Synergistic antibacterial effects of colistin in combination with aminoglycoside, carbapenems, cephalosporins, fluoroquinolones, tetracyclines, fosfomycin, and piperacillin on multidrug resistant \textit{Klebsiella pneumoniae} isolates

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Abstract

Multidrug resistant Enterobacterales have become a serious global health problem, with extended hospital stay and increased mortality. Antibiotic monotherapy has been reported ineffective against most drug resistant bacteria including \textit{Klebsiella pneumoniae}, thus encouraging the use of multidrug therapies as an alternative antibacterial strategy. The present works assessed the antibacterial activity of colistin against \textit{K. pneumoniae} isolates. Resistant isolates were tested against 16 conventional antibiotics alone and in combination with colistin. The results revealed that all colistin resistant isolates demonstrated multidrug resistance against the tested antibiotics except amikacin. At sub-inhibitory concentrations, combinations of colistin with amikacin, or fosfomycin showed synergism against 72.72\% (8 of 11 isolates). Colistin with either of gentamicin, meropenem, cefoperazone, cefotaxime, ceftazidime, moxifloxacin, minocycline, or piperacillin exhibited synergism against 81.82\% (9 of 11 isolates). Combinations of colistin with either of tobramycin or ciprofloxacin showed synergism against 45.45\% (5 in 11 isolates), while combinations of colistin with imipenem or ceftolozane and tazobactam displayed 36.36\% (4 of 11 isolates) and 63.64\% (7 of 11 isolates) synergism. In addition, combinations of colistin with levofloxacin was synergistic against 90.91\% (10 of 11 isolates). The results revealed that combinations of colistin with other antibiotics could effectively inhibit colistin resistant isolates of \textit{K. pneumoniae}, and thus could be further explore for the treatment of multidrug resistant pathogens.
Introduction

The spread of multidrug resistant bacteria has become a public health emergency that threatens the continued usage of antibiotics chemotherapy. Infections due to carbapenemase-producing Enterobacterales are fast spreading across the globe, rendering the healthcare systems ineffective. It is estimated that infections associated with drug-resistant pathogens currently cause about 700,000 deaths annually, and if the status quo prevails could increase to 10 million annual deaths by 2050 [1]. *Klebsiella pneumoniae* is a Gram-negative Enterobacterales member commonly associated with hospital acquired infections. In the past, carbapenems were used as choice drugs for the treatment of *K. pneumoniae* infection. However, with the emergence and spread of carbapenemase-producing Enterobacterales, colistin is considered a last-resort treatment option for infections caused by carbapenem resistant bacterial isolates.

Colistin also known as polymyxin E is a cationic peptide that targets the negatively charged lipopolysaccharide (LPS) of Gram-negative bacteria. Colistin competitively displaces divalent cations (Ca$^{2+}$ and Mg$^{2+}$) from the phosphate groups of membrane lipids, resulting in the rupture of cell membrane and leakage of intracellular components [2]. Emergence of colistin resistance in Enterobacterales including *K. pneumoniae* has been reported in many parts of the globe [3, 4] and is fast spreading [5]. Colistin resistance was generally thought to be mediated by alterations and modification of the LPS target through addition of positively charged 4-amino-4-deoxy-L-arabinose (L-Ara4N) and phosphoethanolamine (pEtN) cationic molecules, responsible for decrease in bacterial outer membrane negative charge and reduced interaction with colistin [6]. However, recent scientific enquiries have reported the role of efflux pumps [7–10] and plasmid encoded *mcr* 1–9 genes [4, 11, 12] in colistin resistance.

As the fight against multidrug resistance continues, concerted efforts by agencies, health care systems and biomedical scientist are restlessly exploring possible alternatives that might suffice pending the discovery and development of novel antibacterial agents that could effectively curb the spread of antibiotics resistance. The use of antibiotics combinations [13–15], efflux pump inhibitors [16–18], and resistance modifying agents [19, 20] are suggested as temporary control measures to reverse microbial resistance or enhance the inactivation of resistant bacterial isolates. Antibiotics combination therapy is proposed as a reliable option with demonstrated results against multidrug resistant bacterial isolates.

The present study tested the antibiotic susceptibility of 85 *K. pneumoniae* isolates obtained from hospitals in the southern region of Thailand against colistin. The resistant isolates were further tested against several groups of antibiotics, and the synergistic antibacterial effects of combinations of colistin with other antibiotics were evaluated. The study presents *In vitro* experimental antibacterial data from broth micro-dilution technique and the checkerboard assay but did not monitor the time-kill kinetics.

Materials and methods

Chemicals and media

All culture media were purchased from Becton Dickinson & Co. Difco™ (Franklin Lakes, NJ, USA). Colistin sulfate, piperacillin, minocycline hydrochloride, tobramycin, and moxifloxacin hydrochloride were obtained from Sigma-Aldrich, (Saint Louis, MO, USA). Ciprofloxacin, cefotaxime, and levofloxacin were purchased from Siam Bheasach Co., Ltd. (Bangkok, Thailand). Tigecycline and ceftaroline fosamil were Pfizer Inc. (Philadelphia, PA, USA). Ceftazidime was obtained from Reyoun Pharmaceutical Co., Ltd. (Shandong, China). Imipenem was obtained from Merck Sharp & Dohme Corp. (Elkton, VA, USA). Meropenem was obtained from M&H manufacturing Co., Ltd. (Samutprakarn, Thailand). Cefoperazone and
sulbactam was obtained from L.B.S. Laboratory Ltd. (Bangkok, Thailand). Ceftolozane and tazobactam was obtained from Steri-Pharma, LLC (Syracuse, NY, USA). Fosfomycin was obtained from Meiji seikakaisna, Ltd. (Tokyo, Japan).

Bacterial strains
Isolates collected from hospitalized patients unresponsive to antibiotics chemotherapy, with prolonged hospital stay in 8 hospitals located in Southern Thailand were used in this study. All isolates were identified to species level using standard biochemical tests and MALDI-TOF-MS. A total of 85 *K. pneumoniae* isolates that exhibited colony on MacConkey agar supplemented with 6 μg/mL imipenem were taken as resistant to carbapenems. The surveillance study was conducted post antibiotics treatments. *Escherichia coli* ATCC 25922 was used as a quality control. All the bacterial cultures were stored in tryptic soy broth (TSB supplemented with 40% glycerol and kept at -80˚C.

Antimicrobial susceptibility testing
Minimum inhibitory concentrations (MICs) were determined by the broth microdilution method in according to Clinical and Laboratory Standards Institute (CLSI) guidelines [21]. Briefly, serial two-fold dilutions of antibiotics were prepared in cation-adjusted Mueller-Hinton II broth (CA-MHII). To investigate the effect of each antibiotic, an aliquot of 100 μL of diluted bacterial suspension (1x10⁶ CFU/ml) was mixed with 100 μL antibiotic into each well and incubated at 37˚C for 18 h. MIC was expressed as the lowest concentration of the antibiotic that inhibits visible growth after incubation. Following European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints, isolates with a colistin MIC ≤ 2 μg/mL were categorized as susceptible and those with a colistin MIC ≥ 4 μg/mL were categorized as resistant [22]. To determine the MBC, MIC and supra-MIC dilutions were spotted on an agar plate and incubated overnight at 37˚C. Bacterial growth was observed, and MBC was defined as the lowest concentration that showed no visible bacterial regrowth.

