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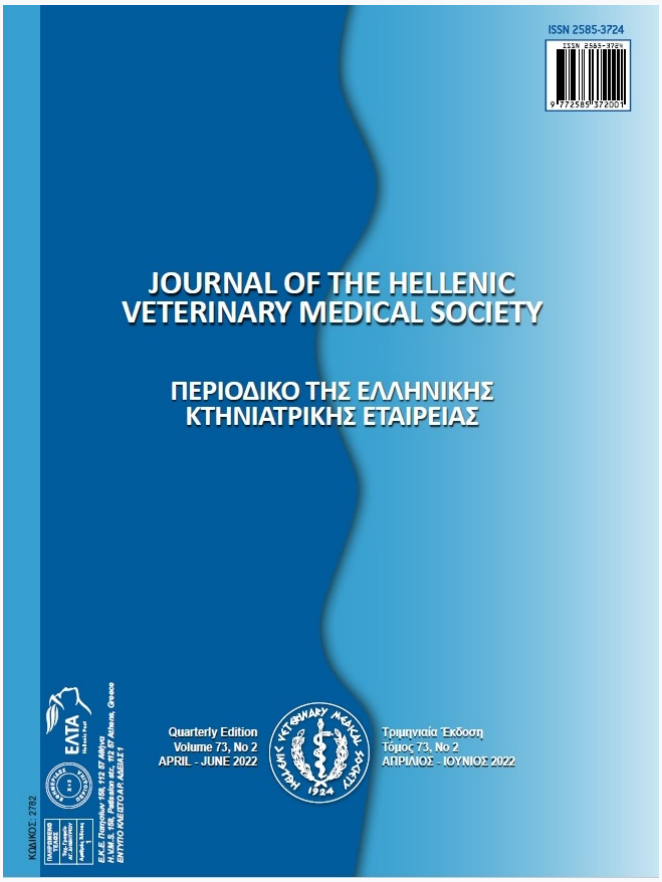

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Antimicrobial susceptibility testing of *Arcobacter butzleri* and *Arcobacter cryaerophilus* isolated from buffalo milk with subclinical mastitis: A different approach

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ABSTRACT: The aim of to our study was the minimum inhibitory concentrations (MICs) of nine antimicrobials in current use and three potentially new alternatives against *Arcobacter* spp. isolated from dairy buffalos with subclinical mastitis, and to evaluate these parameters instead of pharmacokinetic parameters. The *Arcobacter* spp. isolates were isolated from milk samples collected from dairy buffalos with subclinical mastitis. The susceptibility of *Arcobacter*spp. strains to antimicrobials were performed according to the guidelines by the NCCLS. The MIC value of vancomycin,erythromycin and tetracycline were not determined, and MIC value of ceftiofur, spiramycin and gentamicin have showed wide variations for isolated strains. However, cefquinome, tylosin, enrofloxacin and florfenicol were determined the best-performing agents against these strains. Antibiotics show concentration and time dependent killing, and studies have demonstrated the AUC/MIC, Cmax/MIC and t MIC ratios to be the best diviner of antibacterial effect. In the present study, based on the MIC values determined for selected antimicrobial agents, and pharmacokinetic parameters, amoxicillin, ceftiofur, cefquinome, enrofloxacin and florfenicol may be appropriate for the treatment of mastitis infections caused by susceptible *Arcobacter* spp. in buffalos.

Keywords: *Arcobacter* spp., buffalos, MIC, pharmacokinetic, pharmacodynamic

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INTRODUCTION

Mastitis is an important inflammatory disease of the mammary gland, caused by a large number of pathogens with economic loss for the dairy industry. The mastitis occurs in two forms, as clinical and subclinical based on the intensity of inflammation (Fagiolo and Lai, 2007). Clinical mastitis is described by the existence clinical manifestations such as changes in structure and composition of milk, abnormal mammary gland (swollen and painful), and changes in animal status (fever, in appetite, dehydration, decrease in milk production). On the contrary, there are no major unusualness in the mammary gland and milk in sub-clinical mastitis (Adkins and Middleton, 2018; Martins et al., 2019). Economical losses due to mastitis contain significant milk losses, physical, chemical and microbiological changes in milk, removal of chronically infected animals from the herd (Seegers et al., 2003; Ashraf and Imran, 2018).

