Research Article

Antibiotic sensitivity of microbial isolates and therapy of otitis externa in dog

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Abstract: This study aimed to determine the antibacterial sensitivity of various microorganisms' isolates and evaluate the efficacy of usual therapy in dogs with otitis externa. Of 976 examined dogs, 117 were infected (12%). Ear swabs were inoculated on different media for microbiological examination. The most prevalent microorganism isolated from ear swab was *Staphylococcus aureus* (35.17%) and *Pseudomonas aenaginosa* (26.7%). Based on the results, penicillin, erythromycin and gentamycin were highly effective against the isolated bacteria and mycostatin against yeast and fungi. The response to the treatment in this study suggests that early diagnosis with a specific treatment is great importance.

Keywords: Dogs, Otitis, Microbial Isolates, Antibiotic, Antifungal.

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Introduction

Otitis externa is a common problem among small animal practitioners. The term otitis, which implies inflammation of the ears, describes abnormalities ranging from simple inflammation to chronic exudative Otorrhoea of the external ear canal (Ettinger et al. 2016). Its incidence in dogs is high in some areas in Baghdad by 12% (Rana et al. 2009). This disease in dogs normally ranges from 3.1 to 9.4% (Hosmer et al. 2013; Bajwa 2019; Dan et al. 2021). Many predisposing factors are responsible for Otitis externa in dogs; these are the shape of the ear canal, retention of debris, foreign bodies, allergic response, Octoparasites, trauma and excessive moisture (David & Michael 2008; Dan et al. 2021).

The most frequent microorganisms in severe and chronic cases of otitis externa in dogs are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus* spp., and *Candida* spp. (Rana et al. 2009; Mekić et al. 2011; Bourély et al. 2019). Various antibiotics have been used for treating otitis externa in dogs, such as penicillin, streptomycin, oxytetracycline, neomycin, and chloramphenicol due to *Staphylococcal* infection (Bajwa 2016).

Recently, erythromycin, cephalosporins, and lincomycin have been recommended (Medleau & Hnilica 2008). However, Garamycin was very effective against bacterial isolates for otitis externa (Lyskova et al. 2007; Moyaert et al. 2019). Some researchers have used Nystatin to treat otitis externa in cases with fungal infections (Crespo et al. 2000; Rana et al. 2009). This study aimed to determine the antibacterial sensitivity of various microorganisms' isolates and evaluate the efficacy of usual therapy in dogs with otitis externa.

Material and Methods

Ears swabs were collected from both ears of 200 dogs of different ages and sexes. Then, they were examined and clinically diagnosed with otitis externa. The swabs were inoculated in Brain Heart Infusion (BHI) and incubated at 37°C for 24-48 hours for bacterial isolation. For yeast isolation, the same Table 1. Microorganisms isolated from otitis ears of dogs.

Microorganisms	No. of cases	%
Staphylococcus aureus	74	47.4
B-Hemolytic Streptococci	7	4.5
Ps. Aeruginosa	26	16.7
Proteus spp.	6	3.8
E. Coli	3	1.9
Candida Spp.	40	25.6
No growth	44	
Total	200	

Isolates	No.	Sensitivity to antibacterial agent %							
		AM	PN	CSI	С	E	FD	CN	Р
Staphylococcus aureus	74	55	100	100	87.8	67.6	100	79.7	100
B. hemolytic Staphylococcus	7	100	62	57	50	60.2	77.7	100	90
Pseudomonas aeruginosa	26	0	0	38.5	61.5	0	0	88.5	0
E. Coli	3	100	80	100	100	20	0	100	0
Proteus spp.	6	50	100	100	75	30	25	100	0

AM: Amoxicillin 25ug; PN: Ampicillin 10ug; CSI: Cephalordin 30ug

C: Chloramphenicol 30ug; E: Erythromycin 15ug; FD: Sodium fucidate 10ug

CN: Gentamycin 10ug; P: Penicillin 10µg

swabs were inoculated onto Sabouraud broth and incubated at 37°C for 7 days. Blood, McConkey, Mannitol, and Sabouraud agar plates were streaked from broth culture and incubated as mentioned above.

