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IN VITRO SENSITIVITY AGAINST BACTERIAL PATHOGENS ISOLATED FROM PNEUMONIC LUNGS OF CAMELS IN JORDAN

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ABSTRACT

Antimicrobial resistance to 15 antimicrobials were determined *in vitro* for 20 *Escherichia coli*, 11 *Klebsiella* spp., 11 *Staphylococcus* spp., 9 *Pseudomonas aeruginosa*, 5 *Arcanobacterium pyogenes*, 5 *Mannheimia haemolytica* and 4 *Streptococcus* spp., incriminated as the causative agents of pneumonia of camels in Jordan. Susceptibility was determined qualitatively by the agar diffusion method. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) values of 5 antimicrobials were determined by the microdilution method. The majority of the isolates were most susceptible to ciprofloxacin and enrofloxacin. Only 40 and 60% of the *A. pyogenes* isolates were sensitive to ciprofloxacin and enrofloxacin, respectively. In addition 18% of *Klebsiella* spp. were resistant to enrofloxacin. Ciprofloxacin minimum inhibitory concentration (MIC_{50}) and minimum bactericidal concentration (MBC) were 0.125 and 0.25 µg/ml and 0.5 and 1 µg/ml for *P. aeruginosa* and *S. aureus*, respectively. With exception of *M. haemolytica* and *A. pyogenes* more than 82% of the isolates were found to be sensitive to gentamicin with MIC_{50} and MBC of 0.25 and 0.5 µg/ml, respectively for *S. aureus* and 1 and 2 µg/ml, respectively for *E. coli* and *Klebsiella* spp. Flumequine was highly effective against *M. haemolytica* isolates, whereas, 15% of *E. coli* isolates were resistant. S*treptococcus* isolates were 100% sensitive to doxycycline, whereas other isolates displayed resistance with 20% and 67% for *M. haemolytica* and *P. aeruginosa*, respectively. The bacterial isolates showed variable resistance ranged between 9 to 100% to penicillin, ampicillin, amoxicillin, tetracycline, lincomycin, erythromycin, colistin, co-trimoxasole, streptomycin and neomycin. Multiple resistance of 4 and up to 11 different antimicrobials were displayed for *E. coli*, *P. aeruginosa*, and *Klebsiella* spp. and the most common resistance pattern was penicillin, ampicillin, amoxicillin, tetracycline, doxycycline, linocomycin, and erythromycin.

Ciprofloxacin, enrofloxacin and gentamicin appear to have a great potential to control bacterial respiratory infections in camels, with the appropriate dosage that based on pharmacokinetic/pharmacodynamic studies.

Key words: Antibiotics, minimum inhibitory concentrations, pneumonia, sensitivity

Pneumonia is a major disease of domestic animals. Outbreaks occur in camel as well as other animals worldwide (Selman and Wiseman, 1983, Al-Doughaym *et al*, 1999). Pneumonia may be caused by bacteria, viruses, parasites and fungi (Schwartz and Dioli, 1992; Al-Ani, 2004). The economic losses due to pneumonia in camels are represented by loss of weight, losses due to condemnations during meat inspection and mortality rate (Mahmoud *et al*, 1988; Al-Ani, 1990; Mohamed *et al*, 1990). In Jordan, the pneumonia prevalence was around 10% among camel lungs examined in two studies (Al-Rawashdeh *et al,* 2000; Al-Tarazi, 2001). *Escherichia coli, Staphylococcus* spp., *Klebsiella* spp., *Pseudomonas aeruginosa*, *Mannheimia haemolytica*, *Actinomyces pyogenes*, and *Streptococcus* spp. are among the most prevalent causes of camel pneumonia (El-Magawry *et al*, 1986; Mahmoud *et al*, 1988, Al-Doughaym *et al,* 1999; Al-Rawashdeh *et al,* 2000; Al-Tarazi, 2001).

