In vitro activity of antimicrobial agents with and without sulbactam, EDTA and sulbactam-EDTA on ESBLs-producing Escherichia coli isolated from healthy Tibetan yaks

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Research

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Abstract

Background

The spread of ESBLs-producing bacteria has been strikingly rapid in many regions of the world and it causes therapeutic difficulties in everyday practice. The aims of this study were to investigate the prevalence and susceptibilities of ESBLs-producing *Escherichia coli* isolates from healthy Tibetan yaks in China, to evaluate the activity of drug combinations on ESBLs-producing *E. coli* isolates.

Methods

From July 2018 to August 2019, a total of 750 nasal swab samples were tested for the presence of *E. coli* and ESBLs-producing strains. The MICs of 11 antimicrobial agents alone and combinations with sulbactam, EDTA or sulbactam-EDTA against 240 ESBLs-producing E.coli strains were determined by the broth microdilution method.

Results

Overall, 59.87% (n = 449) of the samples were positive for *E. coli*, 240 (53.45%) of 449 *E. coli* isolates were confirmed to be ESBLs-producing. The addition of sulbactam to the third generation cephalosporins, amikacin and fosfomycin for all isolates resulted in low MICs, increasing the level of susceptibility from 0, 0 and 0% to 50 ~ 87.5, 4.2 and 100% respectively. The addition of EDTA to fluoroquinolones, doxycycline, florfenicol, amikacin and fosfomycin, showed improved activities and resulted in low MICs, increasing the level of susceptibility from 0, 0, 8.3, 0 and 0% to 4.2 ~ 29.2, 33.3, 33.3, 66.7 and 45.8%, respectively. All other antibacterials (except fluoroquinolones, doxycycline and florfenicol), when combined with sulbactam-EDTA, were found to be more active than combinations only with sulbactam or with EDTA against most of isolates, with lower MIC_{50}s and MIC_{90}s.

Conclusion

In conclusion, ESBLs-producing *E. coli* isolates were widespread in healthy Tibetan yaks in China. ESBLs-producing *E. coli* isolates exhibited varying degrees of multidrug resistance. This study these findings suggested that sulbactam can enhance activity of β-lactams and some non-β-lactams of antimicrobial agents and had a synergistic effects with EDTA in improving activities of some families of antimicrobials.

Background

Nowadays, the growing frequency of antibiotic resistances is a universal problem. The emergence of multidrug resistant (MDR) strains poses several challenges to the clinical facilities [1]. The production of

β-lactamases is still the main mechanism for resistance of gram-negative bacilli to β-lactams [2]. Resistance to third generation cephalosporins is mediated by extended-spectrum β-lactamases (ESBLs), which are derivatives of narrow spectrum TEM and SHV β-lactamases [3]. Such enzymes are most commonly found in E.coli isolates from chickens and have been recently detected at high frequency in China chicken farms [4–6]. A typical characteristic of ESBLs is their ability to hydrolyze cephalosporins and aztreonam while being inhibited by β-lactamases inhibitors [7]. β-lactamase inhibitor sulbactam can greatly enhance the antibacterial activity of β-lactams [8], but MICs of their combinations against ESBLs producers were still higher than MICs against non-ESBLs-producing strains [5, 6]. Additionally, MICs of combinations on induced ESBLs producers were higher than MICs on strains before induction [9, 10]. These data suggested that β-lactam combinations with β-lactamase inhibitors could not completely resolve the problem of MICs rise that caused by ESBLs-producing and ESBLs-producing was not the only reason for MICs rise. There may be other reasons, such as AmpC β-lactamases production, membrane permeability droping, the role of active efflux, and so on. The suicide inhibitor sulbactam and cephalosporins combination has been assessed in vitro and in vivo on ESBLs-producing bacteria [8, 11–13]. Studies about effects of membrane permeability enhancers

ethylenediamine tetraacetic acid (EDTA) on activity of the antibacterial drugs have also been described [14, 15]. However, there is paucity of data on effects of sulbactam on activity of non-β-lactams and whether there is interaction between sulbactam and EDTA in affecting activity of the antibacterial drugs is unknown.

Because these antimicrobials are a significant part of drug therapy to some human bacterial infections, resistance to these drugs could ultimately pose a significant threat to human health [16]. The spread of ESBLs-producing strains has been strikingly rapid in many regions of the world and it causes therapeutic difficulties in everyday practice, therefore it would thus seem necessary to find new therapeutic weapons against this growing threat [3]. Because of the close relationships between humans and animals, thus, investigating prevalence and researching drug combinations of ESBLs-producing *E. coli* isolates in healthy Tibetan yaks is paramount for establishing guidelines for veterinary and human clinical medication use, it would be of particular significance for medical science.

