Evidence for possible clinically relevant antifungal resistance in Malassezia pachydermatis: 10 cases

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Introduction

Malassezia pachydermatis is a component of the normal cutaneous flora in the dog. However, alterations of the cutaneous microclimate and/or host defence mechanisms may allow the yeast to multiply and become pathogenic. This can lead to a localised or generalised pruritic dermatosis, often with concurrent yeast otitis. Some dogs may present with yeast otitis only.1

Canine Malassezia dermatitis and otitis have been reported responsive to a wide variety of antifungal medications including a variety of azole derivatives, nystatin, amphotericin B, terbinafine, in addition to other antiseptics and topical therapies such as lime sulphur, acetic acid with and without boric acid, piroctone olamine, silver sulphadiazine, chlorhexidine 3%, povidone iodine, alcohol and an astringent spray (Dermacool, Virbac).1-6 In clinical practice however, three of the authors (DR, GB, RB) had found that in a limited number of cases of yeast otitis (and less commonly dermatitis) there was a failure to respond to azole antifungals, and that other causes for failure of therapy appeared to be ruled out, suggesting possible antifungal resistance being exhibited by the yeast.

Clinical resistance to antifungals by Malassezia spp has only rarely been reported in veterinary or human medicine, and while a number of studies have shown little evidence for in vitro antifungal resistance, multiple reports have demonstrated occasional very high anti-fungal MICs in individual Malassezia species and strains (summarized in Robson 2007).7 Furthermore, resistance by M. pachydermatis has been shown to develop in vitro with multiple passage at near MIC concentrations of antifungals, suggesting that the cellular machinery exists in this species for development of possible clinically relevant resistance.8

One problem in assessing antifungal resistance in Malassezia species though is the ongoing lack of standardisation in in vitro susceptibility testing, though this is currently in development by the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards [NCCLS]).9 In vitro broth microdilution susceptibility tests have been standardised for Candida spp. and other yeast (National Committee for Clinical Laboratory Standards document M27-A2) but are not applicable to most yeasts of the genus Malassezia owing to their obligatory lipophilic nature. In the literature there are a number of papers which have developed MIC methodology for Malassezia species isolated from both humans and animals but there has been limited interlaboratory agreement.10-13 In all papers the methodology has been altered from the original CLSI protocols in order to overcome four main problem areas involved in testing this yeast:

• Finding a suitable growth medium for Malassezia, especially the lipophilic species. This growth medium is often supplemented with a dispersing agent (mild detergent) to overcome the problem of cellular clumping which occurs due to the butyrous nature of this yeast.
• Increasing the inoculum size to counteract the slower growth rate of Malassezia compared to that of Candida species.
• Increasing the incubation time to up to 72 hours, again to counteract the slower growth rate of Malassezia compared to that of Candida species.
• Altering the definition of the MIC end point. Many authors refer to the MIC breakpoint as the level of 50% inhibition of growth, whilst others use a 90% or 100% inhibition level. When testing the azole group of anti-fungal agents, there is also the well known trailing end point problem with MIC determination. Trailing occurs when the turbidity continually decreases as the drug concentration increases but the suspension fails to become optically clear (partial inhibition of growth over an extended range of antifungal concentrations).

Researchers in general have followed two paths when altering the MIC broth micro-dilution methodology to counteract these problems – one in which growth inhibition is measured by modifying growth medium, inoculum size and incubation conditions (modified CLSI protocol) and one where metabolic activity is
measured, usually via a colorimetric assay utilizing a growth medium with an indicator, such as modified Christensen’s urea broth.

The susceptibility of the yeast isolates to anti-fungal agents via disk diffusion methodology has also been standardised though CLSI document M44-A2 2008 but similar limitations exist. Furthermore, correlation between any in vitro and in vivo results for Malassezia spp. in humans OR animals has not been established for any antifungal agent. Nonetheless, it seems probable that high antifungal MICs could correlate with a poor therapeutic response, as they have for Candida spp.

The aim of this study was to examine in vitro antifungal susceptibility of Malassezia spp. isolated from cases of canine otitis where possible antifungal resistance was suspected as a cause for failure, using a variety of in vitro techniques. The hypothesis was that these Malassezia strains would show evidence of in vitro resistance to the empirically selected antifungals they had failed to respond to clinically, when compared with clinically responsive control strains.

