



CAMiProt Companion Animal Microbiology Protocols

Bacterial Culture of Clinical Specimens from Ear Infections



Issued by ENOVAT - The European Network for Optimization of Veterinary Antimicrobial Treatment (COST Action CA18217)

CAMiProt | As initiative of the European Network for Optimization of Veterinary Antimicrobial Treatment (ENOVAT)

Background, Aim and Users of CAMiProt

Primarily, the CAMiProt are intended as a general resource for practicing professionals in the field of small animal clinical diagnostic microbiology and infection specialties worldwide. The resources are intended to support and harmonise the microbiology diagnostic approach to bacterial culture of veterinary pathogens across different veterinary laboratories. CAMiProt also provide small animal clinicians with information about suitable clinical specimens to submit for culture and antimicrobial susceptibility testing, the most common pathogens isolated, and the standard of laboratory services they may expect for the investigation of ear infection in their patients. The documents also attempt to provide users with guidance on processing clinical specimens and selection of bacterial isolates with likely clinical significance, with the ultimate goal of limiting unnecessary reporting and antimicrobial susceptibility testing of organisms whose clinical significance may be irrelevant, thus promoting prudent antibiotic use in companion animal practice.

The CAMiProt comprise a collection of recommended procedures covering all stages of the investigative process in veterinary microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Protocols are presented for veterinary microbiology investigations of different body sites and apparatuses, with emphasis given to differences in clinical specimens processing where appropriate. Recommendations may be supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Standardisation of the veterinary diagnostic process through the application of the CAMiProt helps to assure the equivalence of investigation strategies in different laboratories processing companion animal samples across the world, and is essential for animal and public health surveillance, research and development activities.

Legal Declaration

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Suggested Citation

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Building protocols and the consultation process

Drafting the consensus protocols is based on sharing veterinary microbiology protocols (or standard operating procedures, SOPs) for processing clinical specimens from companion animals from at least 2-3 European veterinary microbiology diagnostic laboratories. This generates an initial draft derived from practical experience, expert knowledge in the field and/or evidence-based scientific guidance. The draft protocols disseminated for a consultation process within the wider CAMiProt network, leading to consensus protocols. These protocols will be then made available for public consultation and upon completion, they will be published on the CAMiProt webpage at University of Liverpool - <https://www.liverpool.ac.uk/infection-veterinary-and-ecological-sciences/research/vetclin-amr/methodology-harmonisation-toolkit/>

Please find a list of the CAMiProt author contributors at the end of this document.



SS. CYRIL AND METHODIUS UNIVERSITY IN SKOPJE



Introduction

Otitis externa in pets

Otitis externa is an inflammatory disease of the external ear canal, that may include the ear pinna, and is a common problem in dogs and cats. Otitis externa may be acute or chronic (persistent or recurrent otitis lasting for 3 months or longer). Chronic inflammation of the external ear canal may lead to alterations such as glandular hyperplasia, glandular dilation, epithelial hyperplasia, and hyperkeratosis (1). These changes usually cause increased cerumen production along the external ear canal, contributing to increase in local humidity and pH of the external ear canal, thus predisposing the ear to secondary infection. Many infections are polymicrobial, including mixed infections of bacteria (rods or cocci) and yeasts. The bacteria most commonly isolated from ear canals of small animals affected by otitis are *Staphylococcus* spp. (2). Other bacteria commonly associated with otitis include *Pseudomonas*, *Proteus*, *Enterococcus*, *Streptococcus*, *E. coli* and *Corynebacterium* (Table 1, adapted from [3]). Some bacteria may produce biofilm, notably *Staphylococcus* and *Pseudomonas*, which can lead to persistence of infection despite adequate therapy. *Malassezia* yeast is another common component of otitis externa in dogs. Some dogs seem to develop an allergic response to *Malassezia* spp., leading to considerable discomfort and pruritus. Importantly, the vast majority of organisms implicated in otitis in companion animals are opportunistic pathogens. As a result, the distinction between commensal and pathogenic organisms can often be blurred, with substantial overlap at both the genus and species levels.

Acute and uncomplicated otitis externa can often be treated successfully, but chronic or recurrent otitis externa is more challenging. Typically, underlying primary factors as well as predisposing and perpetuating factors are at play, facilitating secondary infection. Repeated bouts of inflammation and infection can cause secondary changes in the ear canal that can ultimately lead to further lack of success in treating otitis, and possible end-stage ear disease.

