

# The Effect of Double-carbapenem Therapy on Mortality Rates and Microbiological Cure Rates in Patients Diagnosed with Carbapenem-resistant *Klebsiella pneumoniae* Infections in Comparison to Monotherapy and Currently Used Combinations of Antibiotics: A Meta-analysis

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## Abstract

**Introduction:** Infection with the bacteria carbapenemase-producing *Klebsiella pneumoniae* (PKP) represents a significant cause of mortality in hospitalized patients. These multidrug-resistant bacteria are resistant to currently used antibiotics as a result of carbapenemase production. Dual-carbapenem therapy (DCT) has been proposed as a valid therapeutic option, this therapy combines two carbapenem antibiotics, with one acting as a suicide inhibitor allowing the subsequent carbapenem to exert a bactericidal effect. **Aim:** The aim of this meta-analysis was to determine if DCT had a significant effect on mortality rate and microbiological cure rate in patients diagnosed with carbapenemase-PKP infections in comparison to standard antibiotic therapies. **Methods:** The search terms “(dual OR double) carbapenem (therapy OR treatment) AND *Klebsiella pneumoniae*” were used to search databases and inclusion and exclusion criteria were applied to retrieved papers, a total of seven studies were identified for inclusion in the meta-analysis. The quality of included studies was assessed using the cochrane tool for risk of bias assessment and funnel plots were produced to determine the influence of publication bias. A random effects model was used to assess the outcomes; mortality rate and microbiological cure rate. **Results:** DCT had a time dependent effect on patient mortality rates. DCT significantly lowered mortality rates in patients in comparison to standard antibiotic therapy, especially in comparison to monotherapy treatment regimens. In addition, DCT significantly improved microbiological cure rate in patients when compared to standard antibiotic treatment regimens. Demonstrating the possible clinical applications of a dual carbapenem antibiotic regimen in the treatment of carbapenemase-PKP infections.

**Keywords:** Double/Dual-carbapenem therapy, Carbapenem-resistant/producing, *Klebsiella pneumoniae*

## Introduction

### Carbapenemase-producing *Klebsiella pneumoniae* (PKP) infection and dual carbapenem therapy (DCT)

*K. pneumoniae* is one of the most common Gram-negative causes of nosocomial infection.<sup>[1]</sup> Carbapenem antibiotics are usually effective in the

treatment of multidrug-resistant bacterial strains. For this reason, carbapenem antibiotics are usually the last line of defense to treat infections caused by *K. pneumoniae*. Recently, however, there has been a growing trend in the number of carbapenem-resistant (CR)/PKP isolates. The increasing emergence of CRKP possesses a threat to health

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globally and has been associated with increased health care costs, prolonged hospital stays, treatment failure, and increased mortality, thus the need for novel treatment strategies is evident.<sup>[1]</sup> In an effort to fight this multidrug-resistant bacterium a new treatment was put forward: Double DCT.<sup>[2]</sup> DCT utilizes a dual carbapenem antibiotic combination, the first carbapenem acts as a suicide inhibitor of the carbapenemase enzyme, allowing the second carbapenem to have a bactericidal effect.

### *K. pneumoniae*: The emerging threat

*K. pneumoniae* isolates are associated with a variety of infections, including infections of the Urinary tract infection (UTIs) and infections of the bloodstream infection, frequently occurring at multiple body sites concurrently. Infections are also commonly associated with underlying comorbidities such as malignancy or previous organ transplantation.<sup>[1]</sup> CRKP are currently classified as critical according to the World Health Organization's "priority list of pathogens for research and discovery of new antibiotics," this list describes the bacteria which pose the greatest threat to health, as determined by; the fatality rate associated with their infection, the length of hospital stay, the rate of resistance to current antibiotics, and the availability of current treatments.<sup>[3]</sup> In addition, the European Centre for Disease Prevention and Control reported that currently more than one-third of *K. pneumoniae* isolates are resistant to at least one antibiotic.<sup>[4]</sup> Consequently, the need for an effective treatment of CPKP infections is vital.

### Current treatments

The antibiotics colistin belonging to the polymyxin antibiotic class, tigecycline belonging to the glycylcycline antibiotic class, fosfomycin belonging to the phosphonic antibiotic class, and antibiotics form the aminoglycoside class, are currently used to treat infections caused by *K. pneumoniae*, either in mono- or combination-therapy, other example combination antibiotic strategies include ceftazidime-avibactam and meropenem-vaborbactam.<sup>[5]</sup> However, limitations exist to the wide-spread use of these antibiotics. Due to increased usage, there is an increasing incidence of CPKP resistance to these antibiotics.<sup>[6]</sup> In addition, the potentially fatal adverse effect, dose-dependent nephrotoxicity is commonly associated with colistin administration.<sup>[7]</sup> Carbapenem antibiotics are used

as a salvage treatment in *K. pneumoniae* infections, post failure of other antibiotics. The most common clinically administered carbapenem antibiotics include imipenem, meropenem, ertapenem, and doripenem. Recently developed carbapenems include tebipenem the first oral carbapenem, tomopenem whose bactericidal properties appear more potent than older carbapenems such as imipenem and meropenem as evidenced by *in vitro* and *in vivo* studies, and Sanfetrinem the first trinem carbapenem possessive of broad spectrum bactericidal activity.<sup>[8]</sup> Trinem carbapenems encompass tricyclic beta-lactams that possess a penicillin, cephalosporin, or carbapenem backbone. Due to the increasing resistance of *K. pneumoniae* isolates to these antibiotics, new treatment options are of vital importance.<sup>[9]</sup>

