UNIVERSITY OF LIVERPOOL

TREATMENTS OF NOSOCOMIAL PNEUMONIA IN THE ERA OF ANTIMICROBIAL RESISTANCE

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy

by

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ABSTRACT

Nosocomial pneumonia, the most common type of healthcare-associated infection, frequently involves multidrug-resistant bacteria, like carbapenem-resistant and third-generation cephalosporin-resistant *Klebsiella pneumoniae*, carbapenem-resistant *Acinetobacter baumannii* and *Stenotrophomonas maltophilia*. Due to the limited choice of antibiotics, therapies for such infections are primarily based on traditional drugs, like tigecycline, polymyxins and trimethoprim-sulfamethoxazole. With differences in antibiotic availability, prescription culture and antibiotic stewardship, therapeutic strategies vary considerably in different institutions. Moreover, no standardized regimens have been proposed for these infections, further complicating clinical therapy. Evidence supporting specific regimens is derived mainly from *in vitro* antibiotic susceptibility testing studies; therefore, clinical evidence is urgently required. This thesis aims to contribute to the build-up of such clinical evidence for this particular type of infection. All clinical studies have been conducted in China, so to put that in perspective, similarities and differences between China and other areas in the world regarding the prevalence and treatments of these infections have been addressed in the introduction chapter.

Chapter 2 contains a nationwide survey about management strategies for nosocomial pneumonia conducted in tertiary hospitals in China, and mainly aimed to understand current antibiotic treatments for those resistant pathogens. The results indicate that tigecycline-based therapy was the most prevalent regimen in treating carbapenem-resistant *K. pneumoniae* and *A. baumannii*, as well as a popular alternative therapy for *S. maltophilia*. However, despite the wide use of tigecycline in these hospitals, its dosage in clinical practice has not been standardized yet. Some hospitals used the standard-dose, while others adopted the high-dose regimen.

To assess whether the high-dose regimen would lead to better clinical outcomes, I have conducted a systematic review and meta-analysis, which is presented in **Chapter 3**. The results demonstrate that the high-dose tigecycline was associated with lower mortality, higher clinical and microbiological cure than the standard-dose regimen and other controls. Therefore, high-dose tigecycline is recommended when tigecycline is the clinical choice.

With these promising results of the high-dose tigecycline regimen in mind, a retrospective study was conducted to evaluate whether intravenously adding polymyxin B to the high-dose tigecycline regimen could bring extra benefit for pneumonia caused by carbapenem-resistant *K. pneumoniae* and *A. baumannii* and the results are described in **Chapter 4**. Unfortunately, the study did not detect any benefit of the combination therapy. Therefore, treating carbapenem-resistant *K. pneumoniae* and *A. baumannii*, combining tigecycline with polymyxin B is not recommended.

Although tigecycline has been widely used as an alternative therapy to treat *S. maltophilia* pneumonia in China, clinical evidence is still limited. To assess the effectiveness of this therapy, I have performed a multicentre retrospective study; described in **Chapter 5**. The results indicate that compared with fluoroquinolone therapy, treatment with the standard-dose of tigecycline was associated with lower clinical and microbiological cure. Therefore, the standard-dose of tigecycline is not recommended in treating pneumonia caused by *S. maltophilia*.

The survey described in chapter 2 indicates that carbapenems and piperacillin-tazobactam are the most commonly used regimens against pneumonia caused by extended-spectrum β-lactamase producing *Enterobacteriaceae*. As there was no clinical evidence regarding the use of piperacillin-tazobactam in such infections, a retrospective study was conducted in **Chapter 6** to facilitate the prudent use of this carbapenem-sparing regimen. Compared with carbapenem therapy, piperacillin-tazobactam therapy resulted in similar 28-day mortality, 14-day clinical and

microbiological cure in patients with pneumonia due to extended-spectrum β-lactamase producing *K. pneumoniae*. Therefore, piperacillin-tazobactam could be used as a carbapenem-sparing regimen in treating such infections.

Findings from this thesis could serve as an essential complement to current clinical guidelines in the management of nosocomial pneumonia, bridging gaps between clinical practice and unanswered questions in these guidelines. These results could be directly used as evidence to support or to discourage specific antibiotic regimens in these infections. However, considering the limitations and possible biases in the present thesis, well-designed randomized trials to confirm these findings are still warranted. Furthermore, studies regarding therapeutic regimens other than those proposed in this thesis are also highly welcomed because they could help enrich our armaments in combating pathogens with antimicrobial resistance.

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I am also grateful to Professors **Neil French** and **Elmer V Villanueva** for their patience in teaching me about study design, statistical analysis and, more importantly, scientific thinking. Although each manuscript sent to them should be revised two to three times, the process was always supportive and encouraging, and I really learnt a lot. Their selfless spirit like this has deeply influenced me. In the future, I will do what they did to help and guide my own students.

