Azidothymidine produces synergistic activity in combination with colistin against antibiotic-resistant Enterobacteriaceae

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Running title: Azidothymidine combination with colistin

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Abstract

Bacterial infections remain the leading killer worldwide which is worsened by the continuous emergence of antibiotic resistance. In particular, antibiotic-resistant Enterobacteriaceae is prevalent and extremely difficult to treat. Reusing existing drugs and rejuvenating the therapeutic potential of existing antibiotics represent an attractive novel strategy.

Azidothymidine (AZT) is an antiretroviral drug which is used in combination with other antivirals to prevent and to treat HIV/AIDS. AZT is also active against Gram-negative bacteria but has not been developed for that purpose. Here we investigated in vitro and in vivo efficacy of AZT in combination with colistin against antibiotic-resistant Enterobacteriaceae including extended-spectrum beta-lactamase (ESBL), New Delhi metallo-beta-lactamase 1 (NDM) or the mobilized colistin resistance (mcr-1) producing strains. Minimum inhibitory concentration was determined using the broth microdilution method. The combinatory effect of AZT and colistin was examined using the checkerboard method and time-kill analysis. A murine peritoneal infection model was used to test the therapeutic effect of the combination of AZT and colistin. Fractional inhibitory concentration index from checkerboard assay demonstrated that AZT synergized with colistin against 61% and 87% of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae*, respectively, 100% of NDM-1-producing strains and 92% of mcr-1 producing *E. coli*. Time-kill analysis demonstrated significant synergistic activities when AZT was combined with colistin. In the murine peritoneal infection model, AZT in combination with colistin showed augmented activities of both drugs in the treatment of NDM-1 *K. pneumoniae* and mcr-1 *E. coli* infections. AZT and colistin combination poses a potential to be used coherently to treat antibiotic-resistant Enterobacteriaceae infections.

**Keywords:** Enterobacteriaceae, azidothymidine, colistin, ESBL, NDM-1, mcr-1
INTRODUCTION

Bacterial infection remains a leading killer worldwide (1) and antibiotic resistance continues to plague the effective control of this pandemic health problem (2, 3). In particular, there is an urgent global threat with an increasing prevalence of multidrug-resistant Enterobacteriaceae, especially carbapenem-resistant Enterobacteriaceae (CRE) such as New Delhi Metallo-beta-lactamase-1 (NMD) carriers (4-8) which are extremely resistant to almost all of our antibiotics (3, 9). As a result, our ability to treat serious community and nosocomial acquired infections is rapidly diminishing (10). Unfortunately, the number of new antibiotics reaching the market annually is unable to keep up with the development of bacterial antibiotic resistance (11-14). The drug discovery process itself is arduous and costly and it is almost impossible to produce a large group of effective antibiotics within a short period of time to combat antibiotic resistance. Therefore, a different therapeutic approach is needed to replenish our antibiotic reservoir against resistant bacteria and the most promising of such strategies is to reuse existing drugs and to restore the therapeutic potencies of existing antibiotics (15, 16).

Azidothymidine (3'-azido-3'-deoxythymidine AZT) is an antiretroviral drug which is used in combination with other antivirals to prevent and to treat HIV/AIDS. It inhibits viral reverse transcriptase and was the first effective treatment for HIV/AIDS (17) entering the US market in 1986. AZT is also active against Gram-negative bacteria (18-22) but has not been developed or approved for that purpose. It is thought to inhibit bacterial DNA replication by chain termination. Resistance to AZT occurs in bacteria and has been attributed to two mechanisms, one of which is unknown and the other is a deficiency of thymidine kinase which phosphorylates inactive AZT into the active triphosphate form (23).

The rapid emergence of CRE which are often resistant to many other antibiotics, has left the world with colistin as the last resort treatment option. The use of colistin has led to high
rates of colistin resistance in patients with infections due to *K. pneumoniae* carbapenemases (KPC) - producing strains (24). A recent study also found that approximately 10% of NDM-1 producing CRE were colistin resistant in the UK (25) and plasmid-born colistin resistance was also found recently in animals and humans (26). Hence it is crucial to boost the effectiveness of colistin against colistin resistant bacteria. However, treatment with colistin has been associated with both nephron- and neurotoxic adverse effects (27). It is not known if AZT can synergistically act with colistin to treat multidrug-resistant Enterobacteriaceae infections which allows the administration of both drugs at lower doses to achieve a desired therapeutic effect while minimising the side effects and to prevent emergence of antibiotic resistance (15, 28). In this study, we performed the first study to retrospectively test the *in vitro* activities of AZT in combination with colistin against 74 antibiotic-resistant Enterobacteriaceae including NDM-1, *mcr*-1 and ESBL producing strains. In addition, the therapeutic effectiveness of AZT plus colistin was tested using a mouse peritoneal infection model.

