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Research Article

Ear Infections in Animals in Bareilly: Common causes and Effective Antimicrobials

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Abstract

Ear infections are one the most common ailments in pet dogs and sometimes in horses affecting their normal behaviour and utility. The study conducted on bacterial causes of ear infections and effective antimicrobials revealed that ear infections are more commonly reported in dogs than in other animals. Though bacteria belonging to 21 genera were isolated in an association of ear infections in animals, the most common were *Staphylococcus* spp., *Pseudomonas* spp. and *Proteus* spp. responsible for more than two-third cases. Isolation of *Raoultella terrigena, Erwinia mallotivora, Sphingomonas echinoides*, and *Vibrio alginolyticus* in association to ear infection in the study have rarely been reported earlier. Most of the antibiotics commonly prescribed to patients with ear infection were not effective and might be responsible for frequent treatment failure. Among the herbal antimicrobials cinnamon oil and ajowan oil have shown the potential for alternatives to antibiotics for formulations of ear drops and further studies are required to develop suitable formulations.

Keywords: *Staphylococcus; Pseudomonas; Proteus; Raoultella; Erwinia; Sphingomonas echinoides; Vibrio alginolyticus*

Introduction

Ear infections affect about 10-20 % of the dogs and are one of the most common health problems of the dogs [1]. Ear infections are usually caused by yeast, ear mites and bacteria, with bacteria being the most common cause. Ear problem in doge are usually identified by observing head shaking/ tilting, smelly ears, ear scratching, lack of balance, unusual back-and-forth eye movements, redness inside the ear, swelling of the ear and/or brown, yellow, or bloody discharge from the ear. The problem may be in one or both the ears at any age. Ear infections are usually caused by *Staphylococcus, Pseudomonas* and *Streptococcus* species [2,3]. *Pseudomonas* is not the only major cause of otorrhea in dogs [4] but also in other animals and humans, causing more than one-fourth of the ear infection cases [5]. The most common bacterial causes of otitis media in animals and human beings include *Escherichia coli*, *Proteus* spp., *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Moraxella* (*Branhamella*) *catarrhalis*, and *Haemophilus influenzae* [6-8]. Besides *Staphylococcus intermedius*, *Staphylococcus hyicus*, *Corynebacterium* spp., and *Enterobacter* spp., isolates, *Proteus mirabilis* was identified as the most frequent cause of otitis in dogs [9].

In cases of bacterial infections of ear, antibiotics are often the first option to treat otorrhoea, mostly for local application and instillation and but in chronic cases oral or systemic antimicrobial therapy is recommended [10]. Besides antibiotics, herbal antimicrobials like oregano oil, apple cider [3] and tea tree oil [9] have been claimed effective. In a previous study [9], 60.5% Gram-positive and 70% Gram-negative bacteria isolated from cases of otitis in dogs were susceptible to gentamicin but all to tea tree oil. Another study revealed that 72% *Staphylococcus aureus* causing otitis were susceptible to tea tree oil, in concentrations lower than 2% [11]. In the present study, antimicrobial susceptibility testing data of 123 strains of bacteria isolated from cases of ear infections and otorrhea in animals was analysed to understand the causes and effective antimicrobials so that clinicians may get an overview of the problem in Bareilly region for instituting the most suitable antimicrobial treatment.

Materials and Methods

Antimicrobial susceptibility assay data of all 123 isolates of bacteria from cases of ear infections in animals in last three years were retrieved from Clinical Epidemiology Database of the Division of Epidemiology and transferred to Microsoft Office Excel 2007 worksheet for analysis.

In the study, deep ear swab samples collected by a clinician from referred ear infection cases of animals at Referral Veterinary Polyclinic of Indian Veterinary Research Institute were submitted within an hour of collection to Epidemiology Laboratory for identification and antibiotic susceptibility testing of the bacteria. Swab samples were processed as per standard protocol for isolation, identification and classification of the bacteria [12-14]; briefly, swab samples were inoculated into buffered peptone water (BBL, Diffco, USA) and incubated at 37°C for 6h, growth was streaked on to Blood agar and MacConkey agar (BBL, Diffco) and incubated at 37°C for 24-48 h. Isolated colonies were picked up and tested for morphological, staining, growth and biochemical characteristics.