This present study aimed at evaluating the activity of 11 antibacterial agents alone and in combination with sulbactam, EDTA and sulbactam-EDTA against ESBLs-producing E.coli strains respectively, analyzing the interactions among β-lactamase inhibitor sulbactam, membrane permeability enhancers EDTA and antimicrobial agents from phenotype, and providing theory basis for designing more effective antibiotic combinations for treating ESBLs-producing strain infections.

Results

Isolation and identification of *E. coli*

A total of 449 (59.87%) *E. coli* isolates were recovered from 750 nasal swab samples in healthy Tibetan yaks between 2018 and 2019.

Prevalence of ESBLs

In screening for ESBLs, 282 *E. coli* isolates were identified as suspected ESBLs producers. The detection results showed that 240 (53.45%) of 449 *E. coli* isolates were confirmed to be ESBLs-producing. Their zones produced by the discs with clavulanic acid were ≥5 mm larger than those without inhibitor in ceftazidime, ceftazidime/clavulanic acid or cefotaxime, cefotaxime/clavulanic acid. Thus, according to the CLSI criterion, 240 *E. coli* isolates produced ESBLs.

Antimicrobial susceptibility testing

The results of the antimicrobial susceptibility of 240 ESBLs-producing *E. coli* strains are listed in Table 1-4. These antimicrobial agents alone and in combinations with sulbactam, EDTA or sulbactam-EDTA, respectively, expressed as the range of MICs and the MICs at which 50% (MIC_{50}) and 90% (MIC_{90}) of strains are inhibited.

As shown in table 1, the majority of 240 ESBLs-producing *E.coli* isolates were highly resistant to fluoroquinolones (gatifloxacin, levofloxacin, enrofloxacin, ciprofloxacin), the third generation cephalosporins (ceftriaxone, cefotaxime), doxycycline, amikacin, florfenicol and fosfomycin, with MICs at which 50% of the isolates are inhibited (MIC_{50}s) of 32 to >512 µg/mL, MIC_{90}s of 64 to >512 µg/mL, and susceptibility rates of 0, 0, 0, 0, 8.3 and 0%, whereas 95.8% of strains were susceptible to the fourth generation cephalosporin cefepime (MIC_{50} and MIC_{90} 4 and 8 µg/mL, respectively). Based on MIC_{90}s, cefepime when tested alone was the most potent agent, followed by gatifloxacin, levefloxacin and doxycycline (64 µg/mL), enrofloxacin and ciprofloxacin (128 µg/mL), cefotaxime and florfenicol (256 µg/mL). Amikacin and fosfomycin were less active, exhibiting high, out-of-range MIC_{90}s. Based on findings reported above, ESBLs-producing *E. coli* strains from yaks expressed multidrug resistance (MDR).

As shown in table 2, the addition of β-lactamase inhibitors sulbactam to the third generation cephalosporins resulted in low MICs for all ESBLs-producing isolates, increasing the level of susceptibility from 0 to 50-87.5% respectively, showed that sulbactam may improve much activity of third generation cephaloporins against ESBLs-producing *E.coli* isolates in vitro. However, some among ESBLs-producing *E.coli* isolates was still found to be intrmediated (50% to Ceftriaxone, 8.3% to Cefotaxime) or resistance(4.2% to Cefotaxime). We have examined the effect of sulbactam to activity of non-β-lactam antibacterials against ESBLs-producing *E.coli* strains. To our knowledge, this is the first time that antimicromial activity of non-β-lactam antimicromials against ESBLs-producing *E.coli* of animal origin, when combined with sulbactam, have been determined. Our studies revealed that, depending on the classes of non-β-lactam antimicrobials tested, sulbactam can have synergy or no effect when it is used in combination with non-β-lactam antibacterials. Amikacin, fosfomycin and florfenicol were found to be more active against ESBLs-producing *E.coli* isolates with some exceptions, when combined with sulbactam, whereas fluoroquinolones, doxycycline was found to have similar activies. The addition of sulbactam to amikacin, or fosfomycin resulted in low MICs for all ESBLs-producing isolates, increasing

the level of susceptibility from 0 and 0% to 4.2 and 100% respectively. Although the addition of sulbactam to florfenicol also had the effect of lowering the MICs in all but very few strains on which florfenicol alone and in combinations with sulbactam were found to yield the same MIC, the susceptibility level remained unchanged. Compared with the drugs alone, MICs of fluoroquinolones and doxycycline combinations with sulbactam had no significant changes and their MIC_{50}s and MIC_{90}s on all tested isolates when combined with sulbactam was either identical or within one doubling dilution of the MIC_{50}s and MIC_{90}s of drugs alone. The reason for this phenomenon could be due to the intrinsic activies of sulbactam and synergistic effect between sulbactam and amikacin, fosfomycin and florfenicol.

