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Colistin monotherapy versus colistin/rifampicin combination therapy in pneumonia caused by colistin-resistant *Acinetobacter baumannii*: A randomised controlled trial

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ABSTRACT

Objectives: The aim of this study was to confirm the synergistic effect of colistin/rifampicin combination therapy compared with colistin monotherapy in pneumonia caused by colistin-resistant *Acinetobacter baumannii* (CoRAB). The utility of the Etest was also assessed.
Methods: Nine subjects with pneumonia caused by CoRAB were enrolled from 20 July 2016 to 21 June 2018. Subjects were randomised to colistin/rifampicin combination therapy or colistin monotherapy. After exclusion of one patient who dropped out, the microbiological response (MR) and clinical response (CR) on Day 14 and mortality on Day 30 were assessed. Etest was conducted using CoRAB isolated at study enrolment.
Results: The MR rate in the colistin/rifampicin combination group (100.0%) was better than that in the colistin group (40.0%), however the difference was not statistically significant ($P = 0.196$). The CR rate was not significantly different between the two groups. The MR (100.0%) and CR (100.0%) rates in subjects with 'partial synergy' as shown by Etest were higher than those (25.0% and 50.0%, respectively) in subjects with 'indifferent' results (i.e. no synergistic effect), however the difference was not statistically significant ($P = 0.143$ and 0.429, respectively). Mortality occurred in two subjects with 'indifferent' results by Etest.
Conclusions: Colistin/rifampicin combination therapy may have potential to achieve MR in pneumonia caused by CoRAB; however, achieving CR with this treatment is doubtful. 'Partial synergy' of colistin and rifampicin, as shown by Etest, may be a good prognostic factor [ClinicalTrial.gov ID: NCT03622918].

1. Introduction

There are insufficient data for the treatment of colistin-resistant *Acinetobacter baumannii* (CoRAB) infection and nowadays treatment of this condition represents a real challenge. Some studies have shown effective combination therapies for CoRAB in vitro and in vivo [1–3]. Bae et al. reported a 100% synergistic effect of colistin/rifampicin combination against nine clinical CoRAB isolates in vitro [4]. Leite et al. showed a 100% synergistic effect of colistin/rifampicin in seven CoRAB isolates in vitro [1]. Hong et al. conducted a relatively large study including 41 CoRAB isolates and reported a 80.5% synergistic effect of colistin/rifampicin in vitro [5]. Oliva et al. have reported successful treatment using colistin plus vancomycin plus rifampicin combination therapy in a patient with ventilator-associated pneumonia (VAP) caused by CoRAB [2]. Although there is some evidence showing that colistin/rifampicin combination will be beneficial in treating infection with CoRAB, a randomised clinical trial confirming the effects of this combination therapy has not been conducted to date.

The synergy test has been used in many previous studies to determine the degree of synergistic effects induced by antimicrobial combinations and to predict clinical outcomes of combination therapy [4,6]. Bremmer et al. showed that the synergy test may have clinical utility in predicting the treatment prognosis of

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infection with CoRAB [7]. However, as this previous study was designed as a retrospective study, it did not conduct any intervention to prove the results. Thus, there is insufficient evidence for the utility of the synergy test in identifying which combination therapy should be used to treat infections with CoRAB.

In the current study, a randomised controlled trial was conducted to confirm the synergistic effect of colistin/rifampicin combination therapy in pneumonia caused by CoRAB. In addition, the utility of the Etest was assessed.

