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A simple, fast and sensitive screening LC-ESI-MS/MS method for antibiotics in fish

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ABSTRACT

The objective of this study was to develop and validate a fast, sensitive and simple liquid chromatographyelectrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) method for the screening of six classes of antibiotics (aminoglycosides, beta-lactams, macrolides, quinolones, sulfonamides and tetracyclines) in fish. Samples were extracted with trichloroacetic acid. LC separation was achieved on a Zorbax Eclipse XDB C18 column and gradient elution using 0.1% heptafluorobutyric acid in water and acetonitrile as mobile phase. Analysis was carried out in multiple reaction monitoring mode via electrospray interface operated in the positive ionization mode, with sulfaphenazole as internal standard. The method was suitable for routine screening purposes of 40 antibiotics, according to EC Guidelines for the Validation of Screening Methods for Residues of Veterinary Medicines, taking into consideration threshold value, cut-off factor, detection capability, limit of detection, sensitivity and specificity. Real fish samples (n=193) from aquaculture were analyzed and 15% were positive for enrofloxacin (quinolone), one of them at a higher concentration than the level of interest (50 μ g kg⁻¹), suggesting possible contamination or illegal use of that antibiotic.

1. Introduction

Aquaculture is one of the food-producing systems with the highest growth in the world and today it accounts for nearly 50% of the world's food fish [1]. However, intensive systems of animal food production are favorable to the spread of infectious diseases due to high population density. This is specially so in aquaculture, as the aquatic environment is prone to disease proliferation. In addition, abrupt physico-chemical changes in the aquatic environment and inappropriate management practices can directly affect the health of the fish [2]. For these reasons, the use of antibiotics in aquaculture is a common practice in the treatment of diseases. In addition, antibiotics can be used as prophylactic agents to avoid or prevent diseases and also as a feed additive to promote growth and increase feed efficiency [3–5]. Many antibiotics are allowed for use in aquaculture worldwide, and varying classes are permitted in different countries. As examples, tetracycline, oxytetracycline (tetracyclines), oxolinic acid, flumequine, enrofloxacin (quinolones), amoxicylin (β -lactam), erythromycin (macrolide), sulfadimethoxine (sulfonamide), ormetoprim (diaminopyrimidine) and florfenicol (amphenicol) can be cited. The first two are the most widely used [6,7]. Antibiotics are administered through the diet or are released directly into surface waters and, after metabolism, antibiotics and/or their metabolites can end up in tissues or can be excreted through urine and feces. Therefore, there can be accumulation of antibiotics in water and sediments which can contaminate the aquatic ecosystem [8,9]. In addition, some antibiotics from intensive livestock can also be released into the environment and reach water resources [9–12].

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Abbreviations: CCβ, detection capability; CE, collision energy; CRLs, Community Reference Laboratories; CXP, collision cell exit potential; DP, declustering potential; ELISA, enzyme-linked immunosorbent assay; ESI, electrospray ionization; FDA, Food and Drug Administration; HFBA, heptafluorobutyric acid; HPLC, high-performance liquid chromatography; HRMS, high-resolution mass spectrometry; LC, liquid chromatography; LOD, limit of detection; MRL, maximum residue limit; MRM, multiple reaction monitoring; MS, mass spectrometry; MS/MS, tandem mass spectrometry; PVDF, polyvinylidene fluoride; STC, screening target concentration; TCA, trichloroacetic acid; Tv, threshold value; UHPLC, ultra-high performance liquid chromatography; UV, ultraviolet

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Table 1

Antibiotics included in the study and respective Maximum Residue Limit (MRL), screening target concentration and concentrations of stock solutions.

Class/analyte	Concentration						
	$\frac{MRL}{(\mu g k g^{-1})}$	Screening target (µg kg ⁻¹)	Stock solution (μ g mL ⁻¹)				
Aminoglycosides							
Amikacin	500 ^a	250	200				
Apramycin	500 ^a	250	200				
Dihydrostreptomycin	500 ^e	250	200				
Gentamicin	500 ^a	250	200				
Hygromycin	500 ^a	250	200				
Kanamycin	500 ^a	250	200				
Neomycin	500^{b}	250	200				
Paromomycin	500 [°]	250	200				
Spectinomycin	500^{b}	250	200				
Streptomycin	500 ^c	250	200				
Tobramycin	500 ^a	250	200				
Beta-lactams							
Ampicillin	50 ^a	25	200				
Cefazolin	50^{a}	25	200				
Oxacillin	300 [°]	150	200				
Penicillin G	50^{a}	25	200				
Penicillin V	25 ^a	12.5	200				
Macrolides							
Clindamycin	$100^{\rm b}$	50	100				
Erythromycin	$100^{\rm b}$	50	100				
Lincomycin	200^{b}	100	100				
Spiramycin	200 ^c	100	100				
Tilmicosin	100°	100	100				
Tylosin	100 ^e	100	100				
Virginiamycin	$200^{\rm b}$	100	100				
Quinolones							
Ciprofloxacin	100 ^a	50	100				
Danofloxacin	$100^{\rm b}$	50	100				
Difloxacin	300 ^a	150	100				
Enrofloxacin	100^{a}	50	100				
Flumequine	600 ^a	300	100				
Marbofloxacin	$100^{\rm b}$	50	100				
Nalidixic acid	20 ^a	20	100				
Norfloxacin	100 ^b	50	100				
Oxolinic acid	20 ^a	20	100				
Sarafloxacin	30 ^a	15	100				
Sulfonamides							
Sulfachloropyridazine	100^{a}	50	250				
Sulfadiazine	100^{a}	50	250				
Sulfadimethoxine	100^{a}	50	250				
Sulfadoxine	100^{a}	50	250				
Sulfamerazine	100^{a}	50	250				
Sulfamethazine	100^{a}	50	250				
Sulfamethoxazole	100^{a}	50	250				
Sulfamethoxypyridazine	100^{a}	50	250				
Sulfaphenazole (IS)	-	-					
Sulfaquinoxaline	100 ^a	50	250				
Sulfathiazole	100 ^a	50	250				
Sulfisoxazole	100^{a}	50	250				
Tetracyclines							
Chlortetracycline	200ª	100	200				
Doxycicline	200ª	100	200				
Oxytetracycline	200ª	100	200				
Tetracycline	200*	100	200				

IS-internal standard.

^a Brasil [44].

^b Codex [45].

° EC [43].

The inappropriate and abusive use of antibiotics can be a potential public health hazard once their residues can remain in the fish muscle [13]. For example, residues of tetracyclines and sulfonamides [14], chloramphenicol [5,15], oxytetracycline [16,17], enrofloxacin [18,19] and florfenicol [16,17,20] have been detected in fish. Furthermore, it can remain in the water and sediment from aquaculture systems. Indeed, Monteiro et al. [16,17] detected oxytetracycline, tetracycline and florfenicol in different fish farms and tetracycline antibiotics were

found in river sediments.

Among health hazard issues to man, antibiotics in food can induce allergic reactions in some sensitive individuals. Furthermore, it can compromise human intestinal and immune systems, lead to the appearance of bacterial resistance in humans and animals, and affect the environment selecting the most resistant bacteria [4,5,13]. Several regulatory agencies established Maximum Residue Limits (MRL) for antimicrobials in food of animal origin (Table 1), and concentrations above the MRL are inappropriate for human consumption.

In order to warrant public health safety and to maintain competitiveness in international trade, the monitoring of antibiotics in fish and other foods of animal origin is needed. Therefore, sensitive and reliable analytical methods for the determination of multi-antibiotics in food are required. The effective control of antibiotics in foods requires the combination of cost effective and high sample throughput screening methods, followed by confirmation and quantification of suspect samples [5,21]. Liquid chromatography coupled to mass spectrometry in tandem (LC-MS/MS) has been used in the analysis of multiantibiotics in food, both for screening and quantitative methods [14,17,22-32]. Analytical methods using bioassay techniques or sensitive microorganisms are widely used as screening methods [33]. However, the use of LC-MS/MS for screening purposes is becoming popular as it can provide good specificity, sensitivity, and low rate of false-positive samples [23,29,34-36]. Through determination of the cut-off factor in a screening method, it is possible to evaluate if the sample contains or not the antibiotic in a concentration above MRL [37]. Since in most of the cases the samples are expected to comply, reports can be issued faster for samples which comply, whereas samples with cut-off factor above MRL should be further analyzed by quantitative methods [21].

LC-MS/MS methods for the analysis of more than five classes of antibiotics are available for milk [22,25,28,29,31], eggs [29], honey [38], meat [26,29,30,39], liver [27] and fish [20,33,40–42]. However, most of the multiclass methods available for the screening of antibiotics in fish are, in general, laborious and limited to a few antimicrobials. Therefore, the objective of this study was to develop a simple, sensitive and fast screening method for multiple classes of antimicrobials in fish muscle.

2. Experimental

2.1. Material

2.1.1. Chemicals and reagents

LC-MS grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany); heptafluorobutyric acid (HFBA) was from Fluka (Buchs, Switzerland) and trichloroacetic acid (TCA) was from Vetec (Rio de Janeiro, Brazil). Ultra-pure water was obtained from a Milli-Q purification apparatus (Millipore, Bedford, MA, USA).

All the antibiotics were of high purity grade (>99.0%). They included aminoglycosides, beta-lactams, macrolides, quinolones, sulfonamides, and tetracyclines, in a total of 49 compounds. They were purchased from Sigma-Aldrich (St. Louis, MO, USA), Fluka (Buchs, Switzerland) and Dr. Ehrenstorfer (Augsburg, Germany). Sulfaphenazole, the internal standard, was purchased from Sigma-Aldrich (St. Louis, MO, USA). The shelf-lives of the antibiotics were carefully considered and varied from 3 to 12 months.

Each standard was accurately weighed and transferred to a 50-mL volumetric flask and used to prepare methanolic stock solutions (Table 1) at concentrations varying from 100 to 250 μg mL⁻¹. Beta-lactams and aminoglycosides were dissolved in ultra-purified water, and 1 mL of 1 mol L⁻¹ NaOH was added to quinolone standard solutions to enhance solubility. Individual stock solutions were stored at -10 °C.

Working standard solutions were obtained by dilution of each stock solution in ultra-purified water, at concentrations varying from $0.125 \ \mu g \ mL^{-1}$ to $3.0 \ \mu g \ mL^{-1}$. The internal standard (sulfaphenazole) solution was prepared at $0.5 \ \mu g \ mL^{-1}$ in ultra-purified water. All the working solutions were kept at $-10 \ ^{\circ}$ C and prepared fresh monthly, except beta-lactams, which were prepared weekly.

2.1.2. Samples

Blank samples of Nile tilapia used in the validation process were collected at two farms from the state of Minas Gerais, Brazil, where none of the studied antimicrobials were used. A total of 193 fish muscle samples from fish farms under federal inspection were obtained: 172 from the state of Minas Gerais and 21 from the state of Pará, Brazil. The samples from Minas Gerais included 149 Nile tilapia (*Oreochromis niloticus*) and 23 trout (*Oncorhynchus mykiss*); whereas the samples from Para included 9 Nile tilapia (*Oreochromis niloticus*) and 12 tambaqui (*Colossoma macropomum*).

2.2. LC-MS/MS analysis

Liquid chromatography was performed in an Agilent 1200 Series HPLC (Agilent Technologies Inc., Santa Clara, CA, USA) coupled to a Triple Quadrupole Mass Spectrometer detector API 5000 AbSciex (Life Technologies Corporation, CA, USA). A Zorbax Eclipse XDB C18 (150×4.6 mm, 1.8μ m, Agilent Technologies, CA, USA) column was used. To establish optimum conditions for the chromatographic separation of all compounds and to achieve a short running time, several chromatographic parameters were investigated, including composition and flow rate of the mobile phase, gradient elution, injection volume and column temperature.