Treatment of pneumonia often requires antimicrobial therapy. The decision of antimicrobial therapy depends on the sensitivity of the targeted microorganism (Minimum Inhibitory Concentrations, MIC) and the pharmacokinetics of the drug to achieve the desired therapeutic concentration at the site of infection and thus clinical efficacy (Mckellar *et al,* 2004). Although antimicrobial agents including penicillins, tetracyclines, aminoglycosides and recently fluoroquinolones are frequently used for treatment of pneumonia in camels, there is paucity of information on the susceptibility of respiratory tract bacterial pathogens in camels to these drugs. To best

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of our knowledge, only Al-Doughaym *et al* (1999) was reported the MICs of antimicrobial agents for bacterial pathogens isolated from camel lungs. Therefore, this study was aimed to investigate the *in vitro* activity; qualitatively by the agar diffusion method, quantitatively by MICs and the multiple resistance patterns of the most widely used antibacterial agents against the bacterial pathogens incriminated as a causative agents of camel pneumonia in Jordan.

Materials and Methods

Bacterial isolates

A total of 65 bacterial isolates accumulated at the Research Microbiology Laboratory, Faculty of Veterinary Medicine at Jordan University of Science and Technology from affected lungs of camel in Jordan during the period July 2000 to February 2001. The isolates comprised 20 *E. coli*, 11 *Klebsiella* spp., 11 *Staphylococcus* spp. with 8 identifying *S. aureus*, 9 *P. aeruginosa*, 5 *A. pyogenes*, 5 *M. haemolytica*, and 4 hemolytic *Streptococci* were tested for their susceptibility to 15 antimicrobial agents. Three reference strains from the American Type Culture Collection (ATCC) were also used in order to validate the sensitivity method; *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) and *P. aeruginosa* (ATCC 27853). All isolates were stored at – 20° C until tested.

Escherichia coli, *Klebsiella* spp., *Staphylococcus* spp. and *P. aeruginosa* were subcultured on Mueller-Hinton agar plates (Fluka Chemie Gmbh, Taufkirchen, Germany) and were incubated aerobically for 24 hours at 37° C. Haemolytic *Streptococci*, *M. haemolytica* and *A. pyogenes* were subcultured in Mueller-Hinton agar plates supplemented with 5% (v/v) sterile defibrinated horse blood. The first two bacterial species. were incubated aerobically for 24-48 hours at 37° C while the third spp. was incubated in atmosphere containing 5% CO_2 at 37° C for 48 hours.

After incubation, bacterial suspensions of each isolate were washed three times and prepared in 0.9% NaCl solution until the turbidity matched 0.5 McFarland turbidity standard (i.e., ~ 10^8 cfu/ml). These were diluted to give 10^6 cfu/ml and was used in the agar diffusion method. For the microbroth dilution method, the inoculum size was prepared by inoculating Mueller-Hinton broth (Difco, Detroit, MI, USA) with the tested bacteria and adjusted to a concentration of 10^7 cfu/ml. For testing *P. aeruginosa* against gentamicin, streptomycin, neomycin and tetracycline, Ca^{++} 25 mg/litre and Mg^{++} 12.5 mg/litre were added to Mueller-Hinton broth before use. The inoculums of Haemolytic Streptococci, *A. pyogenes* and *M. haemolytica* were prepared by collecting these bacteria from Mueller-Hinton agar plates supplemented with 5% (v/v) sterile defibrinated horse blood and dissolved in Mueller-Hinton broth.

Antimicrobial agents

Antimicrobial discs to penicillin (10 U), ampicillin (10 mcg), amoxicillin (25 mcg), tetracycline (30 mcg), doxycycline (30 mcg), streptomycin (10 mcg), gentamicin (10 mcg), neomycin (30 mcg), enrofloxacin (5 mcg), ciprofloxacin (5 mcg), flumequine (30 mcg), co-trimoxasole (25 mcg), erythromycin (15 mcg), lincomycin (2 mcg) and colistin sulfate (10 mcg) {Arab Company for Medical Diagnostics (Arcomex), Amman, Jordan} were used for agar diffusion test.