2. Materials and methods

2.1. Study subjects

The inclusion criteria were as follows: (i) subjects aged >18 years who were admitted to Gangnam Severance Hospital (Seoul, South Korea); (ii) subjects who were diagnosed as having active pneumonia caused by CoRAB based on the following criteria: (a) new-onset respiratory symptoms (such as cough, sputum and dyspnoea) or rales or desaturation; (b) new-onset or progressive infiltrative lesion on chest radiography suggestive of pneumonia; (c) fever (≥38.0 °C) or hypothermia (≤35.0 °C) for >48 h or abnormal white blood cell count (≥10 000/mm^3 or ≤4500/mm^3); and (d) CoRAB documented by a culture study of sputum samples (>10^6 CFU/mL), airway aspirates (>10^5 CFU/mL) or bronchoalveolar fluid (>10^4 CFU/mL); (iii) non-pregnant women agreeing to avoid pregnancy during the study; and (iv) subjects who fully understand the provided explanation regarding the study and agreed to participate in the study.

Exclusion criteria were as follows: (i) subjects who have experienced hypersensitivity reaction to colistin or rifampicin; (ii) subjects who had been treated with colistin or rifampicin in the previous 15 days; (iii) subjects who had evidence of colistin-resistant *A. baumannii* in the previous 15 days; and (iv) subjects who are vulnerable to the side effects of drugs [creatinine clearance (CL_{Cr}) <15 mL/min, aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >3× upper limit of normal (ULN), total bilirubin >1× ULN, haemoglobin <7 g/dL, absolute neutrophil count (ANC) < 500/mm^3, platelets <50 000/mm^3].

The inclusion criterion was not limited to VAP. However, VAP may affect clinical outcome, including mortality, therefore whether the pneumonia was VAP was determined. VAP was defined as patients receiving mechanical ventilation for ≥24 h with a first positive bacterial culture finding after ventilator start date [8].

2.2. Study protocol

Patients with pneumonia caused by CoRAB in Gangnam Severance Hospital from 20 July 2016 to 21 June 2018 were prospectively searched for. A total of 18 subjects were enrolled, of whom 9 were excluded for various reasons (3 refusal to enrol, 3 vulnerable to the side effects of drugs, 2 previous rifampicin or colistin use and 1 negative conversion of *A. baumannii* before enrolment). Thus, nine subjects were finally enrolled and randomised in a blinded fashion using a computerised random number generator (permuted-block randomisation) for treatment with colistin/rifampicin combination therapy or colistin monotherapy at a ratio of 1:1. Colistin (100 mg colistin sodium methanesulfonate; SCD Pharm, Seoul, South Korea) was administered intravenously with 100 mL of normal saline every 8 h. Rifampicin (600 mg; Yuhan, Seoul, South Korea) was administered orally daily. Treatment was administered daily for ≥7 days and up to 28 days. The duration of treatment with antibiotics was determined through discussion with pulmonology and infection specialists (MKB and SHH). Use of other antibiotics and other medications was permitted if clinicians judged that these were necessary. One subject dropped out because he was transferred to another hospital as per the patient's request on Day 2. Thus, eight subjects completed the study and were included in the analysis (Fig. 1).