Mass spectrometer parameters were also optimized for each compound separately by direct infusion of individual standard solutions at concentrations ranging from 50 to $100 \ \mu g \ L^{-1}$ in MeOH. The best precursor and product ions, declustering potential (DP), collision energy (CE) and collision cell exit potential (CXP) were established. Electrospray ionization (ESI) generated the ions in a positive mode. Multiple reaction monitoring (MRM) was used and two transitions were selected: the most intense transition for quantifications and the second most intense for confirmation purposes. Each chromatographic run was divided into scan events with a scan time of 90 s for each transition. The analytical system control, acquisition and data processing were performed using Analyst software, version 1.5.1, from AbSciex (Life Technologies Corporation, CA, USA).

2.3. Sample preparation

The method used for extraction of the antibiotics from the samples was adapted from that described by Gaugain-Juhel et al. [22]. A schematic diagram for sample preparation is indicated in Fig. 1. Briefly, 2.0 g (wet weight) of ground and homogenized fish muscle was weighted in a 50-mL polypropylene centrifuge tube. Then, 200 μ L of internal standard (sulfaphenazole at 0.5 μ g mL⁻¹) and 800 μ L of deionized water were added. The sample was vortexed for 30 s and after standing for 10 min at room temperature, 8 mL of 5% TCA was added. The sample was homogenized in an ultra-turrax for 20 s, placed in a shaker for 10 min, and centrifuged at 2700×g for 12 min at 4 °C. The extract was filtered through a PVDF membrane with 0.45 μ m pore size (Millipore, Bedford, MA, USA) immediately prior to LC-MS/MS analysis.

2.4. Validation of the method

The fitness of the screening method optimized for the analysis of antibiotics in fish was evaluated according to the Guidelines for the Validation of Screening Methods for Residues of Veterinary Medicines (Initial Validation and Transfer)-Community Reference Laboratories (CRLs) 20/1/2010 [37]. The following parameters were evaluated: threshold value (T_v), cut-off factor (F_m), detection capability (CC β),



Fig. 1. Sample preparation for screening analysis of six classes of antimicrobials in fish muscle.

limit of detection (LOD), sensitivity and specificity.

2.4.1. Threshold value

The threshold value (T_v) was determined by analyzing twenty blank samples of fish muscle extracted according to the procedure described in item 2.3. The analytical response (chromatographic peak area) of the blank sample at the retention time ($\pm 10\%$) of each analyte was determined in each chromatogram for both quantitation and confirmation transitions. The mean and the estimated standard deviation of the noise were calculated. T_v was calculated according to Eq. (1).

$$\Gamma_{\rm v} = \mathbf{B} + 1.64 \times \mathbf{S}_{\rm B} \tag{1}$$

where B and S_B are, respectively, the mean and the standard deviation of the chromatographic peak areas of blank samples at the retention time of each analyte.

2.4.2. Cut-off factor

The cut-off factors (F_m) were calculated by using twenty blank samples of fish muscle spiked with the screening target concentration (STC), which is half of the MRL concentration based on Brazilian legislation for fish and other matrices (chicken, pork and meat) when not available for fish and European legislations [43–45], except for nalidixic acid, oxolinic acid, tilmicosin and tylosin (STC=1.0×MRL) (Table 1). The samples were analyzed at the same day and this step was repeated in a different day to obtain forty independent data. Peak area was determined for each analyte (n=40) for both transitions of quantification and confirmation. Means and estimated standard deviations were calculated for each analyte and the cut-off factor was estimated according to Eq. (2).

$$F_{\rm m} = D - 1.64 \times \rm{Sd} \tag{2}$$

where D and Sd are, respectively, the mean and the standard deviation of the chromatographic peak areas. It means statistically that 95% of the samples spiked at the level of interest should give an analytical response above this value.

2.4.3. Detection capability

The detection capability (CC β) was estimated from the comparison of threshold values and cut-off factors. When the cut-off factor is above the threshold value, CC β is considered as definitely below the level of interest (0.5×MRL, in this case). On the other hand, when the cut-off factor is below the threshold value, more than 5% of the samples will be considered as negative samples and, consequently, CC β is really above the level of interest [37].

2.4.4. Limit of detection (LOD)

The limit of quantification (LOD) was estimated by extracting and analyzing by LC-MS/MS 20 blank samples of fish muscle. LODs for each analyte (one for each m/z transition – quantification and confirmation) were calculated as the mean concentration of the blank samples in the retention time of each analyte plus three times the standard deviation of the blank concentration. The LOD for each analyte was ascribed as the higher of the two values, in most cases from the confirmation m/z transition.

2.4.5. Sensitivity and specificity

To calculate the sensitivity (%), twenty samples were spiked with all antibiotics at 0.5 x MRL concentration, extracted and analyzed by LC-MS/MS. The instrument response for peak area (R_{an}) for each analyte was compared to the cut-off factor and if $R_{an} > F_m$, the sample was considered non-compliant (positive), i.e., it contains a concentration above 0.5×MRL. However, if $R_{an} < F_m$, the sample was considered compliant (negative), i.e., it contains a concentration below 0.5×MRL.

The method sensitivity was estimated from Eq. (3) and it must be higher than 95% to ensure a β error below 5%. In this case, all the samples are positive because they were spiked at a 0.5×MRL concentration.

$$Sens.(\%) = \frac{Number of samples considered positive}{Number of samples really positive (20)} x100$$
(3)

To determine specificity of the method, e.g. its ability to detect unambiguously a specific analyte from a complex matrix, the blank chromatograms at the retention time of each studied analyte were carefully evaluated in order to verify possible interferences.

3. Results and discussion

3.1. Optimization of the LC-MS/MS procedure

The optimized spectrometric parameters and the retention time windows (equal to retention time \pm 5%) for each analyte individually are shown in Table 2. The chromatographic conditions for the screening method were optimized to provide the shortest possible run of all analytes of interest with appropriate resolution. The mobile phase composition which provided best results was phase A – 0.1% of heptafluorobutyric acid (HFBA) in water and phase B – acetonitrile at a gradient elution of: initial time – 90% A; 7.0 min – 50% A; 11.0 min – 50% A; 12.0 min – 90% A; and 15 min – 90% A at a constant flow rate of 600 µL min⁻¹. The flow rate and injection volume were 0.6 mL min⁻¹ and 10 µL, respectively and the column temperature was set at 35 °C. Total chromatographic run lasted 15 min

The presence of two chromatographic peaks, one for each m/z transition – quantification and confirmation, eluting at the same retention time allowed the unequivocal identification of each analyte. Each chromatographic peak presented a signal-to-noise ratio (S/N) equal to 3 under these conditions [23]. As can be noticed, several sulfonamides exhibit the same quantification and confirmation ions. However, as the precursor ion differs among them, distinction of each of them is allowed. Sulfadimethoxine and sulfadoxine had the same quantification and confirmation ions but they had also similar precursor ions (311.1 and 311.0, respectively), which could lead to mistaken identification of these two substances. However, because of

the different retention time windows observed for these compounds (9.17–9.60 and 8.15–8.57, respectively), the correct identification of each antibiotic was possible.

The total ion chromatograms obtained for all analytes in solvent (water) and in the fish matrix are indicated in Fig. 2. The run had a total time of 15 min and all analytes eluted within 12 min. The shortest retention time was observed for sulfadiazine (5.58-6.00 min), which has highest affinity with the aqueous phase and lowest interaction with the stationary phase. On the other hand, the longest retention time was observed for oxacicillin (11.00-11.60 min).

The high specificity and sensitivity of the triple quadrupole mass analyzer allowed the detection of the 40 analytes in only one chromatographic run. To assess specificity, 20 blank samples of fish muscle of different origins were analyzed and no chromatographic peak was detected in these samples at the retention time corresponding to each analyte, indicating a specificity of 100% for all the analytes. Both quantification and confirmation transitions (m/z) were used to confirm promptly a positive response. The extraction procedure proposed provided good quality chromatograms, suggesting its efficiency for the extraction and the analytes concentration.

3.2. Screening method validation

During validation of a screening method, it is important to find global conditions to detect all of the analytes simultaneously. The method has to present sufficient sensitivity to detect all the targeted analytes at least at the level of interest, which is $0.5 \times MRL$. Furthermore, qualitative methods of analysis must have the capability of a high sample throughput and the ability to detect all targeted analytes with a false-compliant rate below 5% (β error) at the level of interest. In the case of suspected non-compliant results, these must undergo confirmation by a confirmatory method [46].

The results of CC β , LOD, sensitivity, and the comparison between threshold value and cut-off factor (F_m/T_v) are presented in Table 3. The cut-off factor (the analytical response – peak area in this case – indicating that a sample contains a substance with a concentration equal to or higher than the level of interest) was compared to threshold value (the minimal analytical response above which the sample will be truly considered positive) to evaluate CC β .

According to the protocol for validation of screening methods [37], detection capability (CC β) of screening methods can be evaluated only when the cut-off factor is above the threshold value. When this condition is achieved, CC β is considered as definitely below the level of interest (0.5×MRL, in this case). On the other hand, when the cut-off factor is below the threshold value, more than 5% of the positive samples will be considered as negative samples and, consequently, CC β is really above the level of interest and the analyte cannot be analyzed by the method with 95% of confidence.

Among the 48 antibiotics analyzed, 40 attended the criteria established by EC [46] and EC [37], e.g., CC β was truly below the level of interest tested during validation (0.5×MRL) and the screening method was efficient in detecting all 40 analytes which presented F_m > T_v, with 95% of significance and a false-compliant rate of 5%. In general, all these analytes showed low LODs values (minimum concentration of a given analyte that can be detected with a reasonable statistical confidence), indicating that the method is capable of detecting low concentrations of these antibiotics.

The eight antibiotics which did not attend EC [46] and EC [37] included erythromycin, spiramycin, tylosin, virginiamycin, ampicillin, oxacillin, penicillin G and penicillin V. These compounds did not have cut-off factors above threshold value (e.g., $F_m < T_v$), which indicates that CC β values for these analytes were higher than 0.5×MRL and also that more than 5% of the non-compliant samples can show a compliant result (false negative). Although sensitivities for these analytes at 0.5×MRL concentration were satisfactory (>95%), most of them had high LODs values (sometimes above the MRL). Therefore, even though

Table 2

Optimized spectrometric conditions – precursor ion, confirmation transition (C) and quantification transitions (Q), declustering potential (DP), entrance potential (EP), collision energy (CE), collision cell exit potential (CXP) and retention time windows (RTW) – for each analyte in the screening method.