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LIST OF ABBREVIATIONS AND SYMBOLS

AMR	Antimicrobial resistance
3GC-R	Third-generation cephalosporin-resistant
3GCs	Third-generation cephalosporins
APACHE II	Acute physiology and chronic health evaluation II
AUC	Area under the receiver operating characteristic curve
BSI	Bloodstream infections
CAP	Community-acquired pneumonia
CCI score	Charlson comorbidity index
CHINET	China antimicrobial surveillance network
CI	Confidence interval
cIAI	Complicated intra-abdominal infections
CR	Carbapenem-resistant
CRAB	Carbapenem-resistant *Acinetobacter baumannii*
CRE	Carbapenem-resistant *Enterobacteriaceae*
CRGNB	Carbapenem-resistant Gram-negative bacteria
CRKP	Carbapenem-resistant *Klebsiella pneumoniae*
CRPA	Carbapenem-resistant *Pseudomonas aeruginosa*

cSSTI	Complicated skin and soft tissue infection
CTX-M	Cefotaxime hydrolysing β-lactamase-Munich
DALYs	Disability-adjusted life-years
ECDC	European centre of disease prevention and control
ERAS-Net	European antimicrobial resistance surveillance network
ESBL	Extended-spectrum β-lactamase
ESCMID	European society of clinical microbiology and infectious diseases
EUCAST	European committee on antimicrobial susceptibility testing
FDA	Food and drug administration
GDP	Gross domestic product
GNB	Gram-negative bacteria
HAI	Healthcare-associated infection
HAP	Hospital-acquired pneumonia
HDT	High-dose tigecycline
ICU	Intensive care unit
IDSA	Infectious diseases society of America
IIAT	Inappropriate initial antibiotic therapy
IMP	Imipenemase metallo-β-lactamase
INFORM	International network for optimal resistance monitoring

IPTW	Inverse probability of treatment weighting
KPC	*Klebsiella pneumoniae* carbapenemase
MBL	Metallo-β-lactamases
MDR	Multidrug-resistant
MIC	Minimum inhibitory concentration
NDM	New Delhi metallo-β-lactamase
NOS	Newcastle-Ottawa scale
OR	Odds ratio
OXA	Oxacillin carbapenemases
PD	Pharmacodynamic
PDR	Pan-drug resistant
PK	Pharmacokinetic
RCT	Randomized controlled trial
RIS	Required information size
SAPS	Simplified acute physiology score II
SDT	Standard-dose tigecycline
SHV	Sulfhydryl variant of the TEM enzyme
SMD	Standardized mean differences
SOFA	Sequential organ failure assessment

TEM	Temoneira class A extended-spectrum β-lactamase
US	The United States
UTI	Urinary tract infections
VAP	Ventilator-associated pneumonia
VIM	Verona integron-encoded metallo-β-lactamase
WHO	World Health Organization
XDR	Extensive-drug resistant

1 CHAPTER 1 – INTRODUCTION

1.1 THE "ERA OF ANTIMICROBIAL RESISTANCE"

Since the first antibiotic, penicillin, was discovered in 1928, healthcare services have been revolutionized thoroughly (Gould, 2016). Common, but fatal diseases, like pneumonia and wound infections, were no longer deadly; moreover, with the help of antibiotics, advanced surgeries and procedures in modern medicine became possible, like heart surgery, organ transplantation, and chemotherapy (Hutchings et al., 2019). However, due to the overuse and misuse of antibiotics, bacteria have progressively less susceptibility to antibiotics, causing bacterial antimicrobial resistance (AMR), which is one of the major threats to public health in the 21st century (Gould, 2016, Hutchings et al., 2019, Nadeem et al., 2020).

In 2014, the World Health Organization (WHO) released its first global report on antibiotic resistance, reporting the global spread of AMR in bacteria that cause common healthcare-associated and community-acquired infections in all WHO regions (WHO, 2014). For example, *Escherichia coli*, a pathogen commonly causing bloodstream infections (BSI), urinary tract infections (UTI) and complicated intra-abdominal infections (cIAI), the rate of resistance to third-generation cephalosporins (3GCs), the first-line antibiotics for such infections, was reported to

be as high as 16% to 68% in the South-East Asia region, 0% to 48% in the region of Americas, and 3% to 82% in the European region. For *Klebsiella pneumoniae*, another bacterium frequently involved in nosocomial respiratory tract infections, BSI and UTI, the resistance rates to 3GCs exceeding 50% were reported in all WHO regions. Moreover, resistance to carbapenems, a class of potent antibiotics in treating multidrug-resistant bacteria, has been observed in *K. pneumoniae* in all WHO regions, with two areas reporting a rate exceeding 50%.

