DOI : 10.5958/2277-8934.2015.00032.6 *Vol 22 No 2, p 199-208*

DANOFLOXACIN AND MARBOFLOXACIN ACTIVITY ON SOME BACTERIAL ISOLATES FROM CAMELS (*Camelus dromedarius*)

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ABSTRACT

Bacterial diseases in camel cause morbidity, mortality, suffering and significant economic losses. This study assessed the types of bacteria involved in different types of lesions encountered in camel's lung and liver slaughtered at municipal slaughter houses of Rabat, Morocco. Bacterial species isolated and identified included *Staphylococcus aureus* (19.2%), *Staphylococcus* sp (46.1%), *Streptococcus* sp (7.6%), *Corynebacterium* sp (15.3%) and *Acinetobacter* sp (11.5%). The percentage of resistance of isolates to the antibiotics varied from 0 to 84.6%. The most frequent resistance was to Ampicillin and Penicillin G (84.6%) followed by Gentamycin and Tetracycline (11.5. %), while all the isolates were susceptible to Cephalothin and Ciprofloxacin. Minimum inhibitory concentrations (MIC) of danofloxacin and marbofloxacin were determined against these bacteria. Time-kill curves against *staphylococcus aureus* (MIC=0.25µg/ml), *Streptococcus* sp (MIC=0.5µg/ml), *Corynebacterium* sp (MIC=0.5µg/ml) for both danofloxacin and marbofloxacin, and *Acinetobacter* sp (MIC=0.125µg/ml for marbofloxacin and 0.25 µg/ml for danofloxacin) were then determined according to a broth microdilution test. The pharmacodynamic parameters as lowest effective concentration (LEC) and optimal bactericidal concentration (OBC) were determined. Optimal values of surrogate markers predicting the antimicrobial effect and preventing the development of resistance were widely reached.

Key words: Camel, danofloxacin, marbofloxacin, MIC, time-kill curves

Bacterial diseases in camel cause morbidity, mortality, suffering, and significant economic losses (Mc Grane and Higgins, 1985). Lesions located in the internal organs are among the most prominent emerging problems of camels causing considerable losses in production and varying mortality rates (BeKkele, 1999). Generally, they are only detected after the animals are slaughtered, because even hundreds of small abscesses or several large abscesses rarely cause clinical manifestation (Nasgarja and Chengappa, 1998). Antibacterial drugs are therefore used in both treatment and prevention programs in this species.

Fluoroquinolones are antimicrobial drugs that generally have very good activity against a broad spectrum of aerobic bacteria, including *Pasteurella* sp. and *mycoplasma* (Giles *et al,* 1991; Gutierrez and Rodriguez, 1993 and Hannan *et al,* 1997). Furthermore, fluoroquinolones used in treatments have good pharmacological characteristics such as large volumes of distribution, low plasma protein binding, and relatively low MIC against susceptible target microorganisms (Brown, 1996). Extensive research is needed to develop and implement appropriate specific dosing regimens that can maximise their clinical efficacy for use in production animals and reduce the risk of selection of resistant pathogens, particularly because these drugs are used for the treatment of multidrug-resistant infection in humans (Zubair *et al,* 2000). Danofloxacin and marbofloxacin are synthetic antibacterial agents of the fluoroquinolone group, developed specifically for use in veterinary medicine. Danofloxacin shares with marbofloxacin a wide spectrum of activity, a large volume of distribution and activity at low concentration (Spreng *et al,* 1995 and Brown, 1996). The determination of clinically optimal dosage schedules requires knowledge of both drug pharmacokinetics and pharmacodynamics. The latter includes spectrum of activity, potency, whether the drug is bactericidal or only bacteriostatic and the type of activity, for example, whether the drug acts

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by a time-dependent or concentration-dependent mechanism.

As with all antimicrobial agents, pharmacodynamic initial investigations begin with minimum inhibitory concentration (MIC) determinations. The commonly used method of bactericidal activity assessment is that of time–kill studies, which are often carried out using set multiples of the drug's MIC. Afterwards, the calculation of optimal bactericidal concentration (OBC) produces a single value per bacterial strain/antibacterial agent combination that represents overall bactericidal activity at clinically relevant concentrations.

The aim of the present work was to isolate and identify the types of bacterial species involved in lesions in apparently healthy camels slaughtered in Rabat, Morocco and to evaluate the potential of danofloxacin and marbofloxacin to support a possible use of these antibiotics in the camel. For this purpose, the pharmacodynamic characteristics (minimum inhibitory concentration and bactericidal activity using time–kill studies) against susceptible camel's bacteria were determined.

