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Research Articles

Antibiogram of bacterial isolates associated with respiratory infection in dogs

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Abstract

The study aimed to determine the susceptibility of clinical isolates of bacteria obtained from respiratory tract infection in dogs towards various antimicrobials. Susceptibility of each isolated bacteria to a panel of 21 antibiotics (Enrofloxacin, Levofloxacin, Ceftriaxone, Oxytetracycline, Ofloxacin, Gentamicin, Neomycin, Cefpodoxime, Ceftriaxone + Tazobactum, Moxifloxacin, Amikacin, Cefoperazone, Ceftriaxone + Sulbactam, Amoxicillin + Clavulanic acid , Ampicillin, Amoxicillin-30, Cefotaxime, Kanamycin, Amoxicillin-10, Cefalexin, Chloramphenicol) was assessed. The agar disc diffusion method was used to test antimicrobial susceptibility. The sensitivity of 52 bacteria isolated from 36 nasal swab samples of dogs having respiratory tract infection was determined. Gentamicin showed the best susceptibility pattern; 94.2% of isolates were susceptible to this antibiotic followed by Chloramphenicol (69.2%), Enrofloxacin (55.7%), Levofloxacin (53.8%) and ampicillin showed the least susceptibility pattern (1.9%). Out of 52 isolates, 51 isolates were found to be multidrug- resistant (resistant to three or more than three antibiotics belonging to different antibiotic groups. Among these multidrug-resistant bacteria, five isolates were found to be extreme drug resistant (sensitive to two or less than two antibiotics belonging to different groups).

Keywords: Respiratory tract infection, Antimicrobial susceptibility, Bacteria isolated

It is widely accepted today that the intensive or inappropriate use of antimicrobial agents, such as antibiotics, in veterinary medicine may lead to an increased risk of bacterial resistance which may ultimately have a potential impact upon human health (CVMP, 1999). Maintaining the efficacy of antimicrobials has become a global public health concern and there are regular calls for recommendations for the prudent use of antibiotics in animal health (CVMP, 1999). Antibiotic therapies for veterinary diseases need to retain their efficacy in eliminating pathogens from an infected animal but should also prevent the spread of these organisms to other animals and of resistance and eliminate the risk of transmission to humans. Guidelines for the responsible use of antimicrobials have been developed by international organizations and veterinary associations (Battersby, 2011). Regular reporting of bacterial sensitivity would facilitate a better understanding of antimicrobial resistance and trends in this process over time to ensure long-term efficacy of the antibacterial products (EMA, 2011).

This article gives information about the antibiotic susceptibility of common bacteria isolated from respiratory tract infection in dogs. The isolated organisms for which antibiotic sensitivity was determined include *Streptococcus* spp., *Staphylococcus* spp., *Salmonella* spp., *E. coli*, *Klebsiella* spp., *Bacillus* spp., *Pseudomonas* spp. and *Actinomyces* spp.

Materials and Methods

Isolates obtained from nasal swabs of dogs suffering from respiratory ailments were processed for antimicrobial sensitivity testing. The agar disc diffusion method was used to determine the antimicrobial susceptibility pattern. With the help of a platinum loop, a small amount of test culture was transferred into a tube of brain heart infusion broth and incubated for 2-5 h at 37°C, to obtain turbidity. With the help of a sterile cotton swab, the broth culture was then evenly spread by smearing over the surface of BA/Mueller-Hinton agar plates. The antimicrobial discs were placed on the agar and gently pressed. These were then incubated at 37°C for 24 h. Twenty one antibiotics that are commonly used in small animal practice including Amikacin, Amoxicillin-10, Amoxicillin-30, Amoxicillin + Clavulanic acid, Ampicillin, Ceftriaxone + sulbactam, Cefalexin, Chloramphenicol, Cefpodoxime, Cefoperazone, Cefotaxime, Ceftriaxone, Enrofloxacin, Gentamicin, Kanamycin, Levofloxacin, Moxifloxacin, Neomycin, Oxytetracycline, Ofloxacin, Ceftriaxone + Tazobactum were selected and the antibiotic susceptibility of the most frequently isolated bacteria

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was evaluated for these antimicrobials. The sensitivity was observed on the basis of zone size interpretation chart, provided by the manufacturer. According to the diameter of the inhibitory zones, bacteria were classified as 'susceptible',' intermediate' or 'resistant' to a certain antibiotic. For interpretation of antibiotic susceptibility, intermediate isolates were considered resistant.