The pathogens responsible for udder infections are bacteria and fungi (Martins et al., 2019).The recognition of pathogens related to mastitis allows for the proper diagnosis and treatment of the disease (Royster and Wagner, 2015). The most important causal microorganisms that reason mastitis are Bacteria (about 90%). Different bacteria can be isolated from the infected cattle and buffalo with mastitis, including *Arcobacter* spp., *Staphylococcus* spp., *Enterococcus*, *Escherichia*, *Streptococcus*, *Lactococcus* (Yesilmen et al., 2014; Vásquez-García et al., 2017; Patel et al., 2019).

The genus *Arcobacter* belongs to the family *Campylobacte r*in the class *Epsilon proteobacteria* originally isolated from aborted bovine and porcine foetuses.Among the well-known *Arcobacter* species; *Arcobacter butzleri* (*A. butzleri*), *Arcobacter cryaerophilus* (*A.cryaerophilus*) and *Arcobacter skirrowi* (*A.skirrowi*) cause serious infections in humans and animals and are therefore of prime clinical and veterinary importance (Collado and Figueras, 2011).In last years, *Arcobacter* spp. strains have become significant since they are considered as probably zoonotic agents. *Arcobacter*spp., have been related with a variety of human and animal diseases including gastrointestinal disorders (Ferreira et al., 2014), abortion (On et al., 2002), septicaemia (On et al., 1995), mastitis (Yesilmen et al., 2014).

Correct diagnosis and appropriate drug selection constitute the basis of the therapeutic success of mastitis (MK, 2017). Because of the wide range of potential pathogens, antimicrobial agents are widely used to treat and control disease that cause mastitis in the dairy industry (Gomes and Henriques, 2016; Ruegg, 2018). However, inappropriate and inaccurate antibiotic use leads to treatment failures such as the development of antimicrobial resistance , which is considered one of the greatest public health problems of the21st century and residue problems (Prestinaci et al., 2015).It is recommended to decide the appropriate and correct antimicrobial treatment after to make susceptibility testing of pathogens causing diseases in addition to identified the pathogen at the species level for successful antibacterial mastitis treatment (Leekha et al., 2011). Antibiotic susceptibility tests (AST) are broadly used to guide the decision of clinically effective antibiotic therapy and to estimate therapeutic results (Jorgensen and Ferraro, 2009). Various methods are used to have an opinion about the antimicrobial susceptibility of pathogens, including disc diffusion assays, broth or agar dilution methods (Wiegand et al., 2008; Jorgensen and Ferraro, 2009).

The minimum inhibitory concentration (MIC) is described as the indicator of the lowest effective antibiotic concentration required to inhibit bacterial growth and is used to assign whether the pathogenic bacterium is susceptible or resistant to an antibiotic (Jorgensen and Ferraro, 2009; Bauer et al., 2014; Pyörälä et al., 2014; Thomas et al., 2015).Additionally, MIC is an important pharmacodynamic (PD) parameter and this value is indicative of the lowest effective drug concentration for the pathogen in the target tissue. For an effective treatment, the antibiotic is required to remain above a certain concentration for a certain time in the target tissue (Toutain et al., 2002). To achieve this goal, PD and pharmacokinetic (PK) parameters should be evaluated together (Ahmad et al., 2016; Luo et al., 2019a).While the most valuable PD parameter used to evaluate the antibacterial effect is the MIC value, the area under the concentration curve (AUC) and the highest drug concentration (C_{max}) are the most valuable PK parameters.

In this study, we aimed to (1) determine the MIC of selected antimicrobial agents against *Arcobacter* spp. isolated from milk buffalos with subclinical mastitis, (2) integrate the MIC value obtained from this study and some PK parameters obtained from previous studies.

MATERIAL AND METHODS

Sample collection and processing: The milk sam-

ples were collected from the lactating buffalos which were not exposed to antibacterial treatment for at least 3 weeks in five different small holdings in and around Diyarbakir (Turkey). A total of 120 milk samples were gathered in sterilized screw caped test tubes. These samples were examined for CMT (California Mastitis Test) mastitis, and then the fifty milk samples declared positive for sub-clinical mastitis were transported to the laboratory at 4 ± 2°C.