Diagnosis of external otitis is based on ear canal palpation, visual inspection of ears, including otoscopic examination, and cytological analysis of otic contents. The external ear canal may exhibit presence of hyperemia, ulceration, ceruminous or suppurative discharge, masses, stenosis, glandular changes, or foreign bodies. The nature of the aural discharge can hint as to the likely organism: in yeast and staphylococcal infections, the discharge becomes lighter than normal brown dog cerumen and looks moist. In *Pseudomonas* spp. infection, the discharge is often green, but can also be tarry, black and mucoid, whereas in other Gram-negative rod infections the discharge tends to be creamy and yellow.

Cytological evaluation of ear contents is the single most informative diagnostic test helping the treatment of otitis. Cytology also allows monitoring the response to therapy. Sometimes, bacterial culture sampling from the ear canal may be used to help determine treatment options and for selection of systemic antibiotic therapy, if indicated. Systemic antibiotics are only indicated following bacterial culture and susceptibility testing in case of a ruptured tympanic membrane and/or otitis media (4) when osteomyelitis is suspected or confirmed based on neurological clinical signs or imaging. Mixed bacterial and bacterial/yeast cultures are commonly obtained and need to be carefully interpreted for clinical significance. As antimicrobial susceptibility testing results indicate attainable drug levels in plasma, topical therapy does not always correlate with culture results. However, topical therapies can achieve levels exceeding 100 - 1000xs those that can be achieved in plasma, thus bacteria are exposed to higher levels of drug. Therefore, susceptibility testing may not always be clinically relevant. Common reasons indicating the need for culturing include cytological detection of (Gram-negative) rods as *Pseudomonas* spp. and/or (Gram-positive) cocci particularly when there is a suspicion of methicillin-resistant staphylococci (MRS), copious purulent discharge without organisms being seen, pyogranulomatous inflammation, in case of treatment failure and of ruptured tympanic membrane in which case topical antibiotics cannot be applied. Culture should always be performed prior to starting systemic therapy.

Middle ear effusion (MEE) of dogs is characterized by the presence of fluid in the tympanic bullae, most common in Cavalier King Charles spaniels and other brachycephalic breeds, which shares similarities with “glue ear” of children (5). Inflammation is not invariably present in these cases and association with bacterial infection is suspected in small animal patients .

Otitis media in pets

Otitis media consists of the inflammation of the middle ear structures and is usually caused by an infection extending from the external ear canal or by penetration of the eardrum by a foreign object; chronic or recurring otitis externa may be an indication of otitis media. The spread of infection through the bloodstream to middle ear structures is also possible, but it is rare. The most common bacterial organisms associated to otitis media in small animals are *Staphylococcus pseudintermedius*, *Pseudomonas aeruginosa*, *Streptococcus*, *Proteus*, *Klebsiella*, *Escherichia coli*, anaerobes such as *Bacteroides*, *Fusobacterium* and, particularly in cats, *Mycoplasma* spp. *Malassezia* yeasts are often seen on cytological preparations but would not be reported from microbiology laboratories.

The signs of otitis media may be similar to those of otitis externa; however, progression from otitis media to the inner ear structures (otitis interna) can cause additional neurological signs and deafness. Upon clinical examination in cases of otitis media, the tympanic membrane may be found intact, perforated or ruptured; it may be difficult to diagnose otitis media when the eardrum is intact, which is the case more than 70% of times (6).

Cytologic examination of middle ear specimens is required for diagnosis, to determine the type and number of organisms present. Cytology may not reveal bacteria because they are often protected from the cytology stains by mucus. Many cytologically negative specimens have been reported as culture-positive. Culture and antimicrobial susceptibility testing are not a routine part of the diagnostic plan but should be carried out for cases of otitis media or severe otitis externa associated to rod bacteria when systemic therapy has to be prescribed. Microbial examination of samples from different regions of the canine ear may reveal heterogeneity of isolates and susceptibility patterns (7); therefore, if concurrent otitis media and externa are present, efforts should be made to collect and culture the exudate of the middle ear separately, notwithstanding that a degree of contamination by the external ear canal can be very difficult to avoid while obtaining a bulla sample.