AmaxianSeriesSeriesSeriesSeriesAnayanjan840160/246/0.821082.10.282-8.5.1Aphynotynoby84080.0/246/0.801080.210.282-8.5.1Aphynotynoby84382.00/17.0/0.101080.210.284-8.5.2Aphynotynoby85310.310.210.480.381.884-8.5.2Aphynotynoby85310.310.480.310.480.480.4.2Aphynotynoby8540.0.310.410.480.410.4.280.4.2Aphynotynoby8540.0.310.410.410.410.410.410.4Aphynotynoby8520.0.310.410.410.410.410.410.4Aphynotynoby8520.0.310.410.410.410.410.410.410.4Aphynotynoby8520.0.310.410.410.410.410.410.410.4Aphynotynoby8520.0.310.410.410.410.410.410.4Aphynotynoby8520.0.310.410.410.410.410.410.410.4Aphynotynoby8520.0.310.41	Class/analyte	Precursor ion (m/z)	Quantification/confirmation ion (m/z)		EP	CE	СХР	Retention time windows RTW ^a (min)
Amband paranycinB86103 (0)/247 (C)601057/217/278.9-8.13Dalydorsteptonycin844253 (0)/240 (C)1201214/274.5-7.57Gerbanicin643.03252 (0)/150.2 (C)10102/2012/1274.1-8.92Hygronycin852252 (0)/150.2 (C)120102/212/11-8.0227.1-8.63Mamorycin103.310.13 (0)/253.50 (C)12010101012027.8-8.21Normycin103.2253 (0)/24.0 (C)1010101012027.8-8.21Steppinoynin852263 (0)/24.0 (C)10101012127.8-8.21Tohmanycin852253 (0)/15.0 (C)1010101217.7-8.10Cefonin455323 (0)/15.0 (C)1010101217.1-7.48Concallin455323 (0)/15.0 (C)10101217.117.1-7.48Cefonin455323 (0)/15.0 (C)101011.912.117.1-7.48Cefonin455323 (0)/15.0 (C)101011.912.117.1-7.48Cefonin45515.2 (0)/75.7 (C)61047.217.017.0-1.00Panidlin42.214.4 (0)/72.0 (C)71017.217.217.2Cefonin45.214.4 (0)/72.0 (C)61047.217.217.2Panidus45.214.4 (0)/72.0 (C)10<	Aminoglycosides							
Aperagy Diphyotory into Diphyotory into <b< td=""><td>Amikacin</td><td>586</td><td>163 (Q)/245 (C)</td><td>60</td><td>10</td><td>53/21</td><td>14/20</td><td>7.80-8.13</td></b<>	Amikacin	586	163 (Q)/245 (C)	60	10	53/21	14/20	7.80-8.13
Displantion884263 (0)/240 (C)101047/4127.43-7.53Gertamicin452 (0)/170 (C)501027/212/127.43-7.63Hygronycin528 (0)/023 (C)101027/212/127.43-7.63Noonycin15.310.3 (0)/235 (C)101041/388.0-9.01Pornoonycin15.320.3 (0)/1.63 (C)101013/1812/127.47-7.09Spectionnycin36120.3 (0)/2.64 (C)101013/1812/127.47-7.09Spectionnycin46310.3 (0)/2.62 (C)101020/2112/127.77-8.10Cafoalin4530.3 (0)/1.64 (C)501010/1211/1211.07-11.69Cafoalin45010.6 (0)/1.60 (C)501011/1211.07-11.69Cafoalin45010.6 (0)/1.60 (C)501011/1211.07-11.69Cafoalin45010.6 (0)/1.50 (C)501011/1211.07-11.69Cafoalin45210.6 (0)/1.50 (C)601011/1211.07-11.69Cafoalin45110.1 (0)/1.27 (C)601011/1211.07-11.69Cafoalin45210.1 (0)/1.27 (C)601011/1211.07-11.69Cafoalin45210.1 (0)/1.27 (C)611011/1211.07-11.69Cafoalin45210.1 (0)/1.27 (C)611011/1211.07-1.69Cafoalin10.1 (0)/1.27 (C)	Apramycin	540	217 (Q)/378 (C)	82	10	35/25	12/12	8.22-8.54
Germannian40-4.332.2.6 (0)/170 (1)501020/2012/208.41-8.92Hygromycin45.810.3 (0)/20.5 (1)7.01037.512/127.81-7.63Kanamycin45.810.3 (0)/20.5 (1)7.01037.512/127.81-7.63Paromonycin16.220.31 (0)/16.2 (1)101033.7512/127.97-8.10Stepetinonycin45.220.3 (0)/24 (1)10101045.7512/127.97-7.83Stepetinonycin45.220.3 (0)/24 (1)10101012/127.97-8.10Stepetinonycin46.220.0 (0)/24 (1)10101012/127.97-8.10Canadian40.220.0 (0)/24 (1)10101012/121.07-1.6.0Canadian40.220.0 (0)/24 (1)101010/1211/121.07-1.6.0Canadian40.220.0 (0)/24 (1)101012/121.07-1.6.0Canadian40.220.0 (0)/24 (1)101012/121.07-1.6.0Canadian40.220.0 (0)/24 (1)101012/121.07-1.6.0Canadian40.220.0 (0)/24 (1)101012/121.00-1.1.0Canadian40.220.0 (0)/24 (1)101012/121.00-1.1.0Canadian40.220.0 (0)/24 (1)101010/1211/121.00-1.0.0Canadian40.220.0 (0)/24 (1)101010/12	Dihydrostreptomycin	584	263 (Q)/246 (C)	120	10	42/54	12/12	7.43-7.75
Hygencycin528522 (0)/77 (C)501027/27731-763Neamycin45.5161.3 (0)/205 (C)701017.88.212.98.88.1Neamycin61.5.3161.3 (0)/235 (C)911010.331.518.701.98.1Specinomycin351207 (0)/189 (C)157103.75.518/101.95.93.97.97.9Specinomycin381207 (0)/189 (C)1571010101010.22.82.87	Gentamicin	464.3	322.6 (Q)/160.2 (C)	50	10	20/20	12/12	8.41-8.92
Konnycin48563 (0)/203 (C)70003/535/127.88-8.21Paromonycin616.22931 (0)/163.2 (C)10041/358/108.19-8.61Spectinonycin852253 (0)/163.2 (C)66031/3312/127.47-7.91Streptonycin852256 (0)/244 (C)10010020/2012/85.74-7.09Streptonycin852256 (0)/244 (C)10010020/2012/85.74-7.69Tobranycin453252 (0)/150 (C)50101012/812/127.77-8.10Casalilin453232 (0)/150 (C)501010/1812/127.77-8.10Casalilin G335.4176.3 (0)/162 (C)601011/1811/101.50-11.00Panicillin G335.4176.3 (0)/162 (C)601013/1211/1811/101.50-11.00Panicillin G335.4176.3 (0)/162 (C)601013/1211/1811/101.50Spiramycin42.51264 (0)/377.2 (C)601013/2612/127.97-8.1Endomycin74.51262 (0)/359 (C)601013/2612/1214/1410/10-11.0Infoncin20.612613/2711/1810.10-10.8011/1811/1811/1811/1811/1811/1811/1811/1811/1811/1811/1811/1811/1811/1811/1811/1811/1811/1811/18<	Hygromycin	528	352 (O)/177 (C)	50	10	25/25	12/12	7.31-7.63
Neumonycin615.3161.3161.317923.50170017.818/108.194-8.50Paromonycin35120710/1381 (C)157037.5518/108.194-8.50Spectinonycin35120710/1381 (C)157037.5518/1017.93-7.83Tohranycin4680.63 (0)/24 (C)157016717.1217.0327.83-7.83Tohranycin4580.63 (0)/160 (C)50017.217.1217.167.84Cacadilin4500.60 (0)/24 (C)70017.217.1017.5-7.48Cacadilin402160 (0)/24 (C)70017.217.109.9-1.0.4Penicillin G35.116.12 (0)/37.7 (C)701017.217.109.9-3.5Penicillin G35.112.64 (0)/37.7 (C)661017.217.217.409.9-3.5Parkonycin47.512.64 (0)/37.7 (C)671017.217.217.409.9-3.5Parkonycin47.512.64 (0)/37.7 (C)661017.217.23.9-7.2Linconycin40712.64 (0)/37.2 (C)75103.17.217.83.9-9.7Parkonycin47.412.64 (0)/37.2 (C)761017.217.23.9-8.7Parkonycin47.417.44 (0)/7.2 (C)15163.9.33.72.1217.16Parkonycin45.217.44 (0)/7.2 (C)15163.9.412	Kanamvcin	485	163 (O)/205 (C)	70	10	35/35	12/12	7.88-8.21
Paramanyain spectinonyain <b< td=""><td>Neomycin</td><td>615.3</td><td>161.3 (O)/293.50 (C)</td><td>120</td><td>10</td><td>41/35</td><td>8/18</td><td>8.50-9.01</td></b<>	Neomycin	615.3	161.3 (O)/293.50 (C)	120	10	41/35	8/18	8.50-9.01
Spectromycin Spin 207 (0)/187 (G) 66 10 10,3 12/12 674-709 Tobramycin 468 103 (0)/24 (C) 100 10 10 2/2 739-783 Tobramycin 468 103 (0)/160 (C) 50 10 10/2 727-810 Casollin 402 106 (0)/160 (C) 50 10 12/2 715-748 Casollin 402 106 (0)/140 (C) 50 10 15/1 8/12 715-748 Casollin 402 106 (0)/142 (C) 60 10 15/1 8/12 10.0-11.60 Macrolidar 425.3 126 (0)/172 (C) 75 10 43/27 1/8 10.0-11.60 Lincomycin 475.3 126 (0)/377 (C) 60 10 43/27 1/8 10.0-11.60 Spiramycin 425.3 126 (0)/377 (C) 60 10 43/27 1/8 10.0-11.60 Spiramycin 425.4 174 (0)/772 (C) 15 10 5/37 1/8 <	Paromomycin	616.2	293.1 (O)/163.2 (C)	91	10	33/55	18/10	8.19-8.50
Sireprovenia582233 (0)/246 (C)1071040712/127.37-R3Tohramyvini458136 (0)/234 (C)101020/2012/127.17-R4Ampicillin553233 (0)/156 (C)50101012/127.17-R4Cefacilin455233 (0)/156 (C)501015.1812/1211.0011.60Cenacillin455233 (0)/162 (C)60101011.8112/1211.0011.60Penicillin (C)335.415.63 (0)/162 (C)70101011.8112/1210.0011.60Penicillin (C)10.64 (0)/172.4 (C)611010.4712/1210.0010.00Penicillin (C)10.64 (0)/172.4 (C)611040.410.0010.0010.00Cilndanycin74.512.84 (0)/10.3 (C)611081.823.0023.00Cilndanycin20.517.44 (0)/172.4 (C)1151053.462.088-10.80Upsin20.517.44 (0)/172.4 (C)1151053.462.088-10.80Upsin20.517.44 (0)/172.4 (C)1151053.462.088-10.80Upsin20.517.44 (0)/172.4 (C)1151053.462.088-10.80Upsin20.520.1211.810.020.1288-82.6Upsin20.520.1220.1220.1288-82.6Upsin20.220.1220.12<	Spectinomycin	351	207(0)/189(C)	66	10	31/33	12/12	6.74-7.09
