

Mucky ears, swabs and smears: A guide to interpreting diagnostics for otitis externa

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Disclosure Ariane:

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Disclosure Marta:

Full-time Employee of IDEXX

The information contained herein is intended to provide general guidance only. As with any diagnosis or treatment you should use clinical discretion with each patient based on a complete evaluation of the patient, including history, physical exam and presentation, and laboratory data. With respect to any drug therapy or monitoring program, you should refer to a product insert, for complete description of dosage, indications, interactions, and cations, Diagnosis, treatment, and monitoring should be patient specific and is the responsibility of the veterinarian providing primary care.



ILOs

+ Review primary, secondary,predisposing and perpetuating factors

 Develop an understanding of ear cytology, and the use of culture and microbiology results in otitis diagnosis

+ Identify treatment strategies for chronic otitis



Primary causes:

- Hypersensitivity *
- Parasitic *
- Space occupying lesions *
- Foreign bodies *
- Endocrinopathies
- Immune system pathology
- Congenital abnormalities



Secondary Causes:

Infections (bacterial and fungal) are secondary and represent **DYSBIOSIS**

Perpetuating factors:

Chronic acquired changes that prevent resolution

- hyperplasia and thickening
- ear canal stenosis
- occlusion, fibrosis and mineralization
- otitis media
- cholesteatoma

Predisposing factors:

- Anatomy and conformation
- Life style and management



Primary causes:

- Hypersensitivity *
- Parasitic *
- Space occupying lesions *
- Foreign bodies *
- Endocrinopathies
- Immune system pathology
- Congenital abnormalities



Drive the inflammation

Allergies common!

Otitis can be ONLY clinical sign

Food relative common

Need to identify & address to avoid relapse

Further tests needed

Overgrowth only possible due to PPP

Often cocci (Staph pseudintermedius

+/- yeast (Malassezia spp)

Other also possible

Pseudomonas particularly difficult to manage

Identify by doing cytology

When rods seen → C&S

Biofilm!



Secondary Causes:

Infections (bacterial and fungal) are secondary and represent **DYSBIOSIS**

Predisposing factors:

- Anatomy and conformation

- Life style and management



Present prior to onset of otitis

Most cannot be changed (conformation

Life-style changes (swimming) may be helpful

Caused by chronic otitis
Cause self perpetuation

Anti-inflammatory meds often needed

Imaging if OM suspected



Perpetuating factors:

Chronic acquired changes that prevent resolution

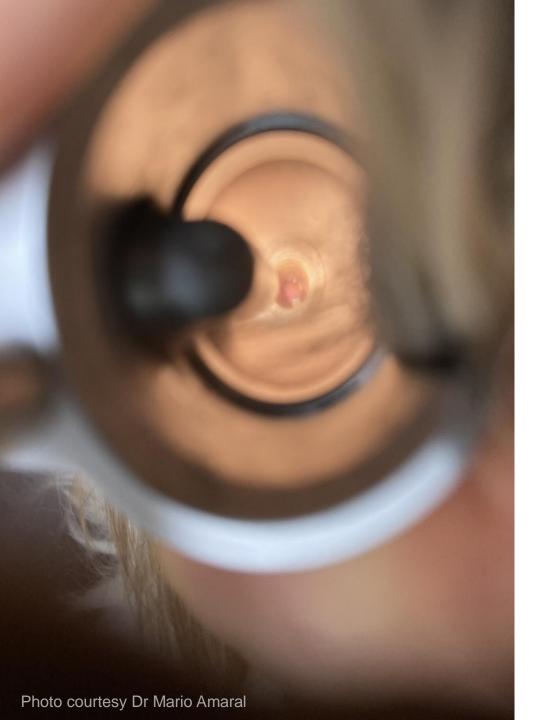
- hyperplasia and thickening
- ear canal stenosis
- occlusion, fibrosis and mineralization
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- cholesteatoma





Ear anatomy

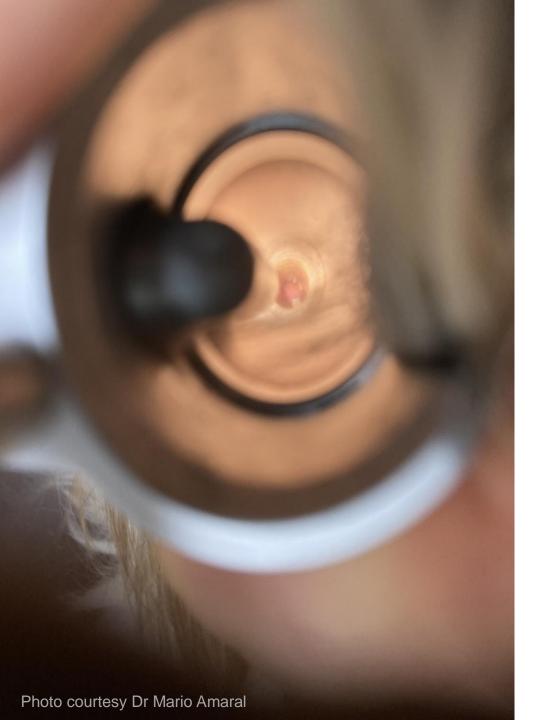
- Explain to owners
- Epithelial migration → self cleaning process
 - Gets overwhelmed in a diseased ear



Ear exam and smear examination is essential in otitis externa

- Evaluate for primary, predisposing and perpetuating factors;
- Evaluate the amount and type of exudate in the ear canals;
- Estimate the amount inflammation;
- Identify hyperplasia, masses, and foreign bodies;
- Determine the status of the tympanic membrane
- Get clues for S





Ear exam: palpate, look, smell, look deeper...

- Evaluate for stenosis, hardening of ear canal
- Evaluate for other signs of generalized & dermatological disease
- Get clues for possible Ps
- Get help formulating therapy (nature of otic discharge, status of TM)

Ear cytology

- + Provides information that can help guide treatment
- + Most important test!
- + Quick
- + Inexpensive
- + EVERY otitis patient & BOTH ears: initial consult & follow up
- + Sometimes allows identification of primary causes (e.g. parasitic) or rule out ceruminous otitis only
- + Most will have variable numbers of:
 - + Keratinaceous material
 - + Yeast or bacterial organisms
 - + Variable inflammation
- + Quantification and monitoring of response to treatment



How?

Cotton bud/gloves







Sample processing

DiffQuick



How to...