As shown in table 3, the addition of EDTA to fluoroquinolones, doxycycline and florfenicol showed improved activities and resulted in low MICs for nearly all tested isolates (MIC_{50} changes were from 32-64, 32 and 128 to 4-8, 8 and 16 μg/mL, respectively), increasing the level of susceptibility from 0, 0, 8.3% to 4.2-29.2, 33.3 and 33.3% respectively. Of 240 ESBLs-producing isolates, 200 (83.3%) and 180 (75%) exhibited amikacin and fosfomycin combination with EDTA low MICs (MIC_{50} changes were from from >512 and >512 to 16 and 128 μg/mL for fosfomycin, respectively), susceptibility level rose from 0 and 0% to 66.7 and 45.8% respectively. Although the addition of EDTA to the third generation cephalosporins showed improved activities and resulted in low MICs for some isolates, the susceptibility level remained unchanged.

As shown in table 4, in the present study, we also investigated the activity of sulbactam-EDTA based combinations against ESBLs-producing *E.coli* strains. All these antibacterial agents, when combined with sulbactam-EDTA, were more active than combinations only with sulbactam against most of the tested isolates, with lower MIC_{50}s and MIC_{90}s. With the exception of fluoroquinolones, doxycycline and florfenicol, all other drugs (the third generation cephaloporins, amikacin and fosfomycin) when combined with sulbactam-EDTA were also found to be more active than combinations only with EDTA against most of the tested isolates, with lower MIC_{50}s and MIC_{90}s. Among the various combinations containing EDTA, susceptibility to fluoroquinolones, doxycycline and florfenicol combinations with sulbactam-EDTA was greater than that to respective combination only with EDTA (the susceptibility level increased of from 4.2-29.2, 33.3 and 33.3% to 4.2-50, 79.2 and 70.8%, respectively), but their MIC_{50}s and MIC_{90}s on all tested isolates when combined with sulbactam-EDTA was either identical or within one doubling dilution of the MIC_{50}s and MIC_{90}s of combinations only with EDTA.

Discussion

This is the first study to investigate the prevalence and drug combinations on ESBLs-producing *E. coli* isolated from healthy Tibetan yaks. As shown in table 1, the emergence of multidrug resistant (MDR) strains, these finding is in accordance with our previous studies [4–6]. This MDR is due to the coexistence of genes encoding drug resistance to other antibiotics on the plasmids which encode ESBLs [17]. In our study, varying range of resistance, from 83.3 to 100% for third generation cephaloporins has been observed. This resistance is due to the hydrolysis of β-lactam ring of lactam antibiotics by the action of

ESBLs. Other mechanism of drug resistance to β-lactam group of antibiotics are loss of outer membrane protein, active efflux action and AmpC β-lactamase production. Amongst the mechanisms of resistance to third generation cephaloporins in gram-negative bacilli, production of ESBLs and AmpC β-lactamase are the most common [18, 19]. In general, ESBLs resulted in very high levels of extended spectrum cephaloporins resistance, whereas hyperproduction of AmpC β-lactamase and/or impermeability/efflux function gave lower levels resistance [20].