Characterised isolates were tested for their sensitivity to different conventional antimicrobials including amoxycillin, amoxycillin+clavulanic acid, amoxycillin+sulbactum, ampicillin, ampicillin+sulbactam, azithromycin, aztreonam, cefepime, cefixime, cefotaxime, cefoxitin, cefpodoxime, ceftriaxone+sulbactum, ceftazidime, ceftazidime+clavulanic acid, ceftriaxone, ceftriaxone+tazobactam, chloramphenicol, ciprofloxacin, cloxacillin, cotrimoxazole, ertapenem, erythromycin, gentamicin,

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Table 1: Bacteria associated with ear infections in animals and their multiple antimicrobial drug resistance indices (MARIs) and multiple herbal antimicrobial resistance indices (MHARIs).

Genus	Species of bacteria (source animal)	MHARI	MARI	Carbapenem resistant
Acinetobacter (2)	*A. ewofflii* 2 (dogs)	0	0.087	1
Aerococcus (1)	*A. sanguinus* 1 (dogs)	0.6	0.419	1
Alcaligenes (1)	*A. denitrificans* 1 (dogs)	0.857	0.75	1
Avibacterium (1)	*A. avium* 1 (dogs)	0.077	0.455	1
Bacillus (8)	*B. alvei* 1, *B. firmus* 2, *B. licheniformis* 1, *B. stearothermophilus* 1, *B. subtilis* 1, *Bacillus* spp. 2 (dogs)	0.283	0.306	2
Citrobacter (1)	*C. amalonaticus* 1 (dogs)	0.462	0.13	0
Enterobacter (2)	*E. agglomerans* 2 (dogs)	0.154	0.451	0
Enterococcus (1)	*E. gallinarum* 1 (dogs)	0.182	0.357	1
Erwinia (1)	*E. mallotivora* 1 (dogs)	0.308	0.261	0
Escherichia (5)	*E. coli* 5 (dogs 4, horse 1)	0.443	0.415	0
Klebsiella (1)	*K. pneumoniae* 1 (dogs)	0.385	0.217	0
Micrococcus (3)	*Micrococcus* spp. 3 (dogs)	0.477	0.201	0
Moraxella (1)	*M. osloensis* 1 (dogs)	1	0.077	0
Proteus (11)	*P. mirabilis* 10 (dogs 9, horse 1), *P. penneri* 1 (dogs)	0.642	0.593	1
Providencia (1)	*P. stuartii* 1 (horse)	0.6	0.5	1
Pseudomonas (16)	*P. aeruginosa* 12, *Pseudomonas* spp. 4 (dogs)	0.749	0.694	8
Raoultella (1)	*R. terrigena* 1 (dogs)	0.857	0.429	0
Sphingomonas (2)	*S. echinoides* 2 (dogs)	0.714	0.232	0
Staphylococcus (56)	*S. aureus* 5, *S. auricularis* 4 (dogs 3, horse 1), (dogs), *S. capitis* 6 (cat 1, dogs 5), *S. caseolyticus* 2, *S. delphini* 1, *S. epidermidis* 2, *S. felis* 1, *S. haemolyticus* 8, *S. hyicus* 1, *S. intermedius* 16, *S. lentus* 2, *S. lugdunerisii* 1, *S. schleiferi* 2, *S. sciuri* 1, *S. simulans* 1 (dogs), *Staphylococcus* spp. 3 (dogs 3, Rhino 1)	0.359	0.303	8
Streptococcus (6)	*S. equi* ssp. *zooepidemicus* 3, *S. porcinus* 1, *S. pyogenes* 2 (dogs)	0.227	0.113	0
Vibrio (2)	*V. alginolyticus* 2 (dogs)	0.357	0.138	0