Tacharany Beta-Jacamy Beta-Jacamy163 (Q):224 (C)10010010020012/828/8Anginilino350106 (Q)/160 (C)50101015/217.77.15-7.4Cohaolin402160 (Q)/243 (C)50101018/1812/1211.011.6Pencillin G335.4176.3 (Q)/160.2 (C)601011.910.101.95-10.40Pencillin G35.4176.3 (Q)/160.2 (C)601043/2721.00.90-9.35Endinoty734.5158.4 (Q)/37.2 (C)601043/2712.01.00-11.60Incomycin734.5158.2 (Q)/356.7 (C)601043/2712.02.39-72.68Spitzmycin422.5174.4 (Q)/66.5 (C)561031/2510.83.9-97.2Tilmicosin916.6174.4 (Q)/66.5 (C)101035.410.20-10.50Typinamycin32.5 (Q)/107.4 (C)151054.710.88.8-9.40Urginamycin36.6 (Q)/29.1 (C)101035.410.28.8-1.80Danofoxacin3636 (Q)/29.0 (C)101035.410.08.8-9.30Danofoxacin3636 (Q)/29.0 (C)101035.410.08.8-9.30Danofoxacin3636 (Q)/29.0 (C)101035.410.08.8-9.30Danofoxacin3636 (Q)/29.0 (C)101035.410.08.8-9.30Danofoxacin	Streptomycin	582	263 (0)/246 (C)	157	10	45/51	12/12	7.39–7.83
Beta-farmsinininininininAmpicitin35323 (Q)/156 (C)501010/2312/127.7.8.10Cacalin402160 (Q)/243 (C)501011/812/1211.00-11.60Penicillin G335.417.6.3 (Q)/160 (C)501012/1210.01.0.6Penicillin V351.11601 (Q)/192 (C)601012/1210.00-11.00MacroildeHH10.00/12 (C)601043/271/4810.10-10.80MacroildeHH10.00/12 (C)601043/271/4810.10-10.80Eythronycin74.515.8.2 (Q)/787. (C)601043/271/4810.10-10.80Einomycin40712.6 (Q)/359 (C)601043/271/4810.10-10.80Spiranycin422.517.44 (Q)/72.4 (C)151055/36/209.88-10.80Spiranycin32.6 J32.6 (Q)/19 (C)61103/371/1410.20-10.50OutoioneHH1023/471/1410.1011.811.8Difoxacin332314 (Q)/22.6 (C)61103/361/148.8-36Difoxacin32346 (Q)/29.0 (C)1010108.8-33Anadosacin363345 (Q)/20.2 (C)44102/1410.4-10.80Difoxacin323316 (Q)/19.1 (C)301011/108.8-32<	Tobramycin	468	163(0)/324(C)	100	10	20/20	12/8	8.27-8.58
magnation magnetic35010610/10501021/207.7.7.8.10Coraculin402160 (0)/243 (C)501013/2121.7.27.1.5.7.48Coaculin402160 (0)/243 (C)701011/2121.0.011.60Penicillin G335.4176.3 (0)/160 (C)701021/2111.0.011.00Marcolitis5010.116/110/122 (C)601013/118/1210.0<-11.00	Beta-lactams	100	100 (Q)/021 (0)	100	10	20/20	12/0	
Cefaodin 455 322 (Q)/156 (C) 50 10 15/23 12/12 7.15-7.48 Oxadilha 402 160 (Q)/243 (C) 50 10 18/18 12/12 11.00-11.60 Penicillin G 355.4 175.3 (Q)/160.2 (C) 66 10 18/17 8/12 10.00-11.10 Penicillin G 355.4 126.4 (Q)/377.2 (C) 75 10 43/27 27.10 9.09-9.35 Endmyrin 74.5 158.2 (Q)/576.7 (C) 66 10 43/27 17.48 10.10-10.80 Endmyrin 474.5 158.2 (Q)/576.7 (C) 66 10 40/26 12.12 7.39-7.68 Spiramyrin 822.5 157.4 (Q)/695.5 (C) 76 10 51/68 9.33-9.72 Tilnicosin 869.5 174.4 (Q)/724.1 (C) 115 10 53/47 10.40 11.00-10.80 Quinolonez U U 51.44 10.00 34/20 12.2 8.48-10.80 Quinolonez U U 53.44 10.00 35/40 10.00 8.48-20.5 Difloacin 35.2	Ampicillin	350	106(0)/160(C)	50	10	20/20	12/12	7 77-8 10
Dacalline 402 160 (Q)/24 (C) 50 10 18/18 12/12 11.00-11.60 Penidlin G 351.4 176.3 (Q)/160 2 (C) 70 10 18/18 12/12 1/10 9.59-10.40 Penidlin Y 351.1 1601 (Q)/192 (C) 66 10 15/17 1/10 9.59-10.40 Macro Cilindanycin 425.3 126.4 (Q)/377.2 (C) 66 10 43/27 1/48 10.10-10.80 Linconycin 734.5 158.2 (Q)/57.7 (C) 66 10 43/27 1/38 9.39-7.63 Spiramycin 422.5 17/4.3 (Q)/10.13 (C) 56 10 31/25 16/8 9.33-9.72 Tilhincosin 896.5 17/4.4 (Q)/72.4 (C) 115 10 55/47 20/10 8.18-826 Virginiamycin 326.5 355.2 (Q)/190 (C) 76 10 23/47 21/2 8.03-8.33 Dandosacin 380 340 (Q)/231 (C) 61 10 35/40 10/10 8.98-9.30 Eurofosaci	Cefazolin	455	323(0)/156(0)	50	10	$\frac{15}{23}$	12/12	7 15–7 48
Peniallin G 335.4 176.3 (Q) 160.2 (C) 70 10 21/21 10/10 559-16.40 Peniallin V 351.1 1001 (Q) 1/22 (C) 66 10 11/17 8/12 10.00-11.10 Macrolida 2 126.4 (Q) 3757 (C) 67 10 43/27 22.10 9.09-9.35 Eythemycin 743.5 126.4 (Q) 3757 (C) 66 10 43/27 1/48 10.10-10.80 Eythemycin 743.5 126.4 (Q) 3757 (C) 66 10 43/27 1/48 10.10-10.80 Spiranycin 722.5 174.3 (Q) 10.1 (C) 76 10 43/27 1/48 10.10-10.80 Tilnicosin 86.9.5 174.4 (Q) 727.4 (C) 15 10 63/57 1/34 10.20-10.50 Quinoloxatin 332 34 (Q) 1/231 (C) 61 10 30/47 1/21 8.48-10.80 Olifoxatin 352 34 (Q) (23C) (C) 71 10 30/40 1/21 8.42-8.72 Dinofoxatin 352 32 (Q	Oxacillin	402	160(0)/243(0)	50	10	18/18	$\frac{12}{12}$	11 00-11 60
Penicillin V S1.1 16.1 (D)(192 (C) 66 10 15/17 8/12 10.00-11.1 Macro U U U U 10.00-11.1 0.00-11.1 Macro U 13/27 12.6 (U)(377.2 (C) 66 10 43/27 12/4 10.00-11.0.80 Linconycin 73.4 5 158.2 (U)/57.6 (C) 66 10 43/27 12/4 10.10-1.80 Spiramycin 422.5 174.3 (U)/10.1 (C) 56 10 31/25 10/6 33.9 -72 Tilnicosin 80.5 174.4 (U)/72.4 (C) 115 10 55/37 6/20 9.88-10.80 Virginiamycin 52.6 (S) 35.2 (Q)/109 (C) 10 10 35/47 6/10 8.15-11.80 Virginiamycin 52.6 (S) 35.2 (Q)/120 (C) 10 10 35/51 10/10 8.16-11.80 Virginiamycin 32.6 36.0 34.2 (Q)/23.0 (C) 10 30/47 12/12 8.33 Dindokoxin 40.0 35.6 (Q)/28.0 (C)	Penicillin G	335.4	$176.3(\Omega)/160.2(\Omega)$	70	10	21/21	10/10	9 59-10 40
InitianityJoin (a) 10, 10, 10, 10, 10, 10, 10, 10, 10, 10,	Penicillin V	351 1	160.1(0)/192(0)	66	10	15/17	8/12	10.00_11.10
Interval 425.3 126.4 (0)/377.2 (C) 75 10 43/27 22/10 9.09-9.35 Erythronycin 734.5 158.2 (0)/376.7 (C) 66 10 43/27 1/48 10.10-10.80 Erythronycin 472.5 174.3 (0)/10.13 (C) 56 10 31/25 16/8 9.33-9.72 Spiramycin 425.5 174.4 (0)/772.4 (C) 15 10 5/57 10/34 10.20-10.50 Tybsin 916.6 174.4 (0)/72.4 (C) 11 10 5/54 6/20 9.88-10.80 Quinolones - - 10 30/47 12/18 8.03-8.33 Difloxacin 332 346 (0)/25 (C) 60 10 35/50 10/10 8.18-13.80 Difloxacin 400 356 (0)/29 (C) 10 10.35/5 10/10 8.98-30 Everfoxacin 363 346 (0)/20 (C) 72 10 30/50 12/12 8.48-72 Plumequine 26.1 244 (0)/20 (C) 44 10 25/41 </td <td>Macrolides</td> <td>551.1</td> <td>100.1 (Q)/172 (C)</td> <td>00</td> <td>10</td> <td>10/1/</td> <td>0/12</td> <td>10.00 11.10</td>	Macrolides	551.1	100.1 (Q)/172 (C)	00	10	10/1/	0/12	10.00 11.10
Cumany chi42.0 (10)104 (0)10	Clindamycin	495.3	$126.4(\Omega)/377.2(\Omega)$	75	10	43/27	22/10	0.00-0.35
Ly nu ony char in problem19-12 10-1219-12 10-1219-12 10-12 10-12 10-1219-12 10-12 10-12 10-12 10-1219-12 10-12 10-12 10-12 10-1219-12 10-12 10-12 10-1219-12 10-12 10-12 10-1219-12 10-12 10-12 10-1219-12 10-12 10-12 10-1219-12 10-12 10-12 10-1219-12 10-12 10-12 10-1219-12 10-12 10-12 10-1219-12 10-12 10-12 10-1219-12 10-12 10-12 10-1219-12 10-12 10-12 10-1219-12 10-12 10-12 10-12 10-1219-12 10-12 10-12 10-12 10-12 10-1219-12 10-12 10-12 10-12 10-12 10-12 10-1219-12 10-12 	Emthromycin	734.5	120.4 (Q)/577.2 (C) 158.2 (Q)/576.7 (C)	66	10	43/27	14/8	9.09-9.33 10.10-10.80
LinkCuryUni4D1D(D) (D) (D)(D)1D4D12/1212/121.337-7.48Tilmicosin869.5174.4 (Q)/696.5 (C)561063/7210/4410.20-10.50Tilmicosin916.6174.4 (Q)/72.4 (C)1151055/436/209.88-10.80Virginamycin526.5355.2 (Q)/109 (C)761025/4726/108.15-11.80Quinolome111030/4712/128.03-8.33Danofloxacin38340 (Q)/255 (C)1001035/6010/108.18-8.26Difloxacin360356 (Q)/290 (C)1001035/6011/108.18-8.26Bumequine26.1244 (Q)/286 (C)701030/4712/128.42-8.72Plumequine363345 (Q)/320 (C)701030/2210/107.89-7.98Naidixis aid233215 (Q)/187 (C)421030/5312/1210.40-10.80Norfloxacin363345 (Q)/220 (C)601033/5012/128.29-9.15Suffadixine aid262244 (Q)/216 (C)501033/5012/127.89-8.20Norfloxacin36603/348 (C)501023/3712/128.58-6.00Suffadixine aid262244 (Q)/186 (C)501023/3712/128.58-6.00Suffadixine aid251156 (Q)/108 (C)601025/3712/128.58-6.00	Lincomucin	407	136.2 (Q)/370.7 (C)	60	10	40/26	19/19	7 20 7 69
Spin any off Var. S (2) / 103 (C) 50 10 63/57 10/4 10/20 - 10.50 Tibnicosin 89.65 174.4 (Q)/724. (C) 115 10 63/57 10/4 10/20 - 10.50 Virginiamycin 52.65 355.2 (Q)/109 (C) 10 25/47 63/57 10/10 8.15 - 11.80 Quinolones - - 10 30/47 12/12 8.03-8.33 Dandloxacin 32 314 (Q)/231 (C) 60 10 33/50 10/10 8.18 - 8.26 Difloxacin 36.0 342 (Q)/286 (C) 72 10 30/51 12/12 8.40 - 8.31 Pumequine 262.1 244 (Q)/202 (C) 44 10 25/45 12/12 1.04 - 11.00 Marbdioxacin 36.3 345 (Q)/187 (C) 70 10 30/35 12/12 1.04 - 11.00 Marbdioxacin 36.3 360 (Q)/31 (C) 60 10 30/35 12/12 1.04 - 11.00 Norfloxacin 30.6 302 (Q)/31 (C) 50 <td< td=""><td>Spiramyein</td><td>407</td><td>120(Q)/339(C) 174.3(O)/101.3(C)</td><td>56</td><td>10</td><td>31/25</td><td>16/8</td><td>7.39-7.00 0.33-0.72</td></td<>	Spiramyein	407	120(Q)/339(C) 174.3(O)/101.3(C)	56	10	31/25	16/8	7.39-7.00 0.33-0.72
