Comparison of microbial isolates isolated from external ear canal of sheep and their susceptibility to antibiotics

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Summary

One hundred sheep with bilateral otitis externa were studied one year and a half .The exudates of both external ears obtained using sterile swabs, and microorganisms were isolated according to standard microbiological techniques. There was bacterial and fungal growth in many of the samples. The total numbers of isolates from right ear was 104, and the most common pathogens isolated in right ear were Staphylococcus aureus 30.76 %, then Staphylococcus epidermidis 9.61% and Pseudomonas aeruginosa 9.61% while E.coli, Proteus mirabilis and Streptococcus pyogenes were 7.69% for each of them, Candida albicans 5.76%, Klebsiella pneumonia 3.84%, Nocardia spp 3.84%, Pasteurella multucida 3.84% , Mannheimia haemolytica 3.84% , Aspergillus spp. 3.84% and Streptomyces spp. 1.23%. The total number of isolates from left ear was 96, the most pathogens were Staphylococcus aureus 27.08% while Pseudomonas aeruginosa 12.5% and Streptococcus pyogenes were 12.5%, Klebsiella pneumonia 8.33 % and Proteus mirabilis 8.33 %, Staphylococcus epidermidis, Nocardia spp 6.25 %, then Streptomyces spp, Pasteurella multucida and Mannheimia haemolytica were 4.16%, the last E.coli, Aspergillus spp and Candida albicans were 2.08 % for each of them . The result were showed significant differences at level P <0.05 between bacteria and fungi isolates, and have no significant differences at level P> 0.05 between right and left ear. High susceptible rates of gram positive Staphylococcus aureus to (Amoxicillin + Clavulaunic acid) and Chloramphenicol were 100 %, while high susceptible rates of gram negative Pseudomonas aeruginosa was 90.90 % to Ciprofloxacin, (Amoxicillin+ Clavulaunic acid) and Chloramphenicol.

مقارنة العزلات الجرثومية المعزولة من التهاب الاذن الخارجية في الاغنام وحساسيتها تجاه المضادات الحياتية

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الخلاصة

تم في هذه الدراسة عزل وتشخيص الاجناس البكترية والفطرية من الاذن الخارجية اليمنى واليسرى المصابة بالتهابات خمجية في الاغذام ومدى حساسيتها للمضادات الحياتية تم اخذ العينات من الاذن المصابة وفحصها وتشخيص انواع الجراثيم باستخدام الاختبارات الكيموحيوية ثم دراسة حساسيتها للمضادات الحياتية . اظهرت الناع عدد العزلات الجرثومية الكلي في الاذن اليمنى هو 104 عزلة واكثرها اهمية هي المكورات العنقودية الذهبية 6.70 % ثم تليها المكورات العنقودية البشروية والزوائف الزنجارية المسحيات و المسجيات والزوائف الزنجارية 1.0 % لكل منها ، تليها الاشريشيا القولونية والمتقلبات و المسجيات القيحية والزوائف الزنجارية 19.6 % لكل منها ، تليها الاشريشيا القولونية والمتقلبات و المسجيات القيحية والزوائف الزنجارية 19.6 % لكل منها ، تليها الاشريشيا القولونية والمتقلبات و المسبحيات القيحية بنسبة 7.69 % لكل منها ، تليها الاشريشيا القولونية والمتقلبات و المسبحيات القيحية والزوائف الزنجارية والمسبحيات القيحية بنسبة 12.5 % وتليها الم نسبة العزلات الجرثومية في الاذن اليسرى كان اهمها العنقوديات الذهبية بنسبة 7.69 % لكل منها ، تليها الاشريشيا القولونية والمتقلبات و المسبحيات القيحية بنسبة 7.69 % لكل منها الما نسبة العزلات الجرثومية في الاذن اليسرى كان اهمها العنقوديات الذهبية بنسبة 7.69 % وتليها الزوائف الزنجارية والمسبحيات القيحية بنسبة 12.5 % . وتبين انه توجد فروق معنوية بين العز لات بين العز لات البكتيرية والعزلات الفطرية بمستوى 20.5 P ، بينما لا توجد فروق معنوية بين العز لات الجرثومية في الاذن اليمنى والاذن اليسرى بمستوى 20.5 P ، ينما لا توجد فروق معنوية الحرثومية في الاذن المولية بين العز لات P مض الكلافيولونك الد والغان المالية بكتريا العزلات الجرثومية في الاذن اليمنى والاذن اليسرى بمستوى 10.5 P ، ينما لا توجد فروق معنوية بين العز لات الجرثومية في الاخرارية العنوديات الذهبية بين العز لات P من العزلات الحمر ومية وي الاذن اليمنى والان المسرى بمستوى 20.5 P ، ينما لازوائف مازز الإلم كاسبين العزلات العمويي والاذن اليمنى والاذن اليسرى بمستوى والكلورام الفينيكول كان بنسبة 20.6 % ما ماليولولونك اليم والفينيول كان بنسبة 100 % ما ماليور والفي الزوائف الزم والوكسلين العرم ما الكلافيولونك العمولي الولي العموي والول الغومي والالورام المويييكول كان بنسبة 20.6 % مال