### *K. pneumoniae* resistance mechanisms

CPKP are resistant to carbapenem antibiotics as a result of carbapenemase production. This enzyme is capable of hydrolyzing carbapenem antibiotics, thus rendering them inactive.<sup>[10]</sup> The carbapenemase enzyme is encoded for by extended spectrum beta-lactamase (KPC) (*bla* (KPC)) genes, evidencing this, Yan *et al.* found that 38.5% of CPKP isolates possessed *bla*-KPC carbapenemase-encoding-genes.<sup>[11]</sup> In accordance, Logan and Weinstein, found similar results, noting that the minimum inhibitory concentration (MIC) of a carbapenem antibiotic was related to *bla*-KPC copy number.<sup>[12]</sup> Additional CPKP resistance mechanisms include the production of AmpC cephalorinase enzymes. OmpK 35/36 is porins present in the cell membrane of *K. pneumoniae* isolates; antibiotics exploit these porins to enter the bacterial cell and exert their bactericidal effect. Insertions or deletions in the OmpK 35/36 encoding genes, *OmpK35/36*, results in the inactivation of, or altered structure of, these porins, thus conferring additional resistance to CPKP isolates. The loss of these porins, particularly ompK 35, increases the MIC of the antibiotic, as reduced concentrations of antibiotic are able to enter the bacterial cell, generating increased resistance as a result of decreased phagocytosis. In addition, porin loss is able to significantly increase the production of the polysaccharide capsule surrounding the bacterium, which forms a physical barrier against the entry of antimicrobial agents as well as preventing the formation of the membrane attack complex. The absence of functional porins also acts to increase the lipopolysaccharide content



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in the outer membrane of the CPKP bacteria, aiding in evasion from the host complement system and protecting against phagocytosis.<sup>[13,14]</sup>

### DCT

In an effort to fight this multidrug-resistant bacterium a new treatment was put forward: DCT. Initially proposed by Bulik and Nicolau,<sup>[2]</sup> DCT has been shown to be an effective treatment for CPKP infections, when other antibiotic regimes have failed, even in cases when single carbapenems used in monotherapy have shown no bactericidal activity. DCT combines two carbapenem antibiotics, ertapenem with either meropenem or doripenem. Ertapenem is administered before the second carbapenem. Ertapenem dosage is lower than that of the second carbapenem. The carbapenemase enzyme produced by CPKP has a greater affinity for ertapenem in comparison to other carbapenem antibiotics, this is exploited in DCT.<sup>[2]</sup>

### Importance of this study

Much of the research into the effectiveness of DCT is positive. DCT appears to be an effective alternative to monotherapy, lowering the mortality rate of patients, as well as improving both microbiological and clinical outcomes. However, the majority of these studies are case studies, or retrospective observational studies, which are characterized by small sample sizes. Meta-analyses regarding DCT and its effectiveness as a treatment for CPKP infections are currently lacking. The addition of a meta-analysis in this area would allow for the determination of the statistical significance in the effectiveness of DCT in treating infections caused by CPKP, allowing for the grouping together of existing data to generate an increased sample size, addressing the issues posed by the previous studies, leading to greater generalizability of findings. Such research could be used to inform clinical decisions regarding the use of DCT. The aim of this meta-analysis is to determine if DCT has a significant effect on mortality rates in patients diagnosed with infections due to CPKP in comparison to monotherapy and alternate antibiotic combination regimens. An additional aim of this meta-analysis is to determine if there is a significant difference in the effect of DCT on mortality rates at different time points post initiation of treatment. Furthermore, this meta-analysis aims to determine if DCT significantly alters the microbiological cure rate in

CPKP infected patients in comparison to standard antibiotic regimes.

## Methods

### Study selection

The search terms “(dual OR double) carbapenem (therapy OR treatment) AND *Klebsiella pneumoniae*” were used to search the following databases for relevant papers; PubMed, ScienceDirect, web of science, Wiley online library, Cochrane controlled trials register, and current controlled trials. In addition, the search terms were used to search the following conferences; Interscience Conference on Antimicrobial Agents and Chemotherapy 2011–2019, and the European Congress of Clinical Microbiology and Infectious Diseases 2014–2019. The reference sections of retrieved papers were searched for further relevant papers. The inclusion criteria applied were patients diagnosed with CR *K. pneumoniae* infection, patients in the experimental group must be receiving DCT, CR *K. pneumoniae* infections at all body sites were included, studies from all countries were included, all patients aged over 18 years of age were included in the study, only studies in which a control group was present were included, and only studies published pre 2020 were included in the study. The exclusion criteria applied were patients diagnosed with multiple infections caused by bacteria other than *K. pneumoniae*, animal studies were excluded from the study, studies lacking a control group were excluded from the study, studies published in languages other than English were excluded from the study, and any study published post January 1, 2020 was excluded from the study.

### Data extraction

The following data were extracted for each study; authors, year of publication, treatment received, sample size, number of deaths among patients receiving DCT, total number of patients receiving DCT, and either the number of deaths amongst patients receiving monotherapy and the total number of patients receiving monotherapy, or the number of deaths amongst patients receiving combination therapy and the total number of patients receiving combination therapy. The microbiological cure rates in patients treated with DCT and the microbiological cure rates in patients treated with standard antibiotic regimes were extracted from applicable studies.



### Quality assessment of studies

To ensure study quality, all papers included in the meta-analysis were subject to peer-review. To ensure methodological quality, papers selected for inclusion were assessed for risk of bias in accordance with the Cochrane tool for assessing the risk of bias in non-randomized studies of interventions.<sup>[15,16]</sup> To be determined as having a low overall risk of bias, the study must be judged to be at low bias in all domains, and to be determined as having a moderate overall risk of bias the study must be judged to be at low or moderate risk of bias in all domains.<sup>[15,16]</sup> Only papers with a low or moderate risk of bias were included in the analysis. Funnel plots were produced to assess the impact of publication bias.

### Definitions

DCT consisted of an antibiotic regimen containing two carbapenem antibiotics. Monotherapy consisted of a single active antibacterial agent. Combination therapy consisted of two or more active antibacterial agents used in combinations other than a dual carbapenem combination. Standard antibiotic therapy (SAT) consisted of appropriate antibiotic regimens, both monotherapy and combination therapies, exempting DCT. Mortality rate was determined as the number of deaths occurring in the population of patients with CPKP infections post initiation of treatment with DCT, monotherapy, or other antibiotic combination therapies at the following time points (where applicable for each study); 14 days mortality, 28–30 day mortality, 60–90 days mortality, and the mortality rate at the latest follow-up time point within each study, termed the overall mortality rate. Microbiological cure was defined as cultures negative for the original pathogen, collected from the original site of infection, post antibacterial treatment.