imipenem, lincomycin, linezolid, meropenem, methicillin, moxalactam, nalidixic acid, nitrofurantoin, oxacillin, penicillin, piperacillin, piperacillin+tazobactam, polymyxin-B, spectinomycin, streptomycin, tetracycline, tigecycline and vancomycin through disc diffusion assay as per guidelines of CLSI [15,16]. All antimicrobial discs were purchased from BBL, Diffco. Bacterial isolates were also tested for their susceptibility to herbal antimicrobials using disc diffusion assay as described earlier [17]. For making discs of herbal antimicrobials >98% pure herbal compounds were used to make 6mm discs cut from Whatman filter paper No.3, each disc contained 1mg of herbal compound [17]. In the study discs were prepared for carvacrol, cinnamaldehyde, citral, tea tree oil (from Sigma, USA), guggul oil (from ICAR-Indian Institute of Natural Resins and Gums, Namkum, Ranchi), *Ageratum conizoides* essential oil, ajowan oil, betel leaf essential oil, cinnamon oil, holy basil oil, lemongrass oil, patchouli (*Pogostemon cablin*) essential oil, sandalwood oil, thyme oil and *Zanthoxylum rhetsa* essential oil (from Shubh Flavours and Fragrance Ltd, New Delhi). A reference sensitive *E. coli* strain (E-382) available in the laboratory was used as control.

Determination of Minimum Inhibitory Concentration (MIC) of herbal antimicrobial for microbes

The MIC of Holy Basil Essential Oil (HBO), carvacrol, cinnamon oil, thyme oil, Sandalwood Oil (SWO), Tea Tree Essential Oil (TTO), Patchouli Essential Oil (PEO), citral, ajowan oil, lemongrass oil, guggul oil and *Zanthoxylum rhetsa* essential oil, for different bacteria was determined using agar well diffusion assay [18]. To determine MIC, nine wells of 6mm diameter each were cut in suitable MHA plates under sterile environment and bottoms of wells were sealed with the same medium. Culture prepared for antimicrobial sensitivity assay for test microbe (described earlier) was swab inoculated and wells were filled with 50μL of serially diluted herbal antimicrobial in sterile dimethyl sulphoxide (DMSO, SDFCL, India) so that well number one to nine contained 10, 20, 40, 80, 160, 320, 640, 1280μL and 2560μg of the PEO, respectively. Plates were incubated under appropriate growth conditions for 2h without inversion to get contents of the well absorbed in the medium and then overnight after inversion in an appropriate environment required for the optimum growth of the microbe. Measurable zone of growth inhibition around the well containing the highest dilution of herbal antimicrobial was marked as MIC value for the microbe. Tests were conducted in triplicate for confirmation.

The results were analysed in Microsoft Excel 2007 worksheet using shorting, filtration, correlation, and χ^2 test tools.

Results and Discussion

The analysis of data revealed that the 10 most common bacteria associated with ear infections in dogs (117) and other animals (cat 1, horses 4 and rhino 1) were of *Staphylococcus* (56), *Pseudomonas* (16), *Proteus* (11), *Bacillus* (8), *Streptococcus* (6), *Escherichia* (5), *Micrococcus* (3), *Acinetobacter* (2), *Enterobacter* (2) and *Sphingomonas* (2) species (Table 1). In earlier studies too [6-9] similar types of bacteria have been reported to be associated with ear infections in animals. However, the most common causal organisms reported in earlier studies *Pseudomonas* species [6-8] or *Proteus mirabilis* [9], were outnumbered by *Staphylococcus* species in our study. It might be due to weather conditions, seasonality and geographic and several other social and biological variations in Bareilly in comparison to other areas targeted in earlier studies. Moreover, *Staphylococcus* being a skin commensal might have also come to samples as contamination during sample collection.