Checkerboard technique
The synergistic effects of colistin and any of sixteen other antibiotics (amikacin, gentamicin, tobramycin, imipenem, meropenem, cefoperazone, cefotaxime, ceftazidime, ceftolozane and tazobactam, ciprofloxacin, levofloxacin, moxifloxacin, minocycline, tigecycline, fosfomycin, and piperacillin) on *K. pneumoniae* isolates were tested using the checkerboard technique. Each antibiotic was diluted to concentrations ranging from 1/64 MIC to 8 MIC of the previously determined MIC. Briefly, 50 μL of colistin and each antibiotic were assessed by adding 100 μL diluted bacterial suspension (1x10⁶ CFU/mL) into a well containing 50 μL colistin and 50 μL one of sixteen other antibiotics. The test was then read after 18 h of incubation at 37˚C. Each value was mean of triplicates from three independent experiments. The effects of the antimicrobial combinations were defined according to the fractional inhibitory concentration index (FICI) as following equation:

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

The FICI results for each combination were interpreted as follows: FICI ≤ 0.5, synergism; 0.5 < FICI < 1, additive; 1 ≤ FICI < 2, indifference; and FICI ≥ 2, antagonism. *Escherichia coli* ATCC 25922 was used as standard control strains for the assays [23].
Ethical statement

This retrospective study was approved by the Institutional Review Board (IRB) of the Faculty of Medicine, Prince of Songkla University, Thailand (EC: 54-080-14-1-2.). The researchers were granted permission to extract the data from the database with waiver of consent because of the observational nature of the study. All data were fully anonymized before the researcher accessed and analyzed.

Results and discussion

Distribution of *K. pneumoniae* isolates

In this study *K. pneumoniae* isolates obtained from patients receiving treatment in tertiary hospitals were tested against colistin. The isolates were collected and tested for antibiotics susceptibility due to patient’s unresponsiveness to antibiotics treatments and suspicion of opportunistic role of drug-resistant bacterial colonizers in aggravating health conditions of immunocompromised patients. Table 1. shows the distribution of the isolates, collected from eight public hospitals located in the southern region of Thailand based on sample type and location of the hospital. The results revealed that all nine isolates from Narathiwat hospital were resistant to colistin, while one out of the three isolates from Songkhla and the only isolate from Trang were also resistant to colistin. Demographic data, clinical characteristics, and outcomes of the patients with colonization due to colistin-resistant and carbapenem-resistant *K. pneumoniae* are presented in S1 Table. The samples were collected from adult patients between the ages of 25–94 years, who were admitted in the ICU units of the hospitals. Most of the patients had underlining health conditions including diabetes mellitus, hypertension, dyslipidemia, coronary artery disease, cerebrovascular disease, chronic kidney disease, and chronic obstructive pulmonary disease.

Antibacterial activities of colistin against *K. pneumoniae* isolates

A total of 85 isolates were tested for susceptibility to colistin using broth microdilution assay (Table 2 and S2 Table). The results showed that 74 isolates (87.05%) were susceptible to colistin with MIC values ranging from 0.5 to 2.0 µg/mL and MBC values ranging from 0.5 to 4.0 µg/mL. However, 11 isolates (12.94%) demonstrated resistance to colistin with MIC and MBC values ranging from 256 to >1024 µg/mL and 512 to >1024 µg/mL, respectively. Colistin resistant *K. pneumoniae* has been reported in various countries and regions including Netherlands, America, Nigeria as well as Thailand [24–26]. Recently 213 of 280 (79.1%) *K. pneumoniae* isolates obtained from humans in Thailand showed colistin resistance [27]. The emergence of colistin resistance *K. pneumoniae* presents a major threat to public health, since colistin represents the last line drug of choice against carbapenem resistant *K. pneumoniae*.

Table 1. Distribution of *K. pneumoniae* isolates based on hospital location and sample type.

| Hospitals     | Gastric content | Throat | Rectal | Endotracheal tube | Environmental | Total resistant |
|---------------|-----------------|--------|--------|-------------------|---------------|----------------|
| Hat Yai       | 4               | 6      | 6      | 4                 | 0             | 0              |
| Narathiwat    | 3(R)            | 2(R)   | 3(R)   | 1(R)              | 0             | 9              |
| Pattani       | 5               | 5      | 8      | 3                 | 1             | 0              |
| Phatthalung   | 4               | 5      | 4      | 1                 | 2             | 0              |
| Songkdanagarind | 2               | 1      | 4      | 0                 | 0             | 0              |
| Songkhla      | 2               | 0      | 1(R)   | 0                 | 0             | 1              |
| Satun         | 2               | 1      | 4      | 0                 | 0             | 0              |
| Trang         | 0               | 0      | 1(R)   | 0                 | 0             | 1              |
| Total         | 22              | 20     | 31     | 9                 | 3             | 11             |

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Antibiogram of colistin resistant *K. pneumoniae* isolates

The eleven resistant isolates were evaluated for antibiogram against sixteen conventional anti-biotics and colistin (Table 3). Although the agar dilution method is recommended as reference method for the determination of Fosfomycin MICs, using the reference agar dilution method in a checkerboard analysis is practically difficult. Thus, the broth microdilution with glucose-6-phosphate (G-6-P) was used [28]. Results of the assay were interpreted based on CLSI and EUCAST breakpoint standards [22, 29]. All colistin resistant isolates exhibited multi-drug resistance to aminoglycosides, carbapenems, cephalosporins, fluoroquinolones, fosfomycin, tetracyclines, and piperacillin, but were susceptible to amikacin with MIC ranging from 4 to 16 μg/mL. Previous researchers have reported 69.57 and 64.1% susceptibility of *K. pneumoniae* to amikacin [30, 31]. Resistance to carbapenems (imipenem and meropenem) was observed for all colistin resistant isolates. Two isolates (1SK5R and 1TR5R) were susceptible to cefoperazone, ceftolozane and tazobactam, levofloxacin, fosfomycin, and piperacillin. In addition, isolate 1TR5R was susceptible to tobramycin, ceftazidime, and minocycline. Multidrug resistant *K. pneumoniae* has been reported by previous researchers [32, 33], and is globally spreading unabated. This might lead to increased hospital stay and *K. pneumoniae* associated mortality.

Synergistic effects of colistin-antibiotics combinations

While the search for alternative antimicrobial agents that can effectively control the spread of multidrug resistance continues, various temporary measures are been employed for the treatment of infections caused by drug resistant bacterial isolates. Antibiotic combination therapy

Table 2. Minimum inhibitory and minimum bactericidal concentrations of colistin on *K. pneumoniae* isolates.

| Isolates (n) | MIC | MBC |
|-------------|-----|-----|
| Susceptible (74) | 0.5–2.0 | 0.5–4.0 |
| Resistant (11) | 256 – >1025 | 512 – >1025 |

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**Table 3. Minimum inhibitory concentrations of colistin resistant *K. pneumoniae* isolates against conventional antibiotics.**

| Isolates | Aminoglycosides | Carbenapenem | Cephalosporins | Fluoroquinolones | Tetracycline |
|----------|-----------------|--------------|----------------|-----------------|--------------|
| COL | AMI | GEN | TOB | IMI | MER | CEFD | CEFT | CIP | LEV | MOX | MIN | TIG | FOS | PIP |
| INT4Ng/I | 256 | 16 | 128 | 32 | 256 | >1024 | >1024 | >1024 | >256 | 512 | 512 | 128 | 8 | >1024 | >1024 |
| INT6Ng/I | 256 | 16 | 128 | 32 | 256 | >1024 | >1024 | >1024 | >256 | 512 | 512 | 128 | 8 | >1024 | >1024 |
| INT6Tu/1 | 256 | 8 | 256 | 64 | 128 | >1024 | >1024 | >1024 | >256 | 512 | 512 | 128 | 8 | >1024 | >1024 |
| INT8R | 256 | 16 | 128 | 32 | 256 | >1024 | >1024 | >1024 | >256 | 512 | 512 | 128 | 8 | >1024 | >1024 |
| INT7R | 256 | 8 | 128 | 32 | 256 | >1024 | >1024 | >1024 | >256 | 512 | 512 | 128 | 8 | >1024 | >1024 |
| INT8Th | 256 | 16 | 64 | 32 | 128 | >1024 | >1024 | >1024 | >256 | 512 | 512 | 128 | 8 | >1024 | >1024 |
| INT6Ng (CCU)/I | 512 | 16 | 128 | 16 | 128 | >1024 | >1024 | >1024 | >256 | 512 | 512 | 128 | 8 | >1024 | >1024 |
| INT6Th(CCU)/I | 512 | 16 | 128 | 16 | 128 | >1024 | >1024 | >1024 | >256 | 512 | 512 | 128 | 8 | >1024 | >1024 |
| INT6R(CCU) | 512 | 16 | 128 | 32 | 128 | >1024 | >1024 | >1024 | >256 | 512 | 512 | 128 | 8 | >1024 | >1024 |
| 1SK5R | >1024 | 4 | 4 | 8 | 128 | 256 | 4 | 512 | 256 | 4 | 32 | 0.50 | 4 | 16 | 16 | 32 | 16 |
| 1TR5R | >1024 | 4 | 0.50 | 1 | 32 | 32 | 2 | 4 | 0.25 | 1 | 4 | 0.25 | 4 | 0.25 | 8 | 0.5 | 0.5 |