**MATERIALS AND METHODS**

**Bacterial strains and growth conditions.** The bacterial strains used were 74 antibiotic-resistant Enterobacteriaceae strains including 7 strains harboring the *bla*\textsubscript{NDM} plasmid which were ATCC BAA-2468 (*Enterobacter cloacae*), ATCC BAA-2469 (*E. coli*), ATCC BAA-2470 (*K. pneumoniae*), ATCC BAA-2471 (*E. coli*), ATCC BAA-2472 (*K. pneumoniae*) and ATCC BAA-2473 (*K. pneumoniae*) and NCTC13443 (*K. pneumoniae*), 13 colistin resistant *E. coli* containing *mcr*-1 plasmid (Table S1) (29-32), 54 antibiotic-resistant Gram-negative strains (23 *E. coli* and 31 *K. pneumoniae*) isolated in the hospitals in Hong Kong, Taiwan, Thailand, Korea, India, Singapore, Malaysia, Philippines and St George’s Hospital, London. The bacterial isolates were grown in nutrient broth (Oxoid, UK), on tryptone soya agar plates
Susceptibility tests of antibiotics and AZT. The minimum inhibitory concentrations (MIC) of antibiotics and AZT were determined using the broth microdilution method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (33). MIC was performed using 96-well polystyrene micro-titre plates (Fisher Scientific, UK). The antibiotics were diluted with two-fold serial dilutions in triplicate followed by addition of a standard bacterial suspension of 1-5 × 10^5 CFU/mL in cation adjusted Mueller Hinton Broth (CA-MHB, Sigma-Aldrich, UK). After 16 - 20 hours of incubation at 37°C, the optical density (OD) readings were determined using an absorbance microplate reader (ELx800, BioTek).

The lowest concentration of an antibiotic which produced a similar OD reading as the control (medium only) was determined as MIC value. The MIC for each agent was identified as the lowest concentration required to inhibit bacterial growth. The MIC_{50} and MIC_{90} values were calculated to investigate the lowest concentrations required to inhibit growth in 50% and 90% of the strains, respectively.

Detection of ESBLs in the antibiotic-resistant Gram-negative isolates. Detection of the multidrug-resistant Enterobacteriaceae producing extended spectrum β-lactamases were performed according to the UK standard for microbiology investigations (34) using CHROMID ESBL (bioMérieux, UK) (35), double-disc synergy test (DDST) (36) and combination disc test (CDT) (34). Detection of ESBL genes were performed by polymerase chain reaction (PCR) using the primers (Table S2) followed by DNA sequencing of the PCR fragments (DNA Sequencing & Services, University of Dundee).

Checkerboard assays to determine combination effects of AZT with antibiotics. Combination of AZT and antibiotic was prepared using 96 well polystyrene micro-titre plates with drug concentrations starting two-fold higher than their MIC values, and were then
serially diluted in a two-fold manner. The two drugs were mixed in a 96 well plate followed by addition of a standard bacterial suspension at 1-5 x 10^5 CFU/mL in CA-MHB. After incubation for 16 - 20 hours at 37°C, the OD values were read using the ELx800 absorbance microplate reader (BioTek). The combinatory effects were determined by calculating the fractional inhibitory concentration index (FICI) of the combination as follows: 

\[
\text{FICI} = \frac{\text{MIC of Drug A, tested in combination}}{\text{MIC of Drug A, tested alone}} + \frac{\text{MIC of Drug B, tested in combination}}{\text{MIC of Drug B, tested alone}}
\]

Synergy was defined as a FICI ≤0.5, no interaction was identified with an FICI >0.5 but <4 and antagonism if the FICI was >4 (37).

**Time-Kill analysis of antibiotics alone and in combination with AZT against log-phase bacteria.** A range of different concentrations of colistin and AZT was chosen according to the checkerboard evaluation as a synergistic combination. The drugs was prepared in a two-fold serial dilution and was added in combination or alone to log phase bacterial cultures suspension containing 1 x 10^7 CFU/mL (38) in CA-MHB, and incubated at 37°C. Viability expressed as log CFU/mL was determined at 0, 2, 4, 8, 24 and 48 hours of incubation by plating out 100 µL of serial dilutions of the cultures onto tryptone soy agar (Oxoid) plates. The colonies on the agar plates were counted using an aCOLyte colony counter (Synbiosis) and analysed with the counter’s software. Synergistic activity was confirmed as a≥2-log_{10} decrease in CFU counts at 24 hours of the combination compared to the antibiotic alone, in addition to a ≥2-log_{10} decrease compared to the zero hour count (39).