The effect of β-lactam/β-lactamase inhibitor combinations varied depending on the subtype of ESBLs present[17]. Some TEM-derived β-lactamases which are resistant to β-lactamase inhibitor (inhibitor-resistant TEMs or IRTs) have beeen described [21, 22]. Also, there is limited clinical experience with use β-lactam/β-lactamase inhibitor in treating animal infection with ESBLs-producing organisms. Because of these variables, β-lactam/β-lactamase inhibitor combinations should not be considered as first line of therap and it would seem necessary to find new therapeutic weapons against this growing threat of MDR in ESBLs-producing isolates. The antibacterial activies of sulbactam was not determined in the present study and its intrinsic activies against ESBLs-producing E.coli isolates should not be excluded. The intrinsic activies of β-lactamase inhibitors, has already been described for some important human pathogens [23, 24], with sulbactam being the most effective, but there is still paucity of data on animal pathogens. The phenomenon of sulbactam's enhancing activity of some non-β-lactam antimicromial agents was particularly interesting from a scientific perspective, as it may indicate a basis for designing more effective antibiotic combinations for treating ESBLs-producing bacterial infections. Further investigations, such as measuring the fractional inhibitory concentration (FIC) index of each combination by checkerboard testing and the killing curve test, should be carried out to understand interactions between sulbactam and non-β-lactam antimicromials and to explore potential clinical implications in veterinary medicine.

In the present study, synergism between EDTA and many antimicrobials have been widely reported against P. aeruginosa and E. coli [14], but activities of the combinations with EDTA against ESBL-producing organisms and their clinical experience is still limited [15]. Our results showed that EDTA may be involved in improved susceptibility of ESBL-producing isolates to antimicrobial agents and MICs reductions are because of the loss of the barrier function of the outer membrane. There are many hydrophilic protein channels located throughout the cytoderm and cell membrane of bacteria, which are nonselective for hydrophilic substances through the cell. It is very important for the hydrophilic drugs (e.g. levofloxacin and doxycycline) to cross into or out of the cell. Moreover, the declination of the outer membrane permeability and the increase of the drug efflux pump have been involved in the mechanism of the MDR [25]. Meanwhile, EDTA can chelate the divalent cations (e.g. Ca^{2+} Mg^{2+}), which are the essential component to maintain the structure and function of cell membrane, to result in the increase of the cell mobility and permeability, this explains the mechanism of its anti-multiple drug-resistance. The work suggested combinations of antimicrobials with EDTA may be beneficial in the treatment of infections caused by ESBLs-producing E.coli strains. Further clinical studies or animal models of infection are needed to confirm efficacy and safety of antimicrobials-EDTA combinations.

In this study, these findings suggested that sulbactam and EDTA had synergism in improved activities of antimicrobials mentioned above (cephaloporins, amikacin and fosfomycin). Meanwhile, these results presented here also implied that there was synergy between specific resistant mechanism (producing β-lactamase) and nonspecific resistant mechanism (the declination of the outer membrane permeability). This synergy is worth further investigation to elucidate the precise mechanism responsible for this effect and to explore its therapeutic potential in veterinary clinical.

Conclusion

This study presents the first insight into the prevalence, antimicrobial resistance, and drug combinations on ESBLs-producing *E. coli* isolated from healthy Tibetan yaks. The high prevalence and ESBLs-producing of *E. coli* highlights the importance of effective animal hygiene measures to prevent the further spread of *E. coli*. It is important to consider the prevalence of *E. coli* in yaks and the risk of its transmission through the food chain. In a word, we have demonstrated that sulbactam can enhance activity of β-lactams and some non-β-lactams of antimicrobial agents (amikacin and fosfomycin) and had a synergistic effects with EDTA in improving activities of the third generation cephaloporins, amikacin and fosfomycin. Future epidemiological investigations should be conducted with larger number of strains and samples.

Methods

Collection of samples

From July 2018 to August 2019, a total of 750 nasal swab samples from healthy Tibetan yaks in eight large-scale farms were collected. The samples were immediately transported to the laboratory under required preservation conditions (in a cooler with ice) within 6 h of collection, and processed within 2 h for samples to test the presence of *E. coli* [16].

Isolation and identification of E. coli

Isolation and identification of *E. coli* were performed by enrichment and sequential plating onto selective plates, as previously described [16]. The samples were incubated in LB Broth (Beijing Land Bridge Technology Co., Ltd, Beijing, China) at 37 °C overnight for 12 h, and draw the line on MacConkey agar plateafter dipping the culture the next day. All presumptive *E. coli* colonies were identified using VITEK 2 compact automated identification system (BioMérieux, Marcy-l'Etoile, France). Reference strains *E. coli* ATCC 25922, *K. pneumoniae* ATCC 25923 and K. pneumoniae ATCC 700603 were used as quality control strains. For all the strains, 3 μl bacteral culture incubated at 37 ℃ for 12 h were diluted in Mueller-Hinton broth to obtain a starting inoculum at 10^5 cfu/mL.