Table 1. Common organisms found in normal and diseased canine and feline ears (Adapted from [3]).

^ Please note that the vast majority of microbes implicated in otitis in companion animals are **opportunistic pathogens**. As a result, the distinction between commensal and pathogenic organisms can often be blurred, with substantial overlap at both the genus and species levels (e.g. *Staphylococcus schleiferi*)

Organisms found in^	
Normal ears	Ears with otitis externa
<i>Malassezia pachydermatis</i> *	<i>Malassezia</i> spp.*
Lipid-dependent <i>Malassezia</i> spp. (e.g. <i>Malassezia furfur</i> and <i>Malassezia obtusa</i>)	<i>Staphylococcus pseudintermedius</i> *
<i>Staphylococcus pseudintermedius</i> *	<i>Staphylococcus schleiferi</i> subsp. <i>coagulans</i> and coagulase-negative staphylococci
<i>Staphylococcus schleiferi</i> subsp. <i>coagulans</i>	<i>Pseudomonas aeruginosa</i> *
Coagulase-negative staphylococci†	<i>Proteus mirabilis</i> *
<i>Bacillus</i> spp.*	Beta-haemolytic streptococci (e.g. <i>S. canis</i>)
<i>Corynebacterium</i> spp.†	<i>Corynebacterium</i> spp.
<i>Streptococcus</i> spp.	<i>Enterococcus</i> spp.
<i>Micrococcus</i> spp.†	<i>Escherichia coli</i>
	Ears with otitis media (in addition to the above)
	Anaerobes (<i>Fusobacterium</i> , <i>Bacteroides</i> spp.)
	<i>Mycoplasma</i> spp. ¹

* Common organisms

† May not be reported by some laboratories as regarded normal flora

¹ *Mycoplasma* spp. especially in cats

Specimen Collection, Transport and Storage

- ◇ Use aseptic technique for sample collection.
- ◇ Collect specimens before antimicrobial therapy where possible.
- ◇ Collect swabs into appropriate leak-proof collection devices (see below) with or without transport medium and transport in sealed plastic bags.
- ◇ Unless otherwise stated, swabs for bacterial (and fungal) culture and susceptibility should be placed in appropriate transport medium, e.g. Copan's Liquid Amies Elution (E-Swab®) or charcoal transport medium (however, cytological artefacts are commonly encountered using charcoal medium).
- ◇ Specimens should be transported and processed as soon as possible. If processing is delayed, refrigeration is preferable to storage at ambient temperature.
- ◇ Compliance with postal, transport and storage regulations is essential.
- ◇ Numbers and frequency of specimen collection are dependent on clinical condition of patient.

Table 2. Type of specimens, collection devices, sampling methods and specific recommendations.

Specimen Type	Collection Device	Sampling method	Other Recommendations
<u>Otitis externa:</u> Outer ear swabs	Transport media swabs (ex. charcoal or E-swabs) Or Sterile universal tubes for ear canal scrapings	Examine with otoscope Clean debris from canal with saline For sampling the ear canal, a cotton swab is inserted in the ear to collect any pus or exudate, usually without the need for sedation (required for some cases). Reach the junction between the horizontal and vertical ear canals for a more representative sample and rotate several times. Guidance on how to collect, process and examine ear samples for cytology and bacterial culture from cases of otitis externa is provided by Shaw, 2016 (3).	Avoid plain swabs without transport media (only used for PCR). Gently pull the ear pinna up and forward to straighten the ear canal taking care to prevent iatrogenic damage to the tympanic membrane during collection. For investigation of fungal infection, scrapings of material from the ear canal are preferred although swabs can also be used
<u>Otitis media:</u> Middle ear swabs Middle ear effusion	1) Ruptured tympanic membrane: Transport media swabs (ex. charcoal or E-swabs)	When the tympanic membrane is ruptured, there is a discharge into the ear canal or bullae filled with debris. In this	Myringotomy technique: see Cole and Nuttal, 2021 (10).