AMI, Amikacin; CCU, Cardiac Care Unit; CEFD, Cefazidime; CEFT, Cefotaxime; CEFZ, Cefoperazone; CIP, Ciprofloxacin; COL, Colistin; FOS, Fosfomycin; GEN, Gentamicin; IMI, Imipenem; LEV, Levofloxacin; MER, Meropenem; MIN, Minocycline; MOX, Moxifloxacin; PIP, Piperacillin; TIG, Tigecycline; TOB, Tobramycin.

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Combinations of antibiotics effectively inhibit microbial proliferation through a multi-target approach resulting in the microbial death. Hence, the synergistic effects of colistin with conventional antibiotics (amikacin, gentamicin, tobramycin, imipenem, meropenem, cefoperazone, cefotaxime, ceftazidime, ceftolozane and tazobactam, ciprofloxacin, levofloxacin, moxifloxacin, minocycline, tigecycline, fosfomycin, and pipercillin) against *K. pneumoniae* isolates were evaluated and classified based on FICI parameter (S3 Table).

**Combination of colistin with aminoglycosides**

Table 4 presents the checkerboard results of colistin in combination with aminoglycoside (amikacin, gentamicin, and tobramycin) against the eleven colistin resistant *K. pneumoniae* isolates. The FICI ranged from 0.125 to 0.500 for *K. pneumoniae* isolates, except for isolates 1NT6R and 1NT6Th (CCU)/1 with amikacin FICI values of 0.078 and 0.062, respectively. However, combination of colistin with amikacin or tobramycin showed no synergy against isolate 1NT7R. Antibacterial synergistic effects have been demonstrated for gentamicin and amikacin combinations with polymyxin B and ceftazidime-avibactam [37, 38].

**Combination of colistin with carbapenems**

The antibacterial activities of colistin in combination with carbapenems (imipenem and meropenem) is presented in Table 5. The results demonstrated synergism with FICI values ranging from 0.250 to 0.500 for most isolates, except isolates 1SK5R and 1TR5R. Combinations of colistin and imipenem however showed no effects on isolates 1NT6Tu/1, 1NT8Th, 1NT6Ng (CCU)/1, 1NT6Th (CCU)/1, and 1NT6R (CCU). The antibacterial activities of fosfomycin and meropenem combination and colistin with meropenem combinations effective inhibited NDM and carbapenemase producing *K. pneumoniae* [34, 39]. In addition, relebactam-imipenem combinations showed enhanced antibacterial activity against colistin resistant *K. pneumoniae* with potentials of restoring bacteria susceptibility to imipenem [40, 41].

**Combination of colistin with cephalosporins**

Colistin–cephalosporins (Cefoperazone, Cefotaxime, Ceftazidime and Ceftolozane and Tazobactam) combinations were also assessed for synergism against colistin resistant *K. pneumoniae* isolates Table 6. Combinatory effect of colistin with cephalosporins revealed FICIs ranging from 0.187 to 0.375 for all isolates, except 1SK5R and 1TR5R, while combination with ceftolozane and tazobactam were ineffective against 1NT8Th and 1NT6R (CCU). The results suggested collaborative disruption of the cell wall since both colistin and cephalosporins targets strategic components of the cell wall. Ceftazidime-avibactam in combination with colistin was previous reported to be effective against colistin non-susceptible strains of multidrug resistant (MDR) *Pseudomonas aeruginosa* [42, 43]. In addition, combinations of ceftazidime/avibactam and colistin, tobramycin, or tigecycline were effective against OXA-48-producing *Enterobacteriales* strains [44].

**Combination of colistin with fluoroquinolones**

The effects of colistin combination with fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin) are presented in Table 7. The FICIs (0.093 to 0.500) indicated synergistic effects against most isolates. However, combinations of colistin with all three antibiotics were ineffective on isolate 1TR5R, while ciprofloxacin or moxifloxacin were also not effective on isolate 1SK5R.
Table 4. Chequerboard results of colistin in combination with aminoglycoside (amikacin, gentamicin and tobramycin) against colistin resistant *K. pneumoniae* isolates.

| Isolates          | Antibiotics | Combined MIC (μg/mL) | FICI  | Fold reduction | Outcome |
|-------------------|-------------|----------------------|-------|----------------|---------|
| 1NT4Ng/1          | COL         | 8                    | 0.281 | 32             | Synergy |
|                   | AMI         | 4                    |       | 4              |         |
|                   | COL         | 32                   | 0.375 | 8              | Synergy |
|                   | GEN         | 32                   |       | 4              |         |
|                   | COL         | 64                   | 0.500 | 4              | Synergy |
|                   | TOB         | 8                    |       | 4              |         |
| 1NT6Ng/1          | COL         | 8                    | 0.281 | 32             | Synergy |
|                   | AMI         | 4                    |       | 4              |         |
|                   | COL         | 8                    | 0.281 | 32             | Synergy |
|                   | GEN         | 32                   |       | 4              |         |
|                   | COL         | 64                   | 0.500 | 4              | Synergy |
|                   | TOB         | 8                    |       | 4              |         |
| 1NT6Tu/1          | COL         | 64                   | 0.265 | 4              | Synergy |
|                   | AMI         | 0.12                 |       | 64             |         |
|                   | COL         | 8                    | 0.281 | 32             | Synergy |
|                   | GEN         | 64                   |       | 4              |         |
|                   | COL         | ND                   | ND    | ND             | ND      |
|                   | TOB         | ND                   |       |                |         |
| 1NT6R             | COL         | 16                   | 0.078 | 16             | Synergy |
|                   | AMI         | 0.25                 |       | 64             |         |
|                   | COL         | 16                   | 0.125 | 16             | Synergy |
|                   | GEN         | 8                    |       | 16             |         |
|                   | COL         | 32                   | 0.375 | 8              | Synergy |
|                   | TOB         | 16                   |       | 4              |         |
| 1NT7R             | COL         | ND                   | ND    | ND             | ND      |
|                   | AMI         | ND                   |       |                |         |
|                   | COL         | 32                   | 0.375 | 8              | Synergy |
|                   | GEN         | 32                   |       | 4              |         |
|                   | COL         | ND                   | ND    | ND             | ND      |
|                   | TOB         | ND                   |       |                |         |
| 1NT8Th            | COL         | 16                   | 0.312 | 16             | Synergy |
|                   | AMI         | 4                    |       | 4              |         |
|                   | COL         | 64                   | 0.500 | 4              | Synergy |
|                   | GEN         | 16                   |       | 4              |         |
|                   | COL         | ND                   | ND    | ND             | ND      |
|                   | TOB         | ND                   |       |                |         |
| 1NT6Ng(CCU)/1     | COL         | 16                   | 0.281 | 32             | Synergy |
|                   | AMI         | 4                    |       | 4              |         |
|                   | COL         | 64                   | 0.250 | 8              | Synergy |
|                   | GEN         | 16                   |       | 8              |         |
|                   | COL         | 64                   | 0.375 | 8              | Synergy |
|                   | TOB         | 4                    |       | 4              |         |
| 1NT6Th(CCU)/1     | COL         | 8                    | 0.062 | 32             | Synergy |
|                   | AMI         | 0.5                  |       | 32             |         |
|                   | COL         | 32                   | 0.375 | 8              | Synergy |
|                   | GEN         | 32                   |       | 4              |         |
|                   | COL         | 16                   | 0.312 | 16             | Synergy |
|                   | TOB         | 4                    |       | 4              |         |