***Identification of Arcobacter spp.isolates*:** The milk samples (1 mL) were homogenized in sterile tubes containing of *Arcobacter* spp. broth (9 mL) (Oxoid Ltd.) supplemented with a mix of cefoperazone, amphotericin B, and teicoplanin as a selective supplement at room temperature. These tubes were incubated at 30 ° C in microaerophilic environment for 48 hour.At the end of incubation, the 0.2 mL volume of each milk sample was filtered on Millipore filters (47-mm diameter and 0.45-µm pore size; Millipore Corporation, Billerica, MA). The filter was inoculated onto blood agar base medium (Oxoid CM271) with 5% defibrinated sheep blood with the membrane filtration method for 5 ± 2 days at 30 ° C in microaerophilic environment. Filters were removed from all samples one hour after inoculation.

DNA extraction was performed by EZ-10 Spin Column Genomic DNA Minipreps Kit, Bacteria (Bio Basic Inc., BS624, Canada) according to the kit manufacturer's instructions and extracted DNAs were stored at -20 oC until they used. Primer sequences for 16S rRNA gene were taken from a previous study (Figueras et al., 2008). Primer sequences used are as follows; forward 5'-AAC ACA TGC AAG TCG AAC GA-3' and reverse 5'-GTC GTG AGA TGT TGG GTT AA-3' (Figueras et al. 2012).

PCR mixtures were prepared in a 20 µl reaction volume containing 1X Taq Buffer, 1,5mM MgCl, 200 µM each deoxynucleotide (dNTPs), 200 nM of each primer and 1.5 U Taq DNA polymerase (Thermo Scientific). Amplification was performed with ThermoArctric thermal cycler (Thermo Fisher Scientific Oy, Finland). PCR conditions were as follows: 5 min at 94 oC, followed by 30 amplification cycles, each consisting of 94 oC for 30 s, 52 oC for 30 s and 72 oC for 90 s. Final extension were performed at 72 oC for 10 min. PCR products were separated with 1.5% agarose gel in 0.5 X TBE with 100 bpGeneRuler (Thermo Scientific) and photographed. DNA fragments of 1026 bp were considered as positive for *Arcobacter* spp. Amplified PCR products were digested with FastDigestMseI restriction endonuclease at 65 oC for 10 min.. Restricted fragments separated on 15% polyacrylamide gel electrophoresis in 1X TBE buffer at constant 20 mA with 50 bp Gen Ruler. Gels were stained with ethidium bromide and photographed (Figueras et al., 2012).

***Antimicrobial agents*:** Antimicrobial agents used in veterinary or human medical field, or both, such as amoxicillin clavulanic acid, ceftiofur, cefquinome, vancomycin, tylosin, spyramicin, gentamicin, enrofloxacin, ciprofloxacin, marbofloxacin, levofloxacin, and florfenicol in ranges of concentrations between 0.005 and 10.24 µg/mLwere subjected to testing susceptibility.All drug solutions were prepared immediately prior to use in stock solutions of 1.000 µg/mL.

***MIC Determination*:** MIC data of beta lactams and macrolides selected against two *Arcobacter* spp. strains isolated from mastitis affected buffalos were determined. Antimicrobial susceptibility testing were made for the *Arcobacter* spp. isolates obtained from subclinical mastitis (n=50) cases sent to the laboratory. Initial isolation was performed on 5% sheep blood agar plates and Mueller-Hinton agar under microaerophilic conditions at 30°C for 48 h.The susceptibility was determined using a microdilution method according to the protocol of the National Committee for Clinical Laboratory Standards (NCCLS, 2008). Inoculum suspension of several *Arcobacter* spp. colonies were freshly prepared in a tube containing 5 mL of Mueller-Hinton broth (MHB) and standardized to a turbidity equivalent to that of a McFarland 0.5 standard.The final bacterial inoculum was standardized at 5×10^5cfu/mL by adding 100 µL of a calibrated bacterial suspension to each well.The U-shaped 96-well microdilution plates containing 100 µl of the diluted inoculum suspension was inoculated with 100 µl of the drug concentration. Antibiotic free cultures were used as positive controls and bacteriafree cultures were used as negative controls.The microdilution plates were incubated at 30°C at 48 h in a microaerophilic atmosphere. After 48 h of incubation at 35 °C, MIC values were defined as the lowest drug concentration that completely inhibited visible growth in turbidity compared to drug-free growth control.