Materials and Methods

Sample collection

The study was conducted between February and June 2012. The samples were taken from the municipal slaughter house of Rabat where, on average, four camels (*Camelus dromedarius*) are slaughtered every Saturday. Camels were aged 6 to 8 years old and brought from all regions of Morocco. Camels were found to be apparently healthy at the ante-mortem examination. Samples were collected by swabbing or by organ fragment. The swabs were put in separate sterile test tubes into which 3 ml Tryptone soya broth was added (Carter, 1984), labeled and kept in a cool box, and transported to the (Institut agronomique et vétérinaire) laboratory for immediate incubation at 37°C for 24 hours (Quinn *et al,* 1994). Using sterile scissors and thumb forceps, about 10g of each sample was transferred into sterile screw-capped universal bottle containing 3 ml tryptone soya broth. After labeling, these were transported in an icebox to the laboratory and incubated aerobically at 37°C for 24 hours.

Isolation and identification studies:

After 24 h of incubation, a loopful of the broth culture was taken and streaked over identified petri-plates, containing blood agar base supplemented with 7% sheep blood (Quinn *et al*, 1994). The plates were labeled and incubated aerobically at 37 °C for 24-48 h. After taking note of cultural characteristics, these were subjected to Gram's staining to study staining reaction and cellular morphology. For further analysis, pure cultures of single colony type, were transferred on to nutrient agar-slants for a series of primary tests: catalase (Hydrogen peroxide, Fisher Chemical, UK), oxidase (TM-p phenylenediamine dihydrocholoide, Merck Co., Germany) and fermentative/oxidative (OF Basal Medium, Titan Biotech Ltd, India); and secondary tests: urease (urea and urease, Labort Co., India), coagulase (rabbit plasma), indole (Peptone water, Merck Co., Germany) and H_2S (Triple Sugar Iron Agar, Merck co., Germany), following standard procedures (Carter, 1984; Quinn *et al,* 1994).

Antibiogram studies of identified bacteria

The antibiotic susceptibility tests for identified microorganisms were applied with multidiscs containing Ampicillin (10µg), Penicillin G (10U), Ticarcillin (75µg), Cephalothin (30µg), Gentamycin (10µg), Trimethoprim+sulfamethoxazole (1.25/23.75µg), Sulfonamides (200µg), Tetracycline (30µg), Ciprofloxacin (5µg), Norfloxain (10µg) and Nitrofuratoines (300µg) (CLSI, 2011 and EUCAST, 2015).

Susceptibility and bacterial killing studies

Antibacterial agents

The investigated antimicrobial agents danofloxacin (VETRANAL, analytical standard 33700-Fluka) and marbofloxacin (VETRANAL, analytical standard 34039-Fluka) were obtained as reference powders with known potency. Stock solutions were prepared according to the CLSI procedure (CLSI, 2012) in distilled water with a minimum amount of 0.1M NaOH required to dissolve the quinolone, dispensed into sterile vials and stored at - 20°C until analysis.

MIC determination

Mueller-Hinton broth (MHB) was used as the optimal growth medium for MIC determination in accordance with the CLSI guidelines. The susceptibility of isolated bacteria to danofloxacin and marbofloxacin was evaluated using the macrodilution broth method according to CLSI M7 A9 (CLSI, 2012). *E. coli* ATCC 25922 (MIC range: 0.008 to 0.03 µg/ml) was used as reference strain for MIC quality control (CLSI, 2008). The final inoculum tested was 5×10^5 colony-forming units per mL (CFU/mL). The

MIC was defined as the lowest concentration of antimicrobial agent that inhibits visible growth of organisms after 16–20 h of incubation at 35°C.

Time-kill assay methodology

Time-kill analysis was performed in Mueller-Hinton broth (MHB) in accordance with the NCCLS guidelines M26-A (NCCLS, 1999). A saline suspension (NaCl, 9 g dissolved in 1 L of Milli-Q water, pH adjusted to 7.3 and autoclaved at 121 °C for 15 min) was used to standardise strain inoculum, for serial dilutions of the sample during the time-kill kinetics. Trypticase soy agar (TSA) was used for isolation of strains and TSA containing 10 g/L activated charcoal was used for viable counts during the killing kinetic studies to prevent the risk of carry-over.

