UNIVERSITY OF VETERINARY MEDICINE BUDAPEST

DEPARTMENT OF SMALL ANIMAL INTERNAL MEDICINE

“A study into the prevalence of antibiotic resistance in bacteria cultured from the ears of a sample of dogs in Budapest “

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Abstract

“Antimicrobial resistance (AMR) is one of the top ten global public health threats facing humanity”, according to the WHO. AMR is recognized as a One Health concern as it extends across humans, animals, and their environment. The potential for AMR transmission between animals and humans coexisting in the same environment is a major concern. The challenge of antibiotic resistance ranks among the most pressing threats to the treatment of infectious diseases. Numerous studies have demonstrated the increasing prevalence of AMR in companion animals. AMR impacting bacterial isolates in the ear canal microbiome has been documented in many countries and is a common occurrence in bacteria associated with otitis. In the ear canal, multidrug-resistant bacteria have also been identified, especially in cases involving Pseudomonas aeruginosa. The increasing evidence of AMR is attributed to empirical and prolonged antibiotic therapies used for treating otitis externa or media without prior susceptibility testing.

The focus of our study was to assess the incidence of antibiotic resistance within the ear canal microbiome of a sample of dogs treated at the University of Veterinary Medicine Budapest for various ear diseases. We analysed the bacterial strains present in the ear canal samples submitted for microbiological examination at the Duo-Bakt Veterinary Microbiology Laboratory, compared to bacteria commonly associated with otitis externa . We investigated the AMR present within the isolated bacterial strains through antimicrobial susceptibility testing. These results were then compared to existing literature of the prevalence of AMR in ear canal microbiome. Our study confirmed the existence of AMR in all bacterial isolates, with the exception of *Pseudomonas aeruginosa*. Beyond this, we established the prevalence of multi-drug resistance in these bacterial isolates. Additionally, our analysis revealed the presence of several strains of Methicillin-Resistant Staphylococcus pseudintermedius in the sampled specimens.

Absztrakt

A WHO szerint „az antimikrobiális rezisztencia (AMR) az emberiséget fenyegető tíz legnagyobb globális közegészségügyi fenyegetés egyike". Az AMR a “One Health” egyik fontos területe és problémája, mivel kiterjed az emberekre, állatokra és környezetükre. Komoly aggodalomra ad okot az AMR transzmissziójának lehetősége az azonos környezetben élő állatok és emberek között. Az antibiotikum-rezisztencia a fertőző betegségek kezelésének legfontosabb kihívásai közé tartozik. Számos tanulmány kimutatta az AMR növekvő előfordulását a társállatokban. Számos országban született publikáció az AMR-el kapcsolatban, amely hatással van a hallójárat mikrobiomában lévő baktériumaira, és gyakori az otitishez társuló baktériumokban. A hallójáratban multirezisztens baktériumoktörzseket is azonosítottak, különösen a *Pseudomonas aeruginosa* esetében. Az AMR egyre növekvő prevalenciája a külső- és középfülgyulladás kezelésére előzetes érzékenységi vizsgálat nélkül alkalmazott empirikus, és hosszan tartó antibiotikum-terápiáknak tulajdonítható.

Vizsgálatunk során a Budapesti Állatorvostudományi Egyetemen különböző fülbetegségek miatt kezelt kutyák fülváladékaiból vett mintákat vizsgáltuk az antibiotikum rezisztencia előfordulását illetően. A Duo-Bakt Állatorvosi Mikrobiológiai Laboratóriumban mikrobiológiai vizsgálatra beküldött hallójáratmintákban jelenlévő baktériumtörzseket elemeztük, összehasonlítva a szakirodalmi adatokkal. Az érzékenységi vizsgálatok eredményei alapján elmeztük az izolált baktériumtörzsekben jelenlévő AMR-t. Vizsgálatunk megerősítette, hogy a *Pseudomonas aeruginosa* kivételével minden bakteriális izolátumban megfigyelhető AMR. Ezen túlmenően megállapítottuk a több gyógyszerrel szembeni rezisztencia előfordulását ezekben a baktériumizolátumokban. Emellett elemzésünk feltárta a meticillinrezisztens Staphylococcus pseudintermedius számos törzsének jelenlétét a mintákban.

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List of Abbreviations

ACD- Allergic contact dermatitis

AMR- Antimicrobial resistance

CKCS- Cavalier King Charles Spaniel

CoNS- Coagulase Negative *Staphylococcus*

CoPS- Coagulase positive *Staphylococcus*

CT- Computerized tomography

DNA- Deoxyribonucleic Acid

EFSA- European food safety authority

ET- Endotracheal

EU- European Union

FAD- Flea allergy dermatitis

GCC- Glucocorticoid

MRI- Magnetic resonance imaging

OE- Otitis externa

OM- Otitis media

PCR- Polymerase chain reaction

PFU- Plaque forming units

Phage- Bacteriophage

PSOM- Primary secretory otitis media

TB- Tympanic bullae

TECA- Total ear canal ablation

TM- Tympanic membrane

WHO- World Health Organisation

1. Introduction

The World Health Organisation (WHO) has declared that "Antimicrobial resistance is one of the top ten global public health threats facing humanity" [1]. According to the WHO (2021), antimicrobial resistance (AMR) is considered the presence of resistance in infectious agents such as bacteria, viruses, parasites and fungi to antimicrobial medicines [2]. There is increasing evidence of the rapid spread of multi-drug resistant pathogens that are resistant to antimicrobial medicines in many different countries [2]. It accounts for 33,000 deaths per year in the EU and 4.95 million worldwide [3]. AMR is considered a one health problem as it encompasses humans, animals and the environment, and the ability of AMR to be transmitted between animals and humans that share the same environment [4]. Governments worldwide under the WHO have established surveillance programmes in food producing animals with the objective to monitor and control the AMR prevalence in zoonotic and commensal bacteria and reduce the use of antibiotics in these animals that enter the food chain [4].

Companion animals have not been the focus in these surveillance programmes and there is a lack of information present on an epidemiological level of the AMR present in companion animals [4]. With more than 60 million dogs and cats in the European Union (EU), Marco-Fuertes et al (2022) considers this lack of information could pose a serious health concern due to the close contact between these companion animals and humans, and the permitted use of antibiotics reserved for humans in companion animal medicine [4]. Information on AMR in companion animals has been conducted in different studies by research groups on a smaller scale with only few countries- Denmark, Finland, France, Norway, Germany, Sweden and Switzerland, reporting on a national level according to the European food safety authority (EFSA). These studies can be hard to compare due to the lack of harmonisation on account of different study design, populations and methods [5]. The EU plans to launch EARS- Vet, the European Antimicrobial Resistance Surveillance Network in Veterinary Medicine, to analyse the current situation of AMR in companion animals and establish standardised European monitoring systems and develop an assessment of the risk of AMR transmission from animals to humans via non-food-borne routes, such as direct contact with companion or food-producing animals [3, 4].

Many studies have illustrated the increasing prevalence of AMR in companion animals. Common resistance found in canines include Methicillin-Resistant Staphylococcus aureus Methicillin-Resistant Staphylococcus pseudintermedius, *E. coli* extended spectrum beta-lactamase, vancomycin-resistant *Enterococci*, and carbapenemase-producing gram negative bacteria [6]. AMR affecting bacterial isolates within the ear canal microbiome has been observed in many countries, and frequently encountered in bacteria associated with otitis externa [4]. In the ear canal, multidrug-resistant bacteria, particularly in the case of *Pseudomonas aeruginosa*, have also been identified [7].

This study aims to assess the incidence of AMR within the ear canal microbiome of a sample of dogs treated at the University of Veterinary Medicine Budapest for various ear diseases.

2. Literature Review

2.1. Anatomy and physiology of the canine external ear

2.1.1. External ear anatomy

The canine ear consists of the pinna or auricle, the external ear canal, the middle ear and the inner ear [8]. The canine external ear is composed of the auricle with the auricular cartilage and muscles, the annular cartilage, the external acoustic meatus and the tympanic membrane (TM) [9]. The auricle of the external ear largely varies in shape and size between the different canine breeds [10]. It can be pendulous or erect depending on the breed [11]. The pinna is composed of auricular cartilage, covered in skin on both surfaces with hair follicles, sweat glands and sebaceous glands [11].The auricle has a convex and concave surface and its opening faces dorsolaterally [11]. The lateral boundary is formed by the tragus which is a quadrangular plate of cartilage [11]. Caudal to the tragus, there is an elongated, thin piece of cartilage called the antitragus, which is separated from it by the intertragic incisure as indicated in **(Figure 1)** [11]. The intertragic notch is important as it is the area used to guide the speculum of the otoscope into the external ear canal for examination [11]. On the medial wall of the pinna, there is a low transverse ridge, called the antihelix [11].

The auricle is considered a sound gathering structure [10]. The external ear canal has the important function in directing and transmitting soundwaves gathered by the pinna through the TM to the auditory ossicles in the middle ear [9]. The auricular cartilage of the pinna is funnel shaped at the entrance of the external ear canal which helps in its sound gathering role [8]. The external ear canal has a vertical and horizontal portion [8]. The distal portion of the ear canal is funnel shaped and forms the vertical ear canal [8]. The vertical canal then turns medially to form the horizontal ear canal [8]. When the ear is in its normal position, there is a prominent cartilaginous ridge which separates the vertical and horizontal portions of the ear canal [11]. This cartilaginous ridge can make otoscopic examinations difficult [11]. Lifting the pinna with gentle traction, thus causing the ear canal to straighten out due to the annular cartilage being pliable and flexible can alleviate this difficulty [11]. The TM separates the external acoustic meatus from the middle ear [9]. The TM has three layers and it is semi- transparent [11]. It is divided into two portions; the pars flaccida which is a smaller, dorsally located section and the pars tensa which is larger section located ventrally [11].

Most breeds of dogs have hair present in the external ear canal that decrease in number proximally, with very few hairs found close to the TM [8]. Although, some dogs can have a tuft of hair present in front of the TM, which can be useful for locating the TM during otoscopic examination or flushing [12]. The external ear canal also contains ceruminous and sebaceous glands in differing amounts, depending on the breed [8]. Ceruminous glands are modified apocrine glands, which produce cerumen. Cerumen is an emulsion that covers the canal and consist of desquamated keratinized squamous epithelial cells and the secretions of the mentioned glands such as lipids, proteins, amino acids and carbohydrates [11, 13]. Cerumen provides a physical barrier and is believed to play a role in protecting the canal against pathogens, as well as having a part in microbial defence through lysozymes and immunoglobulins [13].

The external ear has a self-cleaning mechanism called epithelial migration [8]. Cerumen is transported from the TM outwards, centripetally toward the opening of the canal [11]. This prevents accumulation of cerumen and debris in the canal which could hinder sound transmission and also acts as a repair mechanism for lesions and lacerations on the TM [11, 14].

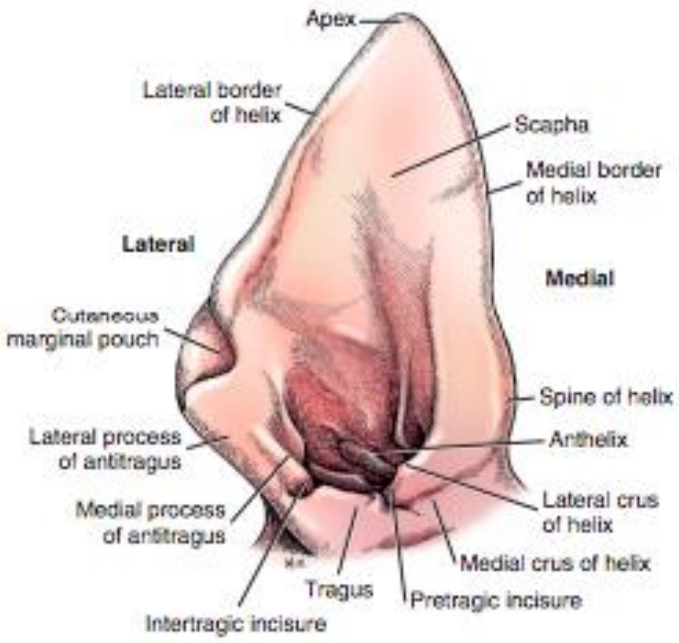

Figure 1: The anatomy of the external ear [10].

2.1.2. Middle ear anatomy

The middle ear is found in the temporal bone and consists of the tympanic cavity, the auditory ossicles and the auditory or eustachian tube [9, 12][9, 15]. As mentioned previously, the middle ear is separated from the external acoustic meatus by the TM as demonstrated in **(Figure 2)**. The tympanic cavity is a small air filled space, that is lined with a thin mucous membrane [15]. It is divided into three parts; dorsal, middle and ventral [15]. The dorsal portion (epitympanicum) is located above the TM and contains the auditory ossicles and their associated muscles [15]. The facial nerve also passes along the wall of the epitympanicum [9]. The middle portion (mesotympanicum) contains the TM in its lateral wall and is connected rostrally to the nasopharynx via the eustachian tube [15]. The ventral portion (hypotympanicum) is known as the tympanic bullae (TB) and it is an enlarged bulbous extension of the temporal bone [15].

The auditory ossicles- the malleus, incus and stapes, mediate the vibration of sound waves in the tympanic cavity and magnify them [9]. The tensor tympani muscle and the stapedial muscle are antagonistic muscles that contribute to the enhancement mechanism of the ossicles [9]. The most lateral of the ossicles is the malleus, and its handle is embedded in the medial surface of the TM and it can be seen as a light band in the TM when examined with an otoscope [15]. There are two windows in the middle ear, the vestibular and cochlear window [15]. The vestibular window connects the tympanic cavity with the vestibule located in the inner ear, the base of the stapes is located here [9, 15].

The cochlear window leads to the cochlear cavity [9]. The cochlea is located in a bony prominence the protrudes from the medial wall of the tympanic cavity called the promontorium [9]. Auditory tube is a narrow tube that connects the tympanic cavity to the nasopharynx, it is osseous closer to the tympanic cavity and cartilaginous towards the pharynx [9]. The auditory tubes function is to equalise the atmospheric pressure on both sides of the TM and so it opens and closes when yawning or swallowing [9]. The middle ear has a normal microflora that according to Leonard (2023) is similar to the microflora of the external ear canal, which is discussed in detail below. *Staphylococcus spp*., *Streptococcus spp*., and *Corynebacterium spp*. are common bacteria present in the middle ear [16].

2.1.3. Internal ear anatomy

The inner ear is composed of a closed system of small membranous ducts and cavities called the membranous labyrinth, found within the temporal bone in the osseus labyrinth [15]. It contains endolymph, which its movement inside the membranous labyrinth stimulates the sensory cells in its membranous wall [15]. The utriculus and sacculus are two enlargements in

the centre of the membranous labyrinth [15]. The vestibular labyrinth is comprised of the utriculus and sacculus along with the semi-circular ducts [9]. The three semi-circular ducts originate from the utriculus and are associated with balance within the ear [15]. The maculae are two receptor areas located in the walls of the utriculus and sacculus [15]. There is also a cochlear labyrinth in the inner ear, consisting of the organ of Corti within the cochlear duct and is considered the organ of hearing [9]. The cochlear duct originates from the sacculus [15]. The cochlea is spiral shaped and is divided into three membranous ducts which is filled with endolymph and is connected to the vestibular labyrinth by the ductus reuniens [9]. The organ of Corti has sensory hair cells and supporting cells immersed in endolymph [8, 9]. These different structures are involved in the transmission and transduction of sound impulses in the inner ear to the cochlear nerve branch of the vestibulocochlear nerve [8].