The excess mortality and costs are the most significant impacts of AMR on patients and health systems (Papadimitriou-Olivgeris et al., 2017, Zhen et al., 2019, Awasthi et al., 2022, Tansarli et al., 2013, Rivera-Espinar et al., 2020, Blot et al., 2007). Compared with patients infected by antibiotic-susceptible bacteria, those infected with their resistant counterparts had a significantly higher all-cause mortality and attributable mortality (Papadimitriou-Olivgeris et al., 2017, Rivera-Espinar et al., 2020). Moreover, the economic cost was also higher in infections caused by resistant bacteria, mainly due to the extended hospital stay and more intensive care unit (ICU) requirements (Zhen et al., 2019, Tansarli et al., 2013). According to The Review on Antimicrobial Resistance, commissioned by the UK government in 2014, AMR was estimated to cause 10 million deaths every year and a reduction of 2% to 3.5% in Gross Domestic Product (GDP) by 2050 (O'Neill, 2014). Although some experts criticized this review (Piddock, 2016, de Kraker et al., 2016), the latest study estimating the global burden of AMR supported its possibility, in which 4.95 million (3.62 to 6.57) deaths were estimated to be associated with AMR in 2019, including 1.27 million (0.91 to 1.71) attributable deaths (Murray et al., 2022).

Facing such problematic prospects, the WHO, adopted the Global Action Plan on Antimicrobial Resistance in May 2015, in which the “one-health” approach was highlighted (WHO, 2015). Two critical drivers contribute to the increasing situation of AMR: the selection of resistant bacteria by misuse and overuse of antibiotics and factors promoting the spread of these resistant bacteria among humans, animals,

and the environment (Hernando-Amado et al., 2019). Therefore, to address the complexity of AMR, the WHO suggested to take the "one-health" approach, which is a collaborative approach coordinating efforts from various sectors aiming to minimize the impact of the two drivers.

The first recommendation is to improve the awareness of AMR among the public, doctors, veterinarians, and farmers through education, training, and policies, thereby reducing the inappropriate use of antibiotics in humans and animals, especially those antibiotics used for growth promotion in stockbreeding and aquaculture industry. Second, improve sanitation and hygiene, and apply effective infection prevention and control measures to reduce the rate of infections and lower the spread of these resistant pathogens among humans, animals, and the environment. Third, improve knowledge of AMR through active surveillance and basic research, thus understanding the development and spread of resistance and supporting the development of new antibiotics for those newly emerged resistances. Fourth, increase investment in new antibiotics, diagnostic tools, and vaccines for resistant bacteria.

Although progress has been observed in many aspects since the initiation of the Global Action Plan, things have not gone as well as expected thus far, especially in the development of new antibiotics. As of March 2021, there were only 13 new antibiotics in phase 3 clinical trials, and no more than half of these are expected to be approved for clinical use (Trusts, 2021). Considering the overwhelmingly increasing AMR and the dwindling antibiotic pipeline, many scholars warned that the "post-antibiotic era" is approaching if no significant actions have been taken worldwide (Chandra et al., 2021, Kwon and Powderly, 2021). To reflect that the times we are living in are dire but not yet as gloomy as depicted by others, I have used the term "the era of antimicrobial resistance" in my thesis title.

1.2 WHO PRIORITY LIST OF ANTIBIOTIC-RESISTANT BACTERIA

In support of the Global Action Plan on Antimicrobial Resistance, the WHO released its first-ever priority list of antibiotic-resistant pathogens in 2017, aiming to direct research and development of new antibiotics for those resistant pathogens that urgently need new antibiotics (WHO, 2017). The priority of individual bacteria was rated by experts based on the weights of the criteria that were determined through the potentially all pairwise rankings of all possible alternatives methods. Among all included criteria, mortality, healthcare burden, treatability and the prevalence of resistance were weighted as the most important ones (Tacconelli et al., 2018). Finally, 20 bacteria with 25 resistant patterns were listed on the priority list and were divided into three categories (critical, high, and medium priority) based on their mean total score (Figure 1-1). Pathogens in the critical priority group are commonly involved in nosocomial infections, like nosocomial pneumonia, catheter-related UTI, and catheter-related BSI, and usually lead to worse clinical outcomes (Mancuso et al., 2021, Juliana. et al., 2020). They include carbapenem-resistant *Acinetobacter baumannii* (CRAB), carbapenem-resistant *Pseudomonas aeruginosa* (CRPA), third-generation cephalosporin-resistant (3GC-R) and carbapenem-resistant *Enterobacteriaceae* (CRE), that all urgently ask for new and effective therapeutic strategies.

