



Short Communication

Activity of ceftolozane/tazobactam against *Pseudomonas aeruginosa* and Enterobacterales isolates recovered from intensive care unit patients in Spain: The SUPERIOR multicentre study



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ABSTRACT

Patients in intensive care units (ICUs) present a high risk of developing an infection caused by multidrug-resistant bacteria. Consequently, new antimicrobials and combinations are required. In this study, the activity of ceftolozane/tazobactam (C/T) was evaluated against Enterobacterales ($n=400$) and *Pseudomonas aeruginosa* ($n=80$) clinical isolates collected from patients in Spanish ICUs with complicated urinary tract infections (cUTI) and complicated intra-abdominal infections (cIAI). Overall susceptibility to C/T in *P. aeruginosa* isolates by infection type was 95.7% in cUTI (MIC_{50/90}, 1/4 mg/L) and 85.3% in cIAI (MIC_{50/90}, 1/64 mg/L). Activity against *P. aeruginosa* was maintained regardless of its resistance pattern, confirming that C/T is one of the best antipseudomonal agents along with colistin and amikacin. Susceptibility to C/T in Enterobacterales by infection type was 79.5/81.9% and 89.3/92.3% (EUCAST/CLSI) in cIAI and cUTI isolates, respectively. Activity was excellent against wild-type organisms, with 100% susceptible and inhibited at MIC ≤ 1 mg/L. Nevertheless, C/T susceptibility decreased against extended-spectrum β -lactamase (ESBL)-producing isolates: *Escherichia coli* (80.4/84.8% susceptible by EUCAST/CLSI) and *Klebsiella pneumoniae* (59.1/77.3% susceptible by EUCAST/CLSI). No activity of C/T was observed in carbapenemase-producing isolates. The *in vitro* activity of C/T observed in this surveillance study suggests that this agent can be considered as a therapeutic option for cUTI and cIAI due to Enterobacterales and *P. aeruginosa* in ICU patients, particularly when carbapenemase-producing isolates are not involved.

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1. Introduction

Multidrug-resistant (MDR) bacteria represent not only a worldwide problem in terms of increased patient morbidity and mortality but also a huge extra cost to healthcare systems [1]. Deaths attributed to antimicrobial resistance are expected to surpass those

caused by cancer by 2050, with a total estimate of more than 10 million deaths [2]. Percentages of resistant bacteria in intensive care units (ICUs) vary between countries and hospitals, but surveillance reports from the European Centre for Disease Prevention and Control (ECDC) give an idea of the dimension of the problem in ICUs: oxacillin resistance in 23.1% of *Staphylococcus aureus*; ceftazidime resistance in 23.7% of *Pseudomonas aeruginosa*; and carbapenem resistance in 11.3% and 23.7% of *Klebsiella* spp. and *P. aeruginosa*, respectively. These figures may be higher in Spain [3].

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Patients in ICUs are at increased risk of developing an infection caused by antimicrobial-resistant bacteria owing to prolonged hospital stay, use of invasive devices and antibiotic exposure [4]. This supports the use of new drugs or combinations [5]. Ceftolozane/tazobactam (C/T), a new β -lactam/ β -lactamase inhibitor combination, combines a cephalosporin (ceftolozane), which is less affected by AmpC β -lactamases, porin loss or efflux systems in *P. aeruginosa*, and a β -lactamase inhibitor (tazobactam) that improves the activity against extended-spectrum β -lactamase (ESBL)-producing Enterobacterales. This combination has been approved for complicated urinary tract infections (cUTI), including pyelonephritis, and complicated intra-abdominal infections (cIAI), the latter in combination with metronidazole [6,7].

The aim of the SUPERIOR study was to assess the *in vitro* activity of C/T and comparator antimicrobials against *P. aeruginosa* and Enterobacterales clinical isolates prospectively collected from ICU patients with cUTI and cIAI in Spain.

2. Materials and methods

2.1. Study design and hospital participants

A prospective multicentre study was designed to assess the *in vitro* activity of C/T and comparator antimicrobials against clinical isolates of Enterobacterales and *P. aeruginosa* prospectively recovered in Spanish ICUs between April 2016 and April 2017. Eight hospitals participated in the study, namely Universitario Ramón y Cajal (co-ordinator laboratory) (Madrid), General Universitario Gregorio Marañón (Madrid), Hospital Clínic (Barcelona), Universitario Virgen Macarena (Seville), Universitario Marqués de Valdecilla (Santander), Complejo Hospitalario Universitario (A Coruña), General Universitario (Valencia) and Universitario Son Espases (Palma de Mallorca).

A total of 480 Enterobacterales and *P. aeruginosa* isolates from intra-abdominal and urine specimens of patients admitted to ICUs with a clinical diagnosis of cIAI or cUTI were sent to the co-ordinator laboratory to confirm identification by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (Bruker Daltonik GmbH, Bremen, Germany) and to perform standard antimicrobial susceptibility testing. The study was approved by the Ethical Committee of Hospital Universitario Ramón y Cajal and the Spanish Medicines Agency.

2.2. Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) of C/T and comparators were determined by the standard broth microdilution method using frozen 96-well plates (Thermo Fisher Scientific, Cleveland, OH). The antimicrobial concentrations tested were as follows: amikacin (AMK), 8–32 mg/L; amoxicillin/clavulanic acid, 8/2–16/2 mg/L; aztreonam, 0.12–32 mg/L; cefepime (FEP), 0.12–64 mg/L; cefotaxime, 0.12–64 mg/L; ceftazidime (CAZ), 0.12–64 mg/L; C/T, 0.12/4–64/4 mg/L; ciprofloxacin, 0.06–2 mg/L; colistin (COL), 2–4 mg/L; fosfomycin, 32–128 mg/L; gentamicin, 2–8 mg/L; imipenem (IPM), 0.12–32 mg/L; meropenem (MEM), 0.12–32 mg/L; piperacillin/tazobactam (TZP), 8/4–64/4 mg/L; tigecycline (TGC), 1–2 mg/L; and tobramycin, 2–8 mg/L. The results were interpreted using European Committee on Antimicrobial Susceptibility Testing (EUCAST) [8] and Clinical and Laboratory Standards Institute (CLSI) breakpoints [9].

Escherichia coli ATCC 25922, *E. coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 700603 and *P. aeruginosa* ATCC 27853 were used as quality control strains for antimicrobial susceptibility testing, and results were according to published EUCAST and CLSI ranges [8,9].

