Enterobacteriaceae cause different types of community- and hospital-acquired infections. Moreover, the spread of multidrug-resistant Enterobacteriaceae is a public health problem and the World Health Organization pointed them among the pathogens in which the search of new antibiotics is critical. The objective of this study was to analyze the in vitro activity of pentamidine alone and in combination with gentamicin, tobramycin, amikacin, tigecycline, rifampicin, or doripenem against eight clinical strains of carbapenemase-producing and/or colistin-resistant Enterobacteriaceae: five carbapenemase-producing Klebsiella pneumoniae, one carbapenemase-producing Escherichia coli, and two colistin-resistant Enterobacter cloacae. MIC and MBC were determined following standard protocols. MIC results were interpreted for all the antibiotics according to the EUCAST breakpoints but for rifampicin in which the French FSM breakpoint was used. Bactericidal and synergistic activity of pentamidine alone and in combination with antibiotics at concentrations of 1xMIC was measured by time-kill curves. For one selected strain, K. pneumoniae OXA-48/CTX-M-15 time-kill curves were performed also at 1/2xMIC of pentamidine. All studies were performed in triplicate. Pentamidine MIC range was 200–800 µg/mL. The 50, 12.5, 62.5, 87.5, and 62.5% of the strains were susceptible to gentamicin, tobramycin, amikacin, tigecycline, rifampicin, or doripenem, respectively. Only the two E. cloacae strains were susceptible to rifampicin. Pentamidine alone at 1xMIC showed bactericidal activity against all strains, except for the E. cloacae 32 strain. The bactericidal activity of pentamidine alone was also observed in combination. The combinations of pentamidine were synergistic against E. cloacae.
INTRODUCTION

Carbapenem-resistance in Enterobacteriaceae is a world health problem that has made the World Health Organization (WHO) to point it as a priority in the list of bacteria for which new antibiotics are urgently needed (World Health Organization, 2017). Moreover, these pathogens have spread globally in the past years, and are associated with carbapenemase production as the most important resistance mechanism (Cantón et al., 2012). Therapeutic options for infections caused by these kinds of pathogens are scarce and colistin is often the only remaining treatment option. However, the appearance of colistin-resistant strains has risen dramatically in the last decade due to antibiotic pressure in both human treatment and its use in agriculture (Kempf et al., 2016; Olaitan et al., 2016). In a recently published study (Hong et al., 2018), the in vitro activity of colistin was analyzed against 356 clinical strains of Enterobacter spp. from eight Korean hospitals, founding that 23.9 and 4.2% of E. cloacae and E. aerogenes strains, respectively, were resistant to colistin.

In this context, clinical experience on the most effective treatment for infections caused by these pathogens is still scarce (Akova et al., 2012). Currently, the majority of clinical studies conclude that the combined treatment with two or more antimicrobials is the better option in terms of increase the survival (Trecarichi and Tumbarello, 2017). Numerous studies, both in vitro and in vivo, have tested the efficacies of antimicrobials combinations against these kinds of pathogens (Pachón-Ibáñez et al., 2018; Wang et al., 2018). Nevertheless, the best combination depends on the susceptibility pattern of the strains and there is no one combination that we could qualify as optimal.

Therefore, the increase in the rates of antimicrobial resistance, the difficulty to find an optimal and effective treatment for infections caused by these pathogens, and the lack of the development of new families of antimicrobials by the pharmaceutical industry (Spellberg and Rex, 2013), make urgent the search for new approaches to combat the problem caused by multi-resistant strains of Gram-negative bacilli (GNB) and, specifically, by carbapenemase-producing and/or colistin-resistant Enterobacteriaceae.

As a new treatment strategy, the repurposing of drugs for the treatment of infections caused by these kinds of pathogens seems especially interesting (Younis et al., 2015). This new approach reduces the time, cost, and risk associated with the development of antimicrobial molecules de novo. In addition, its effectiveness has been demonstrated in different medical areas, such as infectious diseases (Debnath et al., 2012). Despite of that several drugs have been recycled for other clinical indications; none has been used for the treatment of bacterial infections.

