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BRIEF COMMUNICATION

In vitro activity of colistin sulfate against *Enterobacteriaceae* producing extended-spectrum β-lactamases

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The widespread multidrug-resistant *Enterobacteriaceae* pose a serious therapeutic challenge. Colistin and tigecycline are potential antimicrobial agents for treating infections caused by extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*. We evaluated the *in-vitro* activity of colistin sulfate against 253 ESBL producers isolated from patients admitted to a medical center in southern Taiwan (*Escherichia coli*, $n = 82$; *Klebsiella pneumoniae*, $n = 102$; *Enterobacter cloacae*, $n = 34$; and *Serratia marcescens*, $n = 35$). Colistin showed promising *in-vitro* activity against *E. coli*, *K. pneumoniae*, and *E. cloacae*, but not *S. marcescens*. One ESBL-producing *K. pneumoniae* strain with resistance to carbapenems (ertapenem, imipenem, and meropenem) was selected for time-killing studies. A combination of colistin and tigecycline showed synergism, but there was an inoculum effect. In conclusion, colistin was active against most ESBL-producing *Enterobacteriaceae*, and a combination of colistin with tigecycline was synergistic against some highly resistant strains, even those with carbapenem resistance.

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Introduction

Multidrug-resistant *Enterobacteriaceae* are ubiquitous and pose a serious therapeutic challenge. The production of plasmid-mediated extended-spectrum β-lactamases (ESBLs) by *Enterobacteriaceae* subsequent to extensive use of broad-spectrum antibiotics, has limited the use of antibiotics to carbapenems, which are used for serious infections.[1] However, increased use of carbapenems leads to the selection of carbapenem-resistant *Enterobacteriaceae*. Colistin and tigecyclin have been suggested as alternatives to carbapenems for the treatment of infections caused by ESBL-producing *Enterobacteriaceae* in order to avoid selecting for carbapenem resistance.

Colistin, discovered in 1949, was gradually phased out from clinical use in the early 1980s because of its high incidence of nephrotoxicity.[2] Two forms of colistin are commercially available: Colistin sulfate for oral and topical use, and colistimethate sodium (also called sodium colistin methanesulfonate) for parenteral use.[2] Colistin sulfate is stable, while colistimethate sodium is readily hydrolyzed to methanesulfonated derivatives. Colistin sulfate is therefore used in antimicrobial susceptibility tests.[3,4] There are very few studies investigating colistin use for the treatment of infections caused by ESBL-producing *Enterobacteriaceae* in Taiwan. One study reported that the 22 isolates of *Escherichia coli* and 16 isolates of *Klebsiella pneumoniae* in northern Taiwan that produce ESBLs were most susceptible to colistin (91% and 100%, respectively).[5] However, another study from central Taiwan reported that colistin showed poor *in-vitro* activity against three members of the *Enterobacteriaceae* family (*Enterobacter cloacae*, *Citrobacter freundii*, and *Serratia marcescens*).[6] In this study, we evaluated the *in-vitro* activity of colistin sulfate against ESBL-producing *E. coli*, *K. pneumoniae*, *E. cloacae*, and *S. marcescens* isolates from southern Taiwan. We also used time-killing studies to evaluate the synergism of colistin and tigecycline activity against one strain of ESBL-producing *K. pneumoniae* (designed Kp340) with resistance to carbapenems.

Materials and methods

Isolates

The ESBL-producing isolates of *Enterobacteriaceae* were collected from the Chi Mei Medical Center, Taiwan, in 2009 and were identified on the basis of routine microbiologic methods (Phoenix system, Becton Dickinson Company, Baltimore, MD, USA). All isolates were subcultured and frozen at −70°C. ESBL producers were tested using the phenotypic confirmatory disc diffusion method described by the Clinical Laboratory Standard Institute (CLSI).[4]

Antimicrobial susceptibility testing

Minimal inhibitory concentrations (MICs) for colistin sulfate (Sigma Chemical Company, St. Louis, MO, USA) and tigecycline (Wyeth, Puerto Rico, USA) were determined by the standard agar dilution method described by CLSI.[3] There is no CLSI recommendation for colistin susceptible breakpoints against *Enterobacteriaceae*, while the CSLI recommendations for *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are as follows: (susceptible, ≤2 μg/mL; intermediately resistant, 4 μg/mL; resistant, ≥8 μg/mL and susceptible, ≤2 μg/mL; resistant, ≥4 μg/mL, respectively).[4] According to the British Society for Antimicrobial Chemotherapy Working Party on Susceptibility Testing, the susceptible MIC breakpoint for colistin against *Enterobacteriaceae* is ≤4 μg/mL, and the strain should be considered resistant if the MIC > 4 μg/mL.[7] We applied the British Society MIC breakpoints to our results.