Materials and Methods

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 June 2006 to June 2009. Criteria for trial admission included the presence of clinically diagnosed otitis externa and/or Malassezia dermatitis, yeast identified on vertical canal (otitis) or tape (skin) cytology and failure to respond to typically clinically effective empirically selected antifungal therapies. Exclusion criteria included determination of any other clinical causes for possible treatment failure, including underdosing, otitis media, compliance failures, excessive otic exudate or excessive inflammation.

Sample acquisition
Swabs were collected from the vertical canal of affected ears, or by rubbing on affected skin and placed into Amies transport media for transportation to The University of Queensland Veterinary Diagnostic Laboratory (UQVDL) via express post. The UQVDL laboratory staff were blinded to clinical outcomes.

Isolation and Storage
Swabs were inoculated onto Sabouraud Dextrose Agar with chloramphenicol (SC) and incubated in a moist atmosphere at 35°C in air for a period ranging from 3 to 10 days (mean period of 5 days). For short term storage a heavy inoculum of each Malassezia strain was added to a vial of Modified Dixon’s Broth with 20% Glycerol and stored at -80°C. Isolates selected for subsequent sensitivity testing were subcultured onto Modified Dixon’s Agar (MDA) a minimum of three times in preparation for anti-fungal testing and long-term storage. Long term storage was achieved through lyophilisation with 2% skim milk broth.

Identification
Colonies resembling Malassezia (small, smooth, round, matt, friable, cream to buff colour) were examined microscopically and identified as Malassezia pachydermatis if they displayed the following observations and growth characteristics:

- a characteristic small “bottle/acorn” shaped yeast with a wide septum between mother and daughter cells
- growth on standard media without the requirement for supplementation with long chain fatty acids after 3 to 5 days incubation at 35°C
- more luxuriant growth on media containing lipids (Modified Dixon’s Agar Medium)

Further molecular characterisation of the selected strains was obtained through the amplification, extraction, purification and sequencing of the ITS1-5.8S ribosomal RNA gene-ITS2 region of each strain. The resulting sequences were assembled and compared to GenBank nucleotide sequences by using the BLASTN program.

Disk Diffusion Sensitivity Testing
The susceptibility of the yeast isolates to anti-fungal agents via a disk diffusion methodology was assessed following disk diffusion guidelines and interpretive zone sizes based on the CLSI standard published by Rosco Diagnostica A/S using their disk diffusion disks (Neo-Sensitabs™). Certain aspects of this guideline were modified to overcome problems associated with the colony characteristics, slower growth and incubation requirements of Malassezia species compared to Candida species in that:

- the inoculum density was increased in order to produce semi-confuent growth
- a dilute suspension of Tween 80 was added to the inoculum to act as a surfactant
• the use of sterile glass beads and vigorous and continuous vortexing was essential to produce an even inoculum suspension
• incubation conditions were extended to 72 hours (normally 48 hours for the control strains) in a moist atmosphere.

Three antifungal drugs were tested against the test isolates and two clinically responsive control isolates; nystatin (50µg), miconazole (10µg) and clotrimazole (10µg). Control strains (Candida parapsilosis ATCC 22019 and Candida krusei ATCC 6258) were also tested against amphotericin B as an internal quality control step. All tests were conducted in triplicate.

To measure the zones of inhibition, the plates were held above a black, non-reflecting background, illuminated with reflecting light. For the two polyene class drugs (amphotericin B and nystatin) the zones of inhibition were read as the distinct clear zone where no visible growth is present. For the two azole class drugs (miconazole and clotrimazole) if a distinct clear zone was not present, the zone was measured up where colonies of normal size commenced. Interpretation of the zones was as per table 1.

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<th>Antibiotic</th>
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<th>Intermediate</th>
<th>Resistant</th>
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<td>14-10</td>
<td>≤ 10</td>
</tr>
<tr>
<td>Nystatin</td>
<td>≥ 15</td>
<td>14-10</td>
<td>No zone</td>
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<tr>
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<td>≥ 20</td>
<td>19-12</td>
<td>≤ 11</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>≥ 20</td>
<td>19-12</td>
<td>≤ 11</td>
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</tbody>
</table>

Table 1: Interpretation of zones of inhibition for Neo-sensitabs

Broth Micro-Dilution Susceptibility Testing
For broth micro-dilution susceptibility testing (BMDST), the turbidity (representing yeast growth) of 96 well trays containing serially diluted concentrations of antifungal medications are typically read at 24-48 hours (depending on the organism) and compared with that of the positive growth (drug-free) control. The MIC for amphotericin B or nystatin is considered to be the lowest concentration where the well is optically clear. However, the MIC for the azoles are determined to be the well where there is 50% inhibition of growth (prominent decrease in turbidity). CLSI have published interpretative MIC breakpoints for Candida and Cryptococcus species against fluconazole, itraconazole and 5-fluorocytosine only.