***PK/PD analyses*:** The PK/PD integration was achieved by using the MIC values of selected antimicrobial drugs obtained in this study and the recommended dose, volume of distribution (V_d), terminal elimination half-life ($t_{1/2\beta}$), dose interval, C_{max}, AUC parameters acquired from previously published stud-

ies for Amoxicillin (Ozdemir et al., 2019), ceftiofur (Gorden et al., 2016), cefquinome (Ahmad et al., 2015), tylosin (Avci and Elmas, 2014), gentamicin (Gurpreet Kaur, 2014), enrofloxacin (Rantala et al., 2002), ciprofloxacin (Rantala et al., 2002), marbofloxacin (Schneider et al., 2004), and florfenicol (Ruiz B. et al., 2010). Since the relevant pharmacokinetic parameters of some antibiotics in cows could not be reached, PK / PD analyses were not performed of these antibiotics (spiramycin, levofloxacin). PK / PD analysis could not be performed because the relevant MIC values could not be obtained for some antibiotics, such as, vancomycin, erythromycin, and tetracycline.

The percentage of the duration of time the drug serumconcentrations exceed the MIC (T > MIC) was calculated as = ln [Dose/ (Vd × MIC)] × (t½ /ln2) × (100/DI), as previously described (Turnidge, 1998).

RESULTS

***MIC analyse*:** Overall seven isolates were obtained from subclinical mastitis cases in buffalos. The MICs for the 12 antimicrobial compounds tested against *A. butzleri* and *A. cryaerophilus* are displayed in Table 1.

The antimicrobial resistance to vancomycin, erythromycin and tetracycline was found for *Arcobacter* spp. isolates (MIC > 10.24 μg/mL).

The most active beta-lactam evaluated was amoxicillin / clavulanic acid; it was highly active against *A. butzleri* (0.01- 0.16 μg/mL). Strains of *A. cryaerophilus* were slightly more susceptible to ceftiofur with MIC ranges of 0.04 - 0.08 than strains of *A.butzleri*. Cefquinome was the most active cephalosporin against all *Arcobacter* spp. isolates with MIC values of 0.01- 0.32 μg/mL.The MIC of tylosin was 0.01-0.08 μg / mL for *A. butzleri* and was 0.02-0.16 μg / mL for *A. cryaerophilus*. With gentamicin, MIC was

TABLE 1. The MIC of different antimicrobial agents tested against *Arcobacter butzleri* and *Arcobacter cryaerophilus* isolated from buffalo's milk with subclinical mastitis

Antimicrobial agent	*Arcobacter butzleri*		*Arcobacter cryaerophilus*	
	MIC range (μg /mL)	Upper MIC (μg / mL)	MIC range (μg /mL)	Upper MIC (μg /mL)
Amoxicillin / Clavulanic Acid	0.01 to 0.16	0.16	0.04 to 0.32	0.32
Vancomycin	1.28 to 10.24	> 10.24	0.64 to > 10.24	> 10.24
Ceftiofur	0.32 to 2.56	2.56	0.04 to 0.08	0.08
Cefquinome	0.08 to 0.16	0.16	0.01 to 0.32	0.32
Erythromycin	> 10.24	> 10.24	> 10.24	> 10.24
Tylosin	0.01 to 0.08	0.08	0.02 to 0.16	0.16
Spiramycin	0.02 to > 10.24	> 10.24	0.02 to 0.31	5.12
Tetracycline	> 10.24	> 10.24	> 10.24	> 10.24
Gentamicin	0.04 to 2.56	2.56	1.28 to 5.12	5.12
Enrofloxacin	0.04 to 0.08	0.08	0.02 to 0.64	0.64
Ciprofloxacin	0.64 to 5.12	5.12	0.16 to 2.56	2.56
Marbofloxacin	0.16 to 0.32	0.32	0.16 to 2.56	2.56
Levofloxacin	0.64 to 2.56	2.56	0.08 to 0.64	0.64
Florfenicol	0.04 to 0.08	0.08	0.08 to 0.32	0.32