The time-kill kinetics were performed for one strain of *staphylococcus aureus* (MIC=0.25µg/ml), *Streptococcus* sp (MIC=0.5µg/ml), *Corynebacterium* sp (MIC=0.5µg/ml) for both danofloxacin and marbofloxacin, and *Acinetobacter* sp (MIC=0.125µg/ml for marbofloxacin and 0.25 µg/ml for danofloxacin). The killing curves were determined using a microdilution test with a final broth volume of 1 ml. Each strain was isolated on TSA and incubated overnight at 35 °C. Danofloxacin and marbofloxacin stock solutions were thawed and diluted to get final concentrations corresponding to the following multiples of the obtained MIC for each strain (0.5 x MIC, 1 x MIC, 2 x MIC, 4 x MIC, 8 x MIC, 16 x MIC and 32 x MIC). Aliquots of 100 µL of the tested concentration of each antibiotic were added to 900 µL of the bacterial suspension (10^6-10^7 CFU/mL of each strain) into propylene tubes. A tube antibiotic free was used as a control. All tubes were incubated at 35 °C. Sampling for colony count was performed at 0 (before incubation), 0.5, 1, 2, 4, 6 and 24 h after the start of incubation. To determine viable count, serial six-fold dilutions were made in (MBH + 0.02% tween 80) solution. Of these solutions, two subcultures of 10 µl of each dilution were further made into TSA containing activated charcoal. Colonies were counted after 24 h incubation at 35°C. For all the strains tested, the number of viable count in CFU/mL was plotted against time for each concentration of marbofloxacin and danofloxacin tested (Renard *et al,* 1996).

Pharmacodynamic parameters

Data obtained from viable counts were evaluated by the determination of an index of survival bacteria (ISB) between 0–4, 6 and 24 h as previously described by (Garraffo *et al,* 1990 and Garraffo, 1994). The area under the curve (AUC_T) between 0 and final time (4, 6 and 24 h) of measure (T) was determined by the trapezoidal rule. ISBT (%) was obtained from the following equation:

$$\text{ISBT (\%)} = \frac{AUC_T \times 100}{I_0 \times T}$$

Where I_0 is the initial inoculum.

The lowest effective drug concentration (LEC) was equivalent to the first concentration inducing a value of the ISB less than 100%. The optimal bactericidal concentration (OBC) was equivalent to the antibiotic concentration yielding the lowest ISB value. All parameters relating to the calculation of ISB were expressed as a MIC ratio to the strain tested rather than in µg/ml.

Results

The observation of lesions on animals slaughtered shows that 90 % lesions were observed in the lungs and only 10% in the liver, the dominant lesion type was atelectasis (28.5%) (Table 1). The main bacterial isolates in different proportions were *Staphylococcus aureus*, *Staphylococcus* sp, *Streptococcus* sp, *Corynebacterium* sp and *Acinetobacter* sp. In samples of lung, the bacterial pathogens associated with major pulmonary lesions were Staphylococci, Streptococci, Corynebacteria and Acinetobacter, while in liver, Staphylococci and Acinetobacter were associated with abscesses (Table 2).

Table 1. Frequency of lesion types observed in camel.

Lesion	Organ	Frequency
Atelectasis	Lung	6 (28.5%)
Abscesses	Lung	4 (19.0%)
Bronchopneumonia	Lung	3 (14.2%)
Congestion	Lung	4 (19.0%)
Bronchial ganglions	Lung	2 (9.5%)
Abscesses	Liver	2 (9.5%)

The percentage of resistant of the isolates per the antibiotics varied from 0 to 84.6% (Table 3). The most frequent resistance was to Ampicillin and Penicillin G (84.6%) followed by Gentamycin and Tetracycline (11.5%), Trimethoprim/ Sulfamethoxazole and Sulfonamides (3.8%), while all the isolates were susceptible to Cephalothin and Ciprofloxacin.

The *in vitro* susceptibility measurement of isolated bacteria and *E. coli* ATCC 25922 to danofloxacin and marbofloxacin are illustrated in Table 2. The MIC of both danofloxacin and

Table 2. Frequency of isolates bacterial genera from camel lesions and *in vitro* MIC of Marbofloxacin and Danofloxacin.

Bacteria	Lung (n=19)	Liver (n=2)	Frequency	MIC (µg/ml)	
				Marbofloxacin	Danofloxacin
Staphylococcus aureus	4	1	5 (19.2%)	0.125-0.5	0.25-0.5
Staphylococcus sp	11	1	12(46.1%)	0.125-0.5	0.25-1
Streptococcus sp	2	0	2(7.6%)	0.5	0.5
Corynebacterium sp	4	0	4(15.3%)	0.016-0.5	0.063-0.5
Acinetobacter sp	2	1	3(11.5%)	0.125-0.25	0.25
E. coli ATCC 25922*				0.016	0.016

* Reference strain for MIC quality control.

Table 3. Antimicrobial resistance profiles of bacteria Isolated from camel samples.