2.3. Phenotypic classification

Enterobacterales isolates were classified into different phenotypes according to their susceptibility to β -lactam antibiotics. EUCAST epidemiological cut-off values (ECOFFs) (<http://www.eucast.org>) were used to separate wild-type (WT) from non-WT isolates (those with acquired resistance mechanisms). The following phenotypes were defined: (i) ESBL phenotype, MIC \geq 2 mg/L for cefotaxime, ceftazidime and/or cefepime in addition to a positive phenotypic confirmation with the double-disk synergy test; (ii) carbapenemase phenotype, MICs higher than or equal to carbapenem ECOFFs in addition to positive carbapenemase results using the ROSCO KPC/MBL and OXA-48 Confirm Kit (Rosco Diagnostica A/S, Taastrup, Denmark); and (iii) AmpC-like (AmpC hyperproduction or plasmid AmpC) phenotype, reduced susceptibility to extended-spectrum cephalosporins in isolates not characterised as ESBL or carbapenemase phenotypes. Cefoxitin resistance was used to confirm this phenotype [10].

P. aeruginosa isolates were classified as susceptible or non-susceptible (NS) to antipseudomonal agents according to EUCAST interpretative criteria. In addition, the following resistance phenotypes were defined: TZP/CAZ-NS; TZP/CAZ/MEM-NS; and MDR (combined resistance to all agents tested, with the exception of AMK and/or COL).

2.4. Carbapenemase detection

In *P. aeruginosa* isolates displaying a C/T resistance pattern and in Enterobacterales isolates with a carbapenemase phenotype, carbapenemase genes were investigated using the Cepheid Xpert® Carba-R Assay (Cepheid, Sunnyvale, CA).

3. Results

3.1. Bacterial isolates

A total of 400 Enterobacterales isolates and 80 *P. aeruginosa* were collected from different ICUs in Spain. Regarding the infection type, 280 isolates (58.3%) were recovered from cUTI (83.6% Enterobacterales and 16.4% *P. aeruginosa*) and 200 (41.7%) were recovered from cIAI (83.0% Enterobacterales and 17.0% *P. aeruginosa*). The distribution by source in cIAI isolates was peritoneal fluid (47%), abdominal abscess (16.5%), bile (14%), wound (10.5%), abdominal drainage (10%) and liver abscess (2%).

3.2. *Pseudomonas aeruginosa* isolates

Among the 80 *P. aeruginosa* isolates, overall C/T susceptibility was 91.3% (MIC_{50/90}, 1/4 mg/L), with COL (95.0% susceptible) and AMK (88.8/93.8% susceptible by EUCAST/CLSI) being the most active agents tested (Table 1). Moreover, based on MIC_{50/90} values, C/T was at least four two-fold dilutions more active than TZP, at least three two-fold dilutions more active than CAZ and FEP, and at least one two-fold dilution more active than IPM. The activity of C/T and other comparators by resistance phenotype is shown in Table 2. Among isolates resistant to different antipseudomonal agents, between 78.8% and 87.9% were susceptible to C/T, whilst susceptibility to comparators did not exceed 25%, with the exception of COL that was active in >91% of the isolates. Against MDR isolates, C/T displayed a susceptibility rate of 88.2%. C/T was inactive against 7 isolates (8.8%): CAZ/MEM-NS ($n=3$; MIC > 64 mg/L); TZP/CAZ/MEM-NS ($n=2$; MIC > 64 mg/L); and MDR isolates ($n=2$; MIC range 16 mg/L to >64 mg/L); 6 of the 7 expressed a VIM metallo- β -lactamase (MBL) phenotype (MIC range 64 mg/L to >64 mg/L). The only C/T-resistant isolate that was not a MBL-producer expressed an MIC of 16 mg/L. Resistance rates to C/T were 0.0% in

Table 1

Antimicrobial activity of ceftolozane/tazobactam (C/T) and comparator antimicrobials tested against *Pseudomonas aeruginosa* isolates ($n = 80$) from patients in Spanish intensive care units.

| Antimicrobial agent | MIC (mg/L) | | | EUCAST | | | CLSI | | |
|---------------------|-------------------|-------------------|--------------|----------------|------|------|------|------|------|
| | MIC ₅₀ | MIC ₉₀ | Range | %S | %I | %R | %S | %I | %R |
| AMC | >16 | >16 | ≤8 to >16 | – ^a | – | – | – | – | – |
| TZP | 16 | >64 | ≤8 to >64 | 60.0 | – | 40.0 | 60.0 | 17.5 | 22.5 |
| C/T | 1 | 4 | 0.25 to >64 | 91.3 | – | 8.8 | 91.3 | 0.0 | 8.8 |
| Ceftazidime | 8 | 64 | 1 to >64 | 55.0 | – | 45.0 | 55.0 | 13.8 | 31.3 |
| Cefotaxime | >64 | >64 | ≤0.12 to >64 | – | – | – | – | – | – |
| Cefepime | 8 | 32 | ≤0.12 to >64 | 58.8 | – | 41.3 | 58.8 | 22.5 | 18.8 |
| Aztreonam | 8 | 32 | ≤0.12 to >32 | 2.5 | 67.5 | 30.0 | 55.0 | 15.0 | 30.0 |
| Imipenem | 2 | 32 | ≤0.12 to >32 | 55.0 | – | 40.0 | 50.0 | 5.0 | 45.0 |
| Meropenem | 1 | 32 | ≤0.12 to >32 | 58.8 | 17.5 | 23.8 | 58.8 | 5.0 | 36.3 |
| Ciprofloxacin | 0.5 | >2 | ≤0.06 to >2 | 57.5 | 7.5 | 35.0 | 65.0 | 1.3 | 33.8 |
| Gentamicin | ≤2 | >8 | ≤2 to >8 | 63.8 | – | 36.3 | 63.8 | 3.8 | 32.5 |
| Tobramycin | ≤2 | >8 | ≤2 to >8 | 65.0 | – | 35.0 | 65.0 | 1.3 | 33.8 |
| Amikacin | ≤8 | 16 | ≤8 to >32 | 88.8 | 5.0 | 6.3 | 93.8 | 3.8 | 2.5 |
| Colistin | ≤2 | ≤2 | ≤2 to >4 | 95.0 | – | 5.0 | 95.0 | 2.5 | 2.5 |
| Fosfomycin | >128 | >128 | ≤32 to >128 | – | – | – | – | – | – |
| Tigecycline | >2 | >2 | ≤1 to >2 | – | – | – | – | – | – |

MIC, minimum inhibitory concentration; MIC_{50/90}, MIC that inhibits 50% and 90% of isolates, respectively; EUCAST, European Committee on Antimicrobial Susceptibility Testing; CLSI, Clinical and Laboratory Standards Institute; S, susceptible; I, intermediate; R, resistant; AMC, amoxicillin/clavulanic acid; TZP, piperacillin/tazobactam.