In this study, two different modified protocols were used to assess BMDST of the test and both clinical and C. krusei ATCC 6258 and C. parapsilosis ATCC 22019 control isolates. The first protocol was based on the paper of Eichenberg et al. 16 (mSA broth microdilution method). This method was devised to follow as closely as possible the CLSI guidelines whilst still allowing for the distinct biochemical and growth requirements of M. pachydermatis. The second protocol was based on the paper of Nakamura et al 13 (mCU broth microdilution method). This method is based on the urease activity of yeast, with the result that urea in the test medium (modified Christensen’s urea broth) will be converted to ammonia, resulting in an alkaline change in the pH of the medium proportional to the number of surviving yeast, with a visible colour change from yellow/clear to pink using a phenol red pH indicator. Turbidity of the growth media could also be measured, and this was essential for the Candida control strains that were urease negative.

Four antifungal drugs were tested using these two protocols; nystatin, miconazole and clotrimazole. The range of drug concentrations tested for all the anti-fungal agents was 32 to 0.0625µg/ml. Control strains (Candida parapsilosis ATCC 22019 and Candida krusei ATCC 6258) were also tested against amphotericin B using the standard CLSI method (mRP) and modified protocols as an internal quality control step. For all test isolates tested against amphotericin B and nystatin the end-point with all BMDST methodologies was specified to be the lowest drug concentration that prevented any discernable growth (i.e. the well was optically clear). For clotrimazole and miconazole the end-point was the lowest drug concentration where the optical density was less than or equal to 50% of the drug free (positive growth) control. All tests were conducted in triplicate.

Results
Case Selection
Of 33 swabs taken from cases of yeast otitis suspected of displaying clinically relevant resistance, 10 were selected for further identification and culture and sensitivity testing (Samples 1-9). Four of these were paired samples from two dogs but from different body locations (Samples 2, 2a, 6 & 6a). A further two cases were
also included where response to empirically selected therapies occurred and no resistance was suspected. (Samples C1 and C2). These and all other results below are summarised in table 2.

Identification
All samples selected for further testing were characterised as Malassezia pachydermatis based on colony and growth characteristics. Molecular testing confirmed these findings with all samples showing 99-100% correlation with one of three known Genbank sequences – AB118940, AY743637 and EU915453. Both cases of paired samples from the same dogs but different body locations revealed the same strain.

Disk Diffusion Sensitivity Testing: Repeatability and Quality Control
Disk diffusion sensitivity testing (DDST) was generally repeatable with only 6 of 48 tests showing greater than 6mm variation in zone of inhibition diameters between triplicate tests. Interestingly these occurred only with samples 4, 6 and 6a against both the azole antifungals, and may be representative of the greater difficulty in interpreting the edge where a distinct clear zone was not present. None of these affected the interpretation of the test as sensitive, intermediate or resistant.

The results for the control strain Candida krusei ATCC 6258 were within the acceptable normal range, but the Candida parapsilosis ATCC 22019 isolate performed unexpectedly poorly against amphotericin B with a zone of inhibition of 15-16mm where 20-26mm was expected.

Another issue was that despite the modifications in the test procedure, it was difficult to obtain semi-confluent growth for all the strains. It was clear that the strains varied in their growth characteristics, with differences in both the size of the colonies and the vigour of their growth following incubation. Studies on the viable count and absorbance readings at 600nm of each strain indicated that they could be subjectively be classified into two broad groups; those considered “good growers” and strains that were considered to be “poor growers”. The “good growers” produced a heavy growth of moderately sized colonies on MDA within 48 hours of incubation. Semi-confluent growth was achieved by using an inoculum density equivalent to an absorbance at 600nm of 1.0 to 1.2. The “poor growers” produced only a light growth of small colonies after 72 hours incubation on MDA and required a heavier inoculum equivalent to an absorbance at 600nm of 1.2 to 1.5 to produce semi-confluent growth for disk diffusion studies. These strains grew very poorly on SC agar plates even after 72 hours incubation.