<p>Tympanic bulla lavage fluid</p> <p>Ear canal biopsy</p>	<p>for exudate and discharge</p> <p>Or</p> <p>Sterile universal tubes for middle ear effusions, tympanic bulla lavage fluids and ear canal biopsies</p> <p>2) Intact tympanic membrane:</p> <p>Sterile universal tubes for middle ear effusions, tympanic bulla lavage fluids and ear canal biopsies</p>	<p>case, samples for cytology and bacterial culture should be obtained from the middle ear cavity using a sterile swab introduced into the horizontal ear canal. For samples from the horizontal ear canal or from the bulla, the patient needs to be anaesthetised and samples should be obtained under video-otoscopic guidance where possible.</p> <p>Alternatively, inserting two sterile urinary catheters threaded into one another to avoid contamination from the external ear canal, sample is aspirated with a syringe or suction apparatus from the middle ear cavity (8). If no liquid is in the bulla, 1 mL of sterile saline can be infused and suctioned back.</p> <p>If the tympanic membrane is not ruptured, it is necessary to perform a myringotomy (incision into the tympanum) to obtain samples and allow flushing and drainage of the middle ear cavity. It would be advisable to obtain pre- and intra-operative samples for comparison of bacterial culture results when performing total ear canal ablations combined with lateral</p>	
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		<p>bullae osteotomies (TECA-LBOs), common surgical procedures to treat end-stage otitis externa and to remove ear canal masses in both dogs and cats as infection is a common post-operative complication (9).</p>	
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- ◇ Containment Level 2.
- ◇ Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological

Specimen Laboratory Processing - Generalities

safety cabinet.

- ◇ The above guidance should be supplemented with local SOPs, COSHH and risk assessments.

Standard Gram staining of clinical specimens' direct smear should be performed following processing of specimens for bacterial culture (unless glass slides are sterile).

1) Microscopy

Table 3. Direct smear methodology.

Specimen Type	Direct Smear Technique
Ear swab	Smear directly onto clean microscope slides after plating out on all non-selective and selective agar media, ensuring the cotton tip is rolled over to allow contact of the whole surface with the glass slide.
Middle ear effusions Tympanic bulla lavage fluids	Handle using a sterile pipette, placing one drop of fluid onto a clean microscope slide and spreading it with a sterile loop to make a thin smear.
Ear canal biopsies Ear scrapings	Gently impress directly onto a clean microscope slide.

Reporting Microscopy

- ◇ Urgent microscopy results to be telephoned or sent electronically as soon as available.
- ◇ For written reports, 12-24 hr.
- ◇ For all specimen types, report on presence of inflammation (WBCs) and organisms detected.
- ◇ It is essential to evaluate direct smear findings in conjunction with bacterial culture results and viceversa.
- ◇ Quantification of the organisms detected on direct smear preparations is more valuable for clinical specimens obtained from normally sterile body sites (e.g. middle ear effusion) than for those collected from normally contaminated body sites, where commensal flora is expected (e.g. external ear canal). Nevertheless, the presence of elevated numbers of organisms by direct microscopic observation can be suggestive of overgrowth and/or infection. Evidence of phagocytosis and intracellular bacteria within neutrophils is highly indicative of ongoing active infection.
- ◇ In order to report on the number of bacterial organisms present on a direct smear, this shall be examined entirely under the light microscope or, at least, in 10 microscopic fields at high magnification (1000x) with immersion oil, however quantification of organisms and its correlation with clinical significance is highly controversial among dermatologists for bacteria and yeasts (as below).
- ◇ Guidelines on the correlation between semi-quantitative assessment and clinical significance of *Malassezia* spp. yeasts have been proposed by some authors, such as ≥ 1 to 5-10 yeasts per HPF in several microscopic fields considered an abnormal finding (11,12). However, these proposed guidelines are not generally accepted because there can be overlap in yeast densities in skin samples from healthy and diseased dogs and there is the potential for relatively small numbers of organisms to generate skin disease in sensitised individuals (13).

2) Bacterial Culture

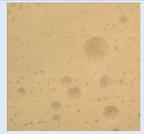
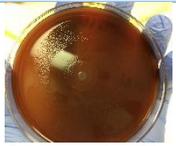
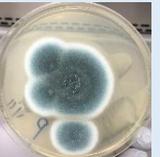
◇ Use appropriate culture set up method depending on specimen type:

Table 4. Culture set up methodology.