Pentamidine (in the form of isethionate) is an antiprotozoal agent effective in trypanosomiasis, leishmaniasis, and some fungal infections (Nguewa et al., 2005). To our knowledge, pentamidine has never been used in clinic as antimicrobial agent. Nevertheless, in a recent study Stokes et al. found that pentamidine is able to disturb the outer membrane of GNB, due to the interaction with membrane lipopolysaccharides (Stokes et al., 2017). Moreover, they concluded that pentamidine in combination with antimicrobials typically used for Gram-positive cocci had synergistic activity in vitro against different GNB and in a mice sepsis model by Acinetobacter baumannii.

The aim of this study was to evaluate in vitro the activity of pentamidine alone and in combination with different antimicrobials against clinical strains of carbapenemase-producing and/or colistin-resistant Enterobacteriaceae.

MATERIALS AND METHODS

Bacterial Strains

Eight clinical strains of carbapenemase-producing and/or colistin-resistant Enterobacteriaceae were studied: (1) five strains of carbapenemase-producing Klebsiella pneumoniae: Kp07, a VIM-1 ST 1603 clone producer from Spain (Miró et al., 2013); Kp21, co-producing VIM-1 and AmpC type beta-lactamase DHA-1 ST 11 clone from Spain (Miró et al., 2013); Kp28, co-producing OXA-48 ST11 clone and the extended spectrum beta-lactamase (ESBL) CTX-M-15 from Spain (Oteo et al., 2015); a Kp29, co-producing KPC-3 ST512 clone with the extended spectrum beta-lactamases TEM-1 and SHV-11 from Spain (López-Cerero et al., 2014); Kp31, a NDM-1 producer from Kenya; (2) Ec271, a Escherichia coli NDM-1 producer from Australia Docobo-Pérez et al., 2012; (3) two strains of Enterobacter spp. from Spain, E. cloacae 32 and E. cloacae 297, both resistant to colistin. Identification of these isolates was confirmed by a Microflex LT-MALDI Biotyper mass spectrometer (Ruiz-Aragón et al., 2018) (Bruker Daltonics GmbH, Bremen, Germany).

The presence of carbapenemase genes, and genes coding for other beta-lactamases was confirmed by PCR and sequencing as described previously.

Keywords: Enterobacteriaceae, colistin-resistant, carbapenemase producers, pentamidine, in vitro activity
Drugs
All the drugs tested were used as standard laboratory powders (Sigma-Aldrich, Madrid, Spain): pentamidine, aminoglycosides (gentamicin, tobramycin, and amikacin), tigecycline, rifampicin, and doripenem.

Antimicrobial Susceptibility Testing
The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values were tested. MICs of antibiotics were determined by broth microdilution as recommended by the (Clinical and Laboratory Standards Institute, 2012), using Mueller Hinton broth II (MHB) (Becton Dickinson & Co., Sparks, MD, United States). MIC results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (http://www.eucast.org/clinical_breakpoints/) breakpoints for all antibiotics (European Committee on Antimicrobial Susceptibility Testing, 2018), but rifampicin, for which the French Society for Microbiology breakpoint was used (Soussy, 2012). Pentamidine has not susceptibility breakpoints defined.

The MIC value was the lowest concentration of antimicrobials that completely inhibited the bacterial growth. To determine the MBC values, 5-µL aliquots from the wells with no visible growth were spread on agar plates. The MBC value was the lowest concentration at which no colony formation occurred. Heteroresistance in the studied strains was also evaluated by reading the MIC at 24 and 48 h of incubation. Heteroresistance was defined when a fraction of the inoculum was able to grow two dilutions above the MIC value previously determined (Ferreira et al., 2015). All assays were performed in triplicates to ensure reproducibility.

Time-Kill Curves
The concentrations used for pentamidine and the different antimicrobials tested corresponded to the MIC value obtained by microdilution. Moreover, with the Kp28 OXA-48/CTX-M-15 strain the assay was also performed at 1/2×MIC of pentamidine. Experiments were carried out with a starting inoculum of 5 × 10^5 cfu/mL and the drugs alone or in combination. Tubes were incubated at 37°C, with shaking, and samples were taken at 0, 2, 4, 8, and 24 h, serially diluted and plated (Pournaras et al., 2011; Souli et al., 2011). Bactericidal activities of single drugs or combination were defined as a decrease ≥ 3 log_{10} cfu/mL from the starting inoculum, bacteriostatic effect was defined as no change respect to the initial bacterial concentration during the 24 h. Synergy was defined as a decrease ≥ 2 log_{10} cfu/mL for the drugs combination compared with the most active single agent (Pachon-Ibáñez et al., 2018). Experiments were performed three times on separate occasions.