Table 2: Antimicrobial resistance pattern of common Gram-positive (GPBs) and Gram-negative (GNBs) bacteria isolated as cause of ear infections in animals in Bareilly area.

Antimicrobial tested	Number bacterial isolates resistant to different antibiotics with respect to their types and sources									
	Total (123)	GPBs (75)	GNBs (48)	Dogs (117)	Other animals (6)	*E. coli* (5)	*Proteus* (11)	*Pseudomonas* (16)	*Staphylococcus* (56)	*Streptococcus* (6)
Amoxycillin	62	25	37	59	3	5	6	15	18	1
Amoxycillin + clavulanic acid	44	16	28	39	5	4	5	12	14	0
Amoxycillin + sulbactam	28	6	22	27	1	2	1	15	5	0
Ampicillin	68	29	39	63	5	5	7	15	24	0
Ampicillin + sublactam	6	5	1	6	0	0	0	0	2	0
Azithromycin	38	16	22	34	4	2	8	7	14	0
Aztreonam	19	0	19	17	2	2	6	6	0	0
Cefepime	26	17	9	25	1	0	4	3	10	1
Cefixime	18	10	8	18	0	1	1	4	9	0
Cefotaxime	40	21	19	38	2	1	5	11	17	1
Cefoxitin	37	13	24	35	2	1	4	14	7	1
Cefpodoxime	24	12	12	24	0	1	2	6	10	0
Ceftriaxone + sulbactam	2	1	1	2	0	0	0	1	1	0
Ceftazidime	63	44	19	58	5	2	5	7	34	2
Ceftazidime + Clavulanic acid	42	34	8	39	3	1	0	5	24	4
Ceftriaxone	27	9	18	25	2	2	6	8	7	0
Ceftriaxone + tazobactam	1	0	1	1	0	0	0	1	0	0
Chloramphenicol	25	6	19	24	1	1	3	14	5	0
Ciprofloxacin	41	27	14	38	3	3	6	3	25	0
Cloxacillin	27	17	10	24	3	2	3	3	12	1
Cotrimoxazole	67	36	31	64	3	2	10	15	28	2
Ertapenem	12	7	5	12	0	0	1	4	5	0
Erythromycin	18	18	0	17	1	0	0	0	14	0
Gentamicin	39	21	18	36	3	3	8	4	20	1
Imipenem	0	0	0	0	0	0	0	0	0	0
Lincomycin	20	20	0	18	2	0	0	0	14	1
Linezolid	1	1	0	1	0	0	0	0	1	0
Meropenem	17	7	10	16	1	0	0	6	5	0
Methicillin	26	16	10	26	0	1	2	4	14	0
Moxalactam	30	17	13	26	4	1	1	8	11	1
Nalidixic acid	30	0	30	29	1	4	6	15	0	0
Nitrofurantoin	35	7	28	33	2	0	6	15	5	0
Oxacillin	42	30	12	40	2	1	3	4	22	0
Penicillin	52	27	25	48	4	3	4	11	23	1
Piperacillin	23	9	14	21	2	2	3	9	6	0
Piperacillin + tazobactam	14	6	8	13	1	2	1	5	6	0
Polymyxin-B	11	0	11	9	2	0	6	0	0	0
Spectinomycin	30	20	10	29	1	0	3	5	18	0
Streptomycin	18	6	12	16	2	2	2	8	5	0
Tetracycline	46	17	29	42	4	1	9	15	17	0
Tigecycline	14	0	14	13	1	0	1	11	0	0
Vancomycin	28	28	0	26	2	0	0	0	25	0

Table 3: Herbal antimicrobial resistance pattern of common Gram-positive (GPBs) and Gram-negative (GNBs) bacteria isolated as cause of ear infections in animals in Bareilly area.