### Data analysis

The outcomes assessed were mortality rate and microbiological cure rate. Microbiological cure rates post-antibacterial treatment was assessed. Mortality rates post-initiation of treatment at the latest follow-up time point within each study was assessed. Mortality rates were then stratified according to the following time points; 14 days, 28–30 days, and 60–90 days, post-initiation of treatment. A random effects model was used to assess these outcomes. The odds ratio (OR) and

95% confidence interval (CI) were calculated for all included studies. The overall OR and 95% CI were calculated for each analysis. The overall effect (Z) and significance (p) were also calculated for each analysis. The  $I^2$  statistic was used as the test of heterogeneity. The  $I^2$  value was interpreted as follows:  $I^2 < 50\%$ : Low heterogeneity;  $I^2 = 50–75\%$ : Moderate heterogeneity; and  $I^2 \geq 75\%$ : High heterogeneity.<sup>[4]</sup>  $P < 0.05$  was considered significant. The software Review Manager (RevMan) version 5.3 was utilized to carry out this meta-analysis.<sup>[5]</sup>

## Results

### Study inclusion

The search strategy of online databases identified 599 studies; an additional study was identified through searching of conference databases. 600 studies were screened for eligibility for inclusion based on title and abstract content, and the inclusion and exclusion criteria previously outlined were applied. Thirty duplicate studies were removed. Thirty studies were subject to full text review and the inclusion and exclusion criteria were applied. A total of seven studies were identified for inclusion in the meta-analysis.<sup>[17]</sup> Figure 1 illustrates the study selection.

### Study characteristics

A total of seven studies were included in the meta-analysis, with a total sample population of 1849 patients included in the analysis, individual study population ranged from 36 to 595 patients. In total, 585 patients in this analysis received DCT, in comparison to 725 patients receiving monotherapy, and 539 patients receiving alternate antibiotic combination regimens. The studies included were published between 2015 and 2019. All included studies were retrospective, observational, and cohort studies. Sites of CPKP infections included UTI, blood stream, respiratory tract, intra-abdominal, and soft tissue. The DCT regimen most commonly administered in the included studies was ertapenem and meropenem at varying doses. Antibiotics administered in monotherapy regimens included colistin, gentamicin, tigecycline, aminoglycosides, meropenem, imipenem, cefepime, aztreonam, ceftazidime, and chloramphenicol. Antibiotics administered in combination regimens included meropenem plus another agent, rifampicin



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plus another agent, colistin plus another agent, tigecycline plus another agent, and gentamicin plus another agent, as shown in Table 1.

### Risk of bias assessment of included studies

The studies selected for inclusion were assessed for risk of bias and all included studies were determined

**Table 1: Characteristics of studies included in meta-analysis**

Author	Year	Study design	Country	Site of infection	Treatment	Number in study
De Pascale <i>et al.</i>	2017	Case-Controlled, observational, two-centre study	Italy	RTI, IAI, UTI, BSI, Multiple site infection	DCT (meropenem 6g every 8h, ertapenem 2g every 12h) Monotherapy (Colistin 9MIU every 12h, Gentamicin 5mg every 24h, Tigecycline 100mg every 12h)	144
Cancelli <i>et al.</i>	2018	Single centre, observational study	Rome	RTI, UTI, soft tissue	DCT (ertapenem 1g every 24h, meropenem 6g every 24h) Monotherapy (colistin, aminoglycosides)	55
Gutierrez-Gutierrez <i>et al.</i>	2017	Retrospective cohort study	10 different countries	BSI	DCT Monotherapy (colistin, meropenem, imipenem, cefepime, aztreonam, ceftazidime, tigecycline, aminoglycosides, chloramphenicol)	245
Tumbarello <i>et al.</i>	2015	Retrospective cohort study	Italy	BSI, RTI, IAI, UT	DCT (meropenem and ertapenem) Monotherapy (colistin, tigecycline, gentamicin)	315
Onal <i>et al.</i>	2019	Retrospective study	Turkey	UTI	DCT Monotherapy	100
Tumbarello <i>et al.</i>	2015	Retrospective cohort study	Italy	BSI, RTI, IAI, UT	DCT (meropenem and ertapenem) Combination therapy (meropenem plus another agent, rifampicin plus another agent)	362
Giannella <i>et al.</i>	2018	Retrospective, multicentre, observational cohort study	Italy	BSI	DCT Combination therapy (colistin plus another agent, tigecycline plus another agent, gentamicin plus another agent)	595
Venugopalan <i>et al.</i>	2017	Single centre, retrospective, observational cohort study	USA	BSI	DCT (doripenem 2g every 8h and ertapenem 1g every 24h 30 min prior to the doripenem infusion) Combination therapy (colistin plus a carbapenem)	36

RTI: Respiratory tract infection, IAI: Intra-abdominal infection, UTI: Urinary tract infection, BSI: Bloodstream infection, DCT: Dual carbapenem therapy, g: Grams, mg: Milligrams, mIU: Milli-international units, h: Hours, De Pascale *et al.*,<sup>[18]</sup> Cancelli *et al.*,<sup>[19]</sup> Gutierrez-Gutierrez *et al.*,<sup>[20]</sup> Tumbarello *et al.*,<sup>[21]</sup> Onal *et al.*,<sup>[22]</sup> Giannella *et al.*,<sup>[23]</sup> and Venugopalan *et al.*,<sup>[24]</sup>



to have a moderate risk of bias, with the exception of the study conducted by Gutierrez-Gutierrez *et al.*, 2017<sup>[20]</sup> and that conducted by Venugopalan *et al.*, 2017<sup>[24]</sup> which were determined to have a low risk of bias. The studies conducted by De Pascale *et al.*, 2017<sup>[18]</sup> Cancelli *et al.*, 2018,<sup>[19]</sup> Tumbarello *et al.*, 2015,<sup>[21]</sup> Onal *et al.*, 2019,<sup>[22]</sup> and Gianella *et al.*, 2018<sup>[23]</sup> had a moderate risk of bias due to confounding, whereas the studies conducted by Gutierrez-Gutierrez *et al.*, 2017<sup>[20]</sup> and Venugopalan *et al.*, 2017<sup>[24]</sup> were determined to have a low risk of bias due to confounding. All studies included in the analysis were determined to have a low risk of bias in selection of participants into the study.<sup>[18-24]</sup> Similarly, all included studies were determined to have a low risk of bias due to deviations from intended interventions and in bias due to classification of interventions.<sup>[18-24]</sup> The study conducted by De Pascale *et al.*, 2017,<sup>[18]</sup> was determined to have a moderate risk of bias due to missing data, all other included studies were determined to have a low risk of bias due to missing data.<sup>[19-24]</sup> In addition, all studies had low risk of bias in both measurements of outcomes and in selection of the reported result,<sup>[18-24]</sup> as shown in Table 2.

