FACTORS ASSOCIATED WITH TREATMENT OUTCOMES OF PATIENTS WITH EXTENSIVELY DRUG-RESISTANT GRAM-NEGATIVE INFECTIONS TREATED WITH COLISTIN COMBINATION THERAPY: A PILOT STUDY

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Abstract. Due to high mortality among patients with extensively drug-resistant (XDR) gram-negative bacterial infections within 7 days at our institute, we aimed to determine the clinical factors associated with treatment outcomes after one week of colistin combination therapy (CCT) in patients with such infections. We conducted a pilot prospective observational study among all inpatients aged >18 years from October 2014 to January 2016 with an XDR gram-negative bacterial infection who received CCT at Ramathibodi Hospital, Bangkok, Thailand. Clinical data, maximum plasma colistin levels, and minimal inhibitory concentration ratios (Cmax/MIC) were obtained for each subject over a 14-day period. Gram-negative bacteria of interest isolated were *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. A total of 34 patients were included in the study. Univariate statistical analysis was performed. The average (±standard deviation) duration of CCT was 10(±4) days. Sixty-six point seven percent of patients achieved a target colistin level. There was a trend towards higher mortality at day 7 among the meropenem–CCT group (3/8 patients) but this was not statistically significant. All bacteremic and urinary-tract-infection patients had 100% microbiological treatment success while the others did not (p <0.0001). A higher APACHE II score was associated with a higher mortality (p =0.031). In our study, infection site and APACHE II score were predictors of survival whereas having an optimal Cmax/MIC ratio for colistin was not a predictor of clinical outcome. In conclusion, treatment of XDR gram-negative bacterial infection is challenging. Further studies are necessary to determine non-significant findings in our study.

Keywords: colistin plasma concentration, extensively drug-resistant gram-negative bacilli, survival, site of infection, APACHE II score

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INTRODUCTION

Healthcare-associated infections (HAI) are a major concern worldwide. In Thailand, gram-negative pathogens account for the majority of HAI, in par-

ticular *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (Danchaivijitr *et al*, 2007; Chaisathaphol and Chayakulkeeree, 2014). These two bacteria are often resistant to multiple antimicrobial regimens, including carbapenems (Chaisathaphol and Chayakulkeeree, 2014). This has resulted in the need to use antibacterials previously discarded from clinical practice, such as colistin (Falagas and Kasaikou, 2005; Li *et al*, 2006). At our hospital, all isolates of these two bacteria are sensitive to colistin (Division of Microbiology, Department of Pathology, 2013-2014). However, colistin monotherapy is not recommended because of the rapid development of resistance and risk of nephro- and neurotoxicity at higher dosages (Petrosillo *et al*, 2008). Colistin is often used in combination with imipenem or ceftazidime because of their favorable synergistic effects (Petrosillo *et al*, 2008; Nation and Li, 2009; Kempf *et al*, 2012).

Plasma drug levels are often a key factor used to determine clinical efficacy (Levison, 2004; Abdul-Aziz *et al*, 2012; Wong *et al,* 2014; Reardon *et al*, 2015). Some clinical studies have reported higher plasma concentrations of colistin are associated with better microbiological outcomes (Vicari *et al*, 2013). Hence, having an optimal plasma colistin level/minimal inhibitory concentration (Cmax/MIC) ratio might be considered important for treatment outcome but there is conflicting data regarding the optimal value, whether this should be >4 or 8 for best clinical outcome (Levison, 2004; Markou *et al*, 2008; Bergen *et al*, 2010; Michalopoulos and Falagas, 2011). However, only a minority of critically ill patients are able to achieve the target Cmax/MIC despite appropriate dosing (Plachouras *et al*, 2009; Garonzik *et al*, 2011; Karnik *et al*, 2013). A study of 18 critically ill patients found the maximum steady state plasma concentrations of colistin, dosed according to manufacturer's recommendation was 2.3 mg/l, which was only slightly above the susceptibility breakpoint of 2 mg/l for *P. aeruginosa* and *A. baumannii* according to the Clinical and Laboratory Standards Institute and the European Committee on Antimicrobial Susceptibility Testing (Nation and Li, 2009; Plachouras *et al*, 2009; Walkty *et al*, 2009; CLSI, 2014). Increasing the daily dose may increase nephrotoxicity as this occurs in up to 30% to 50% of patients taking colistin therapy, as well as a concern of neurotoxicity (Lim *et al*, 2010; Pike and Saltiel, 2014; Ortwine *et al*, 2015). A plasma colistin concentration >4 mg/l (equivalent to a serum colistin concentration/MIC >4) is associated with complete bactericidal activity against *P. aeruginosa* (Daikos *et al*, 2010). However, a Cmax/MIC ratio ≥8 is considered optimal against both *P. aeruginosa* and *A. baumannii* in all isolates (Gurja, 2015). However, in one *in vivo* study on variety of pharmacodynamic and pharmacokinetic parameters in association with treatment outcomes of biofilm infections, the area under the curve/minimal inhibitory concentration (AUC/MIC) ratio has been suggested as the parameter that is best associated with successful treatment of lung infections (Hengzhuang *et al*, 2012).