Broth Micro-Dilution Susceptibility Testing (mSA): Repeatability and Quality Control
A one-fold dilution difference is regarded as acceptable when comparing MIC results. Of the 56 MICs performed on the test and control isolates using mSA only 3 showed unacceptable repeatability.

The control strains C. krusei ATCC 6258 and C. parapsilosis ATCC 22019 were subjected to the same conditions as the test strains. Both strains exhibited excellent growth in the modified Sabouraud broth and readings taken at 24 hours remained the same as those taken at 48 hours. However, while C. krusei ATCC 6258 showed expected results against all antibiotics and acceptable results between the CLSI method and modified Sabouraud broth, the C. parapsilosis ATCC 22019 strain showed unacceptable variation between the different techniques for amphotericin B, miconazole and clotrimazole. The result for amphotericin B (2µg/ml) was still considered to be in the acceptable range according to CLSI breakpoints.

Broth Micro-Dilution Susceptibility Testing (mCU): Repeatability and Quality Control
Nakamura stated that a good correlation was found between the number of fungal cells and the optical density of modified Christensen’s urea broth, and that urease activity could be measured instead of counting viable cells of Malassezia. This assumption was not supported in this study. The tested isolates varied greatly in their ability to produce a distinct colour change (data not shown). For the strains that produced the strongest colour change (e.g. number 1) all the wells with added yeast turned bright pink, preventing any visual assessment of MIC cut-off and also causing problems with the spectrophotometric assessment. Strain number 7 (a very strong urease producer) was particularly difficult to assess using this method and even after four attempts, no results could be obtained against clotrimazole and miconazole. The negative control well for these strains produced an unusually high absorbance reading; in some strains this reading was almost the same as that for the positive control well, although the wells showed no turbidity. Some urease producing strains though, such as strain 11 producing trays with a strong colour change and excellent visual cut-off points. Weaker urease producing strains required extended incubation (72 hours) to produce a discernable weak colour change; however a visual cut-off for some of the antifungal agents was possible. For these reasons, MICs were determined using spectrophotometric rather than visual assessments of the incubated trays. Unfortunately, there seemed to be no set pattern in how the strains would respond. In addition, repeatability of the mCU method was not as good as the mSA with 8/56 MICs showing unacceptable variability between triplicate tests.
The control strains *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 were subjected to the same conditions as the test strains. However these strains are urease negative and no colour change was discerned in the 96 well trays, however turbidity in the wells was measured spectrophotometrically and MIC cut-off points could be detected. For *C. krusei* ATCC 6258, evaluation of the results of the mCU broth microdilution method when compared with the mSA broth microdilution method and the CLSI method showed surprisingly similar results for amphotericin B, nystatin, miconazole and clotrimazole; with all testing methodologies within a one fold dilution difference. For *C. parapsilosis* ATCC 22019, comparison of the results of the mCU broth microdilution method when compared with the mSA broth microdilution method also showed similar MIC levels (within a one fold dilution difference) for each of the anti-fungal agents but when the results for both test methods are compared to the CLSI method, there was an unacceptable two fold difference against amphotericin B, miconazole and clotrimazole.

**In-vitro Sensitivity of Tested Isolates**

Unfortunately there are no CLSI MIC interpretive guidelines for amphotericin B. The CLSI guidelines state that most *Candida* species will have MICs that cluster between 0.25 and 1.0µg/ml, and that isolates that appear to be clinically resistant to amphotericin B when tested have a MIC of greater than 1.0µg/ml. If this was extrapolated to the test *Malassezia* strains, strains 2a, 6, 6a, 7 and 8 could be classified as resistant if the mSA broth microdilution method is used. Only strains 6a and 7 could be classified as having a MIC for amphotericin B at the resistant level if the mCU broth microdilution method is used. All the test strains as well as the control strains were susceptible to amphotericin B by the disk diffusion method using Rosco Neo-Sensitabs™.

Regarding nystatin, with no available interpretive breakpoints a judgement cannot be made as to the susceptibility or otherwise of test or control strains though MICs were within two dilutions for all except two test and control isolates; using the mCU method MICs were 2 to 8 (median 8)µg/mL and using mSA MICs were 4 to 16 (median 8)µg/mL. All the test strains as well as the control strains were, however, susceptible to nystatin by the disk diffusion method using Rosco Neo-Sensitabs™. The mean diameter of the zone of inhibition for these sensitive strains against miconazole was 18mm.