Introduction

Otitis externa is similar to skin and soft tissue infection elsewhere. Two major type of external Otitis: acute and chronic; acute external otitis may be localized or diffuse .Acute localized disease occurs in the form of a pustule or furuncle and typically result from Staphylococcus aureus. Erysipelas caused by group A streptococci may involve the external ear canal and the soft tissue of the ear itself (1). The normal flora of external ear are rather sparse, similar to flora of the conjunctival sac qualitatively except pneumococci, Staphylococcus aureus and Enterobacteriacae, Pseudomonas aeruginosa and non-Candida albicans (2). Many factors can predispose sheep and goat to Otitis externa, the first is the anatomic orientation of the ear canal itself and cause an inflammation of the outer ear and ear canal (3). Otitis externa has multifactorial etiology and bacteria play an important role in otic diseases (3, 4) lesions on ear pain, balance alterations, itch, hemorrhagic lesions, ear orifice surrounding mucous discharge yellowish in color (5). These are clinical signs of significance in most cases and may indicate the presence of pathogenic infections (6, 7). Many studies have used samples collected from only one ear per animal (8), others have used samples collected from 1 or both ears and considered them as different samples (9). The purpose of this study was bacterial and fungal isolation patterns of samples collected from both ears to study the antimicrobial susceptibility pattern on the prevalent strains.

Material and Methods

Hundred samples were taken from infected ear of sheep right and left ears from the Veterinary Clinic farms around veterinary college in Baghdad, the age between 6 months -2years old, in this study both numbers of female and male were 50. Both ears swabbed by sterile cotton swabs then inoculated in nutrient broth as a primary bacterial isolation and incubated at 37 °C for 24 hours after that cultured by streaking method on MacConkey agar then incubated aerobically at 37(10)+, while blood agar under CO₂ condition for 24 hours (11). Routine bacterial culture was performed in all samples, then made the biochemical test to identified bacterial species, also the fungus were isolated by Sabaouroaud dextrose agar (12). For sensitivity test we have prepared Sterile Muller Hinton agar media then poured in sterile petri plates under aseptic condition used for susceptibility of antibiotic sensitivity disc. (13, 14) included; Ampicillin, Amikacin, Clarithromycin, Gentamycin . Amoxicillin. Ciprofloxacin, Cefotaxime, Augmantin, Trimethoprim Sulfamethoxazol +and Chloramphenicol.

The values were considered statistically significant ANOVA (Analysis of variance) for least significant differences (15).

Results

The result were showed that there was significant differences at level P <0.05 between bacterial and fungal isolates, the total number of bacterial and fungal species isolated from right external ear canal was 104 included : *Staphylococcus aureus* the most prevalent 30.76 %, followed by *Staphylococcus epidermidis* and, *Pseudomonas aeruginosa*, 9.61 % for each of them, but *E.coli*, *Proteus mirabilis*, *Streptococcus pyogenes were* 7.69 % for each of them , while *Candida albicans* 5.76 %, then *Klebsiella pneumonia*, *Nocardia spp. Pasteurella multucida*, *Mannheimia haemolytica Aspergillus spp* were 3, 84 % for each of them, the last *Streptomyces spp* was1.23 %. The total number of isolates from left ear was 96 included : *Staphylococcus aureus* 27.8 %, *Pseudomonas aeruginosa*, *Streptococcus pyogenes* 12.5 % per each ,while *Klebsiella pneumonia*, and *Proteus mirabilis* 8,33 %, then *Staphylococcus epidermidis*, *Nocardia spp* 6.25%, *Streptomyces spp*, *Pasteurella multucida* and *Mannheimia haemolytica* were 4.16%, the last *E.coli*, *Aspergillus spp* and *Candida albicans* were 2.08 % per each (table 1 ,2). There were no significant differences at P> 0.05 between right and left infected ear.