Detection of ESBLs-producing strains

ESBLs were detected by the reference double-disc diffusion test method by the Clinical and Laboratory Standards Institute (CLSI) [26], using discs of cefotaxime (30 µg) and ceftazidime (30 µg) and discs of cefotaxime plus clavulanic acid (30 and 10 µg) and ceftazidime plus clavulanic acid and discs of cefotaxime plus clavulanic acid (30 and 10 µg) and ceftazidime plus clavulanic acid (0.5 McFarland inoculum size) of suspected ESBLs producing clinical isolates on Mueller-Hinton Agar (MHA). *E. coli* ATCC 25922 was used as the negative control and *K.pneumoniae* ATCC 700603 was used as the ESBLs positive control. ESBLs production was inferred if the inhibition zone increased by 5 mm towards the cefotaxime plus clavulanic acid disc or ceftazidime plus clavulanic acid disc in comparison to the third generation cephalosporin disc alone.

Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) of 11 antimicrobial agents against the 240 ESBLs-producing *E. coli* strains, were determined by the broth microdilution method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) guidelines [26]. The stock solutions of all antimicrobial compounds were prepared to a final concentration of 5120 µg/mL. Each antimicrobial solution was sterilized by filtration using 0.2 µm-pore size filters. MICs for gatifloxacin, levofloxacin, enrofloxacin, ciprofloxacin, ceftriaxone, cefotaxime, cefepime, doxycycline, amikacin, florfenicol, fosfomycin alone and their respective combinations with either sulbactam or EDTA or sulbactam-EDTA were determined. Sulbactam was added to these antimicrobial drugs at a fixed ratio of 2:1 and EDTA at a fixed concentration of 2560 µg/mL which was a twofold concentration below the respective MIC for all isolates.

E. coli ATCC 25922 was used as a reference strain for quality control in the MIC determinations. The MIC_{50} and MIC_{90} were determined which represent concentrations of the relevant antibiotics which inhibited growth of the bacteria by 50% or 90% respectively. The MIC breakpoints for most antimicrobial agents were in accordance with CLSI [26, 27].

Data analysis

The 240 isolated strains were categorized as sensitive (S), resistant (R) based on the MIC values and the CLSI interpretive criteria.

Abbreviations

E. coli
Escherichia coli; CLSI:clinical and laboratory standards institute; ESBLs:extended-spectrum β-lactamases; MICs:minimum inhibitory concentrations; MDR:Multidrug resistant; EDTA:ethylenediamine tetraacetic acid

Declarations

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Availability of data and materials

The data supporting the findings of this study are included within the manuscript and its supporting information.

Authors' contributions

BGL, MSM and EPX participated in study conception, design and prepared the manuscript. MB, XLY, BGL and YHC participated in sample collection and performed the experiments. DDH, XHL and GZH analyzed result and reviewed the manuscript. MSM and EPX revised the manuscript and coordinated the whole project. All authors read and reviewed the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

With regards to our study's use of animals, this study protocol was reviewed and approved by the Henan University of Chinese Medicine animal ethics committee, and the experiment was performed in accordance with the regulations and guidelines established by this committee. The owners of the farm animals from which samples were taken gave permission for their animals to be used in this study.

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Tables

Table 1. In vitro activities of 11 antimicrobial agents alone against ESBLs-producing *E.coli* strains (*n*=240).

Antimicrobial agents	MIC (μg/mL)			S (%)	I (%)	R (%)
	MIC range	MIC_{50}	MIC_{90}			
Gatifloxacin	16-256	64	64	0%	0%	100%
Levofloxacin	8-256	64	64	0%	0%	100%
Enrofloxacin	16-256	32	128	0%	0%	100%
Ciprofloxacin	16-256	64	128	0%	0%	100%
Ceftriaxone	128->512	256	512	0%	0%	100%
Cefotaxime	16-512	128	256	0%	16.7%	83.3%
Cefepime	0.5-16	4	8	95.8%	4%	0%
Doxyeyeline	16-128	32	64	0%	0%	100%
Amikacin	128->512	>512	>512	0%	0%	100%
Florfenicol	2->512	128	256	8.3%	0%	91.7%
Fosfomyein	256->512	>512	>512	0%	0%	100%

Note: The initial concentration of all the antibacterial agents were 5120 μg/mL

Table 2. In vitro activities of 11 antimicrobial agents combined with sulbactam against ESBLs-producing

ESBLs-producing *E.coli* strains (*n*=240).