Microorganisms were isolated in pure culture and identified by conventional methods for bacteria (Barrow & Feltham 2004) and yeast (Bajwa 2019). Staphylococcus aureus was further identified as a pathogenic or nn-pathogenic strain using a tube coagulation test. An antibacterial sensitivity test was done using the agar diffusion technique (disc method, Oxoid). After incubation at 37°C for 24 h, the results were evaluated. Lack and the presence of definite and inhibition of growth zones were recorded as "resistant" and "Sensitive", respectively (Moyaert et al. 2019). Infected dogs were divided into six groups according to causative agents and were treated with a number of antibiotics and antifungal agents either locally or systematically, or both. These agents were sodium fucidate. Gentamicin, erythromycin, procaine penicillin, amoxicillin, Chloramphenicol, cephaloridine, nystatin and clotrimazole, Canesten 1% cream (Bayer). The owners were advised to bring their dogs after three months for a final examination regarding recovery or after ten days in the case of incurable ears. The prognosis was checked through several visits within the last three months after the start of treatment.

Results

Microbe identification: Table 1 summarizes the study's microbiological findings of the 100 positive cases. Among them, *S. aureus* was the most frequent pathogen, followed by *Candida* spp., *Pseudomonas aeruginosa*, and beta-Hemolytic *Streptococci* spp. *Proteus* spp. and *Escherichia coli* were isolated from 22% of infected ears.

In vitro antibiotic sensitivity test: Susceptibility of the isolated microorganisms to various antibiotics is given in Table 2, showing all *Staphylococcus* isolates are highly sensitive to most antibiotics used in this study. *Pseudomonius aruginose* showed the highest resistance to most antibiotics, and Gentamicin showed the best antibiotic action on the bacterial isolates.

Treatment and complement: The affected ears were treated according to laboratory studies (Table

Group of dogs Treatm		nent	Clinical response %				End result %			
	Microorganisms	Local	Systemic	days	Excellent	Good	Fair	Poor	Healthy	Reoccurrence
А	Staph.aureus	Fucidin	Penicillin	4	100	-	-	-	100	-
		Gentamycin	-	6	100	-	-	-	100	-
		Erythromycin	-	6	-	60	40	-	72.7	2.3
В	B-hemolytic Streptococci	Gentamycin	Amoxycillin	4	100	-	-	-	100	-
С	Pseudomonas aeruginosa		Cephalordine	6	100	-	-	-	100	-
				6	25	75	-	-	66.7	33.3
			-	6	-	75	25	-	-	100
D	E.Coli	Gentamycin	Cephalordine	4	100	-	-	-	100	-
Е	Proteus spp.	Gentamycin	Cephalordine	4	100	-	-	-	100	-
F	Candid spp.	Nystatin	Nystatin	6	-	60	20	20	80	20
		Clotrimazol		6	100	-	-	-	100	-

Table 3. Therapy results in dogs with otitis externa.

3). This included the local application on drops or ointments of sodium fusidate. gentamicin, Erythromycin, Chloramphenicol, Mycostatin three times daily and oral administration of ampicillin, cephaloridine every six hours and mycostatin daily while procaine penicillin was injected intramuscularly every 24 hours (Table 3). The results showed four clinical categories responses, including 'excellent', 'good', 'fair', and "poor." Most of the premium classes included treated dogs systematically and locally at the same time and considered healthy after three months, unlike this deal only locally which also included the most frequent cases, especially in group C (Table 3).