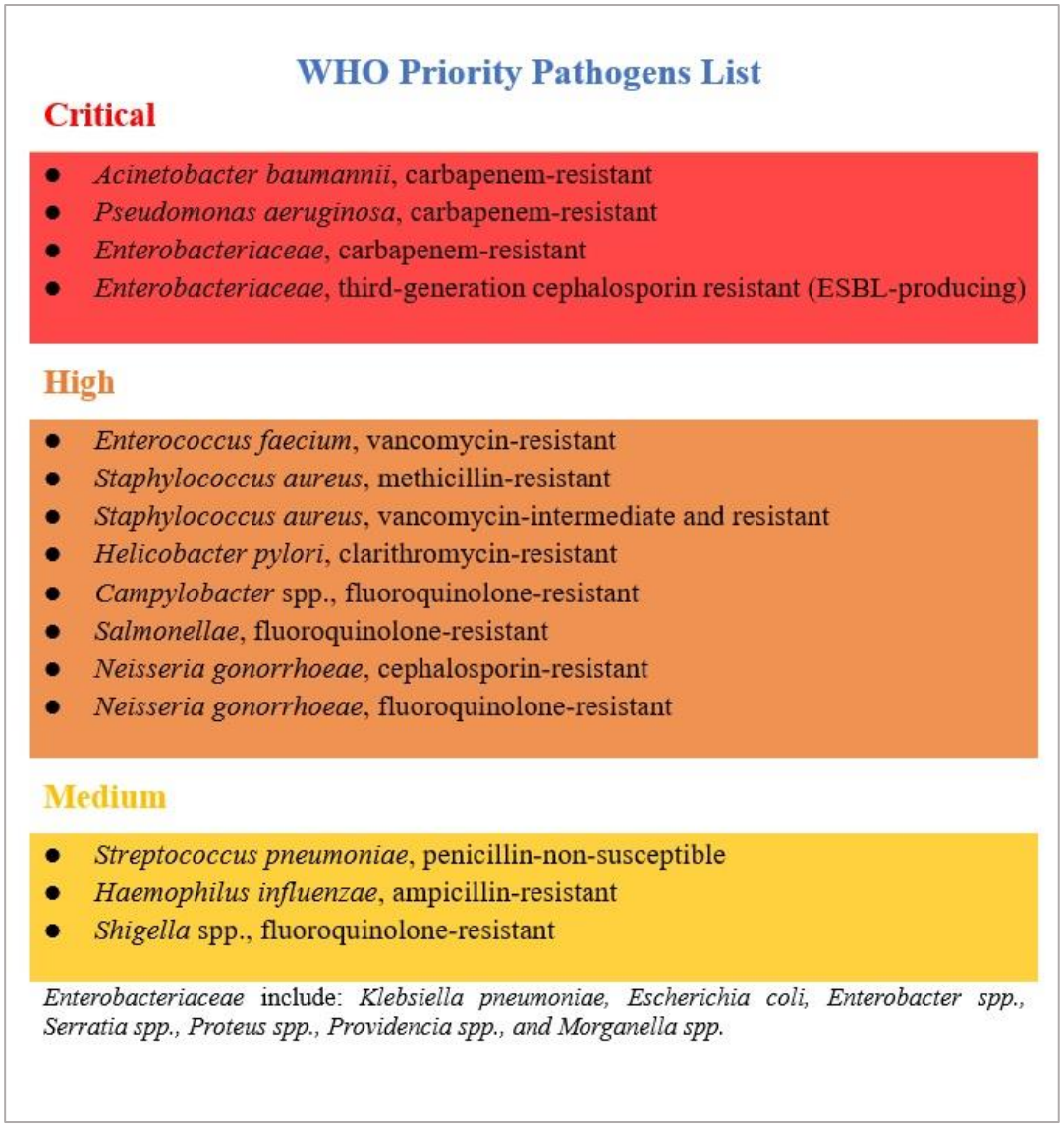

Figure 1-1. The WHO priority list of antibiotic resistant bacteria. (Adapted from Tacconelli et al., 2018)

1.3 Carbapenem-resistant Gram-negative bacteria

Carbapenems, a class of broad-spectrum antibiotics, have long been used as the last line of defence against multidrug-resistant (MDR) Gram-negative bacteria (GNB) due to their potent activity in binding to penicillin-binding proteins, which are essential for cell wall synthesise in these bacteria (El-Gamal et al., 2017, Papp-Wallace et al., 2011, Nicolau, 2008). The chemical structures of meropenem,

imipenem and ertapenem, commonly used carbapenems in the clinic to fight pneumonia and other infections, are presented in Figure 1-2.