A large proportion of patients infected with *P. aeruginosa* and *A. baumannii* at Ramathibodi Hospital died within 7 days of admission. Therefore, we conducted a pilot prospective observational study of a variety of colistin combination therapies (CCTs) among patients infected with extensively drug-resistant (XDR) gram-negative bacteria at Ramathibodi Hospital. We aimed to determine the optimal colistin Cmax/MIC ratio in regards to: 1) microbiological treatment success, 2) site

of infection, and 3) other parameters associated with treatment outcome of infection after 7 days of treatment. The data will allow a more rational, evidence-based decision when prescribing CCT for patients with XDR bacterial infections in order to achieve a good treatment outcome.

MATERIALS AND METHODS

Definitions

The definition of XDR bacteria was modified from Magiorakos *et al* (2012) as a bacterial pathogen that is susceptible only to colistin but is resistant to other classes of antibiotics. Acute kidney injury (AKI) definition was based on RIFLE criteria (risk, injury, failure, loss of kidney function, and end-stage kidney disease) (Ma *et al*, 2008; Magiorakos *et al*, 2012). Microbiological treatment success was defined as clearance of bacteria by 7 days, and microbiological treatment failure was defined as persistence of bacteria by 7 days. The optimal Cmax/MIC ratio was set at ≥8 (Gurja, 2015). Immunocompromized status was defined as having one of the following: presence of a hematological malignancy, metastatic solid tumor, HIV, AIDS, receipt of long term corticosteroid treatment, immunosuppressive therapy or cytotoxic chemotherapy. A seven-day mortality rate was defined as death occurring within 7 days of admission or enrollment.

Patients

We conducted a prospective observational study at Ramathibodi Hospital, an academic tertiary care medical center in Bangkok, Thailand from October 2014 to January 2016. Study subjects were those aged ≥18 years diagnosed with XDR *Pseudomonas aeruginosa* or *Acinetobacter baumannii* infection. Bacteria were isolated from the blood, respiratory aspirate, urine, intra-abdominal fluid and/or discharge from deep structures of skin or soft tissue. Bacteria were cultured and identified by standard conventional methods. The antibiograms are shown in Table 1. Study subjects were treated with CCT according to Garonzik *et al* (2011), consisting of colistin combined with one of the following: ceftazidime 2 grams IV every 8 hours, piperacillin/tazobactam 4.5 grams IV every 6 hours, tigecycline 50 mg IV every 12 hours after 100 mg IV given as a loading dose, imipenem cilastatin 1 gram IV every 8 hours, meropenem 2 grams IV every 8 hours, or doripenem 1 gram IV every 8 hours in patients with a glomerular filtration rate (GFR) ≥70 ml/min and renally adjusted doses prescribed among individuals with a GFR <70 ml/min by Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (Levey *et al*, 2009). Treating physicians were not aware of plasma colistin levels and colistin doses were adjusted solely based on renal function throughout the course of treatment. The type of CCT chosen was based upon the treating physician's decision for each patient; study investigators had no influence in regimen selection. Exclusion criteria were patients who: 1) received only intravenous colistin as monotherapy, 2) received nebulized colistin, 3) discontinued CCT ≤72 hours after beginning, 4) receiving colistin treatment during the 3 days prior to study enrollment, or 5) who were co-infected with other pathogens than *P. aeruginosa* or *A. baumannii*. Individuals undergoing hemodialysis were excluded from RIFLE analysis for AKI.