Results and Discussion

Antibiogram pattern in any area is reflected by common antimicrobials used in that area. More and indiscriminate use of antibiotics results in their resistance after a certain period of time as mentioned by Guardabassi *et al.,* (2004). Overall sensitivity irrespective of isolates revealed maximum sensitivity towards Gentamicin (94.2%) followed by Chloramphenicol (69.2%), Enrofloxacin (55.7%), Levofloxacin (53.8%), Oxytetracycline (44.2%), Ofloxacin (44.2%), Cefpodoxime (44.2%), Ceftriaxone + Tazobactum (40.3%), Moxifloxacin (40.3%), Amikacin (36.5%), Ceftriaxone + Sulbactam (34.6%), Cefoperazone (32.6%), Ceftriaxone (30.7%), Neomycin (28.8%), Amoxicillin + Clavulanic acid (28.8%), Amoxicillin-30 (25%), Cefotaxime (23%), Kanamycin (23%), Amoxicillin-10 (15.3%), Cefalexin (9.6%) and least sensitivity was observed for Ampicillin (1.9%). Our findings of Gentamicin to be highly sensitive are in agreement with Johnson *et al*. (2013). In the present study least sensitivity was observed for Ampicillin (1.9%) which is similar to Epstein *et al*., 2010 and Rheinwald *et al*., 2015.

Antibiotic susceptibility pattern of *Streptococcus* spp. isolates showed towards maximum sensitivity towards Gentamicin (87.5%) followed by Chloramphenicol & Moxifloxacin (75%), levofloxacin (68.7%) and least towards antibiotic belonging to penicillin groups. Similar to our results higher sensitivity of *Streptococcus* spp. towards Chloramphenicol was observed by Schwarz *et al.* (2007) and Morrissey *et al.* (2016). On contrary Epstein *et al.* (2010) found Cefotaxime and Amoxicillin + Clavulanic acid combination to be most effective against *Streptococcus* spp. isolates obtained from respiratory tract infection

Table I. Antimicrobial sensitivity pattern of different bacterial isolates from nasal swabs of dogs affected with respiratory tract infection

Sr. No.	Antimicrobials used		Sensitivity (%)
1	Tetracyclines	Oxytetracycline	23 (44.2%)
2	Penicillins	Ampicillin	1 (1.9%)
3		Amoxicillin/10	8 (15.3%)
4		Amoxicillin/30	13 (25%)
5		Amoxicillin + clavulanic acid	15 (28.8%)
6	Fluoroquinolones	Enrofloxacin	29 (55.7%)
7		Ofloxacin	23 (44.2%)
8		Moxifloxacin	21(40.3%)
9		Levofloxacin	28 (53.8%)
10	Aminoglycosides	Gentamicin	49 (94.2%)
11		Amikacin	19 (36.5%)
12		Neomycin	15 (28.8%)
13		Kanamycin	12 (23%)
14	Cephalosporins	Ceftriaxone	16 (30.7%)
15		Cefotaxime	12 (23%)
16		Cefpodoxime	23 (44.2%)
17		Cefalexin	5 (9.6%)
18		Ceftriaxone + sulbactum	18 (34.6%)
19		Cefoperazone	17 (32.6%)
20		Ceftriaxone + tazobactam	21 (40.3%)
21	Chloramphenicol	Chloramphenicol	36 (69.2%)

Table II. Antimicrobial susceptibility of each bacterial isolate from respiratory tract infection for 21 different antibiotics