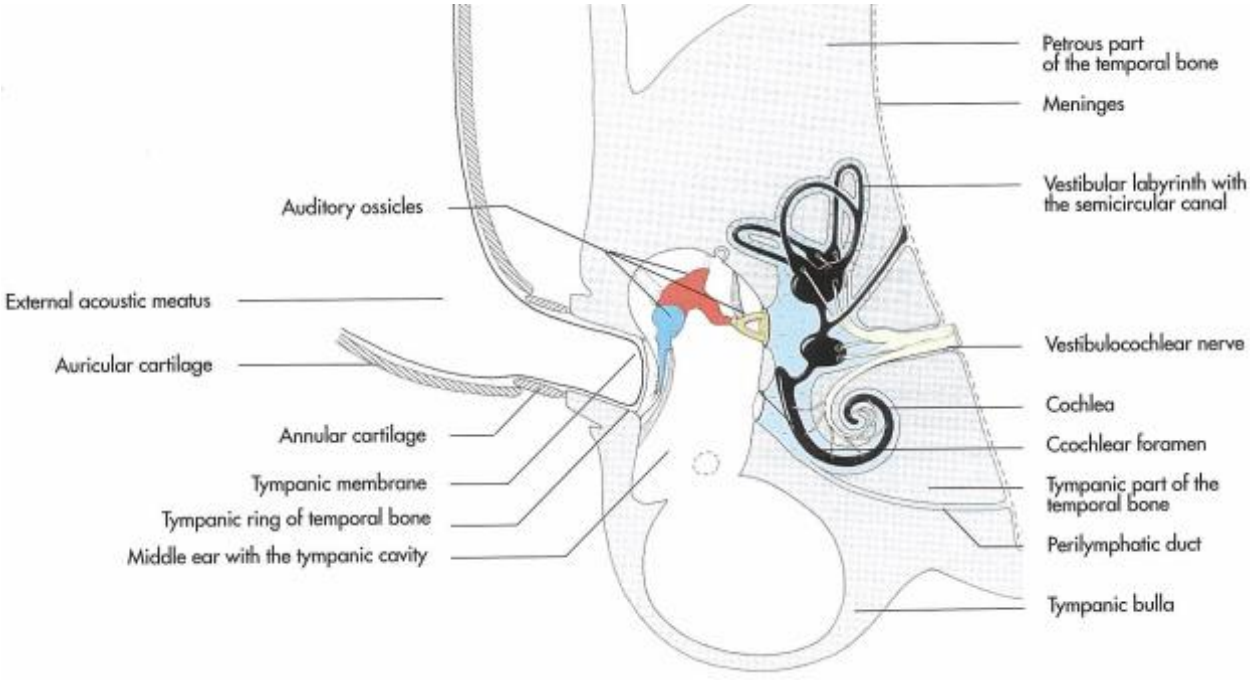

Figure 2: Schematic illustration of the external acoustic meatus, the middle and internal ear of the dog [9].

2.1.4. The microbiome of the external ear canal

The healthy canine external ear has a natural microbiome present that consist of different bacteria and yeasts [17]. Small numbers of bacteria are considered a normal finding, *Staphylococcus pseudintermedius* in particular [18]. *Malassezia pachydermatis* is considered a common yeast found in normal ears [18].

Three major studies have been conducted investigating the normal ear microbiota and its alteration in disease. When the ear canal was clinically affected all studies found a change in the diversity and number of the microbiome. They noted a dysbiosis of the ear microbiome, which occurs when one microorganism grows is in abundance and causes a lack of balance within the microbiome [17, 19, 20].

A study in 2020 by the Veterinary department in A&M University Texas analysed healthy and clinically affected ears microbiomes. They found in healthy ears the microbiome consisted mainly of the bacteria *Cutibacterium acnes, Staphylococcus pseudintermedius and Streptococcus sp.* [17]. When clinically affected, they found a loss of microbial diversity and an overgrowth of certain microbes [17]. When compared to the samples of the healthy ear canals they found that 78.3% of the clinically affected ear samples had an microbial overgrowth, 69.8% had a bacterial overgrowth and 16.3% had a fungal overgrowth and 7% had both a fungal and bacterial overgrowth [17]. They also found that the microbial taxa most abundant in the clinically affected samples were *Malassezia pachydermatis, Staphylococcus pseudintermedius, Staphylococcus schleiferi*, and a few anaerobic bacteria such as *Finegoldia magna, Peptostreptococcus canis,* and *Porphyromonas cangingivalis*.

In 2018, the Université de Liége, the University of Veterinary Medicine in Belgium also did a study on the ear canal microbiota in healthy dogs and dogs with atopic dermatitis. In healthy ears the most prevalent bacteria genera were *Escherichia* which ranged from 0.08%-35.6% in the different samples. *Conchiformibius, Cornybacterium* and *Staphylococcus* were also prevalent [19]. When clinically effected with atopy, the yeast, *Malassezia pachydermatis,* was the main pathogen cultured from the ear microbiota. The bacteria *S. pseudintermedius* was the second most common pathogen and it represented over 70% of the bacteria cultured. The *Staphylococcus* family was the most common genus in their samples ranging from 2.1% to 94.3% with a mean of 43.6%. Besides *Staphylococcus, Cornebacterium, Escherichia* and *Propionibacterium* were predominantly cultured from

their samples. This study confirmed that an atopic dog differed microbiologically to dogs with healthy ears [19].

Similarly, the Toho University School of Medicine performed a study where they analysed changes in the ear canal microbiota in dogs with otitis externa. In healthy ears they found Proteobacteria to be the most prevalent, followed by *Firmicutes*, *Cyanobacteria* and *Actinobacteria* as indicated in **(Figure 3)** [20]. They found that there was a significant decrease in microbial diversity in the group affected with otitis externa compared to the healthy group. Staphylococcus was the primary bacteria cultured in the affected group (43.3%) and its phylum, firmicutes had a relative abundance that was significantly higher than the healthy control group (14.7%) [20]. This study found that the genera *Corynebacterium*, *Staphylococcus* and *Pseudomonas* were found in both healthy and affected ears, which suggests that opportunistic infections of these common bacteria can be responsible for otitis externa [20]. The relative abundance of *Proteobacteria*, *Bacteroides* and *Cyanobacteria* were significantly lower than that of the control group. The dogs were graded with the otitis index score (3 scale) and were marked as none, mild, moderate and severe. They examined if an increase in the severity of otitis externa had an impact on the microbiome of the ear canal. Interestingly they found that the severity of the otitis had no impact on the otic microbiome like they had expected [20]. There were no differences in the diversity or abundance of the bacteria found between the severe and mild subgroups.

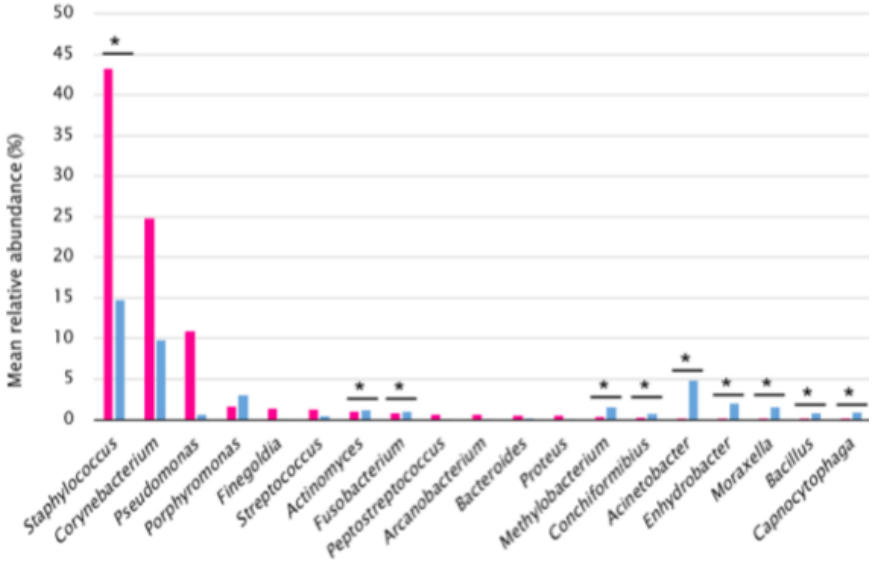

Figure 3: Relative abundance of the predominant taxa in the otic microbiota. Pink column- Clinically affected ears. Blue column- Control/healthy ears. [20].

2.2. Common canine ear diseases- Clinical signs, diagnosis and management

2.2.1. Otitis externa

Otitis externa (OE) is a multifactorial inflammatory disease of the external ear canal and the pinna, and accounts for up to 20% of consultations in small animal practice [21, 22]. A study done in 2021 in the United Kingdom found that OE was prevalent in 7.30% of all dogs under veterinary care in a one-year period [23].

The causative factors of OE are classified into four categories: predisposing, primary, secondary and perpetuating factors, illustrated in **(Figure 4)** [21]. Conformation of the pinna and external ear canal, increased temperature or moisture, obstruction or stenosis, and trauma are considered predisposing factors of OE These factors increase the risk of OE but don't normally cause the disease directly itself [20].

The primary factors of OE can be neoplasia, polyps, foreign bodies such as grass awns, hypersensitivities such atopic and contact dermatitis, autoimmune and immune mediated diseases and ectoparasites such as *Otodectes, Demodex*, *Sarcoptes* or *Neotrombicula autumnalis* [20]. *Otodectes cynotis* has be reported to cause up to 50% of the OE cases in cats and 5-10% of OE in dogs [22]. The primary causes normally initiate the inflammation in the ear canal allowing secondary factors to exacerbate the otitis [21]. The secondary factors of OE include bacterial and fungal infections [21]. Cytology and bacterial culture are commonly used diagnostic tools to diagnose OE, and it has been shown that *S. pseudintermedius, Enterococcus spp., Pseudomonas aeruginosa, Streptococcus spp., Corynebacterium spp.* and *Escherichia coli* are the main bacteria identified in OE [19].

Perpetuating factors are pathological changes to the ear canal, TM or middle ear due to the chronic inflammation that occurs during OE and normally prevent the successful treatment of the disease [21]. These perpetuating factors can be stenosis of the ear canal, otitis media, cartilage fibrosis or mineralisation, or erosions or ulcerations and altered epithelial migration [21, 22].

A retrospective study done by the University of Veterinary Medicine Thessaly investigated the aetiology of canine OE in 100 dogs. They found primary causes were the most common, with 43% of the cases caused by allergic dermatitis, 10% caused by grass awns, and 7%

caused by ear mites [21]. Secondary causes were also common; 66% of the cases had *Malassezia spp.*, 38% had cocci present and 22% had rods [21]. Perpetuating factors such as ear canal stenosis was found in 38% of cases, as well as TM perforation- otitis media in 25% [21].

Primary Causes	Secondary Infections	Predisposing Factors	Perpetuating Factors
Ectoparasites *Otodectes* *Demodex* *Neotrombicula autumnalis* *Sarcoptes* *Ticks*	Bacterial *Staphylococci* spp *Streptococci* *E.coli* *Proteus* spp *Pseudomonas* spp *Corynebacterium*	Increased ambient temperature	Overcleaning
Foreign bodies	Fungal *Malassezia* *Candida* *Aspergillus* spp *Dermatophyte*	Abnormal anatomical confirmation Congenital stenosis Increased epidermal folds Hirsute ear canal	Stenosis
Atropic dermatitis		Hypothyroidism	Fibrosis and calcification of the ear cartilages
Adverse food reactions		Hyperadrenocorticism	Cholesteatoma
Neoplasia			Otitis media
Autoimmune and immune-mediated diseases			Contact irritant or allergic reactions to topical medications

Figure 4: Causes of otitis in the dog [24].

Clinical signs of acute OE vary due to the different combinations of the aetiologies discussed. There can be a wide range seen from scratching, head shaking, purulent or ceruminous discharge, varying degrees of erythema of the pinna, and lining of the ear canal, malodour, swelling, pain, self-trauma and excoriations [22]. In chronic cases of OE, hyperplastic changes of the external ear canal can be seen, as well as stenosis and fibrosis and mineralisation of the soft tissue [22]. When these proliferative changes occur, medical treatment is normally unsuccessful and surgical treatment such as total ear canal ablation (TECA) is needed [22].

During a clinical exam, an accurate history of the patient is important when evaluating a patient with otitis externa, especially when presented with chronic OE. An additional dermatological history as shown in appendix 1 is useful to help find the underlying cause of the OE or the recurrence [22]. A full dermatological exam of the skin can show underlying skin conditions such as atopic dermatitis, allergic contact dermatitis (ACD), food allergy dermatitis, ectoparasites such as scabies or flea allergy dermatitis (FAD), based on the

locations of the pruritis or lesions, as the underlying cause of the presenting OE [22]. A thorough otoscopic exam is also essential for diagnosis [25]. Some animals may require an ear flush or cleaning before examination if excessive ceruminous discharge or debris is present in order to be able to evaluate the ear canal adequately [12]. It requires adequate restraint and animals with painful ears may need sedation in order for it to be performed [12]. A video otoscope can be useful in sedated animals for ear flushing procedures, demonstrated in **(Figure 5)** [12]. The diameter of the ear canal, the integrity of the TM, the presence of foreign bodies, parasites, tumours, and amount and type of exudate present should be assessed with a sterile speculum [25].

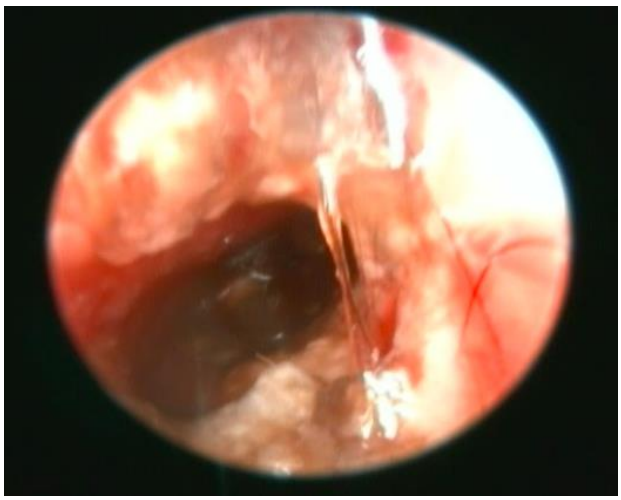

Figure 5: Video otoscopy of the external ear canal showing purulent & ulcerative otitis externa. Courtesy of Dr. Márton Balogh DVM, University of Veterinary Medicine Budapest, 2020.

With OE, cytology is considered the pre-eminent diagnostic tool, an example is illustrated in **(Figure 6)** [25]. A sample should be taken from the horizontal ear canal and examined on a stained slide for the number and morphology of bacteria, the number of yeasts, presence of parasites, number and type of leucocytes and the presence of neoplastic cells, excessive cerumen and keratinaceous debris [25]. In normal ears, large, flattened keratinocytes should predominate with only a small number of cocci and *Malassezia* [18]. As mentioned previously, the ear canal has a normal microbiome of bacteria and yeasts, so taking into account the number or amount of the bacteria present and the presence of inflammatory cells can help distinguish bacterial or fungal otitis from the microbiome [25]. A culture and sensitivity is recommended when rods are seen on cytology, otitis media is suspected or the

first treatment fails [25]. The most suitable microbial treatment can only be properly chosen if the pathogen causing secondary inflammation is identified [18].