The results of antibiotic sensitivity test to bacterial isolates from otitis externa in sheep showed that *Mannheimia haemolytica* have been a significant differences at level P < 0.05

among different antibiotics, the highest were Amikacin, Gentamycin, ciprofloxacin, Augmantin and Chloramphenicol have 100% and the lowest was Ampicillin (table -8).

Bacterial isolation from right	Number of	Percentage %
0		rercentage 70
ear	isolates from right	
	ear	
Staphylococcus aureus	32	30.76
Staphylococcus epidermidis	10	9.61
Pseudomonas aeruginosa	10	9.61
Escherichia coli	8	7.69
Proteus mirabilis	8	7.69
Streptococcus pyogenes	8	7.69
Candida albicans	6	5.76
Klebsiella pneumonia	4	3.84
Nocardia spp.	4	3.84
Pasteurella multucida	4	3.84
Mannheimia haemolytica	4	3.84
Aspergillus spp.	4	3.84
Streptomyces spp.	2	1.23
	104 total	

 Table 1: Numbers of bacteria and fungus species isolates from infected right

 ear in sheep

 Table 2: Numbers of bacteria and fungus species isolates from infected

 Left ear in sheep

Bacterial isolation from left ear	Number of	Percentage %
infected	isolates from left	-
	ear	
Staphylococcus aureus	26	27.08
Pseudomonas aeruginosa	12	12.5
Streptococcus pyogenes	12	12.5
Klebsiella pneumonia	8	8.33
Proteus mirabilis	8	8.33
Staphylococcus epidermidis	6	6.25
Nocardia spp	6	6.25
Streptomyces spp.	4	4.16
Pasteurella multucida	4	4.16
Mannheimia haemolytica	4	4.16
Escherichia coli	2	2.08
Aspergillus spp.	2	2.08
Candida albicans	2	2.08
	96	
	total	

The high percentage of gram positive bacterial isolates from right ear was *Staphylococcus aureus* 61.5 % (table-3).

Gram- Positive Bacteria	Number of isolates from right ear	Percentage %
Staphylococcus aureus	32	61.5
Staphylococcus epidermidis	10	19.2
Streptococcus pyogenes	8	15.38
Streptomyces spp.	2	3.84
	52 total	

Table 3: Numbers of gram positive bacteria from infected right ear in sheep

Table 4: Numbers of gram positive bacteria from infected left ear in sheep

Gram- Positive Bacteria	Number of isolates from left ear	Percentage %
Staphylococcus aureus	26	54.16
Streptococcus pyogenes	12	25
Staphylococcus epidermidis	6	12.5
Streptomyces spp.	4	8.33
	48 total	

The most gram negative bacteria isolated from right ear was *Pseudomonas aeruginosa* 26.315 % (table -5), while in the left ear was 26.315 % (table - 6).

Table 5: Numbers of gram negative bacteria from infected right ear in sheep

Gram- negative bacteria	Number of isolates from right ear	Percentage %
Pseudomonas aeruginosa	10	26.3
Escherichia coli	8	21.05
Proteus mirabilis	8	21.05
Klebsiella pneumonia	4	10.5
Pasteurella multucida	4	10.5
Mannheimia haemolytica	4	10.5
	38 total	

Gram- negative bacteria	Number of isolates from left ear	Percentage %
Pseudomonas aeruginosa	12	31.5
Klebsiella pneumonia	8	21.05
Proteus mirabilis	8	21.05
Pasteurella multucida	4	10.5
Mannheimia haemolytica	4	10.5
Escherichia coli	2	5.26
	38 total	

Table 6: Numbers of gram negative bacteria from infected left ear in sheep

Table:7 Biochemical tests were include: Biochemical test for bacterial species isolates from otitis externa in sheep

Bacterial species	SIM	catalase	oxidase	Coagulase	pigments	MR	VP	SC	Urea hydrolysis	TSI
Staphylococcus aureus		+	_	+	Yellow		+	/	+	/
Staphylococcus epidermidis		+	_	_	White		+	/	+	/
Pseudomonas aeruginosa	••+	+	+	/	Green			+	+	K/K
Escherichia coli	• + •	+	-	/	/	+	-	_	-	Y / Y gas+
Proteus mirabilis	+ • +	+	_	/	/	+	-	+	+	K / A H2S+
Streptococcus pyogenes		-	-	/	/	/	/	/	/	/
Klebsiella pneumonia		+	_	/	mucoid	-	+	+	+	A / A gas +
Pasteurella multucida	/+/	+	+	/	/				-	/
Mannheimia haemolytica	/-/	+	+	/	/				-	/
Streptomyces spp.		-		/	branching Arial filament	/	/		±	/