**Mouse peritoneal infection model.** Female ICR mice (five to six weeks old, body weight 24 - 26 g) were used (Harlan UK Ltd) for the mouse peritoneal infection model (40). Human medicines of AZT (Retrovir® 10 mg/ml, ViiV Healthcare UK Ltd) and colistin methanesulfonate (CMS) (Colomycin® injection, Forrest) were used in the mouse study.
Mice were infected intraperitoneally with two hundred microliter bacterial suspension containing 10^7 CFU of the NDM-1 *K. pneumoniae* BAA2472 and the *mcr-1* *E. coli* strain Af40 (Table S1). After 30 minutes of infection, AZT (2, 5 or 10 mg/kg) and CMS (10, 20 or 30 mg/kg) singly or in combination was injected intravenously into the mice. A group of mice was treated with saline as a control group. At 30 minutes after infection (treatment starting), 2 and 6 hours after treatment, 4 mice in each group were sacrificed and 1 ml sterile PBS was injected intraperitoneally followed by gently massaging of the abdomen. Peritoneal fluid was sampled aseptically. The fluid was diluted in a serial of 10-fold dilutions and 100 µl of each dilution were plated onto tryptone soy agar (Oxoid) plates. Viability was defined as Log CFU/ml of peritoneal fluid.

The animal husbandry guidelines and all animal experiments were performed according to the Animals Scientific Procedures Act, 1986 (an Act of the Parliament of the United Kingdom 1986 c. 14) (Home Office Project licence Number 70/7077) with approval from St George’s, University of London ethics committee.

**Statistical analysis.** The significance of differences between experimental groups was determined by Student’s t test. P values <0.05 were considered significant.

**RESULTS**

**In vitro** susceptibility of AZT and colistin against 74 antibiotic-resistant Enterobacteriaceae. The MICs for aztreonam, amoxicillin, piperacillin, cefotaxime, ceftriaxone, ceftazidime, meropenem, amikacin, gentamicin, tobramycin, ciprofloxacin, levofloxacin, trimethoprim, nitrofurantoin, rifampicin, tigecycline, colistin, polymyxin B, and AZT were determined against the 7 NDM-1 strains. As seen in Table 1, compared with the antibiotic breakpoints (41) resistance was found in all strains for nearly all antibiotics. Only certain strains were susceptible to a number of antibiotics such as nitrofurantoin (BAA-2469), amikacin (BAA-2471) and tigecycline (BAA-2469, BAA-2470 and BAA-2471).
However, all NDM-1 strains were susceptible to colistin (41). AZT MIC ranged from 2 to 4 mg/L.

The MICs for the antibiotics and AZT were also determined against the 54 antibiotic-resistant isolates, *E. coli* and *K. pneumoniae*. As shown in Table 2 and Table S3, these strains were resistant to monobactam, penicillins and cephalosporins but were susceptible to carbapenems. Resistance was seen for gentamicin, ciprofloxacin and trimethoprim. 90% of the strains were susceptible to tigecycline and colistin. The MIC for AZT ranged from 0.25 to 64 for *E. coli* and 2 to 32 for *K. pneumoniae*. The 54 multidrug-resistant *E. coli* and *K. pneumoniae* were tested for ESBL production using commercial ESBL-testing systems and demonstrated that these were ESBL producing strains (Table S3).

For colistin resistant strains, the MIC for AZT ranged from 8 to 64 with MIC50 at 8 mg/L and MIC90 at 64 mg/L. The range of MIC for colistin was 2 to 8 mg/L with MIC50 at 4 mg/L and MIC90 at 8 mg/L.

**Checkerboard analysis of combination effects.** The effects of combining AZT with colistin were determined using checkerboard assays against all the 74 strains. As shown in Table 3, the combination of AZT with colistin showed synergistic activity with FIC index ≤0.5 against 60.87% of the ESBL *E. coli*, 87.1% of the ESBL *K. pneumoniae*, 100% of NDM-1 strains and 92.31% of colistin resistant (*mcr-1*) *E. coli*. With the concentration of AZT range from 0.25 to 16 mg/L, the MICs of colistin were significantly reduced from 32 to 256-fold against the seven NDM-1 strains, 2 to 64-fold against ESBL *E. coli*, 2 to 512 fold against ESBL *K. pneumoniae* and 4 to 256 fold against *mcr-1* containing *E. coli*.