Herbal antimicrobial tested	Number of bacterial isolates resistant* to different herbal antimicrobial with respect to their types and sources									
	Total (123)	GPBs (75)	GNBs (48)	Dogs (117)	Other animals (6)	*E.coli* (5)	*Proteus* (11)	*Pseudomonas* (16)	*Staphylococcus* (56)	*Streptococcus* (6)
Ageratum conizoides oil	17	4	13	17	0	1	5	4	4	0
Ajowan oil	8	0	8	8	0	0	2	6	0	0
Betel leaf oil	21	13	8	21	0	0	1	7	11	0
Carvacrol	8	0	8	8	0	0	0	8	0	0
Cinnamaldehyde	3	3	0	3	0	0	0	0	3	0
Cinnamon oil	8	5	3	8	0	0	0	2	5	0
Citral	19	8	11	19	0	1	1	8	7	0
Guggul oil	86	45	41	81	5	5	11	16	33	2
Holy basil oil	27	15	12	25	2	1	1	9	10	1
Lemongrass oil	50	22	28	48	2	0	8	11	18	1
Patchouli (*Pogostemon cablin*) oil	64	27	37	62	2	5	10	14	20	1
Sandalwood oil	48	18	30	45	3	4	7	10	13	2
Tea Tree oil	40	28	12	35	5	2	3	5	20	2
Thyme oil	8	1	7	8	0	0	0	7	1	0
Zanthoxylum rhetsa essential oil	27	17	10	23	4	1	4	3	13	0

Minimum inhibitory concentration of resistant isolates for different herbal compounds varied from 1280µg/mL to >2560µg/mL.

In the study, few bacteria not reported or rarely reported in earlier studies were also identified causing otitis in dogs including *Bacillus* species (Table 1), *Raoultella terrigena*, *Erwinia mallotivora*, *Sphingomonas echinoides*, and *Vibrio alginolyticus*. These bacteria are known to cause several invasive and topical infections [14] but their isolation from cases of otorrhoea of dogs revealed the expanding plurality of causes.

Of the 123 isolates of bacteria identified, 25 had resistance to one or more carbapenem drugs (meropenem, imipenem, ertapenem). All the bacteria positive for Carbapenem Resistance (CR) were among the most common causes of ear infections reported earlier [1,6-9]. However, CR bacteria have rarely been reported earlier as a cause of ear infections [1,6-9,19].

High MARI and MHARI of pseudomonads indicated that ear infections associated with *Pseudomonas* spp., may lead to persistence of infection despite the best antimicrobial used. Nowadays chronic and persistent ear infections are becoming common [9] and might be due to high levels of drug resistance in bacteria. In the study, *Alcaligenes denitrificans* and *Moraxella osloensis* causing ear infection in dogs had the highest MARI and MHARI (Table 1) indicating that such infections may be even more dangerous than due to pseudomonads.

Of the 56 staphylococcal isolates, 7 (12.5%) were resistant to methicillin (MRS) and oxacillin and certainly a big threat as MRSA are grouped under ESKAPE pathogens commonly able to evade all the common treatments [20]. Methicillin-resistant staphylococci have been reported to be associated with otitis in dogs [9]. Of the 7 MRS strains, six were vancomycin-resistant (VRS) but none of *Enterococcus* and *Streptococcus* isolates from ear infections were resistant to vancomycin. Besides *S. aureus*, other members of ESKAPE group including *E. agglomerans, K. pneumoniae* and *P. aeruginosa* were also detected as an important cause of ear infections in dogs. Isolation of ESKAPE bacteria have been reported commonly associated with animal infections including in dogs, horses and cats in Bareilly area [20].

