.4) of the flush or to a 1/5 and 1/25 dilution in PBS of the lotion. Control suspension consisted of the organisms in PBS (pH = 7.4) only. The mixtures were held at room temperature and 0.1-mL aliquots were removed after 1, 2, 4, 8, 16, and 30 minutes and spread-plated on sheep blood agar plates (Remel, Lenexa, KS) for *S. intermedius* and *P. aeruginosa* or Sabouraud’s dextrose agar plates for *M. pachydermatis*. Plates were then incubated as previously described. Colony counts were performed on plates containing *S. intermedius* and *P. aeruginosa* after 48 hours and on plates containing *M. pachydermatis* after 72 hours. The studies were repeated three times for validity, and each product was evaluated with ten independent isolates of each test organism.

#### RESULTS
Undiluted and 1/5 dilution of the flush completely killed all three organisms at all sampling times within 1 minute as evidenced by the lack of visible growth on plating media after incubation. The lotion diluted 1/5 also killed all test or-
organisms within 1 minute of contact time. This same lotion at 1/25 dilution killed *S. intermedius* and *P. aeruginosa* within 1 minute, whereas 125 CFU/mL of *M. pachydermatis* were counted. The number of viable cells of *M. pachydermatis* dropped to zero within 2 minutes of contact time. Control studies contained counts of $1 \times 10^7$ CFU/mL and remained at this level longer than the 30-minute sampling time.

**DISCUSSION**

To avoid bias in evaluating the in vitro activity of the two topical products against *S. intermedius*, *P. aeruginosa*, and *M. pachydermatis*, organisms were obtained from canine referral patients submitted to various veterinary diagnostic laboratories in different geographic locations. The two products evaluated were effective against the test organisms as determined by the rate at which the organisms were killed. Their clinical efficacy in canine ear and skin disease remains to be evaluated. Recently, Lloyd and Lamport reported the importance of the specific formulation of chlorhexidine rather than its concentration for efficacy against these organisms. Although the results reported here are in agreement with the report by Lloyd and Lamport, concentration is also important as evidenced by the effect of a 1/25 dilution of lotion on *M. pachydermatis*. Based on the results, a contact time of at least 2 minutes is desirable for the elimination of *M. pachydermatis* in vitro using a 1/25 dilution of the same lotion.

Several reports have revealed the emergence of a greater proportion of strains with resistance to agents commonly used to treat otitis externa and cutaneous pyoderma. It will be interesting to identify those resistant strains and test the activity of these two products on those isolates.

**CONCLUSION**

Two topical formulations (Hexadene Flush and ResiCHLOR Lotion with Spherulites) evaluated in this study are active against *S. intermedius*, *P. aeruginosa*, and *M. pachydermatis* and may be useful in their clinical management.

**ACKNOWLEDGMENT**

This study was supported by the Virbac Corporation.

**REFERENCES**