^a – indicates no published interpretative criteria.

Table 2

Activity of ceftolozane/tazobactam (C/T) and comparator antimicrobials by resistance phenotype in *Pseudomonas aeruginosa* isolates from patients in Spanish intensive care units.

| Resistance phenotype | C/T | | TZP | | CAZ | | MEM | | COL | |
|---------------------------------------|----------------------|------|----------------------|------|----------------------|------|----------------------|------|----------------------|------|
| | MIC _{50/90} | %S |
| All <i>P. aeruginosa</i> ($n = 80$) | 1/4 | 91.3 | 8/64 | 55.0 | 8/64 | 55.0 | 1/32 | 58.8 | ≤2/≤2 | 95.0 |
| TZP-NS ($n = 32$) | 2/16 | 87.9 | – | – | 32/>64 | 6.3 | 8/>32 | 21.9 | ≤2/≤2 | 96.9 |
| CAZ-NS ($n = 36$) | 2/64 | 80.6 | 64/>64 | 16.7 | – | – | 8/>32 | 25.0 | ≤2/≤2 | 91.7 |
| MEM-NS ($n = 33$) | 2/64 | 78.8 | 8/>64 | 12.1 | 32/>64 | 18.2 | – | – | ≤2/≤2 | 93.9 |
| TZP/CAZ-NS ($n = 30$) | 2/16 | 86.7 | – | – | – | – | 8/>32 | 23.3 | ≤2/≤2 | 96.7 |
| TZP/CAZ/MEM-NS ($n = 23$) | 2/>64 | 82.6 | – | – | – | – | – | – | ≤2/≤2 | 95.7 |
| MDR ($n = 17$) | 2/16 | 88.2 | 64/>64 | 0 | 32/>64 | 0 | 8/>32 | 0 | ≤2/≤2 | 100 |

TZP, piperacillin/tazobactam; CAZ, ceftazidime; MEM, meropenem; COL, colistin; MIC_{50/90}, minimum inhibitory concentration that inhibits 50% and 90% of isolates, respectively; %S, percent susceptible; NS, non-susceptible; MDR, multidrug-resistant.

four of eight participant hospitals, and resistance ranged from 5.3% to 25.0% in the remaining centres (data not shown).

Overall C/T susceptibility in *P. aeruginosa* isolates by infection type was 95.7% in cUTI (MIC_{50/90}, 1/4 mg/L) and 85.3% in cIAI (MIC_{50/90}, 1/64 mg/L).

3.3. Enterobacteriales isolates

The overall distribution of species was as follows: *E. coli* ($n = 209$; 52.3%); *Klebsiella* spp. group ($n = 95$; 23.8%); *Enterobacter* spp. ($n = 33$; 8.3%), *Proteus* spp. ($n = 19$; 4.8%); *Morganella morganii* ($n = 16$; 4.0%); *Citrobacter* spp. ($n = 9$; 2.3%); *Serratia marcescens* ($n = 9$; 2.3%); *Hafnia alvei* ($n = 5$; 1.3%); *Providencia stuartii* ($n = 3$; 0.8%); *Kluyvera ascorbata* ($n = 1$; 0.3%); and *Salmonella enterica* ($n = 1$; 0.3%). A summary of MIC data in Enterobacteriales broken down by major species and phenotypes is shown in Table 3. MIC distributions of C/T against Enterobacteriales by resistance phenotype are presented in Supplementary Table S1.

In *E. coli* isolates ($n = 209$), C/T demonstrated good overall activity (MIC_{50/90}, 0.25/1 mg/L; 95.2% and 96.2% susceptible by EUCAST and CLSI, respectively). Based on percentage susceptibility, TGC was the antimicrobial with the highest activity (MIC_{50/90}, ≤1/≤1 mg/L; 100% susceptible), followed by IPM (MIC_{50/90}, ≤0.12/≤0.25 mg/L; 99.5/98.1% susceptible) and MEM (MIC_{50/90}, ≤0.12/≤0.12 mg/L; 98.6/98.6% susceptible). The activity of C/T against isolates displaying an ESBL phenotype ($n = 46$) (MIC_{50/90}, 0.5/16 mg/L; 80.4/84.8% susceptible) was higher than TZP (MIC_{50/90}, ≤8/64 mg/L; 71.7/82.6%

susceptible). Ten *E. coli* isolates (4.8%) were resistant to C/T by EUCAST criteria in which the resistance phenotypes were: ESBL phenotype ($n = 8$; MIC range 2 mg/L to >64 mg/L); ESBL+AmpC-like phenotype ($n = 1$; MIC = 32 mg/L); and carbapenemase phenotype ($n = 1$; MIC = 64 mg/L). C/T-resistant isolates ranged from 4.0% to 15.3% in four of eight hospitals, however resistant isolates were not detected in the four other hospitals (data not shown).

The activity of C/T against *Klebsiella* spp. ($n = 95$) was moderate (MIC_{50/90}, 0.5/64 mg/L; 66.3% and 72.9% susceptible by EUCAST and CLSI, respectively). The antimicrobials with the highest activity were AMC (MIC_{50/90}, ≤8/≤8 mg/L; 95.8/97.9% susceptible), MEM (MIC_{50/90}, ≤0.12/2 mg/L; 91.7/87.5% susceptible), IPM (MIC_{50/90}, 0.25/2 mg/L; 90.6/82.3% susceptible) and TGC (MIC_{50/90}, ≤1/≤1 mg/L; 90.6% susceptible). C/T showed decreased activity against ESBL phenotype isolates ($n = 22$) (MIC_{50/90}, 1/16 mg/L; 59.1/77.3% susceptible) although higher than TZP (MIC_{50/90}, ≤8/>64 mg/L; 50.0/68.2% susceptible). C/T was inactive against 32 isolates (33.7%, EUCAST criteria) with resistance phenotypes as follows: carbapenemase phenotype [$n = 19$; NDM+ESBL ($n = 1$), OXA-48 ($n = 2$), OXA-48+ESBL ($n = 16$); MIC_{50/90}, 32/>64 mg/L; 0.0/5.0% susceptible]; ESBL phenotype ($n = 9$; MIC range 2 mg/L to >64 mg/L); AmpC-like phenotype ($n = 2$; MICs of 4 mg/L and 32 mg/L); and other resistance phenotypes ($n = 2$; MIC = 2 mg/L). *Klebsiella* spp. resistant to C/T were not found in one hospital. Resistance in the other centres ranged from 16.7% to 83.3% (data not shown).