Initiation695.3174.7 (2)/09.3 (C)151005.3710/2410.20-10.30Tylosin916.6174.4 (2)/72.4 (C)1151025/376/209.88-10.80Virginamycin526.5352. (Q)/109 (C)1151025/4726/108.15-11.80Quinoloacin358314 (Q)/235 (C)611030/4712/128.08-8.33Dandloxacin358340 (Q)/255 (C)1001035/4010/108.38-9.30Enrofloxacin360342 (Q)/286 (C)721030/5012/128.42-8.72Flumequine262.1244 (Q)/212 (C)441023/3512/128.42-8.72Nalidixic acid233215 (Q)/187 (C)421030/5112/128.98-9.30Nardhoxacin363345 (Q)/230 (C)601033/5112/128.98-9.30Nardhoxacin363345 (Q)/230 (C)601033/5112/128.98-9.30Nardhoxacin363345 (Q)/216 (C)601033/5112/128.98-9.30Nardhoxacin386368 (Q)/48 (C)601033/5112/128.98-9.30Sulfadarine251156 (Q)/180 (C)501023/3712/128.92-9.55Sulfadarine251156 (Q)/180 (C)531022/3012/128.78-8.66Sulfadorine311156 (Q)/180 (C)601035/3512/128.28-9.65Sulfadorine	Tilmioogin	9422.3	1/4.3 (Q)/101.3 (C) 174.4 (Q)/606.5 (C)	50	10	69/57	10/0	9.33-9.72
Tyrisni510.017.4.7 (U)11.31030.430.209.609.60Virginianycian526.5352 (Q)/109 (C)76025.726/108.1511.80Quinolones </td <td>Tulocin</td> <td>016.6</td> <td>1/4.4 (Q)/090.3 (C) 174.4 (Q)/772.4 (C)</td> <td>115</td> <td>10</td> <td>55/37</td> <td>6/20</td> <td>10.20-10.50</td>	Tulocin	016.6	1/4.4 (Q)/090.3 (C) 174.4 (Q)/772.4 (C)	115	10	55/37	6/20	10.20-10.50
Virginally end326.3335.2 (U) 109 (C)7610 $23/4$ <td>1 yiosini Vinginia musain</td> <td>510.0</td> <td>1/4.4 (Q)/7/2.4 (C)</td> <td>76</td> <td>10</td> <td>35/43</td> <td>0/20</td> <td>9.00-10.00</td>	1 yiosini Vinginia musain	510.0	1/4.4 (Q)/7/2.4 (C)	76	10	35/43	0/20	9.00-10.00
QuintotiesCiprofloxacin332314 (Q)/231 (C)611030/4712/128.03-8.33Dandloxacin358340 (Q)/255 (C)60103/5010/108.98-9.30Birnofloxacin360342 (Q)/286 (C)721030/5012/128.42-8.72Flumequine262.1244 (Q)/202 (C)441025/4512/1210.6-11.00Marbofloxacin363345 (Q)/320 (C)701030/3512/127.89-8.20Natidixic acid233215 (Q)/187 (C)60103/35012/127.89-8.20Nordloxacin320302 (Q)/231 (C)60103/35012/128.92-9.28Sarafloxacin320302 (Q)/231 (C)60103/4012/128.92-9.28Sarafloxacin366368 (Q)/348 (C)501021/3012/128.92-9.15Sulfachloropyridazine285156 (Q)/92 (C)511021/3012/128.92-9.15Sulfachloropyridazine285156 (Q)/108 (C)511021/3012/128.72-8.66Sulfachloropyridazine211156 (Q)/108 (C)60103/5312/128.12-8.67Sulfachloropyridazine256156 (Q)/108 (C)60103/5312/128.12-8.67Sulfachloropyridazine211156 (Q)/108 (C)60103/5312/128.12-8.67Sulfachlorine31.1156 (Q)/108 (C)601		520.5	555.2 (Q)/109 (C)	/0	10	23/4/	20/10	8.13-11.80
Chronoxacin352314 (0)/251 (C)611030/471/128.30-3.53Dandbaxacin400356 (0)/299 (C)1001035/4010/108.18-8.26Difboxacin360342 (0)/286 (C)721030/5012/128.42-8.72Humequine262.1244 (0)/202 (C)411025/4512/1210.6-11.00Marbofoxacin363345 (0)/320 (C)701030/2512/1210.4-10.80Norfloxacin363345 (0)/320 (C)601033/5012/128.29-9.28Naidikira acid233215 (0)/187 (C)601033/5012/128.29-9.28Sarafloxacin386366 (0)/348 (C)501030/4012/128.29-9.28Sarafloxacin386366 (0)/348 (C)501021/3912/127.89-8.20Sulfachioropyridazine251156 (0)/108 (C)511021/3912/127.89-8.20Sulfadiamethoxine311.1156 (0)/108 (C)601025/4012/128.29-15Sulfadiamethoxine311.1156 (0)/108 (C)601025/3512/127.89-8.20Sulfadiamethoxine311.1156 (0)/108 (C)601025/3512/128.58-8.67Sulfadiamethoxine311.1156 (0)/108 (C)601025/3512/128.29-8.68Sulfamethoxine31.1156 (0)/108 (C)601025/3512/128.29-8.68 </td <td>Cinneflausein</td> <td>222</td> <td>214(0)/221(0)</td> <td>61</td> <td>10</td> <td>20/47</td> <td>19/19</td> <td>8.02, 8.22</td>	Cinneflausein	222	214(0)/221(0)	61	10	20/47	19/19	8.02, 8.22
Dationation Dationation358340 (U) 253 (C)601035,7510/108.18~6.26Diffoxacin400356 (Q)/299 (C)101035/4010/108.48~8.72Flumequine262.1244 (Q)/202 (C)441025/4512/1210.6-11.00Marbofloxacin363345 (Q)/320 (C)701030/2210/107.89~7.98Nalidixie acid233215 (Q)/187 (C)601033/5012/127.89~8.20Oxolinic acid262244 (Q)/216 (C)501030/4012/128.82~9.15Sarafloxacin386368 (Q)/348 (C)501021/3912/127.89~8.20Suffachioropyridazine262244 (Q)/216 (C)501021/3912/128.82~9.15Sulfachioropyridazine285156 (Q)/92 (C)511021/3912/127.82~8.26Sulfadiarine311156 (Q)/108 (C)501023/3712/129.17~8.0Sulfadiarine311.1156 (Q)/108 (C)501025/3512/128.28~6.9Sulfadiarine311156 (Q)/108 (C)601035/3512/128.28~6.9Sulfadionazine311156 (Q)/108 (C)601035/3512/128.28~6.9Sulfadinethoxine311156 (Q)/108 (C)601035/3512/128.28~6.9Sulfadinethoxine311156 (Q)/108 (C)601035/3512/128.28~	Demoflowersin	352	314 (Q)/251 (C)	60	10	30/4/	12/12	8.03-8.33
Dunsacin400536 (0)/29 (C)1001053/4010/106.50-9.30Enrofloxacin360342 (0)/286 (C)721030/5012/128.42-8.72Flumequine262.1244 (0)/202 (C)701030/2210/107.89-7.98Marbofloxacin363345 (0)/187 (C)701030/3512/128.04-0.10.80Norfloxacin320302 (0)/231 (C)601033/5012/128.92-9.28Sarafloxacin386366 (0)/48 (C)531021/128.92-9.15Suffachine251156 (0)/108 (C)511021/217.82-8.26Sulfachine251156 (0)/108 (C)511021/217.82-8.26Sulfachine311.1156 (0)/108 (C)501023/3712/127.82-8.26Sulfachine311.1156 (0)/108 (C)501023/3712/128.73-9.40Sulfachine311.1156 (0)/108 (C)601035/3512/128.73-7.11Sulfamethoxine311.1156 (0)/108 (C)601035/3512/126.73-7.11Sulfamethoxine254156 (0)/108 (C)601035/3512/126.73-7.11Sulfamethoxine254156 (0)/108 (C)601025/3612/126.73-7.11Sulfamethoxine254156 (0)/108 (C)601025/3512/126.73-7.11Sulfamethoxine315156 (0)/108 (C) <td< td=""><td>Danonoxacin</td><td>358</td><td>340 (Q)/255 (C) 356 (Q)/200 (C)</td><td>100</td><td>10</td><td>33/50</td><td>10/10</td><td>8.18-8.20</td></td<>	Danonoxacin	358	340 (Q)/255 (C) 356 (Q)/200 (C)	100	10	33/50	10/10	8.18-8.20
Enrioloxatcin500542 (0)/280 (C)721050/5012/126.742-6.72Marbofloxacin262.1244 (0)/202 (C)441025/4512/1210.6-11.00Marbofloxacin363345 (Q)/320 (C)701030/3212/1210.40-10.80Nalidixia acid233215 (Q)/187 (C)421030/3512/127.89-8.20Oxolinia acid262244 (Q)/216 (C)531025/4012/128.92-9.28Sarafloxacin366368 (Q)/348 (C)501030/4012/128.92-9.28Sulfachloropyridazine285156 (Q)/92 (C)511021/3912/127.82-8.26Sulfachloropyridazine285156 (Q)/108 (C)501023/3712/128.78-8.60Sulfachinethxine311.1156 (Q)/108 (C)601025/4012/128.15-8.57Sulfamerazine265156 (Q)/20 (C)601035/3512/128.13-8.57Sulfamerazine279156 (Q)/108 (C)601035/3512/128.23-8.68Sulfamerhoxazole254186 (Q)/108 (C)601035/3512/128.23-8.68Sulfamethoxazole254156 (Q)/108 (C)601035/3512/128.23-8.68Sulfamethoxazole254160 (J)/18 (C)601025/3612/128.53-8.99Sulfamethoxazole254156 (Q)/108 (C)501023/412/12<	Enroflouooin	400	330 (Q)/299 (C)	70	10	35/40	10/10	8.98-9.30
Humedune26.1244 (0)/20 (C)441029/4512/1210.6-11.00Marbofoxacin363345 (0)/320 (C)701030/2210/107.89-798Nalidixic acid233215 (0)/187 (C)421030/3512/1210.40-10.80Norfloxacin320302 (0)/231 (C)601033/5012/127.89-798Oxolinic acid262244 (0)/216 (C)531025/4012/128.92-9.28Sarafloxacin366 (0)/348 (C)501030/4012/128.82-9.15Suffacinic251156 (0)/108 (C)531023/3012/125.58-6.00Sulfadizine251156 (0)/108 (C)531023/3712/129.17-9.60Sulfadizine311.1156 (0)/108 (C)501025/4012/128.15-8.57Sulfadizine279156 (0)/108 (C)501025/3612/126.73-7.11Sulfanethoxine311.1156 (0)/108 (C)501025/3512/126.73-7.11Sulfanethoxine279156 (0)/108 (C)501025/3512/126.73-7.11Sulfanethoxine254108 (0)/92 (C)601035/3512/128.28-8.68Sulfanethoxine256156 (0)/108 (C)5010209.35-9.45Sulfanethoxine31156 (0)/108 (C)5010209.35-9.45Sulfanethoxine301156 (0)/108 (C)5	Elironoxacin	360	342 (Q)/286 (C)	/2	10	30/30	12/12	0.42-0.72