Microbiological tests

Samples were plated onto blood agar and MacConkey agar. Plates were incubated at 37°C. Colonies from the plates were selected for further identification by conventional biochemical tests and the matrix-assisted laser desorption/ionization

time-of-flight (MALDITOF) technique. Antimicrobial susceptibilities were performed using the disk diffusion and E-test methods at the Division of Microbiology, Department of Pathology, Ramathibodi Hospital. MIC results were calculated following Clinical and Laboratory Standard Institute (CLSI) criteria (CLSI, 2014).

Data collection

Data collected included patients' demographics (age, gender, weight), history of underlying disease, length of stay in the medical ward or intensive care unit, need for ventilatory support, site of infection, CCT regimen received and acute physiology, chronic health evaluation (APACHE) II scores on days 0 and 7 of being included in the study, other pertinent clinical parameters and vital signs. Laboratory tests were conducted to assess renal function on days 0, 3, and 7 of being included in the study. Samples were obtained again from the same initial positive collection sites on day 7 of CCT and prior to death. Antibiograms were determined for any cultured bacteria. Clinical outcomes on days 7 and 14 were recorded.

Pharmacokinetics

In order to determine the maximum (Cmax) and minimum (Cmin) colistin concentrations at steady state, EDTA blood samples were collected on day 0 (30 minutes after conclusion of infusion of colistin) and immediately prior to the next dose and at 3, 7, and 10 days of colistin treatment. Plasma samples were stored at -80°C until analyzed. Plasma colistin levels were measured using liquid chromatography coupled to quadruple time-of-flight mass spectrometry and reported as total colistin amount (Ma *et al*, 2008).

Endpoint determination and statistical analysis

Clinical response was assessed on days 7 and 14 of CCT and coded based on treatment regimen. Microbiological response and 7-day mortality were recorded. Means ±standard deviations (SD) were calculated for continuous variables if normally distributed, and as medians and interquartile ranges (IQR) if non-normally distributed. Absolute numbers, relative frequencies and percentages were calculated for categorical variables for patient demographics, overall clinical responses and adverse effects during the study period. Comparisons were determined using the Fisher's exact or chi-square test for categorical variables and non-parametric Mann-Whitney *U* test for continuous variables. The *t*-test was used for continuous data with normal distribution as tested by the Shapiro-Wilk test. A p-value <0.05 was considered statistically significant. Analyses were performed using International Business Machines Statistical Package for the Social Science for Windows (IBM SPSS) version 22.0 (IBM, Armonk, NY).

The study was approved by the Institutional Review Board of Ramathibodi Hospital (approval no. MURA 2014-475) and prior written consent was obtained from all participants or next of kin.

RESULTS

Patient characteristics on enrollment

Thirty-four patients were included in the study. Twenty-four (71%) were female. The mean (±SD) patient age was 55(±20) years. Fifty percent of study subjects had a malignancy, 32% had a cardiovascular condition (hypertension or coronary artery disease) and 18% had diabetes mellitus type 2. None of the subjects were neutropenic. Five patients had chronic kidney disease requiring hemodialysis upon enrollment in the study. Sixty-five percent of subjects had a respiratory tract

Table 1
Antibiograms for all patients with an infection due to the studied organisms at the study hospital during 2013-2014.

Organisms	Number of isolates tested	Susceptibility (%)							
		TZP	CAZ	IPM	MEM	DOR	CIP	AMK	CST
P. aeruginosa	1,742	74	75	63	68	72	72	90	100
A. baumannii	1,105	9	10	9	10	9	7	23	100

TZP, piperacillin/tazobactam; CAZ, ceftazidime; IPM, imipenem cilastatin; MEM, meropenem; DOR, doripenem; CIP, ciprofloxacin; AMK, amikacin; CST, colistin.

infection, 18% had a urinary tract infection, 9% had a blood stream infection; two patients had an intra-abdominal infection and one had a soft tissue infection.

The drugs prescribed with colistin in the CCT were imipenem and meropenem (8 patients each), ceftazidime and tigecycline (7 patients each), doripenem (3 patients), and piperacillin/tazobactam (1 patient).

