Correlation between topical antibiotic selection, \textit{in vitro} bacterial antibiotic sensitivity and clinical response in 17 cases of canine otitis externa complicated by \textit{Pseudomonas aeruginosa}

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\textbf{Introduction}

Bacterial culture and sensitivity has previously been recommended to guide selection of antibiotic therapy in cases of \textit{Pseudomonas} otitis externa.$^{1,4}$ However, \textit{in vitro} sensitivity testing has several limitations with respect to topical antibiotic administration.

These limitations include:

- a failure to take account of the antimicrobial effect of concurrently used cleaners or antiseptics (including the pharmacological effect the pH of the products may have on antibiotic activity)$^{5,9}$
- a failure to account for the high concentrations of antibiotics achieved by topical antibiotics, especially those antibiotics that are concentration dependent$^{9,10}$
- issues with repeatability of identification of cultured organisms, and antibiotic sensitivity of cultured organisms.$^{11,13}$

In the author’s clinic it was found that the majority of cases of otitis dominated by rod bacteria on cytology treated empirically with topical therapies responded well, and that this seemed at odds with the response suggested by \textit{in vitro} sensitivities. The authors hypothesised that \textit{in vitro} sensitivity, as determined by \textit{in vitro} sensitivity testing, would be poorly predictive of the clinical outcome of cases of otitis externa treated with empirically selected therapies.

The purpose of this prospective, double-blinded study was to compare the clinical response to empirically selected topical antimicrobials with the response predicted by disk diffusion antibiotic sensitivity testing.

\textbf{Materials and Methods}

\textbf{Inclusion criteria}

Candidate dogs for inclusion in the trial were patients referred to the Animal Skin Ear and Allergy Clinic at the Melbourne Veterinary Specialist Centre from February 2009 to January 2010. Criteria for trial admission included the presence of clinically diagnosed otitis externa, bacilli identified on vertical canal cytology and \textit{Pseudomonas} sp. subsequently identified on culture of the affected ear(s) as the sole or predominant infectious organism, and client consent for entry into the trial. Exclusion criteria included any neurological or clinical evidence for otitis media.

\textbf{Treatment}

Topical antibiotics, antiseptics, glucocorticoid therapy and the requirement for otic flushing were selected for each case on the basis of cytology and clinical examination only by the examining clinician. Otic flushing was performed if required under general anaesthesia using a video otoscope. Systemic antibiotic therapy was not used. Repeat cytology and examination was performed every 1-2 weeks until the ears were grossly and cytologically normal.

\textbf{Data recording}

For all cases, the duration of disease prior to entry into the trial, the physical examination of the ear canal (including that made under general anaesthesia where applicable), ear canal cytology, complete culture results and all treatments were recorded.

\textbf{Antibiotic Sensitivity}

Antibiotic sensitivity of the isolated \textit{Pseudomonas} sp. was performed at a commercial laboratory using calibrated dichotomous sensitivity (CDS) disk diffusion sensitivity testing (DDST).
Statistics
Treatment was considered successful where no bacteria were found on vertical canal cytology 14 days or less, or if there was clear improvement in bacterial numbers by 14 days, and no bacteria at a subsequent recheck on the same otic antibiotics. Treatment was otherwise considered a failure.

Descriptive statistics were used to analyse the data.

Results
Sixteen dogs with twenty affected ears initially entered the trial. The median duration of disease prior to entry was 5 months (range 4 days to over 12 months). 13/20 ears showed significant ulceration, maceration and purulent exudate without significant stenosis. Only 1/20 ears showed moderate ceruminous hyperplasia leading to mild stenosis. The remaining ears showed milder changes.

Cytology revealed rods (few to numerous) in all cases, neutrophils (few to numerous) in 19/20 ears, cocci (few to moderate) in 7/20 ears and occasional yeast in 4/20 ears.

_Pseudomonas aeruginosa_ was isolated from all ears in a pure (7/20) or mixed culture (13/20). Other isolated organisms included *Staphylococcus* (pseudointermedius (7/13), *Proteus mirabilis* (3/13), β-haemolytic *Streptococcus* (3/13), *Escherichia coli* (2/13), “Mixed skin flora” (2/13), “Mixed anaerobes” (1/13) and “non-fermenting Gram negative bacillus” (1/13).

According to DDST, the _Pseudomonas aeruginosa_ isolates were reported as being all sensitive to polymixin B, variably susceptible to marbofloxacin (14/20), gentamicin (17/20), tobramycin (18/20) and ticarcillin (15/20).

Empirical topical treatments selected are summarised in table 1. All topical medications were compounded, and used either a saline or propylene glycol vehicle with the exception of the gentamicin, which was a commercial product. Triz EDTA, where used, was applied approximately 10 minutes prior to antibiotic administration. In addition, 18/20 ears were also treated with oral prednisolone (0.45-0.71 mg/kg/day), and 12/20 ears were flushed under general anaesthesia.

<table>
<thead>
<tr>
<th>Topical Antibiotic Selected</th>
<th>Antibiotic Only</th>
<th>Antibiotic &amp; Tris EDTA</th>
<th>Antibiotic, Tris EDTA &amp; 0.1% Dexamethasone NaP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5% Enrofloxacin q12h</td>
<td>2</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>6% Ticarcillin-Clavulanic Acid q12h</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0.3% Gentamicin* q24h</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

* Mometamax, Intervet Schering Plough

Table 1: Empirical topical treatment selections.