**Time kill analysis of AZT in combination with colistin against log-phase bacteria.** The synergistic combination of AZT and colistin was performed using time kill assays against 7 NDM-1, 3 ESBL *E. coli* and 3 ESBL *K. pneumoniae* and 3 *mcr-1* *E. coli* which showed an FICI <0.5 for the combination. The characteristics of the 16 strains are shown in Table S4.
A range of different concentrations was used starting from 2 fold or MIC level for each of the two drugs. Data from representative strains are shown to display combinations with the synergistic activities. As shown in Figure 1 for the NDM-1 *K. pneumoniae* BAA2472, colistin at 2 mg/L was bactericidal until 7 hours followed by a regrowth and at 1 mg/L (MIC) inhibited bacterial growth. AZT at 4, 2 (MIC) and 1 mg/L was bactericidal showing dose-dependent kill and regrowth occurred after 8 hours of drug exposure. However when colistin at 2 mg/L combined with 4 and 2 mg/L of AZT, significant killing to the limit of detection of initial bacterial counts was achieved within 4 hours, and the same kill was seen at 8 hours when the same concentration of colistin combined with 1 mg/L of AZT (Figure 1A-1C). When colistin at 1 mg/L was combined with 4, 2 and 1 mg/L of AZT, kill at the level of limit of detection was achieved at 8 hours (Figure 1E-1F). No bacterial regrowth was observed in both 24 (Figure 1) and 48 hours of post-treatment (data not shown).

As shown in Figure 2, for the *mcr-1* *E. coli* strain Af40 (Table S1), colistin at 8 mg/L (MIC) inhibited bacterial growth and at 4 mg/L showed the similar growth pattern as the control. AZT at 4, 2 and 1 mg/L reduced the initial counts till 4 hours and regrowth was seen. When colistin at 8 mg/L was combined with the concentrations of 4, 2 and 1 mg/L AZT, kill to the limit of detection was seen at 8 hours (Figure 2A – 2C). The same effects were seen when colistin at 4 mg/L was combined with 4 mg/L of AZT (Figure 2D). Reduced effects were seen when colistin at 4 mg/L with 4 and 2 mg/L of AZT and kill to the limit of detection was shown at 24 hours (Figure 2E and 2F).

Significant synergistic activity was also observed in other 6 NDM-1 strains (Figure S1-S6), 3 ESBL *E. coli* (Figure S7-S9) and 3 ESBL *K. pneumoniae* (Figure S10-S12) and two colistin resistant *mcr-1* *E. coli* (Figure S13-S14).

**In vivo combination activities of AZT combined with colistin.** The *in vivo* activity of AZT combination with colistin was studied using a murine peritoneal infection model. A dose
range study of the two drugs was performed. For AZT, the minimal dosages (5 mg/kg) was chosen which only inhibited bacterial growth but provide significant enhanced activities when combined with CMS. For CMS, we found that 10 to 30 mg/kg showed no activities against the infected bacteria. Therefore, for the colistin sensitive NDM-1 strain, we used 10 mg/kg of CMS and for the mcr-1 E. coli, we used 20 mg/kg of CMS. The drugs were tested singly or in combination against the NDM-1 K. pneumoniae BAA2472 and the mcr-1 E. coli strain Af40 (Table S4).

As shown in Figure 3A, for strain K. pneumoniae BAA2472, compared with the untreated control, colistin at 10 mg/kg showed no activities at both 2 and 6 hours and AZT at 5 mg/kg inhibited bacterial growth. Combination of colistin with AZT, although only showing inhibition at 2 hours, exhibited 2.72 log kill of the bacterium at 6 hours. The difference of the bacterial numbers between zero hour and 6 hours was significant (P <0.001, n=4). For E. coli strain Af40 (Figure 3B), colistin at 20 mg/kg showed the same growth pattern as the control and AZT inhibited bacterial growth. Combination of colistin with AZT exhibited 1.32 and 2.96 log kill of the bacterium at 2 and 6 hours, respectively. The difference of the bacterial numbers between zero hour and 2 hours or 6 hours was significant (P <0.01 and 0.001, respectively, n=4). In both untreated control groups and the colistin treated group, all animals developed mild clinical signs such as transiently hunched posture at 6 hours after infection. The animals in other treatment groups showed no discomfort with normal and heathy behaviors. All animals were sacrificed at 6 hours after treatment according to the restriction of adverse effects in the project licence.