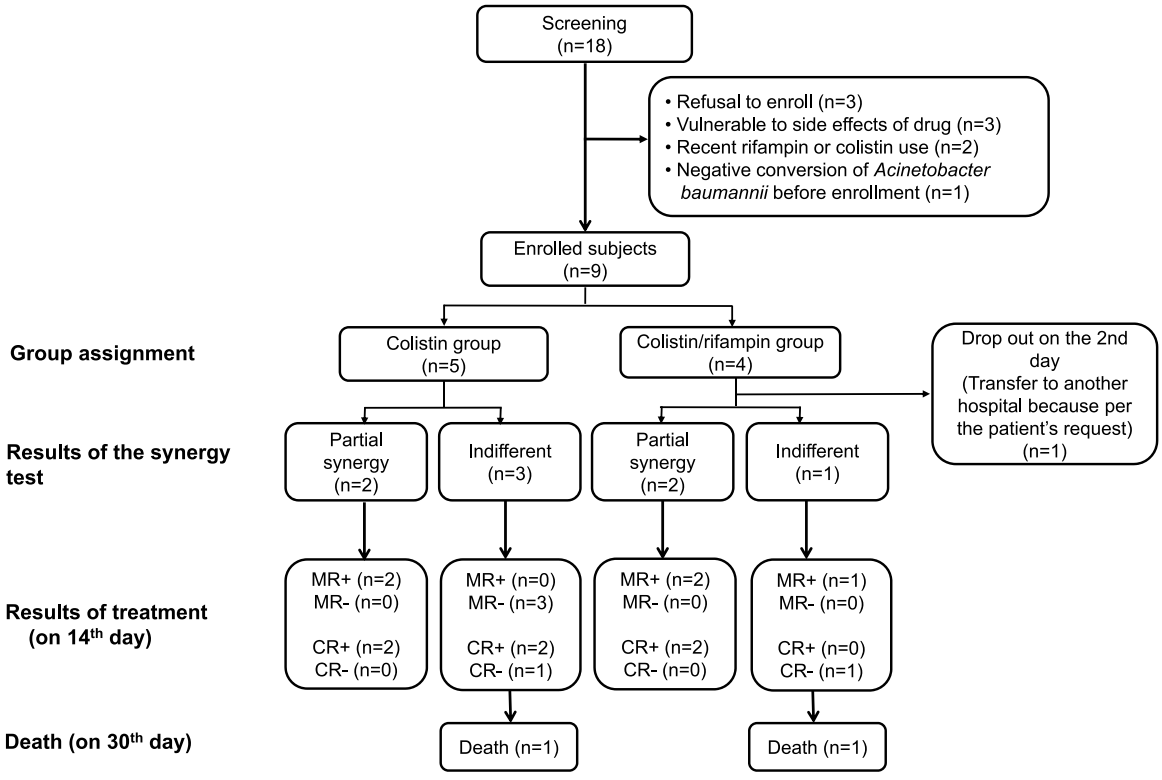

Fig. 1. Study flow as well as microbiological response (MR) and clinical response (CR) of enrolled subjects.

2.3. Synergy test

PCR for the bla_{OXA-51} gene and nucleotide sequencing of the *rpoB* gene were performed to identify *A. baumannii* as described previously [5]. The Etest MIC:MIC ratio method was performed to predict in vitro synergy of colistin and rifampicin combination therapy [6]. Briefly, a CoRAB suspension was inoculated on a Mueller–Hinton agar (Difco Laboratories, Detroit, MI) plate. Colistin and rifampicin Etest strips (bioMérieux, Inc., Durham, NC) were placed on different areas of the plate and were incubated for 1 h at room temperature. The agar was marked at the previously determined MIC and then each Etest strip was removed. New colistin and rifampicin Etest strips were placed on the areas of the previously removed Etest strips as follows: a new rifampicin Etest strip was placed on the area of the previously removed colistin Etest strip so that the rifampicin MIC corresponded with the mark of the colistin MIC; and a new colistin Etest strip was placed on the area of the previously removed rifampicin Etest strip in the same manner. The plates were incubated for 16–20 h at 35 °C and the combination MIC [Σfractional inhibitory concentration (ΣFIC)] was derived. Synergistic effects were classified as follows: synergy, $\Sigma FIC \leq 0.5$; partial synergy, $0.5 < \Sigma FIC \leq 1$; additive, $\Sigma FIC = 1$; indifferent, $1 < \Sigma FIC \leq 2$; and antagonistic, $2 < \Sigma FIC$.

2.4. Study outcomes

The primary outcome was microbiological response (MR), defined as a negative conversion of culture study (eradication) of *A. baumannii* on Day 14. The secondary outcome was clinical response (CR), defined as clinical resolution of fever, symptoms and infectious signs on Day 14 and mortality on Day 30.