### **Mortality rates**

#### **Mortality rates in patients treated with DCT in comparison to monotherapy**

The studies included in this analysis displayed low heterogeneity ( $I^2 = 0\%$ ). Patients receiving DCT

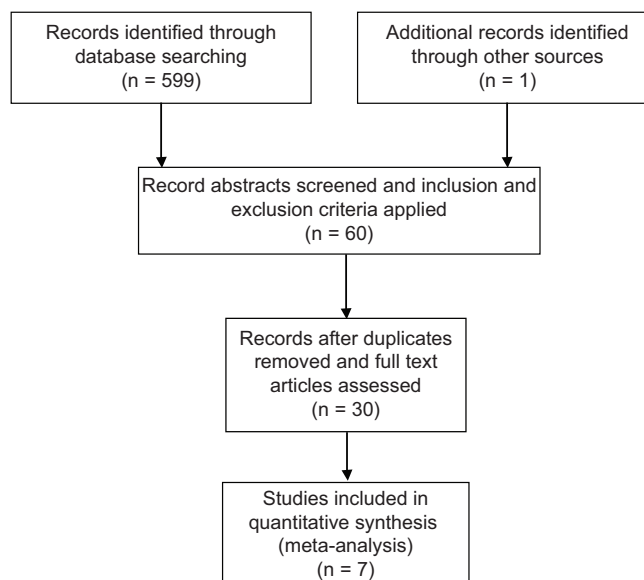
had a lower overall mortality rate in comparison to patients receiving monotherapy (OR = 0.77, 95% CI [0.50, 1.19]). However, the overall effect of DCT on mortality rates was non-significant ( $Z = 1.19$  [ $P = 0.24$ ]), as illustrated in Figure 2.

However, stratifying by time post-initiation of treatment revealed a significant effect. DCT significantly lowered the 28–30 days mortality rate in patients in comparison to patients treated with monotherapy regimes (OR = 0.44, 95% CI [0.17, 1.10],  $Z = 1.76$  [ $P = 0.08$ ]). The studies included in this analysis displayed moderate heterogeneity ( $I^2 = 66\%$ ), as illustrated in Figure 3.

#### **Mortality rates in patients treated with DCT in comparison to alternative antibiotic combinations**

The studies included in the analysis displayed low heterogeneity ( $I^2 = 8\%$ ). Patients receiving DCT had a lower overall mortality rate in comparison to patients receiving alternate antibiotic combinations (OR = 0.71, 95% CI [0.45, 1.14]). However, the overall effect of DCT on mortality rate was non-significant ( $Z = 1.41$  [ $P = 0.16$ ]), as illustrated in Figure 4.

In contrast to the monotherapy subgroup, stratifying the mortality rate by time post-initiation of treatment for patients receiving combination therapy in comparison to those receiving DCT did not yield a significant effect (OR = 0.77, 95% CI [0.52, 1.16],  $Z = 1.23$  [ $P = 0.22$ ]). The studies included in this analysis displayed low heterogeneity ( $I^2 = 0\%$ ), as illustrated in Figure 5.



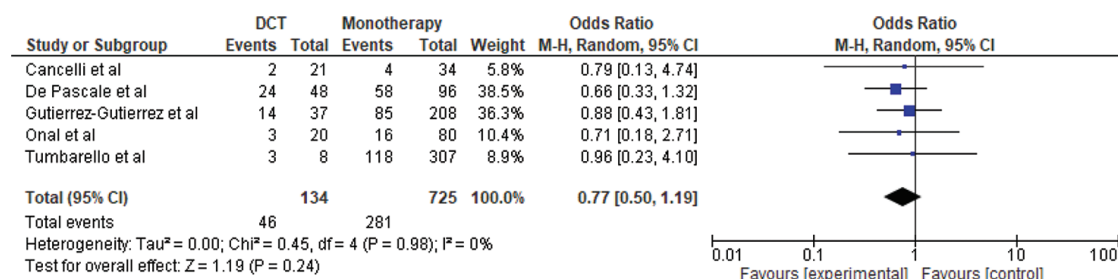
**Figure 1: PRISMA flow diagram illustrating the study selection process**

Table 2: Risk of bias assessment in studies included in the meta-analysis

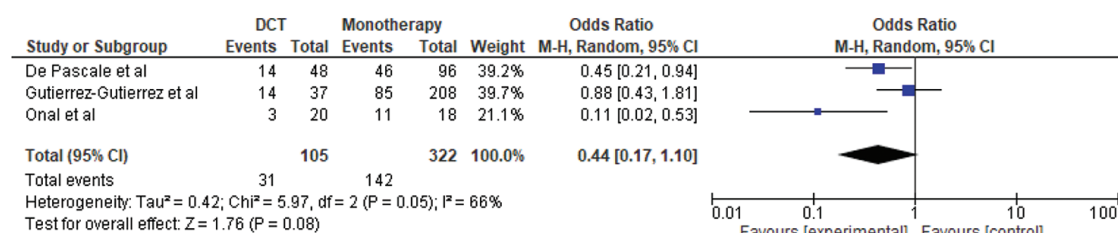
Study	Bias due to confounding	Bias in selection of participants into the study	Bias due to deviations from intended interventions	Bias in classification of interventions	Bias due to missing data	Bias in measurements of outcomes	Bias in selection of the reported result	Overall bias
De Pascale <i>et al.</i> , 2017	Moderate risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Moderate risk of bias	Low risk of bias	Low risk of bias	Moderate risk of bias
Cancelli <i>et al.</i> , 2018	Moderate risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Moderate risk of bias
Gutierrez-Gutierrez <i>et al.</i> , 2017	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias
Tumbarello <i>et al.</i> , 2015	Moderate risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Moderate risk of bias
Onal <i>et al.</i> , 2019	Moderate risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Moderate risk of bias
Giannella <i>et al.</i> , 2018	Moderate risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Moderate risk of bias
Venugopalan <i>et al.</i> , 2017	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias

De Pascale *et al.*,<sup>[18]</sup> Cancelli *et al.*,<sup>[19]</sup> Gutierrez-Gutierrez *et al.*,<sup>[20]</sup> Tumbarello *et al.*,<sup>[21]</sup> Onal *et al.*,<sup>[22]</sup> Giannella *et al.*,<sup>[23]</sup> and Venugopalan *et al.*,<sup>[24]</sup>

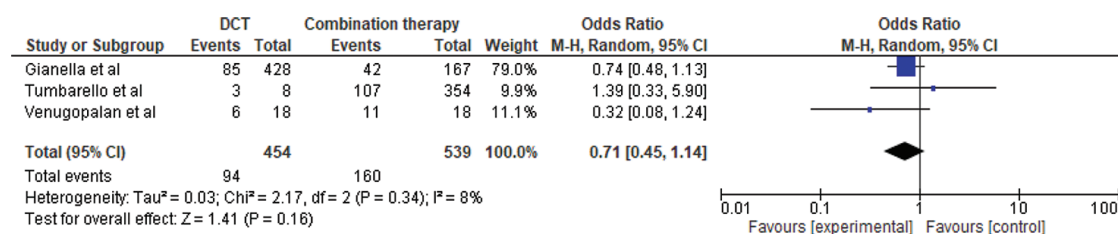




**Figure 2: Overall mortality rates of CPKP infected patients treated with DCT in comparison to patients treated with a monotherapy antibiotic regimen.** Data shown are number of deaths (events) and total number in group (total), data shown are odds ratio and 95% CI, events refer to mortality, CI-confidence interval, df- degrees of freedom, DCT- dual carbapenem therapy, experimental group received DCT treatment, control group received monotherapy,  $p < 0.05$  is significant, De Pascale *et al.*,<sup>[18]</sup> Cancelli *et al.*,<sup>[19]</sup> Gutierrez-Gutierrez *et al.*,<sup>[20]</sup> Tumbarello *et al.*,<sup>[21]</sup> Onal *et al.*<sup>[22]</sup>



**Figure 3: 28-30 mortality rate in patients treated with DCT in comparison to patients treated with monotherapy.** Data shown are number of deaths (events) and total number in group (total), data shown is odds ratio and 95% CI, CI- confidence interval, df- degrees of freedom, DCT- dual carbapenem therapy, experimental group received DCT treatment, control group received monotherapy,  $p < 0.05$  is significant, De pascale *et al.*,<sup>[18]</sup> Gutierrez-Gutierrez *et al.*,<sup>[20]</sup> Onal *et al.*<sup>[22]</sup>



**Figure 4: Overall mortality rates in CPKP infected patients treated with DCT in comparison to patients treated with alternate antibiotic combinations.** Data shown are number of deaths (events) and total number in group (total), data shown are odds ratio and 95% CI, events refer to mortality, CI- confidence interval, df- degrees of freedom, DCT-dual carbapenem therapy, experimental group received DCT treatment, control group received alternate antibiotic combinations,  $p < 0.05$  is significant. Tumbarello *et al.*,<sup>[21]</sup> Giannella *et al.*,<sup>[23]</sup> and Venugopalan *et al.*<sup>[24]</sup>

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### Mortality rates in patients treated with DCT in comparison to standard antibiotic regimens 14 days post-initiation of treatment

The studies included in the analysis displayed low heterogeneity ( $I^2 = 0\%$ ). Patients receiving DCT had a lower 14 days mortality rate in comparison to patients receiving standard antibiotic treatment (OR = 0.79, 95% CI [0.53, 1.16]). However, the overall effect of DCT on 14 days mortality rate was non-significant ( $Z = 1.20$  [ $P = 0.23$ ]), as illustrated in Figure 6.

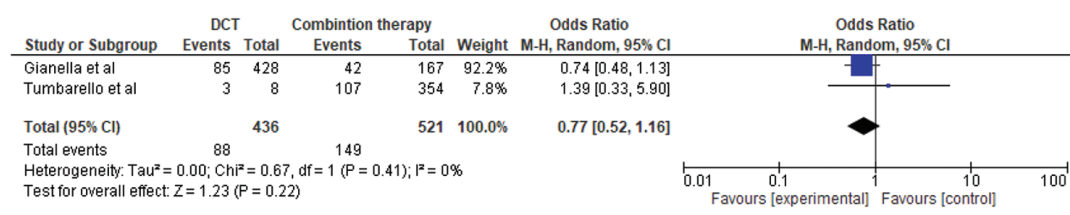
### Mortality rates in patients treated with DCT in comparison to standard antibiotic regimens 28–30 days post-initiation of treatment

The studies included in the analysis displayed low heterogeneity ( $I^2 = 0\%$ ). Patients receiving DCT had a lower 28–30 days mortality rate in comparison to

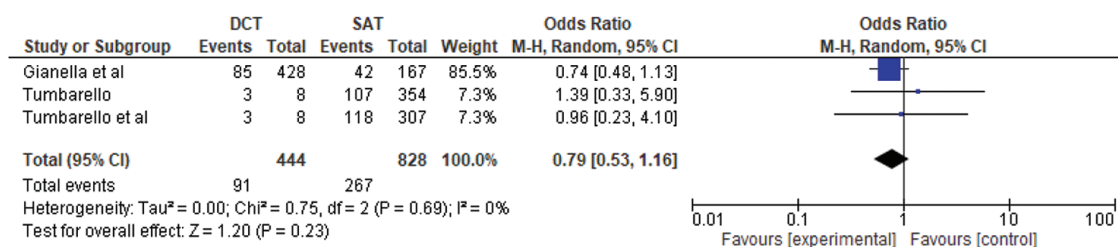
patients receiving standard antibiotic combinations (OR = 0.59, 95% CI [0.38, 0.94]). This difference in 28–30 days mortality rate between patients receiving DCT and patients receiving standard antibiotic treatment was significant ( $Z = 2.24$  [ $P = 0.02$ ]), as illustrated in Figure 7.