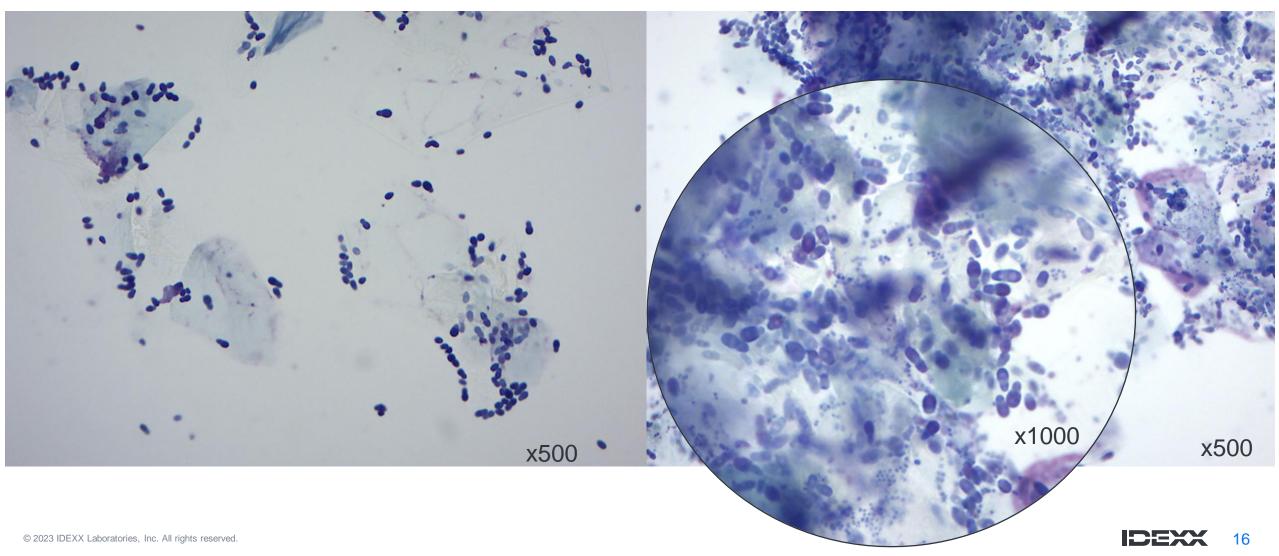
- + Fixation: heat? Solution 1?
- + Air dry
- + Eosin red?
- + Methylen blue!!!



Different ears – different findings

Left ear – Malassezia only

Right ear – Malassezia and cocci





- it gets easier



Examining the smear

+ Start with low power

+ Examine the whole smear with a "battle ship movement"



+ Choose the most cellular / well spread/ well preserved areas

- + Increase to higher power field for identification and quantification of organisms:
- + Look for organisms: cocci, rods, yeast
- + Look for inflammatory cells



Ceruminous Otitis

No/few microorganisms detected

- No parasites

- No/few bacteria

- No/few yeasts

Idiopathic seborrhoea and other keratinization disorders

The excess cerumen will predispose to bacterial and Malassezia overgrowth





Otodectes cynotis

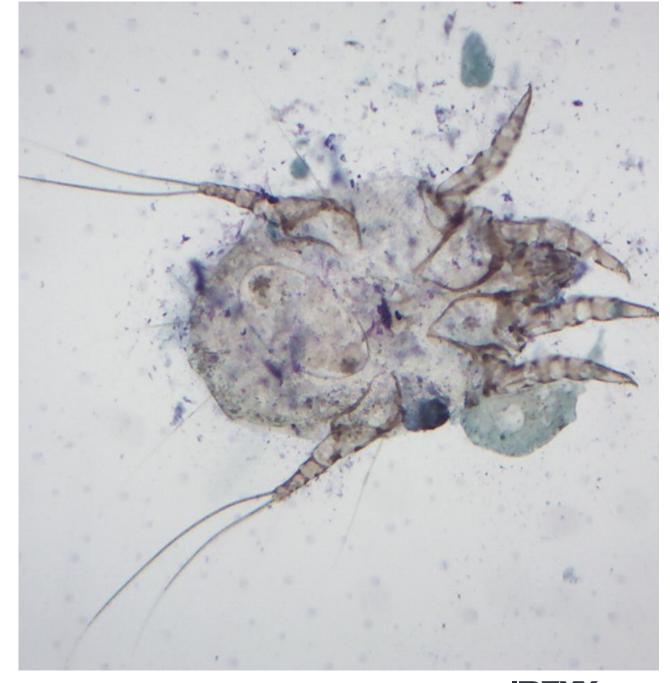
Parasites are primary cause of otitis

May have secondary bacterial overgrowth/infection

Best diagnosed in fresh smears

Up to 85% of feline otitis externa

Reinfestation can occur after treatment (specially from other pets)



Site: LEFT EAR:

Aerobic Culture - Ear

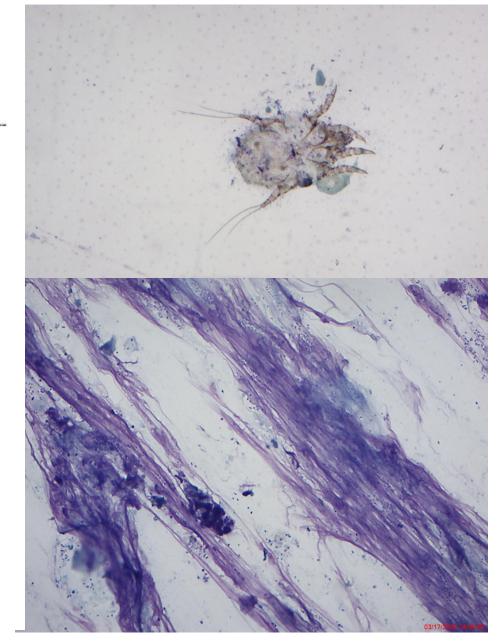
Isolate 1 Profuse growth:Staphylococcus felis

Antibiotic	Result	MIC	Sensit	Sensitivity Range		
Ampicillin (1)	Resistant					
Amoxicillin (1)	Resistant					
Amoxicillin-Clavulanic acid (1)	SENSITIVE					
Cloxacillin (1)	SENSITIVE					
Enrofloxacin (2)	SENSITIVE	<=0.5	0.5	Siir	4	
Marbofloxacin (2)	SENSITIVE	<=0.5	0.5	Ssirr	8	
Pradofloxacin (2)	SENSITIVE	<=0.12	0.12	Ssiirr	4	
Neomycin (2)	SENSITIVE	<=2	2	Sssir	32	
Chloramphenicol (1)	SENSITIVE	<=4	4	Ssirr	64	
Ofloxacin (2)	SENSITIVE					
Fusidic acid (2)	SENSITIVE	<=0.5	0.5	Ssrrrr	32	
Minocycline (1)	SENSITIVE	<=0.5	0.5	Ssssir	16	
Cephalexin (1)	SENSITIVE					
Gentamicin (2)	SENSITIVE	<=0.5	0.5	Ssssir	16	
Ciprofloxacin (2)	SENSITIVE					
Florfenicol (1)	SENSITIVE	<=4	4	Ssrr	32	

Organism identified by MALDI-TOF as Staphylococcus felis (coagulase-negative), which is regarded as a primary pathogen when recovered from urine. It is potentially pathogenic when recovered from skin, wounds, ears, abscess or conjunctiva.

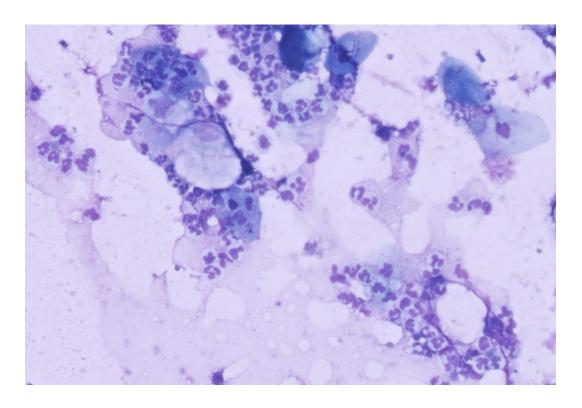
Note:

Standardised susceptibility tests do not reflect in vivo activity of topical antibiotics due to the high levels achieved in the target site with topical administration. Generic antibiotics quoted. The choice of antibiotic and knowledge of any contraindications is the Veterinary Surgeons responsibility. MIC units expressed in ug/ml. Antibiotics without a MIC have been predicted using international guidelines. For more information on interpretation of MICs visit idexx.co.uk/MIC



Neutrophils only

- Inflammation is not infection!
- Remember allergies and foreign bodies!