**In vitro Selection of Resistant Mutants**
Time–kill curves were used. Strains elected strains were incubated with drugs at concentrations 1×MIC, and Kp28 OXA-48/CTX-M-15 also at pentamidine concentration of 1/2×MIC. Furthermore, the possible combinations of pentamidine plus the different studied antimicrobials were tested. Tubes with 20 mL of MHB with an inoculum of 5 × 10^5 cfu/mL of each one of the strains were used. Tubes with the bacterial inoculum and without drugs were used as growth controls. The bacterial growth was counted at 0 and 24 h after incubation at 37°C. Ten-fold dilutions were made and 100 µL was plated on sheep blood agar and incubated for 24 h at 37°C. For the detection of resistant mutants, the MIC of each one of the studied drugs was carried out in triplicate for a maximum of five colonies at each time-point.

**RESULTS**

MIC/MBC and Heteroresistance
Individual MIC/MBC of each drug tested for the different clinical strains are shown in Table 1. All the strains were multidrug-resistant (MDR) (Magiorakos et al., 2012) except Kp28 OXA-48/CTX-M-15 which was resistant to rifampicin and fosfomycin. Heteroresistance was observed with tigecycline for the Kp1 NDM-1 (1 mg/L) and Kp21 VIM-1/DHA-1 strains (1 mg/L) and with doripenem for E. cloacae 32 (1 mg/L). The antibiotic susceptibility profiles are included in the Supplementary material.

**TABLE 1** | MIC/MBC of the different drugs for the eight carbapenemase-producing and/or colistin-resistant Enterobacteriaceae clinical strains.

| Clinical strains | MIC/MBC (mg/L) |
|------------------|----------------|
|                  | PEN  | GEN  | AMK | TOB  | RIF  | TGC | DOR |
| Kp07 VM-1        | 400  | 4/16 | 1/1 | 4/4  | 32/32| 0.5/1| 1/2 |
| Kp21 VM-1/DHA-1  | 400  | 2/2  | 2/4 | 8/16 | >256/>256| 0.25/>4| >4/>4|
| Kp28 OXA-48/CTX-M-15 | 400  | 0.25/0.25 | 1/1 | 0.5/0.5 | 16/16 | 1/4 | 0.5/0.5 |
| Kp29 KPC-3       | 800  | 2/2  | 64/64 | 0.25/0.25 | 32/64 | 1/>8| >4/>4 |
| Kp1 NDM-1        | 400  | >32/>32 | >128/>128 | >32/>32 | >256/>256 | 0.25/>4 | 1/2 |
| Ecc21 NDM-1      | 200  | >32/>32 | >128/>128 | >32/>32 | >256/>256 | 1/1 | >4/>4 |
| E. cloacae 32    | 800  | 8/16 | 2/4 | 4/8  | 8/8  | 0.5/>4 | 0.25/>4 |
| E. cloacae 207   | 400  | 0.5/4 | 0.5/1 | 8/8  | 8/256 | 2/>8 | 0.25/0.25 |

PEN, pentamidine; GEN, gentamicin; AMK, amikacin; TOB, tobramycin; TGC, tigecycline; RIF, rifampicin; DOR, doripenem; * Susceptible, MIC ≤ 2 mg/L and resistant MIC > 4 mg/L; AMK, Susceptible, MIC ≤ 8 mg/L and resistant MIC > 16 mg/L; TGC and DOR; Susceptible, MIC ≤ 1 mg/L and resistant MIC > 2 mg/L and RIF; and resistant MIC > 16 mg/L.
**Time-Kill Curves**

The bactericidal activity of the drugs alone is shown in Table 2. Pentamidine alone at 1xMIC was bactericidal from 2 to 24 h against six of the strains and from 4 to 24 h against Kp21 VIM-1/DHA-1; however, pentamidine alone was no bactericidal against *E. cloacae* 32. Doripenem showed bactericidal activity against three strains, and gentamycin, tobramycin, and amikacin were bactericidal against two strains each. Tigecycline alone was the only antimicrobial that showed not bactericidal effect against any strain.

The in vitro activity of pentamidine in combination with antimicrobials is shown in Table 3. The bactericidal activity of pentamidine alone was also observed in combination. The combinations of pentamidine were synergistic against *E. cloacae* 32 with amikacin, tobramycin and rifampicin at 24 h. Pentamidine plus rifampicin was the combination that showed synergistic activity against more strains: Kp21 VIM-1/DHA-1, Kp29 KPC-3 and *E. cloacae* 297 at 2 h and *E. cloacae* 32 at 24 h. Pentamidine plus doripenem did not show synergy against any strain.