Antimicrobial susceptibility assays (Table 2) against 42 conventional antimicrobial preparations revealed that antibiotic susceptibility of different bacteria varied to a large extent. The most commonly used antimicrobials for treatment of bacterial otitis media are amoxycillin, amoxycillin+clavulanic acid, cephalospins (cefalexin, cefdinir, cefixime, ceftriaxone, cefuroxime), gentamicin and fluoroquinolones (ciprofloxacin, ofloxacin, levofloxacin) azithromycin, clarithromycin, neomycin, ploymyxin-B, and sulphamethoxazole+trimethoprim [21]. However, in our study one of the recommended drugs, ceftriaxone (inhibited about 75% isolates) could make to reach at the 9th place among the 10 most effective antimicrobials, it was preceded by imipenem, ceftriaxone+tazobactam, ceftriaxone+sulbactam, piperacillin+tazobactam, tigecycline, chloramphenicol, meropenem and ampicillin+sulbactam, Only piperacillin (inhibiting 73.8% isolates) was behind it. Among the recommended antibiotics cefixime inhibited only 30% isolates and was at the 36th place in efficacy on bacteria causing otitis. There was only a little variation between effective antibiotics on GPBs and GNBs (Table 2) as ceftriaxone failed to inhibit about 42% of GNBs but was effective on 86% GPBs. However, two of the recommended drugs in otitis [21], polymyxin B (70.3%) and ciprofloxacin (68.9%) stood at 8th and 9th place among the most effective 10 drugs for GNBs causing otitis and one of the most used antibiotic, gentamicin failed to inhibit 39% of the isolates. *Proteus* species isolates, the third most common cause of ear infection in this study and the most common cause at some places [9], 80% isolates were not inhibited by any of the recommended antibiotics for otitis treatment. However,

Table 4: Minimum inhibitory concentration determined through well diffusion assay of different herbal compounds (µg/mL) for bacteria isolated from ear infection cases in animals.

Genus of bacteria (no. of isolates)	Minimum inhibitory concentration of different herbal compounds (µg/mL) determined through well diffusion assay											
	Holy basil oil	Carvacrol	Cinnamon oil	Thyme oil	Sandal wood oil	Tea tree oil	Patchouli oil	Citral	Ajowan oil	Lemon grass oil	Guggul oil	ZREO
Acinetobacter (2)	80, 320	10, 320	10, 640	80, 160	NT	10	NT	1280, 2560	10, 320	10, >2560	320, >2560	10-1280
Aerococcus (1)	NT	160	NT	NT	NT	NT	NT	NT	160	1280	>2560	640
Alcaligenes (1)	320	160	320	640	NT	NT	NT	640	320	640	>2560	>2560
Avibacterium (1)	NT	40	10	NT	NT	NT	NT	NT	320	20	40	80
Bacillus (8)	80-640	40-640	20-640	320-1280	320-1280	NT	10-1280	NT	320-1280	Oct-60	20->2560	80->2560
Citrobacter (1)	640	1280	1280	NT	NT	NT	NT	NT	1280	1280	>2560	2560
Enterobacter (2)	1280	320	320	320	NT	10, 40	NT	1280	320	>2560	>2560	>2560
Enterococcus (1)	1280	1280	640	640	NT	640	NT	>2560	1280	>2560	2560	640
Erwinia (1)	640	160	320	640	NT	640	NT	640	160	320	2560	2560
Escherichia (5)	640	160-640	160-1280	160-320	>2560	640->2560	160->2560	160-1280	160-1280	160->2560	2560->2560	640->2560
Klebsiella (1)	1280	320	640	320	NT	NT	NT	2560	320	2560	>2560	>2560
Micrococcus (3)	>2560	160	1280	320-1280	NT	2560	NT	>2560	NT	160->2560	80->2560	320->2560
Moraxella (1)	320	40	10	320	NT	640	NT	320	NT	320	320	320
Proteus (11)	160->2560	160-320	80-640	320-2560	NT	320->2560	NT	20->2560	10-1280	320-2560	320->2560	320->2560
Providencia (1)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	2560	1280
Pseudomonas (16)	320->2560	10-1280	320-640	320-1280	NT	>2560	NT	1280->2560	160-1280	160->2560	640->2560	80->2560
Raoultella (1)	640	160	320	320	NT	>2560	NT	320	320	640	2560	2560
Sphingomonas (2)	NT	160, 320	NT	NT	NT	320	NT	NT	NT	320	NT	NT
Staphylococcus (56)	640->2560	80-2560	320-1280	40-1280	>2560	160->2560	160->2560	160->2560	320-1280	80-1280	10->2560	80->2560
Streptococcus (6)	320-1280	10-320	10-640	640-1280	640-2560	640->2560	10-1280	NT	320-1280	10-640	10->2560	40->2560
Vibrio (2)	NT	10 , 20	NT	NT	NT	NT	>2560	80, 640	NT	80, 640	2560, >2560	10, 1280