The ear is not sterile...

- Just because we see/grow bacteria does not implicate bacterial otitis
- Consider **DYSBIOSIS**
- High numbers of organisms overgrowth and/or infection
- Phagocytosis and intracellular bacteria ongoing active infection
- If reporting number of organisms present:
 - average of 10 oil immersion (x1000) microscopy fields:

DEBATABLE!

- use semiquantitative assessment



Common organisms found in normal ears and in otitis

Normal ears

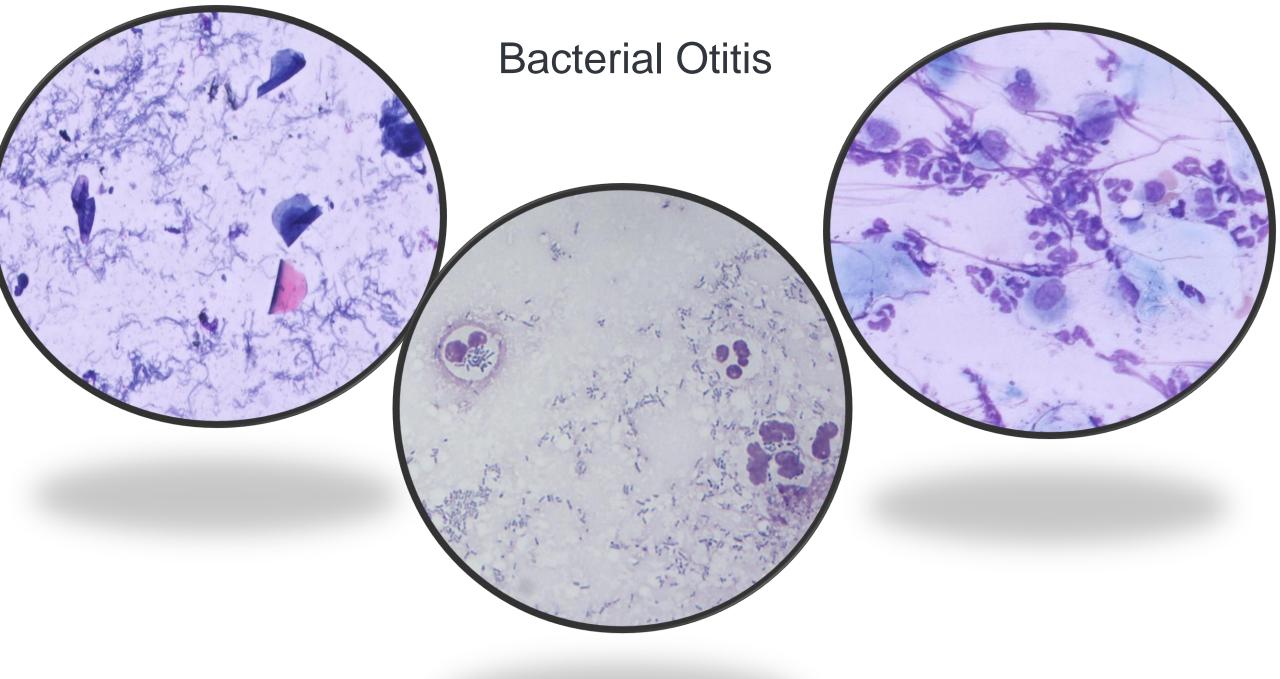
- + Malassezia pachydermatis*, other Malassezia spp. and other yeasts
- + Staphylococcus pseudintermedius*
- + Staphylococcus schleiferi subsp. coagulans
- Coagulase-negative staphylococci
- + Corynebacterium spp. †
- + Streptococcus spp.
- Other species
 - + Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes

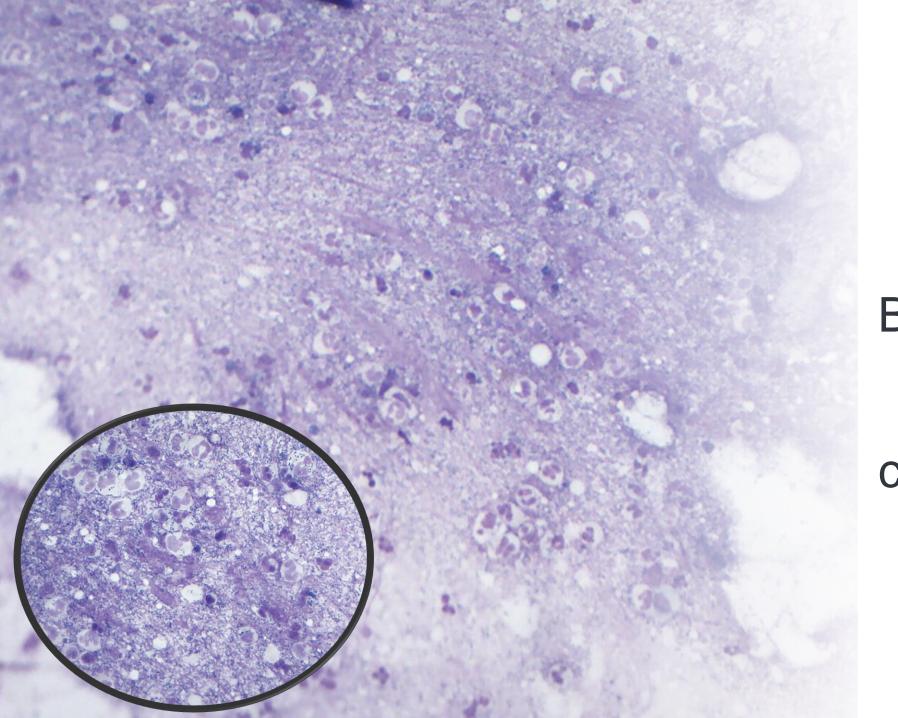
Ears with otitis externa

- + Malassezia spp.*
- + Staphylococcus pseudintermedius* and other staphylococci
- + Pseudomonas aeruginosa*
- + Proteus mirabilis*
- + Beta-haemolytic streptococci (e.g. S. canis)
- + Corynebacterium spp.
- + Enterococcus spp.
- + Escherichia coli