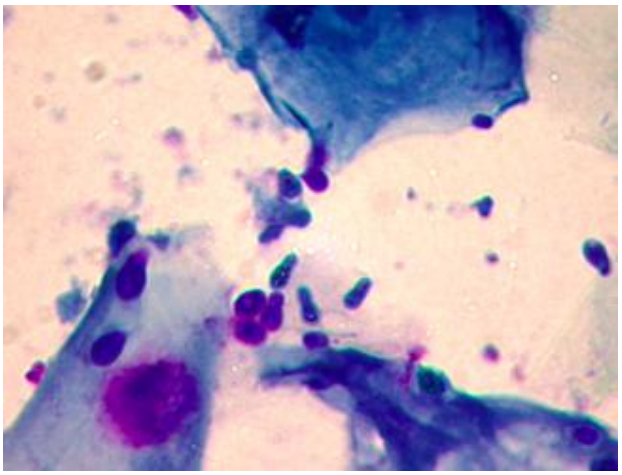

Figure 6: Ear cytology showing a *Malassezia pachydermatis* infection Courtesy of Dr. Noemi Tarpataki DVM and Dr. Peter Vajdovich DVM, Dipl. ECVCP, University of Veterinary Medicine Budapest, 2023.

Treatment of OE should be tailored to each individual case [25]. Ear cleaning is an essential part of treatment, as it facilitates the removal of cerumen, purulent discharge and bacteria and it aids in restoring the microbiome [24]. Reversing inflammation such as oedema and erythema and treating secondary bacterial or fungal infections will help restore epithelial migration [24]. Glucocorticoids (GCC) are recommended in this incidence [24]. Topical antimicrobials and antifungals are only recommended if there are secondary infections present [24]. The quicker the oedema or swelling is treated., the less likely chronic irreversible changes will occur [24] . Systemic GCC may be recommended for a short course to quickly reduce severe swelling and inflammation and subsequently reduce pain, making it easier for treatment at home [24]. There are topical preparations for ears available on the market that contain a combination of GCC, antifungals and antibiotics [24]. Systemic antibiotics are not indicated in OE, as they have limited value in the secondary infections in OE [24].

2.2.2. Otitis media

Otitis media (OM) is an inflammatory disease of the middle ear including the tympanum, tympanic bulla and the eustachian tube [26, 27]. It normally occurs secondary to OE or due a foreign object such as a grass awn, penetrating the TM [26]. OM is more prevalent than previously thought, occurring in 16% of acute OE and up to 50% to 80% of chronic OE cases [28]. It should be suspected in dogs with recurrent or chronic OE [29]. The middle ear can become inflamed by three routes; first, through the tympanic membrane, which is the most common form, second, through the auditory tube and finally by hematogenous spread [29]. Infectious causes of OM include, bacteria, fungi and viruses [27]. According to Logas (2012) viruses are the least common cause [27]. The most common bacteria cultured from the middle ear of dogs with OE include *Staphylococcus pseudintermedius* and *epidermis, Pseudomonas aeruginosa* and *β- haemolytic Streptococci* [27]. OM can also be associated with upper airway disease such as chronic sinusitis as those bacteria can ascend the Eustachian tube [27]. If that is the case, *Mycoplasma* or *Bordetella* can be cultured [27]. Malassezia pachydermatis is the most common fungal cause of OM [27]. *Aspergillus spp*., and *Penicillium spp*., can cause OM but it thought to be infrequent [27]. Non-infectious causes such as neoplastic growth, lymphoid hyperplasia or polyps can also cause OM [27].

Clinical signs of OM can be similar to otitis externa; ear scratching, head shaking, pawing at the affected ear, discharge and inflammatory changes of the ear canal can be seen [26]. Horner's syndrome; constriction of the pupil (miosis), drooping of the eyelid (ptosis), protrusion of the nictitating membrane and keratoconjunctivitis sicca, facial nerve paralysis; drooling, inability to move the ear or lip and a decreased or absent palpebral reflex and auditory deficits can also occur on the same side as the affected ear as the facial and sympathetic nerves run through the middle ear [26, 29, 30]. OM, or rupture of the TM can may lead to inflammation of the inner ear structures, if otitis interna occurs, the patient may show signs of incoordination, ipsilateral head tilt and nystagmus [30].

Primary OM has been reported in some breeds, primary secretory otitis media (PSOM) in Cavalier King Charles Spaniels (CKCS) in particular [30]. According to Cole (2012) this disease may occur due to increased production of viscous or gelatinous mucus in the middle ear and a decrease in drainage of the middle ear through the auditory canal [31]. A study done in the Royal Veterinary College by Mielke et al (2017) found a dorso-ventral flattening

of the tympanic bulla in CKCS, which they suggested these anatomical changes could contribute to auditory tube dysfunction, and therefore be another possible cause for the pathogenesis of PSOM [32]. Another study done by Hayes et al (2010) proposed that CKCS are predisposed to PSOM due to the anomalies of their nasopharynx as a result of their brachycephalic conformation such as an elongated soft palate and a decreased nasopharyngeal dimension that leads to an impairment of the eustachian tube orientation and function and therefore drainage [33].

A study done by Cole (2012) investigated if a bacterial infection played a part in the disease process. They found that 56% of the samples had no bacterial growth and 25% had a positive bacterial culture from the middle ear. The most common bacteria found in the middle ear were Coagulase-negative Staphylococci, Methicillin-Resistant Staphylococci, *Staphylococci pseudintermedius*, *Corynebacterium spp*. and *Moraxella* in low amounts. They posed a question whether the bacteria cultured were part of the normal microbiome and not pathogenic since they were found in low numbers [31].

According to Shell (1998) sometimes OM is overlooked during an otic exam as once the signs of OE are present visualization if the TM becomes more difficult [29]. Another problem with diagnosing OM is examination of the TM is crucial but that can be hindered by the anatomy of the ear canal which makes it difficult to see with the bend between the vertical and horizontal portion [28]. Debris, excessive cerumen, proliferative changes in the ear canal such as stenosis and lack of patient co-operation can make it hard for the examiner to properly visualize the TM [29]. Diagnosis is also difficult if the TM is intact which occurs in around 70% of cases according to Moriello (2023) [26].

Examination of the TM should be done under general anaesthesia in patients with OM [28]. An endotracheal (ET) tube should be placed in case there is a ruptured TM and any material from manipulation or flushing drains through the Eustachian tube into the nasopharynx causing aspiration [28]. If stenosis or pathological changes to the ear canal hinder the visualization of the TM, topical or systemic corticosteroids can be useful to reduce inflammation and allow examination on a further visit [28].

Radiography of the TB, shown in **(Figure 7)** can be useful to determine if fluid is present there or if there is any changes in the bone [28]. Computerized tomography (CT), of the

bullae, demonstrated in **(Figure 8)** can help differentiate bony lesions from soft tissue reactions and be useful for evaluating the horizontal portion of the ear canal and TB when there is stenosis [28]. MRI can also be used to evaluate the middle and inner ear. MRI can detect if there has been a spread of infection into the meninges, so it can be useful when patients present with neurological signs [28]. The most reliable and accurate way to diagnose according to Logas (2012) is by video-otoscopic examination and myringotomy [27].

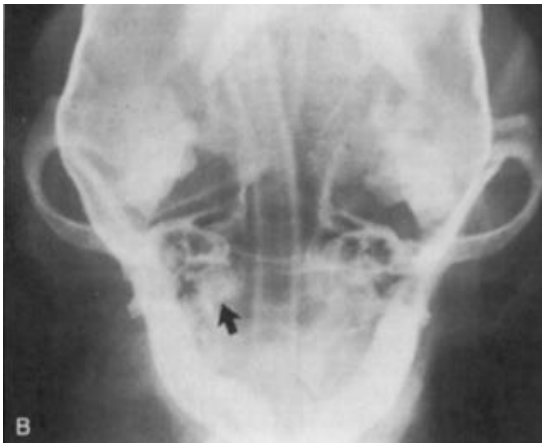

Figure 7: Radiograph of the head, showing purulent exudate in the right ear canal [29].

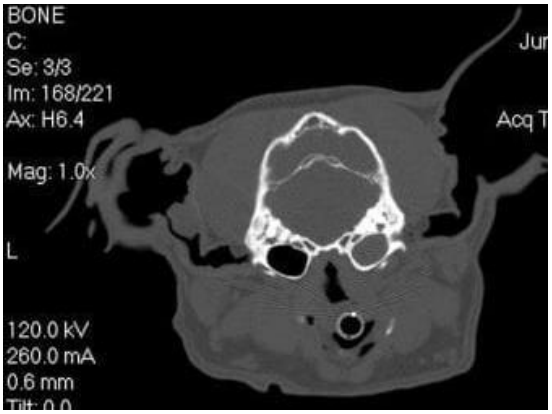

Figure 8: CT of the head, showing Otitis Media in a dog [30].

The first procedure for treatment should be removal of any debris or exudate that has accumulated in the TB under general anaesthesia [27]. Lavaging the middle ear can cause complications such as facial nerve paralysis, Horner's syndrome and hearing loss but they are considered rare in canine patients and more common in cats [27]. Based on cytology and susceptibility testing, either topical or systemic antimicrobial therapy can be chosen to treat

the right pathogen present [27]. The average length of OM takes 2- 3 months to treat, ranging from 30 days to 360 days [27]. If the TM is perforated, it should be noted that topical medications and their chemicals in the ear cleaners or preparations could access the inner ear causing ototoxicity [28]. Damage to the hair cells in the cochlea or the vestibular apparatus can cause loss of hearing and vestibular disease [28]. Chlorohexidine, found in many ear cleaners is ototoxic, especially in cats, so it should be avoided [28]. Polymyxins, aminoglycosides, detergents and most alcohols that are used in different preparations to treat OE are ototoxic [28].

The cost vs benefits should be weighed up when treating OM. For example, the aminoglycoside- tobramycin is considered an effective antibiotic to treat multidrug resistant Pseudomonas spp., but it is associated with potential ototoxic side effects. Still, it is often used in the bulla to treat the infections because of it efficacy [28]. Gentamicin concentrates in the hair cells in the organ of Corti when given parenterally and can cause vestibular signs such as ataxia, circling and a head tilt when given topically [28]. Fluoroquinolones such as ciprofloxacin, ofloxacin and enrofloxacin, aqueous penicillin, carbenicillin, ticarcillin and the cephalosporin, ceftazidime and cefmenoxime are safe to use in the middle ear [28]. The antifungals, miconazole, clotrimazole, tolnaftate and nystatin can be used in the middle ear [28]. The aqueous forms of the anti-inflammatories fluocinolone and dexamethasone can be used in the middle ear too [28]. The cerumenolytic squalene is safe to use along with the flushing agent Tris-edta [28]. According to Morris (2004) OM cannot be treated properly with topical therapy alone and recommends systemic treatment based on culture/sensitivity for a minimum of 6-8 weeks [34]. When giving antimicrobial or anti-inflammatory treatment, rechecks should be done weekly and can be retreated as necessary [28]. Surgery such as TECA and bulla osteotomy may be needed in case of chronic or OM that was unsuccessful with medical treatment [28]. After treatment the TM should regenerate, although in some cases it may not [34].

2.3. Antibiotic resistance development

Antimicrobials include antibiotics, antifungals, antiparasitics and antivirals, and are medicines used to prevent and treat infections in humans and animals. AMR and the increase in multi-resistant bacteria are considered a global phenomenon affecting both public and animal health [1]. According to the WHO (2017) overuse and misuse of antimicrobials are the main reasons in the development of AMR. This overuse of antibiotics has led to bacteria adapting, mutating and creating new strains that are now resistant to these antibiotics [35]. Bacteria use multiple different mechanisms to evolve and evade the effect of antibiotics and survive antibiotic exposure [35]. Some bacteria can even modify or inactivate the components of the antibiotic and make it ineffective through degrading enzymes, others can transport it out of the bacteria and some can modify their outer structure so antibiotics cannot attach to their receptors [35].

There are four commonly characterized mechanisms of AMR:

- **Intrinsic resistance**- Through evolution bacteria have an intrinsic resistance that enable them to survive exposure to antibiotics, by changing their structure or their components, such as the reducing or eliminating the permeability of the bacterial wall to the antibiotic, presence of efflux pumps, inactivation of the antibiotic or lack of target molecules [35, 36].
- **Acquired resistance**- Through genetic mutation bacteria can acquire resistance to an antibiotic which it was previously susceptible to, or it can acquire the resistance from a bacteria that is already resistant as discussed below [35].
- **Genetic change**- The bacteria have the ability to change their DNA and alter their protein production, which gives rise to different components and receptors in the bacteria that make it unrecognizable by the antibiotic [35].
- **Deoxyribonucleic Acid (DNA) transfer**- Bacteria have the ability to share genetic material with each other and so can horizontally transfer the antibiotic resistant DNA to other bacteria. Transformation, transduction and conjugation are three ways bacteria can acquire external genetic material [35].

Biofilms are another important component of AMR especially with *Pseudomonas aeruginosa*, a bacteria commonly found in OE [37]. Biofilms are bacterial clusters held together in a protective matrix that can attach to surfaces and protect these bacteria from

antibiotics and the immune system [37]. In veterinary medicine, improper antibiotic use, whether it involves using them at insufficient dosages, for durations that are too short or too long, or too frequently, or for treating bacteria that aren't susceptible to the chosen antibiotic, or as a broad-spectrum treatment when the cause might not be bacterial, increases the risk of AMR development, as stated by the Irish Health Products Regulatory Authority (HPRA) [38].

2.4. Antibiotic resistance in canines

Common resistance found in canines include Methicillin-Resistant Staphylococcus aureus Methicillin-Resistant Staphylococcus pseudintermedius, *E. coli* extended spectrum beta-lactamase, vancomycin-resistant *Enterococci*, and carbapenemase-producing gram negative bacteria that are of increasing concern to human health [39]. A topic of much research at the moment is the possibility of AMR transmission between companion animals and their owners due to indirect and direct contact such as touching, kissing or licking [40]. This is an escalating concern because of the significant antimicrobial agents employed in companion animal medicine, and the resistance to these agents could potentially pose a significant threat to human medicine. Multiple studies have been carried out in search of an answer to this question with varied conclusive results.

A study by Song et al., 2013 found that people cohabiting with dogs shared a similar skin microbiome and have a higher microbial diversity than people without dogs or with other dogs than their own [41]. A study by this University in 2022 explored the possibility of canine saliva as a possible source of antimicrobial resistance genes and concluded that saliva especially from dog bites may colonise human skin and mucous membranes and several genes containing resistance against important antimicrobial groups may drift to the bacterial genome in humans [42]. A study by Naziri et al, analysed 144 *E.coli* isolates from 28 dog-owner pairs and 16 humans without dogs and found the extended spectrum beta-lactamase producing strains was similar in 71.4% of pairs and the antibiotic resistance profile of *E.coli* isolates were the same in 14.3% of pairs suggesting that sharing resistant bacteria is possible [43]. Interesting to this study, Tanner et al. found evidence of non-invasive transmission of S. intermedius from a canine companion to a female patient with otitis externa [44].