	Staphylococcus aureus (n=5)	Staphylococcus sp (n=12)	Streptococcus sp (n=2)	Corynebacterium sp (n=4)	Acinetobacter sp (n=3)	Per cent of Isolates resistance
Ampicillin (10µg)	5	12	2	3	0	84.6%
Penicillin G (10U)	5	12	2	3	-	84.6%
Ticarcillin (75 µg)	-	-	-	-	0	0
Cephalothin (30µg)	0	0	0	-	-	0
Gentamycin (10µg)	0	0	0	3	0	11.5%
Trimethoprim/ Sulfamethoxazole (1.25/23.75µg)	0	1	0	0	0	3.8%
Sulfonamides (200µg)	0	1	0	-	-	3.8%
Tetracycline (30µg)	0	2	1	0	-	11.5%
Ciprofloxacin (5µg)	0	0	0	-	0	0
Norfloxacin (10µg)	-	-	0	-	-	0
Nitrofuratoines (300µg)	0	0	-	-	-	0

marbofloxacin was 0.016µg/ml against *E. coli* ATCC 25922. The MIC for the two fluoroquinolones demonstrated activity against both gram-negative organisms and gram-positive bacteria. Using internal breakpoints for the interpretation of the MICs established and validated for the aerobic pathogenic Gram-positive or negative bacteria isolated from cattle, pigs and pets (CLSI, 2008), a strain is considered resistant when the MIC is ≥4 mg/L for marbofloxacin, and MIC is ≥8 mg/L for danofloxacin. None of the strains tested in the current study were resistant to these antibiotics.

The time-killing-kinetic curves of marbofloxacin against *Staphylococcus aureus* (MIC=0.25µg/ml), *Streptococcus* sp (MIC= 0.5µg/ml), *Corynebacterium* sp (MIC=0.5µg/ml), *Acinetobacter* sp (MIC=0.125µg/ml), and of danofloxacin against *Staphylococcus aureus* (MIC=0.25µg/ml), *Streptococcus* sp (MIC=0.5µg/ml), *Corynebacterium* sp (MIC=0.5µg/ml) and *Acinetobacter* sp (MIC= 0.25 µg/ml) (Fig 1 & 2).

The effect of increasing concentration showed an initial rapid and extensive degree of bacterial killing with decrease of viable counts, followed by a slower decrease between 6 and 24 h of exposure to the antibiotic. Time-kill-curves for both marbofloxacin and danofloxacin had similar profiles and no major late regrowth of bacteria was observed for both antibiotics.

The strains of *Staphylococcus aureus*, *Streptococcus* sp, *Corynebacyerium* sp and *Acinetobacter* sp were exposed to danofloxacin and marbofloxacin at concentration 0.5 x MIC, these drugs exhibited a slight stationary effect but the bacterium resumed growth at a rate similar to that of the untreated control. As the concentration of the drug was increased above the MIC, there was a decrease in the number of viable organisms. For drug concentration equivalent to 1xMIC, there was a slight decrease in the number of viable organisms but after 24 hours of exposure, the number of viable organisms had increased to more than the initial inoculum except for *Staphylococcus aureus* which was exposed to marbofloxacin where there was no regrowth. This suggests that marbofloxacin and danofloxacin at

concentrations equal to 1xMIC had a bacteriostatic effect on *Staphylococcus aureus*, *Streptococcus* sp, *Corynebacterium* sp and *Acinetobacter* sp.

Up to 2xMIC, danofloxacin and marbofloxacin exerted a very powerful bactericidal effect until 6 h incubation. There was a significant (> 2 log) drop in bacterial population at concentrations ≥ 2xMIC. The rates of killing for concentrations above 2xMIC were almost identical for both antibiotics. For *Acinetobacter* sp, the bacterial killing for both danofloxacin and marbofloxacin did not start immediately after the addition of the antibiotic, and a lag period was observed.

Table 4 showed that LEC at 4 and 24 h were equal to the MIC for *Staphylococcus aureus* and to the MIC plus one or two dilution for *Streptococcus* sp, *Corynebacterium* sp and *Acinetobacter* sp with danofloxacin and marbofloxacin. ISB decreased with increasing danofloxacin and marbofloxacin concentrations. Nevertheless, with higher concentrations, almost similar ISB were observed. This reduction of bactericidal activity at higher concentrations was confirmed by the determined OBC for all strains.