(Continued)
Combination of colistin with fosfomycin, tetracyclines (minocycline, and tigecycline) and penicillin (piperacillin)

In addition, effects of colistin in combination with fosfomycin, tetracyclines (minocycline, and tigecycline) and penicillin (piperacillin) resistant *K*. *pneumoniae* isolates is presented in Table 8. Combination of colistin with fosfomycin showed FICI ranging from 0.062 to 0.500 against most isolates except isolates 1NT6Tu/1, 1NT6R, and 1SK5R while combination of colistin with piperacillin range of 0.046 to 0.375 for most isolates except 1NT7R and 1SK5R. Against all isolates, combination of colistin with tigecycline was not synergistic, whereas combinations with minocycline demonstrated synergistic activity against most isolates with FICI value of 0.093, except for isolate 1NT6R (CCU) with FICI value of 0.062, and were not effective against 1SK5R and 1TR5R.

**Discussion**

The rapid spread of multidrug resistant pathogenic bacteria places an enormous responsibility on global health care systems. Moreover, the search and development of new antibiotics is far too slow with no novel discovery over the last 30 years. This shortage of effective therapeutic agents has encouraged trial of combinations of existing agents for synergistic activities against drug resistant isolates. The present study combined colistin a last line drug for multidrug resistant Enterobacterales with 16 other antibiotics that are not effective against *K*. *pneumoniae*. A total of 11 isolates (12.94%) presented resistance to colistin. Nine out of the 11 resistant isolates were obtained from Narathiwat hospital, with a 100% resistance prevalence. The results suggested high prevalence of resistance amongs *K*. *pneumoniae* in Narathiwat hospital which might be due to local transmission within the hospital. Furthermore, 1 among 3 isolates obtained from Songkhla hospital was resistant, whereas the single isolate obtained from Trang hospital was also resistant. However, due to the few number of isolates used in the study, epidemiological statements on the prevalence of colistin resistant isolates in this regions might be biased. Colistin resistant *K*. *pneumoniae* has previously been reported in various countries and regions including Netherlands, America, Nigeria as well as Thailand [24–26]. Recently 213 of 280 (79.1%) *K*. *pneumoniae* isolates obtained from humans in Thailand showed colistin resistance [27].

All colistin–resistant isolates were also resistant to other antibiotics including aminoglycosides, carbapenems, cephalosporins, fluoroquinolones, fosfomycin, tetracyclines, and piperacillin, but were susceptible to amikacin with MIC ranging from 4 to 16 μg/mL. Previous researchers have reported 69.57 and 64.1% susceptibility of *K*. *pneumoniae* to amikacin [30, 31]. The results suggested that amikacin might be a drug option in the management of drug resistant isolates.
resistant Enterobacterales, and should be further explored. Resistance to carbapenems (imipenem and meropenem) was observed for all colistin resistant isolates. Two isolates (1SK5R and 1TR5R) were susceptible to cefoperazone, ceftolozane and tazobactam, levofloxacin, fosfomycin, and piperacillin. In addition, isolate 1TR5R was susceptible to tobramycin, ceftazidime, and minocycline. Multidrug resistance in *K. pneumoniae* has been reported by previous researchers [32, 33], resulting in increased hospital stay and *K. pneumoniae* associated mortality.

### Table 5. Chequerboard results of colistin in combination with carbapenems (imipenem and meropenem) against colistin resistant *K. pneumoniae* isolates.

| Isolates  | Antibiotics | Combined MIC (μg/mL) | FICI | Fold reduction | Outcome |
|-----------|-------------|----------------------|------|---------------|---------|
| INT4Ng/1  | COL 64      | 0.375                | 4    | Synergy       |
|           | IMI 32      | 8                    |      |               |
|           | COL 64      | 0.375                | 4    | Synergy       |
|           | MER 32      | 8                    |      |               |
| INT6Ng/1  | COL 64      | 0.500                | 4    | Synergy       |
|           | IMI 64      | 4                    |      |               |
|           | COL 64      | 0.375                | 4    | Synergy       |
|           | MER 32      | 8                    |      |               |
| INT6Tu/1  | COL ND      | ND                   | ND   | ND            |
|           | IMI ND      | 4                    | 16   | Synergy       |
|           | COL 16      | 0.312                | 4    |               |
|           | MER 64      | 4                    |      |               |
| INT6R     | COL 64      | 0.500                | 4    | Synergy       |
|           | IMI 32      | 4                    |      |               |
|           | COL 16      | 0.312                | 4    | Synergy       |
|           | MER 64      | 4                    |      |               |
| INT7R     | COL 64      | 0.281                | 4    | Synergy       |
|           | IMI 4       | 64                   |      |               |
|           | COL 32      | 0.250                | 8    | Synergy       |
|           | MER 32      | 8                    |      |               |
| INT8Th    | COL ND      | ND                   | ND   | ND            |
|           | IMI ND      | 4                    | 8    | Synergy       |
|           | COL 64      | 0.500                | 4    | Synergy       |
|           | MER 32      | 4                    |      |               |
| INT6Ng(CCU)/1 | COL ND | ND                   | ND   | ND            |
|           | IMI ND      | 4                    | 8    | Synergy       |
|           | COL 32      | 0.312                | 16   | Synergy       |
|           | MER 64      | 4                    |      |               |
| INT6Th(CCU)/1 | COL ND | ND                   | ND   | ND            |
|           | IMI ND      | 4                    | 8    | Synergy       |
|           | COL 64      | 0.500                | 4    | Synergy       |
|           | MER 32      | 4                    |      |               |
| INT6R(CCU) | COL ND      | ND                   | ND   | ND            |
|           | IMI ND      | 4                    | 8    | Synergy       |
|           | COL 64      | 0.500                | 4    | Synergy       |
|           | MER 32      | 4                    |      |               |

CCU, Cardiac Care Unit; MIC, Minimum inhibitory concentrations; COL, Colistin; FICI, Fractional inhibitory concentration index; IMI, Imipenem; MER, Meropenem; ND, not determined.