Sr. No.	Antimicrobials used		Sensitivity (%)							
			Streptococcus spp. (n=16)	*Staphylococcus* spp. (n=15)	*E.coli* (n=6)	*Salmonella* spp. (n=6)	*Klebsiella* spp (n=3).	*Bacillus* spp. (n=3)	*Pseudomonas* spp. (n=2).	*Actinomyces* spp. (n=1)
1	Tetracyclines	Oxytetracycline	6 (37.5%)	9 (60%)	0%	3 (50%)	2 (66.6%)	2 (66..6%)	0%	1 (100%)
2	Penicillins	Ampicillin	0%	1 (6%)	0%	0%	0%	0%	0%	0%
3		Amoxicillin/10	0%	8 (53.3%)	0%	0%	0%	0%	0%	0%
4		Amoxicillin/30	0%	9(60%)	1 (16.6%)	1 (16.6%)	0%	0 %	1 (50%)	1 (100%)
5		Amoxicillin/ clavulanic acid	4 (25%)	6 (40%)	1 (16.6%)	1 (16.6%)	2 (66.6%)	0%	0%	1 (100%)
6	Fluoroquinolones	Enrofloxacin	10 (62.5%)	7 (46.6%)	3 (50%)	4 (66.6%)	2 (66.6%)	2 (66.6%)	1 (50)%	0%
7		Ofloxacin	7 (43.5%)	8 (53.3%)	1 (16.6%)	3 (50%)	1 (33.3%)	1 (33.3%)	1 (50%)	1 (100%)
8		Moxifloxacin	12 (75%)	8 (53.3%)	0%	0%	0%	1 (33.3%)	0%	0%
9		Levofloxacin	11 (68.7%)	9 (60%)	2 (33.3%)	3 (50%)	1 (33.3%)	1 (33.3%)	1 (50%)	0%
10	Aminoglycosides	Gentamicin	14 (87.5%)	14 (93.3%)	6 (100%)	6 (100%)	3 (100%)	3(100%)	2 (100%)	1 (100%)
11		Amikacin	4 (25%)	9 (60%)	0%	1 (16.6%)	1 (33.3%)	2 (66.6%)	1 (50%)	1 (100%)
12		Neomycin	3 (18.7%)	8 (53.3%)	1 (16.6%)	0%	0%	1 (33.3%)	1 (50%)	1 (100%)
13		Kanamycin	0%	6 (40%)	2 (33.3%)	2 (33.3%)	1 (33.3%)	1 (33.3%)	0%	0%
14	Cephalosporins	Ceftriaxone	7 (43.7%)	2 (13.3%)	1 (16.6%)	3 (50%)	1 (33.3%)	(0%)	1 (50%)	1 (100%)
15		Cefotaxime	3 (18.7%)	5 (33.3%)	0%	1 (16.6%)	1 (33.3%)	1(33.33%)	0%	1 (100%)
16		Cefpodoxime	7 (43.7%)	8 (53.3%)	1 (16.6%)	3 (50%)	1 (33.3%)	2 (66.6%)	0%	1 (100%)
17		Cefalexin	1 (6.2%)	3 (20%)	0%	0%	0%	0%	0%	1 (100%)
18		Ceftriaxone/ sulbactum	4 (25%)	4 (26.6%)	2 (33.3%)	2 (33.3%)	1 (33.3%)	1 (33.3)%	1 (50%)	0%
19		Cefoperazone	4 (25%)	6 (40%)	0%	3 (50%)	2 (66.6%)	0%	1 (50%)	1 (100%)
20		Ceftriaxone/ tazobactum	9 (56%)	5 (33.3%)	2 (33.3%)	3 (50%)	1 (33.3%)	0%	1 (50%)	0%
21	Chloramphenicol	Chloramphenicol	12 (75%)	9 (60%)	5 (83.3%)	5 (83.3%)	2 (66.6%)	1 (33.3%)	1 (50%)	1 (100%)

Table III. In vitro antibiotic sensitivity pattern of different bacterial isolates (1-26 isolates)

Bacterial isolates			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Antibiotics (1-21)	A	1						R		R			R					R	R	R			R		R	R	R	R
within antibiotic	B	2	R	R	R	R	R	R	R	R		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
groups		3				R	R	R		R			R	R	R			R	R	R	R	R		R	R	R	R	R
(A-G)		4					R	R		R				R	R	R	R	R	R	R	R	R		R	R	R	R	R
	C	5						R	R	R						R	R	R		R	R	R		R	R	R	R	R
	D	6	R					R		R			R						R	R			R	R	R	R	R	R
		7						R		R			R				R	R	R	R	R	R	R	R		R	R	R
		8	R		R	R		R		R		R	R				R	R	R	R	R	R			R		R	R
		9				R		R		R			R				R	R	R	R	R	R					R	R
	E	10																		R				R				
		11					R	R		R		R	R	R			R	R	R	R		R		R	R	R	R	R
		12						R	R			R	R	R	R		R	R	R	R	R	R		R	R	R	R	R
		13		R		R	R	R		R		R	R		R		R	R		R	R	R	R	R	R	R	R	R
	F	14				R	R					R	R						R	R	R		R		R	R		R
		15	R	R				R		R			R		R				R	R	R	R	R	R	R	R	R	R
		16			R			R	R	R			R				R	R	R		R	R		R		R	R	R
		17			R			R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
		18	R	R	R			R		R			R			R	R	R		R		R		R	R	R	R	
		19		R	R			R		R							R	R	R	R	R		R	R	R	R	R	R
		20		R	R	R		R	R	R			R	R	R	R	R		R		R			R		R	R	R
	G	21						R		R									R						R	R	R	R

Table IV. In vitro antibiotic sensitivity pattern of different bacterial isolates (27-52 isolates)