NT, not tested; ZREO, *Zanthoxylum rhetsa* essential oil.

many of the antibiotics in market including ampicillin+sulbactam, ceftriaxone+tazobactam, imipenem, ceftriaxone+sulbactam, meropenem, ceftazidime+clavulanic acid, piperacillin+tazobactam, tigecycline, moxalactam, and amoxycillin+sulbactum inhibited majority of the isolates of *Proteus* spp.

Staphylococcus isolates, the most common cause of ear infections in Bareilly region, >83% could be managed with imipenem, tigecycline, linezolid, chloramphenicol, nitrofurantoin, cefriaxone+sulbactam, amoxycillin+sulbactam, piperacillin+tazobactam, ceftriaxone, piperacillin and cefoxitin. However, the most commonly prescribed amoxycillin+clavulanic acid, azithromycin and gentamicin in such cases [21] could inhibit the growth of only 75%, 75% and 64.3% staphylococcal isolates, respectively. The second most common cause, pseudomonads, were sensitive to five out of 10 most commonly prescribed antibiotics including polymyxin B, ciprofloxacin, gentamicin, cefepime, but only first three were able to inhibit >80% isolates while later two failed on >30% of the isolates.

Among herbal antimicrobials, cinnamaldehyde (an antimicrobial ingredient in cinnamon oil) and carvacrol (an antimicrobial ingredient in ajowan oil, thyme oil and oregano oil) inhibited >93% of the total isolates (Table 3). However, 53% pseudomonads were resistant to carvacrol but 100% were sensitive to cinnamaldehyde. Similar herbal antimicrobial resistance pattern among pseudomonads of water and milk origin has been reported in Bareilly region [22]. Staphylococcal isolates were inhibited by carvacrol (100%), ajowan oil (100%), thyme oil (96.2%), cinnamaldehyde (88.5%), cinnamon oil (83.9%), citral (73.1%) and sandalwood oil (70.5%). However, Tea Tree Oil (TTO), often claimed highly effective on staphylococci causing ear infections [9,11] failed to inhibit >91% of the isolates in the study. It might be due to lower TTO concentration used to determine the susceptibility of isolates in our study (1µL/disc) than 2% level used earlier [11] or due to a difference in resistance in isolates of different locality [16,19]. Our results with respect to carvacrol are in concurrence to reported effectiveness of oregano oil (source of carvacrol) in cases of ear infections [3]. In the study, cinnamon oil and cinnamaldehyde appeared as the most promising herbal antimicrobials inhibiting >80% of the bacteria associated with an ear infection in animals and gives a window to explore further for the use of cinnamon oil or cinnamaldehyde in development of effective antimicrobial ear drops.

Observations on MIC of different herbal compounds (Table 4) revealed that MIC of different bacteria varied to a large extent even among strains of the same species and genus. However, MIC of thyme oil, ajowan oil, carvacrol and cinnamon oil was always ≤1280µg/mL (≤0.128%). The MIC of tea tree oil varied from 0.001% to >0.256% and was one of the least efficacious oil on bacteria causing ear infections.

In earlier studies [11] too, tea tree oil could inhibit all bacteria causing ear infections at 2% level. This study indicated that instead of tea tree oil options of adding thyme oil or ajowan oil or carvacrol, an active ingredient of thyme, oregano and ajowan oil or cinnamon oil may be better to be added to ear instillation preparations and these may act even at very low concentrations similar to many antibiotics.

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