In *Enterobacter* spp. isolates ($n = 33$), C/T showed moderate activity (MIC_{50/90}, 0.25/16 mg/L; 66.7% and 72.7% susceptibility

Table 3

Antimicrobial activity of ceftolozane/tazobactam (C/T) and comparator antimicrobials tested against Enterobacterales from patients in Spanish intensivexsx care units, according to major organisms and phenotypes.

| Organism/antimicrobial agent | MIC (mg/L) | | | EUCAST | | | CLSI | | |
|--|-------------------|-------------------|--------------|--------|----------------|------|------|------|------|
| | MIC ₅₀ | MIC ₉₀ | Range | %S | %I | %R | %S | %I | %R |
| All <i>Escherichia coli</i> (n = 209) ^a | | | | | | | | | |
| AMC | 16 | >16 | ≤8 to >16 | 45.9 | – ^b | 54.1 | 45.9 | 12.9 | 41.2 |
| TZP | ≤8 | 32 | ≤8 to >64 | 85.2 | 3.8 | 11.0 | 89.0 | 4.3 | 6.7 |
| C/T | 0.25 | 1 | ≤0.12 to >64 | 95.2 | 0.0 | 4.8 | 96.2 | 1.0 | 2.9 |
| Ceftazidime | 0.25 | 16 | ≤0.12 to >64 | 73.2 | 5.3 | 21.5 | 78.5 | 4.8 | 16.7 |
| Cefotaxime | ≤0.12 | >64 | ≤0.12 to >64 | 72.7 | 1.0 | 26.3 | 72.7 | 1.0 | 26.3 |
| Cefepime | ≤0.12 | 64 | ≤0.12 to >64 | 77.0 | 1.9 | 21.1 | 83.7 | 2.4 | 13.9 |
| Aztreonam | ≤0.12 | >32 | ≤0.12 to >32 | 73.2 | 3.8 | 23.0 | 77.0 | 2.9 | 20.1 |
| Imipenem | ≤0.12 | 0.25 | ≤0.12–32 | 99.5 | 0.0 | 0.5 | 98.1 | 1.4 | 0.5 |
| Meropenem | ≤0.12 | ≤0.12 | ≤0.12–32 | 98.6 | 1.0 | 0.5 | 98.6 | 0.0 | 1.4 |
| Ciprofloxacin | ≤0.06 | >2 | ≤0.06 to >2 | 61.7 | 4.8 | 33.5 | 67.5 | 0.5 | 32.1 |
| Gentamicin | ≤2 | >8 | ≤2 to >8 | 82.3 | 0.5 | 17.2 | 82.8 | 0.5 | 16.7 |
| Tobramycin | ≤2 | >8 | ≤2 to >8 | 74.6 | 6.2 | 19.1 | 80.9 | 3.3 | 15.8 |
| Amikacin | ≤8 | ≤8 | ≤8 to >32 | 97.1 | 1.9 | 1.0 | 99.0 | 0.0 | 1.0 |
| Colistin | ≤2 | ≤2 | ≤2 to >4 | 97.1 | – | 2.9 | – | – | – |
| Fosfomicin | ≤32 | 64 | ≤32 to >128 | 87.1 | – | 12.9 | 94.7 | 1.0 | 4.3 |
| Tigecycline | ≤1 | ≤1 | ≤1 | 100 | 0.0 | 0.0 | – | – | – |
| ESBL phenotype <i>E. coli</i> (n = 46) | | | | | | | | | |
| AMC | >16 | >16 | ≤8 to >16 | 19.6 | – | 80.4 | 19.6 | 15.2 | 65.2 |
| TZP | ≤8 | 64 | ≤8 to >64 | 71.7 | 10.9 | 17.4 | 82.6 | 8.7 | 8.7 |
| C/T | 0.5 | 16 | 0.25 to >64 | 80.4 | 0.0 | 19.6 | 84.8 | 4.3 | 10.9 |
| Ceftazidime | 16 | >64 | 2 to >64 | 0.0 | 19.6 | 80.4 | 19.6 | 17.4 | 63.0 |
| Cefotaxime | >64 | >64 | 4 to >64 | 0.0 | 0.0 | 100 | 0.0 | 0.0 | 100 |
| Cefepime | 64 | >64 | 0.5 to >64 | 8.7 | 4.3 | 87.0 | 28.3 | 10.9 | 60.9 |
| Aztreonam | >32 | >32 | 2 to >32 | 0.0 | 13.0 | 87.0 | 13.0 | 6.5 | 80.4 |
| Imipenem | ≤0.12 | 0.5 | ≤0.12–2 | 100 | 0.0 | 0.0 | 97.8 | 2.2 | 0.0 |
| Meropenem | ≤0.12 | 0.25 | ≤0.12–4 | 95.7 | 4.3 | 0.0 | 95.7 | 0.0 | 4.3 |
| Ciprofloxacin | >2 | >2 | 0.12–4 | 13.0 | 8.7 | 78.3 | 23.9 | 2.2 | 73.9 |
| Gentamicin | ≤2 | >8 | ≤2 to >8 | 67.4 | 0.0 | 32.6 | 67.4 | 2.2 | 30.4 |
| Tobramycin | 4 | >8 | ≤2 to >8 | 45.7 | 4.3 | 50.0 | 50.0 | 6.5 | 43.5 |
| Amikacin | ≤8 | ≤8 | ≤8 to >32 | 91.3 | 6.5 | 2.2 | 97.8 | 0.0 | 2.2 |
| Colistin | ≤2 | ≤2 | ≤2 to >4 | 93.5 | – | 6.5 | – | – | – |