TABLE 2. The PK/PD of different time-depend antimicrobial agents tested against *Arcobacter butzleri* and *Arcobacter cryaerophilus* isolated from buffalo's milk with subclinical mastitis

Antibiotic	***Arcobacter butzleri***		***Arcobacter cryaerophilus***	
	MIC	**t >MIC**	***MIC***	**t >MIC**
Amoxicillin (14 mg/kg, IM, 12 hour)	0.16	117.19	0.32	66.77
Ceftiofur (2.2 mg/kg, IM, 24 hour)	2.56	152.01	0.08	551.41
Cefquinome (1 mg/kg, IV, 8 hour)	0.16	117.61	0.32	91.36
Tylosin (17.5 mg/kg, IM, 24 hour)	0.08	294.22	0.16	208.97

TABLE 3. The PK/PD of different concentration-depend antimicrobial agents tested against *Arcobacter butzleri* and *Arcobacter cryaerophilus* isolated from buffalo's milk with subclinical mastitis.

	Arcobacter butzleri			*Arcobacter cryaerophilus*		
Antibiotic	**MIC**	**AUC/MIC**	**C_{max}/MIC**	**MIC**	**AUC/MIC**	**C_{max}/MIC**
Gentamicin (5 mg/kg, IV)	2.56	17.93	6.21	5.12	8.96	3.1
Tylosin (17.5mg/kg, IM)	0.08	261.88	16.25	0.16	130.94	8.13
Enrofloxacin (5 mg/kg, SC)	0.08	108.75	6	0.64	5.65	0.75
Ciprofloxacin (5 mg/kg, SC)	5.12	1.35	0.04	2.56	2.69	0.09
Marbofloxacin (5 mg/kg, SC)	0.32	23.9	5.06	2.56	2.99	0.63
Florfenicol (20 mg/kg, IM)	0.08	1043	35.75	0.32	260.75	8.94

ranged from 0.04 to 2.56 μg/mL for *A. butzleri* strain, where as MICs for *A. cryaerophilus* was ranged from 1.28 to 5.12 μg/mL. Of the fluoroquinolones for *A. butzleri and A. cryaerophilus*, the range of MIC of enrofloxacin lower than that of the ciprofloxacin, marbofloxacin and levofloxacin. MIC values of florfenicol were ranged 0.04 to 0.32 μg/mL for the all isolate in this study.

***The PK/PD analysis*:** The time above the MIC (T >MIC) for time dependent antibiotic are performed in Table 2. The C_{max}/MIC and AUC/MIC data for selected concentration depended antibiotics are shown in Table 3.

DISCUSSION

The *Arcobacter* spp., which is a foodborne pathogen, can cause serious infections in humans and animals. It has become progressively important as pathogenicity and as a potential food-water -based zoonotic agent has begun to be identified (Ho et al., 2006; Collado and Figueras, 2011). In recent studies, it has been determined that *Arcobacter* spp. play an important role as the cause of mastitis in cattle (Logan et al., 1982; Ramees et al., 2017; Parisi et al., 2019). Although there is a lot of data on the determination of this pathogen in cow milk (Pianta et al., 2007; Cruzado-Bravo et al., 2020; Marta et al., 2020), there is little information that this pathogen isolation in buffalo milk (Yesilmen et al., 2014). These results indicate that raw milk may be a source of possible *Arcobacter* spp. infections in buffalos and humans. For this reason, veterinarians and medical professionals must be aware of the milk transmission of the *Arcobacter*spp. to prevent the possibility of disease and spread of these organisms.

The MIC value is an important clinical laboratory parameter that does not necessarily cause the death of the bacteria and is used in epidemiological monitoring of antibiotic-resistant bacteria (Wiegand et al., 2008). Previous studies have presented the antimicrobial activity of varied antimicrobial agents against *Arcobacter* spp. isolates were isolated from raw milk and of a water buffalo dairy farm (Abay et al., 2012; Serraino et al., 2013; Yesilmen et al., 2014). These studies have used the E test and the agar disk diffusion methods and have evaluated data by classifying isolates as susceptible or resistant, using interpretive criteria that were based on animal treatment regimens (Serraino et al., 2013; Yesilmen et al., 2014). However, considering that each susceptibility test has its own advantages and limitations (Jorgensen and Ferraro, 2009), no study has been encountered to determine the MIC values of *Arcobacter* spp. strains isolated from buffalo milk with subclinical mastitis by microdilution method.