Time-killing study

One ESBL-producing *K. pneumoniae* urine isolate (Kp340) that was resistant to ertapenem, imipenem, and meropenem was recovered from a host who was previously exposed to tigecycline and meropenem. We used the Kp340 isolate to evaluate the synergistic activity of colistin (MIC, 1 μg/mL) and tigecycline (MIC, 4 μg/mL) using 1/2 × MIC for each drug concentration alone or for the combination of both drugs. We also performed time-kill studies using 1/4 × MIC for the combination of both drugs. Serial samples (baseline, 2, 4, 6, 8, 12, 24 h) were obtained over a time period of 24 hours. We used two different final inocula of colony-forming units (CFU)/mL with a 100-fold difference in densities. The standard inoculum was 5×10^5 CFU/mL, and the high inoculum was 5×10^7 CFU/mL. Bactericidal activity was defined as a ≥3-$\log_{10}$ CFU/mL decrease in viable cell counts compared to the original inoculum. The lower limit of detection was 100 CFU/mL.

Results and discussion

A total of 253 ESBL-producing *Enterobacteriaceae* were collected, which included 82 strains of *E. coli* (blood, $n = 7$; sputum, $n = 15$; urine, $n = 37$; wound pus, $n = 15$, ascites,

Table 1 The colistin MIC distribution (n, %) for ESBL-producing *Enterobacteriaceae*

Organisms (n = 253)	MIC ≤ 1 μg/mL	MIC = 2 μg/mL	MIC = 4 μg/mL	MIC 8 μg/mL
Escherichia coli (82)	74 (90%)	8 (10%)	0	0
Klebsiella pneumoniae (102)	41 (40%)	60 (59%)	1 (1%)	0
Enterobacter cloacae (34)	9 (26%)	24 (71)	1 (3%)	0
Serratia marcescens (35)	0	0	0	35 (100%)

ESBL = extended-spectrum β-lactamase; MIC = minimal inhibitory concentration.