2.5. Safety

Drug safety was assessed on the basis of laboratory findings. Decreased CL_{Cr} ($\leq$1/2 from baseline value or <10 mL/min), increased ALT or AST levels (>3 times baseline or >5× ULN), increased bilirubin levels (>3 times baseline or >3× ULN), decreased haemoglobin (<70% from baseline or <7 g/dL) and ANC <500 mm^3 and/or platelets <50 000/mm^3 were considered to be critical signs of side effects of drugs. The causality of the relationship between the administered drug and the adverse drug reaction was assessed by an allergy specialist (HJP) using the World Health Organization (WHO) Uppsala Monitoring Center (UMC) causality assessment algorithm [9,10].

2.6. Statistical analysis

Data are presented as the mean and standard deviation. Mann–Whitney *U*-test and Fisher's exact test were used to identify any statistically significant differences between groups in continuous and categorised variables, respectively. PASW Statistics v.18.0 (SPSS Inc., Chicago, IL) was used for statistical analyses, and a *P*-value of <0.05 was considered statistically significant.

3. Results

3.1. Baseline clinical and laboratory characteristics according to treatment group

Baseline clinical and laboratory findings were not significantly different between treatment groups, except for the prevalence of males (100.0% in the colistin monotherapy group vs. 25.0% in the colistin/rifampicin combination group; $P = 0.048$) and the mean ALT value (13.0 IU/L in the colistin monotherapy group vs. 5.0 IU/L in the colistin/rifampicin combination group; $P = 0.013$) (Table 1).

3.2. Microbiological response and clinical response of enrolled subjects

Among five subjects in the colistin group, two subjects showed 'partial synergy' to the combination of colistin and rifampicin by Etest. Although they were treated with colistin monotherapy, CR and MR were achieved on Day 14. The remaining three subjects showed 'indifferent' results (i.e. no synergistic effect). Although none of them achieved MR, two subjects achieved CR; the other subject who did not achieve CR died on Day 24 (Fig. 1).

Among four subjects in the colistin/rifampicin combination therapy group, one subject dropped out on Day 2. Two subjects showed "partial synergy" by Etest and they both achieved MR and CR. The remaining subject showed an 'indifferent' result and although MR was achieved, CR was not. The patient died on Day 10 (Fig. 1).

3.3. Microbiological response and clinical response according to treatment group and results of the synergy test

MR and CR rates were compared between the groups. The MR rate in the colistin/rifampicin combination therapy group (100.0%)

Table 1
Baseline clinical and laboratory characteristics of patients according to treatment group[a].

Characteristic	Colistin monotherapy ($n = 5$)	Colistin/rifampicin combination ($n = 4$)	*P*-value
Clinical findings			
Age (years)	58.0 ± 39.0	76.0 ± 43.0	0.323
Male sex [*n* (%)]	5 (100.0)	1 (25.0)	0.048[b]
Charlson comorbidity index	4.0 ± 2.5	3.5 ± 1.8	0.893
Coinfection [*n* (%)]	3 (60.0)	2 (50.0)	>0.999
Laboratory findings			
WBC count (cells/mm^3)	9960 ± 21 100	14 585 ± 8530	0.624
Neutrophils (%)	76.3 ± 23.1	80.4 ± 18.2	>0.999
CRP (mg/L)	73.8 ± 249.9	73.3 ± 96.7	>0.999
Haemoglobin (g/dL)	9.1 ± 2.4	10.5 ± 2.2	0.221
Creatinine (mg/dL)	0.4 ± 0.7	0.5 ± 0.5	0.806
AST (IU/L)	21.0 ± 34.5	16.0 ± 13.3	0.319
ALT (IU/L)	13.0 ± 48.5	5.0 ± 4.0	0.013[b]
Protein (mg/dL)	5.4 ± 0.9	5.4 ± 1.4	0.806
Albumin (mg/dL)	2.9 ± 0.7	2.9 ± 1.0	0.711
Prothrombin time (s)	15.5 ± 24.2	13.4 ± 3.3	0.085
aPTT (s)	35.9 ± 33.4	29.4 ± 24.1	0.564

WBC, white blood cell; CRP, C-reactive protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; aPTT, activated partial thromboplastin time.
[a] Data are the mean ± standard deviation unless otherwise stated.
[b] Statistically significant difference between groups.

