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Current Approaches in the Diagnosis and Treatment of Canine Otitis

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ABSTRACT: As a disease with high prevalence in canine, otitis externa (OE) requires a summary of its diagnosis and treatment. Since antimicrobials are predominantly used in OE treatment, their prudent use must be in focus due to significant impact on the spread and development on the antimicrobial resistance (AMR). Furthermore, as AMR is constantly increasing, further studies are required to create novel treatment approaches against causative agents. Therefore, new alternative approaches to antimicrobials and antifungals were investigated to discover an effective approach to OE treatment. In addition to diagnosis and conventional treatment, this review aimed to summarize the findings of various publications related to the usage of essential oils against OE associated pathogens. While there are very few in vivo trials, numerous in vitro studies have demonstrated the effectiveness of essential oils and their potential application in OE treatment. New alternative approaches are leading toward the development of new treatment objectives and alternatives.

Key words: dogs; essential oils; otitis externa

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INTRODUCTION

OE is a common dermatological disease that affects companion animals (Neves et al., 2018), with an incidence as high as 20% of the affected dogs (Angus, 2004, Cole, 2004). It is defined as an inflammatory condition affecting the external auditory canal, which extends from the pinna to the tympanic cavity via the vertical and horizontal ear canals (Pye, 2018). The etiology that can be multifactorial and an extensive list of factors and microorganisms can cause and worsen the course of this disease (Rosser, 2004, Lyskova et al., 2007).

The external ear canals of dogs are not physiologically sterile; therefore, the ear contains a variety of microorganisms living in harmony with their host and among themselves (Kiss et al., 1997) and if the ear structure or defense mechanisms are not compromised, the ear canals remain free of infection. When the balance is disturbed, a new pathogenic flora evolves and disease can develop (Puigdemont et al., 2021). One of the microbes that is characterized as commensal and has the potential to turn pathogenic is *Malassezia* yeast (Metiner et al., 2015) and it represents one of the most common agents in canine OE, with prevalence as the sole causative agent from 8% to 26% (Petrov et al., 2013, Lund et al., 1999, Rougier et al., 2005, King et al., 2018, Tešin et al., 2023a). Of all the fungi that can be isolated, it is the most common cause of fungal otitis (Lyskova et al., 2007). As for bacteria most frequently isolated from otic ears, *staphylococci* stand out. In fact, previous studies have identified *Staphylococcus pseudintermedius* as the most common OE pathogen in dogs with high incidence rate (70%) (De Martino et al., 2016, Scherer et al., 2018). Other bacteria commonly associated with OE include *Pseudomonas spp.*, *Proteus spp.*, *Enterococcus spp.*, *Streptococcus spp.*, and *Escherichia coli* (Lyskova et al., 2007, Bugden, 2013). Although bacteria and fungi can often be identified separately as the cause of infection, polymicrobial infection can frequently be present as well (Little, 1996). Moreover, expression of clinical symptoms is mainly the result of bacterial and/or fungal infestation. The onset of the disease is manifested by inflammation that causes redness of the ear pinna and the skin of the external auditory canal (Vercelli et al., 2021). Subsequently, there may be evident typical clinical indications of OE, such as ear pain and ceruminous or purulent discharge within the ear canal. These symptoms are caused by changes involving glandular hyperplasia, glandular dilation, epithelial hyperplasia, and hyperkeratosis (Ebani et

al., 2023). Ear discomfort can range from pruritus to severe pain that is aggravated by head shaking and ear scratching, causing lesions or aural hematoma due to self-trauma. If inflammation causes sufficient swelling, which may occur in recurring or chronic instances, clinical symptoms may advance to proliferative changes leading to stenosis of external ear canal, and ultimately to occlusion (Guarda, 2013).

OE is not potentially a life-threatening disease (Türkyilmaz, 2008), however if it is not adequately treated, infection can worsen and become more complicated and can be spread to the deeper tissues (Gothelf, 2004). Depending on the type and severity of the infection as well as the causative agents involved, tympanic perforation may occur, leaving the middle and internal ear vulnerable (Neves et al., 2018).