### Mortality rates in patients treated with DCT in comparison to standard antibiotic regimens 60–90 days post-initiation of treatment

The studies included in the analysis displayed low heterogeneity ( $I^2 = 0\%$ ). Patients receiving DCT had a lower 60–90 days mortality rate in comparison to patients receiving standard antibiotic treatment (OR = 0.67, 95% CI [0.35, 1.29]). However, the overall effect of DCT on 60–90 days mortality rate was non-significant ( $Z = 1.20$  [ $P = 0.23$ ]), as illustrated in Figure 8.

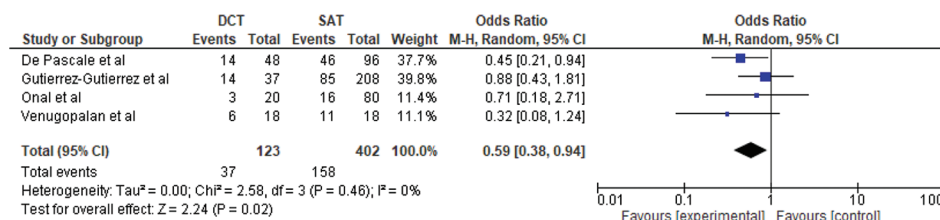


**Figure 5: 14 days mortality rate in patients treated with DCT in comparison to patients treated with combination therapy.** Data shown are number of deaths (events) and total number within group (total), data shown are odds ratio and 95% CI, CI- confidence interval, df- degrees of freedom, DCT- dual carbapenem therapy, experimental group received DCT, control group received combination antibiotic regimens, Gianella *et al.*,<sup>[23]</sup> Tumbarello *et al.*<sup>[21]</sup>

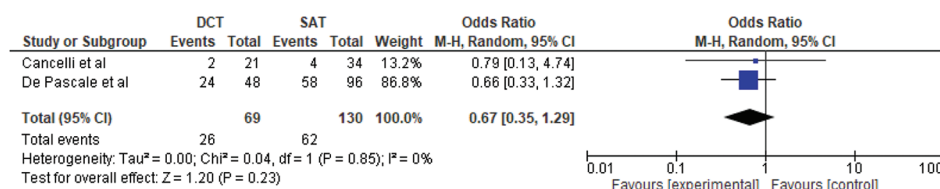


**Figure 6: 14 days mortality rate in CPKP infected patients treated with DCT in comparison to patients treated with standard antibiotic regimens.** Data shown are number of deaths (events) and total number in group (total), data shown are odds ratio and 95% CI, events refer to mortality, CI- confidence interval, df- degrees of freedom, SAT- standard antibiotic therapy, DCT- dual carbapenem therapy, experimental group received DCT treatment, control group received standard antibiotic regimens,  $p < 0.05$  is significant. Tumbarello *et al.*,<sup>[21]</sup> Tumbarello,<sup>[21]</sup> Gianella *et al.*<sup>[23]</sup>





**Figure 7: 28-30 days mortality rate in CPKP infected patients treated with DCT in comparison to patients treated with standard antibiotic regimens.** Data shown are number of deaths (events) and total number in group (total), data shown are odds ratio and 95% CI, events refer to mortality, CI-confidence interval, df- degrees of freedom, SAT-standard antibiotic therapy, DCT-dual carbapenem therapy, experimental group received DCT treatment, control group received standard antibiotic regimens,  $p < 0.05$  is significant. De Pascale *et al.*,<sup>[18]</sup> Gutierrez-Gutierrez *et al.*,<sup>[20]</sup> Onal *et al.*,<sup>[22]</sup> Venugopalan *et al.*<sup>[24]</sup>



**Figure 8: 60-90 days mortality rate in CPKP infected patients treated with DCT in comparison to patients treated with standard antibiotic regimens.** Data shown are number of deaths (events) and total number in group (total), data shown are odds ratio and 95% CI, events refer to mortality, CI-confidence interval, df- degrees of freedom, SAT- standard antibiotic therapy, DCT-dual carbapenem therapy, experimental group received DCT treatment, control group received standard antibiotic regimens,  $p < 0.05$  is significant. De Pascale *et al.*,<sup>[18]</sup> Cancelli *et al.*<sup>[19]</sup>

### Microbiological cure rates in patients treated with DCT in comparison to standard antibiotic regimes

The studies included in this analysis displayed low heterogeneity ( $I^2 = 0\%$ ). Patients receiving DCT had a higher microbiological cure rate in comparison to patients receiving standard antibiotic regimens (OR = 2.14, 95% CI [1.24, 3.71]). This difference in microbiological cure rate between patients receiving DCT and those receiving standard antibiotic regimens was significant (Z = 2.71, [P = 0.007]), as illustrated in Figure 9.

### Publication bias

To investigate potential publication bias funnel plots were produced of the included studies. The funnel plots display symmetry, suggesting that publication bias is not an issue in these analyses, as demonstrated by Figures 10 and 11.

The funnel plot produced for the studies included in the analysis of microbiological cure rate, displays asymmetry, suggesting the possible influence of publication bias, as demonstrated in Figure 12.

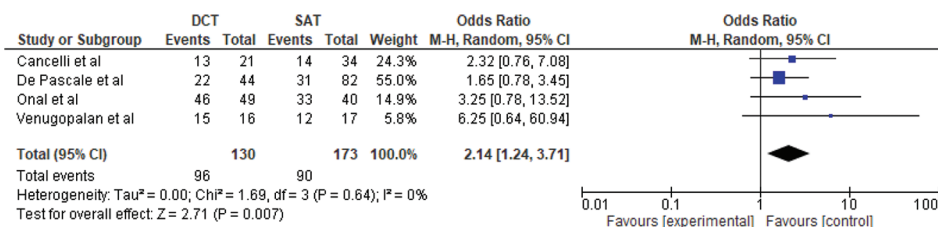
## Discussion

### Impact of the results of the present analysis

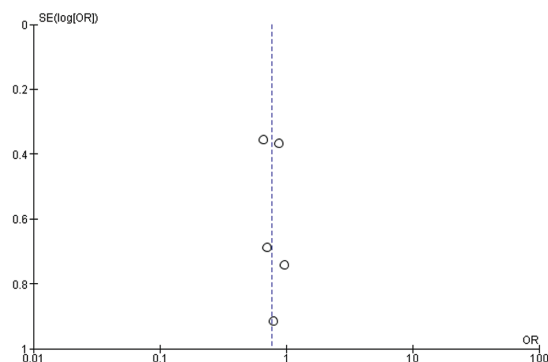
The majority of studies included in the analysis displayed homogeneity. The  $I^2$  statistics were low (<50%), or moderate (66%) as in the case of one analysis, suggesting there is very little inter-study variation in the results. Although the test of heterogeneity was not statistically significant, a random effects model was still selected for this analysis. Although the test of heterogeneity did not reach statistical significance, this may have been due to low power, the observed effect sizes may still vary across a range in studies with a great deal