Discussion

This study assessed the types of aerobic bacteria involved in different types of lesions encountered in camel's lung and liver slaughtered at municipal slaughterhouses of Rabat. Slaughterhouses provide an excellent opportunity for detecting diseases of both economic and public health importance (Raji *et al,* 2000). The most commonly observed pulmonary lesion was atelectasis (28.5%). The prevalence of liver abscesses (9.5%) was slightly higher than those reported by previous investigators in Jordan (1.2%) (Al-Ani *et al,* 1998) and in Iran (0.64%) (Nourani and Salimi, 2013) but lower than that reported in Sudan (13.5%) (Aljameel *et al,* 2014). Our high prevalence rate could be attributed to environmental changes and husbandry practices, mixed herding and sharing of water and pasture.

Major lesions were associated with various bacteria known to be pathogenic. The isolation rate of bacteria experienced in this study was lower than previous studies (Al-Doughaym *et al,* 1999; Zubair *et al,* 2000; Al-Tarazi, 2001; Kane *et al,* 2005 and Tigani *et al,* 2006); which could be due to the little sample size. Bacterial infections are one of the main causes of pneumonia in camels (Rana *et al,* 1993 and Seddek, 2002). Several species of microorganisms were isolated from both apparently healthy and affected respiratory tract of camels as *Staphylococci, Streptococci, Corynebacteria, E. coli, Pasteurella* and *Klebsiella* (El-Mosalami and Ghawi, 1983; Chauhan *et al,* 1987' Rana *et al,* 1993; Fatma *et al,* 2001 and Seddek, 2002). *Staphylococcus* sp, *Streptococcus* sp, and *Corynebacterium pseudotuberculosis* were the most incriminated bacteria in camels liver abscess (Aljameel *et al,* 2014). In this study, *Staphylococcus aureus* was recovered at a rate of 19.2% from pulmonary and liver lesions, this is higher than the results of (Al-Doughaym *et al,* 1999) and lowers than (Al-Tarazi, 2001) where *S. aureus* was isolated at rates of 10.6 and 24.8%, respectively. *Staphylococcus sp*ecies occur as commensals on the skin and mucous membranes. They also occur as environmental contaminants. Staphylococcus infections are opportunistic and associated with trauma, immunosuppression, infections and other stress factors (Quinn *et al,* 1994). Isolation of staphylococci from the lungs and liver of camel may be attributed to the stress of transportation

Table 4. Lowest effective concentrations (LEC) and optimal bactericidal concentrations (OBC) with the corresponding index of surviving bacteria (ISB) for the tested strains after 4 and 24 h.

Bacteria	Bacteria	LEC				OBC			
		4h		24h		4h		24h	
		Marbo	Dano	Marbo	Dano	Marbo	Dano	Marbo	Dano
Staphylococcus aureus (MIC=0.25µg/ml)	Conc µg/ml	0.25	0.25	0.25	0.5	4	8	4	8
	ISB %	34.2	93.2	19	17.2	17	33.4	15.2	15.1
Streptococcus Sp (MIC=0.5µg/ml)	Conc µg/ml	0.5	1	1	1	16	8	8	16
	ISB %	88.2	38	17.3	13 .7	26	18.9	15	13.5
Acinetobacter Sp (MIC=0.125µg/ml)	Conc µg/ml	0.25	0.25	0.5	0.5	4	8	2	4
	ISB %	43.3	91.1	18	18.2	27.2	22.6	14.9	14.5
Corynebacterium Sp (MIC=0.5µg/ml)	Conc µg/ml	1	2	1	1	16	16	8	16
	ISB%	40.3	43.5	23.7	22.5	16.1	16.8	12.3	12

LEC: lowest effective concentration, OBC: optimal bactericidal concentration,
Marbo : Marbofloxacin, Dano: Danofloxacin, ISB: index of survival bacteria