We can't say with absolute certainty that AMR is directly transmitted from dogs to their owners as current research has dividing results, however, emerging evidence and recent studies suggest that this is a viable concern. Given the escalating prevalence of AMR and multi-drug resistance in canines, this warrants closer examination and further investigation.

2.5. Prevalence of antibiotic resistance in ear microbiome

As mentioned previously otitis is a common multifactorial disease of the ear canal in dogs caused by a wide range of bacteria and yeasts. The most common pathogens that cause OE are Staphylococcus spp., Pseudomonas spp., *E. coli, Corynebacterium spp., Proteus spp*., and *Malassezia spp*. [7]. Treatment for otitis in general practice is usually empirical antibiotic therapy based on clinical signs without any susceptibility testing [7]. If a gram stain has been done from an ear swab, and either gram positive cocci or gram-negative rods are present, the choice of antibiotic can be narrowed down to a drug that is likely to work against either [7]. Due to the presence of multi-drug resistance in *P. aeruginosa*, if gram negative rods in abundance are seen on a smear, clinicians might do an antibiotic culture and susceptibility testing then [7].

A study performed by Martins et al in Brazil in 2022 tested the susceptibility of pathogens they isolated from 142 cases of unmedicated otitis externa against fifteen antibiotics and three antifungals. The antimicrobial susceptibility testing was performed by the Kirby-Bauer disk-diffusion method in this study. The antibiotics tested were– the β- Lactams- penicillin G, ampicillin, amoxicillin/clavulanic acid, cephalexin, cefoxitin, and oxacillin, the peptides- polymyxin B, polymyxin E, the aminoglycoside- gentamicin, amikacin, streptomycin, and neomycin, the fluoroquinolones- enrofloxacin, and ciprofloxacin, the macrolides- erythromycin, Rifampins- rifampicin and Sulphonamides-trimethoprim-sulphamethoxazole. Coagulase positive staphylococci (40.2%), Enterobacteriaceae (13%) and Pseudomonas spp., (11.3%) were the most common bacteria cultured [7].

This study found multidrug resistance in 57% of bacterial isolates including, 92% of the *Pseudomonas spp.,* 88.26% of *Enterobacteriaceae*, 84.6% of the *Proteus spp*., 55% of Coagulase negative Staphylococci, 50% of *Streptococcus spp*., 41% of Coagulase positive Staphylococci, 40% of *Bacillus spp*., and 20% of *Corynebacterium spp*. [7]. The lowest antibiotics sensitivity testing was penicillin (39% in gram positive isolates), erythromycin (39.2%), oxacillin (44%), enrofloxacin (48.3%) and trimethoprim- sulphamethoxazole (48.8%) (Martins et al., 2022). High susceptibility rates to amoxicillin/clavulanate (86.4%), ciprofloxacin (85.6%), neomycin (83.3%), polymyxin B (82%), amikacin (81.9%), ampicillin (80%) and gentamicin (79.5%) were found among the isolates, suggesting that aminoglycosides are the better choices for otitis therapy in this region [7]. Though there

were some high susceptibility results in the β- Lactams, overall they found a high resistance in the isolates in this group, bar amoxicillin/clavulanate [7].

A smaller study by Tesin et al. from Serbia in 2023, analysed 40 bacterial isolates from dogs with otitis externa, using the disc diffusion method. The most common bacterial pathogen found in 54.72% of samples was *Staphylococcus pseudintermedius*, with 7.55% of samples containing P. aeruginosa and 5.66% Proteus spp. 28 antibiotics were tested in this study- amoxicillin/clavulanic acid, amikacin, amoxicillin, gentamicin, neomycin, penicillin, sulfamethoxazole-trimethoprim, ceftriaxone, ciprofloxacin, enrofloxacin, tobramycin, doxycycline, clindamycin, azithromycin, tetracycline, erythromycin, levofloxacin, ampicillin, vancomycin, colistin, pradofloxacin, cefoxitin, nitrofurantoin, rifampicin, cefquinome, marbofloxacin, lincomycin, and fusidic acid [45].

S. pseudintermedius had high resistance to penicillin (76%), amoxicillin (69%), clindamycin (66%), lincomycin (62%) and azithromycin (62%). And a low resistance against cefoxitin (3%), amoxicillin/clavulanic acid (10%), ceftriaxone (14%) and enrofloxacin (14%). P. aeruginosa showed total resistance (100%), against many antibiotics, including- amoxicillin/clavulanic acid, amoxicillin, penicillin, sulfamethoxazole-trimethoprim, tetracycline, levofloxacin, ampicillin, vancomycin, cefoxitin, nitrofurantoin, rifampicin and cefquinome, while neomycin was the only antibiotic without resistance (0%). *Proteus spp*. also showed 100% resistance to doxycycline, tetracycline, azithromycin and vancomycin. Proteus spp. had no resistance (0%) to amoxicillin and clavulanic acid, gentamicin, ceftriaxone, ciprofloxacin, enrofloxacin, levofloxacin, pradofloxacin and cefoxitin. All gram-negative bacteria showed total resistance (100%) against tetracycline and vancomycin, which is a worrying result as vancomycin is considered a critically important antibiotic in human medicine. The highest susceptibility overall of gram positive and negative bacteria was enrofloxacin and the lowest was against penicillin and amoxicillin [45].

A large study by Bourély et al in 2019 by the French national surveillance network for AMR, also analysed the antimicrobial resistance of over 7021 bacteria isolated from dogs with otitis using the disk diffusion method over a four-year period. The most common bacteria genera isolated were Coagulase positive Staphylococci, *Pseudomonas spp.*, *Streptococcus spp*., and *Proteus spp*. *S. pseudintermedius* accounted for 33% of the isolates, *P. aeruginosa* accounted for 27.5%, *Streptococci* for 14.1%, *P. mirabilis* for 13.6% and *S. aureus* for 3.9%.

Coagulase positive Staphylococci was tested against gentamicin, cefovecin or cefoxitin, enrofloxacin, trimethoprim-sulfamethoxazole, fusidic acid, erythromycin, Penicillin G, and chloramphenicol. Pseudomonas spp. was tested against gentamicin and enrofloxacin. Proteus spp. was tested against gentamicin, ceftiofur, enrofloxacin, trimethoprim-sulfamethoxazole and amoxicillin. Streptococcus spp. was tested against gentamicin, enrofloxacin, trimethoprim-sulfamethoxazole, erythromycin, chloramphenicol and oxacillin. This study found resistance was high for penicillin in *S. pseudintermedius* (68.5%), *S. aureus* (70.9%) and lower in *P. mirabilis* (28.9%) and *Streptococci* (14.4%). 9.4% of *S. pseudintermedius* was resistant to cefovecin and 10.6% of *S. aureus* was resistant to cefoxitin, indicating a presence of MRSP and MRSA to cephalosporins in the bacterial isolates. The resistance for trimethoprim-sulfamethoxazole ranged from 10.2 %- 22.9% in the bacteria isolated [46].

The resistance in *S. pseudintermedius*, *S. aureus* and *Streptococci* for chloramphenicol and erythromycin were similar and ranged from 25%- 40%. *P. aeruginosa* had the highest resistance to gentamicin (17.9%) and the resistance was lower in *S. pseudintermedius* (13.5%), *S. aureus* (12.9%), *P. mirabilis* (10.3%) and lowest for *Streptococcus spp.* (3.3%). The level of resistance to enrofloxacin was highest in *P. aeruginosa* (67.7%) and *Streptococcus* (62.9%) and lower in *P. mirabilis* (13.2%) and *Staphylococci* isolates (12-13%). Fluroquinolones are considered critically important antibiotics in human medicine so the high levels of resistance in *P. aeruginosa* is considered worrying. Multi-drug resistance was found in 20% of *S. pseudintermedius*, 17% of *S. aureus*, 12.9% of *Streptococcus spp*. and 11.8% of *P. mirabilis*. 15.3% of *P. aeruginosa* was resistant to enrofloxacin and gentamicin [46].

A study by Bugden in 2012 in Australia analysed 3541 bacterial isolates from dogs with otitis externa using disc diffusion. The antibiotics tested in this study were gentamicin, neomycin and polymyxin B. Enrofloxacin was not tested directly and ciprofloxacin and moxifloxacin were used as a surrogate test in two different years. Neomycin was also used as a surrogate used for framycetin. The most common bacteria isolated were *P. aeruginosa* (35.5%), *S. pseudintermedius* (24.3%), *Proteus spp*. (6.8%), β- haemolytic *Streptococci* (6.2%) and *E. coli* (4.2%). Susceptibility to gentamicin was high in *E. coli* (100%), *Proteus spp.* (99%), *S. pseudintermedius* (99%) and *P. aeruginosa* (95%) but the resistance in β-haemolytic *Streptococci* (99%) was very high. The resistance to polymyxin B was very high

in *S. pseudintermedius* (100%), *Proteus spp* (100%), β- haemolytic *Streptococci* (100%) and *E. coli* (60%), though *P. aeruginosa* (93%) was highly sensitive. The susceptibility to enrofloxacin were quite high in *S. pseudintermedius* (98%), *Proteus spp*. (96%), *E. coli* (97%) , with low susceptibility for *P. aeruginosa* (36%) and total resistance in β- haemolytic *Streptococci*. The susceptibility for neomycin/framycetin was high in *S. pseudintermedius* (96%), low in *Proteus spp.* (53%) and *E. coli* (49%) and with almost complete resistance in β- haemolytic *Streptococci* (1%). If cocci are seen in cytology in this country, gentamicin, neomycin/ framycetin or enrofloxacin would be a good option based on the result from this study. If rods are seen on cytology, with no further identification available, the most appropriate choice of antibiotic would be gentamicin as *P. aeruginosa* is the most likely cause based on the number of times it was isolated compared to the other rod shaped bacteria (*Proteus spp, E.coli*) in the study [47].

A study performed by the University of Veterinary Medicine in Tehran in 2010 analysed 92 bacterial isolates of dogs with otitis externa using the Kirby-Bauer disc diffusion method. The antibiotics tested were- amikacin, ampicillin, amoxicillin-clavulanic acid, ceftriaxone, cephalothin, chloramphenicol, enrofloxacin, erythromycin, gentamicin, lincomycin-spectinomycin, oxytetracycline, rifampin, penicillin G, and trimethoprim-sulfamethoxazole. 83.7% of the isolates were gram positive cocci belonging to the *Staphylococcus* genus and 16.3% were gram negative rods including *Pseudomonas spp., Proteus spp., E. coli* and *Pasteurella*. The *Staphylococcus spp*. were all completely susceptible to enrofloxacin, rifampin and amikacin. There was a low resistance present in the genus to ceftriaxone (6.49%), cephalothin (5.5%), and gentamicin (2.6%). The highest resistance occurred in penicillin (61.04%), followed by ampicillin (53.25%), and lower levels of resistance were found in trimethoprim-sulfamethoxazole (37.66%) and oxytetracycline (33.77%) [48].

All *Pseudomonas aeruginosa* isolates were completely susceptible to enrofloxacin and amikacin. There were lower levels of resistance found in gentamicin (10 %), ceftriaxone (10%) and trimethoprim-sulfamethoxazole (40%). High levels of resistance were found in ampicillin (100 %), amoxicillin-clavulanic acid (100 %), cephalothin (100 %), erythromycin (100 %), rifampin (100 %), penicillin G (100 %), lincomycin-spectinomycin (100 %), oxytetracycline (80 %) and chloramphenicol (70 %). The *Proteus mirabilis* isolates were totally sensitive to ceftriaxone, amikacin, enrofloxacin and trimethoprim- sulfamethoxazole. There was complete resistance found in cephalothin, erythromycin, oxytetracycline,

rifampin and penicillin G. Lower levels of resistance were found in ampicillin (33.33%), chloramphenicol (33.33%), lincomycin-spectinomycin (33.33%) and amoxicillin-clavulanic acid (33.33%). *E. coli* was completely susceptible to amikacin, ceftriaxone, chloramphenicol, gentamicin, enrofloxacin, lincomycin-spectinomycin and trimethoprim-sulfamethoxazole. Complete resistance was found in ampicillin, amoxicillin-clavulanic acid, erythromycin, oxytetracycline, rifampin and penicillin G. If gram positive bacteria are seen in cytology in this country, enrofloxacin, amikacin, gentamicin or ceftriaxone would be a good option to use. If gram negative bacteria are seen on cytology, with no further identification possible, the most appropriate choice of antibiotic would be amikacin or enrofloxacin. In mixed cultures based on the results of this study, amikacin or enrofloxacin could be used for both gram positive and negative bacteria [48]. The data from these studies are illustrated in appendix 2.

The varying levels of resistance and susceptibility of the different bacteria isolates tested in the different studies discussed highlights the importance of each country performing their own antibiotic susceptibility testing to evaluate what resistance levels are present there.

2.6. Alternate therapy for otitis

Bacteriophages (phages) are viruses that have the ability to target and kill bacteria (Marco-Fuertes et al., 2022). They are considered to be a semi-parasite as they need bacterial cells to reproduce and survive [49]. A case report by Marza et al (2006), administered 400 PFU of bacteriophages to a patient with chronic bilateral otitis externa that was infected with *Pseudomonas aeruginosa*. The patient had been unresponsive to traditional topical and systemic antibiotic treatment. After application of phages into the right ear, the inflammation in the ear improved dramatically and the left ear was also treated after remission in the right ear [50]. For a period of time, *P. aeruginosa* was still isolated from the ears after treatment. There were periods of relapse and recovery but the condition was less severe than before the phage treatment and no further antibiotics were given. Nine months after the phage therapy both ears were completely recovered with no adverse effects noted and no *P. aeruginosa* was isolated [50].

Another similar study in 2010 by Hawkins et al, administered bacteriophages to ten dogs in a clinical trial with otitis caused by *P. aeruginosa*. This trial administered six different

phages in their $1x10^5$ PFU mixture unlike the one strain in the previous study. All *P. aeruginosa* isolates were partially resistant to two or more anti-pseudomonal agents [51]. Clinical scores for erythema and odour in seven dogs improved, along with improved scores for discharge and ulcerative lesions in five dogs and improved scores for discharge quantity in six. Overall scores improved in all dogs 48 hours after treatment when compared to their baseline result. The follow up in this study was inconclusive as two dogs were euthanised for unrelated reasons but chronic otitis was completely resolved in three dogs and the *P. aeruginosa* component of the otitis was resolved in a further three dogs after treatment [51].

With further testing, bacteriophage therapy could be a successful alternative to antibiotics in otitis in case of resistance or in general to decrease the usage. They could also be used in conjunction with antibiotics to increase the bactericidal efficacy and when combined have shown to destroy biofilms, which would be beneficial in cases with *P. aeruginosa* [49]. If bacteriophage therapy is authorized in the future, responsible use is needed to avoid phage resistance similarly to antibiotics [49].

3. Aim of the Study

The primary objective of this study is to assess the incidence of antibiotic resistance within the ear canal microbiome of a sample of dogs treated at the University of Veterinary Medicine Budapest for various ear diseases in the year 2020.

Based on the findings, this data pertaining to resistance patterns in Budapest may serve as valuable guidance for university clinicians in their future deliberations regarding the selection of antibiotics for otitis treatment protocols.