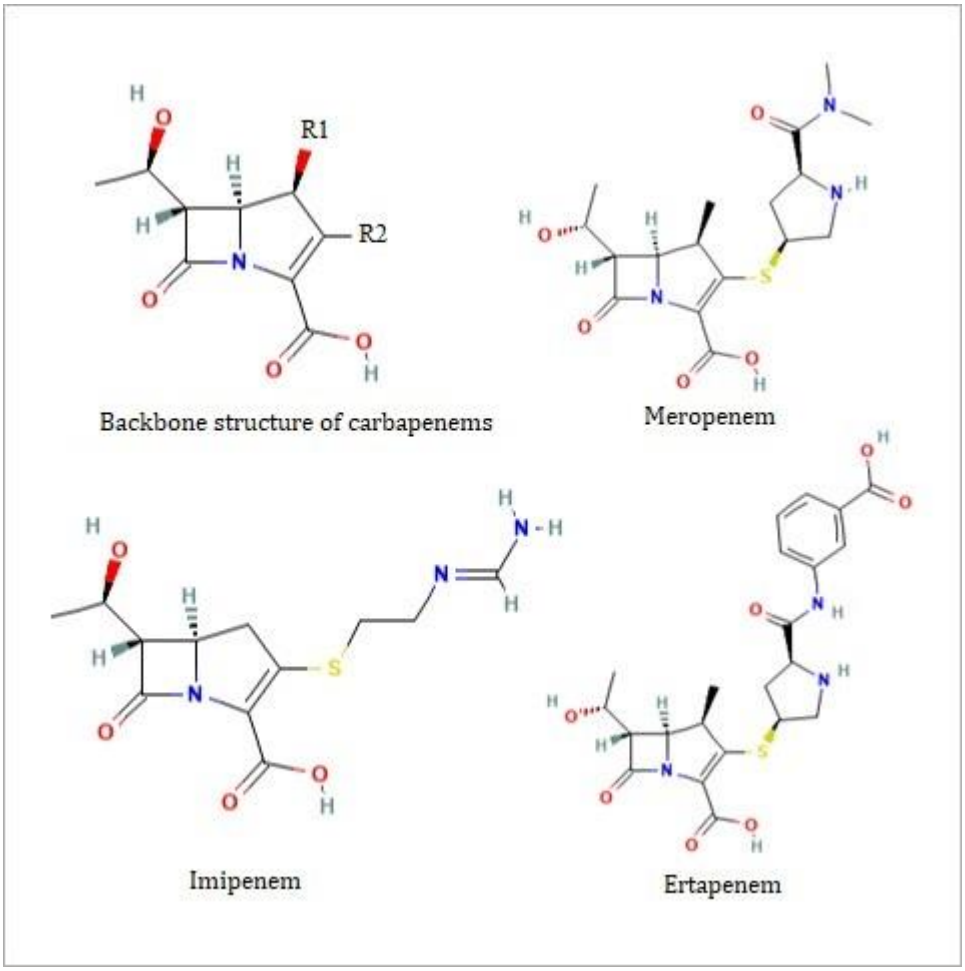

Figure 1-2. Chemical structures of the backbone structure of carbapenems, as well as three clinically commonly used carbapenems (downloaded from https://pubchem.ncbi.nlm.nih.gov/).

In recent years, the efficacy of carbapenems has been challenged considerably because of the occurrence of carbapenemases and other resistant mechanisms (e.g. porin mutation, overexpression of efflux pumps) in infectious bacteria (Pourgholi et al., 2022, Nguyen and Joshi, 2021, Han et al., 2020, Nordmann and Poirel, 2019). The mechanism of action and common resistance mechanisms to carbapenems are illustrated in Figure 1-3.