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Table 6. Chequerboard results of colistin in combination with cephalosporins (cefoperazone, cefotaxime, ceftazidime and ceftolozane and tazobactam) against colistin resistant *K. pneumoniae* isolates.

| Isolates | Antibiotics | Combined MIC (μg/mL) | FICI | Fold reduction | Outcome |
|----------|-------------|----------------------|------|----------------|---------|
| 1NT4Ng/1 | COL 32      | 0.375                | 8    | Synergy        |
|          | CEFZ 256    |                      |      |                |         |
|          | COL 16      | 0.312                | 16   | Synergy        |
|          | CEFX 256    |                      |      |                |         |
|          | COL 16      | 0.312                | 16   | Synergy        |
|          | CEFD 256    |                      |      |                |         |
|          | COL 64      | 0.375                | 4    | Synergy        |
|          | CEF    128  |                      | 8    |                |         |
| 1NT6Ng/1 | COL 32      | 0.375                | 8    | Synergy        |
|          | CEFZ 256    |                      |      |                |         |
|          | COL 16      | 0.312                | 16   | Synergy        |
|          | CEFX 256    |                      |      |                |         |
|          | COL 32      | 0.375                | 8    | Synergy        |
|          | CEFD 256    |                      |      |                |         |
|          | COL 64      | 0.253                | 4    | Synergy        |
|          | CEF    128  |                      | 64   |                |         |
| 1NT6Tu/1 | COL 16      | 0.312                | 16   | Synergy        |
|          | CEFZ 256    |                      | 4    |                |         |
|          | COL 16      | 0.312                | 16   | Synergy        |
|          | CEFX 256    |                      | 4    |                |         |
|          | COL 16      | 0.312                | 16   | Synergy        |
|          | CEFD 256    |                      | 4    |                |         |
|          | COL 32      | 0.187                | 8    | Synergy        |
|          | CEF    128  |                      | 64   |                |         |
| 1NT6R    | COL 16      | 0.312                | 16   | Synergy        |
|          | CEFZ 256    |                      | 4    |                |         |
|          | COL 16      | 0.312                | 16   | Synergy        |
|          | CEFX 256    |                      | 4    |                |         |
|          | COL 16      | 0.312                | 16   | Synergy        |
|          | CEFD 256    |                      | 4    |                |         |
|          | COL 64      | 0.257                | 4    | Synergy        |
|          | CEF    128  |                      | 168  |                |         |
| 1NT7R    | COL 16      | 0.312                | 16   | Synergy        |
|          | CEFZ 256    |                      | 4    |                |         |
|          | COL 16      | 0.312                | 16   | Synergy        |
|          | CEFX 256    |                      | 4    |                |         |
|          | COL 32      | 0.250                | 8    | Synergy        |
|          | CEFD 128    |                      | 8    |                |         |
|          | COL 64      | 0.257                | 4    | Synergy        |
|          | CEF    128  |                      | 128  |                |         |
| 1NT8Th   | COL 64      | 0.281                | 4    | Synergy        |
|          | CEFZ 32     |                      | 32   |                |         |
|          | COL 16      | 0.312                | 16   | Synergy        |
|          | CEFX 256    |                      | 4    |                |         |
|          | COL 16      | 0.187                | 8    | ND             |
|          | CEFD 128    |                      | 8    |                |         |
|          | COL ND      |                      | ND   | ND             |         |
|          | CEF    ND   |                      | ND   |                |         |

(Continued)
While the search for alternative and effective antimicrobial agents continues, various temporary measures are being employed for the treatment of infections caused by drug resistant bacterial isolates. Antibiotic combination therapy is a possible effective option which currently is attracting numerous research attention [34–36]. Combinations of antibiotics effectively inhibit microbial proliferation through a multi-target approach resulting in effective inactivation of cells and microbial death. Hence, the synergistic effects of colistin with conventional antibiotics (aminoglycosides, β-lactams, β-lactamase inhibitors, and fluoroquinolones) against multidrug-resistant *K. pneumoniae* isolates were evaluated and classified based on FICI parameter (S3 Table). Antibiotic combination demonstrated 5- to 64-fold reduction in MIC of colistin and 4-to 512-fold reduction in MIC of tested antibiotics. A summary of the results indicates that colistin with amikacin, or fosfomycin combinations were synergistic against 72.72% (8 of 11 isolates). Colistin with gentamicin, or meropenem, or cefoperazone, or cefotaxime, or ceftazidime, or moxifloxacin, or minocycline, or piperacillin and tazobactam displayed 36.36% (4 of 11 isolates) and 63.64% (7 of 11 isolates) synergism. In addition, combinations of
Table 7. Chequerboard results of colistin in combination with fluoroquinolones (ciprofloxacin, levofloxacin and moxifloxacin) against colistin resistant *K. pneumoniae* isolates.

| Isolates          | Antibiotics | Combined MIC (μg/mL) | FICI   | Fold reduction | Outcome |
|-------------------|-------------|----------------------|--------|----------------|---------|
| 1NT4Ng/1          | COL         | 64                   | 0.257  | 4              | Synergy |
|                   | CIP         | 2                    |        | 128            |         |
|                   | COL         | 16                   | 0.125  | 16             | Synergy |
|                   | LEV         | 32                   |        | 16             |         |
|                   | COL         | 8                    | 0.093  | 32             | Synergy |
|                   | MOX         | 32                   |        | 16             |         |
| 1NT6Ng/1          | COL         | 64                   | 0.257  | 4              | Synergy |
|                   | CIP         | 2                    |        | 128            |         |
|                   | COL         | 16                   | 0.187  | 16             | Synergy |
|                   | LEV         | 64                   |        | 8              |         |
|                   | COL         | 8                    | 0.093  | 32             | Synergy |
|                   | MOX         | 32                   |        | 16             |         |
| 1NT6Tw/1          | COL         | ND                   | ND     | ND             | ND      |
|                   | CIP         | ND                   | ND     | ND             | ND      |
|                   | COL         | 16                   | 0.093  | 16             | Synergy |
|                   | LEV         | 16                   |        | 32             |         |
|                   | COL         | 8                    | 0.093  | 32             | Synergy |
|                   | MOX         | 32                   |        | 16             |         |
| 1NT6R             | COL         | 32                   | 0.375  | 8              | Synergy |
|                   | CIP         | 64                   |        | 4              |         |
|                   | COL         | 16                   | 0.125  | 16             | Synergy |
|                   | LEV         | 32                   |        | 16             |         |
|                   | COL         | 8                    | 0.093  | 32             | Synergy |
|                   | MOX         | 32                   |        | 16             |         |
| 1NT7R             | COL         | 64                   | 0.500  | 4              | Synergy |
|                   | CIP         | 64                   |        | 4              |         |
|                   | COL         | 16                   | 0.125  | 16             | Synergy |
|                   | LEV         | 32                   |        | 16             |         |
|                   | COL         | 16                   | 0.125  | 16             | Synergy |
|                   | MOX         | 32                   |        | 16             |         |
| 1NT8Th            | COL         | 64                   | 0.500  | 4              | Synergy |
|                   | CIP         | 64                   |        | 4              |         |
|                   | COL         | 16                   | 0.125  | 16             | Synergy |
|                   | LEV         | 32                   |        | 16             |         |
|                   | COL         | 16                   | 0.093  | 16             | Synergy |
|                   | MOX         | 16                   |        | 32             |         |
| 1NT6Ng(CCU)/1     | COL         | ND                   | ND     | ND             | ND      |
|                   | CIP         | ND                   | ND     | ND             | ND      |
|                   | COL         | 64                   | 0.250  | 8              | Synergy |
|                   | LEV         | 8                    |        | 8              |         |
|                   | COL         | 128                  | 0.312  | 4              | Synergy |
|                   | MOX         | 2                    |        | 16             |         |
| 1NT6Th(CCU)/1     | COL         | ND                   | ND     | ND             | ND      |
|                   | CIP         | ND                   | ND     | ND             | ND      |
|                   | COL         | 32                   | 0.250  | 8              | Synergy |
|                   | LEV         | 8                    |        | 8              |         |
|                   | COL         | 32                   | 0.156  | 8              | Synergy |
|                   | MOX         | 1                    |        | 32             |         |

(Continued)
Table 7. (Continued)

| Isolates | Antibiotics | Combined MIC (μg/mL) | FICI | Fold reduction | Outcome |
|----------|-------------|----------------------|------|----------------|---------|
| INT6R(CCU) | COL | ND | ND | ND | ND |
| | CIP | ND | ND | ND | ND |
| | COL | 16 | 0.187 | 16 | Synergy |
| | LEV | 64 | 8 | 8 | Synergy |
| | COL | 8 | 0.093 | 32 | Synergy |
| | MOX | 32 | 16 | 16 | |
| ISK5R | COL | ND | ND | ND | ND |
| | CIP | ND | ND | ND | ND |
| | COL | 16 | 0.255 | 64 | Synergy |
| | LEV | 0.12 | 4 | 4 | |
| | COL | ND | ND | ND | ND |
| | MOX | ND | ND | ND | ND |

CCU, Cardiac Care Unit; MIC, Minimum inhibitory concentrations; CIP, Ciprofloxacin; COL, Colistin; FICI, Fractional inhibitory concentration index; LEV, Levofloxacin; MOX, Moxifloxacin; ND, not determined.