Otitis media: Also consider anaerobes





Septic Neutrophilic Inflammation Bacterial Otitis

Biofilm can complicate the picture



Biofilm and Bacilli consider Pseudomonas

Isolate 1	Profuse growth:Pseudor	nonas aeru	iginosa		
Antibiotic	Result	MIC	Sensitivity Range		
Ampicillin (1)	Resistant	N/A		(Intrinsic R)	
Amoxicillin-Clavulanic acid (1) Resistant	N/A		(Intrinsic R)	
Enrofloxacin (2)	SENSITIVE	0.5	0.12	ssSiir	4
Gentamicin (2)	SENSITIVE	<=1	1	Sssir	16
Clindamycin (1)	Resistant	N/A		(Intrinsic R)	
Amikacin (2)	SENSITIVE	<=2	2	Ssssir	64
Tobramycin (2)	SENSITIVE				
Polymyxin B (3)	Intermediate	1	0.25	iilirrr	16
Cephalexin (1)	Resistant	N/A		(Intrinsic R)	
Marbofloxacin (2)	SENSITIVE	1	0.5	sSir	4
Cefovecin (2)	Resistant	N/A		(Intrinsic R)	

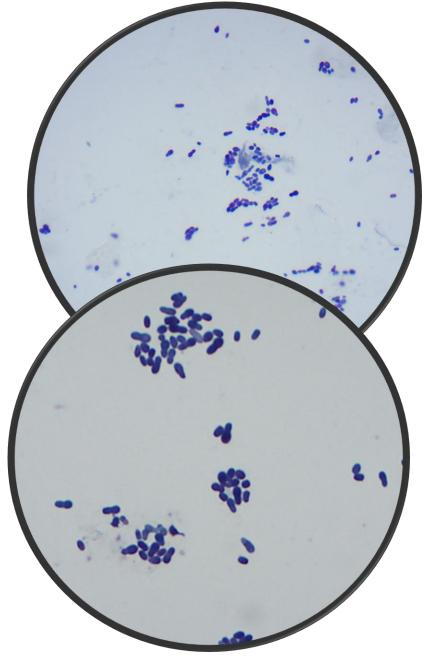
Pseudomonas aeruginosa may develop resistance during prolonged therapy with all antimicrobial agents. Therefore, isolates that are initially susceptible may become resistant within three or four days after initiation of therapy. Testing of repeat isolates may be warranted.

in mixed bacterial growth.

Note:

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Malassezia Otitis

Semi quantitative assessment:

Varies with studies

- dry hpf (40x)
- mean yeasts/hpf $\geq 1 5 10$ abnormal

However:

- overlap in yeast densities in skin samples from healthy and diseased dogs
- relatively small numbers of organisms may lead to skin disease in sensitised individuals

"Factors such as important variations in anatomical site, breed, sampling method and host immune status commonly thwart the interpretation of the clinical significance of an observed population ("XX yeasts in YY fields"); trial therapy is routinely required to establish this."

Bond R, et al Biology, diagnosis and treatment of Malassezia dermatitis in dogs and cats: Clinical Consensus Guidelines of the World Association for Veterinary Dermatology. Vet Dermatol. 2020 Feb;31(1):75. doi: 10.1111/vde.12834. PMID: 31957203.

- May have limited benefit in otitis externa
- Allows to identify if only mixed flora
- Most cases of infection are due to :
 - Malassezia spp
 - Staphylococus spp (cocci)
 - Pseudomonas (bacilli)
- More useful in recurrent cases or with organisms with unusual morphology
 - e.g., coryneform, cocci-bacilli, filaments, yeasts, hyphae, etc
- Direct microscopy findings aid in the determination of clinical significance of isolates e.g., bacterial morphology associated with inflammation and phagocytosis.
 - Always do Cytology before and when doing culture



IDEXX SERVICES: CANA, EARSW
SAMPLES RECEIVED: Pink cap e-swab

MICROBIOLOGY

*Anaerobic Culture ^a Moderate growth mixed anaerobes

*Site: BOTH EARS:

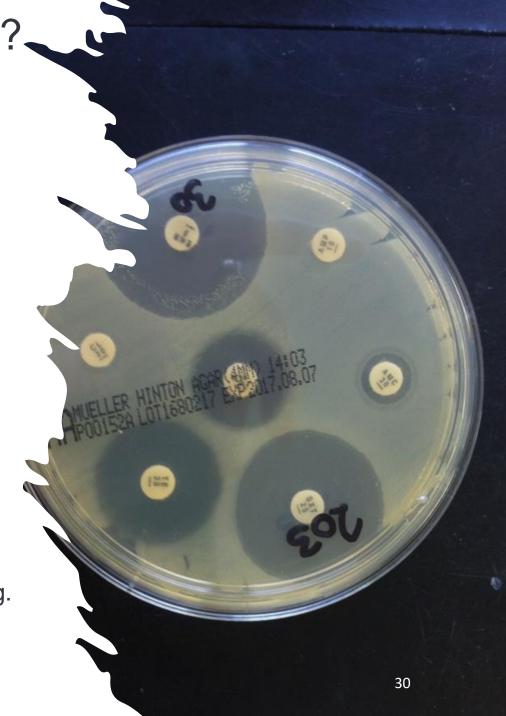
Aerobic Culture - Ear

Isolate 1 Profuse growth:Pseudomonas aeruginosa

Antibiotic	Result	MIC	Sensitivity Range		
*Amikacin	SENSITIVE	<=2	2	Ssssir	64
*Gentamicin	SENSITIVE	<=1	1	Sssir	16
*Ciprofloxacin	SENSITIVE	0.25	0.06	ssSssir	4
*Enrofloxacin	Intermediate	1	0.12	ssslir	4
*Marbofloxacin	SENSITIVE	<=0.5	0.5	Ssir	4
*Polymixin B	SENSITIVE	1	0.25	ssSsrrr	16
*Ofloxacin	SENSITIVE				

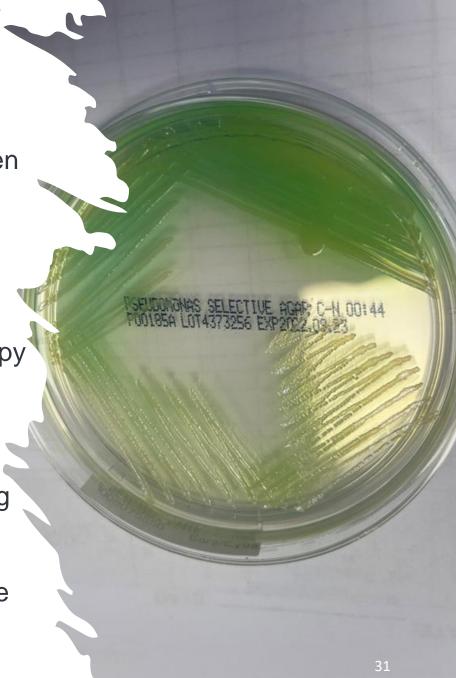
in mixed bacterial growth.