Matronovacient353353354 (1)/320 (C)701030/2210/107.89-7.98Malidixic acid233215 (0)/187 (C)421030,35012/1210.40-10.80Norfloxacin320302 (Q)/231 (C)601033,5012/128.29-9.28Sarafloxacin386386 (Q)/348 (C)501030/012/128.82-9.15Sulfachloropyridazine285156 (Q)/92 (C)511021/3912/127.82-8.26Sulfachloropyridazine285156 (Q)/108 (C)501023/3712/129.17-9.60Sulfachvine311.1156 (Q)/108 (C)501023/3712/128.15-8.57Sulfadmethoxine311.1156 (Q)/108 (C)601025/3612/126.73-7.11Sulfadmethoxacle254156 (Q)/108 (C)601025/3512/126.73-7.11Sulfamethoxacle254156 (Q)/108 (C)601025/3512/126.73-7.11Sulfamethoxacle254156 (Q)/108 (C)601025/3512/127.04-7.42Sulfamethoxacle256156 (Q)/108 (C)501023/4012/129.15-6.51Sulfamethoxacle31515610/101023/3512/127.04-7.42Sulfamethoxacle256156 (Q)/108 (C)501023/4012/129.15-6.51Sulfamethoxacle256156 (Q)/108 (C)501023/4012/12 <td>Flumequine</td> <td>262.1</td> <td>244 (Q)/202 (C)</td> <td>44</td> <td>10</td> <td>25/45</td> <td>12/12</td> <td>10.6–11.00</td>	Flumequine	262.1	244 (Q)/202 (C)	44	10	25/45	12/12	10.6–11.00
National edd233213 (Q)/18/ (C)421030/3512/1210.40-10.80Norfloxacin320302 (Q)/231 (C)601030/35012/128.92-9.28Sarafloxacin386264 (Q)/216 (C)501030/4012/128.82-9.15Suffachioropyridazine286366 (Q)/348 (C)501021/3912/125.82-9.26Sulfachioropyridazine285156 (Q)/92 (C)511021/3912/125.88-6.00Sulfadimethoxine311.1156 (Q)/108 (C)531023/3712/129.17-9.60Sulfadimethoxine311.1156 (Q)/108 (C)601023/3512/126.22-6.59Sulfamethoxine311156 (Q)/20 (C)601035/3512/126.22-6.59Sulfamethoxine279156 (Q)/108 (C)501025/3612/126.23-6.59Sulfamethoxazole254108 (Q)/92 (C)601035/3512/126.23-6.59Sulfamethoxazole254156 (Q)/108 (C)501025/3512/126.23-6.59Sulfanethoxazole31156 (Q)/108 (C)501030129.35-9.45Sulfachinzole256156 (Q)/108 (C)501020/3412/129.14-7.42Sulfachinzole256156 (Q)/108 (C)531020/3412/129.15-6.51Sulfachinzole256156 (Q)/108 (C)531020/3412/129.5-9.	Marbonoxacin	303	345 (Q)/320 (C)	/0	10	30/22	10/10	/.89-/.98
Nortioxacin320302 (0)/231 (C)6010375012/127.89–8.20Oxolinic acid262244 (Q)/216 (C)531025/4012/128.92–9.28Sarafloxacin386368 (Q)/348 (C)511021/3912/127.82–8.26Sulfachloropyridazine285156 (Q)/92 (C)511022/3012/125.58–6.00Sulfadimethoxine311.1156 (Q)/108 (C)531023/3712/128.15–8.57Sulfadimethoxine311.1156 (Q)/108 (C)601035/3512/126.22–6.59Sulfadmethoxazole254156 (Q)/92 (C)601035/3512/126.22–6.59Sulfamethoxazole254156 (Q)/108 (C)501025/3612/128.28–8.68Sulfamethoxazole281156 (Q)/108 (C)601035/3512/128.28–8.68Sulfamethoxazole284108 (Q)/92 (C)601025/3612/128.28–8.68Sulfamethoxazole254156 (Q)/108 (C)601035/3512/128.28–8.68Sulfaquinoxaline301156 (Q)/108 (C)50102012/129.35–9.45Sulfaquinoxaline301156 (Q)/108 (C)501020/3412/129.15–6.51Sulfaquinoxaline301156 (Q)/108 (C)501021/2412/128.55–8.99Sulfaquinoxaline301156 (Q)/108 (C)501021/3412/12	Nalidixic acid	233	215 (Q)/18/ (C)	42	10	30/35	12/12	10.40-10.80
Oxinic acid262244 (Q)/216 (C)531025/4012/128.92-9.28Sarafloxacin386368 (Q)/348 (C)501030/4012/128.92-9.15Sulfachloropyridazine285156 (Q)/92 (C)511021/3912/127.82-8.26Sulfachloropyridazine251156 (Q)/108 (C)531022/3012/125.88-6.00Sulfadimethoxine311.1156 (Q)/108 (C)601025/4012/128.15-8.57Sulfadoxine311156 (Q)/108 (C)601025/3612/126.22-6.59Sulfamethazine279156 (Q)/108 (C)601025/3512/126.22-6.59Sulfamethoxapole254108 (Q)/92 (C)601025/3512/128.38-6.68Sulfaquinoxaline315156 (Q)/108 (C)601025/3512/128.38-6.68Sulfaquinoxaline301156 (Q)/108 (C)601025/3512/128.38-6.68Sulfaquinoxaline301156 (Q)/108 (C)501020/3412/129.35-9.45Sulfaquinoxaline301156 (Q)/108 (C)531020/3412/128.55-8.99Tetracycline268156 (Q)/108 (C)531020/3412/128.55-8.99Sulfaquinoxaline301156 (Q)/108 (C)531020/3412/128.55-8.99Sulfaquinoxaline301156 (Q)/108 (C)531020/3412/12<	Norfloxacin	320	302 (Q)/231 (C)	60	10	33/50	12/12	7.89-8.20
Saraitoxacin386386 (0)/348 (C)501030/4012/128.82–9.15Sulfachorapyridazine285156 (0)/92 (C)511021/3912/127.82–8.26Sulfachoropyridazine251156 (0)/108 (C)501023/3712/129.17–9.60Sulfachinethoxine311.1156 (0)/108 (C)601025/4012/128.15–8.57Sulfachinethoxine311156 (0)/108 (C)601025/3012/126.22–6.59Sulfachinethoxine279156 (0)/108 (C)601025/3512/126.23–8.68Sulfamethoxapole254108 (0)/92 (C)601025/3512/128.23–8.68Sulfamethoxapole254156 (0)/108 (C)601025/3512/128.23–8.68Sulfamethoxapole254156 (0)/108 (C)601025/3512/128.23–8.68Sulfaphenazole (IS)31156 (0)/108 (C)601025/3512/129.35–9.45Sulfaphenazole (IS)30129.35–9.45301020/3412/126.15–6.51Sulfaphenazole (IS)30126 (0)/108 (C)501020/3412/128.55–8.99Sulfactinazole266156 (0)/108 (C)501021/2312/128.55–8.99Sulfactinazole268156 (0)/130 (C)511021/248.55–8.99Sulfactinazole268156 (0)/130 (C)511021/249.1	Oxolinic acid	262	244 (Q)/216 (C)	53	10	25/40	12/12	8.92-9.28
SuironamucesSuifachloropyridazine285156 (Q)/92 (C)511021/3912/125.58~6.00Sulfadiazine311.1156 (Q)/108 (C)501023/3712/129.17~9.60Sulfadimethoxine311.1156 (Q)/108 (C)601025/4012/128.15~8.57Sulfadmerazine265156 (Q)/92 (C)601025/3512/126.22~6.59Sulfamethoxazole279156 (Q)/108 (C)601025/3512/126.73~7.11Sulfamethoxazole254186 (Q)/20 (C)601025/3512/128.23~8.68Sulfamethoxazole254156 (Q)/108 (C)601025/3512/128.23~8.68Sulfamethoxazole281156 (Q)/108 (C)601025/3512/129.35~9.45Sulfaquinoxaline301156 (Q)/108 (C)501030129.35~9.45Sulfathiazole256156 (Q)/108 (C)501021/2312/126.15~6.51Sulfaxoazole268156 (Q)/108 (C)531021/248.55~8.99Fetracyclines156 (Q)/131 (C)461021/2312/128.55~8.99Chorteracycline445428 (Q)/154.2 (C)551025/4012/129.31~9.64Doxycicline445288 (Q)/154.2 (C)551025/4012/129.51~9.82Oxyteracycline445288 (Q)/154.2 (C)551025/4012/	Sarafloxacin	386	368 (Q)/348 (C)	50	10	30/40	12/12	8.82-9.15
Sulfachloropyridazine285156 (Q)/92 (C)511021/3912/127.82-8.26Sulfadiazine251156 (Q)/108 (C)531022/3012/125.58-6.00Sulfadimethoxine311.1156 (Q)/108 (C)501023/3712/128.15-8.57Sulfadimethoxine265156 (Q)/92 (C)601025/3612/126.22-6.59Sulfametazine265156 (Q)/108 (C)501025/3612/126.73-7.11Sulfamethoxazole254108 (Q)/92 (C)601035/3512/128.23-8.68Sulfamethoxazole254108 (Q)/92 (C)601025/3512/128.74-8.66Sulfamethoxazole254108 (Q)/108 (C)601035/3512/129.35-9.45Sulfaquinoxaline301156 (Q)/108 (C)501030129.35-9.45Sulfaquinoxaline301156 (Q)/108 (C)531023/4012/129.19-9.61Sulfaxihiazole268156 (Q)/108 (C)531020/3412/126.15-6.51Sulfaxihiazole268156 (Q)/113 (C)461021/2312/128.55-8.99Tetracycline445428 (Q)/154.2 (C)551025/4012/129.31-9.64Oxytetracycline461.3201.1 (Q)/283.2 (C)411059/5312/128.07-8.40Tetracycline445410 (Q)/427 (C)551027/2512/128.	Sulfonamides	0.05	15((0) (0) (0)	- 1	10	01/00	10/10	E 00, 0 0/
Sulfadiazine251156 (Q)/108 (C)531022/3012/125.58-6.00Sulfadimethoxine311.1156 (Q)/108 (C)501023/3712/129.17-9.60Sulfadoxine311156 (Q)/108 (C)601025/4012/128.15-8.57Sulfamethazine265156 (Q)/92 (C)601035/3512/126.22-6.59Sulfamethoxazole254108 (Q)/92 (C)601035/3512/128.23-8.68Sulfamethoxypridazine281156 (Q)/108 (C)601035/3512/128.23-8.68Sulfamethoxypridazine281156 (Q)/108 (C)601030129.35-9.45Sulfaquinoxaline301156 (Q)/108 (C)501023/4012/129.19-9.61Sulfaxinozole256156 (Q)/108 (C)531020/3412/126.15-6.51Sulfaxinozole268156 (Q)/108 (C)531021/2312/128.55-8.99Tetracyclines268156 (Q)/113 (C)461021/2312/128.55-8.99Tetracycline445428 (Q)/154.2 (C)551025/4012/129.31-9.64Oxytetracycline445428 (Q)/154.2 (C)551025/4012/129.51-9.82Oxytetracycline461.3201.1 (Q)/283.2 (C)411059/5312/128.07-8.40Tetracycline445410 (Q)/427 (C)551027/2512/128.4	Sulfachloropyridazine	285	156 (Q)/92 (C)	51	10	21/39	12/12	7.82-8.26