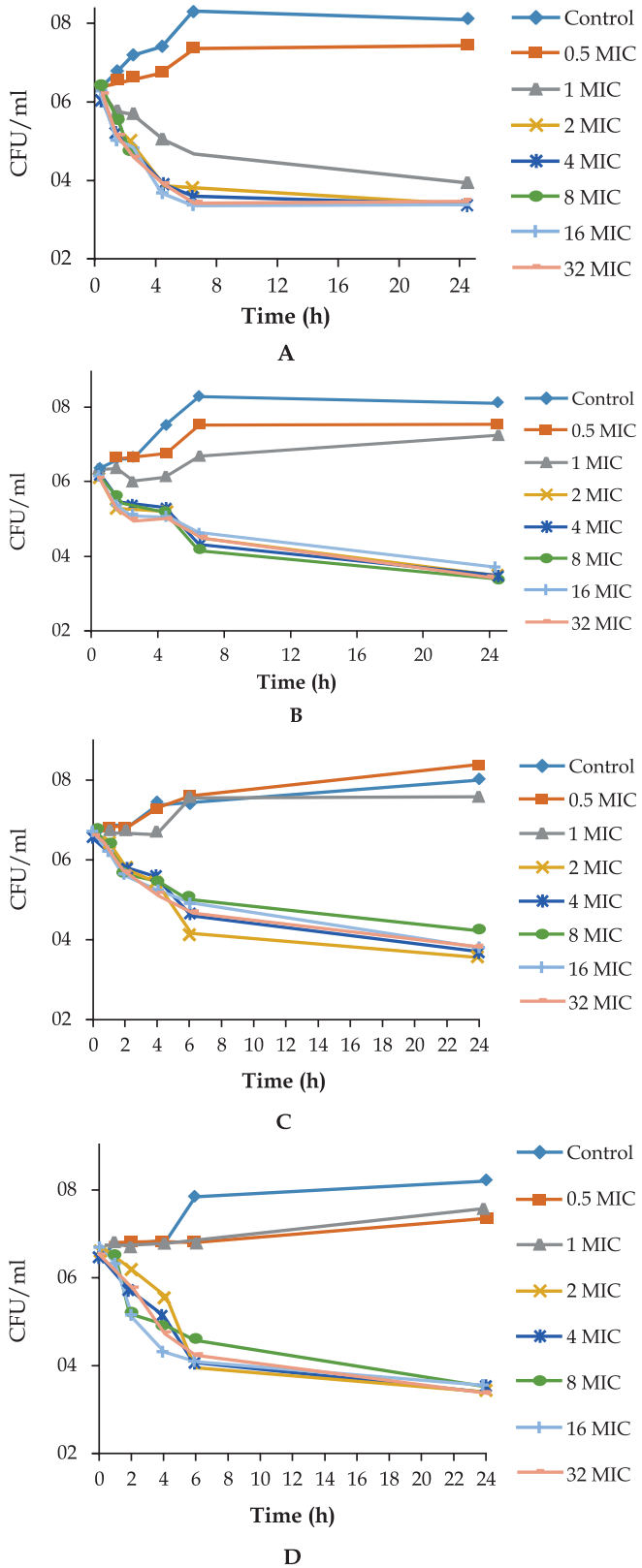

Fig 1. Time-Kill curve of marbofloxacin. A: Against *Staphylococcus aureus* strain (MIC= 0.25ug/ml). B: Against *Streptococcus* sp strain (MIC= 0.5ug/ml). C: Against *Corynebacterium* sp strain (MIC = 0.5ug/ml). D: Against *Acinetobacter* sp strain (MIC=0.125ug/ml).

and confinement. The camels are exposed to dusty conditions for prolonged periods (3 to 4 days) in the lairage without sufficient feed and water.

Streptococcus species were recovered at a rate of 7.6%, which is in agreement with (Zubair *et al,* 2000, Al-Tarazi, 2001) who recovered at a rate of 7% and 5.33%, respectively. However, this finding is lower than rate of 13.9% recovered by Tigani *et al* (2006). Streptococci species are widely distributed in nature and lives as commensals in the respiratory tract of many species of domestic animals, although potentially pathogenic species do exist (Carter, 1984).

Corynebacterium sp was isolated in a percentage of 15.3% of lesions, this was in agreement with previous studies (Kane *et al,* 2005), who reported that this pathogen was involved in pneumonia of camels under condition of stress, poor sanitation and immunosuppression. Further work has isolated *Pasteurella multocida* and *Mannheimia haemolytica* in some cases of pneumonia in camels (Shigidi, 1973). This is not the case in present study. This difference may be due to, among others, the different study areas, the ecology of bacteria, and the fragility of Pasteurella, making isolation difficult from field samples.

Acinetobacter sp was isolated at rate of 11.5%. The clinical role of *Acinetobacter* species has been reviewed previously (Joly-Guillou, 2005; Pelleg *et al,* 2008). These organisms are typical opportunistic pathogens. Infections comprise pneumonia, urinary tract infections, wound infections, skin and soft tissue infections.

The antimicrobial susceptibility tests carried out in this study indicated the high resistance of *Staphylococcus* species to Penicillin G (66%) followed by Ampicillin (45%). The resistance of staphylococci to these β-lactams antibiotics may be attributed to the production of β-lactamase, an enzyme that inactivates penicillin and closely related antibiotics and this may be probably explained by a horizontal transfer of antibiotic resistance gene from the resistant bacterium to another bacterium normally susceptible to this antibiotic. Moreover, this could be associated with the predominant use of Penicillin for treatment of animal diseases; this result agrees with other results regarding the increase in incidence of β-lactam antibiotics resistance (Aleskshun and Levy, 2000). Martonova *et al* (2008) also reported a high incidence of antimicrobial resistance among coagulase-negative staphylococci.