Antimicrobials routinely utilized in small animal practice for both prevention and treatment (Pomba et al., 2017). As a result of wrong antimicrobial treatment primarily in veterinary medicine, but in animal husbandry as well, the problem of AMR arises (Ebani and Mancianti, 2020). In addition to corticosteroids, antibiotics and antifungals are crucial pharmacological supports for the treatment of canine OE (Tešin et al., 2023b, Jacobson, 2002). Considering that OE is a quite common disease in dogs, the consumption of antimicrobial medicines is relatively high (Tešin et al., 2023c). Topical therapy is preferred over systemic therapy; however, it often fails due to the involvement of antibiotic-resistant bacterial strains and resistance of bacteria and yeast pathogens to currently available medicines (Ebani et al., 2023, Ebani et al., 2020). AMR may also be the cause of therapeutic failures (Friedman et al., 2016), in addition to inadequate therapy management and failures to identify the primary causes (Nuttall, 2016). Development of resistance is common in fungal therapy, as well (Khosravi et al., 2016). The application of combined topical preparations in case of individual infection (only by yeast or bacteria) is another area of concern (Nuttall, 2023). The emergence of AMR is a global issue that poses a medical risk to both humans and animals regarding infectious diseases, and it is one of the major health challenges of the 21st century (Silva et al., 2013).

Given the AMR and treatment failures that have been discussed, the growing interest in alternative therapies is not surprising. Therefore, the present review paper will discuss the diagnosis and treatment options of OE along with the prospective topical alternative treatment approaches.

MATERIALS AND METHODS

In this manuscript, a literature review was conducted to examine the current diagnostic and therapeutic approaches to canine OE. The search was conducted using in PubMed and Web of Science databases. Keywords included “canine otitis externa”, “antimicrobial resistance in canine otitis”, “essential oils for OE treatment” and “alternative therapies for OE in dogs”. Studies published within the last 20 years were included, prioritizing peer-reviewed research manuscripts and clinical trials. Studies lacking full-text availability or focusing solely on human otitis were excluded. The data were synthesized to provide an overview of the most relevant diagnostic techniques,

conventional treatments, and emerging alternative therapies for managing OE in dogs.

RESULTS

The following section presents key findings from the literature on the diagnosis and treatment of canine OE. Data from *in vitro* and *in vivo* studies highlight conventional antimicrobial therapies and alternative treatment options, particularly the use of essential oils. The tables below summarize research findings on the antimicrobial activity of various essential oils against OE pathogens, both *in vitro* (Table 1.) and *in vivo* (Table 2.).

Table 1. Antibacterial activity of essential oils against OE pathogens - *in vitro*

Essential oils	OE pathogens	Study
<i>Origanum vulgare</i> , <i>Satureja montana</i> , and <i>Thymus vulgaris</i>	<i>Staphylococcus</i> sp., <i>Streptococcus</i> sp., <i>Pseudomonas aeruginosa</i> , <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , <i>Serratia marcescens</i> and <i>Malassezia pachydermatis</i>	Ebani et al., 2023
<i>Origanum vulgare</i> <i>Origanum majorana</i> , and <i>Rosmarinus officinalis</i>	<i>Malassezia pachydermatis</i>	Waller et al., 2022
<i>Melaleuca alternifolia</i> (also named tea tree oil), <i>Thymus serpillum</i> , <i>Salvia officinalis</i> , <i>Eucalyptus officinalis</i> , <i>Rosmarinus officinalis</i> , <i>Macadamia alternifolia</i> , <i>Lavandula officinalis</i> , and <i>Helianthus annus</i>	<i>M. pachydermatis</i> , <i>Pseudomonas aeruginosa</i> and <i>Staphylococcus pseudintermedius</i> and an ATCC strain of <i>Candida albicans</i>	Vercelli et al., 2021
<i>Angelica archangelica</i> L., <i>Cinnamomum verum</i> J.S. PRESL, <i>Salvia sclarea</i> L. <i>Syzygium aromaticum</i> (L.) MERR et L.M. PERR, <i>Coriandrum sativum</i> L., <i>Foeniculum vulgare</i> var. <i>dulce</i> (MILL) BATT et TRAB, <i>Lavandula hybrida</i> REVERCH, <i>Lavandula angustifolia</i> MILL, <i>Citrus limon</i> L., <i>Cymbopogon citratus</i> (DC.) STAPF, <i>Leptospermum scoparium</i> J.R. et G. FORST, <i>Cymbopogon nardus</i> (L.) RENDLE, <i>Citrus aurantium</i> L. ssp. <i>Amara</i> , <i>Origanum vulgare</i> L., <i>Cymbopogon martinii</i> (ROXB.) J.F. WATSON, <i>Cinnamomum camphora</i> (L.) J.S. PRESL, <i>Pelargonium graveolens</i> L'HERIT ex AIT, <i>Melaleuca alternifolia</i> CHEEL, <i>Thymus vulgaris</i> L., <i>Thymus vulgaris</i> L.c.t. <i>linalool</i> , <i>Thymus vulgaris</i> L.c.t. <i>thymol</i> , <i>Satureja montana</i> L.	<i>Malassezia pachydermatis</i>	Bismarck et al., 2020
<i>Thymus capitatus</i> , <i>Thymus vulgaris</i> , carvacrol, and thymol	Meticillin-susceptible, <i>Staphylococcus pseudintermedius</i> , meticillin-resistant <i>S. pseudintermedius</i> , b-haemolytic <i>Streptococcus</i> spp., <i>Pseudomonas aeruginosa</i> , <i>Proteus mirabilis</i> and <i>Malassezia pachydermatis</i>	Sim et al., 2019b