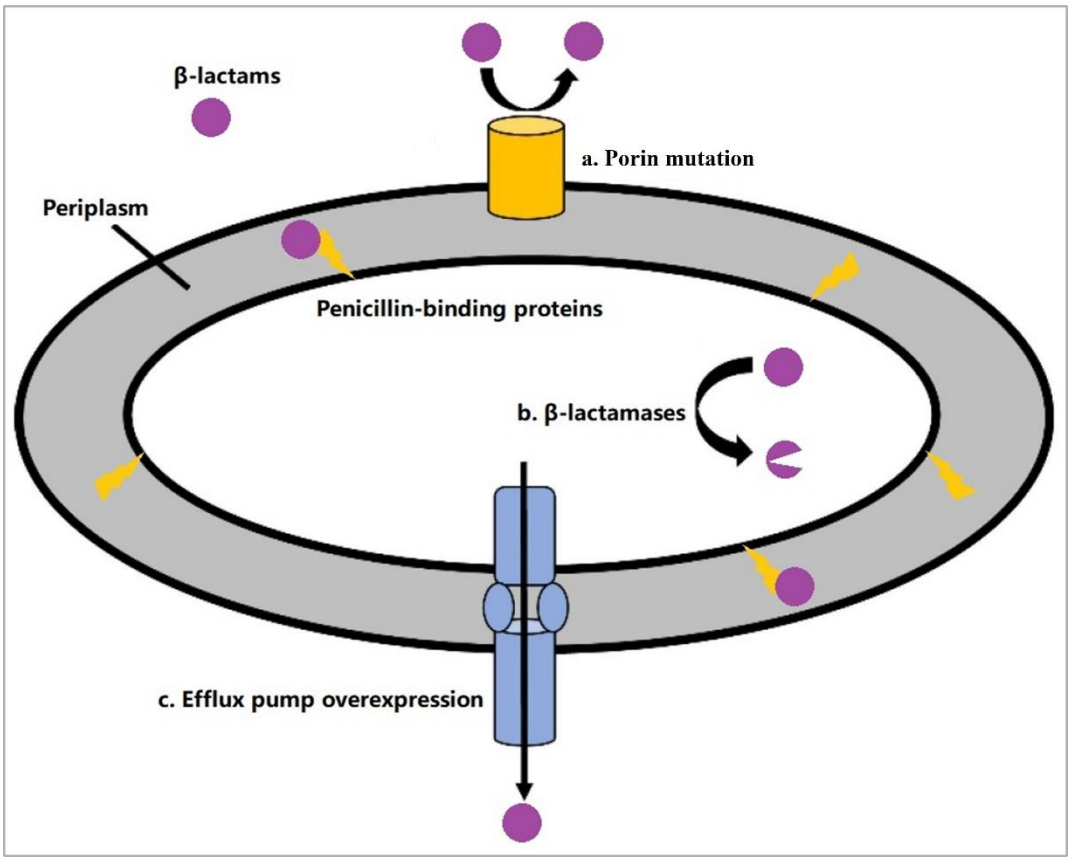

Figure 1-3. Schematic representation of the mechanism of action of β-lactams (including carbapenems) and primary resistance mechanisms to β-lactams in Gram-negative bacteria. β-lactams (purple circles) bind to penicillin-binding proteins (yellow lighting symbol) on the inner membrane to prevent bacterial growth and division by inhibiting bacterial cell wall synthesis. There are three common mechanisms mediating resistance to β-lactams in Gram-negative bacteria: (a) reducing the permeability for β-lactams by mutations in outer membrane porins, (b) inactivating β-lactams by β-lactamases, and (c) increasing efflux of β-lactams by efflux pump overexpression (revised from (Eichenberger and Thaden, 2019)).

Therapeutic options for carbapenem-resistant Gram-negative bacteria (CRGNB) are restricted to antibiotics with uncertain clinical effectiveness, like colistin and tigecycline (Tamma et al., 2022, Paul et al., 2022, Tamma et al., 2021, Karakonstantis et al., 2020, El-Sayed Ahmed et al., 2020, Doi, 2019). Therefore, the WHO put clinically important bacteria (*A. baumannii*, *K. pneumoniae*, *P. aeruginosa*) with carbapenem resistance as the highest priority for research and development of new antibiotics (see Figure 1-1). In addition to bacteria listed on the WHO priority list, *S. maltophilia*, a GNB with natural multidrug resistance and carbapenem resistance, is

another emerging pathogen that needs to be prioritized due to its increasing prevalence and very high rate of mortality involved in nosocomial infections (Ebara et al., 2017, Chang et al., 2015, Brooke, 2012). Those bacteria will all be addressed in the different chapters within this thesis. Therefore, in the following part, I will review the global epidemiology, resistance mechanisms, and therapeutic options for CRAB, CRE (especially *K. pneumoniae*) and *S. maltophilia*.

1.3.1 EPIDEMIOLOGY OF SELECTED CRGNB

CRE, especially extraintestinal pathogenic *E. coli* and *K. pneumoniae*, are the most frequently found GNB to cause healthcare-associated infections (HAI), including ventilator-associated pneumonia (VAP), hospital-acquired pneumonia (HAP), BSI, cIAI, and UTI (Geurtsen et al., 2022, Lutgring, 2019, Perez and Bonomo, 2019, Potter et al., 2016). In 2009, the European Centre of Disease Prevention and Control (ECDC) issued its first Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net) (ECDC, 2009), including data from 28 countries. The characteristics of the ERAS-Net and other surveillance systems cited in this thesis have been summarised in Table 1-1. Based on data from EARS-Net 2009 annual report, the carbapenem resistance rate in *K. pneumoniae* was 6.98%, with 95.9% of the isolates from Greece. Apart from Greece (43.5%), Cyprus (17%), Italy (1.3%), and Belgium (1.2%), resistance rates in other countries were less than 1%, and 13 countries did not isolate any carbapenem-resistant *K. pneumoniae* (CRKP) strains in 2009. However, according to the latest 2022 annual report of EARS-Net (ECDC, 2022), which is based on data from 2020, resistance to carbapenems of *K. pneumoniae* in Europe increased significantly during the surveillance period, from 7.4% in 2016 to 10% in 2020. The highest resistance rate was reported in Greece (66.3%), followed by Romania (48.3%), Italy (29.5%), Bulgaria (28.1%), Cyprus (19.8%), Croatia (19.1%) and Portugal (11.6%), all accounted for more than 10% of the clinical isolates (Figure 1-4). In the United States (US), the percentage of CRKP isolated from central line-associated BSI did not change so much from 2006-2007 to 2015-2017, according to data reported to the National Healthcare Safety Network,

staying at a relatively high level of around 10%; in contrast, the resistance rate to carbapenems in *K. pneumoniae* isolated from patients with VAP increased significantly during the same surveillance period, from 3.6% in 2006-2007 and soaring to 23.3% in 2015-2017 (Weiner-Lastinger et al., 2020, Hidron et al., 2008). In China, an even more dire situation was observed. In 2005, only 3% of *K. pneumoniae* was reported as imipenem resistance according to data from the China Antimicrobial Surveillance Network (CHINET), whereas it dramatically increased to 20.9% in 2017 and peaked at 25% in 2018 (Hu et al., 2018a, Hu et al., 2019). Although the increasing trend was curbed recently in China, resistance rates stayed considerably high, with 23.7% reported in 2019 and 23.3% reported in 2020 (Hu et al., 2020, Hu et al., 2021). The prevalence and resistance trends of CRKP in Europe and China from 2010 to 2020 reported in the EARS-Net and CHINET are summarized in Figure 1-4.