Specimen Type	Culture Technique
Ex. Outer ear swab	Inoculate each agar plate with swab; for the isolation of individual colonies, spread inoculum with a sterile loop.
Middle ear effusions Tympanic bulla lavage fluids	Inoculate using a sterile pipette to transfer a small aliquot of fluid onto each agar plate; for the isolation of individual colonies, spread inoculum with a sterile loop.
Ear canal biopsies Ear scrapings	Impress directly onto the surface of each agar plate using sterile disposable forceps; for the isolation of individual colonies, spread inoculum with a sterile loop.

Culture media, conditions and organisms

Clinical condition	Specimen	Standard media	Incubation			Culture reads	Target organism	Image
			Temp. (°C)	Atmosp.	Time			
Otitis externa Otitis media	All swabs	5% SBA ^a	35-37	Aerobic	48-72 hr	Daily	Aerobic Gram-positive cocci, Corynebacteria and Gram-negative rods	 <i>Pseudomonas aeruginosa</i>
	Middle ear effusion							
	Tympanic bulla lavage fluid	FAA ^b	35-37	Anaerobic	5-7 d	>48 hr	Anaerobes	 <i>Bacteroides</i> spp.
		CNA ^c	35-37	Aerobic	48-72 hr	Daily	<i>S. aureus</i> <i>S. pseudintermedius</i> Enterococci β-haemolytic streptococci	 <i>Streptococcus canis</i>
		MRSA Brilliance	35-37	Aerobic	24-48 hr	Daily	MRSA MRSP	 MRSP
		SABC ^d	35-37	Aerobic	3-5 d	>48 hr	<i>Malassezia</i> spp. and other fungi	 <i>Malassezia</i>

								<i>pachydermatis</i>
		Mycoplasma Broth & Agar media ¹	35-37	Aerobic 5-10% CO ₂	5d	>48-72 hr	<i>Mycoplasma</i> spp.	 <i>Mycoplasma</i> spp.
Otitis media	Biopsy	5% SBA ^a	35-37	Aerobic	48-72 hr	Daily	Aerobic Gram-positive cocci, Corynebacteria and Gram-negative rods	 <i>Proteus mirabilis</i>
		FAA ^b	35-37	Anaerobic	5-7 d	>48 hr	Anaerobes	 <i>Fusobacterium necrophorum</i>
		BHI ^e broth	35-37	Aerobic	24-48 hrs	Daily	Any organism	 Tubes with (R) and without (L) bacterial growth
		SABC ^d	35-37	Aerobic	3-5 d	>48 hr	<i>Malassezia</i> spp. and other fungi	 <i>Aspergillus fumigatus</i>

^a Sheep Blood Agar ^b Fastidious Anaerobe Agar ^c Columbia Nalidixic-Acid Agar ^d Sabouraud Dextrose Agar with Chloramphenicol ^e Brain Heart Infusion Broth; any bacterial growth obtained should be sub-cultured onto 5% SBA and FAA. ¹ Only upon request. Some strains of feline and canine mycoplasmas may grow also on 5% SBA and FAA.

Bacterial Identification

◇ The reader is addressed to further resources below:

1. Veterinary-specific clinical microbiology textbooks:

- Quinn et al., Clinical Veterinary Microbiology, 1994 (14).
- Quinn et al., Veterinary Microbiology and Microbial Disease 2nd Edition, 2011 (15).
- Markey et al., Clinical Veterinary Microbiology 2nd Edition, 2013 (16).

2. Medical clinical microbiology manuals:

- Carroll and Pfaller, eds. Manual of Clinical Microbiology 13th Edition, 2024 (17).

3. Other scientific publications:

- Váradi et al., Methods for the detection and identification of pathogenic bacteria: past,

present, and future, 2017 (18).

- Chauhan and Jindal, Biochemical and Molecular Methods for Bacterial Identification, 2020 (19).