Antibiotics are uncommonly needed in the treatment except in long-term and severe cases, since *Arcobacter*spp. infections are mostly self-limited. However, of concern for animal and human health is the decrease in sensitivity among *Arcobacter* spp. to commonly used antibiotics (Collado and Figueras, 2011). The reduced sensitivity to antibiotics gives researchers a different perspective to investigate new and alternative treatment choices to effectively prevent and control *Arcobacter* spp. in terms of health and food safety (Ramees et al., 2017). In order for an antibiotic to destroy or inactivate an organism, it must reach a certain concentration in the microorganism and remain at that concentration for a certain period of time. Therefore, the MIC value alone may not be sufficient

to establish an effective treatment protocol. There are three important PK / PD markers used to determine the clinical outcome of antibiotics. An effective treatment protocol for antibiotics is established by keeping the drug concentration in the target tissue above the MIC for a certain time, which is achieved by evaluating AUC and C_{max} parameters together (Lees et al., 2004). The AUC / MIC, C_{max} / MIC and T > MIC is the best surrogate marker, respectively, for time-dependent and concentration-dependent drugs (Toutain et al., 2002). Evaluating these PK parameters together with MIC data may allow some antibiotics to be recommended for the treatment of mastitis caused by *Arcobacter* spp. As an indicator of the effectiveness of various antimicrobial agents against *Arcobacter* spp., MIK has been investigated using it alone in most published articles regimens (Abay et al., 2012; Serraino et al., 2013; Yesilmen et al., 2014). To the best of our knowledge, this article represents the first report evaluating the MIC of *Arcobacter* spp. clinical isolates against of the pharmacokinetic parameters of various antimicrobial agents (quinolones, beta-lactams, phenicol, macrolides, and aminoglycosides). For this reason in this study, the MIC of *Arcobacter spp*. obtained from clinical samples from buffalos affected by subclinical mastitis together with the PK parameters of some antibiotics obtained from previous studies were evaluated together.

The MICs for vancomycin,erythromycin and tetracycline were high for most of the strains tested in this study (MIC > 10.24 μg/mL). The high level MIC to vancomycin, erythromycin and tetracycline in *Arcobacter* spp. seen in our study agrees with results obtained by previous studies (Serraino et al., 2013; Yesilmen et al., 2014). These results may indicate that all isolates are resistant to vancomycin, tetracycline and erythromycin against the *Arcobacter* spp. tested. While tetracycline is a widely used antibiotic in cattle, why were high MIC values obtained against *Arcobacter* spp. against antibiotics that are not commonly used such as vancomycin and erythromycin? It is known that exposure to antimicrobials alone is not responsible for the decreased sensitivity to drugs. In fact, a mutation or a certain genetic combination provides the survival of the bacteria even in the presence of certain concentrations of antimicrobial agents. In order to express these clearly, it is necessary to evaluate PK and PD parameters of these drugs together. However, since no data on the pharmacokinetics of these drugs in buffalos were found in the literature reviews, such an evaluation could not be made in this study.

The MIC values obtained for amoxicillin / clavulanic acid, ceftiofur, cefquinome and tylosin selected in this study demonstrated the good sensitivity of these selected drugs against two *Arcobacter* spp. strains isolated from mastitis affected buffalos (Table 1). These results obtained for beta lactams, with the exception of amoxicillin, were similar to those obtained previously for *Arcobacter* spp. (Fera et al., 2003). On the other hand it is desirable that the PK / PD target threshold for time-dependent drugs such as, beta-lactams and macrolides in order to achieve an optimal dosage regimen remain at a concentration above the MIC in the dose range of at least 80% (80% t > MIC) (Toutain et al., 2002; McKellar et al., 2004; Papich, 2014). For this purpose, it is seen that the desired target against *Arcobacter butzleri* can be achieved for amoxicillin (14 mg / kg, IM), ceftiofur (2.2 mg / kg, IM), cefquinome (1 mg / kg, IV), and tylosin (17.5 mg/kg, IM) as a result of the MIC values obtained in this study and the PK / PD evaluations made with predetermined pharmacokinetic parameters. However, according to 80% t > MIC, it is seen that ceftiofur gives more effective results against *Arcobacter cryaerophilus* than amoxicillin, cefquinome and tylosin.According to the obtained MIC values, while cefquinome, amoxicillin, ceftiofur and tylosin seemed effective against *Arcobacter*spp. isolates, according to PK / PD evaluations, it was seen that the most effective results against both *Arcobacter*spp. isolates were obtained with ceftiofur (2.2 mg/kg, IM). (Table 2).