4. Materials and Methods

We conducted a retrospective evaluation of patient data extracted from the online database of endoscopic patients of the Small Animal Clinic at the University of Veterinary Medicine Budapest for the year 2020. This dataset comprised forty-one patients who presented with diverse clinical symptoms related to otitis externa and media. Among these cases, twelve were sampled by the clinicians in the university for microbiological examination and subsequent antibiotic susceptibility testing.

4.1 Patients

Each of the forty-one patients arrived for sedated, endoscopic examination of the external ear canals, and were either referred to the clinic by external clinicians, or from within the Small Animal Clinic of UVMB.

Sedation was induced with 5 mcg/kg dexmedetomidine, 0.25 mg/kg diazepam, and 1mg/kg boluses of propofol, until the patient could be intubated, after which oxygenisation, and maintenance of the anaesthesia was achieved with 2% isoflurane.

Patients were examined with a Karl Storz HOPKINS Telescope 30°, 2.7 mm, 18 cm (Item no: 28719BA) with an attached operational sheath.

Microbiological culture and sensitivity evaluation was performed by Duo Bakt Állatorvosi Laboratórium kft.

4.2 Data

Patient data was recorded into a patient management system (Doki for Vets, Instant System Kft.) and image data was also recorded to a separate personal computer. This data was then harmonized into an online database (https://anyadahalor.org/scopedb), allowing for searching and filtering the data.

4.3 Analysis of data

Our study comprised of three main elements:

Firstly, we analysed the bacterial strains present in the ear canal samples submitted for microbiological examination and compared them to the bacterial species typically associated with canine otitis.

Subsequently, we evaluated of antimicrobial resistance within the isolated bacterial strains through antimicrobial susceptibility testing.

Lastly, we compared the prevalence of antibiotic resistance and susceptibility observed in the bacterial isolates against existing literature on antibiotic resistance within the canine ear microbiome.

Due to the retrospective nature of the study, and the data available, no statistical analysis was performed on the data

5. Results

Microscopic examination of ear canal samples collected from the twelve patients revealed a total of eighteen bacterial isolates, with two bacterial species found in five patients. The most commonly cultured bacteria as shown in **(Figure 9)** were *Staphylococcus pseudintermedius* (33%) and *Pseudomonas aeruginosa* (28%). *Beta haemolytic Streptococcus* (22%) was moderately prevalent in the cultures, while *Klebsiella oxytoca* (11%) and *Coagulase-negative Staphylococcus* (6%) exhibited the lowest prevalence.

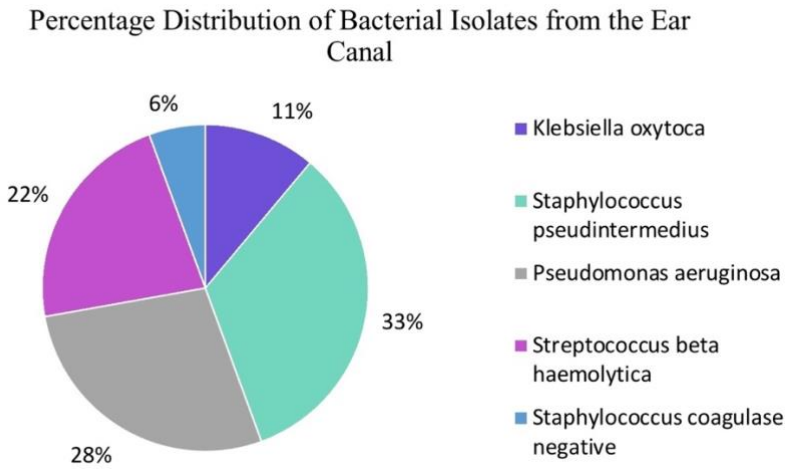

Figure 9: The distribution of bacterial strains isolated from the ear canal samples from cases of the Department and Clinic of Internal Medicine, UVMB in 2020.

Pseudomonas aeruginosa

Pseudomonas aeruginosa was cultured from five patients. Susceptibility testing was performed with ceftazidime, gentamicin, tobramycin, amikacin, ciprofloxacin, marbofloxacin and polymyxin B. All isolates were totally susceptible (100%) to all the antibiotics tested as illustrated in **(Table 1).**

Table 1: Antibiotic resistance profile of *Pseudomonas aeruginosa* cultured from 5 patients. R= Resistant, S sensitive, M - moderately resistant, - = Not tested.

Antibiotic	*Pseudomonas aeruginosa* Isolates					Total Susceptibility
	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	
Ceftazidime	S	S	S	S	S	100%
Gentamicin	S	S	S	S	S	100%
Tobramycin	S	S	S	S	S	100%
Amikacin	S	-	-	-	-	100%
Ciprofloxacin	S	S	S	S	S	100%
Marbofloxacin	S	S	S	S	S	100%
Polymyxin B	S	S	S	S	S	100%

Klebsiella oxytoca

Klebsiella oxytoca was isolated in two patients. The antibiotics tested against these bacterial isolates were amoxicillin, amoxicillin & clavulanic acid, cefalexin, cefuroxime, gentamicin, amikacin, sulfamethoxazole trimethoprim, ciprofloxacin, enrofloxacin, moxifloxacin, pradofloxacin, chloramphenicol, florfenicol and polymyxin B. As demonstrated in **(Table 2)** *Klebsiella oxytoca* was susceptible (100%) to most of the antibiotics tested including, amoxicillin & clavulanic acid, cefuroxime, amikacin, sulfamethoxazole trimethoprim, ciprofloxacin, enrofloxacin, moxifloxacin, pradofloxacin, chloramphenicol, florfenicol and polymyxin B. Moderate susceptibility was demonstrated in gentamicin (75%), while total resistance (0%) was found in two antibiotics: amoxicillin and cefalexin.

Table 2: Antibiotic resistance profile of *Klebsiella oxytoca* cultured from 2 patients. R= Resistant, S sensitive, M - moderately resistant, - = Not tested.

Antibiotic	*Klebsiella oxytoca* Isolates		Total Susceptibility
	Isolate 1	Isolate 2	
Amoxicillin	R	R	0%
Amoxicillin & Clavulanic acid	S	S	100%
Cefalexin	R	R	0%
Cefuroxime	S	S	100%
Gentamicin	S	M	75%
Amikacin	S	-	100%
Sulfamethoxazole Trimethoprim	S	-	100%
Ciprofloxacin	S	S	100%
Enrofloxacin	S	S	100%
Moxifloxacin	-	S	100%
Pradofloxacin	-	S	100%
Chloramphenicol	-	S	100%
Florfenicol	-	S	100%
Polymyxin B	S	S	100%

Coagulase Negative *Staphylococcus*

Coagulase Negative *Staphylococcus (CoNS)* was isolated from a single patient. The antibiotics used in the susceptibility testing for this bacterial strain include, amoxicillin & clavulanic acid, cefalexin, chloramphenicol, florfenicol, gentamicin, clindamycin, clarithromycin, ciprofloxacin, enrofloxacin, and polymyxin B. As shown in **(Table 3)** high susceptibility (100%) was exhibited in the majority of the antibiotics tested, including amoxicillin & clavulanic acid, cefalexin, chloramphenicol, florfenicol, ciprofloxacin, and enrofloxacin. Moderate susceptibility (50%) was found in polymyxin B and complete resistance (0%) was found in gentamicin, clindamycin and clarithromycin.

Table 3: Antibiotic resistance profile of Coagulase Negative *Staphylococcus* cultured from 1 patient. R= Resistant, S sensitive, M - moderately resistant.

Antibiotic	Coagulase Negative *Staphylococcus* Isolate	Total Susceptibility
Amoxicillin & Clavulanic acid	S	100%
Cefalexin	S	100%
Chloramphenicol	S	100%
Florfenicol	S	100%
Gentamicin	R	0%
Clindamycin	R	0%
Clarithromycin	R	0%
Ciprofloxacin	S	100%
Enrofloxacin	S	100%
Polymyxin B	M	50%

Staphylococcus pseudintermedius

Staphylococcus pseudintermedius was the most cultured bacteria, isolated from six patients. The following antibiotics were tested against these bacterial isolates: amoxicillin & clavulanic acid, cefalexin, cefuroxime, ceftazidime, tetracycline, doxycycline, chloramphenicol, florfenicol, amikacin, gentamicin, tobramycin, rifampicin, mupirocin, fusidic acid, clindamycin, clarithromycin, sulfamethoxazole trimethoprim, vancomycin, ciprofloxacin, enrofloxacin, marbofloxacin, moxifloxacin, pradofloxacin, and polymyxin B.

Total susceptibility (100%) of the isolates were found in only four antibiotics, amikacin, vancomycin, mupirocin and fusidic acid. High susceptibility (80%) was demonstrated in polymyxin B. Moderate levels of susceptibility was observed in chloramphenicol (67%), florfenicol (67%), rifampicin (67%), amoxicillin & clavulanic acid (50%), cefalexin (50%),

ciprofloxacin (50%), and enrofloxacin (50%). Low susceptibility levels were observed in clindamycin (33%), clarithromycin (33%), moxifloxacin (33%), pradofloxacin (33%), and gentamicin (17%). Complete resistant (0%) was seen in cefuroxime, ceftazidime, tetracycline, doxycycline, tobramycin, and marbofloxacin, and sulfamethoxazole trimethoprim as indicated in **(Table 4).**

Table 4: Antibiotic resistance profile of *Staphylococcus pseudintermedius* cultured from 6 patients. R= Resistant, S sensitive, M - moderately resistant, - = Not tested.

Antibiotic	*Staphylococcus pseudintermedius* Isolates						Total Susceptibility
	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	
Amoxicillin & Clavulanic acid	R	S	S	R	S	R	50%
Cefalexin	R	S	S	R	S	R	50%
Cefuroxime	-	-	-	R	-	-	0%
Ceftazidime	-	-	-	-	-	R	0%
Tetracycline	R			R		R	0%
Doxycycline	R	-	-	R	-	R	0%
Chloramphenicol	S	S	S	R	R	S	67%
Florfenicol	S	S	S	R	R	S	67%
Amikacin	S			S		S	100%
Gentamicin	R	R	S	R	R	R	17%
Tobramycin	-	-	-	-	-	R	0%
Rifampicin	S	-	-	S	-	R	67%
Mupirocin	S	-	-	S	-	S	100%
Fusidic acid	S	-	-	S	-	S	100%
Clindamycin	R	S	S	R	R	R	33%
Clarithromycin	R	S	S	R	R	R	33%
Sulfamethoxazole Trimethoprim	R	-	-	R	-	R	0%
Vancomycin	S	-	-	S	-	S	100%
Ciprofloxacin	R	S	S	R	S	R	50%
Enrofloxacin	R	S	S	R	S	R	50%
Marbofloxacin	-	-	-	-	-	R	0%
Moxifloxacin	R	-	-	-	S	R	33%
Pradofloxacin	R	-	-	-	S	R	33%
Polymyxin B	-	S	S	S	R	S	80%

Beta Haemolytic *Streptococcus*

Beta Haemolytic *Streptococcus* was isolated from four patients. Penicillin, amoxicillin, amoxicillin & clavulanic acid, cefalexin, cefuroxime, ceftazidime, chloramphenicol, florfenicol, gentamicin, tobramycin, amikacin, clindamycin, clarithromycin,

sulfamethoxazole trimethoprim, ciprofloxacin, enrofloxacin, marbofloxacin, moxifloxacin, pradofloxacin, and polymyxin B were used in the susceptibility test for this bacterial isolate. More than half the antibiotics showed a level of complete susceptibility (100%) including penicillin, amoxicillin, amoxicillin & clavulanic acid, cefalexin, cefuroxime, ceftazidime, chloramphenicol, florfenicol, sulfamethoxazole trimethoprim, moxifloxacin, and pradofloxacin. High susceptibility levels (75%) were illustrated in clindamycin and clarithromycin. A moderate level of susceptibility (50%) was observed in amikacin, ciprofloxacin and enrofloxacin. Lower levels of susceptibility were indicated for the antibiotics tobramycin (25%), marbofloxacin (25%), and gentamicin (12.5%). However, the isolates demonstrated complete resistance (0%) to only one antibiotic- polymyxin B as depicted in **(Table 5).**

Table 5: Antibiotic resistance profile of Beta Haemolytic *Streptococcus* cultured from 4 patients. R= Resistant, S sensitive, M - moderately resistant, - = Not tested.

Antibiotic	Beta Haemolytic *Streptococcus* Isolates				Total Susceptibility
	Isolate 1	Isolate 2	Isolate 3	Isolate 4	
Penicillin	S	S	S	S	100%
Amoxicillin	S	S	S	S	100%
Amoxicillin & Clavulanic acid	S	-	S	S	100%
Cefalexin	S	S	S	S	100%
Cefuroxime	S	-	-	-	100%
Ceftazidime	-	-	-	S	100%
Chloramphenicol	S	S	S	S	100%
Florfenicol	S	S	S	S	100%
Gentamicin	R	R	R	M	12.5%
Tobramycin	-	R	-	M	25%
Amikacin	-	-	-	M	50%
Clindamycin	S	S	S	R	75%
Clarithromycin	S	S	S	R	75%
Sulfamethoxazole Trimethoprim	S	S	S	S	100%
Ciprofloxacin	M	M	M	M	50%
Enrofloxacin	M	M	M	M	50%
Marbofloxacin	-	M	-	R	25%
Moxifloxacin	S	S	S	S	100%
Pradofloxacin	S	S	S	S	100%
Polymyxin B	R	R	R	R	0%

6. Discussion and Conclusion

OE is a multifactorial inflammatory disease of the external ear frequently seen in small animal medicine [21, 22]. The secondary factors of OE include bacteria and fungi and are often involved in the pathogenesis of the disease [21]. Treatment of OE in small animal practice often includes empirical antibiotic treatment without further microbial examination including cytology or antibiotic susceptibility testing which can be attributed to the increase in AMR [7]. The development of AMR poses a risk to both human and veterinary medicine. The issue of antibiotic resistance is regarded as one of the foremost threats to effective disease treatment [1]. Due to their close interaction and shared living environments with their owners, companion animals are often regarded as potential reservoirs of AMR [52]. Investigating the prevalence of AMR in companion animals not only contributes to the betterment of their healthcare but also serves as a protective measure for public health, particularly in case transmission between pets and their owners is confirmed in future studies.

This aim of our study was to assess the incidence of AMR within the ear canal microbiome of dogs treated at the University of Veterinary Medicine Budapest.

Eighteen bacterial strains were isolated from samples during microscopic evaluation. The most commonly cultured bacteria were *Staphylococcus pseudintermedius* (33%) and *Pseudomonas aeruginosa* (28%) which is consistent with findings from the studies of Martins et al[7], Tesin et al[45], Zamankhan et al [48] and Bugden et al[47]. These findings were almost identical to a study by Bourély et al[46], which found *S. pseudintermedius* and *P. aeruginosa* in 33% and 27.5% of isolates respectively. *Beta haemolytic streptococci* (22%) was moderately prevalent in our isolates, a higher percentage than Bugden et al[47] and Bourély et al[46], which found *beta-haemolytic streptococci* in 6.2% and 5.9% of isolates respectively. *Klebsiella oxytoca* was isolated in 11% of the samples. Previously studies discussed did not isolate this species, though Martins et al[7] found its family *Enterobacteriaceae* in 13% of isolates, a different strain *klebsiella ozaenae* was isolated in only one sample. *Coagulase-negative Staphylococcus* (6%) exhibited the lowest prevalence which aligned with the study performed by Tesin et al[45] who also only isolated CoNS in 1.89% of samples, in one patient. A higher prevalence of this species was isolated by Martins et al[7] and Zamankhan et al [48] in 10.3% and 9.8% of isolates, respectively.