For theazole drug miconazole, disk diffusion testing showed half of the test strains (numbers 1, 2, 2a, 3, 5 and 7) to be resistant, whilst the other half (numbers 4, 6, 6a, 8, C1 and C2) were susceptible. Test strains resistant by the disk diffusion method also had a MIC of >32µg/ml. These strains in all but one test of one strain produced no zone of inhibition in the disk diffusion tests. The test strains that were sensitive by the disk diffusion method had MICs ranging from 2 to 16µg/ml, with a mean MIC of 8µg/ml. The mean diameter of the zone of inhibition for these sensitive strains against miconazole was 32mm.

By disk diffusion testing, the majority of the strains were susceptible to clotrimazole with only two strains (numbers 1 and 3) found to be highly resistant producing no zone of inhibition. These were also strains resistant to miconazole. Two strains (number 2 and 7) produced variable results in the intermediate/susceptible range against clotrimazole. The mean diameter of the zone of inhibition for these was 19.8mm. However, all these strains produced a MIC by both BMDST methods of >32µg/ml. The strains classified as susceptible to clotrimazole through disk diffusion tests had MICs ranging from 2 to 32µg/ml, with a mean MIC of 8µg/ml. The mean diameter of the zone of inhibition for these sensitive strains against clotrimazole was 38mm.

**Correlation of Perceived Clinical Resistance with In-vitro Resistance**

Of the nine cases where the antifungal perceived as being clinically ineffective was able to be tested in vitro, five were classified as resistant (4) or sensitive / intermediate (1) on disk diffusion testing and all concurrently showed >32µg/ml MIC on both mSA and mCU broth methods.

Of the two control cases where empirically selected antifungals were clinically effective, neither showed evidence for resistance on disk diffusion testing and only a single test on mCU showed an MIC >32µg/ml, however this test also showed a variability of 2 or more fold differences between triplicates for this isolate.

**Discussion**

In this study, in vitro sensitivity testing showed variable repeatability and reliability. Disk diffusion testing was generally repeatable, though the lack of a clear zone of inhibition with theazole drugs appear to make interpretation more difficult. A second problem was the failure of the control *C. parapsilosis* strain to return amphotericin results as expected. A recent study recognised problems with the lack of correlation of disk diffusion results with broth microdilution CLSI MIC levels for amphotericin B, and found that the Neo-
Sensitab method failed to identify some well documented amphotericin B resistant *Candida* isolates. This has prompted Rosco Diagnostica A/S to stop production of amphotericin B discs until further investigations are carried out. However, the subsequent MIC testing in this study also showed poor results when this particular strain was used, perhaps indicating that the strain no longer was performing as per ATCC parameters.

Another difficulty in disk diffusion testing was the variability of *in vitro* growth of isolated strains. The cause for this difference was not clear but did not seem to be inherent to the different *Malassezia* isolates. While strain AB118940 was a uniformly “good grower”, the other strains AY743637 and EU915453 had examples of both “good” and “bad” growing isolates, including a pair of the same isolate from one dog which showed markedly contrasting growth characteristics.

From a technical point of view the mSA broth microdilution method produced more consistent and reliable results. All strains were able to grow in the broth with minimal differences in the readings between 48 and 72 hours incubation. The negative control well (no drug, no yeast) provided a consistent background value for positive growth comparisons. In comparison results from the mCU broth microdilution method varied greatly from strain to strain in terms of the colour change (level of urease activity) and spectrophotometric readings and the negative control well often produced unusually high absorbance readings. Another recent study also found an absence of urease activity in a significant proportion (34.4%) of Malassezia isolates examined. However, when MIC results for individual strains are compared, the mSA method more often produced a higher MIC value than the mCU method; perhaps the growth conditions of the Christensen’s urea broth were not ideal for all strains.

Correlation between results of testing methods was generally good in ‘resistant’ test strains with both BMDSTs finding MICs to the relevant antibiotics $>$32µg/ml in all cases. This also correlated well with a resistant finding on disk diffusion testing for miconazole, but in only 2/4 samples tested against clotrimazole. The remaining 2/4 isolates both reported sensitive/intermediate for clotrimazole on DDST, suggesting that the interpretation of zones of inhibition may need to alter in light of the different test species and alterations to the test medium. Alternatively, it may be prudent to assume possible resistance in the face of a sensitive/intermediate result for clotrimazole. Further study would be required to clarify this.