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Table 8. Chequerboard results of colistin in combination with fosfomycin, tetracyclines (minocycline, and tigecycline) and penicillin (piperacillin) against colistin resistant K. pneumoniae isolates.

| Isolates | Antibiotics | Combined MIC (μg/mL) | FICI | Fold reduction | Outcome |
|----------|-------------|----------------------|------|----------------|---------|
| INT4Ng/1 | COL | 64 | 0.500 | 4 | Synergy |
| | FOS | 256 | 4 | Synergy |
| | COL | 16 | 0.093 | 16 | Synergy |
| | MIN | 4 | 32 | |
| | COL | ND | ND | ND | ND |
| | TIG | ND | ND | ND | ND |
| | COL | 32 | 0.128 | 8 | Synergy |
| | PIP | 4 | 256 | |
| INT6Ng/1 | COL | 64 | 0.375 | 4 | Synergy |
| | FOS | 128 | 8 | Synergy |
| | COL | 16 | 0.093 | 16 | Synergy |
| | MIN | 4 | 32 | |
| | COL | ND | ND | ND | ND |
| | TIG | ND | ND | ND | ND |
| | COL | 32 | 0.187 | 8 | Synergy |
| | PIP | 64 | 16 | |
| INT6Tu/1 | COL | ND | ND | ND | ND |
| | FOS | ND | ND | ND | ND |
| | COL | 16 | 0.093 | 16 | Synergy |
| | MIN | 4 | 32 | |
| | COL | ND | ND | ND | ND |
| | TIG | ND | ND | ND | ND |
| | COL | 32 | 0.126 | 8 | Synergy |
| | PIP | 2 | 512 | |

(Continued)
| Isolates       | Antibiotics | Combined MIC (μg/mL) | FICI | Fold reduction | Outcome |
|---------------|-------------|----------------------|------|----------------|---------|
| 1NT6R         | COL         | ND                   | ND   | ND             | ND      |
|               | FOS         | ND                   |      |                |         |
|               | COL         | 16                   | 0.093| 16             | Synergy |
|               | MIN         | 4                    |      | 32             |         |
|               | COL         | ND                   | ND   | ND             | ND      |
|               | TIG         | ND                   |      |                |         |
|               | COL         | 64                   | 0.251| 4              | Synergy |
|               | PIP         | 2                    |      | 512            |         |
| 1NT7R         | COL         | 64                   | 0.500| 4              | Synergy |
|               | FOS         | 256                  |      | 4              |         |
|               | COL         | 16                   | 0.093| 16             | Synergy |
|               | MIN         | 4                    |      | 32             |         |
|               | COL         | ND                   | ND   | ND             | ND      |
|               | TIG         | ND                   |      |                |         |
|               | COL         | ND                   | ND   | ND             | ND      |
|               | PIP         | ND                   |      |                |         |
| 1NT8Th        | COL         | 64                   | 0.500| 4              | Synergy |
|               | FOS         | 256                  |      | 4              |         |
|               | COL         | 16                   | 0.093| 16             | Synergy |
|               | MIN         | 4                    |      | 32             |         |
|               | COL         | ND                   | ND   | ND             | ND      |
|               | TIG         | ND                   |      |                |         |
|               | COL         | 64                   | 0.251| 4              | Synergy |
|               | PIP         | 2                    |      | 512            |         |
| 1NT6Ng(CCU)/1 | COL         | 32                   | 0.078| 16             | Synergy |
|               | FOS         | 2                    |      | 64             |         |
|               | COL         | 16                   | 0.093| 32             | Synergy |
|               | MIN         | 2                    |      | 16             |         |
|               | COL         | ND                   | ND   | ND             | ND      |
|               | TIG         | ND                   |      |                |         |
|               | COL         | 16                   | 0.046| 32             | Synergy |
|               | PIP         | 16                   |      | 64             |         |
| 1NT6Th(CCU)/1 | COL         | 8                    | 0.062| 32             | Synergy |
|               | FOS         | 4                    |      | 32             |         |
|               | COL         | 8                    | 0.093| 32             | Synergy |
|               | MIN         | 2                    |      | 16             |         |
|               | COL         | ND                   | ND   | ND             | ND      |
|               | TIG         | ND                   |      |                |         |
|               | COL         | 16                   | 0.066| 16             | Synergy |
|               | PIP         | 4                    |      | 256            |         |
| 1NT6R(CCU)    | COL         | 32                   | 0.375| 8              | Synergy |
|               | FOS         | 256                  |      | 4              |         |
|               | COL         | 8                    | 0.062| 32             | Synergy |
|               | MIN         | 4                    |      | 32             |         |
|               | COL         | ND                   | ND   | ND             | ND      |
|               | TIG         | ND                   |      |                |         |
|               | COL         | 64                   | 0.251| 4              | Synergy |
|               | PIP         | 2                    |      | 512            |         |

(Continued)
colistin with levofloxacin was synergistic against 90.91% (10 of 11 isolates), while colistin and tigecycline combination were overall not synergistic. The results revealed that colistin in combination with fifteen other antibiotics could effectively inhibit colistin resistant isolates of *K. pneumoniae*. The results suggested that combination therapies could be further explore for the treatment of multidrug resistant pathogens.

**Conclusions**

The present study combined colistin a last line drug for multidrug resistant Enterobacterales with 16 other antibiotics that are not effective against *K. pneumoniae*. The results revealed that colistin in combination with fifteen other antibiotics effectively inhibit colistin resistant isolates of *K. pneumoniae*. The results suggested that combination therapies could be further explore for the treatment of multidrug resistant pathogens.

**Supporting information**

S1 Table. Demographic data, clinical characteristics, and outcomes of the patients with colonization due to colistin-resistant and carbapenem-resistant *K. Pneumoniae*. (DOCX)

S2 Table. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of colistin against *Klebsiella pneumoniae* clinical isolates. (DOCX)

S3 Table. Synergistic effects of antibiotics and colistin combination against *Klebsiella pneumoniae* isolates from hospitalized patients. (DOCX)

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**Funding acquisition:** Sarunyou Chusri.

**Investigation:** Julalak C. Ontong, Nwabor F. Ozioma.

Table 8. (Continued)

| Isolates | Antibiotics | Combined MIC (μg/mL) | FICI | Fold reduction | Outcome |
|----------|-------------|----------------------|------|----------------|---------|
| ITRSR    | COL         | 256                  | 0.500| 4              | Synergy |
|          | FOS         | 0.125                |      | 4              |         |
|          | COL         | ND                   | ND   | ND             | ND      |
|          | MIN         | ND                   | ND   | ND             | ND      |
|          | COL         | ND                   | ND   | ND             | ND      |
|          | TIG         | ND                   |      |                |         |
|          | COL         | 128                  | 0.375| 8              | Synergy |
|          | PIP         | 0.125                |      | 4              |         |

CCU, Cardiac Care Unit; MIC, Minimum inhibitory concentrations; COL, Colistin; FICI, Fractional inhibitory concentration index; FOS, Fosfomycin; MIN, Minocycline; PIP, Piperacillin; TIG, Tigecycline; ND, not determined.

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**References**

1. WHO. No Time to Wait: Securing the future from drug-resistant infections. World Health Organization: Geneva, Switzerland. 2019.