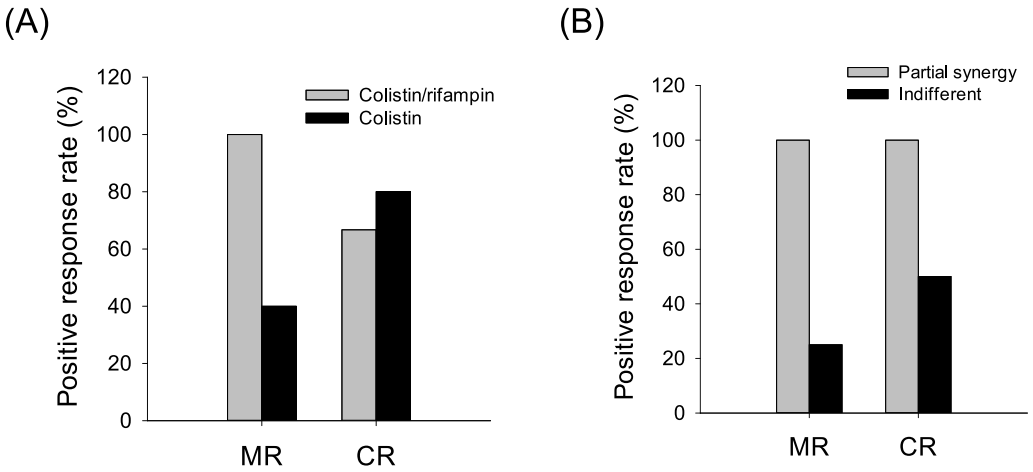

Fig. 2. Microbiological response (MR) and clinical response (CR) according to (A) treatment group and (B) results of the synergy test.

was better than that in the colistin monotherapy group (40.0%), however the difference was not statistically significant ($P = 0.196$). The CR rate in the colistin/rifampicin combination therapy group (66.7%) and the colistin monotherapy group (80.0%) was not significantly different ($P > 0.999$) (Fig. 2A).

MR and CR rates were compared according to the results of the synergy test. The MR (100.0%) and CR (100.0%) rates in subjects with 'partial synergy' by Etest were higher than those in subjects with 'indifferent' results (25.0% and 50.0%, respectively), however the difference was not statistically significant ($P = 0.143$ and 0.429, respectively) (Fig. 2B).

3.4. Clinical characteristics and treatment outcomes of enrolled subjects

Table 2 shows detailed information for the enrolled subjects, including the presence of VAP and combined antibiotic therapy. Both cases that died were VAP. There were many antibiotic combinations, however no significant correlation between combined antibiotics and treatment outcomes was found.

3.5. Safety

Unexpected serious adverse events suspected to be associated with treatment were not observed in this study. In the two patients who died, clinicians maintained antimicrobial administration until the end. Clinicians suspected that these patients died because of uncontrolled pneumonia and multiorgan failure.

4. Discussion

Although this study could not prove that the effect of combination therapy was statistically significant owing to the limited number of enrolled subjects, it did suggest that colistin/rifampicin combination therapy may be helpful in achieving MR in some cases of pneumonia caused by CoRAB. To the best of our knowledge, this is the first prospective randomised controlled trial to reveal the effects of colistin/rifampicin combination therapy on infection with CoRAB. We believe that this pilot study may be helpful for a follow-up, well-designed, large clinical trial to determine the research direction. Management of *A. baumannii* infection has been a great challenge for physicians owing to multidrug-resistant strains. In addition, extensively drug-resistant *A. baumannii* (resistant to carbapenems, i.e. CRAB) and pandrug-resistant *A. baumannii* (resistant to polymyxins and tigecycline, i.e. CoRAB) have emerged [11]. CoRAB has been considered to develop after or during colistin therapy with selection of the colistin-resistant subpopulation [12–15]. However, a recent study has shown that colistin-susceptible *A. baumannii* may become CoRAB via mutation even in an antibiotic-free environment [16]. Recently, there have been no new emerging antibiotics for CoRAB. Many clinicians have tried various combination therapies of well-known antibiotics. Previous studies have shown that colistin/rifampicin combination therapy has potential as a treatment option for infection with CoRAB; however, these were in vitro or case studies [1–3]. This is the first randomised clinical trial to compare the effectiveness of colistin monotherapy versus colistin/rifampicin combination therapy in pneumonia caused by CoRAB.