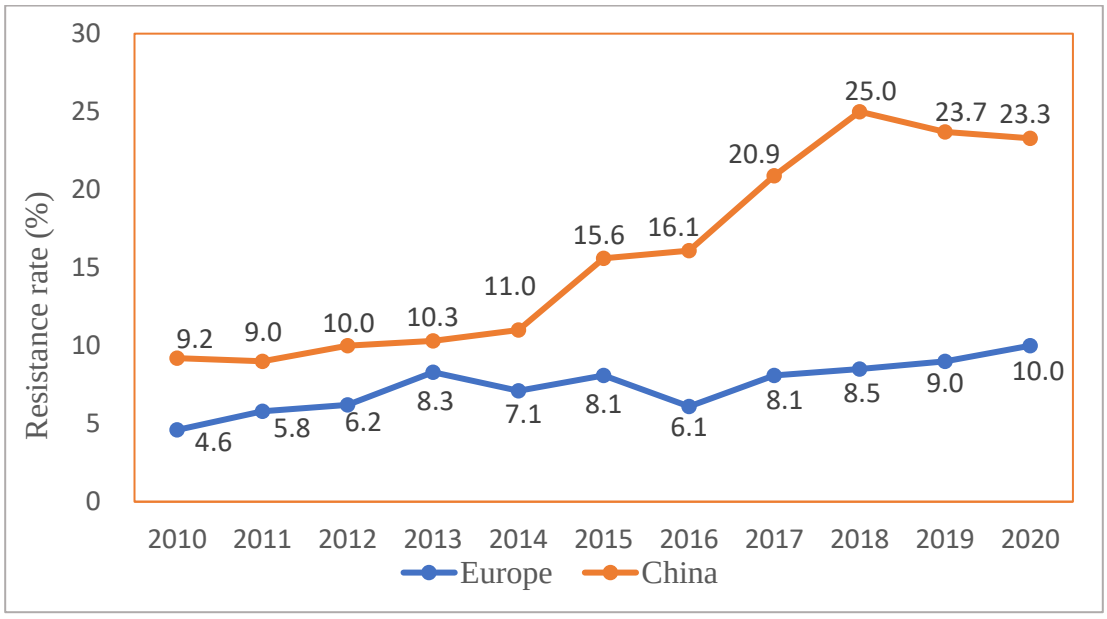

Figure 1-4. The prevalence and resistance trends of carbapenem-resistant *K. pneumoniae* reported in the European Antimicrobial Resistance Surveillance Network (EARS-Net) and the China Antimicrobial Surveillance Network (CHINET) from 2010 to 2020 (data from https://www.chinets.com/ and https://www.ecdc.europa.eu/en).

Table 1-1. Summary of epidemiology surveillance programs and clinical studies cited in this thesis.

Surveillance system or study	**Description**	**Website or reference**
CARSS	The China Antimicrobial Resistance Surveillance System monitors resistance profile of clinical pathogens in China (Started from 2005).	www.carss.cn
CHINET	The China Antimicrobial Surveillance Network monitors resistance profiles of clinical pathogens in China (Started from 2005).	www.chinets.com
CRACKLE-2	A prospective, multicentre study, measuring molecular characteristics of CRE[a] in 49 US hospitals.	(van Duin et al., 2020)
EARS-Net	The European Antimicrobial Resistance Surveillance Network monitors resistance profile of clinical pathogens in European countries (Started from 2009).	www.ecdc.europa.eu
EuSCAPE	A prospective study analyzing carbapenemases in CRE pathogens collected from 36 countries between Nov. 2013 and April 2014.	(Grundmann et al., 2017)
INCREMENT	An observational, multinational study involving 37 hospitals in 11 countries assessing different antibiotic regimens in treating blood stream infections caused by ESBL-E or CRE.	(Gutiérrez-Gutiérrez et al., 2016)
INFORM	The International Network for Optimal Resistance Monitoring is conducted in more than 85 US hospitals (Started from 2012).	(Sader et al., 2018)
MERINO	A multinational RCT assessing the efficacy of piperacillin-tazobactam in treating bloodstream infections due to ceftriaxone resistant *E. coli* or *K. pneumoniae*.	(Harris et al., 2018)
SENTRY	Surveillance conducted in the United States and Europe, parts of Asia, Latin America, and the Western Pacific monitoring resistance profile of clinical pathogens caused pneumonia, urinary tract infections, bloodstream infections and intra-abdominal infections (Started from 1997).	www.jmilabs.com/sentry-surveillance-program/
SMART	The Study for Monitoring Antimicrobial Resistance Trends is conducted worldwide monitoring resistance profile and trends of clinical pathogens (Started from 2002).	(Morrissey et al., 2013)

[a] Abbreviations: CRE, carbapenem-resistant *Enterobacteriaceae*; ESBL, extended-spectrum β-lactamase; RCT, randomised controlled trial.