- ASTs are based on systemic breakpoints
- The results can be poorly predictive of the response to topical treatment.
 - If R on AST may respond in vivo due to high concentration that can be achieved on site
 - If S on AST may not respond in vivo due to local factors (e.g. inflammation, biofilm, ear stenosis, etc)
- May be useful with bacilli infection (e.g. Pseudomonas vs Enterobacterales vs Corynebacteria)



Typical indications for Culture include the following:

- Chronic otitis associated with bacteria (cocci and/or rods) seen on cytology
- Rods (bacilli) seen on cytology
- Organisms with unusual morphology
- Suspected or confirmed cases of otitis media (systemic therapy may be indicated)
- History of multidrug-resistant bacteria
- History of long-term oral or topical antibiotic therapy (including for other conditions
- Bacteria persisting on cytology despite apparently appropriate therapy



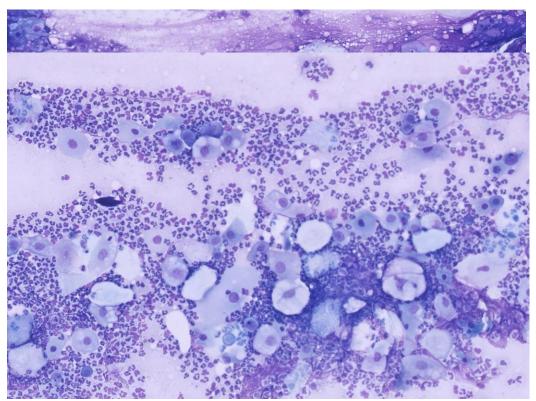
SPECIES	AGE			
Canine	9y 9m (03/06/2014			
BREED	SEX	NEUTERED	ENTIRE	
Basset Hound	F	х		



Please provide history to allow for interpretation (please indicate Differential Diagnosis / Disease Suspected / Specific questions you would like arrawmed)

DOG MAS BEEN ON GENTAMICIN TOPICALLY FOR 4-6

WEEKS.



Ear Aerobic Culture

Isolate 1 Profuse growth:Pseudomon	nas aeruginosa			
Antibiotic	Result	MIC	Sensitivity Range (Intrinsic R)	
Ampicillin (1)	Resistant	N/A		
Amoxicillin-Clavulanic	acid (1)Resistant	N/A	(Intrinsic Ŕ)	
Enrofloxacin (2)	Íntermediate	1	0.12 sssIir´	4
Gentamicin (2)	Resistant	>=16	1 sssiR	16
Clindamycin (1)	Resistant	N/A	(Intrinsic R)	
Amikacin (2)	Resistant	>=64	2 ssssiR	64
Tobramycin (2)	Resistant			
Polymyxin B (3)	Intermediate	1	0.25 iiIirrr	16
Ofloxacin (2)	SENSITIVE			
Cephalexin (1)	Resistant	N/A	(Intrinsic R)	
Marbofloxacin (2)	SENSITIVE	1	0.5 sSir	4
Cefovecin (2)	Resistant	N/A	(Intrinsic R)	
Ciprofloxacin (2)	SENSITIVE	0.5	0.06 sssSirr	4

Pseudomonas aeruginosa may develop resistance during prolonged therapy with all antimicrobial agents. Therefore, isolates that are initially susceptible may become resistant within three or four days after initiation of therapy. Testing of repeat isolates may be warranted.

Standardised susceptibility tests do not reflect in vivo activity of topical antibiotics due to the high levels achieved in the target site with topical administration. Please note that topical treatment with the antimicrobials listed as intermediate (e.g. Polymyxin B) may be effective in this case given the high concentrations achieved at the site.



Take Home MSG

- Physical exam and looking for primary causes essential
- Cytology will provide essential information on initial consult...
- ...and at every subsequent visit until cure is achieved
- Recurrent cases need addressing underlying causes
- Culture may be useful in identifying which organisms are present
 - Overgrowth of normal flora
 - Dysbiosis
 - Infection
 - AST provides limited but valuable information

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Traditional ear cytology can be challenging

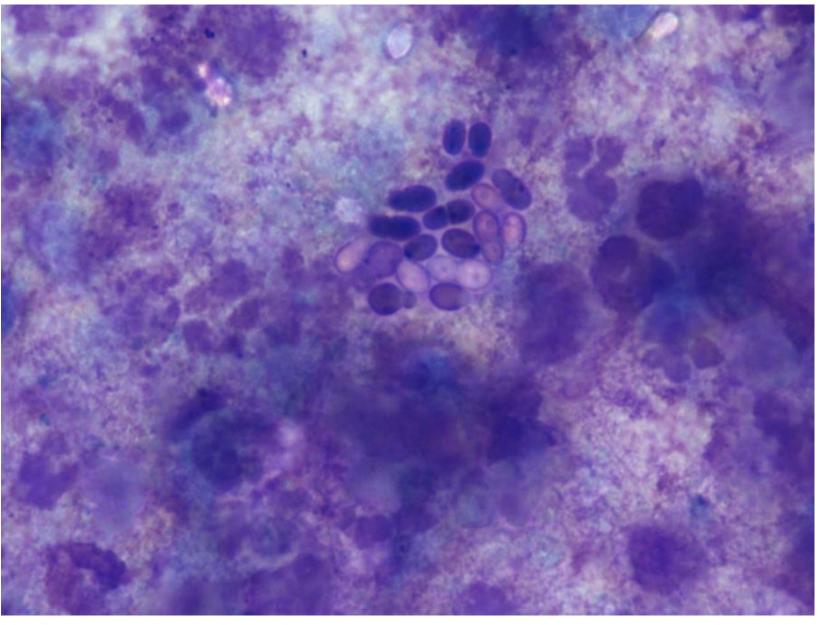
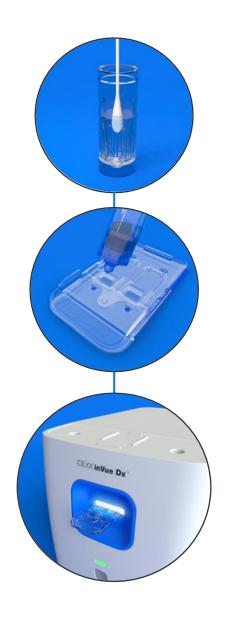


Photo courtesy of Dr. Elizabeth Layne

IDEXX inVue Dx[™] analyzer: ear cytology workflow



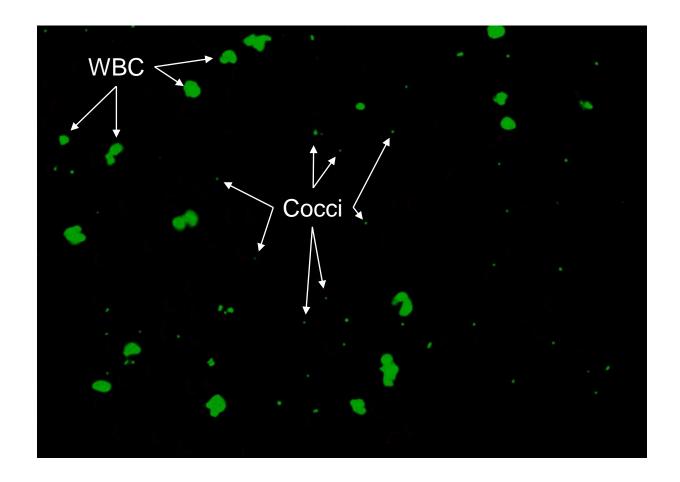
Put sample in the reagent.