4. **Online guides and training:**

- CDC Laboratory Training Resources, available online at: <https://www.cdc.gov/lab-training/site.html> (20)
- Public Health England's UK Standards for Microbiology Investigations, available online at <https://www.rcpath.org/profession/publications/standards-for-microbiology-investigations.html> (21)

◇ Minimum level of identification in the laboratory:

Organism	Minimum ID level
<i>Staphylococcus aureus/pseudintermedius</i>	Species level
Other staphylococci	Coagulase positive/negative staphylococci (CoPS/CoNS)
Streptococci	Lancefield group level
Pseudomonas spp.	"pseudomonads" level
Enterobacterales	"coliform" level
Corynebacterium spp.	Genus level
Yeasts	Genus level
Fungi	Genus level

Reporting Bacterial Culture and Identification

- ◇ Report initial findings (interim reports) after 24-48 hours from sample receipt.
- ◇ Update on yeasts and/or fungal growth at 5 days post-incubation.
- ◇ Bacterial culture may be finalised at 7 days and fungal culture at 21 days (final reports if any slow growing organism was further detected).
- ◇ General guidance reporting bacterial culture and identification findings:
 - 1) Report clinically significant organisms isolated using a semi-quantitative approach (light, moderate or heavy growth), e.g. bacterial genera and/or species relevant to the clinical condition, organisms grown in pure culture and predominant organisms grown in mixed moderate cultures, up to a maximum of three organisms/sample. When possible, correlate bacterial culture findings with Gram-stained smear or cytology findings.
 - 2) Report other growth findings (i.e. non-predominant organisms) semi-quantitatively when potentially clinically significant, e.g. '*Staphylococcus coagulans isolated in lesser amounts*', or as overall other growth making use of comments such as '*clinical significance is doubtful*', '*light mixed growths of likely contaminating and/or commensal organisms*' etc.
 - 3) Report absence of growth.
 - 4) In cases of ambiguous or challenging to interpret bacterial findings, report that clinicians are encouraged to contact the microbiology laboratory for clarification or further discussion. Collaborative communication between clinical teams and the laboratory is essential to ensure accurate interpretation of results and appropriate patient management.

3) Antimicrobial Susceptibility Testing (AST)

Refer to the [Clinical Laboratory and Standards Institute \(CLSI\) Subcommittee on Veterinary Antimicrobial Susceptibility Testing \(VAST\)](#) and/or the [European Committee on Antimicrobial Susceptibility Testing \(EUCAST\)](#) guidelines.

Reporting Antimicrobial Susceptibility Testing (AST)

- ◇ Report susceptibilities as clinically indicated.
 - ◇ AST reports to reinforce the prudent use of antimicrobials according to local and national protocols.
 - ◇ Refer to guidelines from Clinical and Laboratory Standards Institute (CLSI) document [VET09: Understanding Susceptibility Test Data as a Component of Antimicrobial Stewardship in Veterinary Settings](#)
- 1) Report AST results for clinically significant organisms, e.g. bacterial species relevant to the clinical condition, organisms grown in pure culture and predominant organisms grown in mixed moderate cultures, up to a maximum of three organisms/sample.
 - 2) Do not report AST data for organisms that are likely contaminants and/or commensal flora, e.g. organisms not usually relevant to the clinical condition, isolated in light mixed growth.
 - 3) Direct microscopy findings may aid in the determination of clinical significance of isolates that will be selected for AST, e.g., bacterial morphology associated with inflammation and phagocytosis.
 - 4) Report intrinsic resistance (i.e. expected antimicrobial resistance phenotypes, for example *Pseudomonas aeruginosa* Resistant to amoxicillin-clavulanic acid) or mark these combinations as “Not Interpretable” on the AST report. EUCAST offers comprehensive expert rules and expected phenotypes tables online at: https://www.eucast.org/expert_rules_and_expected_phenotypes (22).
 - 5) Ideally, laboratories should have a comment on the reports to explain the epidemiological, treatment and infection control implications of the most important AMR mechanisms suspected or identified through AST testing, including methicillin resistance in staphylococci and extended-spectrum beta-lactamase (ESBL) production in *Enterobacterales*.
 - 6) Report that clinicians are encouraged to contact the microbiology laboratory for discussion of antimicrobial selection. Collaborative communication between clinical teams and the laboratory is essential to ensure accurate interpretation of AST results and appropriate patient management.