Bacterial isolates			27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
Antibiotics (1-21) within antibiotic groups	A	1	R		R	R	R	R	R	R	R			R	R	R	R	R	R			R	R			R	R	R
	B	2	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
		3	R	R	R	R	R	R	R	R	R	R	R		R	R	R	R	R	R	R	R	R	R	R	R	R	R
(A-G)		4	R	R	R	R	R	R	R	R	R	R	R		R	R	R	R	R		R	R	R	R	R	R	R	R
	C	5	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
	D	6	R		R	R	R				R	R					R	R	R			R	R			R	R	R
		7	R		R	R	R	R		R	R	R	R	R	R	R		R	R		R	R	R					R
		8	R		R		R			R	R	R		R		R		R			R	R	R			R	R	R
		9	R		R	R	R	R		R	R	R			R	R		R			R	R	R					R
	E	10				R																						
		11	R		R	R	R		R	R	R	R		R		R	R				R	R		R	R	R	R	R
		12	R		R	R	R	R	R	R	R	R		R		R	R				R	R	R	R	R	R	R	R
		13	R	R	R	R	R	R	R		R	R			R	R	R	R	R		R	R	R	R	R	R	R	R
	F	14	R	R	R	R	R	R			R		R	R	R				R			R	R			R	R	R
		15	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R		R	R			R	R	R
		16	R	R	R	R	R			R	R	R				R	R	R			R	R	R			R	R	R
		17	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R		R	R	R	R	R	R	R	R
		18	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R		R	R	R	R	R				R	R
		19	R	R	R	R	R	R	R	R	R		R	R	R	R	R		R		R	R	R			R	R	R
		20		R			R	R		R	R	R	R	R	R	R		R	R					R				R
	G	21										R	R		R			R	R								R	R

A. Tetracyclines (1-Oxytetracycline)
B. Penicillin (2-Ampicillin; 3-Amoxycillin(10); 4-Amoxycillin(30))
C. Penicillin/β lactamase inhibitor combination (5-Amoxycillin/clavulanic acid)
D. Fluoroquinolones (6-Enrofloxacin; 7-Ofloxacin; 8-Moxifloxacin; 9-Levofloxacin)
E. Aminoglycosides (10-Gentamicin; 11-Amikacin; 12-Neomycin; 13-Kanamycin)
F. Cephalosporins (14-Ceftriaxone; 15-Cefotaxime; 16-Cefpodoxime; 17-Cefalexin; 18-Ceftriaxone/sulbactum; 19-Cefoperazone; 20- Ceftriaxone/tazobactum)
F. Chloramphenicol (21-chloramphenicol)

in dogs. Comparing the sensitivity of enrofloxacin and tetracycline present study revealed 37.5% isolates revealed sensitivity towards tetracycline and 62.5% towards enrofloxacin while Moyaert *et al*. (2019) observed 51.4% isolates to be sensitive towards tetracycline and none of the *streptococci* were observed resistant to enrofloxacin but 31.4% were found to be intermediate sensitive.

In case of Staphylococcal isolates, maximum sensitivity was shown towards Gentamicin (93.3%) and least towards Ampicillin (6%). The lower sensitivity of staphylococcal isolates towards enrofloxacin was recorded as compared to Morrissey *et al.* (2016). In contrary higher sensitivity of staphylococcal isolates was shown towards amoxiclav with 42.8% sensitivity as compared to Morrissey *et al.* (2016) who reported complete resistant of staphylococcal towards amoxiclav.

In the present study, 33.3% isolates of *E.coli* were found susceptible towards enrofloxacin while Morrissey *et al.* (2016) found a higher percentage (83.3%) of isolates susceptible *towards* enrofloxacin. For *Salmonella* spp. isolates maximum sensitivity was observed for Gentamicin (100%) and Chloramphenicol (83.3%) which corresponds with the results of Dutta *et al*. (2008) and Van Duijkeren *et al.* (2002). *Klebsiella* spp. isolates showed 100% sensitivity for gentamicin and the same results were observed by Johnson *et al*., 2013. In the present study, *Pseudomonas* spp. isolates revealed maximum sensitivity for gentamicin while least sensitivity for ampicillin and these findings are well supported by Rheinwald *et al.,* 2015.

Based on the sensitivity pattern, isolates were categorized into MDR, extreme drug- resistant (XDR), and pan drug-resistant. Isolates resistant to three or more antibiotics belonging to different groups were classified as MDR. Among MDR isolates, isolates susceptible to only two antibiotics belonging to two different groups were considered XDR, while resistance to all the antibiotics was considered as pan drug-resistant. In the present study, 51 isolates were found to be multi drug- resistant (resistant to three or more than three antibiotics belonging to different antibiotic groups. Among these multi drug-resistant bacteria, five isolates were found to be extreme drug resistant (sensitive to two or less than two antibiotics belonging to different groups). Multiple drug resistance in pathogens isolated from pets especially dogs may pose public health concern as dogs remain in close vicinity of humans (Guardabassi *et al.,* 2004). Higher prevalence of multi drug-resistant isolates in companion animals draws attention to different mechanisms of attaining resistance. Mechanism of resistance associated in the present study could be explained as acquired because of multiple factors that can be studied in detail in future.

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