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In vitro antimicrobial synergy of colistin with rifampicin and carbapenems against colistin-resistant *Acinetobacter baumannii* clinical isolates

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ABSTRACT

Increased use of colistin in a clinical setting had resulted in the emergence of colistin-resistant (CoR) *Acinetobacter baumannii*. Combination therapy has been studied as a new approach to treat infections caused by *A. baumannii*. Here, we investigated the *in vitro* antimicrobial synergistic activities of several antimicrobial agent combinations against CoR *A. baumannii*. A total of 41 non-duplicate clinical isolates of CoR *A. baumannii* from a tertiary care hospital in Korea were prospectively collected from April 2012 to December 2014. As a control group, 41 carbapenem-resistant but colistin-susceptible (CoS) *A. baumannii* strains were also evaluated. Minimum inhibitory concentrations (MICs) of antimicrobial agents were determined by Etest in triplicate, and *in vitro* synergy tests were performed by the Etest MIC:MIC ratio method. Synergistic activity was determined as the sum of each antimicrobial agent's fractional inhibitory concentration evaluated (ΣFIC): synergy, ≤0.5; indifference, >0.5–4; and antagonism, >4. Synergistic activities were more frequently observed in the CoR group than the CoS group for combinations of colistin-rifampicin (80.5% *vs.* 14.6%, $P< 0.0001$), colistin-meropenem (85.4% *vs.* 4.9%, $P< 0.0001$), and colistin-imipenem (46.3% *vs.* 2.4%, $P< 0.0001$). Combination with rifampicin or meropenem lowered colistin MICs against CoR *A. baumannii* clinical isolates to the susceptible range (≤ 2 μg/mL) more frequently (61.0%, 25/41, both) than combination with imipenem (29.3%, 12/41). Clinical trials are needed to prove the *in vivo* efficacy of those antimicrobial combinations that exhibited significant *in vitro* antimicrobial synergistic effects against CoR *A. baumannii*.

1. Introduction

Colistin (polymyxin E) is a cationic polypeptide antimicrobial agent composed of a cyclic decapeptide linked by an α-amide linkage to a fatty acyl chain (Li et al., 2006). It exerts its antimicrobial effects on Gram-negative bacteria through a two-step mechanism comprising initial binding to and permeabilization of the outer membrane, followed by destabilization of the cytoplasmic membrane (Wiese et al., 2003). Discovered in 1952, colistin was abandoned in the clinical market due to its major complications of neurotoxicity and nephrotoxicity (Falagas and Kasiakou, 2006). As carbapenem-resistant gram-negative pathogens, including carbapenem-resistant *Acinetobacter baumannii*, disseminated around the world (Giske et al., 2008), colistin was reintroduced to clinical practice as a last resort for the treatment of infections with this microorganism (Falagas and Kasiakou, 2005). Unfortunately, increased use of the drug resulted in the appearance of pandrug-resistant (PDR) *A. baumannii* clinical strains, which exhibit resistance to all anti-*Acinetobacter* drugs, including colistin, in clinical settings (Gales et al., 2006; Rodriguez et al., 2009).

Currently, there is no available antimicrobial monotherapy against colistin-resistant (CoR) *A. baumannii* infections in clinical practice. Given the increasing multidrug resistance rates and lack of new drugs, combination therapy could be an alternative option to treat PDR *A. baumannii* (Boucher et al., 2009). Recently, Qureshi et al. reported the clinical outcomes of patients treated with various colistin-based antimicrobial combinations against CoR *A. baumannii* (Qureshi et al., 2015). In this study, the antimicrobial combinations consisted of colistin methansulfonate, doripenem, ampicillin-sulbactam, tigecycline, meropenem, or rifampicin, and patients who received colistin-carbapenem-ampicillin/sulbactam had significantly better 30-day survival outcomes than the other patients. A few studies have also investigated the *in vitro* synergistic effects of antimicrobial combinations against CoR *A. baumannii* isolates (Karaoglan et al., 2013; Nastro et al., 2014; Peck et al., 2012; Principe et al., 2013). Various kinds of *in vitro* synergy testing were performed such as checkerboard, time-kill, or Etest assays using combinations of colistin, carbapenems, rifampicin, doxycycline, and tigecycline. However, only a few CoR *A. baumannii*

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Table 1
Characteristics of the colistin-resistant *Acinetobacter baumannii* clinical isolates.