Cinnamomum zeylanicum	Methicillin-susceptible <i>S. Pseudintermedius</i> , methicillin-resistant <i>S. Pseudintermedius</i> , b-haemolytic <i>Streptococcus</i> spp., antimicrobial-susceptible <i>P. aeruginosa</i> , antimicrobial-resistant <i>P. Aeruginosa</i> , <i>P. mirabilis</i> , and <i>M. pachydermatis</i> <i>S. Pseudintermedius</i> , <i>S. aureus</i> , <i>Staphylococcus hyicus</i> , <i>P. aeruginosa</i> , <i>P. mirabilis</i> , <i>E. coli</i> , <i>Corynebacterium</i> spp., <i>Enterobacter</i> spp., <i>Streptococcus</i> spp., <i>Proteus</i> spp., <i>Candida</i> spp., <i>Malassezia pachydermatis</i>	Sim et al., 2019a
Melaleuca alternifolia		Neves et al., 2018
<i>Citrus bergamia</i> , <i>Cedrus</i> spp., <i>Matricaria recutita</i> , <i>Cinnamomum aromaticum</i> , <i>Syzygium aromaticum</i> , <i>Citrus paradisi</i> , <i>Juniperus communis</i> , <i>Lavandula angustifolia</i> , <i>Origanum vulgare</i> , <i>Pinus sylvestris</i> , <i>Salvia officinalis</i> , <i>Satureja montana</i> , <i>Melaleuca alternifolia</i> , <i>Achillea millefolium</i>		
<i>Anthemis nobilis</i> L., <i>Illicium verum</i> , <i>Lavandula hybrida</i> , <i>Litsea cubeba</i> (Lour.) Pers., <i>Ocimum basilicum</i> L., <i>Origanum vulgare</i> L. subsp. <i>hirticum</i> , <i>Rosmarinus officinalis</i> L., <i>Salvia sclarea</i> L., and <i>Thymus vulgaris</i> L.	<i>Malassezia pachydermatis</i>	Vánczi et al., 2018
<i>Citrus paradisi</i> , <i>Salvia sclarea</i> , <i>Ocimum basilicum</i> , <i>Rosmarinus officinalis</i> , <i>Citrus limon</i> , <i>Anthemis nobilis</i> , <i>Lavandula hybrid</i> , and <i>Thymus vulgaris</i>	<i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> , <i>Staphylococcus pseudointermedius</i> , <i>Aspergillus niger</i> , <i>Aspergillus fumigatus</i> , <i>Aspergillus terreus</i> , <i>Candida albicans</i> , <i>Candida tropicalis</i> , <i>Trichosporon</i> sp., and <i>Rhodotorula</i> sp.	Ebani et al., 2017
<i>Thymus vulgaris</i> , <i>Pelargonium graveolens</i> , <i>Lavandula angustifolia</i> , <i>Melaleuca alternifolia</i> , <i>Origanum majorana</i> , and <i>Eucalyptus globulus</i>	<i>Malassezia pachydermatis</i>	Nardoni et al., 2017
		Kim et al., 2012

Table 2. Antibacterial activity of essential oils against OE pathogens - *in vivo*