Clinical and microbiological outcomes

A variety of patient characteristics and clinical features were evaluated for their association with microbiological treatment success or not by day 7 of CCT (Table 2). The average (±SD) duration of CCT was 10(±4) days. The overall microbiological treatment success rate was 54%. The following factors were not significantly associated with microbiological treatment success: patient age, gender, immunological status, history of underlying disease, including those with and without malignancy, length of stay in the ICU, time on ventilator support, length of hospital stay and APACHE II score. A higher APACHE II score was found to have an association with a higher mortality (p =0.031) regardless of microbiological treatment success.

The colistin MICs had a range of 0.5-1 mg/l (mean ± SD: 0.6±0.3 mg/l) and mean (± SD) colistin Cmax/MIC was 15.6 (±14.9). Of the 28 patients remaining alive on day 7, 9 patients each in microbiological treatment success group and microbiological treatment failure group had achieved a target colistin Cmax/MIC ratio of >8 and the average (±SD) Cmin values were higher in the microbiological treatment success group than the microbiological treatment failure group [5.8(±2.3) g/l *vs* 3.3±(2.2) g/l] but this difference was not significant. The microbiological treatment success rate did not differ significantly by type of partner antibiotic used in the CCT. Eighty-seven percent of patients in the imipenem-CCT treatment group achieved a target Cmax/MIC value compared to 50% of those in the other CCT regimen groups, but this difference was not statistically significant (p =0.126) (data not shown).

The respiratory tract was the most common site of infection, found in 18 of 28 patients still alive by day 7, of these, 8 had microbiological treatment success by day 7 of CCT (Table 2). All 7 patients with bacteremia and/or urinary tract infection had microbiological success (Table 2); of these 7, 5 had reached the target Cmax/MIC

Table 2
Association between study patient characteristics by microbiological treatment outcome.

Characteristics	(Total *N*=28)	Microbiological treatment success on day 7 (*n*=15)	Microbiological treatment failure on day 7 (*n*=13)	*p*-value
Gender, *n* (%)				0.885
Male	9	5 (55)	4 (45)	
Female	19	10 (53)	9 (47)	
Mean patient age in years (±SD)	56 (±20)	58 (±21)	54 (±20)	0.598
Immune status of host, *n* (%)				0.263
Compromised	22	13 (59)	9 (41)	
Competent	6	2 (33)	4 (67)	
Underlying disease, *n* (%)				
Malignancy	13	9 (69)	4 (31)	0.121
Cardiovascular	11	6 (54)	5 (46)	0.934
Diabetes mellitus	6	2 (33)	4 (67)	0.262
Combination of above conditions	12	7 (58)	5 (42)	0.662
Mean (+SD) GFR, ml/min/1.73 m^2	93 (±35)	93 (±40)	93 (±29)	0.986
Mean APACHE II score (±SD)	22 ± 6	22 ± 6	20 ± 6	0.578
Average colistin treatment in days (±SD)	10 ± 4	11 ± 3	13 ± 4	0.116
Infection site, *n* (%)		0.038		
Respiratory tract	18	8 (44)	10 (56)	
Urinary tract	5	5 (100)	0 (0)	
Blood stream	2	2 (100)	0 (0)	
Other sites	3	0 (0)	3 (100)	
Partner antibiotic, *n* (%)				0.654
Imipenem	7	5 (71)	2 (29)	
Ceftazidime	6	4 (67)	2 (33)	
Tigecycline	6	3 (50)	3 (50)	
Meropenem	4	2 (50)	2 (50)	
Doripenem	3	1 (33)	2 (67)	
Piperacillin/tazobactam	1	0 (0)	1 (100)	
On hemodialysis, *n* (%)	3	1 (33)	2 (67)	0.457
Median (IQR) hospital length of stay in days	16 (8-25)	15 (3-25)	17 (8.5-30.5)	0.579
Colistin Cmax/MIC ratio >8 on day 0 (%)	18/27 (67%)	9 /15	9 /13	0.785
Mean colistin Cmin (+SD) at day 7 (mg/l)	4.5 (±3.8)	5.8 (±2.3)	3.3 (±2.2)	0.132

GFR, glomerular filtration rate; APACHE, acute physiology and chronic health evaluation; Cmax/MIC, maximum plasma concentration/minimal inhibitory concentration; Cmin, minimum plasma concentration.

ratio of >8 (data not shown). However, 8 of 21 patients with other sites of infection achieved significantly lower microbiological treatment success (p <0.0001). Of the patients with microbiological treatment success, 5, 4, 3, 2, and 1 had received as the partner drug with CCT imipenem, ceftazidime, tigecycline, meropenem, and doripenem, respectively (Table 2).