A standard antibiotic powder of potency 99%, 100%, 100%, 99.1%, and 92.1% for ciprofloxacin, enrofloxacin, gentamicin, flumequine, and doxycycline respectively, were used in microbroth dilution test {Arcomex and Veterinary and Agricultural Products Manufacturing Co. Ltd, (VAPCO) Amman, Jordan}. Ciprofloxacin, enrofloxacin, gentamicin and doxycycline powder were dissolved and diluted in sterile distilled water, whereas flumequine was dissolved and diluted in phosphate buffer pH 6.0, 0.1 M with gentle heating. All antibiotics were adjusted to 100% potency, and then adjusted to double times of the final concentration required. The microbroth dilution method were carried in standard 96-well flat bottom microtitre plates (Sigma Chemicals, Poole, UK) covering the range from 0.0625 to 512 µg/ml. Control wells without any antimicrobials were also included.

Susceptibility testing procedure

A. Agar diffusion method

Susceptibility was determined qualitatively by the agar diffusion method using antimicrobial discs according to the recommendations of the National Committee for Clinical Laboratory Standards (2002) to the selected antimicrobial agents. Plates containing Mueller-Hinton agar medium were used, each plate was inoculated with 100 µl broth culture by spreading method using glass rod. Plates was left to dry (15 minutes) and antibiotic discs were placed on the surface and then incubated at 37°C/24hrs. The diameters of inhibition zones around each disk were measured to the nearest whole millimetre using a ruler. The results were interpreted on the basis of guidelines of the manufacturer and the bacteria were accordingly

reported as susceptible, intermediate or resistant to the antimicrobial agent tested.

B. Microbroth dilution method

Ciprofloxacin, enrofloxacin, gentamicin, flumequine and doxycyclin were also examined quantitatively by the determination of MIC and MBC for *E. coli*, *Klebsiella* spp., *S. aureus* and *P. aeruginosa* isolates using a two-fold microbroth dilution method with Mueller-Hinton broth medium and a final inoculum size equal to $5X10^5$ cfu/ml (National Committee for Clinical Laboratory Standards 2002). Briefly 100 µl of each prepared antimicrobial dilution was added to the wells of microtitre plates. Each column contained the same concentration of antimicrobial and the 11^{th} wells in each row contained only the tested antimicrobial. The 12^{th} well was served as control well containing only the broth of the tested microorganism. Finally, 100 µl broth culture of each tested microorganism was added, giving a final inoculum of ~ $5 X 10^5$ cfu/ml. The plates were sealed and incubated at 37^oC/24-48 hours. The plates were then examined with an inverted mirror and the growth or absence of growth of the tested microorganism was recorded for each well. The first dilution with no visible bacterial growth was considered as the MIC for that isolate. The MBCs were determined by subculture of 0.1 ml from the contents of the last three wells showed no turbidity on Mueller-Hinton agar and incubated at 37^o C for 24-48 hours. The antimicrobial concentration of the wells which showed no growth were considered as MBCs.

Statistical analysis

SPSS 11 for Windows statistical program (2002) has been used for the calculation of MIC_{50}, MIC90, median and range for the tested antimicrobial agents.

Results

1. The agar diffusion test:

The results of the agar diffusion test indicated that most of the bacterial isolates obtained were sensitive to enrofloxacin and ciprofloxacin, excluding 2 *Klebsiella* spp. and 2 *A. pyogenes* which were resistant to enrofloxacin and one *Klebsiella* spp. and 3 *A. pyogenes* which were resistant to ciprofloxacin. With the exception of *M. haemolytica* and *A. pyogenes*, more than 82% of the isolates were found to be sensitive to gentamicin. For flumequine, 80% of *M. haemolytica* and *E. coli* isolates were sensitive, and 33% of *P. aeruginosa* isolates were resistant thereto. Only S*treptococcus* isolates were 100% sensitive to doxycycline, whereas other isolates were resistant with the range between 20% for *M. haemolytica* isolates and 67% for *P. aeruginosa* isolates. Bacterial isolates