SIM= sulfur Indole Motility, MR=methyl red, VP = vogues proskaour, SC=Simmoncitrate, TSI= Triple sugar iron

				extern	externa in sheep	9				
					Bact	Bacterial species	ies			
Antibiotics	S.aur	S.epider	P.aerugi	E.coli	P.mira	<u>s</u> .	K.pneum	P.multu	M.haemoly	Streptom
3H/	eus	midis	1050	10	bilis	pyogenes	onia	cida	ticus	yces spp.
	58	16	22	isolates	16	20	12	*0	8 isolates	9
	isolates	isolates	isolates		isolates	isolates	isolates	isolates		isolates
	Ν.	N	N.	N.	N.	N.	N.	N.	N.	N.
	9/0	9/6	9,6	9/6	9/6	9/6	9/0	9/6	9/0	9/0
Ampicillin 10	9	10	3	8	14	14	2	5	4 50	4
	27.58	62.5	13.6	80	87.5	70	16.6	62.5		66.6
Amiltacin30	99	13	18	s	14	18	s	8	8 100	5
	86.2	81.2	81.8	80	87.5	90	66.6	100		83.3
Clarithromy	99	16	6	8	12	15	6	7	5	5
cin 15	86.2	100	40.90	S 0	75	75	75	87.5	62.5	83.3
Gentamycin	19	14	18	6	15	†I	8	8	8 100	Ŧ
10	93.1	87.5	81.8	90	93.7	70	66.6	100		66.6
Amoxicillin	91	10	8	7	12	91	†	9	9	9
25	27.58	62.5	36.36	70	75	80	33.3	62.5	62.5	83.3
Ciprofloxaci	54	14	20	10	16	20	ø	8	8 100	9
n.5	93.1	87.5	90.90	100	100	100	66.6	100		100
Cefotaxime	24	14	18	10	15	20	11	7	4	9
30	41.37	87.5	81.8	100	93.7	100	91.6	87.5	87.5	83.3
Augmantin	89	16	20	6	16	20	11	7	8 100	9
30	100	100	6.09	06	100	100	91.6	87.5		100
Trimethopri	57	16	4	10	15	18	11	~	7	9
+ H	98.2	100	18.18	00	93.7	06	91.6	100	87.5	\$3.3
Sulfamethoxazol 25										
Chloramphe	8 8	13	20.	10	15	20	12	4	8 100	8
nicol 30	100	81.5	06'06	100	93.7	100	100	87.5		83.3

antihintic sensitivity from ofitis agente nercentages of hacterial isolation affected and the Table :8 Numbers

Discussion

The purpose of this study to determine the isolation and identification of bacterial and fungal species and antibacterial susceptibility test then compare this isolation pattern from both ears in bilateral otitis externa in sheep. Ear infection in calves and lambs has been associated with concurrent respiratory diseases, also the otitis in goat and sheep compared with the information on cattle and horses (16). the skin lining of the external ear canal has a large numbers of gland, these include modified porcine gland which produce large amounts of secretions which provide good suitable media for irritation and infection (1). The result appears that the total numbers in right infected ears were 104 isolates, while in left ears were 96 isolates, the result have no significant differences at P>0.05 between right and left infected ears. Many microbial infections commonly inhibit the ear canal and can become secondary opportunistic invaders when conditions are favorable (5). And it probably ascended from the pharynx through the auditive tube into the tympanic cavities (17). The most importance gram positive pathogenic bacteria was Staphylococcus aureus that isolated from both ears, while the most one of gram negative bacteria was pseudomonas aeruginosa (5). As expected, low level of sensitive to Ampicillin 27.58 % was observed because of the ß- lactamase enzyme produced by Staphylococcus aureus similar to that described in the literature (18, 19, and 20). Pseudomonas aeruginosa is this study sensitive to Ciprofloxacin, Augmantin and Chloramphenicol 90.90 %. Also polymyxin B ticarcillin ,or enrofloxacin , polymyxin B is inactivated by purulent debris and must be applied only in clean ears (21), either acetic acid 2% is effective against Pseudomonas after 1 minute of contact time, higher concentrations of acetic acid may be irritating ,aluminum acetate is also effective against Pseudomonas when used topically(22). Ear medications are applied after cleaning and drying ,and is usually in form of ointment applied daily or twice daily for one or two weeks and the type of infection must be identified by the veterinarian by examining a sample of ear exudates under a microscope, since bacterial infection will only respond to antibiotics, fungal infection to a fungicide (23).

Management of chronic recurrent otitis externa requires diagnosis and control of predisposing factors with oral and topical antimicrobials.

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