| Fosfomicin | ≤32 | 64 | ≤32 to >128 | 84.8 | – | 15.2 | 93.5 | 2.2 | 4.3 |
| Tigecycline | ≤1 | ≤1 | ≤1 | 100 | 0.0 | 0.0 | – | – | – |
| All <i>Klebsiella</i> spp. group (n = 95) ^c | | | | | | | | | |
| AMC | >16 | >16 | ≤8 to >16 | 46.9 | – | 53.1 | 46.9 | 2.1 | 51.0 |
| TZP | ≤8 | >64 | ≤8 to >64 | 61.5 | 5.2 | 33.3 | 66.7 | 6.3 | 27.1 |
| C/T | 0.5 | 64 | ≤0.12 to >64 | 66.3 | – | 33.7 | 72.9 | 7.3 | 19.8 |
| Ceftazidime | 0.5 | 64 | ≤0.12 to >64 | 50.0 | 5.2 | 44.8 | 55.2 | 7.3 | 37.5 |
| Cefotaxime | 0.25 | >64 | ≤0.12 to >64 | 54.2 | 3.1 | 42.7 | 54.2 | 3.1 | 42.7 |
| Cefepime | 0.5 | >64 | ≤0.12 to >64 | 54.2 | 5.2 | 40.6 | 63.5 | 5.2 | 31.3 |
| Aztreonam | 0.25 | >32 | ≤0.12 to >32 | 52.1 | 4.2 | 43.8 | 56.3 | 3.1 | 40.6 |
| Imipenem | 0.25 | 2 | ≤0.12 to >32 | 90.6 | 4.2 | 5.2 | 82.3 | 12.5 | 5.2 |
| Meropenem | ≤0.12 | 2 | ≤0.12 to >32 | 91.7 | 3.1 | 5.2 | 87.5 | 4.2 | 8.3 |
| Ciprofloxacin | ≤0.06 | >2 | ≤0.06 to >2 | 53.1 | 3.1 | 43.8 | 57.3 | 0.0 | 42.7 |
| Gentamicin | ≤2 | >8 | ≤2 to >8 | 67.7 | 1.0 | 31.3 | 68.8 | 1.0 | 30.2 |
| Tobramycin | ≤2 | >8 | ≤2 to >8 | 58.9 | 1.1 | 40.0 | 60.0 | 3.2 | 36.8 |
| Amikacin | ≤8 | ≤8 | ≤8 to >32 | 95.8 | 2.1 | 2.1 | 97.9 | 1.1 | 1.1 |
| Colistin | ≤2 | 4 | ≤2 to >4 | 87.5 | – | 12.5 | – | – | – |
| Fosfomicin | 128 | >128 | ≤32 to >128 | 12.5 | – | 87.5 | – | – | – |
| Tigecycline | ≤1 | ≤1 | ≤1 to >2 | 90.6 | 7.3 | 2.1 | – | – | – |
| ESBL phenotype <i>Klebsiella pneumoniae</i> (n = 22) | | | | | | | | | |
| AMC | >16 | >16 | ≤8 to >16 | 4.5 | – | 95.5 | 4.5 | 9.1 | 86.4 |
| TZP | ≤8 | >64 | ≤8 to >64 | 50.0 | 18.2 | 31.8 | 68.2 | 13.6 | 18.2 |
| C/T | 1 | 16 | 0.25 to >64 | 59.1 | – | 40.9 | 77.3 | 9.1 | 13.6 |
| Ceftazidime | 16 | 64 | 2 to >64 | 0.0 | 13.6 | 86.4 | 13.6 | 27.3 | 59.1 |
| Cefotaxime | 64 | >64 | 0.25 to >64 | 9.1 | 4.5 | 86.4 | 9.1 | 4.5 | 86.4 |
| Cefepime | 32 | >64 | 1 to >64 | 4.5 | 9.1 | 86.4 | 22.7 | 18.2 | 59.1 |
| Aztreonam | >32 | >32 | 0.5 to >32 | 4.5 | 9.1 | 86.4 | 13.6 | 9.1 | 77.3 |
| Imipenem | ≤0.12 | 0.5 | ≤0.12–1 | 100 | 0.0 | 0.0 | 100 | 0.0 | 0.0 |
| Meropenem | ≤0.12 | ≤0.12 | ≤0.12–1 | 95.5 | 4.5 | 0.0 | 95.5 | 0.0 | 4.5 |
| Ciprofloxacin | >2 | >2 | ≤0.06 to >2 | 13.6 | 0.0 | 86.4 | 13.6 | 0.0 | 86.4 |
| Gentamicin | >8 | >8 | ≤2 to >8 | 45.5 | 0.0 | 54.4 | 45.5 | 0.0 | 54.5 |
| Tobramycin | >8 | >8 | ≤2 to >8 | 27.3 | 0.0 | 72.7 | 27.3 | 4.5 | 68.2 |
| Amikacin | ≤8 | ≤8 | ≤8–16 | 90.9 | 9.1 | 0.0 | 100 | 0.0 | 0.0 |
| Colistin | ≤2 | >4 | ≤2 to >4 | 81.8 | – | 18.2 | – | – | – |
| Fosfomicin | 128 | >128 | 32 to >128 | 9.1 | – | 90.9 | – | – | – |
| Tigecycline | ≤1 | 2 | ≤1 to >2 | 81.8 | 13.6 | 4.5 | – | – | – |
| Carbapenemase phenotype <i>K. pneumoniae</i> (n = 19) ^d | | | | | | | | | |
| AMC | >16 | >16 | >16 | 0.0 | – | 100 | 0.0 | 0.0 | 100 |
| TZP | >64 | >64 | 64 to >64 | 0.0 | 0.0 | 100 | 0.0 | 5.0 | 95.0 |
| C/T | 32 | >64 | 2 to >64 | 0.0 | – | 100 | 5.0 | 20.0 | 75.0 |
| Ceftazidime | 64 | >64 | 2 to >64 | 0.0 | 5.0 | 95.0 | 5.0 | 0.0 | 95.0 |

(continued on next page)