Isolate	Specimen	MIC (μg/mL)						Disk diffusion susceptibility test									Carbapenemase	MLST		PFGE type
		CST	MEM	IPM	RIF	TGC	DOX	TZP	CAZ	FEP	GEN	TOB	AMK	TET	CIP	T/S		ST	CC	
SVCR-01	**Pleural fluid**	**10.7**	**>32**	**>32**	**3.7**	**1.5**	**4**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**OXA-23**	**191**	**92**	**A**
SVCR-02	**Wound**	**16**	**>32**	**>32**	**8**	**1.5**	**256**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**OXA-23**	**191**	**92**	**B**
SVCR-03	**Urine**	**12**	**8**	**>32**	**4**	**3**	**3.3**	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**OXA-23**	**357**	**92**	**A-1**
SVCR-04	**Endotracheal aspirate**	**8**	**12**	**>32**	**>32**	**2**	**3**	**R**	**R**	**R**	**R**	**R**	**S**	**I**	**R**	**R**	**OXA-23**	**191**	**92**	**C**
SVCR-05	**Endotracheal aspirate**	**24**	**>32**	**>32**	**3.7**	**1**	**3**	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**OXA-23**	**191**	**92**	**D**
SVCR-06	**Endotracheal aspirate**	**8**	**>32**	**>32**	**4**	**2**	**4**	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**OXA-23**	**191**	**92**	**C-1**
SVCR-07	**Blood**	**8**	**>32**	**>32**	**4**	**2**	**4**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**OXA-23**	**191**	**92**	**C-1**
SVCR-08	**Sputum**	**24**	**24**	**>32**	**4**	**1.8**	**6**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**OXA-23**	**191**	**92**	**D-1**
SVCR-09	**Wound**	**7.3**	**>32**	**>32**	**4**	**2**	**3**	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**OXA-23**	**191**	**92**	**C-1**
SVCR-10	**Tracheal aspirate**	**10.7**	**>32**	**>32**	**>32**	**1.3**	**2**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**OXA-23**	**191**	**92**	**C-1**
SVCR-11	**Sputum**	**16**	**>32**	**>32**	**>32**	**1.5**	**2**	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**OXA-23**	**191**	**92**	**C-1**
SVCR-12	**Endotracheal aspirate**	**12**	**>32**	**>32**	**>32**	**1.7**	**2**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**OXA-23**	**191**	**92**	**C-1**
SVCR-13	**Tracheal aspirate**	**8**	**>32**	**>32**	**4**	**2**	**4**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**OXA-23**	**191**	**92**	**C-1**
SVCR-14	**Blood**	**16**	**21.3**	**>32**	**2**	**2**	**3**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**OXA-23**	**191**	**92**	**C-1**
SVCR-15	**Endotracheal aspirate**	**7.3**	**>32**	**>32**	**>32**	**1.5**	**3**	**R**	**R**	**I**	**R**	**R**	**R**	**S**	**R**	**R**	**OXA-23**	**191**	**92**	**E**
SVCR-16	**IV catheter tip**	**8**	**24**	**>32**	**>32**	**1.7**	**3**	**R**	**R**	**I**	**R**	**R**	**R**	**R**	**R**	**R**	**OXA-23**	**191**	**92**	**F**
SVCR-17	**Endotracheal aspirate**	**12**	**>32**	**>32**	**4**	**2.3**	**3**	**R**	**R**	**R**	**I**	**S**	**S**	**I**	**R**	**S**	**OXA-23**	**191**	**92**	**F**
SVCR-18	**Blood**	**9.3**	**>32**	**>32**	**4**	**2**	**3.7**	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**OXA-23**	**191**	**92**	**F-1**
SVCR-19	**Endotracheal aspirate**	**6**	**>32**	**>32**	**3**	**8**	**5.3**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**OXA-23**	**191**	**92**	**F-2**
SVCR-20	**Endotracheal aspirate**	**10.7**	**>32**	**>32**	**>32**	**1.7**	**2.7**	**R**	**R**	**I**	**R**	**R**	**R**	**S**	**R**	**R**	**OXA-23**	**191**	**92**	**F-3**
SVCR-21	**Endotracheal aspirate**	**8**	**>32**	**>32**	**>32**	**1.7**	**3**	**R**	**R**	**I**	**R**	**R**	**R**	**S**	**R**	**R**	**OXA-23**	**191**	**92**	**G**
SVCR-22	**Endotracheal aspirate**	**7.3**	**>32**	**>32**	**>32**	**0.3**	**5.3**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**OXA-23**	**191**	**92**	**G**
SVCR-23	**Endotracheal aspirate**	**14.7**	**>32**	**>32**	**>32**	**8**	**2**	**R**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**OXA-23**	**191**	**92**	**G**
SVCR-24	**Endotracheal aspirate**	**12**	**12**	**>32**	**4**	**3**	**9.3**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**OXA-23**	**191**	**92**	**H**
SVCR-25	**Tracheal aspirate**	**16**	**>32**	**>32**	**>32**	**2.7**	**1**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**OXA-23**	**191**	**92**	**G**
SVCR-26	**Sputum**	**16**	**>32**	**>32**	**4.7**	**2**	**2**	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**OXA-23**	**191**	**92**	**G**
SVCR-27	**Endotracheal aspirate**	**16**	**>32**	**>32**	**4**	**2**	**4**	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**OXA-23**	**191**	**92**	**H**
SVCR-28	**Sputum**	**>256**	**>32**	**>32**	**>32**	**6**	**12**	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**OXA-23**	**191**	**92**	**I**
SVCR-29	**Endotracheal aspirate**	**>256**	**26.7**	**>32**	**6**	**6**	**256**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**OXA-23**	**858**	**92**	**J**
SVCR-30	**Endotracheal aspirate**	**32**	**12**	**>32**	**>32**	**2**	**8**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**OXA-23**	**191**	**92**	**K**
SVCR-31	**IV catheter tip**	**24**	**12**	**>32**	**>32**	**1.5**	**7.3**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**OXA-23**	**191**	**92**	**K**
SVCR-32	**Sputum**	**8**	**>32**	**>32**	**>32**	**8**	**3.7**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**OXA-23**	**191**	**92**	**L**
SVCR-33	**Blood**	**8**	**>32**	**>32**	**3.7**	**2**	**3**	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**OXA-23**	**191**	**92**	**M**
SVCR-34	**Endotracheal aspirate**	**6**	**>32**	**>32**	**4**	**1.5**	**256**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**OXA-23**	**872**	**92**	**N**
SVCR-35	**Sputum**	**32**	**>32**	**>32**	**3**	**2**	**2**	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**OXA-23**	**191**	**92**	**N-1**
SVCR-36	**Bronchial washing**	**>256**	**>32**	**>32**	**2.7**	**2**	**2.7**	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**OXA-23**	**191**	**92**	**N-2**
SVCR-37	**Sputum**	**37.3**	**>32**	**>32**	**2**	**2**	**2**	**R**	**R**	**R**	**S**	**S**	**S**	**I**	**R**	**S**	**OXA-23**	**191**	**92**	**O**
SVCR-38	**Sputum**	**9.3**	**>32**	**>32**	**>32**	**2**	**192**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**OXA-23**	**357**	**92**	**P**
SVCR-39	**Sputum**	**9.3**	**24**	**>32**	**>32**	**1.8**	**192**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**OXA-23**	**357**	**92**	**P-1**
SVCR-40	**Sputum**	**4.7**	**>32**	**>32**	**>32**	**1**	**64**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**OXA-23**	**357**	**92**	**R**
SVCR-41	**Blood**	**13.3**	**>32**	**>32**	**3**	**4**	**3**	**R**	**R**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**OXA-23**	**138**	**92**	**S**