Sulfadimethoxine311.1156 (Q)/108 (C)501023/3/12/129.1/~9.60Sulfadoxine311156 (Q)/108 (C)601025/4012/128.15-8.57Sulfametazine265156 (Q)/92 (C)601025/3512/126.22-6.59Sulfamethoxazole279156 (Q)/108 (C)601025/3512/128.23-8.68Sulfamethoxazole254108 (Q)/92 (C)601035/3512/127.04-7.42Sulfamethoxazole (IS)315156 (Q)/108 (C)601023/4012/129.35-9.45Sulfaquinoxaline301156 (Q)/108 (C)501023/4012/129.19-9.61Sulfathiazole256156 (Q)/108 (C)501023/4012/126.15-6.51Sulfathiazole256156 (Q)/108 (C)531020/3412/126.15-6.51Sulfathiazole256156 (Q)/108 (C)531020/3412/126.15-6.51Sulfathiazole256156 (Q)/13 (C)611067/5512/128.78-8.99TetracyclinesTTT7.929.82 (Q)/275 (C)611067/5512/129.31-9.64Doxycicline445428 (Q)/154.2 (C)551025/4012/129.51-9.82Oxytetracycline461.3201.1 (Q)/283.2 (C)411059/5312/128.07-8.40Tetracycline445410 (Q)/427 (C)551027/2	Sulfadiazine	251	156 (Q)/108 (C)	53	10	22/30	12/12	5.58-6.00
Sulfadoxine311156 (Q)/108 (C)601025/4012/128.15-8.57Sulfamerazine265156 (Q)/92 (C)601035/5512/126.22-6.59Sulfamethoxazole279156 (Q)/108 (C)501025/3612/128.23-8.68Sulfamethoxypyridazine281156 (Q)/108 (C)601035/3512/128.23-8.68Sulfamethoxypyridazine281156 (Q)/108 (C)601025/3512/129.35-9.45Sulfaquinoxaline301156 (Q)/108 (C)501023/4012/129.19-9.61Sulfathiazole256156 (Q)/108 (C)531020/3412/126.15-6.51Sulfathiazole268156 (Q)/108 (C)531020/3412/126.15-6.51Sulfathiazole268156 (Q)/108 (C)611027/5312/129.31-9.64Chorteracycline479.298.2 (Q)/275 (C)611067/5512/129.31-9.64Doxycicline445428 (Q)/154.2 (C)551025/4012/129.51-9.82Oxytetracycline461.3201.1 (Q)/283.2 (C)411059/5312/128.07-8.40Tetracycline445410 (Q)/427 (C)551027/2512/128.44-8.77	Sulfadimethoxine	311.1	156 (Q)/108 (C)	50	10	23/37	12/12	9.17–9.60
Sulfamerazine265156 (Q)/92 (C)601035/3512/126.22-6.59Sulfamethazine279156 (Q)/108 (C)501025/3612/126.73-7.11Sulfamethoxazole254108 (Q)/92 (C)601035/3512/128.23-8.68Sulfamethoxypyridazine281156 (Q)/108 (C)601025/3512/127.04-7.42Sulfaphenazole (IS)315156501023/4012/129.35-9.45Sulfaquinoxaline301156 (Q)/108 (C)501023/4012/129.19-9.61Sulfasoazole256156 (Q)/108 (C)531020/3412/126.15-6.51Sulfasoazole268156 (Q)/108 (C)531020/3412/128.55-8.99TetracyclinesTTTT8.25 (Q)/275 (C)611067/5512/129.31-9.64Doxycicline445428 (Q)/154.2 (C)551025/4012/129.51-9.82Oxytetracycline461.3201.1 (Q)/283.2 (C)411059/5312/128.07-8.40Tetracycline445410 (Q)/427 (C)551027/2512/128.44-8.77	Sulfadoxine	311	156 (Q)/108 (C)	60	10	25/40	12/12	8.15-8.57
Sulfamethazine2/9156 (Q)/108 (C)501025/3612/126.73-7.11Sulfamethoxazole254108 (Q)/92 (C)601035/3512/128.23-8.68Sulfamethoxypyridazine281156 (Q)/108 (C)601025/3512/129.35-9.45Sulfamethoxazole (IS)315156501030129.35-9.45Sulfaquinoxaline301156 (Q)/108 (C)501023/4012/129.19-9.61Sulfathiazole256156 (Q)/108 (C)531020/3412/126.15-6.51Sulfathiazole268156 (Q)/113 (C)461021/2312/128.55-8.99TetracyclinesTTTTTTChlortetracycline445428 (Q)/275 (C)611067/5512/129.31-9.64Oxytetracycline461.3201.1 (Q)/283.2 (C)411059/5312/128.07-8.40Tetracycline445410 (Q)/427 (C)551027/2512/128.44-8.77	Sulfamerazine	265	156 (Q)/92 (C)	60	10	35/35	12/12	6.22-6.59
Sulfamethoxazole 254 108 (Q)/92 (C) 60 10 35/35 12/12 8.23-8.68 Sulfamethoxypyridazine 281 156 (Q)/108 (C) 60 10 25/35 12/12 7.04-7.42 Sulfaphenazole (IS) 315 156 50 10 30 12 9.35-9.45 Sulfaphenazole (IS) 301 156 (Q)/108 (C) 50 10 23/40 12/12 9.19-9.61 Sulfaphenazole (IS) 268 156 (Q)/108 (C) 53 10 20/34 12/12 8.55-8.99 Sulfaphenazole (IS) 268 156 (Q)/131 (C) 46 10 21/23 12/12 8.55-8.99 Tetracyclines 79.2 98.2 (Q)/275 (C) 61 10 67/55 12/12 9.31-9.64 Doxycicline 445 428 (Q)/154.2 (C) 55 10 25/40 12/12 9.51-9.82 Oxytetracycline 461.3 201.1 (Q)/283.2 (C) 41 10 59/53 12/12 8.07-8.40 Tetracycline 445 410 (Q)/427 (C) 55 10 27/25 12/12 8.44-8.77 <td>Sulfamethazine</td> <td>2/9</td> <td>156 (Q)/108 (C)</td> <td>50</td> <td>10</td> <td>25/36</td> <td>12/12</td> <td>6./3-/.11</td>	Sulfamethazine	2/9	156 (Q)/108 (C)	50	10	25/36	12/12	6./3-/.11
Sulfamethoxypyridazine281156 (Q)/108 (C)601025/3512/127.04-7.42Sulfaphenazole (IS)315156501030129.35-9.45Sulfaquinoxaline301156 (Q)/108 (C)501023/4012/129.19-9.61Sulfathiazole256156 (Q)/108 (C)531020/3412/126.15-6.51Sulfaxoazole268156 (Q)/113 (C)461021/2312/128.55-8.99 Tetracyclines Chlortetracycline479.298.2 (Q)/275 (C)611067/5512/129.31-9.64Doxycicline445428 (Q)/154.2 (C)551025/4012/129.51-9.82Oxytetracycline461.3201.1 (Q)/283.2 (C)411059/5312/128.07-8.40Tetracycline445410 (Q)/427 (C)551027/2512/128.44-8.77	Sulfamethoxazole	254	108 (Q)/92 (C)	60	10	35/35	12/12	8.23-8.68
Sulfaphenazole (IS)31515650501030129.35-9.45Sulfaquinoxaline301156 (Q)/108 (C)501023/4012/129.19-9.61Sulfathiazole256156 (Q)/108 (C)531020/3412/126.15-6.51Sulfasoxazole268156 (Q)/113 (C)461021/3212/128.55-8.99TetracyclinesChloretracycline479.298.2 (Q)/275 (C)611067/5512/129.31-9.64Doxycicline445428 (Q)/154.2 (C)551025/4012/129.51-9.82Oxytetracycline461.3201.1 (Q)/283.2 (C)411059/5312/128.07-8.40Tetracycline445410 (Q)/427 (C)551027/2512/128.44-8.77	Sulfamethoxypyridazine	281	156 (Q)/108 (C)	60	10	25/35	12/12	7.04–7.42
Sulfaquinoxaline301156 (Q)/108 (C)501023/4012/129.19-9.61Sulfathiazole256156 (Q)/108 (C)531020/3412/126.15-6.51Sulfaoxazole268156 (Q)/113 (C)461021/2312/128.55-8.99TetracyclinesChloretracycline479.298.2 (Q)/275 (C)611067/5512/129.31-9.64Doxycicline445428 (Q)/154.2 (C)551025/4012/129.51-9.82Oxytetracycline461.3201.1 (Q)/283.2 (C)411059/5312/128.07-8.40Tetracycline445410 (Q)/427 (C)551027/2512/128.44-8.77	Sulfaphenazole (IS)	315	156	50	10	30	12	9.35-9.45
Sulfathiazole256156 (Q)/108 (C)531020/3412/126.15-6.51Sulfasxazole268156 (Q)/113 (C)461021/2312/128.55-8.99TetracyclinesChloretracyclineDoxycicline479.298.2 (Q)/275 (C)611067/5512/129.31-9.64Doxycicline445428 (Q)/154.2 (C)551025/4012/129.51-9.82Oxytetracycline461.3201.1 (Q)/283.2 (C)411059/5312/128.07-8.40Tetracycline445410 (Q)/427 (C)551027/2512/128.44-8.77	Sulfaquinoxaline	301	156 (Q)/108 (C)	50	10	23/40	12/12	9.19–9.61
Sulfsoxazole 268 156 (Q)/113 (C) 46 10 21/23 12/12 8.55-8.99 Tetracyclines V Chloretracycline 479.2 98.2 (Q)/275 (C) 61 10 67/55 12/12 9.31-9.64 Doxycicline 445 428 (Q)/154.2 (C) 55 10 25/40 12/12 9.51-9.82 Oxytetracycline 461.3 201.1 (Q)/283.2 (C) 41 10 59/53 12/12 8.07-8.40 Tetracycline 445 410 (Q)/427 (C) 55 10 27/25 12/12 8.44-8.77	Sulfathiazole	256	156 (Q)/108 (C)	53	10	20/34	12/12	6.15-6.51
TetracyclinesChlortetracycline479.298.2 (Q)/275 (C)611067/5512/129.31–9.64Doxycicline445428 (Q)/154.2 (C)551025/4012/129.51–9.82Oxytetracycline461.3201.1 (Q)/283.2 (C)411059/5312/128.07–8.40Tetracycline445410 (Q)/427 (C)551027/2512/128.44–8.77	Sulfisoxazole	268	156 (Q)/113 (C)	46	10	21/23	12/12	8.55-8.99
Chlorettracycline479.298.2 (Q)/275 (C)611067/5512/129.31-9.64Doxycicline445428 (Q)/154.2 (C)551025/4012/129.51-9.82Oxytetracycline461.3201.1 (Q)/283.2 (C)411059/5312/128.07-8.40Tetracycline445410 (Q)/427 (C)551027/2512/128.44-8.77	Tetracyclines							
Doxycicline445428 (Q)/154.2 (C)551025/4012/129.51-9.82Oxytetracycline461.3201.1 (Q)/283.2 (C)411059/5312/128.07-8.40Tetracycline445410 (Q)/427 (C)551027/2512/128.44-8.77	Chlortetracycline	479.2	98.2 (Q)/275 (C)	61	10	67/55	12/12	9.31–9.64
Oxytetracycline 461.3 201.1 (Q)/283.2 (C) 41 10 59/53 12/12 8.07-8.40 Tetracycline 445 410 (Q)/427 (C) 55 10 27/25 12/12 8.44-8.77	Doxycicline	445	428 (Q)/154.2 (C)	55	10	25/40	12/12	9.51-9.82
Tetracycline 445 410 (Q)/427 (C) 55 10 27/25 12/12 8.44–8.77	Oxytetracycline	461.3	201.1 (Q)/283.2 (C)	41	10	59/53	12/12	8.07-8.40
	Tetracycline	445	410 (Q)/427 (C)	55	10	27/25	12/12	8.44-8.77