Table 1. The percentage of resistance patterns of *Escherichia coli* (n=20), *Klebsiella* spp. (n=11), *Staphylococcus* spp. (n=11), *Pseudomonas aeruginosa* (n=9), *Archanobacterium pyogenes* (n=5), *Mannheimia haemolytica* (n=5), and haemolytic *Streptococcus* spp. (n=4) isolates collected from pneumonic lungs of camels in Jordan against 15 antimicrobial agents, using the agar diffusion test

	E. coli			*Klebsiella* spp.			*Staph.* spp.			*P. aeruginosa*			*A. pyogenes*			*M. haemolytica*			*Strept.* spp.v		
	R%	**I%**	**S%**	**R%**	**I%**	**S%**	**R%**	**I%**	**S%**	**R%**	**I%**	**S%**	**R%**	**I%**	**S%**	**R%**	**I%**	**S%**	**R%**	**I%**	**S%**
Penicillin	100	0	0	100	0	0	73	0	27	100	0	0	100	0	0	100	0	0	25	25	50
Ampicillin	85	10	5	82	9	9	82	0	18	100	0	0	80	20	0	80	20	0	50	25	25
Amoxicillin	80	10	10	91	0	9	64	0	36	89	0	11	40	20	40	20	40	40	25	25	50
Tetracycline	70	20	10	46	27	27	73	9	18	67	22	11	80	20	0	60	40	0	50	25	25
Doxycycline	65	0	35	37	27	36	27	0	73	67	0	33	60	20	20	20	20	60	0	0	100
Streptomycin	10	40	50	27	46	27	9	18	73	56	22	22	20	40	40	0	40	60	25	25	50
Gentamicin	0	5	95	0	18	82	0	9	91	0	0	100	40	20	40	0	40	60	0	0	100
Neomycin	10	60	30	27	64	9	9	27	64	56	33	11	60	40	0	40	20	40	0	50	50
Enrofloxacin	0	10	90	18	0	82	0	0	100	0	22	78	0	40	60	0	0	100	0	25	75
Ciprofloxacin	0	5	95	0	9	91	0	0	100	0	0	100	40	20	40	0	0	100	0	0	100
Flumequine	15	5	80	18	9	73	9	18	73	33	11	56	20	20	60	0	20	80	25	25	50
Lincomycin	95	5	0	91	9	0	55	9	36	100	0	0	100	0	0	60	40	0	50	0	50
Erythromycin	50	40	10	73	18	9	36	18	46	67	33	0	80	20	0	60	40	0	25	25	50
Colistin sulfate	40	50	10	9	45	46	46	36	18	11	56	33	60	20	20	20	60	20	75	0	25
Co-Trimoxasole	45	25	30	46	18	36	36	36	28	45	22	33	60	40	0	60	40	0	0	100	0

R: resistant; I: intermediate; S: sensitive; n: number of isolates

showed a wide variation in resistance, that ranged from 9 to 100% to penicillin, ampicillin, amoxicillin, tetracycline, lincomycin, erythromycin, colistin and co-trimoxasole. Streptomycin and neomycin were intermediate in activity with considerable resistance to *Klebsiella* spp., *P. aeruginosa* and *A. pyogenes* isolates as illustrated in (Table 1).

2. Multiple resistance patterns:

All of the *E. coli*, *P. aeruginosa* and nine of 11 *Klebsiella* spp., isolates tested by agar diffusion exhibited multiple resistance patterns range between 4 to 11 different antimicrobials. The different resistance patterns displayed were 14 for *E. coli*, 10 for *Klebsiella* spp. and 7 for *P. aeruginosa*. The most common pattern was penicillin, ampicillin, amoxicillin, tetracycline, doxycycline, lincomycin, and erythromycin. Four of the *M. haemolytica* isolates showed multiple resistance to 8 different antimicrobials. Nine of the 11 *Staphylococcus* spp. isolates showed multiple resistance to 7 different antimicrobials with 8 patterns. The most common pattern was penicillin, ampicillin, amoxicillin, tetracycline, doxycycline, and linocomycin. Five different patterns was displayed for *A. pyogenes* isolates with multiple resistance range between 7 to 11 different antimicrobials. For Streptococcus two isolates showed resistance to 6 antimicrobials and the other two resist 3 different antimicrobials. The tested bacterial isolates displayed multiple resistance mainly to penicillin, ampicillin, amoxicillin, tetracycline, doxycycline, lincomycin, erythromycin and co-trimoxasole.