The activity of pentamidine at 1/2xMIC in combination with antimicrobials against Kp28 OXA-48/CTX-M-15 is showed in Figure 1. Pentamidine 1/2xMIC plus antimicrobials showed synergism at 24 h, but the combination with

| Clinical strains | PEN | GEN | AMK | TOB | RIF | TGC | DOR |
|------------------|-----|-----|-----|-----|-----|-----|-----|
| Kp07 VM-1        | B   | –   | B   | –   | –   | –   | B   |
| (2-24 h)         | (2-24 h) |     | (4-8 h) |     |     |     | (4 h) |
| Kp21 VM-1/DHA-1  | B   | B   | –   | –   | –   | –   | –   |
| (4-24 h)         | (4-24 h) |     |     |     |     |     |     |
| Kp29 KPC-3       | B   | B   | B   | –   | –   | –   | –   |
| (2-24 h)         | (2-24 h) | (4-8 h) |     |     |     |     |     |
| Ec271 NDM-1      | B   | –   | –   | –   | –   | –   | –   |
| (2-24 h)         |     |     |     |     |     |     |     |
| *E. cloacae* 32  | –   | B   | –   | –   | –   | –   | B   |
| (2-24 h)         | (24 h) |     |     |     |     | (8-24 h) |     |
| *E. cloacae* 297 | B   | –   | –   | B   | –   | –   | B   |
| (4-24 h)         |     |     | (8 h) |     |     |     | (8 h) |

**TABLE 2** | Bactericidal activity of drugs alone against eight of carbapenemase-producing and/or colistin-resistant *Enterobacteriaceae* clinical strains.

| Clinical strains | PEN + GEN | PEN + AMK | PEN + TOB | PEN + TGC | PEN + RIF | PEN + DOR |
|------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| Kp07 VM-1        | B         | B         | B         | B         | B         | B         |
| (2-24 h)         | (2-24 h) | (4-24 h) | (2-24 h) | (2-24 h) | (2-24 h) | (2-24 h) |
| Kp21 VM-1/DHA-1  | B         | B         | B         | B         | B + S     | B         |
| (2-24 h)         | (8-24 h) | (8-24 h) | (4-24 h) | (2-24 h) | (2-24 h) | (2-24 h) |
| Kp29 KPC-3       | B         | B         | B         | B         | B + S     | B         |
| (2-24 h)         | (2-24 h) | (2-24 h) | (2-24 h) | (2-24 h) | (2-24 h) | (2-24 h) |
| Kp1 NDM-1        | B         | B         | B         | B         | B         | B         |
| (2-24 h)         | (2-24 h) | (2-24 h) | (2-24 h) | (2-24 h) | (2-24 h) | (2-24 h) |
| Ec271 NDM-1      | B         | B         | B         | B         | B         | B         |
| (2-24 h)         | (2-24 h) | (2-24 h) | (2-24 h) | (2-24 h) | (2-24 h) | (2-24 h) |
| *E. cloacae* 32  | –         | B + S     | S         | –         | B + S     | B         |
| (24 h)           | (8-24 h) | (24 h)   |     | (24 h)   | (24 h)   | (8-24 h) |
| *E. cloacae* 297 | B         | B         | B         | B         | S         | B         |
| (4-24 h)         | (2-24 h) | (4-24 h) | (4-24 h) | (2-24 h) | (2-24 h) | (4-24 h) |

**TABLE 3** | In vitro activity of pentamidine in combination with antimicrobials against eight of carbapenemase-producing and/or colistin-resistant *Enterobacteriaceae* clinical strains.

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PEN, pentamidine; GEN, gentamicin; AMK, amikacin; TOB, tobramycin; TGC, tigecycline; RIF, rifampicin; DOR, doripenem; B, Bactericidal; –: no bactericidal activity found; (): time frame in hours of the in vitro activity found.
In vitro Selection of Resistant Mutants

The high bactericidal activity of pentamidine in combination with antimicrobials, achieving bacterial concentrations close to 0 log cfu/mL did not allow to analyze the selection of resistant mutants after 24 h incubation.