Resistant strain were not observed with ciprofloxacin and none of the strains tested in the current study were resistant to danofloxacin and marbofloxacin. Normally, in the case of fluoroquinolones (and some other antimicrobial classes), resistance to a single representative of this class of antibiotic agent can reasonably be extrapolated to resistance (or reduced susceptibility) to other members of that class (Shwartz *et al,* 2010).

Using *E. coli* ATCC 25922 for quality control of the MIC tests, the found value was almost superior to the value of 0.008 µg/ml found by Ferran *et al* (2007) for marbofloxacin and inferior to the value of 0.03 µg/ml (Nikolina *et al,* 2009) but these were in the recommended range (0.008 - 0.03µg/ml). Marbofloxacin and danofloxacin MICs found were close to the published results on similar bacteria indeed, *Staphylococcus aureus* and Staphylococci strains showed a similar distribution of marbofloxacin MIC, with a susceptible population centred on 0.25 µg/ml. This level of susceptibility to fluoroquinolones is characteristic of *Staphylococcus sp*ecies in cattle, marbofloxacin MIC on *Streptococcus* sp was 0.5 µg/ml, which is less than value centered on 1 µg/ml in cattle (Kroemer *et al,* 2012).

For danofloxacin, the MIC values reported in previous studies against *Staphylococcus aureus* isolated from goat infections (0.12- 1) µg/ml (Marin *et al,* 2010) were almost similar to those obtained in the present study (0.25-0.5) µg/ml. *Corynebacterium* sp isolated from bovine mammary glands showed a danofloxacin MIC range of (0.06-0.5) µg/ml (Jeffrey and Rossbach, 2000) similar to found results.

The ratios C_{max}/MIC and AUC_{24}/MIC are the best parameters for predicting the antimicrobial effect of fluoroquinolones, where C_{max} = peak or maximum plasma concentration following extravascular administration, and AUC_{24} = area under the plasma concentration-time curve from 0 to 24 h. Previous investigations have shown that for fluoroquinolones, C_{max}/MIC >3 produced 99% reduction in bacterial counts and C_{max}/MIC of ≥8 prevented the emergence of resistant organisms (Craig, 1996). Furthermore, AUC_{24}/MIC >100 h should be achieved to give maximum clinical and bacteriological efficacy (Turnidge, 1999).