AKI was found in 19 non-dialysis patients, of whom 18 developed AKI within 7 days of CCT: 6 patients on day 3, 12

Table 3
Risk factors for development of AKI in non-dialysis patients undergoing CCT.

Risk factors	With AKI (n =19)	Without AKI (n =10)	p-value
Age in years (±SD)	62 (±18)	46 (±17)	0.025
Mean GFRc (±SD) (ml/min/1.73 m^2)	84 (±33)	109 (±25)	0.030
Concomitant nephrotoxic agent used[a], n (%)	9 (47)	6 (60)	0.400
APACHE II score (±SD)	22 (±7)	20 (±6)	0.504
Colistin Cmax/MIC (±SD)	16.10 (±8)	14.33 (±9.49)	0.701
Colistin Cmin level (±SD) (g/l)	5.63 (±5.77)	2.69 (±2.85)	0.083
Length of CCT in days (±SD)	11 (±4)	11 (±4)	1.000

[a]Acyclovir, amphotericin B, cisplatin, contrast media, ganciclovir, trimethoprim/sulfamethoxazole, and vancomycin. AKI, acute kidney injury; CCT, colistin combination therapy; GFR, glomerular filtration rate; APACHE, acute physiology and chronic health evaluation; Cmax/MIC, maximum plasma concentration/minimal inhibitory concentration; Cmin, minimum plasma concentration.

patients on day 7 and 1 patient on day 10. Younger age and higher glomerular filtration rate (GFR) at enrollment were significantly associated with a lower incidence of AKI (p <0.05) (Table 3). Risk of AKI was higher among individuals aged ≥65 years (95%CI: 1.05-95.23) and those with a GFR <60 ml/min (95%CI: 1.19-2.20). No significant association was seen between those with a higher incidence of AKI and a higher APACHE II score, colistin Cmax/MIC, Cmin, or concomitant nephrotoxic agent use (Table 3). Those with AKI did not have a significantly greater mortality rate than those without AKI.

Twenty of 34 patients died during hospitalization: 6 during the first 7 days of treatment: 3 among those who received meropenem as the partner group in the CCT and one each in those who received ceftazidime, imipenem, and tigecycline as the partner drug in the CCT. Four of the 6 deaths occurring during the first 7 days had microbiological treatment failure determined on days 4 and 6, 1 of the deaths occurred in a patient with a urinary tract infection who died on day 6 and had a negative urine culture on day 3 of treatment and another death occurred in a patient with a positive blood culture who died on day 4; the blood culture obtained on day 4 was negative. The death of these 6 patients could not be attributed to failure to achieve the target Cmax/MIC ratio >8, since 4 of these 6 patients had reached this target. It is worth noting the mean (± SD) APACHE II score (28±5) of these 6 patients was significantly (p =0.031) higher than the 28 patients who survived to day 7 (21±6).

Another 10 patients died during second week of CCT due to infection. The CCT for those patients who died during the second week were: 4 receiving ceftazidime-CCT, 3 receiving tigecycline-CCT, 2 receiving doripenem-CCT, and 1 each receiving imipenem-CCT, and piperacillin/tazobactam-CCT. Five patients had microbiological failure with continuing positive respiratory cultures at their death. Seventeen of the 20 deaths in our study were due to infection.

DISCUSSION

Colistin is a cationic, multicomponent (including colistin A and B) lipo-

peptide antibiotic that is intravenously administered as its prodrug, sodium colistimethate (Orwa *et al*, 2001; Falagas and Kasiakou, 2005). Colistin is reserved for treatment of infections due to MDR pathogens, generally *P. aeruginosa* and *A. baumannii*, not uncommon in Thailand (Falagas and Kasiakou, 2005; Danchaivijitr *et al*, 2006; Phongpech *et al*, 2010; Division of Microbiology, Department of Pathology, 2014; Chaisathaphol and Chayakulkeerec, 2014). However, there is no consensus on the dosage of colistin, and in view of its nephrotoxicity, therapeutic drug monitoring is suggested to ensure safety as well as to reach the treatment goal, rather than prescribing a fixed dose regimen (Daikos *et al*, 2010). We conducted this observational study to identify factors (colistin Cmax/MIC ratio, CCT regimen, site of infection and APACHE score) associated with treatment outcome at 1 week of treatment.