Essential oils	OE pathogens	Study
<i>Melaleuca alternifolia</i> (also named tea tree oil), <i>Thymus serpillum</i> , <i>Salvia officinalis</i> , <i>Eucalyptus officinalis</i> , <i>Rosmarinus officinalis</i> , <i>Macadamia alternifolia</i> , <i>Lavandula officinalis</i> and <i>Helianthus annus</i>	<i>M. pachydermatis</i> , <i>Pseudomonas aeruginosa</i> and <i>Staphylococcus pseudointermedius</i> and an ATCC strain of <i>Candida albicans</i>	Vercelli et al., 2021
<i>Melaleuca alternifolia</i>	<i>S. Pseudintermedius</i> , <i>S. aureus</i> , <i>Staphylococcus hyicus</i> , <i>P. aeruginosa</i> , <i>P. mirabilis</i> , <i>E. coli</i> , <i>Corynebacterium</i> spp., <i>Enterobacter</i> spp., <i>Streptococcus</i> spp., <i>Proteus</i> spp., <i>Candida</i> spp., <i>Malassezia pachydermatis</i>	Neves et al., 2018
<i>Citrus paradisi</i> , <i>Salvia sclarea</i> , <i>Ocimum basilicum</i> , <i>Rosmarinus officinalis</i> , <i>Citrus limon</i> , <i>Anthemis nobilis</i> , <i>Lavandula hybrid</i> , and <i>Thymus vulgaris</i>	<i>Malassezia pachydermatis</i>	Nardoni et al., 2017

DISCUSSION

Factors involved in the otitis externa etiology

It is of particular importance that veterinarians assess the involvement of causes and factors that can participate in the etiology of OE while examining each individual patient affected by this disease. If all or at least most of these factors are established in time, dealing with the current infection will be easier and there would be fewer chances of repeated ones (Bajwa, 2019). The causes and factors are divided into primary, secondary, predisposing and perpetuating based on the most recent classification system (PSPP system) modified by Miller et al. (2013). The risk of OE is increased or prolonged by predisposing factors such trauma, high cerumen production, excessive humidity, and obstructive ear disorders, but they often do not cause it directly (Saridomichelakis et al., 2007). The unique structure of the ear pinna and external ear canal, especially in some breeds, also may contribute to disease development. On the other hand, primary causes of OE directly induce the disease and include foreign bodies, allergies, autoimmune disease, parasites, and glandular disorders (Jacobson, 2002). Furthermore, when primary causes disturb the ear ecosystem and initiate ear canal inflammation, secondary factors take an opportunity to develop the infection and intensify the inflammatory reaction. This group involves yeast and bacteria that are mostly normal inhabitants of the ear, so these agents only contribute to or cause pathology in combination with predisposing factors. Perpetuating factors prevent the disease resolution and comprise chronic pathological changes of the ear canal, tympanic membrane changes and otitis media (Kasai et al., 2021, Jacobson, 2002, Rosser, 2004, Scott et al., 2001).

Diagnosis of the otitis externa

When there is a suspicion about OE presence, the typical diagnostic procedures must be conducted. These include detailed physical and otoscopic examination of the pinna and external ear canal, as well as tympanic membrane, if visible (Angus, 2004, Rosser, 2004). A careful and thorough review of history and clinical symptoms can help to narrow down the differential diagnosis search, allowing for the cost- and time-effective use of adequate diagnostic steps and treatment (Nuttall, 2023). It is of particular importance that the entire dog is fully examined, not just the ears, making sure to examine both ears even in cases where it appears that only one ear is infected.

Determination of the primary cause and identification of the factors that can be involved in OE etiology represents a major diagnostic challenge (White, 1999). Although bilateral otitis is more common, the factors that predispose the disease can cause the otitis to occur more frequently, or to worsen in one ear compared to the other (Nuttall, 2023). Furthermore, otoscopic examination is significant to determine if there are pathological changes or foreign bodies in the ear canal, to assess the type and volume of discharge and to evaluate the state of the eardrum (Nuttall, 2016, Scott et al., 2001). The appearance of discharge may vary depending on the causative agent present. In fact, it may give a hint to its etiology. The presence of ectoparasites is characterized by dry coffee ground-like debris. Furthermore, staphylococcal and yeast infections are correlated with moist brown discharge, while gram-negative bacteria are associated with purulent creamy to yellow exudates (Scott et al., 2001). Thus, although a particular kind of exudate can increase the index of suspicion for a particular kind of otitis gross, examination alone is inadequate (Jacobson, 2002). Sample from the ear is taken by inserting a swab into the canal with rotating movement, after which it is rolled onto a slide and stained (Lehner et al., 2010). Cytology can give an insight into the number and form of bacteria, number of yeasts, excessive cerumen, keratinaceous debris and neoplastic cells and the number and type of leukocytes (Chickering, 1988). The cytology may also successfully identify the most likely microorganisms in majority of otitis cases, which is especially useful in polymicrobial infection, where culture may discover different organisms with various susceptibility patterns (Nuttall, 2016).