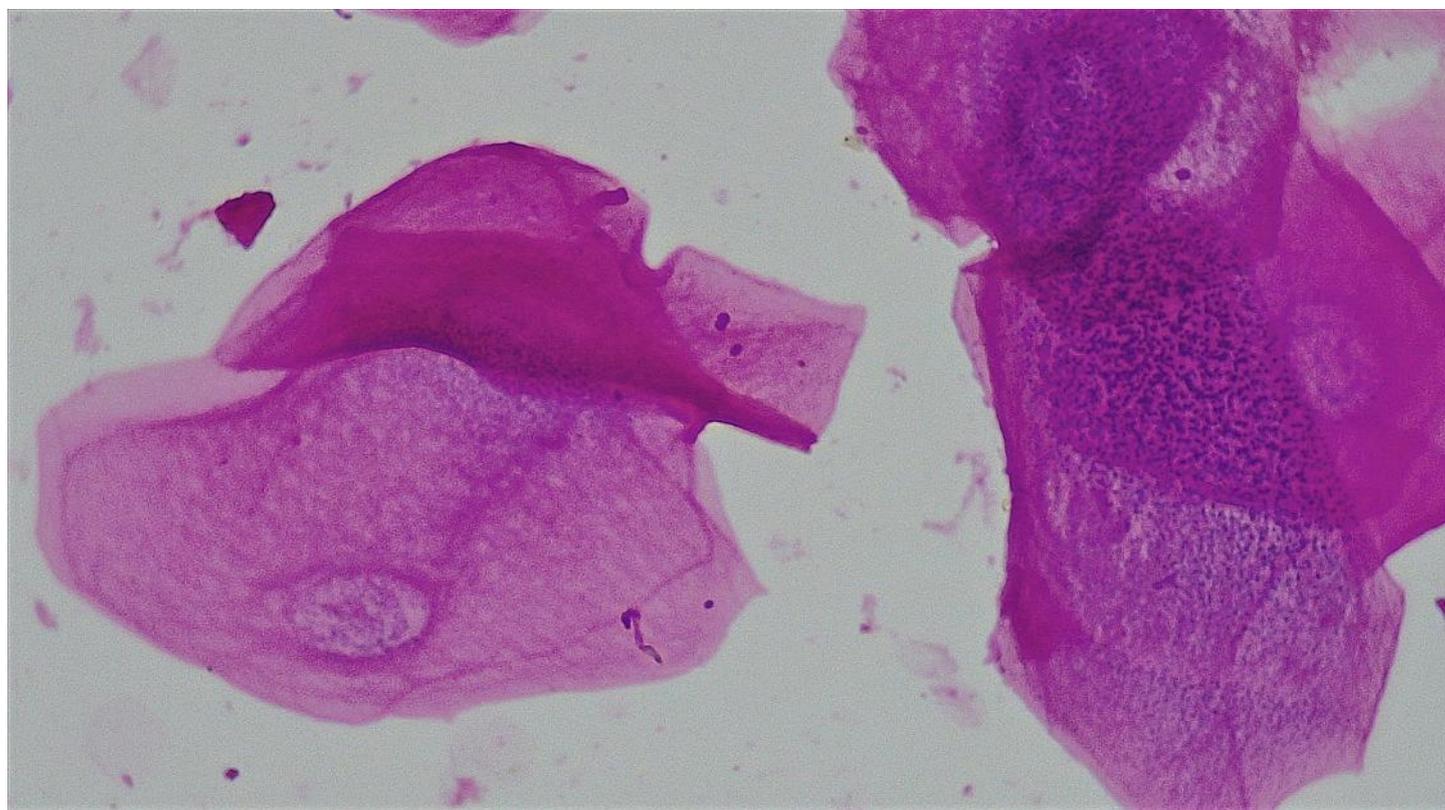
Case Examples

Case 1.