3. Minimum Inhibitory Concentrations:

Ciprofloxacin and gentamicin were the most effective against *E. coli*, *Klebsiella* spp., *P. aeruginosa* and *S. aureus* isolates. With an MIC_{50} of 0.125 and MBC median of 0.25 µg/ml for *P. aeruginosa* and 0.5 and 1 µg/ml for *S. aureus*, for ciprofloxacin. The MIC_{50} and MBC median was 0.25 and 0.5 µg/ml for *S. aureus* and 1 and 2 µg/ml for *E. coli* and *Klebsiella* spp. for gentamicin; followed by enrofloxacin which was the third in effectiveness. The MIC_{50} for flumequine against *E. coli* was 16 µg/ml with the range between 1 and 64 µg/ml. Thirteen of the 20 *E. coli* isolates were resistance to flumequine, MIC_{50} 16 µg/ml, while *Klebsiella* spp., *P. aeruginosa* and *S. aureus* were all resistance. The four bacterial species tested were also resistant to doxycycline, with MIC_{50} > 128 µg/ml (Tables 3 and 4). The MICs range of ciprofloxacin for *E. coli*, *Klebsiella* spp., *P. aeruginosa* and *S. aureus* were from ≤ 0.0625 to 4, ≤ 0.0625 to 2, ≤ 0.0625 to 0.5 and

Table 2. Range of minimum inhibitory concentrations (MICs) (µg /ml) of selected antimicrobial agents against *Escherichia coli, Klebsiella* spp., *Pseudomonas aeruginosa* and *Staphylococcus aureus,* isolated from pneumonic lungs of camels.

Antimicrobial agent concentration (µg/ml)v	E. coli (n = 20)					Klebsiella spp. (n = 11)					P. aeruginosa (n = 9)					S. aereus (n = 8)				
	C	E	G	F	D	C	E	G	F	D	C	E	G	F	D	C	E	G	F	D
=0.0625	3	-	-	-	-	1	-	-	-	-	3	-	-	-	-	-	-	-	-	-
0.125	3	-	-	-	-	4	-	-	-	-	2	-	-	-	-	1	-	-	-	-
0.25	4	3	-	-	-	2	2	2	-	-	-	-	-	-	-	1	1	7	-	-
0.5	4	11	2	-	-	2	4	1	-	-	4	4	5	-	-	2	4	1	-	-
1	3	3	9	4	-	1	3	3	-	-	-	2	1	-	-	-	3	-	-	-
2	2	3	7	1	-	1	1	4	-	-	-	3	1	-	-	-	-	-	-	-
4	1	-	2	1	-	-	1	1	-	-	-	-	1	-	-	2	-	-	-	-
8	-	-	-	1	-	-	-	-	1	-	-	-	1	-	-	1	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	-	-	-	4	-	-	-	-	3	-	-	-	-	-	-	-	-	-	3	-
24	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	-	-	-	2	-	-	-	-	1	-	-	-	-	1	-	-	-	-	2	-
64	-	-	-	2	3	-	-	-	-	-	-	-	-	4	1	1	-	-	1	-
96	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
128	-	-	-	-	-	-	-	-	4	2	-	-	-	4	4	-	-	-	1	2
256	-	-	-	-	7	-	-	-	2	7	-	-	-	-	4	-	-	-	-	5
384	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
≥ 512	-	-	-	-	10	-	-	-	-	1	-	-	-	-	-	-	-	-	1	1

C: ciprofloxacin, E: enrofloxacin, G: gentamicin, F: flumequine, D: doxycycline, n: number of isolates.