^a RTW, retention time \pm 5% (n=20).

the method demonstrates ability to monitor these compounds, it is not capable of detecting them in concentrations below the MRL. Further studies at concentrations above the 0.5xMRL can be undertaken to determine the difference between this level and CC β .

3.3. Screening of farm fish samples

The samples collected from Brazilian fish farms were analyzed using the validated screening method for the presence of the 40 antibiotics that attended the criteria established by EC [46] and EC [37]. Twenty nine samples (15% of 193 fish samples) were positive for enrofloxacin, both tilapia and trout, from the state of Minas Gerais. None of the samples from the state of Para, both Nile tilapia and 'tambaqui', had positive results. This could result from the fish farming practices prevalent in Para. Due to the large availability of fresh water from rivers, the fishes are usually cultivated in cages inside the rivers or in large tanks (lower fish densities), which reduces the risk of spread of diseases, thereby reducing the need of antibiotics. Overall, the low occurrence of antibiotics in farm fishes can reflect the good practices adopted in most of the farms, which results in lower need for the use of antibiotics.

Among the 29 positive samples, three were trout samples from the south of Minas Gerais and 26 samples were Nile tilapia also from Minas Gerais, but different regions (metropolitan region of Belo Horizonte, 'Central Mineira' and 'Zona da Mata'). Only one sample of Nile tilapia had analyte concentration above the cut-off factor, which



Fig. 2. Total ion chromatogram of six classes of antibiotics (a) in water and (b) in the fish matrix extract. Chromatographic conditions: mobile phases A - 0.1% heptafluorobutyric acid (HFBA) in water and B - acetonitrile, at a gradient elution: initial time - 90% A; 7.0 min - 50% A; 11.0 min - 50% A; 12.0 min - 90% A; and 15 min - 90% A at a constant flow rate - 600 μ L min⁻¹.

means that this sample contained enrofloxacin in a concentration higher than the level of interest, which is $50 \ \mu g \ kg^{-1}$. The other 28 samples had trace levels of enrofloxacin (< $50 \ \mu g \ kg^{-1}$) and they should be submitted to a quantitative method for confirmation. These samples were positive for enrofloxacin below the cut-off factor.

Even though the use of enrofloxacin is forbidden in aquaculture in several countries, including Brazil [44,47,48], it was present in fish. Enrofloxacin is a fluoroquinolone antimicrobial agent with broad spectrum of activity available in the market for veterinary use and also allowed for use in aviculture in some countries [44,48]. In 2005, FDA

Table 3

Limit of detection (LOD), detection capability (CC β), sensitivity (sens.) and the comparison of cut-off factor and threshold value (F_m/T_v) for each antibiotic residue in the validated screening method.

Class/analyte	LOD (μ g kg ⁻¹)	Quantification transition			Confirmation transition			
		F_m/T_v	CCβ (µg/kg)	Sens. (%)	F_m/T_v	CCβ (µg/kg)	Sens. (%)	
Aminoglycosides								
Amikacin	1.62^{b}	$F_m > T_v$	< 250	95	$F_m > T_v$	< 250	100	
Apramycin	3.15 ^a	$F_m > T_v$	< 250	100	$F_m > T_v$	< 250	95	
Dihydrostreptomycin	1.91 ^b	$F_m > T_v$	< 250	95	$F_m > T_v$	< 250	95	
Gentamicin	3.50^{b}	$F_m > T_v$	< 250	100	$F_m > T_v$	< 250	100	
Hygromycin	29.16 ^a	$F_m > T_v$	< 250	95	$F_m > T_v$	< 250	100	
Kanamycin	4.11 ^b	$F_m > T_v$	< 250	95	$F_m > T_v$	< 250	95	
Neomycin	3.32^{b}	F > T	< 250	100	F > T	< 250	100	
Paromomycin	3.67 ^a	$F_m > T_v$	< 250	95	$F_m > T_v$	< 250	95	
Spectinomycin	20.29 ^b	$F_m > T_v$	< 250	100	$F_m > T_v$	< 250	100	
Streptomycin	6.98 ^b	F > T	< 250	100	F > T	< 250	95	
Tobramycin	2 49 ^a	$F_m > T_n$	< 250	100	F > T	< 250	100	
Beta-lactams		1 m / 1 v	1200	100	1 m / 1v	1200	100	
Ampicillin	0.83 ^b	F < T	> 25	100	F < T	> 25	100	
Cefazolin	1.88 ^b	F >T	< 25	100	F >T	< 25	100	
Oracillin	95 77 ^b	F < T	> 150	100	$\mathbf{F} < \mathbf{T}$	> 150	100	
Paniaillin C	110 60 ^b	$F_m < T_v$	> 150	100	$r_m < r_v$ E < T	> 150	100	
Ponicillin V	26 80 ^b	$\Gamma_m < \Gamma_v$ F < T	> 19 5	100	$\Gamma_{m} < \Gamma_{v}$ F < T	> 19 5	100	
Magrolidas	20.09	$\Gamma_{\rm m} < \Gamma_{\rm v}$	>12,5	100	$\Gamma_{m} < \Gamma_{V}$	/12,0	100	
Clindamycin	0.40 ^b	F \T	< 50	100	F \T	< 50	100	
Emethnomic	5.94ª	$\Gamma_m > \Gamma_v$	< 50 > 50	100	$\Gamma_m > \Gamma_v$	< 50 > 50	100	
Lincomucin	1.60b	Fm VIV	> 100	100	Fm VIV	> 100	100	
Contraction of the second seco	1.00 74.94 ^a	$\Gamma_m > \Gamma_v$	< 100	100	$\Gamma_m > \Gamma_v$	< 100 > 50	100	
Tiluisesia	/ 4.24	$r_m < I_v$	> 30	100	$r_m < r_v$	> 50	100	
Thinicosin Thata at a	1.22	$\Gamma_m > I_v$	< 100	95	$F_m > I_v$	< 100	95	
Tylosin Vinaini anno ain	13.29	$F_m < I_v$	> 100	100	$F_m < I_v$	> 100	95	
	22.80	$r_m < 1_v$	>100	100	$\mathbf{r_m} < \mathbf{I_V}$	>100	100	
Quinciones	o cch	F . T	. 50	05	Б. Л	. 50	05	
Cipronoxacin	0.56	$F_m > I_v$	< 50	95	$F_m > I_v$	< 50	95	
Danofloxacin	1.74	$F_m > T_v$	< 50	100	$F_m > T_v$	< 50	100	
Difloxacin	3.42	$F_m > T_v$	< 150	95	$F_m > T_v$	< 150	100	
Enrofloxacin	1.24	$F_m > T_v$	< 50	100	$F_m > T_v$	< 50	100	
Flumequine	9.09	$F_m > T_v$	< 300	95	$F_m > T_v$	< 300	95	
Marbofloxacin	10.02 ^a	$F_m > T_v$	< 50	95	$F_m > T_v$	< 50	95	
Nalidixic acid	0.82	$F_m > T_v$	< 20	95	$F_m > T_v$	< 20	100	
Norfloxacin	0.50 ^b	$F_m > T_v$	< 50	100	$F_m > T_v$	< 50	95	
Oxolinic acid	6.28 ^a	$F_m > T_v$	< 20	100	$F_m > T_v$	< 20	100	
Sarafloxacin	1.71 ^a	$F_m > T_v$	< 15	95	$F_m > T_v$	< 15	100	
Sulfonamides								
Sulfachloropyridazine	6.06 ^a	$F_m > T_v$	< 50	95	$F_m > T_v$	< 50	100	
Sulfadiazine	0.39 ^b	$F_m > T_v$	< 50	100	$F_m > T_v$	< 50	100	
Sulfadimethoxine	1.20^{a}	$F_m > T_v$	< 50	100	$F_m > T_v$	< 50	95	
Sulfadoxine	0.20^{a}	$F_m > T_v$	< 50	100	$F_m > T_v$	< 50	100	
Sulfamerazine	1.19 ^a	$F_m > T_v$	< 50	95	$F_m > T_v$	< 50	95	
Sulfamethazine	0.19 ^a	$F_m > T_v$	< 50	95	$F_m > T_v$	< 50	100	
Sulfamethoxazole	1.30 ^a	$F_m > T_v$	< 50	100	$F_m > T_v$	< 50	95	
Sulfamethoxypyridazine	0.54 ^b	$F_m > T_v$	< 50	100	$F_m > T_v$	< 50	100	
Sulfaquinoxaline	0.55 ^b	$F_m > T_v$	< 50	95	$F_m > T_v$	< 50	95	
Sulfathiazole	0.71 ^b	$F_m > T_v$	< 50	100	$F_m > T_v$	< 50	95	
Sulfisoxazole	$1.78^{\rm b}$	$F_m > T_v$	< 50	100	$F_m > T_v$	< 50	100	
Tetracyclines								
Chlortetracycline	34.76 ^a	$F_m > T_v$	< 100	100	$F_m > T_v$	< 100	100	
Doxycicline	2.69 ^b	$F_m > T_v$	< 100	100	$F_m > T_v$	< 100	95	
Oxytetracycline	2.60 ^a	$F_m > T_v$	< 100	95	$F_m > T_v$	< 100	95	
Tetracycline	3.64 ^b	$F_m > T_v$	< 100	95	$F_m > T_v$	< 100	100	

Analytes that do not meet the requirements for inclusion in the screening method are shown in bold.

^a Estimated from the data arising from the quantification m/z transition.

 $^{\rm b}$ Estimated from the data arising from the confirmation m/z transition.

[6] withdrew approval of its use in poultry because it could select for fluoroquinolone resistant Campylobacter. However, enrofloxacin is still approved for use in some food producing animals and companion animals [47]. It is important to consider that there could be several sources of fish contamination with antibiotics besides its administration. In the case of enrofloxacin, its use as a veterinary antibiotic, in aviculture for example, can result in its release in the environment through waste streams by which fish may be contaminated. Another source could be the direct use of enrofloxacin in aquaculture, either due to misinformation or on purpose. However, the source of contamination should be determined and educational programs implemented to warrant fish quality. Due to the health hazard associated with antibiotics abuse, there should be continuous monitoring of antibiotics in fish to warrant human health and international trade.

4. Conclusions

A screening LC-MS/MS method was optimized for the simultaneous determination of 40 antibiotics from six different classes, including aminoglycosides, beta-lactams, macrolides, quinolones, sulfonamides and tetracyclines, in fish muscle. Extraction was performed with TCA. A C18 column was used along with a gradient elution of 0.1% HFBA in water:acetonitrile. A single run of 15 min was capable of determining the presence of the compounds.

Sample preparation was simpler and faster when compared with other methods for multiclass antibiotic analysis in fish found in literature, which is desirable for routine methods. The developed method was validated according to the Guidelines for the Validation of Screening Methods for Residues of Veterinary Medicines (Initial Validation and Transfer)-Community Reference Laboratories (CRLs) 20/1/2010 and it satisfactorily fulfilled the established criteria for 40 antibiotics in fish. The method was successfully applied to real samples. Twenty nine (15%) of the 193 samples analyzed were positive for one of the 40 antibiotics (enrofloxacin), which is not allowed for use in aquaculture in Brazil. Only one sample had a concentration of enrofloxacin above the cut-off factor (50 µg kg⁻¹). This sample should proceed to quantification using a quantitative method to verify its real concentration. The low occurrence of antibiotics in farm fish suggests that there is a responsible management of aquaculture.

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