Opinions about the use of antibiotic susceptibility testing in the case of OE are divided. Numerous studies emphasize how important it is to consider how susceptible bacteria are to the microbiological agents employed in otitis treatment, both in terms of culture and susceptibility testing (Zamankhan Malayeri et al., 2010, Hariharan et al., 2006, Lyskova et al., 2007). It is certain that bacterial culture and susceptibility testing determines the bacteria which causes the infection (Nuttall, 2016). On the other hand, Morris (2004) points out that antibiotic susceptibility testing is not applicable in all types of otic infections referring to the poor passage of systemic antimicrobials through the mucosa of the external ear, as well as to the fact that susceptibility testing is performed using serum rather than tissue concentrations. Moreover, when

susceptibility testing of OE in dogs is done using standard discs for serum concentration, the results of *in vivo* and *in vitro* studies may differ because topical therapy can achieve greater concentrations (Morris, 2004).

Imaging techniques also represents helpful diagnostic tool in the assessment of the inflamed ear (Grosi et al., 2003). Radiography can be used in refractory of repeated OE cases, when there is a doubt about presence of the otitis media and/or mineralization and in cases where surgical intervention is being considered (Murphy, 2001). However, radiographic studies have some limitations, such as technical challenges, time-consuming and difficulties in interpretation of the eardrum under general anaesthesia, while, normal radiographic findings do not exclude disease of the middle ear (Murphy, 2001, Lorek et al., 2020). Computed tomography (CT) and magnetic resonance imaging (MRI) are other imaging modalities that can be used for diagnosis of ear diseases (Lorek et al., 2020). CT compared to radiography, provides superior image contrast and represents a noninvasive diagnostic method that easily can evaluate middle ear disease and chronic changes of the ear canal (Murphy, 2001). It is preferred in assessing the osseous structures of the ear, whereas MRI better detect changes in the soft tissues surrounding the tympanic bullae (Lorek et al., 2020).

Treatment of the otitis externa

A limited number of antimicrobials agents available in the topical form, treatments recommended without indications, as well as the presence of mixed infections in the ear and various factors that contribute to the disease disabled the establishment of a solid literature data that indicates which specific treatment is most effective in which specific situations (Jacobson, 2002). The key role in the successful long-term clinical management of OE is to identify, diagnose and control the prime cause and to provide appropriate education of the owner, as well as regular examination (Murphy, 2001, Jacobson, 2002). Management of predisposing and primary factors differs depending on the cause. Infections caused by yeast or/and bacteria cannot be found in a normal ear but they occur as a product of inflammation caused by primary triggers, usually combined with perpetuating and predisposing factors. The treatment of infections is important, but if treated regardless of the cause, recurrence is inevitable (Paterson, 2016). In fact, treating the primary cause alone is in most cases enough to treat the sec-

ondary microbial infections. For example, foreign bodies, such as grass awns is a frequently occurrence in veterinary patients causing peracute, painful type of otitis that rapidly could cause severe self-inflicted trauma, and even damage to the tympanum (Paterson, 2016). However, if discovered in time, these conditions easily can be treated. On the other hand, allergic skin diseases are the most common reasons for chronic OE in dogs (Murphy, 2001). Veterinarians should examine each case individually and should recognize as many underlying causes and factors as possible that play a part in the disease (Scott et al., 2001, Little, 1996). Lack of success to recognize and control primary cause is one of the most frequent reasons for recurrent otitis and why acute cases progresses to chronic, often irreversible, disease (Paterson, 2016). Identifying a primary cause is more important in chronic and repeated ear infections (Jacobson, 2002), because most chronic cases have minimum one cause and at least one other factor contributing to the otitis, and lack of success to identify and rectify these may lead to failures in treatment (Scott et al., 2001). In cases like this, susceptibility test results do not guarantee a favorable outcome of the treatment because susceptibility testing does not account for variables such as active inflammation, biofilm, earwax, canal stenosis, and others that may affect the test's effectiveness (Nuttall, 2023). Great challenges in the treatment of OE also represents the ability of some bacteria, such as *Staphylococcus* and *Pseudomonas*, to create biofilm because despite appropriate therapy, it may lead to perseverance of infections, so biofilms must be broken for the therapy to be efficient (Bajwa, 2019). In addition to therapy, biofilms have a significant impact on AMR because they may trigger the formation of resistance mutations in bacteria, particularly in Gram-negative bacteria that progressively produce these mutations in response to concentration-dependent medicines (Nuttall, 2016).