Table 3. Minimum inhibitory concentrations (MIC_{50} and MIC_{90}) (µg/ml) of selected antimicrobial agents against *Escherichia coli, Klebsiella* spp., *Pseudomonas aeruginosa,* and *Staphylococcus aureus* isolated from pneumonic lungs of camels.

	***E. coli* (n = 20)**		***Klebsiella* spp. (n = 11)**		***P. aeruginosa* (n = 9)**		***S. aureus* (n = 8)**	
	MIC_{50}	**MIC_{90}**	**MIC_{50}**	**MIC_{90}**	**MIC_{50}**	**MIC_{90}**	**MIC_{50}**	**MIC_{90}**
Ciprofloxacin	0.25	2	0.25	1.8	0.125	0.5	0.5	8
Enrofloxacin	0.5	2	0.5	2	1	2	0.5	1
Gentamicin	1	3.8	1	3.6	0.5	8	0.25	0.5
Flumequine	16	60.8	128	256	64	128	32	128
Doxycycline	192	256	256	486	128	192	256	512

n: number of camel isolates MIC: Minimum Inhibitory Concentration
Data are expressed as MIC_{50} and MIC_{90} indicating the concentration that is required to inhibit 50 and 90% of isolates, respectively.

Table 4. Minimum Bactericidal Concentrations (MBC Range and Median, µg/ml) of different antimicrobial agents against *Escherichia coli, Klebsiella* spp., *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolated from pneumonic lungs of camels.

	***E. coli* (n = 20)**		***Klebsiella* spp. (n = 11)**		***P. aeruginosa* (n = 9)**		***S. aureus* (n = 8)**	
	Range	**Median**	**Range**	**Median**	**Range**	**Median**	**Range**	**Median**
Ciprofloxacin	0.0625 - 4	0.75	0.125 - 4	0.5	0.0625 – 1	0.25	0.25 - 128	1
Enrofloxacin	0.5 – 4	1	0.5 – 8	1	1 – 4	2	0.5 – 2	1
Gentamicin	1 – 8	2	0.5 – 8	2	0.5 -16	1	0.5 – 1	0.5
Flumequine	2 – 128	32	16 – 512	256	64 – 256	128	32 - 768	64
Doxycycline	64 – 512	384	256 - 512	512	128 -512	256	> 512	512

n: number of isolates

Table 5. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values (µg /ml) obtained for 3 (ATCC) reference bacterial strains tested against 5 antimicrobial agents.

	Ciprofloxacin		**Enrofloxacin**		**Gentamicin**		**Flumequine**		**Doxycycline**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
E. coli										
(ATCC 25922)	<0.0625	<0.0625	0.125	0.25	0.25	0.5	0.125	2	0.5	4
P. aeruginosa										
(ATCC 27853)	0.0625	0.125	1	2	0.5	1	64	256	2	32
S. aureus										
(ATCC 25923)	8	32	1	2	0.25	0.5	2	16	0.25	4

from 0.125 to 8 µg/ml, respectively. The other MICs range are displayed in Table 2. The MIC_{50} and MIC_{90} results are illustrated in Table 3. The MICs and MBCs of the tested reference strains of *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) and *S. aureus* (ATCC 25923) which was used as control are displayed in Table 5.