Table 2
Clinical characteristics and treatment outcomes of enrolled subjects.

Variable	Colistin monotherapy group					Colistin/rifampicin combination group		
	a	b	c	d	e	f	G	h
Age (years)/sex	30/M	44/M	58/M	78/M	74/M	88/M	74/F	32/F
VAP	N	N	Y	Y	Y	Y	Y	Y
Ever used antibiotic therapy for 30 days	TZP, LVX, TEC, CAZ, SXT	MFX, MTR	CIP, MEM, TEC, FEP, LVX, SAM, MFX	TZP, TEC, SXT, SAM, MEM	MEM, VAN, SAM, TEC, MFX, TZP, LVX,	ATM, MFX, TEC	MEM, TEC, TZP, MTR, CAZ	MEM, TEC, LVX, CAZ
Results of the synergy test (Etest)	Indifferent	Partial synergy	Indifferent	Indifferent	Partial synergy	Indifferent	Partial synergy	Partial synergy
MR	N	Y	N	N	Y	Y	Y	Y
CR	Y	Y	Y	N	Y	N	Y	Y
Mortality at Day 30	Alive	Alive	Alive	Dead	Alive	Dead	Alive	Alive

VAP, ventilator-associated pneumonia; N, no; Y, yes; TZP, piperacillin/tazobactam; LVX, levofloxacin; TEC, teicoplanin; CAZ, ceftazidime; SXT, trimethoprim/sulfamethoxazole; MFX, moxifloxacin; MTR, metronidazole; CIP, ciprofloxacin; MEM, meropenem; FEP, cefepime; SAM, ampicillin/sulbactam; VAN, vancomycin; ATM, aztreonam; MR, microbiological response; CR, clinical response.

While the effectiveness of colistin/rifampicin combination therapy for CoRAB has limited evidence, scientific evidence does exist for the effectiveness of colistin/rifampicin combination therapy for CRAB. Durante-Mangoni et al. showed the effectiveness of colistin/rifampicin combination therapy in CRAB through a well-designed, multicentre, randomised clinical trial [17]. Although they could not prove the clinical benefit of combination therapy, the *A. baumannii* eradication rate of combination therapy (60.6%) was significantly higher than that of colistin monotherapy (44.8%) ($P = 0.034$) [17], and this observation is in accordance with the results of the current study. In addition, Nordqvist et al. suggested that colistin/rifampicin combination therapy can prevent transformation of colistin-susceptible *A. baumannii* into CoRAB [18]. Colistin/rifampicin combination therapy may be helpful in eradicating or preventing CoRAB.

Colistin acts as a cationic detergent disturbing the bacterial cell membrane, increasing permeability and inducing cell death; it also exhibits bactericidal activity against *A. baumannii* [19]. CoRAB can develop if colistin-resistant subpopulations grow selectively [12,13,20]. Colistin-resistant subpopulations have increased susceptibility to some antibiotics that are normally ineffective against Gram-negative bacteria. The possible mechanism of this action may be a substantial change in the outer membrane of CoRAB. Disruption of the membrane of CoRAB would allow enhanced penetration of antibiotics, making different antibiotics, which are originally ineffective, effective [21]. In fact, Nordqvist et al. have shown extreme susceptibility of CoRAB to rifampicin, which was originally considered to be ineffective against *A. baumannii* [18]. However, a lack of controlled clinical trials of combination therapy for infection with CoRAB makes treatment selection difficult. This study may guide in selecting colistin/rifampicin combination therapy for infection with CoRAB.