The reason for these minor differences is not know currently to the author if this work, but it can be related to a variety of reasons, such as differences in climate on the site of sample collection between the studies, different general antibiotic protocols, but a so far undiscovered case is also possible, that needs to be investigated.

Pseudomonas aeruginosa

Total susceptibility was demonstrated in 100% of *Pseudomonas aeruginosa* isolates for all antibiotics tested. This is in contrast to other *P. aeruginosa* results in the studies discussed which found high levels of resistance in this species. Contrarily Tesin et al[45] found 100% resistance in amoxicillin & clavulanic acid, ampicillin, levofloxacin, nitrofurantoin, penicillin, rifampicin, Sulfamethoxazole-trimethoprim, and vancomycin. In the antibiotics we investigated, they found lower levels of resistance- 25% in tobramycin, gentamicin, ciprofloxacin, marbofloxacin and 50% in amikacin. Ceftazidime wasn't tested in this study but other cephalosporins showed varied levels of resistance, cefoxitin and cefquinome showed 100% resistance, while ceftriaxone displayed a lower resistance of 25%. The studies by Bourély et al[46] and Bugden et al[47] did not test all of the antibiotics we used. However, they found high levels of resistance in enrofloxacin in 67.7% and 36% of isolates respectively. They also found lower level of resistance in gentamicin in 17.9% and 5% of isolates. Bugden et al[47] found a low resistance in polymyxin B in 7% of isolates.

Zamankhan et al[48] found 100% resistance in amoxicillin & clavulanic acid, erythromycin, Lincomycin-Spectinomycin, penicillin, and rifampicin. In the antibiotics used in our test, they found 0% resistance in amikacin which corroborates our findings and 10% resistance in gentamicin. Ceftazidime was also not tested in this study but other cephalosporins showed different resistance levels, there was 100% of resistance in cephalothin and 10% of ceftriaxone. Martins et al[7] tested the *Pseudomonas spp*., not just the *P. aeruginosa* species specifically. Martins et al [7] found 100% resistance in erythromycin and 96% resistance in rifampicin. In the antibiotics employed in our study, resistance rates were observed in 32% of gentamicin, 27.3% in amikacin, 12.5% in polymyxin B, and 8.3% in ciprofloxacin. They found resistance in one cephalosporin-85% in cefalexin. No resistance in our results displayed very different findings to current literature of *P. aeruginosa.*

This remarkable difference between our research and the literature data can be caused by a variety of reasons. One possible explanation could be that our dataset contained naïve patients without prior antibiotic treatment, this is however not the case, as several underwent

prior topical and systematic antibiotic treatment. A possible contamination of the samples with a *Pseudomonas aeruginosa* strain inhabiting the clinic could also be a cause, however this would mean that in 2020 there were no patients arriving to the clinic with Pseudomonas otitis. Also, the difference between the dates these samples were collected, and intermittent samples with the same technique showing no Pseudomonas at all also makes this theory unlikely. A final theory might suggest a possible endemic nature of *Pseudomonas aeruginosa* but this so far remains unproven and requires further examination using data from more cases and clinical years.

Beta Haemolytic *Streptococcus*

Complete susceptibility was found in the majority of antibiotics tested against our *beta haemolytic Streptococcus* isolate except for polymyxin B- 0%, gentamicin- 12.5%, tobramycin- 25%, marbofloxacin- 25%, amikacin- 50%, ciprofloxacin- 50%, enrofloxacin- 50%, clindamycin- 75%, clarithromycin- 75%. Bugden et al[47] only tested their *beta haemolytic streptococcus* isolate with gentamicin and enrofloxacin. They found 100% resistance in enrofloxacin which is contrasting to our finding of 100% susceptibility. The prevalence of resistance in gentamicin was 99%, which was higher than our finding of 87.5%. Martins et al[7] and Bourély et al[46] pooled their results for the *Streptococcus spp* isolates. Comparable to our results Martins et al[7] found no resistance in amoxicillin and clavulanic acid, cefalexin, penicillin, and sulfamethoxazole trimethoprim. Both studies demonstrated higher resistance to enrofloxacin- 62.9%[46] and 100%[7]. Compared to our findings, Martins et al[7] had a very low level of resistance to gentamicin- 3.3%, while Bourély et al[46] displayed a higher level of 50%. Martins et al[7] found 33.3% of resistance to polymyxin B, while we had complete resistance to that antibiotic. Both our study and Martins et al[7] showed no resistance to sulfamethoxazole trimethoprim, while Bourély et al[46] displayed a low resistance of 20.7%. While our susceptibility to chloramphenicol was 100%, Bourély et al[46] demonstrated a resistance of 33.3%. No resistance was found in ciprofloxacin in Martins et al[7] study while we displayed a moderate resistance level of 50%. Martins et al[7] also found the same resistance- 50% to amikacin compared to our results.

The difference between the susceptibility toward phenicol type drugs can be related to the fact that it is a relatively underutilized antibiotic in Hungary in 2020. As for the rest of the differences, possible explanations include those mentioned in the paragraphs above.

Coagulase negative *Staphylococcus*

100% susceptibility of coagulase negative *Staphylococcus* was displayed in most antibiotics tested, bar polymyxin B that was moderately susceptible- 50% and complete resistant in gentamicin, clindamycin and clarithromycin. In contrast to our findings with complete susceptibility, Martins et al[7] displayed resistance levels in amoxicillin & clavulanic acid- 4.6%, cefalexin- 27.3%, ciprofloxacin- 10%, and enrofloxacin- 83.4%. Similarly we also found resistance in gentamicin, though our results showed complete resistance while Martins et al[7] displayed a lower level in 28.6%. In the study by Zamankhan et al[48] they has the highest levels of resistance in amoxicillin & clavulanic acid- 16.88%. While our finding for chloramphenicol was completely susceptible, Zamankhan et al[48] found a low level of resistance in 11.69% of isolates. Both our study and Zamankhan et al[48] found complete susceptibility to enrofloxacin. Our results displayed total resistance to gentamicin while their study found resistance in only 2.6%.

These differences are once again possibly related to differences in antibiotic protocols used by clinicians in Hungary.

Klebsiella oxytoca

There was total susceptibility in all antibiotics tested against this bacterial species except for three. There was complete resistance in amoxicillin and cefalexin and 25% resistance was found in gentamicin. The studies previous discussed did not isolate *klebsiella oxytoca* in their samples. Though Martins et al[7] found its family *Enterobacteriaceae* in 13% of isolates, a different strain, *k. ozaenae* was isolated in only one sample. This study produced AMR profiles for *Enterobacteriaceae* in general. Both our study and Martins et al[7] found the same resistance to gentamicin- 25%. While we found complete resistance to amikacin and cefalexin, this study found 38.5% and 53.9% resistance levels respectively. We found complete susceptibility to amoxicillin and clavulanic acid, ciprofloxacin, enrofloxacin, polymyxin B, Sulfamethoxazole-trimethoprim. This study found resistance in 12.5% of amoxicillin and clavulanic acid, 14.3% of ciprofloxacin, 57.2% of enrofloxacin, 20% of polymyxin B, and 38.5% of Sulfamethoxazole-trimethoprim. A study by Lee et al[53] investigated the prevalence of antimicrobial resistance of Klebsiella spp. isolated from clinically ill companion animals in various animal hospitals, forty three strains were isolated and 11 were *k. oxytoca,* though only one of those was isolated from the ear canal [53]. They found a higher resistance to gentamicin at 36.4% in their *k. oxytoca* isolates than our

findings. They also had a resistance to ciprofloxacin at 36.4% while our isolate was completely susceptible. While they didn't test for cefalexin or cefuroxime like we did in our study, they tested ten different cephalosporins and found resistance in cefazolin and cephalothin in both at 54.5%. Cefoxitin and cefpodoxime has resistance levels of 27.3%, while ceftriaxone, cefotaxime and ceftazidime had lower resistance results of 18.2% [53]. This study didn't find complete susceptibility to a cephalosporin like we demonstrated in our study.

These differences likely relate to factors mentioned in chapter above.

Staphylococcus pseudintermedius

Resistance was found in all antibiotics tested against *S. pseudintermedius* except for vancomycin, amikacin, mupirocin and fusidic acid. Zamankhan et al[48] also corroborated this finding as they also had 0% resistance in amikacin. In contrast Tesin et al [45] and Bourély et al [46] displayed resistance to fusidic acid with 28% and 6.1% respectively. Tesin et al [45] contrastingly found resistance to vancomycin in 31%. High susceptibility- 80% was demonstrated in our results polymyxin B while Bugden et al [47] found the opposite with 100% resistance. Our findings displayed resistance of 33% in chloramphenicol which was higher than the study by Zamankhan et al [48]-13% and lower than the findings of Bourély et al [46]- 38.9%. We found moderate levels of susceptibility to rifampicin- 67%, while Zamankhan et al [48] found complete susceptibility. We also found moderate levels of resistance to amoxicillin & clavulanic acid- 50%, which Tesin et al [45] and Zamankhan et al [48] displayed results with less resistance at 10% and 17.7% respectively. Our levels of resistance to ciprofloxacin- 50% were a lot higher than Tesin et al [45], that found resistance in 21%. Our study also demonstrated the highest levels of resistance to enrofloxacin- 50% compared to Tesin et al [45], Bourély et al [46], and Bugden et al [47] which found resistance in 14%[45], 13%[46] and 2%[47] respectively. Zamankhan et al [48] found the opposite to these studies as they had no resistance to this antibiotic. In all studies gentamicin had varying levels of resistance, our findings were the highest at 83%, while Tesin et al [45], found resistance at 31%. Bourély et al [46] and Bugden et al [47], Zamankhan et al [48] displayed lower levels of resistance in 13.5%, 1% and 3% respectively. Tesin et al [45] found similar levels of resistance in clindamycin- 67% to our results at 66%. Half of our isolates were classified as MRSP by the laboratory, with resistance to amoxicillin & clavulanic acid and cefalexin, with some isolates also resistant to cefuroxime or ceftazidime. Lower levels of resistance

was found in the cephalosporins tested in the others studies with none finding complete resistance. The highest resistance found was by Tesin et al [45] in 31% in cefquinome.
Our results also found complete resistance to several antibiotics including tetracycline, doxycycline, tobramycin, marbofloxacin, and sulfamethoxazole trimethoprim which was in contrast to the other studies which found low to moderate levels of resistance in these antibiotics.
The selective and low susceptibility of this bacteria, especially in contrast to historical data makes principles of antibiotic stewardship even more paramount.

Fortunately, low levels of resistance were found in most of the bacterial isolates, giving a good range of choice when choosing antimicrobial treatment for otitis in the university clinic. All antibiotics could be chosen for treatment of *P. aeruginosa*. Most antibiotics bar amoxicillin, cefalexin and gentamicin could be recommended for treatment where *K. oxytoca* is the pathogen present. Only four antibiotics- gentamicin, clindamycin, clarithromycin and polymyxin B would not be recommended for treating otitis where coagulase negative *Staphylococcus* is prevalent. Higher prevalence of resistance was found in *S. pseudintermedius* and beta haemolytic *Streptococcus*, making the treatment choice of antibiotics more difficult. Though there were nine antibiotics to which beta haemolytic *Streptococcus* was resistant to, eleven were still related to complete susceptibility, giving a good range of choice for treatment to these bacteria such as penicillin and amoxicillin. With the presence of MRSP in some bacterial isolates of *S. pseudintermedius*, these bacteria would be the most difficult to treat with the varying levels of resistance found in twenty antibiotics, though amikacin and fusidic acid are 100% susceptible. Vancomycin was completely susceptible, but since it is a critically important antibiotic in human medicine other antibiotic therapy should be chosen instead. The gold standard for prudent use of antibiotic is susceptibility testing on all bacterial samples to identify which antibiotics are most suited to choose for treatment.

As mentioned before, variations in research findings regarding the different bacterial strains present in otitis and their antibiotic susceptibility can be attributed to the data origin, influenced by the geographical location in which the study was carried out, variations in antibiotic prescription, overuse, misuse, and patient adherence to treatments in the different countries. The genetic diversity of the bacterial strains along with microbiome differences

can also influence AMR. The time period in which the study was carried out, the data collection, testing methods and analysis can also influence the findings.

The varying levels of resistance and susceptibility of the different bacteria isolates tested in the different studies discussed and our study in Budapest highlights the importance of each country performing their own antibiotic susceptibility testing to evaluate what resistance levels are present there.

7. Limitations

These AMR findings were gathered from a very small sample of dogs in Budapest, making it inaccurate to apply them to a broader region within Hungary. To obtain a more precise understanding of the prevalence on a larger scale and across the canine population as a whole, a more extensive sample, from various regions across Hungary, is essential.

The samples were also collected within a one-year period, which may limit the accuracy of our analysis, as it shows AMR at a specific point in time, and does not take into account evolving AMR or changes since 2020. To enhance the accuracy of our research, further retrospective sample collection from previous years and continuous collection should be considered. The reason for the above limitation is that the online database currently only holds data for the 2020 caseload. As more years are added, a detailed, year-by-year breakdown of resistance data should provide a better understanding of the changing of AMR.

These results provide valuable insights into AMR of the ear microbiome, but they do not evaluate the overall occurrence of AMR in canines, which will vary in the microbiomes of the gastrointestinal tract, skin, or bladder. To obtain a more complete picture of AMR in canines throughout Hungary, a broader-scale, long-term study is needed.