3/20 ears were excluded because of compliance failure or lack of follow up. Of the remaining ears, 11/17 were reported as resistant to empirically selected antibiotics, but 10/11 were successfully treated clinically. However, 1/10 ears relapsed following an initial cytological response to 1.5% enrofloxacin in propylene glycol with Tris EDTA. This subsequently responded to a second topical antibiotic reported as resistant (6% ticarcillin-clavulanic acid). The 1/11 ‘resistant’ ears that initially failed to respond to empirical topically selected therapy (1.5% enrofloxacin in propylene glycol) subsequently responded to a second topical antibiotic also reported as resistant (6% ticarcillin-clavulanic acid).

6/17 ears tested susceptible to empirically selected antibiotics and 5/6 responded. The 1/6 ‘susceptible’ ears that initially failed to respond to empirically selected topical antibiotics (0.3% gentamicin with Triz EDTA), subsequently responded to a second topical antibiotic reported as resistant (6% ticarcillin-clavulanic acid).

Discussion
In this case series, all cases of otitis externa complicated by _Pseudomonas aeruginosa_ were successfully treated using only topical therapies, with a mean time to bacteriological remission on cytology in most cases of less than two weeks. This strongly suggests that use of systemic antibiotics is not mandatory in at some if not most cases of otitis externa complicated by _Pseudomonas aeruginosa_, and also where neutrophils have been found on cytology, as has been suggested by some authors previously. There is only a single study that involved treating _Pseudomonas_ otitis with a systemic antibiotics alone. Of 54 cases of otitis externa treated with oral marbofloxacin 5 mg/kg q24h for either 21 or 42 days depending on response and following saline flushing, 27.8% of cases resolved, 42.6% improved and 29.6% did not improve. All cases were...
sensitive to marbofloxacin in vitro. This shows clearly that even in sensitive cases, oral antibiosis is unreliable as a sole therapy of otitis externa, but was of benefit in about 70% of cases. While it is unknown if the speed of response in the current study would have been improved by the concurrent use of systemic antibiotics, given the rapid response in the current study compared with use of oral antibiotics alone, it seems unlikely. Further studies are required to clarify this. It is also important to note that only 1/17 ears in this study showed any chronic canal changes, and the presence of these may affect the success rate of topical medications if they failed to penetrate to the full length of the ear canal.

In this case series also, all cases initially reported as being ‘resistant’ to empirically selected therapies ultimately responded to topical antibiotics reported as being ‘resistant’. While the exact reasons cannot be confirmed based on the data in this study, as previously suggested it seems probable that the use of concurrent topical antiseptics and the higher concentration of topically applied antibiotics achieved compared with that available with systemic antibiotics were significant factors. In addition, improvement of the otic microenvironment by control of exudate, ulceration and inflammation may have further contributed to control of Pseudomonas, which normally prefers a moist habitat. Lastly, the possibility of overestimation of reported resistance needs to be considered.

Previous studies comparing DDST with minimum inhibitory concentrations have found that DDST (using CLSI methodology) can both over- and underestimate resistance. Furthermore, the emphasis on correct reporting of susceptibility in the CDS methodology of DDST means that there are no ‘intermediate’ results reported, and that there is a tendency to over-report resistance.

In this study, the rate of reported resistance to enrofloxacin was surprisingly high. A recent Australian survey using CDS reported only 36% of Pseudomonas resistance to enrofloxacin. Susceptibilities to gentamicin (99.1%) and polymixin B (100%) in that report were similar to the current study. The cause for this variation is unknown.

In the study 1/6 cases where the Pseudomonas was reported susceptible failed to respond clinically. The possible explanations for this (as well as the failure of the single ‘resistant case’) include owner compliance, antibiotic inactivation in an inflammatory environment, and inadequate dosing. In general practice, all these causes for possible failure for topical therapies may be increased where more marked otic stenosis exists. A false report of susceptibility is unlikely under CDS, but cannot be ruled out given the inherent variability in culture and sensitivity.

The single case where infection relapsed from cytological negativity while still on the initial empirical therapy may have been a case where clinically relevant resistance developed in vivo to the empirically selected antibiotic, though other possible causes (e.g. compliance) can never be entirely ruled out.

In conclusion, in this study, there was a poor correlation between in vitro antibiotic resistance reported from DDST and in vivo response to empirically selected therapies for Pseudomonas otitis. As a consequence, in practice, an over-reliance on culture and sensitivity based on DDST may lead to overuse of 2nd or 3rd line antibiotics, which ironically may increase the incidence of general resistance in the long term.

So what role is there for otic culture and sensitivity? It has been suggested that otic culture may assist in rational selection of topical empirical therapy, though this was not needed in this study. Sensitivity (and ideally MIC) may be more relevant guiding topical antibiotic selection in cases where topical empirical therapy has failed, there is a uniform (rather than mixed) bacterial population on cytology, and there are no other causes for failure present, as these cases may be more likely to display in-vivo antibiotic resistance, and the uniform population may reduce variability in culture and sensitivity results. Sensitivity is also more likely to be useful (given the limitations of the test) where systemic antibiotic therapies are to be used, though outside otitis media, their benefit in many cases remains questionable. Further studies are required in all these areas.

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