Generally, AUC / MIC and C_{max} / MIC predicted the clinical outcome of drugs with a concentration-dependent killing activity such as aminoglycosides and fluoroquinolones. From previous studies with the fluoroquinolones and aminoglycoside, it has been proposed that treatment should be optimized by providing a breakpoint for AUC/MIC and Cmax/MIC at least 100 and 8, respectively (Turnidge, 1999; Toutain et al., 2002). In our analysis, AUC/MIC and C_{max}/MIC of enrofloxacin were 108.75 and 6 at the dosage of 5 mg/kg, respectively, for *Arcobacter butzleri* (Table 3). However, it was determined that these values did not reach the desired levels for marbofloxacin, ciprofloxacin dosage. Although low MIC values were obtained for enrofloxacin, ciprofloxacin, marbofloxacin, and gentamicin against *Arcobacter* spp. isolates, it was seen that the desired therapeutic success could not be achieved with these drugs (enrofloxacin 5 mg/

kg, SC; ciprofloxacin 5 mg/kg, SC; marbofloxacin 5 mg/kg, SC; and gentamicin 5 mg/kg, IV) as a result of PK / PD evaluation. More information on the pharmacokinetics of antimicrobials is needed to interpret MIC levels.

The MIC value of florfenicol was determined as 0.08 μg / mL for *Arcobacter butzleri* and 0.32 μg / mL for *Arcobacter cryaerophilus* tested. MIC value of florfenicol against *Arcobacter* spp. have not been reported in cattle up to now. Since there was no MIC value for florfenicol previously reported in buffalos, this value was found to be lower compared to the MIC values obtained from humans (Riesenberg et al., 2017). The T>MIC parameter is often used to formulate the dosage regimen of florfenicol due to their bactericidal effects (Luo et al., 2019b). However, since the half-life is long, the most suitable PK / PD parameter recommended to be used for florfenicol is AUC/MIC (Pelligand et al., 2019). The surrogate marker C_{max} / MIC and AUC / MIC ratios were calculated using the pharmacokinetic parameters previously obtained from cows (Ruiz B. et al., 2010) and the MIC values obtained for *Arcobacter* spp. from this study (0.08 and 0.32 μg / mL) (Table1). In this study, AUC/MIC and Cmax/MIC ratios were achieved to 125 and 10, respectively, for MIC value of *Arcobacter* spp., after IM 20 mg/kg dosing (table 3). In conclusion, when florfenicol was used at a dose of 20 mg / kg in buffalos, it was seen that it exceeded the C_{max} / MIC and AUC / MIC ratios required to obtain optimum bactericidal activity against *Arcobacter* spp. However, further to develop an effective dose determination against mastitis infections caused by *Arcobacter* spp. in buffalo, additional data on pharmacokinetic parameters of florfenicol are needed in cow with mastitis.

In conclusion, the aim of our study was to estimate the most appropriate antibiotics to be effective in the treatment of *Arcobacter* spp. mastitis in water buffalos. Antimicrobial therapy of *Arcobacter* spp. mastitis based on in vitro susceptibility results has its own limitations. As discussed, breakpoints for antimicrobials are determined by expected pharmacokinetic (C_{max}, AUC) and pharmacodynamic (MIC) after systemic administration. In the current study, the antimicrobial agents, except for vancomycin, ciprofloxacin and levofloxacin, tested represent compounds that are currently used for bovine. More information on the pharmacokinetics of antimicrobials is needed to interpret MIC levels. This is particularly relevant to the use of the new antimicrobials tested in animals.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

FA experimental design, laboratory analysis, data interpretation, and corresponding author. SYA sample collection, laboratory analysis, scientific, and language editing of the manuscript.

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