Table 3 (continued)

| Organism/antimicrobial agent | MIC (mg/L) | | | EUCAST | | | CLSI | | |
|--|-------------------|-------------------|--------------|--------|------|------|------|------|------|
| | MIC ₅₀ | MIC ₉₀ | Range | %S | %I | %R | %S | %I | %R |
| Cefotaxime | >64 | >64 | 16 to >64 | 0.0 | 0.0 | 100 | 0.0 | 0.0 | 100 |
| Cefepime | 64 | >64 | 8 to >64 | 0.0 | 0.0 | 100 | 10.0 | 5.0 | 85.0 |
| Aztreonam | >32 | >32 | 4 to >32 | 0.0 | 5.0 | 95.0 | 5.0 | 0.0 | 95.0 |
| Imipenem | 2 | >32 | 1 to >32 | 55.0 | 20.0 | 25.0 | 15.0 | 60.0 | 25.0 |
| Meropenem | 2 | >32 | 0.25 to >32 | 65.0 | 10.0 | 25.0 | 45.0 | 20.0 | 35.0 |
| Ciprofloxacin | >2 | >2 | 0.5 to >2 | 0.0 | 10.0 | 90.0 | 10.0 | 0.0 | 90.0 |
| Gentamicin | >8 | >8 | ≤2 to >8 | 15.0 | 5.0 | 80.0 | 20.0 | 0.0 | 80.0 |
| Tobramycin | >8 | >8 | ≤2 to >8 | 5.3 | 5.3 | 89.5 | 10.5 | 0.0 | 89.5 |
| Amikacin | ≤8 | ≤8 | ≤8 to >32 | 94.7 | 0.0 | 5.3 | 94.7 | 5.3 | 0.0 |
| Colistin | ≤2 | >4 | ≤2 to >4 | 80.0 | – | 20.0 | – | – | – |
| Fosfomicin | >128 | >128 | 32 to >128 | 10.0 | – | 90.0 | – | – | – |
| Tigecycline | ≤1 | 2 | ≤1 to >2 | 80.0 | 15.0 | 5.0 | – | – | – |
| All <i>Enterobacter</i> spp. (n = 33) ^e | | | | | | | | | |
| AMC | >16 | >16 | ≤8 to >16 | 6.1 | – | 93.9 | 6.1 | 0.0 | 93.9 |
| TZP | ≤8 | 32 | ≤8 to >64 | 75.8 | 3.0 | 21.2 | 78.8 | 12.1 | 9.1 |
| C/T | 0.25 | 16 | ≤0.12 to >64 | 66.7 | – | 33.3 | 72.7 | 15.2 | 12.1 |
| Ceftazidime | 0.5 | 64 | ≤0.12 to >64 | 63.6 | 6.1 | 30.3 | 69.7 | 3.0 | 27.3 |
| Cefotaxime | 0.5 | >64 | ≤0.12 to >64 | 63.6 | 6.1 | 30.3 | 63.6 | 6.1 | 30.3 |
| Cefepime | ≤0.12 | 64 | ≤0.12 to >64 | 81.8 | 3.0 | 15.2 | 84.8 | 0.0 | 15.2 |
| Aztreonam | ≤0.12 | >32 | ≤0.12 to >32 | 69.7 | 3.0 | 27.3 | 72.7 | 0.0 | 27.3 |
| Imipenem | 0.5 | 2 | ≤0.12 to >32 | 97.0 | 0.0 | 3.0 | 78.8 | 18.2 | 3.0 |
| Meropenem | ≤0.12 | 0.5 | ≤0.12 to >32 | 93.9 | 3.0 | 3.0 | 93.9 | 0.0 | 6.1 |
| Ciprofloxacin | ≤0.06 | 0.5 | ≤0.06 to >2 | 78.8 | 0.0 | 21.2 | 81.8 | 3.0 | 15.2 |
| Gentamicin | ≤2 | >8 | ≤2 to >8 | 84.8 | 3.0 | 12.1 | 87.9 | 0.0 | 12.1 |
| Tobramycin | ≤2 | >8 | ≤2 to >8 | 81.8 | 6.1 | 12.1 | 87.9 | 0.0 | 12.1 |
| Amikacin | ≤8 | ≤8 | ≤8 to >32 | 93.9 | 0.0 | 6.1 | 93.9 | 0.0 | 6.1 |
| Colistin | ≤2 | >4 | ≤2 to >4 | 60.6 | – | 39.4 | – | – | – |
| Fosfomicin | 128 | >128 | 32 to >128 | 18.2 | – | 81.8 | – | – | – |
| Tigecycline | ≤1 | ≤1 | ≤1–2 | 97.0 | 3.0 | 0.0 | – | – | – |
| <i>Proteus</i> spp. (n = 19) ^f | | | | | | | | | |
| AMC | ≤8 | >16 | ≤8 to >16 | 63.2 | – | 36.8 | 63.2 | 10.5 | 26.3 |
| TZP | ≤8 | ≤8 | ≤8 | 100 | 0.0 | 0.0 | 100 | 0.0 | 0.0 |
| C/T | 0.5 | 1 | 0.5–1 | 100 | 0.0 | 0.0 | 100 | 0.0 | 0.0 |
| Ceftazidime | ≤0.12 | ≤0.12 | ≤0.12–2 | 94.7 | 5.3 | 0.0 | 100 | 0.0 | 0.0 |
| Cefotaxime | ≤0.12 | 1 | ≤0.12–4 | 94.7 | 0.0 | 5.3 | 94.7 | 0.0 | 5.3 |
| Cefepime | ≤0.12 | 1 | ≤0.12–4 | 94.7 | 5.3 | 0.0 | 100 | 0.0 | 0.0 |
| Aztreonam | ≤0.12 | ≤0.12 | ≤0.12 | 100 | 0.0 | 0.0 | 100 | 0.0 | 0.0 |
| Imipenem | 2 | 4 | 0.25–4 | 63.2 | 36.8 | 0.0 | 21.1 | 78.9 | 0.0 |
| Meropenem | ≤0.12 | 1 | ≤0.12–1 | 94.7 | 5.3 | 0.0 | 94.7 | 0.0 | 5.3 |
| Ciprofloxacin | ≤0.06 | >2 | ≤0.06 to >2 | 68.4 | 0.0 | 31.6 | 68.4 | 10.5 | 21.1 |
| Gentamicin | ≤2 | 8 | ≤2–8 | 78.9 | 5.3 | 15.8 | 84.2 | 0.0 | 15.8 |
| Tobramycin | ≤2 | >8 | ≤2 to >8 | 84.2 | 5.3 | 10.5 | 89.5 | 0.0 | 10.5 |
| Amikacin | ≤8 | 16 | ≤8–16 | 84.2 | 15.8 | 0.0 | 100 | 0.0 | 0.0 |
| Colistin | >4 | >4 | >4 | 0.0 | – | 100 | – | – | – |
| Fosfomicin | 32 | >128 | 32 to >128 | 47.4 | – | 52.6 | – | – | – |
| Tigecycline | >2 | >2 | ≤1 to >2 | 10.5 | 10.5 | 78.9 | – | – | – |
| <i>Morganella morganii</i> (n = 16) ^g | | | | | | | | | |
| AMC | >16 | >16 | >16 | 0.0 | – | 100 | 0.0 | 0.0 | 100 |
| TZP | ≤8 | ≤8 | ≤8–16 | 93.8 | 6.3 | 0.0 | 100 | 0.0 | 0.0 |
| C/T | 0.25 | 0.5 | ≤0.12–4 | 93.8 | – | 6.3 | 93.8 | 6.3 | 0.0 |
| Ceftazidime | 0.5 | 4 | ≤0.12–32 | 68.8 | 25.0 | 6.3 | 93.8 | 0.0 | 6.3 |
| Cefotaxime | 1 | 8 | ≤0.12–32 | 62.5 | 25.0 | 12.5 | 62.5 | 25.0 | 12.5 |
| Cefepime | ≤0.12 | 1 | ≤0.12–1 | 100 | 0.0 | 0.0 | 100 | 0.0 | 0.0 |
| Aztreonam | ≤0.12 | 2 | ≤0.12–4 | 87.5 | 12.5 | 0.0 | 100 | 0.0 | 0.0 |
| Imipenem | 4 | 4 | 1–4 | 31.3 | 68.8 | 0.0 | 6.3 | 93.8 | 0.0 |
| Meropenem | 0.25 | 0.25 | ≤0.12–0.5 | 100 | 0.0 | 0.0 | 100 | 0.0 | 0.0 |
| Ciprofloxacin | ≤0.06 | >2 | ≤0.06 to >2 | 75.0 | 0.0 | 25.0 | 87.5 | 0.0 | 12.5 |
| Gentamicin | ≤2 | 8 | ≤2 to >8 | 81.3 | 6.3 | 12.5 | 87.5 | 6.3 | 6.3 |
| Tobramycin | ≤2 | 8 | ≤2 to >8 | 87.5 | 0.0 | 12.5 | 87.5 | 6.3 | 6.3 |
| Amikacin | ≤8 | 16 | ≤8–32 | 87.5 | 6.3 | 6.3 | 93.8 | 0.0 | 6.3 |
| Colistin | >4 | >4 | >4 | 0.0 | – | 100 | – | – | – |
| Fosfomicin | >128 | >128 | >128 | 0.0 | – | 100 | – | – | – |
| Tigecycline | ≤1 | >2 | ≤1 to >2 | 75.0 | 12.5 | 12.5 | – | – | – |