2. Biswas S, Brunel J-M, Dubus J-C, Reynaud-Gaubert M, Rolain J-M. Colistin: an update on the antibiotic of the 21st century. Expert Review of Anti-Infective Therapy. 2012; 10(8):917–34. https://doi.org/10.1586/er.12.78 PMID: 23030331

3. Roy S, Das P, Das S, Roy S, Pal S, Joy VM, et al. Detection of the emergence of mcr-1-mediated colistin-resistant *Escherichia coli* and *Klebsiella pneumoniae* through a hospital-based surveillance in an oncology center in eastern India. Infection Control & Hospital Epidemiology. 2020; 41(3):378–80.

4. Gelbicova T, Kolackova I, Krutova M, Karpiskova R. The emergence of mcr-1-mediated colistin-resistant *Escherichia coli* and *Klebsiella pneumoniae* in domestic and imported turkey meat in the Czech Republic 2017–2018. Folia Microbiologica. 2020; 65(1):211–6. https://doi.org/10.1007/s12223-019-00709-z PMID: 31001764

5. Lalaoui R, Bakour S, Livnat K, Assous MV, Diene SM, Rolain J-M. Spread of Carbapenem and Colistin-resistant *Klebsiella pneumoniae* ST512 clinical isolates in Israel: a cause for vigilance. Microbial Drug Resistance. 2019; 25(1):63–71. https://doi.org/10.1089/mdr.2018.0014 PMID: 30129868

6. Trent MS. Biosynthesis, transport, and modification of lipid A. Biochemistry and Cell Biology. 2004; 82(1):71–86. https://doi.org/10.1139/o03-070 PMID: 15052329

7. Sun S, Gao H, Liu Y, Jin L, Wang R, Wang X, et al. Co-existence of a novel plasmid-mediated efflux pump with colistin resistance gene mcr in one plasmid confers transferable multidrug resistance in *Klebsiella pneumoniae*. Emerging Microbes & Infections. 2020;(just-accepted):1–41. https://doi.org/10.1080/22221751.2020.1768805 PMID: 32401163

8. Srinivasan VB, Rajamohan G. KpnEF, a new member of the *Klebsiella pneumoniae* cell envelope stress response regulon, is an SMR-type efflux pump involved in broad-spectrum antimicrobial resistance. Antimicrobial Agents and Chemotherapy. 2013; 57(9):4449–62. https://doi.org/10.1128/AAC.02284-12 PMID: 23836167

9. Singh SK, Mishra M, Sahoo M, Patole S, Mohapatra H. Efflux mediated colistin resistance in diverse clones of *Klebsiella pneumoniae* from aquatic environment. Microbial Pathogenesis. 2017; 102:109–12. https://doi.org/10.1016/j.micpath.2016.11.024 PMID: 27914962

10. Naha S, Sands K, Mukherjee S, Roy C, Rameez MJ, Saha B, et al. KPC-2-producing *Klebsiella pneumoniae* ST147 in a neonatal unit: Clonal isolates with differences in colistin susceptibility attributed to AcrAB-ToLC pump. International Journal of Antimicrobial Agents. 2020; 55(3):105903. https://doi.org/10.1016/j.ijantimicag.2020.105903 PMID: 31954832

11. Salloum T, Panossian B, Bitar I, Hrabak J, Araj GF, Tokajian S. First report of plasmid-mediated colistin resistance mcr-8.1 gene from a clinical *Klebsiella pneumoniae* isolate from Lebanon. Antimicrobial Resistance & Infection Control. 2020; 9(1):1–7. https://doi.org/10.1186/s13756-020-00759-w PMID: 32586402

12. Nabti LZ, Sahli F, Ngaiganan EP, Radji N, Mezaghibha W, Lupande-Mwenebitu D, et al. Development of real-time PCR assay allowed describing the first clinical *Klebsiella pneumoniae* isolate harboring plasmid-mediated colistin resistance mcr-8 gene in Algeria. Journal of Global Antimicrobial Resistance. 2020; 20:266–71. https://doi.org/10.1016/j.jgarm.2019.08.018 PMID: 31746749

13. Wistrand-Yuen P, Olsson A, Skarp K-P, Friberg LE, Nielsen EJ, Lagerbäck P, et al. Evaluation of polymyxin B in combination with 13 other antibiotics against carbapenemase-producing *Klebsiella pneumoniae* in time-lapse microscopy and time-kill experiments. Clinical Microbiology and Infection. 2020. https://doi.org/10.1016/j.cmi.2020.03.007 PMID: 32224200
14. Bakthavatchalam YD, Shankar A, Muthuirulandi Sethuvel DP, Asokan K, Kanthan K, Veeraraghavan B. Synergistic activity of fosfomycin–meropenem and fosfomycin–colistin against carbapenem resistant Klebsiella pneumoniae: an in vitro evidence. Future Science OA. 2020; 6(4):FSO461. https://doi.org/10.2144/fsoa-2019-0074 PMID: 32257374

15. Aye SM, Galani I, Yu H, Wang J, Chen K, Wickremasinghe H, et al. Polymyxin triple combinations against polymyxin-resistant, multidrug-resistant KPC-producing Klebsiella pneumoniae. Antimicrobial Agents and Chemotherapy. 2020. https://doi.org/10.1128/AAC.00246-20 PMID: 32393492

16. Sundaramorothy NS, Mohan HM, Subramaniam S, Raman T, Ganesan SS, Sivasubamanian A, et al. Ursolic acid inhibits colistin efflux and curtails colistin resistant Enterobacteriaceae. AMB Express. 2019; 9(1):27. https://doi.org/10.1186/s13568-019-0750-4 PMID: 30778773

17. Ni W, Li Y, Guan J, Zhao J, Cui J, Wang R, et al. Effects of efflux pump inhibitors on colistin resistance in multidrug-resistant Gram-negative bacteria. Antimicrobial Agents and Chemistry. 2016; 60(5):3215–8. https://doi.org/10.1128/AAC.00248-16 PMID: 26953203

18. Baron SA, Rolain J-M. Efflux pump inhibitor CCCP to rescue colistin susceptibility in mcr-1 plasmid-mediated colistin-resistant strains and Gram-negative bacteria. Journal of Antimicrobial Chemotherapy. 2018; 73(7):1862–71. https://doi.org/10.1093/jac/dky134 PMID: 29718423

19. Witherell KS, Price J, Bandaranayake AD, Olson J, Call DR. Circumventing colistin resistance by combining colistin and antimicrobial peptides to kill colistin-resistant and multidrug-resistant Gram-negative bacteria. Journal of Global Antimicrobial Resistance. 2020. https://doi.org/10.1016/j.jgar.2020.05.013 PMID: 32512236

20. Hashemi MM, Rovig J, Weber S, Hilton B, Forouzann M, Savage PB. Susceptibility of colistin-resistant, Gram-negative bacteria to antimicrobial peptides and ceragenins. Antimicrobial Agents and Chemistry. 2017; 61(8). https://doi.org/10.1128/AAC.00292-17 PMID: 28584137

21. CLSI. Performance standards for antibiotics susceptibility testing. M100S. CLSI Wayne, PA; 2016.

22. EUCAST. “The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. European Society of Clinical Microbiology and Infectious Diseases. Version 10.0; 2020.

23. Kheshti R, Pourabbas B, Mosayebi M, Vazin A. In vitro activity of colistin in combination with various antibiotics against Acinetobacter baumannii species, a report from South Iran. Infection and Drug Resistance. 2019; 12:129. https://doi.org/10.2147/IDR.S182585 PMID: 30644341

24. Weterings V, Zhou K, Rossen J, van Stenis D, Thewessen E, Kluytmans J, et al. An outbreak of colistin-resistant Klebsiella pneumoniae carbapenemase-producing Klebsiella pneumoniae in the Netherlands (July to December 2013), with inter-institutional spread. European Journal of Clinical Microbiology & Infectious Diseases. 2015; 34(8):1647–55.