Abbreviations: S = susceptibility; I = intermediate; R = resistant; TZP = piperacillin-tazobactam; CAZ = ceftazidime; FEP = cefepime; CTX = cefotaxime; IPM = imipenem; MEM = meropenem; CST = colistin; GEN = gentamicin; TOB = tobramycin; AMK = amikacin; TET = tetracycline; CIP = ciprofloxacin; T/S = trimethoprim-sulfamethoxazole; TGC = tigecycline; RIF = rifampicin; DOX = doxycycline; MLST = multilocus sequence typing; ST = sequence type; CC = clonal complex.

isolates were evaluated in these previous studies, making it difficult to draw strong conclusions about which combinations consistently showed effective antimicrobial synergistic activities.

Here, we evaluated the *in vitro* effectiveness of various combinations of colistin and several commonly-used anti-*Acinetobacter* agents against a large number of clinical isolates of CoR *A. baumannii*.

2. Materials and methods

2.1. Bacterial isolates

A total of 41 non-duplicate *A. baumannii* clinical isolates exhibiting resistance to both carbapenems and colistin were collected from a tertiary care hospital in Seoul, Korea from April 2012 to December 2014. For comparison, *A. baumannii* clinical isolates (n = 41) resistant to carbapenems but susceptible to colistin, selected randomly from the same period, were included. *rpoB* gene sequencing and bla_{OXA-51} gene PCR were performed to identify *Acinetobacter baumannii*.

2.2. Multilocus sequence typing

Multilocus sequence typing (MLST) with the Bartual scheme was performed using partial sequences of seven housekeeping genes (*cpn60*, *gdhB*, *gltA*, *gpi*, *gyrB*, *recA*, and *rpoD24*) to determine the sequence types (STs) of *A. baumannii* isolates as described in a previous report (Kim et al., 2014). Each ST number was assigned by comparing the allele sequences to those in the MLST database (http://pubmlst.org/abaumannii). Clonal complex (CC) was defined as a group of STs sharing ≥5/7 alleles, and was determined by eBURSTv.3 (http://eburst.mlst.net).

2.3. Pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis (PFGE) was performed with SmaI-digested genomic DNA extracted from the AB clinical isolates using a CHEF-DRII device (Bio-Rad, Hercules, *CA*, USA). The conditions of PFGE were 6 V/cm for 20 h with pulse times of 3–10 s at a temperature of 11 °C. PFGE band patterns were analyzed with Molecular Analyst Fingerprinting Software Ver. 3.2 (Bio-Rad). Interpretation of genetic relatedness of PFGE profiles was done with the criteria of Tenover et al. (1995).

2.4. Antimicrobial susceptibility testing

Antimicrobial susceptibilities were tested by the disk diffusion method following the recommendations of the Clinical and Laboratory Standards Institute (CLSI) guideline M100-S26 ("Clinical and Laboratory Standards Institute. Performance standards for antimicrobial