Among our study subjects, only 67% reached the treatment goal level of a Cmax/MIC ratio >8 (Garonzik *et al*, 2011). Our findings are similar to previous studies who reported approximately 40% of patients did not achieve target drug levels (Plachouras *et al*, 2009; Garonzik *et al*, 2011). Our study also shows having a Cmax/MIC >8 was not significantly associated with microbiological treatment success. This suggests adequate colistin levels are not the key factor in treatment success of XDR pathogen infections. Petrosillo *et al* (2008) states only two of three studies reviewed showed carbapenems exhibit a synergistic effect in CCT of MDR *A. baumanni* and *P. aeuginosa*. One study reported a synergistic effect between colistin and imipenem but an antagonistic effect between colistin and meropenem (Pongpech *et al*, 2010). In our study, the slightly higher microbiological treatment success rate with colistin-imipenem than colistin-meropenem could support the antagonistic effect between colistin and meropenem. Leu *et al* (2014) reported a syngergistic effect of colistin-imipenem against imipenem-non-susceptible MDR *A. baumannii* was more evident at a colistin concentration of 1 mg/l than 0.5 mg/l. In our study, the efficacy of colistin-imipenem treatment was slightly greater than other regimens to treat MDR *A. baumannii* and *P. aeruginosa* but this difference was not statistically significant, possibly due to the small sample size.

In our study, subjects with bacteremia and urinary tract infections had greater microbiological treatment success rates than those with other types of infection. This could be explained by pharmacokinetics and pharmacodynamics of colistin, which has low protein binding and is poorly distributed in bone and lung parenchyma but has a better distribution in the liver, muscle and kidneys (Michalopoulos and Falagas, 2011; Gurja, 2015). Biofilm formation caused by bacteria in the lung is likely a major obstacle to a good clinical outcome (Hengzhuang *et al*, 2012). A study by Lu *et al* (2010) in an animal model found no detectable colistin in the lung tissue after intravenous injection at recommended doses. The infection site should be considered when prescribing CCT.

The Cmax and Cmin were not significantly associated with AKI, although our study population was too small to determine this with assurity. There was a slightly (non-significant) greater rate of AKI among study subjects with higher Cmax and Cmin levels. In our study, age ≥65 years old and a glomerular filtration rate <60 ml/min at baseline were associated with AKI. Other studies here also reported other risk factors for AKI includ-

ing male gender, concomitant calcineurin inhibitor use, hypoalbuminemia and hyperbilirubinemia (Kwon *et al*, 2010). In our study, a higher APACHE II score was associated with a greater mortality rate regardless of treatment regimen, similar to a study by Zimmerman *et al* (2006).

There were limitations to our study: 1) the data might be biased in view of the limited number of available patients and the diversity of CCT prescribed by the attending physicians; 2) since two microorganisms were included in our study, the outcome assessments might be affected by microbiological treatment success and mortality; 3) combining different sites of infection studies in assessing outcomes could have limited the ability to discern infection site as a role in treatment outcome; 4) the 7-day time point assessment of microbiological treatment efficacy might not be valid to assess pneumonia treatment outcomes in some circumstances. Six patients had died by day 7, of whom 4 with respiratory infection had achieved an optimal Cmax/MIC ratio and all cultures from respiratory infection sites remained positive by day 7; 5) there was a disproportionately large number of patients with respiratory infections receiving meropenem-CCT; and 6) heteroresistance to colistin may have had an impact on clinical outcomes. This was not included in our analyses.

Although we had a limited number of study subjects, the results highlight several issues regarding the use of CCT to treat XDR bacterial infections. First, the plasma colistin Cmax/MIC ratio was not a good indicator for assessing treatment efficacy clinically or microbiologically. Second, the synergistic effect of the partner antimicrobial agent with CCT should be considered in the drug selection. Third, CCT efficacy depends on the infection site. Fourth, the APACHE II score is an important prognostic factor for survival regardless of treatment regimen. In conclusion, treatment of XDR gram-negative bacterial infection remains challenging. Further studies are needed with a larger study population to better clarify factors that reached non-significancy in our study.

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