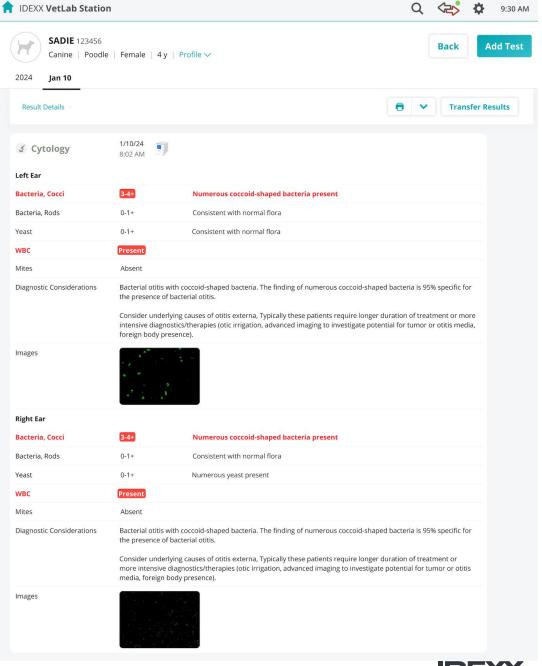
2 Drop sample into cartridge.

Insert and press the Start button.



IDEXX inVue Dx[™] analyzer: Ear cytology







Thank you all for your attention!
Questions?



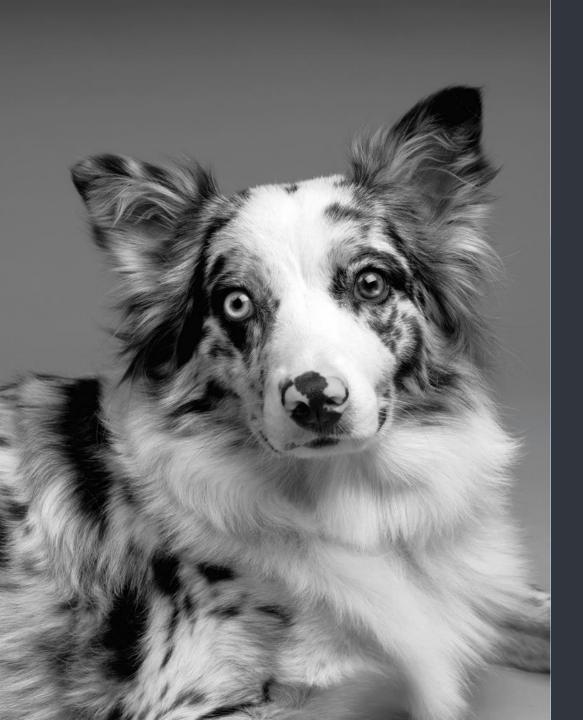
Questions?



References

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- Angus JC. Otic cytology in health and disease. Vet Clin North Am Small Anim Pract. 2004 Mar;34(2):411-24. doi: 10.1016/j.cvsm.2003.10.005. PMID: 15062616.
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- + Tang S, Prem A, Tjokrosurjo J, et al. The canine skin and ear microbiome: a comprehensive survey of pathogens implicated in canine skin and ear infections using a novel next-generation-sequencing-based assay. Vet Microbiol. 2020;247:108764. doi:10.1016/j.vetmic.2020.108764
- + Bond R, Morris DO, Guillot J, Bensignor EJ, Robson D, Mason KV, Kano R, Hill PB. Biology, diagnosis and treatment of Malassezia dermatitis in dogs and cats: Clinical Consensus Guidelines of the World Association for Veterinary Dermatology. Vet Dermatol. 2020 Feb;31(1):75. doi: 10.1111/vde.12834. PMID: 31957203.





Tricks for tricky ears: treatment, complications and management of chronic otitis

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ILOs

+ To identify strategies to avoid complications from otitis

+ To understand what information is provided in the microbiology report and how it can be used in treatment of otitis

+ To develop treatment regimens adapted to the individual patient

+ To recognize the role of analgesia in the management of ear disease





Otitis

Very common clinical presentation

Common reason to change practice

Acute vs chronic
Unilateral vs bilateral
Can be extremely painful!

→ fear aggression/avoidance common!



Otitis clinical signs

Head shaking Smell Scratching ear Acute moist dermatitis behind ear Head tilt

Increased discharge

Rubbing ear

Pain Fear agression Behavious hanges → Get it right first time!





Dermatological Examination



Palpate ear canal







Otic discharge

- Brown coffee ground
 - Ear mites
- Pale yellow waxy
 - cocci, yeast, demodex
- Pale brown waxy
 - cocci, yeast
- Purulent maloderous
 - Pseudomonas
- Black watery or thick
 - Pseudomonas
- Thick chocolate colour
 - cornification defect/Malassezia & biofilm

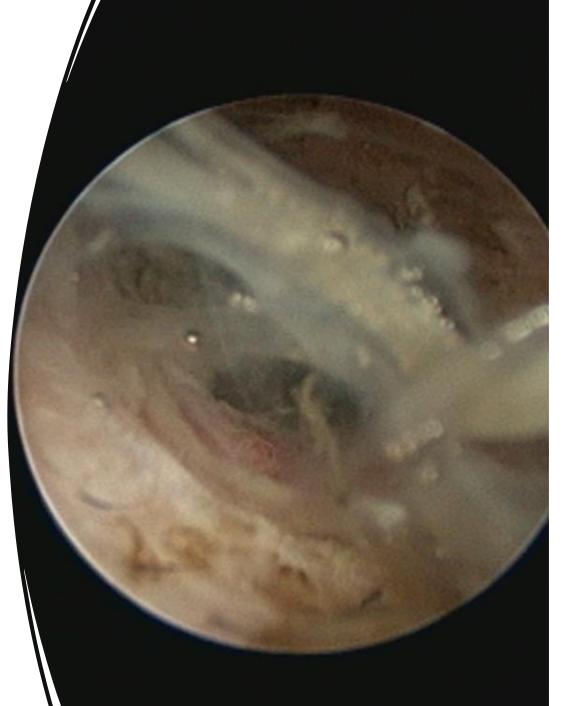


Otoscopy

- Important way to examine patients with otitis
- Ask owners to train dogs from puppyhood
- Use in conjunction with other techniques (cytology)
- Warm up cone
- Nose down, pinna up
- Might require analgesia/sedation/GA if painful
- Pre-treatment with GCS to "open up" if needed



Pseudomonas











Good quality microscope
Stain (Romanowsky-type, Methylene blue)
Immersion oil
Slides, cover slips
Cotton buds



Gloved finger

If patient head shy/fear aggressive

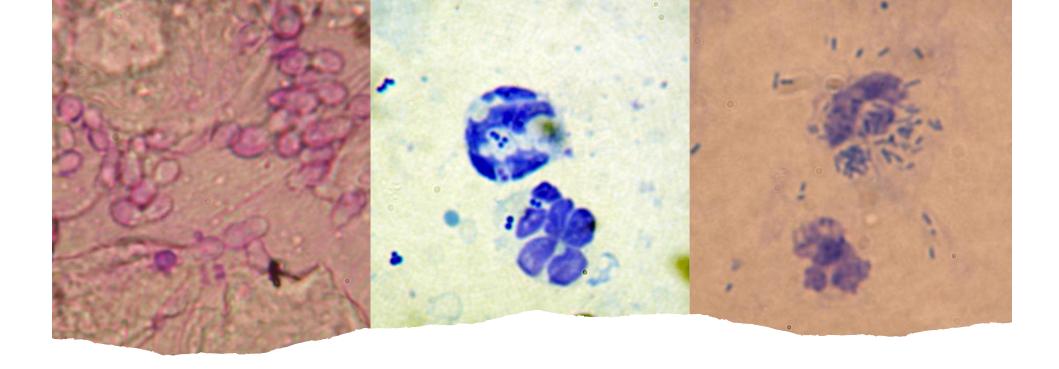
Massage ears

Distract

Slip finger in

Dab onto slide





Organisms

Which type?