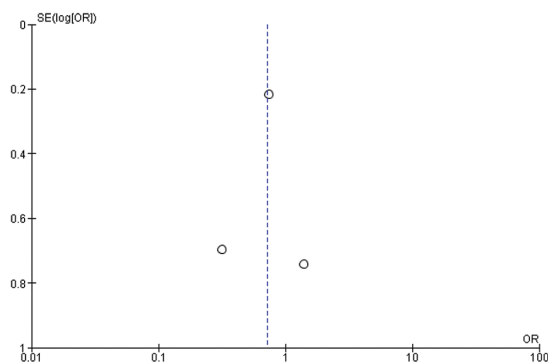
## Moody: DCT for the treatment of CPKP infections



**Figure 9: Microbiological cure rate in CPKP infected patients treated with DCT and those treated with standard antibiotic regimes. Data shown are number of cases achieving microbiological cure (events) and total number in group (total), data shown are odds ratio and 95% CI, events refer to microbiological cure, CI- confidence interval, df- degrees of freedom, SAT- standard antibiotic therapy, DCT- dual carbapenem therapy, experimental group received DCT treatment, control group received standard antibiotic regimes,  $p < 0.05$  is significant. Cancelli *et al.*,<sup>[19]</sup> De Pascale *et al.*,<sup>[18]</sup> Onal *et al.*,<sup>[22]</sup> Venugopalan *et al.*<sup>[24]</sup>**



**Figure 10: Publication bias of studies included in the analysis of overall mortality rates in patients treated with DCT in comparison to patients treated with monotherapy. OR-odds ratio, SE(log[OR])- standard error (log[OR]), De Pascale *et al.*<sup>[18]</sup>, Cancelli *et al.*<sup>[19]</sup>, Gutierrez-Gutierrez *et al.* (20), Tumbarello *et al.*<sup>[21]</sup>, Onal *et al.*<sup>[22]</sup>**

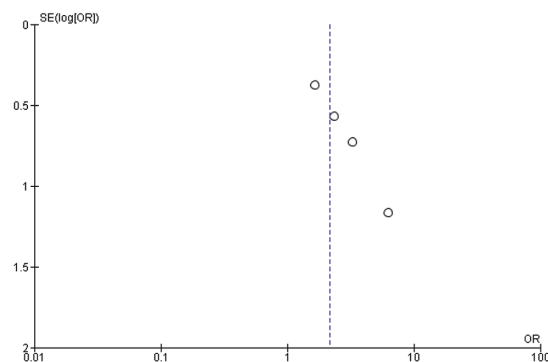


**Figure 11: Publication bias in the studies included in the analysis of overall mortality rates in patients treated with DCT in comparison to patients treated with alternate antibiotic combinations. OR- odds ratio, SE(log[OR])- standard error (log[OR]), Tumbarello *et al.*<sup>[21]</sup>, Giannella *et al.*<sup>[23]</sup>, and Venugopalan *et al.*<sup>[24]</sup>**

or error, for example. The ORs with respect to each study were subject to variation ( $OR = 0.11$ – $6.25$ ), and for this reason a random effects model was selected. An additional benefit of utilizing a random effects model is that this model allows greater generalizability of the results, beyond the scope of the studies included in the analysis allowing the conclusions drawn from this analysis to be generalized into clinical practice.

The funnel plots produced for the studies included in the analysis of microbiological cure rate displayed asymmetry, suggesting the influence of publication bias in this analysis. Asymmetry may be ascribed

to poor methodological study design, typically a feature of studies with a small sample size, and resulting in an overestimation of the treatment effect.<sup>[25]</sup> However, all the studies included in the present analysis have relatively large sample sizes, suggesting another factor as the causative agent for the asymmetry. Heterogeneity in the studies is another causative factor of asymmetry in funnel plots, similarly; however, the included studies were determined to be homogenous, thus heterogeneity is an unlikely cause of the asymmetry. Funnel plots become unreliable methods of investigating publication bias when the number of studies is



**Figure 12: Publication bias in the studies included in the analysis of microbiological cure rate in patients treated with DCT in comparison to patients treated with standard antibiotic therapy. OR-odds ratio, SE (log[OR])- standard error(log[OR]), Cancelli *et al.*<sup>[19]</sup>, De Pascale *et al.*<sup>[18]</sup>, Onal *et al.*<sup>[22]</sup>, Venugopalan *et al.*<sup>[24]</sup>**

small, as is the case with the present analysis, thus uncertainties remain as to the true effect of publication bias in the analysis of the microbiological cure rate.

Evidencing the symbiotic and bactericidal effects of DCT, patients treated with DCT had a significantly higher microbiological cure rate in comparison to the microbiological cure rate in patients treated with standard antibiotic regimens. Carbapenem antibiotics are beta-lactamase inhibitors, exerting their bactericidal effects through binding of penicillin-binding-proteins present on the bacterial cell wall, inhibiting the synthesis of peptidoglycan, an essential component of the cell wall in many bacterial species including *K. pneumoniae*, resulting in a weakening, and subsequent lysis of the bacterial cell due to osmotic pressure, and thus cell death.<sup>[26,27]</sup> The synergistic action of the dual carbapenem combination explains the significantly greater microbiological cure rate seen in patients to whom DCT was administered.