Regardless of colistin monotherapy or combination therapy, 'partial synergy' as shown by Etest showed potential for use as a good prognostic factor for infection with CoRAB. Subjects with 'partial synergy' showed 100.0% MR and CR rates regardless of the treatment regimen. Among subjects with 'indifferent' results, two subjects died. This implies that the result of the synergy test is more important than the regimen of combination therapy selected. Other antibiotics were not excluded and subjects were enrolled who used the study drug combined with other drugs such as carbapenems or glycopeptides, which have been used as a combination therapy regimen in previous studies. We suggest that partial synergy associated with the combination of rifampicin and colistin causes increased permeability of the cell membrane, thereby allowing for better antibiotic effects; utilises the synergistic effects of other combination regimens; and finally leads to a good prognosis. However, this speculation should be confirmed via further studies.

Etest was conducted in this study to prove the utility of this method in clinical settings. The Etest MIC:MIC ratio method, which is widely used as it is an easy protocol, can assess only inhibitory activity [22]. Time–kill assay can measure bactericidal activity but is difficult to perform [23]. Etest can assess bactericidal activity and shows good correlation with the time–kill assay and requires less time and labour. The results of the present study suggest that the Etest can be applied to predict the prognosis for infection with CoRAB in real clinical settings.

There are insufficient data on treatment and prognostic factors for pneumonia caused by CoRAB because it is a very rare disease and it is difficult to enrol subjects with this condition in clinical trials. Many clinicians have used various combination therapies for infection with CoRAB in real clinics because they have no other options. There was an urgent need to perform a clinical study to prove the significant role of combination therapy for infection with CoRAB. We showed the potential benefit of colistin/rifampicin combination therapy for pneumonia caused by CoRAB. Although a significant benefit in clinical improvement was not shown, we can choose this regimen to eradicate CoRAB. In addition, we can predict the prognosis of CoRAB infection based on the result of the synergy test. Although the Etest has not been widely used because of insufficient evidence of utility, we showed for the first time the utility of the Etest in CoRAB infection. The Etest is easy to perform and its results can identify the optimal treatment regimen.

The study has some limitations. First, a limited number of subjects were enrolled and the results were statistically insignificant. However, it is very difficult to enrol a sufficient number of subjects because pneumonia caused by CoRAB is extremely rare and fatal. Furthermore, as we believe that it is urgent to show scientific evidence proving the effectiveness of combination therapy for CoRAB, we decided to conduct the study quickly with a limited number of subjects. However, further large clinical studies will be needed to corroborate the conclusions. Second, the allowance of other antibiotic regimens can act as a confounding factor in interpretation of the study results. However, we could not discontinue other antibiotics because some patients had co-infection, and in other patients antibiotics had to be used to prevent other bacterial infections. Third, MR is easily affected by complex factors, including collection method, quality of the specimen and quantification techniques. MR might not be a good and reliable outcome. Lastly, the MIC for rifampicin and serial changes of the synergy test will be needed to corroborate the results of this study.

5. Conclusions

To date, there has been no definite treatment option for CoRAB. Colistin/rifampicin combination therapy may be helpful to achieve MR in some cases of pneumonia caused by CoRAB; however, its effectiveness in achieving CR appears doubtful. This regimen may be helpful in eradicating CoRAB, preventing the acquisition of further resistance, and preventing the further spread of CoRAB. In addition, the utility of the Etest in CoRAB infection was demonstrated for the first time. 'Partial synergy' of colistin and rifampicin, as shown by Etest, can be a good prognostic factor for MR and CR in pneumonia caused by CoRAB.

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Competing interests

None declared.

Ethical approval

The study protocol was approved by the Institutional Review Board of Gangnam Severance Hospital, Yonsei University Health System (Seoul, South Korea) [approval no. 3-2016-0125].

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