Antimicrobial agents	MIC (μg/mL)			S (%)	I (%)	R (%)
	MIC range	MIC_{50}	MIC_{90}			
Gatifloxacin/sulbactam (2:1)	16-256	64	64	0%	0%	100%
Levofloxacin/sulbactam (2:1)	8-128	32	64	0%	0%	100%
Enrofloxacin/sulbactam (2:1)	16-256	32	128	0%	0%	100%
Ciprofloxacin/sulbactam (2:1)	8-256	32	64	0%	0%	100%
Ceftriaxone/sulbactam (2:1)	4-32	8	16	50%	50%	0%
Cefotaxime/sulbactam (2:1)	2-64	8	16	87.5%	8.3%	4.2%
Cefepime/sulbactam (2:1)	0.5-8	2	4	100%	0%	0%
Doxyeyeline/sulbactam (2:1)	16-128	32	32	0%	0%	100%
Amikacin/sulbactam (2:1)	16-128	64	64	4.2%	12.5%	83.3%
Florfenicol/sulbactam (2:1)	2-256	32	64	8.3%	12.5%	78.2%
Fosfomyein/sulbactam (2:1)	16-64	32	64	100%	0%	0%

Note: The drug concentration is calculated as the first drug

Table 3. In vitro activities of 11 antimicrobial agents combined with EDTA against ESBLs-producing *E.coli* strains (*n*=240). [a] EDTA at a fixed concentration of 2560 μg/mL which was a twofold concentration below the respective MIC for all isolates (2:1)

Antimicrobial agents	MIC (μg/mL)			S (%)	I (%)	R (%)
	MIC range	MIC_{50}	MIC_{90}			
Gatifloxacin/EDTA[a]	2-32	4	8	29.2%	33.3%	37.5%
Levofloxacin/EDTA	2-16	8	16	12.5%	8.3%	79.2%
Enrofloxacin/EDTA	2-32	8	32	8.3%	12.5%	79.2%
Ciprofloxacin/EDTA	1-64	8	8	4.2%	8.3%	91.7%
Ceftriaxone/EDTA	128-512	128	256	0%	0%	100%
Cefotaxime/EDTA	16-256	64	64	0%	0%	100%
Cefepime/EDTA	1-8	4	8	100%	0%	0%
Doxyeyeline/EDTA	1-16	8	8	33.3%	58.6%	8.3%
Amikacin/EDTA	4->512	16	>512	66.7%	0%	33.3%
Florfenicol/EDTA	1-64	16	32	33.3%	37.5%	29.2%
Fosfomyein/EDTA	16->512	128	512	45.8%	29.2%	25.0%

[a] EDTA at a fixed concentration of 2560 μg/mL which was a twofold concentration below the respective MIC for all isolates (2:1)

Table 4. In vitro activities of 11 antimicrobial agents combined with sulbactam-EDTA against ESBLs-producing *E.coli* strains (*n*=240). [b] EDTA at a fixed concentration of 2560 μg/mL which was a twofold concentration below the respective MIC for all isolates (2:1:1)

Antimicrobial agents	MIC (μg/mL)			S (%)	I (%)	R (%)
	MIC range	MIC_{50}	MIC_{90}			
Gatifloxacin/sulbactam/EDTA[b]	1-32	2	16	50%	12.5%	37.5%
Levofloxacin/sulbactam/EDTA	1-32	4	16	20.8%	41.7%	37.5%
Enrofloxacin/sulbactam/EDTA	1-64	8	16	16.7%	0%	83.3%
Ciprofloxacin/sulbactam/EDTA	1-32	8	16	4.2%	29.2%	66.7%
Ceftriaxone/sulbactam/EDTA	0.5-16	1	8	91.7%	8.3%	0%
Cefotaxime/sulbactam/EDTA)	0.25-16	1	4	95.8%	4.2%	0%
Cefepime/sulbactam/EDTA	0.25-4	0.5	2	100%	0%	0%
Doxyeyeline/sulbactam/EDTA	2-8	4	8	79.2%	20.8%	0%
Amikacin/sulbactam/EDTA	0.5-32	8	32	83.3%	16.7%	0%
Florfenico/sulbactaml/EDTA	0.5-32	8	16	70.8%	20.8%	8.3%
Fosfomyein/sulbactam/EDTA	1-32	8	32	100%	0%	0%

[b] EDTA at a fixed concentration of 2560 μg/mL which was a twofold concentration below the respective MIC for all isolates (2:1:1)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementarymaterialTABLES1.pdf
- Coverletter.doc