DCT significantly lowered the 28–30 days mortality rate in patients in comparison to standard antibiotic therapies, this effect may be explained by the action of ertapenem and the second carbapenem acting synergistically. The carbapenemase enzyme produced by CPKP has a greater affinity for ertapenem in comparison to other carbapenem antibiotics, as such ertapenem is administered first, and due to its high affinity for

the carbapenemase enzyme, it binds with ease to the enzyme, resulting in a depletion of the enzyme, sufficient time for this reaction to occur elapses before the second carbapenem, meropenem or doripenem, is administered. Due to depletion of the carbapenemase enzyme in the reaction with ertapenem, higher concentrations of the second carbapenem are established in the environment surrounding the bacteria which would otherwise be concentrated with the carbapenemase enzyme. Alternatively, ertapenem may act as a suicide inhibitor. Ertapenem may bind to the carbapenemase enzyme and be hydrolyzed. The second carbapenem is then administered, again allowing sufficient time for the reaction between ertapenem and the carbapenemase enzyme to occur. The carbapenemase enzyme is bound to ertapenem, thus there is a lower concentration of enzyme available to hydrolyze the second carbapenem; consequently, this carbapenem can exert its bactericidal effect.<sup>[2,6,26]</sup>

DCT did not significantly lower the 60–90 days mortality rate in patients diagnosed with CPKP infections, in comparison to standard antibiotic treatments. The failure of DCT to sustain effectiveness at the 60–90 days time point may be attributed to unmeasured confounders occurring so long post-treatment. The fact that all the included studies were retrospective observational studies, and therefore have the possibility to be influenced by confounding variables, such as underlying comorbidities, strengthens this hypothesis.

DCT also had no significant effect on the 14 days mortality rate in comparison to standard antibiotic treatments. This is suggestive of DCT's need for accumulation of efficacy over a prolonged course of therapy to have a significant effect. This theory is supported by the findings of Wiskirchen *et al.*<sup>[28]</sup> Wiskirchen *et al.* reported DCT demonstrated significantly enhanced efficacy when compared to monotherapy in the treatment of murine CPKP infections, but only 72 h post-infection, not at prior time points. Taken together, this suggests that DCT may take time to have a significant effect, 14–28 days, and, additionally, that while DCT may be initially effective in treating CPKP infections, such effectiveness is not maintained, up to 60 or 90 days follow-up.

DCT failed to significantly lower the overall mortality rate in patients diagnosed with CPKP infections in comparison to patients treated with monotherapy antibiotic regimes.



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Similarly, DCT failed to significantly lower the overall mortality rate in patients diagnosed with CPKP infections in comparison to alternative antibiotic combination regimens. One possible explanation for the failure of DCT to significantly lower the overall mortality rate in patients may be the uncertainty surrounding DCT's mechanism of action. Varying hypothesis exists to explain the mechanisms underpinning the possible effectiveness of DCT; the establishment of higher concentrations of the carbapenem antibiotic in the microenvironment of the bacteria which would otherwise be concentrated with carbapenemase enzyme may play a role, alternately the lower concentration of enzyme available to hydrolyze the carbapenem antibiotic may be responsible. Failure to elucidate these mechanisms prevents accurate predictions regarding the effectiveness of a DCT regimen in the treatment of infections due to CPKP.

Nonetheless, DCT did significantly lower the 28–30 days mortality rate, in particular, when compared to monotherapy antibiotic regimens. This effect may be due to the synergism of the two carbapenems antibiotics, which is an advantage in comparison to monotherapy antibiotic regimens. These results are in line with those obtained by Oliva *et al.*, who determined the combination of ertapenem and meropenem to be synergistic and bactericidal in activity against *K. pneumonia*.<sup>[29]</sup>

In contrast, DCT failed to significantly lower the 14 days mortality rate in patients in comparison to patients treated with other antibiotic combination regimens. This suggests that whilst DCT may be significantly more effective in treating CPKP infection than monotherapy regimens, DCT is not more effective than other antibiotic combinations. Oppositely, the causative factor for these results may be the accumulation of efficacy over a prolonged time period required to yield a significant effect within a DCT regimen. These results support the finding that DCT failed to significantly lower the 14 days mortality rate when compared to standard antibiotic regimens, which included both monotherapy and combination regimens. Thus, these results strengthen the hypothesis that DCT has a time dependent effect on mortality rates.

Such findings indicate that DCT may have a time-dependent effect on mortality rates. Evidencing that while DCT may be initially effective at lowering the mortality rate in patients, such a response is not maintained in the long term. However, failure to maintain effectiveness may be less to do with

an inherent flaw in DCT but rather due to the influence of confounding variables not controlled for in the included studies. Thus, there is a need for randomized controlled trials to determine if DCT's effectiveness can be sustained when confounding variables are controlled for.

The findings presented here indicate that, up to 2 weeks post-initiation of treatment, there was no significant difference in the mortality rates of patients receiving DCT in comparison to patients receiving SAT. Interestingly, however, 28–30 days post-initiation of antibiotic treatment, there was a significantly lower mortality rate in the cohort of patients to whom DCT was administered in comparison to those receiving SAT. However, 60–90 days post-initiation of treatment, this significant difference was absent, and no significant difference existed in the mortality rates in the cohort of patients who had been receiving SAT, compared to that in the cohort of patients who had received DCT.

## Conclusion

DCT significantly improved the microbiological cure rate in CPKP infected patients in comparison to standard antibiotic treatments. DCT failed to significantly lower the overall mortality rate in comparison to the overall mortality rates in patients treated with either monotherapy or alternate combination therapies. However, when stratified by time post initiation of treatment, DCT significantly lowered the 28–30 days mortality rate in patients in comparison to the 28–30 days mortality rate in patients treated with monotherapy. Contrastingly, when stratified according to time post-initiation of treatment, DCT still had no significant effect on the 14 days mortality rate in comparison to the 14 days mortality rate in patients treated with alternate combination therapies. DCT did not significantly lower the 14 days mortality rate in comparison to standard antibiotic regimens. DCT did, however, significantly lower the 28–30 days mortality rate in patients in comparison to standard antibiotic regimens. DCT did not significantly lower the 60–90 days mortality rate in comparison to standard antibiotic regimens. These results indicate a time dependent response in the effectiveness of a DCT regimen. Overall, DCT demonstrated efficacy in the treatment of CPKP infection, significantly lowering the 28–30 days mortality rate and significantly improving the microbiological cure rate, in comparison to standard antibiotic treatments, and thus may be a valid therapeutic option for the treatment of CPKP infections.



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