DISCUSSION

This study evaluates the use of pentamidine as antimicrobial agent against clinical strains of carbapenemase-producing and/or colistin-resistant Enterobacteriaceae, finding a strong in vitro activity both with pentamidine alone and combined with other antimicrobials, as aminoglycosides, tigecycline, doripenem, and rifampicin. Additionally, pentamidine was synergistic against selected strains in combination with some of these antimicrobials, especially when was studied combined with rifampicin.

Pentamidine showed a MIC range against eight clinical strains of carbapenemase-producing and/or colistin-resistant Enterobacteriaceae from 200 to 800 mg/L. Due to its use as an antiprotozoal agent (Nguewa et al., 2005), no susceptibility breakpoints for pentamidine are defined. However, the MIC values obtained are in accordance to those reported analyzing the in vitro activity of pentamidine and five pentamidine analogs against a E. coli strain, with MIC values ranging from 100 to
Moreover, we found that pentamidine at MIC concentration is bactericidal against seven of the eight tested strains; furthermore, at 1/2xMIC was bactericidal against the Kp28 OXA-48/CTX-M-15 producer strain.

Besides the robust bactericidal effect found with pentamidine alone, more important is that its combinations with the different antimicrobials tested potentiates the effect of these antimicrobials alone against the clinical strains using the time-kill assay. The in vitro activity observed with pentamidine in combination with antibiotics suggests there is strong possibility to repurpose it for antibacterial use against these difficult to treat MDR GNB (Pachón-Ibáñez et al., 2018; Rodriguez-Baño et al., 2018). These results are in accordance to the ones reported by Stokes et al. in which they found that the combination of pentamidine with rifampicin, novobiocin, erythromycin, or vancomycin potentiated the antimicrobials alone against a wild-type E. coli strain using checkerboard broth microdilution assays (Stokes et al., 2017). It is noteworthy that synergistic activity was observed when pentamidine was combined with amikacin, tobramycin, tigecycline, and/or rifampicin against the colistin-resistant E. cloacae 32, strain against which pentamidine alone did not show bactericidal activity. We would also like to mention, that no more synergistic effect with pentamidine in combination was observed due to the excellent bactericidal activity found with pentamidine alone at MIC concentration.

Pentamidine plus rifampicin was the combination that showed synergism against more of the tested strains (five out of eight). This excellent activity was also pointed out in the Stokes et al. study, in which pentamidine synergized with rifampicin against a wide phylogenetic distribution of antibiotic-resistant strains, including naturally polymyxin-resistant Serratia species (Stokes et al., 2017). The combination of rifampicin with other antimicrobials has been proved to be useful, both in vitro and in vivo, against other MDR GNB as Acinetobacter baumannii (Pachón-Ibáñez et al., 2010), as other example of repurposing a drug, such as rifampicin, previously used in staphylococcal and mycobacterial infections.

In summary, these results suggest that pentamidine, alone or in combination, may be a new alternative for the treatment of infections caused by carbapenemase-producing and/or colistin-resistant Enterobacteriaceae. To investigate further the possible usefulness of pentamidine new data from pharmacokinetics and pharmacodynamics and in vivo efficacy in experimental models of infection, including the dosage and safety, are required.

**AUTHOR CONTRIBUTIONS**

MP-I has planned and coordinated the experiments, analyzed the results, and written the manuscript. GL-H and TC-C had performed the in vitro experiments. YS had reviewed the manuscript. RÁ-M, EC-M, and JP had reviewed the manuscript and the experiments.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2018.00363/full#supplementary-material
Pentamidine Activity Against Clinical Enterobacteriaceae

Nguewa, P. A., Fuertes, M. A., Cepeda, V., Iborra, S., Carrion, J., Valladares, B., et al. (2005). Pentamidine is an antiparasitic and apoptotic drug that selectively modifies ubiquitin. *Chem. Biodivers.* 2, 1387–1400. doi: 10.1002/cbdv.20059011

Olaitan, A. O., Morand, S., and Rolain, J. M. (2016). Emergence of colistin-resistant bacteria in humans without colistin usage: a new worry and cause for vigilance. *Int. J. Antimicrob. Agents* 47, 1–3. doi: 10.1016/j.ijantimicag.2015.11.009

Oteo, J., Ortega, A., Bartolome, R., Bou, G., Conejo, C., Fernandez-Martinez, M., et al. (2015). Prospective multicenter study of carbapenemase-producing Enterobacteriaceae from 83 hospitals in Spain reveals high *in vitro* susceptibility to colistin and meropenem. *Antimicrob. Agents Chemother.* 59, 3406–3412. doi: 10.1128/AAC.0086-15