An important and indispensable step in the treatment of OE is cleaning and removing impurities from the ear canal. Cleaning and drying preparations are an integral part of treatment, maintenance, and prevention, as well (Jacobson, 2002, Tešin et al., 2023c). Furthermore, due to excessive amount of discharge, topical therapy may be ineffective because direct contact between the topical medicines and the ear epithelium and causative agents is prevented (Neves et al., 2018). Cleaning provides an adequate visual examination of the ear by removing impurities, toxins, and enzymes produced by pathogens, plus it reduces the

microbial cultures and allows topical agents to exert their effectiveness (Jacobson, 2002). In addition, radical flushing and aspiration can physically disrupt and eliminate biofilm from the ear (Nuttall, 2016).

Since topical medicines can affect the causative agent directly, topical therapy is more often indicated than systemic one in the case of OE (Rougier et al., 2005). Antimicrobials applied systemically will only get to the inner side of the tympanic membrane, so it can only lead to a higher chance of AMR (Woodwars, 2022). In addition, it is likely that topical therapy has less impact to the microbiota than systemic one because local administration to limited surfaces, such as the external ear, will cause less harm to the microbiota on the opposite side (Nuttall, 2023). Therefore, systemic antimicrobial use (AMU) is indicated in case of the otitis media because the middle ear comprises a well blooded mucous membrane layer that provides higher medicine diffusion from the blood vessels to the middle ear area (Morris, 2004).

Antimicrobials from the aminoglycosides group such as gentamicin and neomycin can be used in form of topical agents and are considered as first-line antibiotics against OE infections (Spinoza et al., 2006). However, because of their unwanted effects such as ototoxicity (Morris, 2004), their use is not allowed when the tympanic membrane is damaged (Jacobson, 2002). In cases such as this, applications of other topical medicines are more appropriate, such as fluoroquinolones (enrofloxacin and marbofloxacin) which are considered as second-line antibiotics (Nuttall, 2016). Antimicrobials such as polymyxin B, fusidic acid, and florfenicol are available for topical use as well (Nuttall, 2016, Morris, 2004).

In 2020, The World Small Animal Veterinary Association (WSAVA) published the List of Essential Medicines for Cats and Dogs. This list should provide clinicians an adequate prevention and therapy options of the most common diseases in companion animals with the goal of improving regulatory oversight to secure adequate medicines availability, their use and quality, as well as pharmacovigilance, while reducing the increasing black/counterfeit market of pharmaceutical preparations (Steagall et al., 2020). Moreover, the research conducted in the UK, Malaysia, Serbia, and Thailand has shown evidence of substandard formulations of antimicrobials used in veterinary medicine (Pelligand et al., 2023). This could compromise equitable access to acceptable quality essential veterinary medicines worldwide since antimicrobial

formulations of substandard quality have negative consequences of efficacy in patients and potentially promote AMR (Pelligand et al., 2023).

According to this list, fusidic acid should be the first-choice medicine in infections associated with staphylococci, while florfenicol represents an increasingly used antimicrobial for the management of staphylococcal otitis, usually as part of a combined treatment that includes antifungal and corticosteroid medicines. Furthermore, aminoglycosides such as gentamicin and neomycin are the first choices for resolving OE infections caused by *Pseudomonas aeruginosa* and other Gram-negative bacteria, whereas enrofloxacin or marbofloxacin are first choices for the treatment of otitis media and an adequate replacement to aminoglycosides for management of OE caused by Gram-negative bacteria. Polymyxin B is useful as an alternative to aminoglycosides and fluoroquinolones, for management of OE caused by Gram-negative bacteria, and it is often used in combination with miconazole, an antifungal agent with antibacterial activity. Topical agents for the treatment of superficial yeast, principally *Malassezia* include antifungal medicines like azole derivatives which lead to depletion of lanosterol, the primary sterol of the fungal cell wall. Topical azoles include miconazole, econazole, clotrimazole and enilconazole. Terbinafine is an allylamine fungicidal agent that inhibits fungal squalene epoxidase to interrupt synthesis of ergosterol (Steagall et al., 2020). Besides these antifungals recommended by WSAVA list, in the treatments against *M. pachydermatis*, nystatin can be used as well (Vercelli et al., 2021).