DISCUSSION

In this study, we demonstrated for the first time that AZT synergized with colistin against 74 antibiotic-resistant Enterobacteriaceae including NDM-1, ESBL and colistin resistant
strains. The antibiotic-resistant Enterobacteriaceae isolates used in the study covered a broad geographic distribution. The colistin resistant strains were from some European countries and South Africa (29-31). The 7 NDM-1 strains represented the most resistant type of Enterobacteriaceae.

The clinical efficacy of AZT has been demonstrated to reduce morbidity and mortality in patients with asymptomatic or acute human immunodeficiency virus (HIV) disease (43, 44). In patients, the oral dosage is 250 – 300 mg twice daily and intravenous infusion is 0.8 – 1 mg/kg every 4 hours for up to 2 weeks. It has been shown that 120 mg iv dosing produced an AUC of 0.0014 mg.h/L and a Cmax of 0.0015 mg/L while 200 mg oral dosing gave rise to an AUC of 0.0017 mg.h/L and a Cmax of 0.0018 mg/L (45). AZT has been shown to be active against Gram-negative bacteria (18-20), it is not known if the concentrations used clinically are sufficient to treat bacterial infections in humans.

Colistin is effective against multidrug-resistant but colistin susceptible Pseudomonas aeruginosa, K. pneumoniae, Acinetobacter (46) and importantly NDM-1 carrying Enterobacteriaceae (8). There is increasing evidence to show that colistin resistance is on the rise, especially the discovery of plasmid born colistin resistance worldwide (26, 47, 48).

It is critically important to preserve and prolong the life of the last resort of antibiotic by enhanced combination therapy. Here we have shown that in combination with AZT, colistin MIC was significantly reduced, especially against mcr-1 containing colistin resistant strains. The enhanced activity of colistin in combination with AZT was confirmed with time kill assays which provided dynamic measures of bactericidal activities of the combination over time. In colistin mono exposure, complete eradication of the NDM-1 K. pneumoniae BAA2472 or mcr-1 E. coli AT40 strains required much higher concentrations of the drug (data not shown), however, more than 4 to 16-fold lower concentrations of colistin when combined with AZT achieved the same effect. This is significant as enhancement of colistin
combination with AZT will likely reduce the dose of colistin but retain maximal therapeutic
efficacy hence minimize its toxic profile. These data suggest that further clinical
development of a colistin plus AZT combination may be able to achieve an effective lower
dose colistin therapy against colistin-sensitive and colistin-resistant infections.

Bacterial infections caused by carbapenem resistant strains are life threatening and
effective treatment is difficult to achieve. The last resort treatment option is to use colistin
(9, 49). Previous studies have shown that in bacteria AZT needs to be converted to the
nucleotide to inhibit bacterial DNA replication (50) and that bacterial thymidine kinase is
responsible for the initiation of the activation process - phosphorylation of AZT (23, 50).

Other antibiotics such as ciprofloxacin also inhibit DNA replication by blocking GyrA. But
comparison of the resistance profiles of ciprofloxacin and AZT are very different (see Table
3). This suggests that AZT has a different mechanism of action to other antibacterial agents
which are in the market. Rather, AZT is likely to act on a new target in bacteria. Further
studies on how AZT acts against Gram-negative bacteria are underway in our laboratories
by analysis of AZT mutants with next-generation sequencing and investigation of the AZT
effect on bacteria by performing Bacterial Cytological Profiling (BCP).

The therapeutic effectiveness of AZT and colistin combinations was also examined using a
mouse peritoneal infection model. As a potential therapeutic agent, AZT has been used to
treat HIV. Its bactericidal activity has been reported in vivo (19). Here we demonstrate that
AZT at 5 mg/kg inhibited the NDM-1 K. pneumoniae BAA2472 and the mcr-1 E. coli strain
Af40 growth in the mouse peritoneal infection. However, the combination of AZT with
colistin improved the therapeutic activities of each single agent with significant kill of the
bacteria at 2 or 6 hours in mouse peritoneal cavity. Most importantly, when colistin
methanesulfonate was completely ineffective up to 6 hours of treatment, the addition of
AZT was able to significantly reduce bacterial counts and attenuate the clinical signs in the
animals. Here we used colistin methanesulfonate instead of colistin sulfate. The reason was that colistin methanesulfonate is used clinically and is less toxic than colistin sulfate in mice (51). Colistin methanesulfonate is a prodrug which needs to convert to the active form of colistin (52). The conversion normally delays the activity of the drug (52). Here we demonstrated that with the addition of AZT, the effect of colistin methanesulfonate and AZT was significantly increased. Collectively, the data show that the application of AZT and CMS combination therapy in vivo offers the potential to increase both colistin and AZT activities against antibiotic-resistant Enterobacteriaceae.