Bacteria/yeast?

Cocci/rods?

Normal numbers?

Infection/overgrowth?



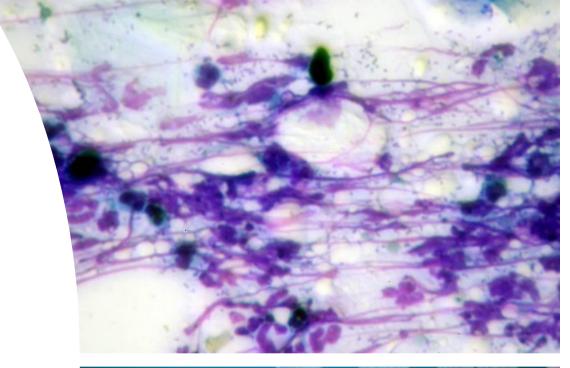
Cytology vs culture/sensitivity

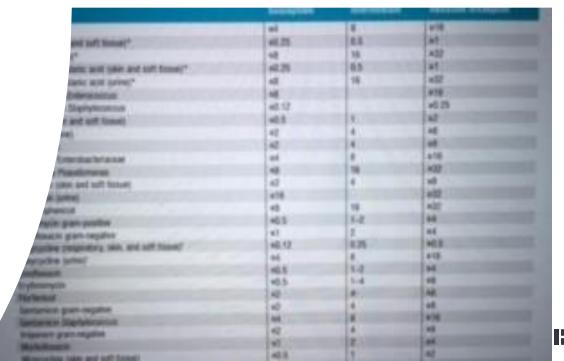
Cytology: for (almost) every case

C&S:

- when rods are seen
- when Tx not effective
- When middle ear disease present (systemic AB)
- With unusual organism morphology

C&S cannot replace cytology





We can have discordant results ...

Bacteria +/ WBC+ Culture negative

- Other causes of inflammation
- Bacteria nonviable
 - AM use
 - Extreme conditions (e.g. Temp; pH)
 - Lack of growth media
 - WBC inhibition
- "Pseudobacteria"
- Contaminated reagents
- Non-significant growth

Bacteria - / WBC-Culture positive

- Low bacterial numbers on cytology
- Lack of inflammation/reduced WBC migration
- Bacteria obscured by debris
- Growth of contaminants/ commensal flora
- Culture is more sensitive



Is Culture Beneficial?

IDEXX SERVICES: CANA, EARSW SAMPLES RECEIVED: Pink cap e-swab

MICROBIOLOGY

*Anaerobic Culture ^a Moderate growth mixed anaerobes

*Site: BOTH EARS:

Aerobic Culture - Ear

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*Marbofloxacin	SENSITIVE	<=0.5	0.5	Ssir	4		
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*Ofloxacin	SENSITIVE						

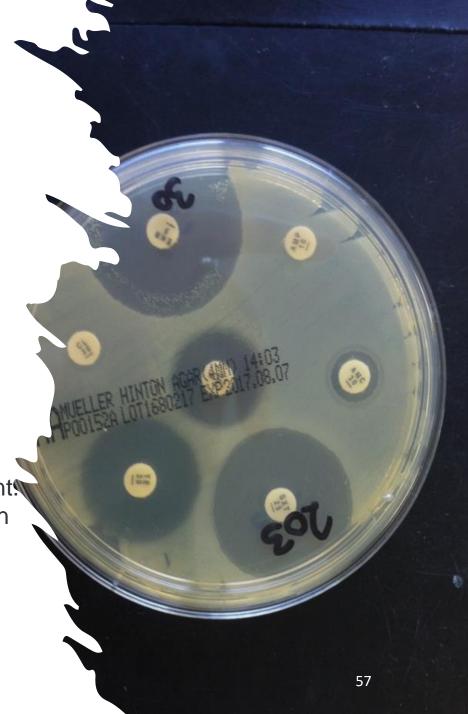
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The results can be poorly predictive of the response to topical treatment.

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- May be useful with bacilli infection (e.g. Pseudomonas vs Enterobacterales vs Corynebacteria)



Antimicrobial Susceptibility Testing

- + Why do we do it?
 - + To predict outcome of therapy
 - + But often we already started therapy "why is it not working?"
 - + Two types of resistance
 - + Intrinsic/innate/inherent PSEUDOMONAS have many....
 - + Acquired
 - + Because we know AMR is increasing
- + Disclaimer
 - + Testing is still only a guideline to treatment
 - + Not all organs/systems behave the same
 - + Not all patients have the same AM distribution/metabolism
 - + Patient response ultimately confirms adequacy of treatment



Is Culture Beneficial?

0.5

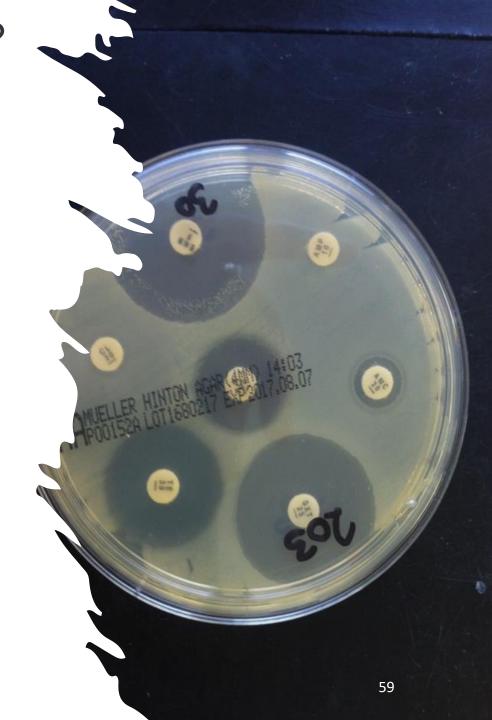
Ear Aerobic Culture

Ciprofloxacin (2)

Isolate 1 Profuse growth:Pseudomonas aeruginosa Antibiotic Result MIC Ampicillin (1) Resistant N/A Amoxicillin-Clavulanic acid (1)Resistant N/A Enrofloxacin (2) Intermediate Gentamicin (2) Resistant >=16 Clindamycin (1) Resistant N/A Amikacin (2) Resistant >=64 Tobramycin (2) Resistant Polymyxin B (3) Intermediate 1 Ofloxacin (2) SENSITIVE Cephalexin (1) Resistant N/A Marbofloxacin (2) SENSITIVE Cefovecin (2) Resistant N/A

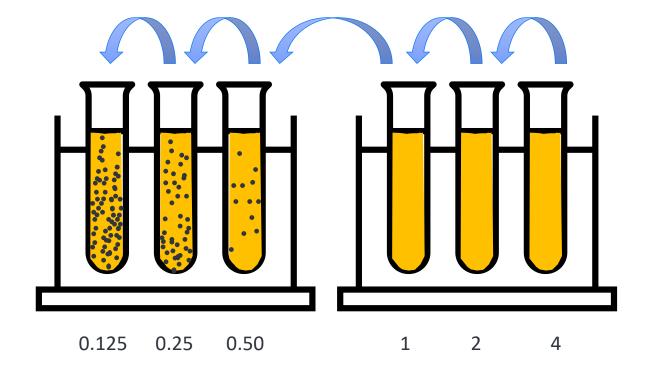
Standardised susceptibility tests do not reflect in vivo activity of topical antibiotics due to the high levels achieved in the target site with topical administration. Please note that topical treatment with the antimicrobials listed as intermediate (e.g. Polymyxin B) may be effective in this case given the high concentrations achieved at the site.