Discussion

This study demonstrated that most of the bacterial isolates obtained from pneumonic camel lungs were sensitive to ciprofloxacin and enrofloxacin. The MICs for enrofloxacin, when tested against *E. coli*, *Klebsiella* spp., *P. aeruginosa* and *S. aureus* were = 2 µg/ml except for one *Klebsiella* isolate (MIC = 4 µg/ml) which may indicate emergence of resistance when compared with MIC (0.125 µg/ml) against *E. coli* reference strain (ATCC 25922) (Table 5). The sensitivity of *P. aeruginosa* and *Klebsiella* spp. isolates (MICs range = 0.0625 to 0.5 µg/ml and = 0.0625 to 2 µg/ml, respectively) was satisfactory against ciprofloxacin. However, only one *E. coli* isolate was resistant (MIC 4 µg/ml) when compared with the MIC < 0.0625 µg/ml against *E. coli* (ATCC 25922). The bimodal distribution of MICs of ciprofloxacin for *S. aureus* isolates indicate emergence of resistance (Table 2). Nonetheless, the MIC_{50} of ciprofloxacin and enrofloxacin ranged between 0.125 and 1 µg/ml for tested bacterial species isolates. Conversely, flumequine, a first generation drug of the fluoroquinolone group, was not effective against all *Klebsiella* spp., *P. aeruginosa*, and *S. aureus* isolates as determined by MIC. In addition, the bimodal distribution of the MICs for *E. coli* indicates emergence of resistance in 65% of the isolates against flumequine

(Table 2). Most of the tested isolates were sensitive to gentamicin, except for *A. pyogenes*. Our results are in agreement with Al-Doughaym *et al* (1999) who was found gentamicin effective against both Gram positive and Gram negative bacteria. The microbroth dilution results confirm an excellent sensitivity of gentamicin against *E. coli* isolates (MICs range 0.5 - 4 µg/ml; MIC_{50} = 1 µg/ml), *Klebsiella* spp. (MICs range 0.25 - 4 µg/ml; MIC_{50} = 1 µg/ml), *P. aeruginosa* (MICs range 0.5 - 8 µg/ml; MIC_{50} = 0.5 µg/ml) and *S. aureus* (MICs range 0.25 - 0.5 µg/ml; MIC_{50} = 0.25 µg/ml). On the other hand, streptomycin and neomycin were not satisfactory effective against all the tested organisms. In contrast to the finding of Al-Doughaym *et al* (1999), most of the tested isolates in our study displayed multiple resistance to erythromycin, lincomycin, co-trimoxasole, penicillin, ampicillin, amoxycillin and doxycycline. Therefore, it is not advisable to use these drugs in treatment of camel pneumonia caused by bacterial pathogens.

Our results showed that Gram negative isolates were resistant to tetracycline and doxycycline. This observation was consistent with previous reports indicating a cross-resistance between tetracyclines (Pijpers *et al,* 1989). Therefore, it can be predicted that resistance may exist against other group members such as oxytetracycline that is extensively used for treatment of pneumonia in farm animals.

The high rates of resistance against most of the antimicrobials tested and emergence of resistance of one *E. coli* isolate against ciprofloxacin (MIC ≥ 4 µg/ml) and one *Klebsiella* spp. isolates against enrofloxacin found in this study can be explained by the extensive and misuse of antimicrobial agents for animal treatment, prophylactic supplements and/or growth promoters. In Jordan, circumstantial evidence indicates that antimicrobial use is not strictly under veterinary prescription control. Such misuse of antimicrobials has created enormous pressure for the selection of antimicrobial resistance among bacterial pathogens and endogenous microflora (WHO, 2000).

The multiple resistance patterns from 4 and up to 11 antibiotics found in the current study is due to the results of numerous complex interactions among antimicrobials, micro-organisms and the surrounding environment (Levin, 2001; McDermott *et al,* 2002). The appearance of such multiple resistance to different bacterial pathogens, is a problem of major concern of public health and suggests the need for a continuous surveillance and for more prudent use of antimicrobials by physicians, veterinarians and farmers. Prevention strategies are needed as well as epidemiological studies that characterise the mechanisms of resistance and spread of resistance strains.

Conclusions

From the current investigation it can be concluded that ciprofloxacin, enrofloxacin and gentamicin appear to show more promise for the control of respiratory infections in camels, provided that dosage recommendations are based on pharmacokinetic/pharmacodynamic studies. In addition, the present study showed the importance of camels as a potential source of single and multiple resistant bacterial pathogens to different antimicrobials that are used in different field of veterinary medicine as well as in the public health sector for treatment of different bacterial diseases in Jordan.

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