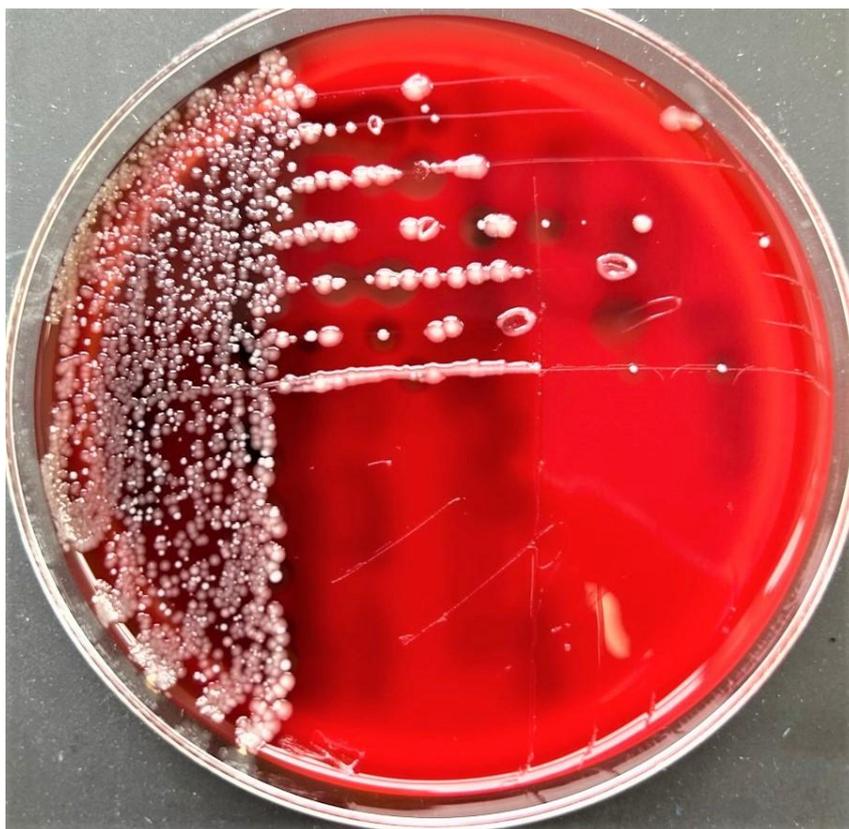
Clinical history: 7-year-old male neutered Cocker Spaniel underwent left TECA-LBO for therapeutic management of chronic otitis externa and otitis media.

Sample submitted: fresh epithelial tissue collected intra-operatively from the left tympanic bulla. The tissue was embedded in a sterile surgical gauze moistened with saline solution and placed in a sterile universal tightly sealed container. Submission for routine bacteriological examination and AST.

Direct smear findings: Numerous debris and keratinocytes with occasional neutrophils observed; 2+ (between 4-10 organisms on average per High Power Field) Gram-positive cocci, mostly arranged in pairs or very small clusters seen extracellularly.



Bacterial culture findings: Moderate to heavy mixed growths were obtained on the non-selective media 5% SBA and FAA incubated aerobically and anaerobically, respectively, at 24 hours post-incubation. Mixed growths consisted of *Staphylococcus pseudintermedius* and *Pluribacter georgoviae* (both identified by MALDI-TOF MS with scores ≥ 2) isolated in comparably equal amounts. Heavy growth of *S. pseudintermedius* was recorded also on selective CNA and light growth of *P. georgoviae* on SDA with chloramphenicol. MRSA Brilliance Agar was negative for bacterial growth after 48 hrs.



Interpretation and Reporting of bacterial culture results:

- *S. pseudintermedius* is one of the most clinically relevant organisms to canine otitis.
- Despite it being frequently encountered as canine skin, ear and mucous membranes commensal, its isolation in moderate to heavy growths from a body site which shall normally be sterile (middle ear) can suggest higher bacterial loads than what may be regarded as commensal.
- Evidence of *S. pseudintermedius* was found under the microscope (despite extracellular).
- Unfortunately, no information on whether the tympanic membrane was be found intact, perforated or ruptured in this patient.
- For this reason, contamination with *P. georgoviae* (formerly *Enterobacter gergoviae*, belonging to the family *Enterobacterales*) cannot be entirely ruled out.
- Nevertheless, *Enterobacterales* are also commonly associated to canine otitis and this organism was isolated in equal amounts to *S. pseudintermedius* from the middle ear tissue.
- In addition, isolation of the combination *S. pseudintermedius* and *P. georgoviae* from cases of otitis media and externa is reported in the literature (23–25).
- For these reasons, both organisms isolated may warrant selection for subsequent AST.

Acknowledgments

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<https://enovat.eu/about/>

<https://ecvmicro.org/>

The CAMiProt is developed, reviewed and revised by members of the ENOVAT Working Group (WG) 1: *Mapping microbiological diagnostics and treatment guidelines*, led by Dr. Dorina Timofte DVM, PhD, Dipl. ECVM, MRCVS.

<https://enovat.eu/working-groups/>

<https://www.liverpool.ac.uk/veterinary-science/staff/dorina-timofte/>

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged.

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