However, it has been noted that there are differences among regional isolates and their susceptibility to various antimicrobial medicines (Wong et al., 2015). Hence, it is necessary that every state establishes its own effective, well-established measures that include the monitoring system of AMR and antimicrobial stewardship based on the national records (Tešin et al., 2023b). As a part of antimicrobial stewardship program, an insight into the antibiotic sale pattern could provide valuable information about the number of prescriptions prescribed by veterinarians that could be used for modified interventions aimed at AMR reduction (Mugoša et al., 2023). Without proper antimicrobial stewardship, further development of ongoing resistance to antimicrobial and antifungal medicine is more likely (Nuttall, 2023). Engagement of Dutch small animal clinics in multifaceted antimicrobial

stewardship program to reduce AMU in small practices showed a positive effect (Hopman et al., 2019). The use of cytology and carefully selected topical therapy will help improve the antimicrobial stewardship (Nuttall, 2023).

Perspectives on the otitis externa treatment

Antibiotic and/or antifungal therapy is not effective in every case of canine OE. On the contrary, it can worsen the process by causing repeated infections (Ebani et al., 2017). On the other hand, treating pets has become more challenging, because owners are more demanding and selective about their way of life and the products they use (Neves et al., 2018). Furthermore, as the growth of AMR is constantly increasing, further studies are necessary to create treatment alternatives against microbial agents (Tešin et al., 2023b). In addition, resistance of new fungi to conventional medicines is on the rise, so identifying novel antifungal agents is important as well (Khosravi et al., 2016).

Medicinal herbs and their essential oils have been used in popular medicine as an alternative treatment of various pathological conditions (Nardoni et al., 2017). Due to satisfactory results, even against resistant pathogens and biofilm formations (Tomanić et al., 2023b), the possible uses of these products against OE pathogens in veterinary practice have been the subject of many studies during the last few years. The focus has been on finding alternative therapeutic methods to replace or reduce AMU. So far, besides *in vitro* studies (Tomanić et al., 2022b, Kovačević et al., 2022b, Tomanić et al., 2022a), in depth *in vivo* research about essential oils use in the treatment of diseased animals was conducted in the mastitis treatment of dairy cows (Tomanić et al., 2023a, Kovačević et al., 2022a, Kovačević et al., 2022b). In the recent years, pharmaceutical formulations of essential oils have been studied and there have been many *in vitro* studies (Table 1) on the efficacy of these herbs and their potential use in OE therapy, while only few of them (Table 2) have dealt with *in vivo* efficacy. The fact that the transfer of *in vitro* results in *in vivo* study models are always demanding, makes this topic even more challenging (Tomanić et al., 2022c).

Different results of bacteriological and mycological effect have been reported. In the *in vitro* study conducted by Ebani et al. (2017) the efficacy of nine essential oils against bacterial and fungal pathogens formerly isolated from canine and feline OE cases

were assessed. It was established that of all essential oils used in this study, *O. vulgare* and *S. sclarea* showed superior antibacterial activity, even though not against all three bacteria (*P. aeruginosa*, *S. aureus*, and *S. pseudointermedius*) evenly, while the *A. nobilis*, *I. verum*, *L. hybrida*, and *L. cubeba* had no activity against the three tested bacteria. *O. vulgare* essential oils inhibited the growth of *S. aureus* and *S. pseudointermedius*, whereas activity against *P. aeruginosa* was not observed. In another study conducted by Ebani et al. (2023) the efficacy of *O. vulgare*, *S. montana*, and *T. vulgaris* essential oils was assessed, as well as a mixture of these three components against bacteria and yeast isolates (Table 1). The results of individually tested essential oils have shown good sensitivity against Gram-positive strains and *M. pachydermatis*, however, they were less effective against Gram-negative isolates, especially against the *P. aeruginosa*. Nevertheless, when these three essential oils were blended, the activity against all bacteria, including *P. aeruginosa* (except for the one strain) was higher. These results indicate that the mixture of several essential oils has better efficacy against OE infections compared to individual ones. Furthermore, in the research conducted by Sim et al. (2019b), oregano and thyme essential oils and their phenolic components (carvacrol, thymol) also showed a good antimicrobial activity against both Gram-negative and Gram-positive OE bacteria with MIC90 values ranging from 0.02% (200 µg/mL) to 0.25% (2,292 µg/mL), as well as antifungal activity against *M. pachydermatis* isolates which were more sensitive to oregano oil and carvacrol, indicating that active ingredients tested in this study could be used for developing novel treatments for sensitive and even resistant microorganisms involved in OE in dogs (Sim et al., 2019b).