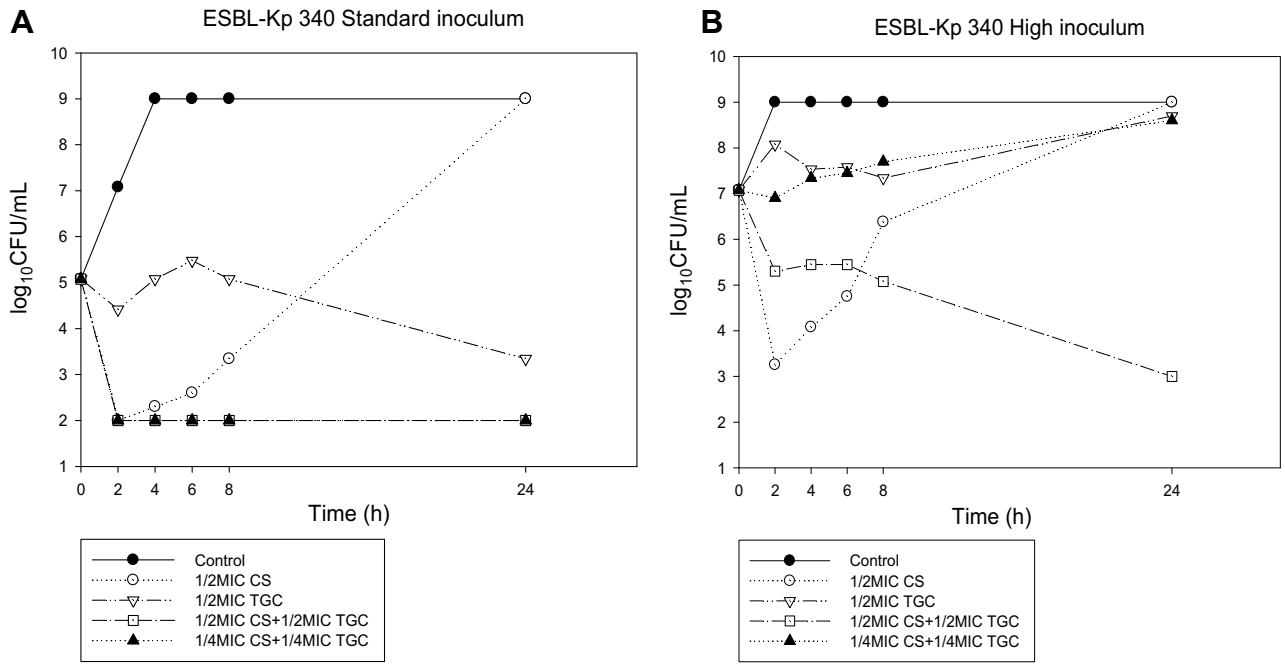

Figure 1. Survival curves of Kp340 standard inoculum (A) and high inoculum (B) using 1/2 × MIC and 1/4 MIC drug concentrations of colistin and tigecycline. CS = colistin; ESBL = extended-spectrum β-lactamase; MIC = minimal inhibitory concentration; TGC = tigecycline.

$n = 8$), 102 strains of *K. pneumoniae* (blood, $n = 15$; sputum, $n = 40$; urine, $n = 30$; wound pus, $n = 17$), 34 strains of *E. cloacae* (blood, $n = 4$; urine, $n = 19$; wound pus, $n = 8$; ascites, $n = 3$), and 35 strains of *S. marcescens* (blood, $n = 12$; sputum, $n = 5$; urine, $n = 18$).

The activity of colistin against the 253 bacterial strains is shown in Table 1. None of the ESBL-producing *E. coli*, *K. pneumoniae*, and *E. cloacae* strains had an MIC > 4 μg/mL, while the ESBL-producing *S. marcescens* strains did. All ESBL-producing *E. coli* strains ($n = 82$) had MICs ≤ 2 μg/mL, with an MIC_{90} value of 1 μg/mL. All the ESBL-producing *K. pneumoniae* ($n = 102$) and *E. cloacae* ($n = 34$) strains had MIC values ≤ 4 μg/ml, and the MIC_{90} values were 2 μg/ml. Colistin showed significant activity against ESBL-producing strains of *E. coli*, *K. pneumoniae* and *E. cloacae* but was less active against ESBL-producing *S. marcescens*, which had an MIC_{100} value > 4 μg/mL. The 100% resistance was predictable since *S. marcescens* is inherently resistant to colistin.[8] Although there are encouraging data describing colistin activity against *Pseudomonas* and *Acinetobacter* isolates,[2] the activity of colistin against multidrug-resistant *Enterobacteriaceae* remains unclear.[8,9] There has also been a rapid and steep increase in colistin resistance in *K. pneumoniae* strains.[10]

We showed that a combination of colistin and tigecycline, at 1/2 × MIC as well as the 1/4 × MIC drug concentrations had synergistic activity against the standard inoculum beginning at 2 hours after inoculation and lasting 24 hours (Fig. 1A). However, although both drug concentrations were less active against the high inoculum, only 1/2 × MIC drug concentration of both drugs exhibited the synergistic, bactericidal effects (Fig. 1B). The standard inoculum of bacterial density treated with colistin alone at 1/2 × MIC drug concentration showed a rapid regrowth of bacteria (Fig. 1A). In contrast, tigecycline alone at 1/2 × MIC drug concentration did not exert a bacteriostatic effect on the high inoculum of bacterial density (Fig. 1B). Our data suggest that a combination of colistin and tigecycline could be an alternative to carbapenem to treat infections caused by the ESBL producers, especially those which are resistant to carbapenem.

In conclusion, colistin has promising *in-vitro* activity against ESBL-producing *Enterobacteriaceae*, including *E. coli*, *K. pneumoniae* and *E. cloacae*. To avoid rapid induction of colistin resistance, a combination of colistin and tigecycline may be considered as an alternative therapeutic option to treat multidrug-resistant *Enterobacteriaceae* infections. However, it is important to keep in mind the inoculum effect.

Conflicts of interest

The authors declare that they have no financial or non-financial conflicts of interest related to the subject matter or materials discussed in the manuscript.

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