8. References

1. World Health Organization Antimicrobial resistance. In: World Health Organization. In: Antimicrobial resistance. In: World Health Organization. https://www.who.int/en/news-room/fact-sheets/detail/antimicrobial-resistance. Accessed 10 Sep 2023

2. World Health Organization Global antimicrobial resistance and use surveillance system (GLASS) report 2021. In: Global antimicrobial resistance and use surveillance system (GLASS) report 2021. https://www.who.int/publications/i/item/9789240027336

3. Mader R, Damborg P, Amat J-P, Bengtsson B, Bourély C, Broens EM, Busani L, Crespo-Robledo P, Filippitzi M-E, Fitzgerald W, Kaspar H, Madero CM, Norström M, Nykäsenoja S, Pedersen K, Pokludova L, Urdahl AM, Vatopoulos A, Zafeiridis C, Madec J-Y, on behalf of EU-JAMRAI (2021) Building the European Antimicrobial Resistance Surveillance network in veterinary medicine (EARS-Vet). Eurosurveillance 26:. https://doi.org/10.2807/1560-7917.ES.2021.26.4.2001359

4. Marco-Fuertes A, Marin C, Lorenzo-Rebenaque L, Vega S, Montoro-Dasi L (2022) Antimicrobial Resistance in Companion Animals: A New Challenge for the One Health Approach in the European Union. Veterinary Sciences 9:208. https://doi.org/10.3390/vetsci9050208

5. Nielsen SS, Bicout DJ, Calistri P, Canali E, Drewe JA, Garin-Bastuji B, Gonzales Rojas JL, Gortazar Schmidt C, Herskin M, Michel V, Miranda Chueca MA, Padalino B, Pasquali P, Roberts HC, Sihvonen LH, Spoolder H, Stahl K, Velarde A, Viltrop A, Winckler C, Guardabassi L, Hilbert F, Mader R, Aznar I, Baldinelli F, Alvarez J (2021) Assessment of animal diseases caused by bacteria resistant to antimicrobials: Dogs and cats. EFS2 19:. https://doi.org/10.2903/j.efsa.2021.6680

6. Pomba C, Rantala M, Greko C, Baptiste KE, Catry B, Van Duijkeren E, Mateus A, Moreno MA, Pyörälä S, Ružauskas M, Sanders P, Teale C, Threlfall EJ, Kunsagi Z, Torren-Edo J, Jukes H, Törneke K (2016) Public health risk of antimicrobial resistance transfer from companion animals. J Antimicrob Chemother dkw481. https://doi.org/10.1093/jac/dkw481

7. Martins E, Maboni G, Battisti R, Da Costa L, Selva HL, Levitzki ED, Gressler LT (2022) High rates of multidrug resistance in bacteria associated with small animal otitis: A study of cumulative microbiological culture and antimicrobial susceptibility. Microbial Pathogenesis 165:105399. https://doi.org/10.1016/j.micpath.2022.105399

8. Cole, LK (2010) Anatomy and physiology of the canine ear. Vet Dermatology 21:221–31. https://doi.org/10.1111/j.1365-3164.2010.00885.x

9. Konig HE, Liebich HG (2009) Veterinary anatomy of domestic animals, 4th ed. Schattauer, Stuttgart, Germany

10. Evans HE, Lahunta AD (2013) Miller's anatomy of the dog. Elsevier, Missouri

11. Njaa BL, Cole LK, Tabacca N (2012) Practical Otic Anatomy and Physiology of the Dog and Cat. Veterinary Clinics of North America: Small Animal Practice 42:1109–1126. https://doi.org/10.1016/j.cvsm.2012.08.011

12. Cole LK (2004) Otoscopic evaluation of the ear canal. Veterinary Clinics of North America: Small Animal Practice 34:397–410. https://doi.org/10.1016/j.cvsm.2003.10.004

13. Lecchi C, Zamarian V, Borriello G, Galiero G, Grilli G, Caniatti M, D'Urso ES, Roccabianca P, Perego R, Minero M, Legnani S, Calogero R, Arigoni M, Ceciliani F (2020) Identification of Altered miRNAs in Cerumen of Dogs Affected by Otitis Externa. Front Immunol 11:914. https://doi.org/10.3389/fimmu.2020.00914

14. Tabacca NE, Cole LK, Hillier A, Rajala-Schultz PJ (2011) Epithelial migration on the canine tympanic membrane. Veterinary Dermatology 22:502–510. https://doi.org/10.1111/j.1365-3164.2011.00982.x

15. Dyce KM, Sack WO, Wensing CJG (2010) Textbook of Veterinary Anatomy, 4th ed. Saunders, St. Louis, Missouri

16. Leonard C, Picavet PP, Fontaine J, Clercx C, Taminiau B, Daube G, Claeys S (2023) The Middle Ear Microbiota in Healthy Dogs Is Similar to That of the External Ear Canal. Veterinary Sciences 10:216. https://doi.org/10.3390/vetsci10030216

17. Tang S, Prem A, Tjokrosurjo J, Sary M, Van Bel MA, Rodrigues-Hoffmann A, Kavanagh M, Wu G, Van Eden ME, Krumbeck JA (2020) 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. Veterinary Microbiology 247:108764. https://doi.org/10.1016/j.vetmic.2020.108764

18. Shaw S (2016) Pathogens in otitis externa: diagnostic techniques to identify secondary causes of ear disease. In Practice 38:12–16. https://doi.org/10.1136/inp.i461

19. Ngo J, Taminiau B, Fall PA, Daube G, Fontaine J (2018) Ear canal microbiota – a comparison between healthy dogs and atopic dogs without clinical signs of otitis externa. Veterinary Dermatology 29:425. https://doi.org/10.1111/vde.12674

20. Kasai T, Fukui Y, Aoki K, Ishii Y, Tateda K (2021) Changes in the ear canal microbiota of dogs with otitis externa. J Appl Microbiol 130:1084–1091. https://doi.org/10.1111/jam.14868

21. Saridomichelakis MN, Farmaki R, Leontides LS, Koutinas AF (2007) Aetiology of canine otitis externa: a retrospective study of 100 cases. Veterinary Dermatology 18:341–347. https://doi.org/10.1111/j.1365-3164.2007.00619.x

22. Rosser EJ (2004) Causes of otitis externa. Veterinary Clinics of North America: Small Animal Practice 34:459–468. https://doi.org/10.1016/j.cvsm.2003.10.006

23. O'Neill DG, Volk AV, Soares T, Church DB, Brodbelt DC, Pegram C (2021) Frequency and predisposing factors for canine otitis externa in the UK – a primary veterinary care epidemiological view. Canine Genet Epidemiol 8:7. https://doi.org/10.1186/s40575-021-00106-1

24. Patel, A (2020) Otitis externa in dogs . In: Otitis externa in dogs. https://www.veterinaryirelandjournal.com/focus/174-otitis-externa-in-dogs. Accessed 29 Aug 2023

25. Jacobson LS (2002) Diagnosis and medical treatment of otitis externa in the dog and cat : review article. J S Afr Vet Assoc 73:162–170. https://doi.org/10.4102/jsava.v73i4.581

26. Moriello KA (2023) Otitis media and Interna in dogs, MSD Veterinary Manual. In: MSD Veterinary Manual. https://www.msdvetmanual.com/dog-owners/ear-disorders-of-dogs/otitis-media-and-interna-in-dogs

27. Logas D (2012) Feline and Canine Otitis Media. In: Monnet E (ed) Small Animal Soft Tissue Surgery, 1st ed. Wiley, pp 121–125

28. Gotthelf LN (2004) Diagnosis and treatment of otitis media in dogs and cats. Veterinary Clinics of North America: Small Animal Practice 34:469–487. https://doi.org/10.1016/j.cvsm.2003.10.007

29. Shell LG (1988) Otitis Media and Otitis Interna. Veterinary Clinics of North America: Small Animal Practice 18:885–899. https://doi.org/10.1016/S0195-5616(88)50088-8

30. Woodward M (2023) Otitis media and Interna in animals - ear disorders. In: MSD Veterinary Manual. In: MSD Veterinary Manual. https://www.msdvetmanual.com/ear-disorders/otitis-media-and-interna/otitis-media-and-interna-in-animals

31. Cole LK (2012) Primary Secretory Otitis Media in Cavalier King Charles Spaniels. Veterinary Clinics of North America: Small Animal Practice 42:1137–1142. https://doi.org/10.1016/j.cvsm.2012.08.002

32. Mielke B, Lam R, Ter Haar G (2017) Computed tomographic morphometry of tympanic bulla shape and position in brachycephalic and mesaticephalic dog breeds. Vet Radiology Ultrasound 58:552–558. https://doi.org/10.1111/vru.12529

33. Hayes GM, Friend EJ, Jeffery ND (2010) Relationship between pharyngeal conformation and otitis media with effusion in Cavalier King Charles spaniels. Veterinary Record 167:55–58. https://doi.org/10.1136/vr.b4886

34. Morris DO (2004) Medical therapy of otitis externa and otitis media. Veterinary Clinics of North America: Small Animal Practice 34:541–555. https://doi.org/10.1016/j.cvsm.2003.10.009

35. Habboush Y, Guzman N (2023) National Center for Biotechnology Information. In: Antibiotic Resistance. https://www.ncbi.nlm.nih.gov/books/NBK513277/

36. Palma E, Tilocca B, Roncada P (2020) Antimicrobial Resistance in Veterinary Medicine: An Overview. IJMS 21:1914. https://doi.org/10.3390/ijms21061914

37. Nesse LL, Osland AM, Vestby LK (2023) The Role of Biofilms in the Pathogenesis of Animal Bacterial Infections. Microorganisms 11:608. https://doi.org/10.3390/microorganisms11030608

38. HPRA (2023) Antibiotic resistance. In: Antibiotic resistance. http://www.hpra.ie/homepage/veterinary/special-topics/antibiotic-resistance

39. Caneschi A, Bardhi A, Barbarossa A, Zaghini A (2023) The Use of Antibiotics and Antimicrobial Resistance in Veterinary Medicine, a Complex Phenomenon: A Narrative Review. Antibiotics 12:487. https://doi.org/10.3390/antibiotics12030487

40. Jin M, Osman M, Green BA, Yang Y, Ahuja A, Lu Z, Cazer CL (2023) Evidence for the transmission of antimicrobial resistant bacteria between humans and companion animals: A scoping review. One Health 17:100593. https://doi.org/10.1016/j.onehlt.2023.100593

41. Song SJ, Lauber C, Costello EK, Lozupone CA, Humphrey G, Berg-Lyons D, Caporaso JG, Knights D, Clemente JC, Nakielny S, Gordon JI, Fierer N, Knight R (2013) Cohabiting family members share microbiota with one another and with their dogs. eLife 2:e00458. https://doi.org/10.7554/eLife.00458

42. Tóth AG, Tóth I, Rózsa B, Dubecz A, Patai ÁV, Németh T, Kaplan S, Kovács EG, Makrai L, Solymosi N (2022) Canine Saliva as a Possible Source of Antimicrobial Resistance Genes. Antibiotics 11:1490. https://doi.org/10.3390/antibiotics11111490

43. Naziri Z, Poormaleknia M, Ghaedi Oliyaei A (2022) Risk of sharing resistant bacteria and/or resistance elements between dogs and their owners. BMC Vet Res 18:203. https://doi.org/10.1186/s12917-022-03298-1

44. Tanner MA, Everett CL, Youvan DC (2000) Molecular Phylogenetic Evidence for Noninvasive Zoonotic Transmission of *Staphylococcus intermedius* from a Canine Pet to a Human. J Clin Microbiol 38:1628–1631. https://doi.org/10.1128/JCM.38.4.1628-1631.2000

45. Tesin N, Stojanovic D, stancic I (2023) Prevalence of the microbiological causes of canine otitis externa and the antibiotic susceptibility of the isolated bacterial strains. Polish Journal of Veterinary Sciences. https://doi.org/10.24425/pjvs.2023.145052

46. Bourély C, Cazeau G, Jarrige N, Leblond A, Madec JY, Haenni M, Gay E (2019) Antimicrobial resistance patterns of bacteria isolated from dogs with otitis. Epidemiol Infect 147:e121. https://doi.org/10.1017/S0950268818003278

47. Bugden D (2013) Identification and antibiotic susceptibility of bacterial isolates from dogs with otitis externa in Australia. Aust Vet J 91:43–46. https://doi.org/10.1111/avj.12007

48. Zamankhan Malayeri H, Jamshidi S, Zahraei Salehi T (2010) Identification and antimicrobial susceptibility patterns of bacteria causing otitis externa in dogs. Vet Res Commun 34:435–444. https://doi.org/10.1007/s11259-010-9417-y

49. Loponte R, Pagnini U, Iovane G, Pisanelli G (2021) Phage Therapy in Veterinary Medicine. Antibiotics 10:421. https://doi.org/10.3390/antibiotics10040421

50. Marza JAS, Soothill JS, Boydell P, Collyns TA (2006) Multiplication of therapeutically administered bacteriophages in Pseudomonas aeruginosa infected patients. Burns 32:644–646. https://doi.org/10.1016/j.burns.2006.02.012

51. Hawkins C, Harper D, Burch D, Änggård E, Soothill J (2010) Topical treatment of Pseudomonas aeruginosa otitis of dogs with a bacteriophage mixture: A before/after clinical trial. Veterinary Microbiology 146:309–313. https://doi.org/10.1016/j.vetmic.2010.05.014

52. Li Y, Fernández R, Durán I, Molina-López RA, Darwich L (2021) Antimicrobial Resistance in Bacteria Isolated From Cats and Dogs From the Iberian Peninsula. Front Microbiol 11:621597. https://doi.org/10.3389/fmicb.2020.621597

53. Lee D, Oh JY, Sum S, Park H-M (2021) Prevalence and antimicrobial resistance of *Klebsiella* species isolated from clinically ill companion animals. J Vet Sci 22:e17. https://doi.org/10.4142/jvs.2021.22.e17

9. Appendices

9.1. Appendix 1

Dermatological history questions [22].

Box 1. Dermatologic history questionnaire [9]

1. How long has your pet had a skin problem?
2. Age of pet when obtained?
3. Age of pet when skin problem started?
4. Where on the body did the problem start?
5. What did it look like initially?
6. If your pet is scratching, did you notice the itching or the skin lesions first?
7. How has it spread or changed?
8. Does the skin condition seem better or worse during any particular season? Which one?
9. Do other pets in your household have skin problems?
10. Do any relatives of your pet have skin problems? Which ones?
11. Do any people in your household have skin problems?
12. Do you use any flea control products? Which ones? How often?
13. Do you bathe your pet? Which products? How often?
14. If your pet is female, are there irregular or abnormal heat cycles? Has she ever been pregnant or had false pregnancies? Has she been spayed? If so, at what age?
15. If your pet is male, does he have a normal interest in female animals? Has he been neutered?
16. Is there any condition or environment that makes the skin problem noticeably worse?
17. Has your pet experienced vomiting, changes in stool character, or disagreement with certain foods?
18. Has your pet ever seemed to be ill from its skin disease (eg, depressed, fever, not eating)?
19. Please indicate if you have noticed any of the following: scratching, biting, or licking; rubbing face on floor or furniture; scratching at ears, rubbing ears, or shaking head; dry skin or coat; greasy skin or coat; scaly skin (dandruff) or crusts on skin; reddening of skin, pimples, or bumps on skin; oozing sores or open bleeding sores; hair loss; darkening or lightening of the skin; thickening of the skin; or fleas.
20. List any medications your pet has received for the condition, including shampoos, lotions, ointments, dips, pills, capsules, and injections (now and previously).
21. Have any of these helped? If so, which ones?
22. Are there any thoughts you have relating to the skin disease? What do you think may be the cause of the skin problem?