The correlation of clinical lack of response or response with *in vitro* ‘resistance’ or ‘sensitivity’ was moderate, with the expected results occurring in seven of eleven samples. The lack of perfect correlation is not surprising because a failure of clinical response may occur because of problems with topical drug administration (possibly an issue in cases 6, 6a and 8 because the tympanic membrane was still able to be observed at the time of sampling despite medication use the previous day; data not shown), host immunity (possibly an issue in case 4 where there was a severe keratinisation defect present; data not shown) and topical pharmacological issues (fungistatic properties, poor absorption, distribution or metabolism) in addition to clinically relevant anti-fungal drug resistance. Another consideration, while not found in this study, is clinical response in the face of *in vitro* resistance. Aside from limitations inherent to *in vitro* testing giving a false resistance, drug synergism (such as that between polymixin B and miconazole) and the high concentration of topically applied antifungal may be able to overcome some resistance mechanisms.

The weight of evidence in this study supports the presence of clinically relevant antifungal drug resistance in at least some of the *Malassezia pachydermatis* isolates in that several strains showed no zone of inhibition to clinically ineffective antifungals in a modified standardised disk diffusion and several strains showed MICs $>$32µg/ml (commonly associated with resistance) to clinically ineffective antifungals. However, the significance of these findings can only be validated when standardised techniques become available as MIC results can vary significantly depending on the testing protocol and conditions.

If the strains identified in this study are truly resistant to miconazole and or clotrimazole, then the question arises is this likely to be intrinsic or acquired resistance? In this study there is was only 1 of 3 strains that was solely represented by ‘resistant’ isolates – AB119940. While this may seem to suggest a degree of intrinsic resistance in this strain, numbers in this study were low, and two isolates of this strain from the same dog showed different patterns of resistance (samples 2 and 2a). The other two strains showed both ‘resistant’ and ‘susceptible’ phenotypes. This suggests that resistance in these cases may be an acquired characteristic.

If this is the case, it would make sense, given the evidence that *Malassezia* may develop higher MICs with passage exposed to sublethal doses of antifungals, that cases involving ‘resistant’ isolates would have had greater previous exposure to relevant antifungal drugs. This supposition is supported in this study, where
dogs with ‘resistant’ isolates having a mean of 4.4 (range 1-9) courses of azole (topically or orally) and / or nystatin (topically) medications and dogs with ‘sensitive’ isolates only a mean 0.8 (range 0-2) courses in the 12 months previous to sampling in the study. Furthermore, in all cases where ‘resistant’ isolates were identified there had been at least one occasion where a clinical failure of an antifungal had been reported prior to the clinical failure that precipitated entry into the trial. This evidence also supports the possibility of acquired resistance being more likely than intrinsic resistance.

The mechanisms of possible resistance in this study remain unknown and only one study previously has defined a possible resistance mechanism in Malassezia pachydermatis. However, numerous investigators have studied the mechanisms of resistance to antymycotics in other yeast species, especially Candida spp. If clinical resistance as suggested in this study is confirmed in Malassezia spp., it seems likely that the mechanisms of resistance will be similar to some of those already characterised in other species. These have been previously summarised.

In conclusion, this study supports the possibility that the failure of some cases of canine Malassezia pachydermatis infection may be due to antifungal resistance, but until standardised testing techniques are developed this remains difficult to confirm.

References
1. Carlotti DN Proceedings 2006 UC Davis George H Muller Veterinary Dermatology Seminar
<table>
<thead>
<tr>
<th>Sample</th>
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<th>GenBank ID</th>
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<td>S 8 4</td>
</tr>
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**Table 2: Summary of Data for All Test Strains**

**Key**
- No definitive result – no spectrophotometric or visual cut-off
- “Poor grower” strains
- Tests not performed
- ^ MIC cut-off was taken at the level of 50% inhibition
- * Two-fold or more difference in results
- # Failed clinical response
- + Successful clinical response
- ? Unconfirmed clinical response
- ❗ Acceptable Range 1.0 - 4.0, CLSI M27-A2, Recommended 48hour MIC limits for quality control strains for Broth Microdilution
- ✤ Acceptable Range 0.5 - 4.0, CLSI M27-A2, Recommended 48hour MIC limits for quality control strains for Broth Microdilution
- mCU protocol based on Nakamura et al. 13
- mSA protocol was based Eichenberg et al. 10
- mRP CLSI reference method
- & Variation in zone of inhibition > 6mm

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