MIC, minimum inhibitory concentration; MIC_{50/90}, MIC that inhibits 50% and 90% of isolates, respectively; S, susceptible; I, intermediate; R, resistant; WT, wild-type; ESBL, extended-spectrum β -lactamase; AMC, amoxicillin/clavulanic acid; TZP, piperacillin/tazobactam.

^a Includes WT isolates (n = 82), ESBL phenotype (n = 46), carbapenemase phenotype (n = 2; OXA-48), AmpC phenotype (n = 4) and other resistant phenotypes (n = 75).

^b –, indicates no published interpretative criteria.

^c *Klebsiella* spp. group includes *Klebsiella pneumoniae* (n = 86), *Klebsiella oxytoca* (n = 8) and *Raoultella ornithinolytica* (n = 1). By resistant phenotypes: WT isolates (n = 42), ESBL phenotype (n = 22), carbapenemase phenotype (n = 19), AmpC phenotype (n = 2) and other resistant phenotypes (n = 10).

^d Carbapenemase phenotype *K. pneumoniae* includes NDM+ESBL (n = 1), OXA-48 (n = 2) and OXA-48+ESBL (n = 16).

^e *Enterobacter* spp. group includes *Enterobacter cloacae* (n = 26), *Enterobacter aerogenes* (n = 5), *Enterobacter kobei* (n = 1) and *E. asburiae* (n = 1). By resistant phenotypes: WT isolates (n = 9), AmpC-hyperproducers (n = 7), ESBL phenotype *E. cloacae* (n = 4), carbapenemase phenotype *E. cloacae* (n = 1; VIM) and other resistant phenotypes (n = 12).

^f *Proteus* spp. includes *Proteus mirabilis* (n = 18) and *Proteus vulgaris* (n = 1). By resistant phenotypes: WT isolates (n = 9) and other resistant phenotypes (n = 10).

^g Includes WT isolates (n = 6), AmpC-hyperproducers (n = 6) and other resistant phenotypes (n = 4).

by EUCAST and CLSI, respectively). The most active agents were TGC (MIC_{50/90}, ≤1/≤1 mg/L; 97.0% susceptible), MEM (MIC_{50/90}, ≤0.12/0.5 mg/L; 93.9% susceptible), AMK (MIC_{50/90}, ≤8/≤8 mg/L; 93.9% susceptible) and IPM (MIC_{50/90}, 0.5/2 mg/L; 97.0/78.7% susceptible). Eleven *Enterobacter* spp. isolates were resistant to C/T. Resistance phenotypes in these isolates were: AmpC hyperproducers ($n = 5$; MIC range 2–8 mg/L); ESBL phenotype ($n = 4$; MIC range 8–64 mg/L); carbapenemase phenotype [$n = 1$ (VIM); MIC > 64 mg/L]; and another resistance phenotype ($n = 1$; MIC = 2 mg/L).

C/T demonstrated excellent overall activity against *Proteus* spp. ($n = 19$) (MIC_{50/90}, 0.5/1 mg/L; 100% susceptible by EUCAST and CLSI) and was the most potent agent together with aztreonam (MIC_{50/90}, ≤0.12/≤0.12 mg/L; 100% susceptible) and TZP (MIC_{50/90}, ≤8/≤8 mg/L; 100% susceptible). *Proteus* spp. isolates resistant to C/T were not found. The activity of C/T against *M. morganii* ($n = 16$) was adequate (MIC_{50/90}, 0.25/0.5 mg/L; 93.8% susceptible by EUCAST and CLSI). Only one isolate of *M. morganii* was resistant to C/T, an AmpC hyperproducer (MIC of 4/4 mg/L). In *Citrobacter* spp. isolates ($n = 9$), C/T showed 77.8% and 88.9% susceptibility by EUCAST and CLSI, respectively, and was inactive against 2 *Citrobacter freundii* AmpC-hyperproducers (MICs of 4/4 and 8/4 mg/L). In *S. marcescens* ($n = 9$), C/T showed 88.9% susceptibility, and 1 isolate was resistant (MIC = 4/4 mg/L). C/T was active in 3/5 *H. alvei* isolates, in 3/3 *P. stuartii* isolates and in the single isolates of *K. ascorbata* and *S. enterica*.