25. Olaitan AO, Diene SM, Kempf M, Berrazeg M, Bakour S, Gupta SK, et al. Worldwide emergence of colistin resistance in Klebsiella pneumoniae from healthy humans and patients in Lao PDR, Thailand, Israel, Nigeria and France owing to inactivation of the PhoP/PhoQ regulator mgbB: an epidemiological and molecular study. International Journal of Antimicrobial Agents. 2014; 44(6):500–7. https://doi.org/10.1016/j.ijantimicag.2014.07.020 PMID: 25264127

26. Marchaim D, Chopra T, Pogue JM, Perez F, Hujer AM, Rudin S, et al. Outbreak of colistin-resistant, carbapenem-resistant Klebsiella pneumoniae in metropolitan Detroit, Michigan. Antimicrobial Agents and Chemistry. 2011; 55(2):593–9. https://doi.org/10.1128/AAC.01020-10 PMID: 21115786

27. Eiamphunpong W, Yainoy S, Jumderm C, Tanarsuwongkul R, Tiengrim S, Thamlikitkul V. Prevalence of the colistin resistance gene mcr-1 in colistin-resistant Escherichia coli and Klebsiella pneumoniae isolated from humans in Thailand. Journal of Global Antimicrobial Resistance. 2018; 15:32–5. https://doi.org/10.1016/j.jgar.2018.06.007 PMID: 29935331

28. Flamm RK, Rhomberg PR, Lindley JM, Sweeney K, Ellis-Grosse E, Shorrtridge D. Evaluation of the bactericidal activity of fosfomycin in combination with selected antimicrobial comparison agents tested against gram-negative bacterial strains by using time-kill curves. Antimicrobial Agents and Chemistry. 2019; 63(5). https://doi.org/10.1128/AAC.02549-18 PMID: 30858207

29. CLSI. Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute Wayne, PA; 2017.

30. Lin L, Xiao X, Wang X, Xia M, Liu S. In Vitro Antimicrobial Susceptibility Differences Between Carba-nem-Resistant KPC-2-Producing and NDM-1-Producing Klebsiella pneumoniae in a Teaching Hospital in Northeast China. Microbial Drug Resistance. 2020; 26(2):94–9. https://doi.org/10.1089/mdr.2018.0398 PMID: 31433255

31. Jacobs MR, Good CE, Hujer AM, Abdelhamed AM, Rhoads DD, Hujer KM, et al. ARGONAUT II study of the in vitro activity of plazomicin against carbapenemase-producing Klebsiella pneumoniae. Antimicrobial Agents and Chemistry. 2020; 64(5). https://doi.org/10.1128/AAC.0012-20 PMID: 32152078
32. Xu H, Huo C, Sun Y, Zhou Y, Xiong Y, Zhao Z, et al. Emergence and molecular characterization of multidrug-resistant *Klebsiella pneumoniae* isolates harboring blaCTX-M-15 extended-spectrum β-lactamases causing ventilator-associated pneumonia in China. Infection and Drug Resistance. 2019; 12:33. https://doi.org/10.2147/IDR.S189494 PMID: 30588046

33. Palmeiro JK, De Souza RF, Schörner MA, Araujo HP, Grazziotin AL, Vidal NM, et al. Molecular epidemiology of multidrug-resistant *Klebsiella pneumoniae* isolates in a Brazilian tertiary hospital. Frontiers in Microbiology. 2019; 10:1669. https://doi.org/10.3389/fmicb.2019.01669 PMID: 31396186

34. Yu L, Zhang J, Fu Y, Zhao Y, Wang Y, Zhao J, et al. Synergistic effects of combined treatment of colistin with meropenem or amikacin on carbapenem-resistant *Klebsiella pneumoniae* in vitro. Frontiers in Cellular and Infection Microbiology. 2019; 9:422. https://doi.org/10.3389/fmicb.2019.00422 PMID: 31921701

35. Kilic U, Koroglu M, Olmez M, Altindis M. Investigation of the In Vitro Effectiveness of Aztreonam/Avibactam, Colistin/Apramycin, and Meropenem/Apramycin Combinations Against Carbapenemase-Producing, Extensively Drug-Resistant *Klebsiella pneumoniae* Strains. Microbial Drug Resistance. 2020.

36. Erdem F, Abulaila A, Aktas Z, Oncul O. In vitro evaluation of double carbapenem and colistin combinations against OXA-48, NDM carbapenemase-producing colistin-resistant *Klebsiella pneumoniae* strains. Antimicrobial Resistance & Infection Control. 2020; 9(1):1–9. https://doi.org/10.1186/s13756-020-00727-4 PMID: 32430058

37. Mikhail S, Singh NB, Kebrina R, Rice SA, Stamper KC, Castanheira M, et al. Evaluation of the synergy of ceftazidime-avibactam in combination with meropenem, amikacin, aztreonam, colistin, or fosfomycin against well-characterized multidrug-resistant *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Antimicrobial Agents and Chemistry. 2019; 63(8):e00779–19. https://doi.org/10.1128/AAC.00779-19 PMID: 31182535

38. Firmo EF, Júnior JBO, Scavuzzi AML, Alves LC, Brayner FA, Veras DL, et al. In vitro activity of polymyxin B in combination with meropenem, amikacin and gentamicin against *Klebsiella pneumoniae* clinical isolates coharboring AMEs, blaNDM-1 and blaKPC-2. Journal of Global Antimicrobial Resistance. 2020.

39. Erturk Sengel B, Altinkanat Gelmez G, Soyletir G, Korten V. In vitro synergistic activity of fosfomycin in combination with meropenem, amikacin and colistin against *Klebsiella pneumoniae*. Journal of Chemotherapy. 2020; 1–7. https://doi.org/10.1080/1120009X.2020.1745501 PMID: 32228228

40. Galani I, Souli M, Nafplioti K, Adamou P, Karaikos I, Giamarelloou H, et al. In vitro activity of imipenem-relebactam against non-MBL carbapenemase-producing *Klebsiella pneumoniae* isolated in Greek hospitals in 2015–2016. European Journal of Clinical Microbiology & Infectious Diseases. 2019; 38 (6):1143–50.

41. Carpenter J, Neidig N, Campbell A, Thomsberry T, Truex T, Fortney T, et al. Activity of imipenem/relebactam against carbapenemase-producing Enterobacteriaceae with high colistin resistance. Journal of Antimicrobial Chemotherapy. 2019; 74(11):3260–3. https://doi.org/10.1093/jac/dkz354 PMID: 31430370

42. Montero M, Ochoa SD, López-Causapé C, VanScy B, Luque S, Sorlí L, et al. Efficacy of Ceftolozane-Tazobactam in Combination with Colistin against Extensively Drug-Resistant *Pseudomonas aeruginosa*, Including High-Risk Clones, in an In Vitro Pharmacodynamic Model. Antimicrobial Agents and Chemistry. 2020; 64(4).

43. Mataraci Kara E, Yılmaz M, İstanbullu Tosun A, Özbek Çelik B. Synergistic activities of ceftazidime-avibactam in combination with different antibiotics against colistin-non-susceptible clinical strains of *Pseudomonas aeruginosa*. Infectious Diseases. 2020:1–9. https://doi.org/10.1080/23744235.2020.1767803 PMID: 32427010

44. Mataraci Kara E, Yılmaz M, İstanbullu Tosun A, Özbek Çelik B. Evaluation of the synergy of ceftazidime/avibactam in combination with colistin, doripenem, levofloxacin, tigecycline, and tobramycin against OXA-48 producing Enterobacteriales. Journal of Chemotherapy. 2020:1–8.