In conclusion, in this proof-of-principle study, we demonstrated the high therapeutic efficacy of AZT-plus-colistin combination therapy against antibiotic-resistant Enterobacteriaceae, including mcr-1, NDM-1 and ESBL strains. ESBL strains were confirmed using commercially-accepted phenotypical methods currently using in clinical practice. The interaction between the genotypic characteristics of ESBL strains and this novel combination therapy deserves further investigation. Importantly, we showed that the combination of AZT with colistin significantly reduced the bacterial burden in vivo. This early groundwork lays the foundation for further validation in clinical trials enabling translation of the combination therapy into clinical benefits for patients.

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REFERENCE


36. The Clinical & Laboratory Standards Institute. 2015. Performance standards for antimicrobial susceptibility testing; Twenty-Fifth Informational Supplement. CLSI document M100-S25. Wayne, PA, USA: CLSI.


Combination concentrations of AZT and colistin are colistin 8 mg/L + AZT 4 mg/L (A), colistin 8 mg/L + AZT 2 mg/L (B), colistin 8 mg/L + AZT 1 mg/L (C), colistin 4 mg/L + AZT 4 mg/L (D), colistin 4 mg/L + AZT 2 mg/L (E) and colistin 4 mg/L + AZT 1 mg/L (F). The dash line is the limit of detection in the assay (20 CFU/ml).

Figure 3. Effects of AZT in combination with colistin against the NDM-1 K. pneumoniae BAA2472 and the mrc-1 E. coli strain Af40 in a mouse peritoneal infection model. A. Mice were infected with strain BAA2472. Treatment was initiated 30 minutes after infection with AZT (5 mg/kg), CMS (10 mg/kg) and AZT plus CMS. B. Mice were infected with strain Af40. Treatment was initiated 30 minutes after infection with AZT (5 mg/kg), CMS (20 mg/kg) and AZT plus CMS. Bacterial counts in the peritoneal cavity were determined from 4 mice for each group at 0 hour before and 2 and 6 hours post-treatment. The data has been repeated once. ** indicates p≤0.01. *** indicates p≤0.001.
Table 1. MIC values of antibiotics and AZT against 7 NDM-1 producing strains

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Table 2. MIC values of antibiotics and AZT against ESBL and mcr-1 producing E. coli and K. pneumoniae

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<th>Antimicrobial</th>
<th>MIC range (mg/L)</th>
<th>MIC50</th>
<th>MIC90</th>
<th>MIC range (mg/L)</th>
<th>MIC50</th>
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<th>MIC range (mg/L)</th>
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<td>0.125 - 128</td>
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-, not tested
Table 3. Combination activities of AZT with colistin

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<tr>
<th>Strains</th>
<th>Combination activity</th>
<th>FICI</th>
<th>AZT + colistin</th>
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<td><strong>ESBL E. coli</strong></td>
<td>synergy ≤ 0.5</td>
<td>14 (60.87%)</td>
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<tr>
<td></td>
<td>no interaction 0.56 -1</td>
<td>9 (39.13%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>antagonism &gt;4</td>
<td>0</td>
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</tr>
<tr>
<td><strong>ESBL K. pneumoniae</strong></td>
<td>synergy ≤ 0.5</td>
<td>27 (87.10%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>no interaction 0.56 -1</td>
<td>4 (12.90%)</td>
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<tr>
<td></td>
<td>antagonism &gt;4</td>
<td>0</td>
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</tr>
<tr>
<td><strong>NDM-1 Strains</strong></td>
<td>synergy ≤ 0.5</td>
<td>7 (100%)</td>
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<tr>
<td></td>
<td>no interaction 0.56 -1</td>
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<tr>
<td></td>
<td>antagonism &gt;4</td>
<td>0</td>
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<tr>
<td><strong>mcr-1 E. coli</strong></td>
<td>synergy ≤ 0.5</td>
<td>12 (92.31%)</td>
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</tr>
<tr>
<td></td>
<td>no interaction 0.56 -1</td>
<td>1 (7.69%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>antagonism &gt;4</td>
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