SENSITIVE



WHAT IS A MIC?

- + Minimum Inhibitory Concentration
- + is the lowest concentration (in µg/ml) of an antibiotic that inhibits the growth of a given strain of bacteria.





WHAT ARE BREAKPOINTS?

- + Cut off between two different populations
 - + Wild type and non-wild type
 - + Susceptible and Resistant isolates
- + Different types
 - +ECOFFs
 - + Clinical breakpoints





What about topical breakpoints?

+ Still not available in Veterinary Medicine

+ EUCAST Guidelines

In the absence of clinical data on outcome related to MIC of infecting organisms, EUCAST has not been able to determine relevant clinical breakpoints for topical use of antimicrobial agents. Laboratories are advised to either use the regular breakpoints or the cut-off values listed below to distinguish between organisms without and with acquired resistance mechanisms.

Organisms	Screening cut-off values for detection and reporting of phresistance. Report resistant isolates with MIC above the value. Otherwise report susce	nenotypic (R) for cut-off	Gentamicin	Tobramycin	Ciprofloxacin	Levofloxacin	<u>Ofloxacin</u>	Chlorampheni col	Colistin (for polymyxin B)	Neomycin (framycetin)
P. aeruginosa	Topical EUCAST	(mg/L)	8	2	0.5	2	2	ND	4	ND
P. aeruginosa	ECOFF EUCAST	(mg/L)	8	2	0.5	2	4	ND	4	ND
	CLSI VET01S-Ed7 S	(mg/L)	≤ 2	≤ 1	NA	≤ 1	NA	NA	NA	NA
	CLSI VET01S-Ed7 R	(mg/L)	≥ 8	≥ 4	NA	≥ 4	NA	NA	NA	NA

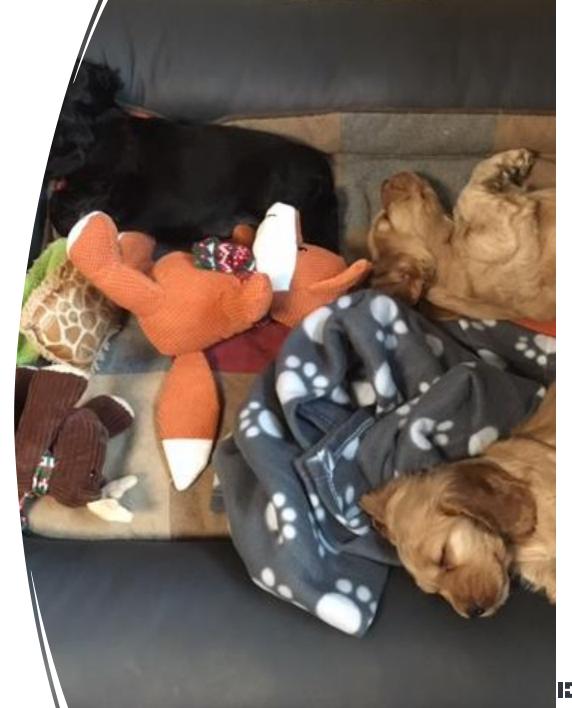
Pseudomonas spp in Canine otitis

- + Not part of the normal flora and not an obligate pathogen
- + Prior dysbiosis may predispose to ear infections

- + Biofilm formation in >40% of cases
- + MDR reported in 13-35% of isolates
- + Rates of resistance vary across countries and change across time

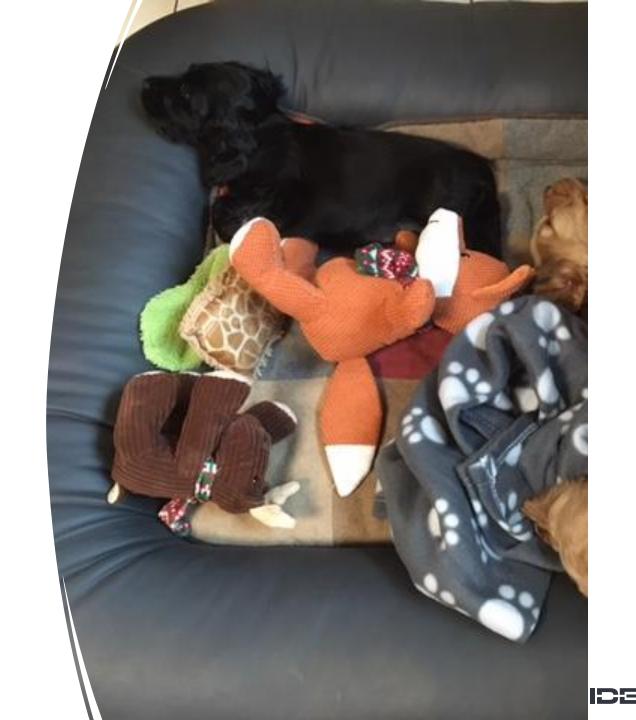
Goals of therapy

Treat infection
Remove discharge
Analgesia
Reduce chronic & perpetuating changes
Avoid relapse
Identify primary disease
Avoid side effects



Analgesia

- Is important!
- Avoids future issues
- Avoids behavioural problems
- Increases compliance, particularly future compliance
- E.g. Gabapentin off label
- Long-acting medications



Determine primary disease

Elimination diet?

- How?
- How long?
 Environmental allergy testing
 Blood tests
 Parasiticidal diagnostic therapy



Ear flush/remove debris

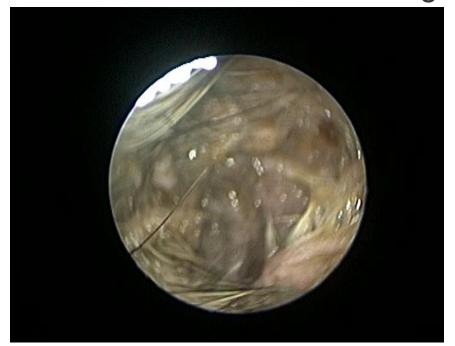
Cleaning at home → most cases

GA flush →

- + if severe disease
- + copious discharge
- + ?TM intact?
- + Biofilm
- + Ceruminolyth

Aim:

- + Remove debris
- + Examine TM
- + Flush middle ear if necessary
- + Diagnostic & therapeutic





Client education/follow up

- Crucial!
- Show how to clean
- Written instructions
- →Increases compliance
- Set expectations
- Allergy: life long diseases
- Treatment duration

- Quality of life!
- Follow up visits depending on severity
- Maintenance therapy
- Long term topical steroids
- Warn about intermittant use of AB drops → resistance/AB stewardship



Thank you all for your attention!
Questions?



Questions?