Although essential oils are GRAS (generally recognized as safe), the use of essential oils should be avoided in cases when ear drum is ruptured, while topical application of solely essential oils could have an irritant effect on canine ear skin. It is necessary to point out that undiluted essential oils should not be dripped into the ears as these types of oils and products with their high concentrations may cause skin irritations and inflammation causing contact dermatitis (Posadzki et al., 2012, Horváth and Ács, 2015). Moreover, as the composition of essential oils differs not only on the basis of the various botanical species, but also on the part of plants that was used, the harvest season, the natural conditions and the extraction methods, before administration, their composition

should be taken into account (Bismarck et al., 2020, Ebani and Mancianti, 2020).

As clinical study are limited, development of the right pharmacological ear drop formulations based on essential oils for external application in canine OE is the subject of many researches. Study conducted by Vercelli et al. (2021) aimed to evaluate an essential oil blend (Otogen®) in treatment of canine OE. Based on the *in vitro* results, a remarkable growth reduction (almost 100%) of *M. pachydermatis* and *C. albicans*, after 15 min. of contact, and *P. aeruginosa*, after 1 h of incubation, was noted, while 50% of *S. pseudintermedius* growth was reduced after 15 min. The efficacy of this blend was also assessed *in vivo* in this study, in addition to *in vitro* testing. The results showed a significant improvement in all clinical signs (particularly head shaking and erythema) in dogs after just one day of administration, and owners agreed that the odor caused by infections had decreased. Another *in vivo* study conducted by Neves et al. (2018) assessed the efficacy of tea tree essential oils ear solution against OE pathogens, and both bacterial and yeast ear infections showed significantly induced remission of clinical symptoms. Interestingly, *M. pachydermatis* ear infection was reduced as much as the nystatin solution used in this study. It seems that essential oils have a better effect on yeast than the bacteria, however good antimicrobial activity and lack of adverse reactions verify the need for further development of essential oil formulations as a viable alternative treatment approach against canine otic infections.

Although essential oils are frequently assessed as a potential replacement to the antimicrobial preparation, their safe use has not yet been confirmed (Kim et al., 2012). Because of the close relationship between their actions and the isolates of bacteria, assessing the *in vitro* effectiveness of different essential oils can help select the most effective natural agent to combat the causative agent (Ebani et al., 2017). On the other hand, there are major concerns about implementations of less irritating and natural original preparations (Packer and Luz, 2007), such as those made

with essential oils (Neves et al., 2018). Since their use is convenient as a remedy for topical therapy, focus should be directed on potential side effects, such as photosensitivity from direct exposure to sunlight. Therefore, dermatitis is the most reported side effect (Ebani and Mancianti, 2020). Though more research is required, it is unlikely that this alternative option will be able to adequately replace prescription medicine; instead, it will probably be used as an additional option in integrated therapy programs that focus on the underlying causes, risk factors, and secondary infections in each case (Nuttall, 2023). Overall, these products have a lot of potential for use in the clinical therapy of OE.

CONCLUSION

One of the aims in therapy of ear infections in dogs is to recognize the underlying factors and causes in time to prevent the disease progression and relapses. With the accurate identifications of the different causative agents responsible for otic infection, rapidly effective targeted therapy can be accomplished. Antimicrobial stewardship programs and AMR monitoring systems must be implemented in every country to reorganize AMU in small animal practice due to the growing resistance to antibiotics. Furthermore, based on the previous *in vitro* and *in vivo* studies, phytotherapy has shown promising results as an alternative treatment approach against OE pathogens. In addition, exploring novel antimicrobial agents, such as essential oils will probably not replace antibiotics and antifungals completely, but they could be promoted as additional therapy. Therefore, essential oils could have a great contribution to reducing the excessive use of different synthetic medicines, as well as AMR.

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CONFLICT OF INTEREST

None declared.

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