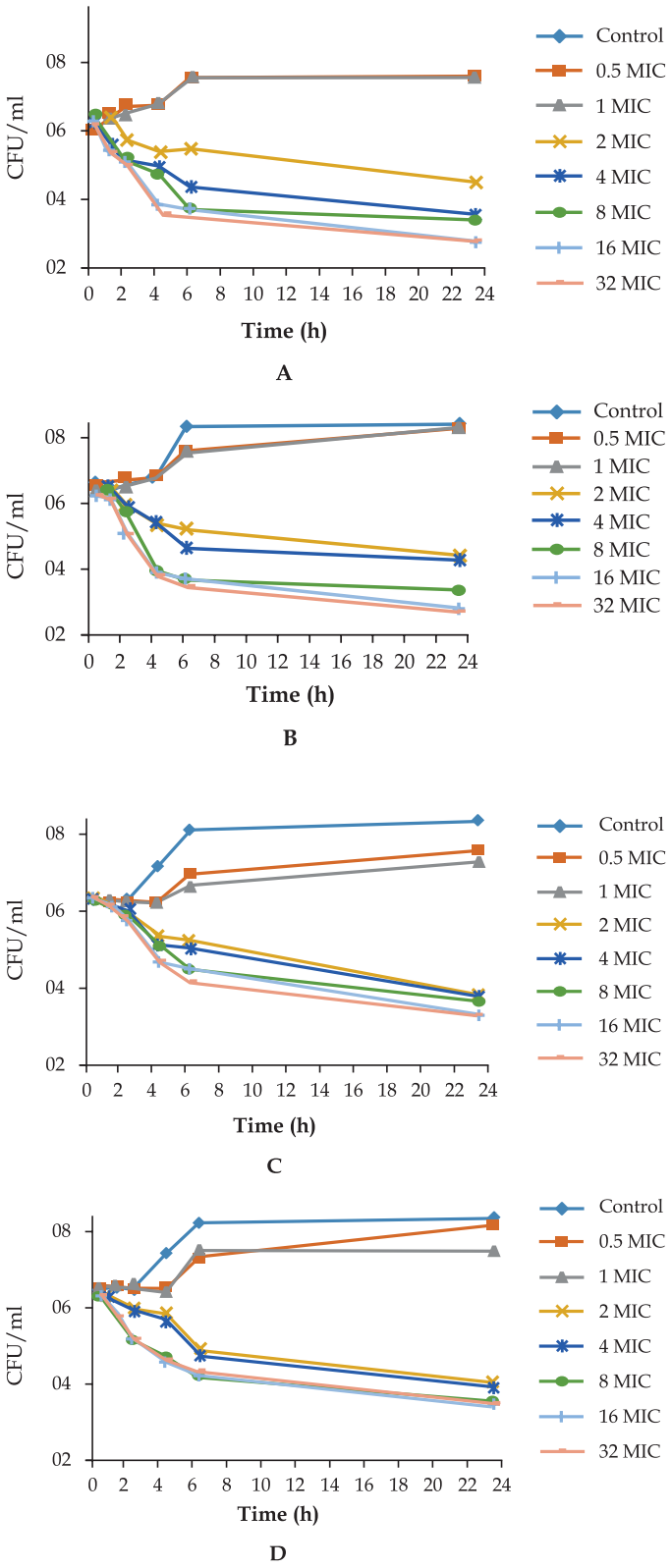

Fig 2. Time-Kill curve of danofloxacin. A: Against *Staphylococcus aureus* strain (MIC= 0.25ug/ml).B: Against *Streptococcus* sp strain (MIC= 0.5ug/ml). C: Against *Corynebacterium* sp strain (MIC= 0.5ug/ml). D: Against *Acinetobacter* sp strain (MIC=0.25ug/ml).

Therefore, if we take into account plasma AUC_{24} and C_{max} parameters from a pharmacokinetic study with danofloxacin and marbofloxacin in camel at a dosage regimen of 6mg/kg and 8mg/kg, respectively (Ait Lachguer *et al,* 2013), the optimal values of C_{max}/MIC >8 and AUC_{24}/MIC >100 h are widely reached in our study. However, it must be noted that the numerical values of C_{max}/MIC and AUC_{24}/MIC, used as surrogate markers to predict optimal therapeutic outcomes, have been generated from experimental infections in laboratory animals or in human clinical trials (Toutain and Lees, 2004), and may be applicable to camel infections or to animal infections in general. Indeed, Lees and Shojaee (2002) showed that the ratio of AUC_{24}/MIC producing bacteriostasis, bactericidal activity and elimination of bacteria with different fluoroquinolones was in all cases lower than 100 to 125 h, for cattle, sheep, goat, and camel. For example, AUC_{24}/MIC values of 673.12h, 641.31h were obtained for *Streptococcus* sp and *Corynebacterium* sp, respectively with danofloxacin and marbofloxacin, showed a good activity of these antibiotics on this camel's bacteria.

One peculiarity of fluoroquinolones is their biphasic concentration-response curve. Indeed, they are considerably less effective against bacterial pathogens at concentrations much higher, as well as lower, than their minimum inhibitory concentrations (MICs). In the first phase, the percentage of killed bacteria increases with concentration; in the second phase, further increase in concentration causes a temporary decrease in the percentage of killed bacteria (Diver & Wise, 1986). With bacterial strains of camel used in the present study, biphasic killing profiles were seen. There was concentration-dependent killing during the initial phase (i.e. the rate of bacterial population reduction could be directly correlated to the drug concentration). However, during the latter phase (>6 h) there seem to be little or no correlation between drug concentration and the reduction rate in the bacterial inoculum. A similar phenomenon was observed in both Gram-positive and Gram-negative bacteria. Such an observation revealed that fluoroquinolone activity could be a complex combination of concentration dependent and independent killing.

For *Acinetobacter* sp, the lag phase corresponds to a stationary phase, before the exponential growth phase, to allow the synthesis of the necessary growth factors, or antibiotic permeation and/or the intermediate steps that exist between antibiotic-receptor binding and expression of cell death are two major possible causes for such lag period (Li *et al,* 1993).

Maximum killing rates achieved at the optimal bactericidal concentration (Mc Grane and Higgins, 1985). OBC's maximum value for danofloxacin and marbofloxacin reaches 16 µg/ml which is much lower than achieved concentrations maximal (C_{max}) in the serum after Subcutaneous (27.61 µg/ml) and intramuscular (39.80 µg/ml) administrations of danofloxacin and marbofloxacin, respectively in camel (Ait Lachguer *et al,* 2013).

In present study danofloxacin and marbofloxacin, showed good *in vitro* activity against *Staphylococcus aureus*, *Staphylococcus* sp, *Streptococcus* sp, *Acinetobacter* sp and *Corynebacterium* sp strains isolated from camel lesions.

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