9.2. Appendix 2

Table 6: Antibiotic resistance results of studies - Bugden et al [47], Bourély et al [46], Martins et al[7], Tesin et al [45], and Zamankhan et al [48] discussed in section 2.5. NT- Not tested

Antibiotics	Staphylococcus spp.	S. pseud-Intermedius	S. aureus	CoPS	CoNS	Pseudomonas spp.	P.aeruginosa	Proteus spp.	Proteus mirabilis	Enterobacteriaceae	E.coli	Streptococcus spp.	Beta-haemolytic streptococci	Corynebacterium spp.	Bacillus spp.
Penicillin	61.04 % [48]	76%[45] 68.5%[46] NT[47]	70.9%[46]	63.9%[7]	87.5%[7]	NT[7]	100%[45] NT[46] NT[47] 100% [48]	67%[45] NT[47] NT[7]	NT[46] 100% [48]	NT[7]	NT[47] 100% [48]	NT[46] 0%[7]	NT[47]	40%[7]	33.3%[7]
Oxacillin	NT [48]	NT[45] NT[46] NT[47]	NT[46]	27.4%[7]	45.5%[7]	87.5%[7]	NT[45] NT[46] NT[47] NT [48]	NT[45] NT[47] 100%[7]	NT[46] NT [48]	100%[7]	NT[47] NT [48]	14.4%[46] 75%[7]	NT[47]	87.5%[7]	80%[7]
Amoxicillin	NT [48]	69%[45] NT[46] NT[47]	NT[46]	NT[7]	NT[7]	NT[7]	100%[45] NT[46] NT[4] NT [48]	67%[45] NT[47] NT[7]	28.9%[46] NT [48]	NT[7]	NT[47] NT [48]	NT[46]	NT[47]	NT[7]	NT[7]
Amoxicillin& clavulanic acid	16.88% [48]	10%[45] NT[46] NT[47]	NT[46]	3.9%[7]	4.6%[7]	NT[7]	100%[45] NT[46] NT[47] 100% [48]	0%[45] NT[47] 16.7%[7]	NT[46] 33.33% [48]	12.5%[7]	NT[47] 100% [48]	NT[46] 0%[7]	NT[47]	10%[7]	20%[7]
Ampicillin	53.25% [48]	NT[45] NT[46] NT[47]	NT[46]	21.4%[7]	19.1%[7]	NT[7]	100%[45] NT[46] NT[47] 0% [48]	67%[45] NT[47] 41.7%[7]	NT[46] 33.3% [48]	68.8%[7]	NT[47] 100% [48]	NT[46] 0%[7]	NT[47]	10%[7]	20%[7]
Cefalexin	NT [48]	NT[45] NT[46] NT[47]	NT[46]	10%[7]	27.3%[7]	85%[7]	NT[45] NT[46] NT[47] NT [48]	NT[45] NT[47] 37.5%[7]	NT[46] NT [48]	53.9%[7]	NT[47] NT [48]	NT[46] 0%[7]	NT[47]	50%[7]	0%[7]
Cephalothin	5.5% [48]	NT[45] NT[46] NT[47]	NT[46]	NT[7]	NT[7]	NT[7]	NT[45] NT[46] NT[47] 100% [48]	NT[45] NT[47] NT[7]	NT[46] 100% [48]	NT[7]	NT[47] 0% [48]	NT[46]	NT[47]	NT[7]	NT[7]
Cefoxitin	NT [48]	3%[45] 10.6% [46] NT [47]	NT[46]	NT[7]	NT[7]	NT[7]	100%[45] NT[46] NT[47] NT [48]	0%[45] NT[47] NT[7]	NT[46] NT [48]	NT[7]	NT[47] NT [48]	NT[46]	NT[47]	NT[7]	NT[7]
Ceftriaxone	6.49% [48]	14%[45] NT[46] NT[47]	NT[46]	NT[7]	NT[7]	NT[7]	25%[45] NT[46] NT[47] 10% [48]	0%[45] NT[47] NT[7]	NT[46] 0% [48]	NT[7]	NT[47] 0% [48]	NT[46]	NT[47]	NT[7]	NT[7]

Antibiotics	Staphylococcus spp.	S. pseud-Intermedius	S. aureus	CoPS	CoNS	Pseudomonas spp.	P. aeruginosa	Proteus spp.	Proteus mirabilis	Enterobacteriaceae	E.coli	Streptococcus spp.	Beta-haemolytic streptococci	Coryne-Bacterium spp.	Bacillus spp.
Cefovecin	NT [48]	NT[45] 9.4[46] NT[47]	NT[46]	NT[7]	NT[7]	NT[7]	NT[45] NT[46] NT[47] NT [48]	NT[45] NT[47] NT[7]	NT[46] NT [48]	NT[7]	NT[47] NT [48]	NT[46]	NT[47]	NT[7]	NT[7]
Ceftiofur	NT [48]	NT[45] NT[46] NT[47]	NT[46]	NT[7]	NT[7]	NT[7]	NT[45] NT[46] NT[47] NT [48]	NT[45] NT[47] NT[7]	2.4%[46] NT [48]	NT[7]	NT[47] NT [48]	NT[46]	NT[47]	NT[7]	NT[7]
Cefquinome	NT [48]	31%[45] NT[46] NT[47]	NT[46]	NT[7]	NT[7]	NT[7]	100%[45] NT[46] NT[47] NT [48]	67%[45] NT[47] NT[7]	NT[46] NT [48]	NT[7]	NT[47] NT [48]	NT[46]	NT[47]	NT[7]	NT[7]
Amikacin	0% [48]	NT[45] NT[46] NT[47]	NT[46]	3.1%[7]	26.3%[7]	27.3%[7]	50%[45] NT[46] NT[47] 0% [48]	33%[45] NT[47] 18.2%[7]	NT[46] 0% [48]	38.5%[7]	NT[47] 0% [48]	NT[46] 25%[7]	NT[47]	12.5%[7]	0%[7]
Gentamicin	2.6% [48]	31%[45] 13.5%[46] 1%[47]	12.9%[46]	17.1%[7]	28.6%[7]	32%[7]	25%[45] 17.9%[46] 5%[47] 10% [48]	0%[45] 1%[47] 8.33%[7]	10.3%[46] 0% [48]	25%[7]	0%[47] 0% [48]	3.3%[46] 50%[7]	99%[47]	0%[7]	0%[7]
Neomycin	NT [48]	24%[45] NT[46] 100%[47]	NT[46]	10%[7]	28.6%[7]	40%[7]	0%[45] NT[46] NT[47] NT [48]	33%[45] 47%[47] 0%[7]	NT[46] NT [48]	0%[7]	51%[47] NT [48]	NT[46] 0%[7]	99%[47]	NT[7]	0%[7]
Tobramycin	NT [48]	52%[45] NT[46] NT[47]	NT[46]	NT[7]	NT[7]	NT[7]	25%[45] NT[46] NT[47] NT [48]	33%[45] NT[47] NT[7]	NT[46] NT [48]	NT[7]	NT[47] NT [48]	NT[46]	NT[47]	NT[7]	NT[7]
Streptomycin	NT [48]	NT[45] NT[46] NT[47]	NT[46]	38.4%[7]	40.9%[7]	76%[7]	NT[45] NT[46] NT[47] NT [48]	NT[45] NT[47] 50%[7]	NT[46] NT [48]	62.5%[7]	NT[47] NT [48]	NT[46] 50%[7]	NT[47]	20%[7]	25%[7]
Oxytetracycline	33.77% [48]	NT[45] NT[46] NT[47]	NT[46]	NT[7]	NT[7]	NT[7]	NT[45] NT[46] NT[47] 80% [48]	NT[45] NT[47] NT[7]	NT[46] 100% [48]	NT[7]	NT[47] 100% [48]	NT[46]	NT[47]	NT[7]	NT[7]

Antibiotics	Staphylococcus spp.	S. pseud-Intermedius	S. aureus	CoPS	CoNS	Pseudomonas spp.	P. aeruginosa	Proteus spp.	Proteus mirabilis	Enterobacteriaceae	E.coli	Streptococcus spp.	Beta-haemolytic streptococci	Coryne-Bacterium spp.	Bacillus spp.
Doxycycline	NT [48]	52%[45] NT[46] NT[47]	NT[46]	NT[7]	NT[7]	NT[7]	75%[45] NT[46] NT[47] NT [48]	100%[45] NT[47] NT[7]	NT[46] NT [48]	NT[7]	NT[47] NT [48]	NT[46]	NT[47]	NT[7]	NT[7]
Tetracycline	NT [48]	52%[45] NT[46] NT[47]	NT[46]	NT[7]	NT[7]	NT[7]	100%[45] NT[46] NT[47] NT [48]	100%[45] NT[47] NT[7]	NT[46] NT [48]	NT[7]	NT[47] NT [48]	NT[46]	NT[47]	NT[7]	NT[7]
Azithromycin	NT [48]	62%[45] NT[46] NT[47]	NT[46]	NT[7]	NT[7]	NT[7]	75%[45] NT[46] NT[47] NT [48]	100%[45] NT[47] NT[7]	NT[46] NT [48]	NT[7]	NT[47] NT [48]	NT[46]	NT[47]	NT[7]	NT[7]
Erythromycin	22.08% [48]	55%[45] 29.8% [46] NT[47]	30.2%[46]	37.5%[7]	53.3%[7]	100%[7]	NT[45] NT[46] NT[47] 100% [48]	NT[45] NT[47] 100%[7]	NT[46] 100% [48]	100%[7]	NT[47] 100% [48]	24.8%[46] 66.7%[7]	NT[47]	33.3%[7]	33.3% [7]
Clindamycin	NT [48]	66%[45] NT[46] NT[47]	NT[46]	NT[7]	NT[7]	NT[7]	NT[45] NT[46] NT[47] NT [48]	NT[45] NT[47] NT[7]	NT[46] NT [48]	NT[7]	NT[47] NT [48]	NT[46]	NT[47]	NT[7]	NT[7]
Lincomycin	NT [48]	62%[45] NT[46] NT[47]	NT[46]	NT[7]	NT[7]	NT[7]	NT[45] NT[46] NT[47] NT [48]	NT[45] NT[47] NT[7]	NT[46] NT [48]	NT[7]	NT[47] NT [48]	NT[46]	NT[47]	NT[7]	NT[7]
Lincomycin-Spectinomycin	19.48% [48]	NT[45] NT[46] NT[47]	NT[46]	NT[7]	NT[7]	NT[7]	NT[45] NT[46] NT[47] 100% [48]	NT[45] NT[47] NT[7]	NT[46] 33.33% [48]	NT[7]	NT[47] 0% [48]	NT[46]	NT[47]	NT[7]	NT[7]
Chloramphenicol	11.69% [48]	NT[45] 38.9%[46] NT[47]	31.1%[46]	NT[7]	NT[7]	NT[7]	NT[45] NT[46] NT[47] 70% [48]	NT[45] NT[47] NT[7]	NT[46] 33.33% [48]	NT[7]	NT[47] 0% [48]	35.3%[46]	NT[47]	NT[7]	NT[7]
Polymyxin B	NT [48]	NT[45] NT[46] 100%[47]	NT[46]	NT[7]	NT[7]	12.5%[7]	NT[45] NT[46] 7%[47] NT [48]	NT[45] 100%[47] 91.7%[7]	NT[46] NT [48]	20%[7]	60%[47] NT [48]	NT[46] 33.3%[7]	100% [47]	11.1%[7]	50%[7]

Antibiotics	Staphylococcus spp.	S. pseud-Intermedius	S. aureus	CoPS	CoNS	Pseudomonas spp.	P. aeruginosa	Proteus spp.	Proteus mirabilis	Enterobacteriaceae	E.coli	Streptococcus spp.	Betahaemolytic streptococci	Coryne-Bacterium spp.	Bacillus spp.
Colistin	NT [48]	NT[45] NT[46] NT[47]	NT[46]	NT[7]	NT[7]	NT[7]	75%[45] NT[46] NT[47] NT [48]	67%[45] NT[47] NT[7]	NT[46] NT [48]	NT[7]	NT[47] NT [48]	NT[46]	NT[47]	NT[7]	NT[7]
Vancomycin	NT [48]	31%[45] NT[46] NT[47]	NT[46]	NT[7]	NT[7]	NT[7]	100%[45] NT[46] NT[47] NT [48]	100%[45] NT[47] NT[7]	NT[46] NT [48]	NT[7]	NT[47] NT [48]	NT[46]	NT[47]	NT[7]	NT[7]
Rifampicin	0% [48]	NT[45] NT[46] NT[47]	NT[46]	14.5%[7]	19.1%[7]	96%[7]	100%[45] NT[46] NT[47] 100% [48]	67%[45] NT[47] 84.6%[7]	NT[46] 100% [48]	86.7%[7]	NT[47] 100% [48]	NT[46] 0%[7]	NT[47]	10%[7]	20%[7]
Fusidic acid	NT [48]	28%[45] 6.1%[46] NT[47]	11.5%[46]	NT[7]	NT[7]	NT[7]	NT[45] NT[46] NT[47] NT [48]	NT[45] NT[47] NT[7]	NT[46] NT [48]	NT[7]	NT[47] NT [48]	NT[46]	NT[47]	NT[7]	NT[7]
Sulfamethoxazole-trimethoprim	37.66% [48]	41%[45] 12.2%[46] NT[47]	10.2%[46]	51.4%[7]	45%[7]	NT[7]	100%[45] NT[46] NT[47] 40% [48]	33%[45] NT[47] 41.7%[7]	22.9%[46] 0% [48]	38.5%[7]	NT[47] 0% [48]	20.7%[46] 0%[7]	NT[47]	70%[7]	40%[7]
Ciprofloxacin	NT [48]	21%[45] NT[46] NT[47]	NT[46]	24.6%[7]	10%[7]	8.3%[7]	25%[45] NT[46] NT[47] NT [48]	0%[45] NT[47] 0%[7]	NT[46] NT [48]	14.3%[7]	NT[47] NT [48]	NT[46] 0%[7]	NT[47]	11.1%[7]	0%[7]
Enrofloxacin	0% [48]	14%[45] 13%[46] 2%[47]	12%[46]	44.1%[7]	83.4%[7]	80%[7]	25%[45] 67.7%[46] 36%[47] 0% [48]	0%[45] 4%[47] 0%[7]	13.2%[46] 0% [48]	57.2%[7]	3%[47] 0% [48]	62.9%[46] 100%[7]	100% [47]	50%[7]	0%[7]
Levofloxacin	NT [48]	24%[45] NT[46] NT[47]	NT[46]	NT[7]	NT[7]	NT[7]	100%[45] NT[46] NT[47] NT [48]	0%[45] NT[47] NT[7]	NT[46] NT [48]	NT[7]	NT[47] NT [48]	NT[46]	NT[47]	NT[7]	NT[7]
Pradofloxacin	NT [48]	24%[45] NT[46] NT[47]	NT[46]	NT[7]	NT[7]	NT[7]	25%[45] NT[46] NT[47] NT [48]	0%[45] NT[47] NT[7]	NT[46] NT [48]	NT[7]	NT[47] NT [48]	NT[46]	NT[47]	NT[7]	NT[7]

Antibiotics	Staphylococcus spp.	S. pseud-Intermedius	S. aureus	CoPS	CoNS	Pseudomonas spp.	P. aeruginosa	Proteus spp.	Proteus mirabilis	Enterobacteriaceae	E.coli	Streptococcus spp.	Beta-haemolytic streptococci	Corynebacterium spp.	Bacillus spp.
Marbofloxacin	NT [48]	21%[45] NT[46] NT[47]	NT[46]	NT[7]	NT[7]	NT[7]	25%[45] NT[46] NT[47] NT [48]	33%[45] NT[47] NT[7]	NT[46] NT [48]	NT[7]	NT[47] NT [48]	NT[46]	NT[47]	NT[7]	NT[7]
Nitrofurantoin	NT [48]	45%[45] NT[46] NT[47]	NT[46]	NT[7]	NT[7]	NT[7]	100%[45] NT[46] NT[47] NT [48]	67%[45] NT[47] NT[7]	NT[46] NT [48]	NT[7]	NT[47] NT [48]	NT[46]	NT[47]	NT[7]	NT[7]

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11. Statements