4. Discussion

The recent list of antibiotic-resistant bacteria published by the World Health Organization (WHO) alerts about the necessity of development of new antibiotics against these pathogens [11]. In this list, *P. aeruginosa* and Enterobacterales represent the highest level of risk. Overall data from the 2016 European Antimicrobial Resistance Surveillance Network (EARS-Net) report show worrisome mean levels of resistance for common treatment options against *P. aeruginosa*: TZP, 16.3%; fluoroquinolones, 15.0%; ceftazidime, 13.0%; and carbapenems, 15.0%. If resistance is combined to three or more antimicrobial groups, 10.3% of isolates match these criteria. This resistance is higher in Spain (14.5%) [12], reflecting that the research and development of new therapeutic options remains a matter of concern. This is also particularly highlighted in the ECDC annual epidemiological report of healthcare-associated infections acquired in ICUs in the European Union. In this report, 23.0% and 26.4% of *P. aeruginosa* isolates were resistant to third-generation cephalosporins and carbapenems, respectively [3]. This is also not a minor matter in Enterobacterales. In *E. coli* and *K. pneumoniae* isolates, fluoroquinolone resistance reaches levels of 21.0% and 24.6%, respectively. Regarding third-generation cephalosporins, *E. coli* shows 12.4% resistance, whilst in *K. pneumoniae* 25.7% of isolates are resistant. With carbapenems, overall EARS-Net data reflect mean resistance rates in Europe of <0.1% and 6.1% in *E. coli* and *K. pneumoniae*, respectively [12]. However, resistance to third-generation cephalosporins and carbapenems is higher in ICUs, with figures reaching 42.9% and 11.3%, respectively, in *Klebsiella* spp. [3].

Results of previous studies show susceptibility levels to C/T in *P. aeruginosa* isolates up to 95% [13]. Moreover, slight variations are obtained when isolates are categorised as resistant with regard to different antipseudomonal agents [7]. Activity in Enterobacterales depends on resistant patterns; susceptibility is high (>95%) if isolates are pooled [14] but might decrease if ESBL-producers are analysed separately [14,15]. Resistance of *P. aeruginosa* and Enterobacterales isolates to C/T has been particularly associated with carbapenemase-producers [7]. Moreover, in vivo resistance development has also recently been described [16].

The results obtained in this study in *P. aeruginosa* isolates from ICUs confirm that C/T is one of the best antipseudomonal

agents with the highest susceptibility rates, along with COL and AMK. Comparing the activity of C/T (91.3% susceptible) with other β -lactams, the activity of C/T was higher (TZP, 60.0%; FEP, 58.8%; and CAZ, 55.0%), as previously described in isolates recovered from different locations in hospitalised patients in US hospitals [17]. This *in vitro* activity of C/T against *P. aeruginosa* is maintained regardless of its resistance pattern (TZP-NS, 87.9% susceptible; CAZ-NS, 80.6%; and MEM-NS, 78.8%), and C/T maintains good activity even when the micro-organism is resistant to more than one antipseudomonal agent (TZP/CAZ-NS, 86.7%; TZP/CAZ/MEM-NS, 82.6%) or in isolates classified as MDR (88.2%). However, C/T was inactive in seven *P. aeruginosa* isolates, six of which exhibited a MBL phenotype. Carbapenemase production together with overexpression of AmpC or mutations in OprD represent limitations of this agent against *P. aeruginosa* [16,18].

The activity of C/T in Enterobacterales is more variable. It depends both on the species level and the resistance mechanism. As we previously described [19], the activity of C/T is excellent against WT organisms (MIC_{50/90} ranges, 0.25–0.5 mg/L and 0.25–1 mg/L, respectively) and 100% inhibited at MIC ≤1 mg/L (Supplementary Table S1). However, the activity decreased against ESBL-producing *K. pneumoniae*, as noted in other studies [15]. In the isolates in the current study, susceptibilities of 80.4/84.8% and 59.1/77.3% were obtained in *E. coli* and *Klebsiella* spp. with an ESBL phenotype when using EUCAST and CLSI criteria, respectively. These figures were higher for IPM (100%), MEM (95.7%), AMK (91.3%) and COL (93.5%) in ESBL-producing *E. coli* as well as in ESBL-producing *Klebsiella* spp. [IPM (100%), MEM (95.5%), AMK (90.9%) and COL (81.8%)]. No activity of C/T was expected in carbapenemase-producers, as previously reported [7]. In fact, the moderate activity against *K. pneumoniae* in the current study is due to the high prevalence of carbapenemases in our environment [20].

Different resistance rates to C/T between hospitals were found. Interestingly, in four of eight hospitals neither *P. aeruginosa* nor *E. coli* C/T-resistant isolates were found. However, *Klebsiella* spp. isolates resistant to C/T are more concerning, with resistance rates up to 83.0%. Unfortunately, the clonal relatedness between isolates was beyond the scope of this study, therefore the presence of outbreaks cannot be ruled out.

Of note, great activity of C/T was obtained when *P. aeruginosa* isolates were pooled by infection type: 95.7% and 85.3% susceptibility in cUTI and cIAI, respectively. In Enterobacterales, 79.5/81.9% (EUCAST/CLSI) of cIAI and 89.3/92.3% of cUTI were susceptible, representing a therapeutic alternative in ICU patients.

A limitation of this study is that only the presence of carbapenemase genes in isolates displaying a C/T resistance pattern was investigated, but different resistance mechanisms affecting C/T might remain unidentified. In summary, the *in vitro* activity of C/T observed in this multicentre study suggests that this agent should be considered as a therapeutic option in cUTI and cIAI due to Enterobacterales and *P. aeruginosa* in ICU patients, excluding carbapenemase-producers.

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Competing interests

RC and GB have participated in educational programmes organised by MSD and Pfizer. All other authors declare no competing interests.

Ethical approval

This study was approved by the Ethical Committee of Hospital Universitario Ramón y Cajal (Madrid, Spain) [Ref. 087-16] and the Spanish Medicines Agency [Ref. MSD-CEF-2016-01].

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.02.004.

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