Pachón-Ibáñez, M. E., Docobo-Perez, F., Lopez-Rojas, R., Dominguez-Herrera, J., Jimenez-Meijas, M. E., Garcia-Curiel, A., et al. (2010). Efficacy of rifampin and its combinations with imipenem, sulbactam, and colistin in experimental models of infection caused by imipenem-resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother.* 54, 1165–1172. doi: 10.1128/AAC.00367-09

Pachón-Ibáñez, M. E., Labrador-Herrera, G., Cebreiro-Canguero, T., Diaz, C., Smani, Y., Del Palacio, J. P., et al. (2018). Efficacy of colistin and its combination with rifampin *in vitro* and in experimental models of infection caused by carbapenemase-producing clinical isolates of *Klebsiella pneumoniae*. *Front. Microbiol.* 9:912. doi: 10.3389/fmicb.2018.00912

Pourraras, S., Vrioni, G., Neou, E., Dendrinos, J., Dimitroulia, E., Poulou, A., et al. (2011). Activity of tigecycline alone and in combination with colistin and meropenem against *Klebsiella pneumoniae* carbapenemase (KPC)-producing Enterobacteriaceae strains by time-kill assay. *Int. J. Antimicrob. Agents* 37, 244–247. doi: 10.1016/j.ijantimicag.2010.10.031

Rodríguez-Baño, J., Gutiérrez-Gutiérrez, B., Machuca, I., and Pascual, A. (2018). Treatment of infections caused by extended-spectrum-beta-lactamase-, ampC-, and carbapenemase-producing enterobacteriaceae. *Clin. Microbiol. Rev.* 31, e00799–e00017. doi: 10.1128/CMR.00799-17

Ruiz-Aragón, M., Ballestero-Téllez, M., Gutiérrez-Gutiérrez, B., de Cueto, M., Rodríguez-Baño, J., and Pascual, Á. (2018). Direct bacterial identification from positive blood cultures using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry: A systematic review and meta-analysis. *Enferm. Infecc. Microbiol. Clin.* 36, 484–492. doi: 10.1016/j.eimc.2017.08.012

Souli, M., Galani, I., Boukova, S., Gourgoulis, M. G., Chryssouli, Z., Kanellopoulou, K., et al. (2011). *In vitro* interactions of antimicrobial combinations with fosfomycin against KPC-2-producing *Klebsiella pneumoniae* and protection of resistance development. *Antimicrob. Agents Chemother.* 55, 2395–2397. doi: 10.1128/AAC.01086-10

Sousy, C. J. (2012). *Antibiogram Committee of the French Society for Microbiology, Recommendations*. Available online at: http://www.sfm-microbiologie.org/UserFiles/files/casfm/CASFM_2012.pdf

Spellberg, B., and Rex, J. H. (2013). The value of single-pathogen antibacterial agents. *Nat. Rev. Drug Discov.* 12, 963. doi: 10.1038/nrd3957-c1

Stokes, J. M., MacNair, C. R., Ilyas, B., French, S., Cote, J. P., Bouwman, C., et al. (2017). Pentamidine sensitizes Gram-negative pathogens to antibiotics and overcomes acquired colistin resistance. *Nat. Microbiol.* 2:17028. doi: 10.1038/nmicrobiol.2017.28

Trecarichi, E. M., and Tumbarello, M. (2017). Therapeutic options for carbapenem-resistant Enterobacteriaceae infections. *Virology* 8, 470–484. doi: 10.1080/21505594.2017.1292196

Wang, J., He, J. T., Bai, Y., Wang, R., and Cai, Y. (2018). Synergistic activity of colistin/fosfomycin combination against carbapenemase-Producing *Klebsiella pneumoniae* in an *in vitro* pharmacokinetic/pharmacodynamic model. *Biomed Res. Int.* 2018:5720417. doi: 10.1155/2018/5720417

World Health Organization (2017). *Global Priority List of Antibiotic-resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics*. Available online at: http://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/

Younis, W., Thangamani, S., and Seleem, M. N. (2015). Repurposing non-antimicrobial drugs and clinical molecules to treat bacterial infections. *Curr. Pharm. Des.* 21, 4106–4111. doi: 10.2174/1381612821666150506154434

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