Discussion

Otitis externa in dogs is a mild form of the disease and might be neglected to its treatment (Bajwa 2019). The most reported microorganisms that cause otitis externa are *S. aureus*, beta-hemolytic *Streptococcus* spp., *P. aeruginosa*; *E. coli*, and yeast. (Jacobson 2002; Rana et al. 2009; Bajwa 2019; Bourély et al. 2019), that is in agreement with our findings. In this study, *S. aureus* was the most frequent isolate (47.4%), followed by *Candida* spp. (25.6%), and *P. aeruginosa* (16.7%). Nuttall & Bensignor (2014) found a nearly similar percentage of infection with yeast followed by *Pseudomonas* spp. *S. aureus* and *Proteus* spp. The frequency of *S. aureus* isolates in our study was greater than those reported from Ireland (Bourély et al. 2019) and Iraq (Rana et al. 2009). The finding of a high incidence of *P. aeruginosa* isolates from dogs with otitis externa is of special interest because otitis externa has been isolated from dogs with chronic disease.

The results also were consistent with the findings of other investigators (Ettinger et al. 2016; Moyaert et al. 2018; Catlin 1990). Boone et al. (2021) recorded that *P. aeruginosa* and *Proteus* spp. were more common in dogs' exhibiting a pale-yellow discharge, similar to the current study. In addition, *Proteus* spp. and *E. coli* were isolated from a smaller proportion of cases, accounting for 3.8 and 1.9%, respectively. Fraser et al. (1969) suggested that ear infection is caused by most strains of *Proteus* and *E. coli* found in fecal.

The results showed that yeast such as *Candida* occurred more frequently than other microorganisms. Candidiasis was associated more commonly with dogs with chronic otitis externa, previously infected with *S. aureus*. However, Boone et al. (2021) reported that yeast (*Pityrosprum canis*) is a common inhabitant of dog ears with chronic ears cases. Kasai et al. (2021) also stated that ear infections primarily caused by fungi and yeast appear to be rare and their importance in the infection is usually a result of prolonged use of antibiotics.

The results showed that *S. aureus* and *Streptococci* spp. have the major sensitive strain, especially to penicillin. Baba and Fukata (1981)

reported that all staphylococci and streptococci tested were highly susceptible to ampicillin and less susceptible to penicillin, while other studies (Blue & Wooley 1977; Bourély et al. 2019) found that *S. aureus* isolates become resistant to penicillin and others antibiotics.

Blue & Wooley (1977), Bourély et al. (2019), and Moyaert et al. (2019) showed that P. aeruginosa is resistant to many commonly used antibacterial agents. The results of the sensitivity test of bacterial isolates to gentamycin showed that gentamycin may be the antibiotic of choice for its treatment (Lyskova et al. 2007; Moyaert et al. 2019), that our results confirm this finding. Antibiotics were chosen based on the isolates' antibiotic sensitivity test results for treating infection locally and /or systematically till the recovery of the animals, as we did in our study. In the current work, four to six days of treatment was found to be efficient for recovery. Jacobson (2002) suggested continuing treatment for one to two weeks, and gentamicin has proven to be the most effective antibiotic used as drops and ointment for local treatment of otitis externa in dogs.

Also, penicillin and amoxicillin were the most effective against positive bacteria in our work, while cephaloridin is effective on gram-negative bacteria when used systemically. The recurrence of infection in some cases and fair response to treatment in others was shown in cases treated locally, especially in groups C and F in this work. This could be attributed to the prolonged treatment with a broad spectrum of antibiotics before referral to the clinic, which are compatible with other studies (Jacobson 2002; Catlin 1990; Ettinger et al. 2016).

In this study, Clotrimazole as a local cream was used for the first time to treat otitis externa in dogs. Excellent response to treatment was observed in 100% of the cases, while nystatin gave a poor response in some cases, although it was used both locally and systemically. Boone et al. (2021) and Kasai et al. (2021) pointed out that the yeast, in all instances, was sensitive to nystatin therapy. The response to the treatment in this study suggests that early diagnosis with a specific treatment is of great importance. The ideal use of an antibacterial sensitivity test must be recommended before commencing treatment of cases of ear infections in dogs.

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