Like *K. pneumoniae*, *Acinetobacter spp.*, especially *A. baumannii*, are frequently isolated in HAIs (Nguyen and Joshi, 2021, Pogue et al., 2013, Garnacho-Montero and Timsit, 2019). Compared with *Enterobacteriaceae*, the resistance rate to carbapenems in *Acinetobacter spp.* is higher. According to data from the EARS-Net in 2020, the average resistance rates of *Acinetobacter spp.* to carbapenems in European countries were hovering at a high level of 32.6% in 2016 and 38% in 2020 (ECDC, 2022). Moreover, eight countries reported a rate of carbapenem resistance in *Acinetobacter spp.* over 80%, with the highest resistance rate of 96.4% in Croatia, followed by Greece (94.6%), Romania (93.3%) and Lithuania (91.1%), were all exceeding 90% (ECDC, 2022). In terms of subregional analysis, the trend of resistance in the Eastern Europe increased sharply from 49.7% in 2013 to 72.6% in 2015 and then turned to be the region with the highest rate of carbapenem resistance in 2016 and 2017; in contrast, the Northern and Western regions of Europe had the lowest rates of carbapenem resistance during the same surveillance period, with the non-susceptible rate of no more than 10% of *Acinetobacter spp.* isolates (Ayobami et al., 2020). The prevalence of *Acinetobacter spp.* with carbapenem resistance reported in the EARS-Net from 2015 to 2020 is presented in Figure 1-5.

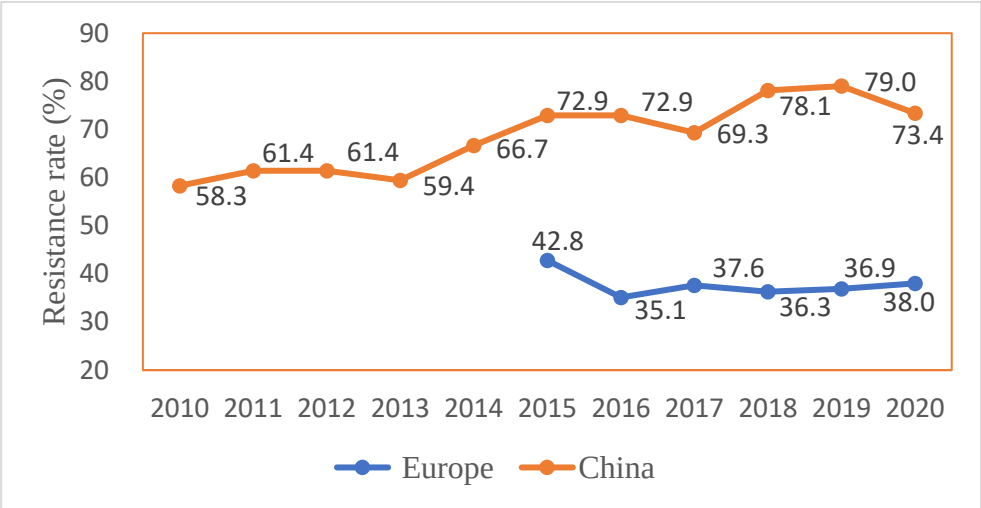

Figure 1-5. The prevalence and resistance trends of carbapenem-resistant *A. baumannii* reported in the European Antimicrobial Resistance Surveillance Network (EARS-Net) and the China Antimicrobial Surveillance Network (CHINET) from 2010 to 2020 (data from https://www.chinets.com/ and https://www.ecdc.europa.eu/en).

Regarding results from the SENTRY Antimicrobial Surveillance Program (1997-2016), significant decreases in the susceptibility to carbapenems of *Acinetobacter spp.* were observed in all regions (Gales et al., 2019). In the Asia-Pacific, the susceptibility rate of *Acinetobacter spp.* to meropenem was as high as 88.9% during 1997-2000 but reached the bottom in 2009-2012, with the susceptibility rate of only 18.5%, and then stayed around a lower level of 21% in 2013-2016. In Europe, the susceptibility rate continuously decreased from 55.7% to 22.2% during the 20 years of the surveillance period. Similar trends were also documented in Latin America and North America. The prevalence of *Acinetobacter spp.* isolates susceptible to meropenem in the SENTRY surveillance program from 1997 to 2016 is summarized in Figure 1-6.

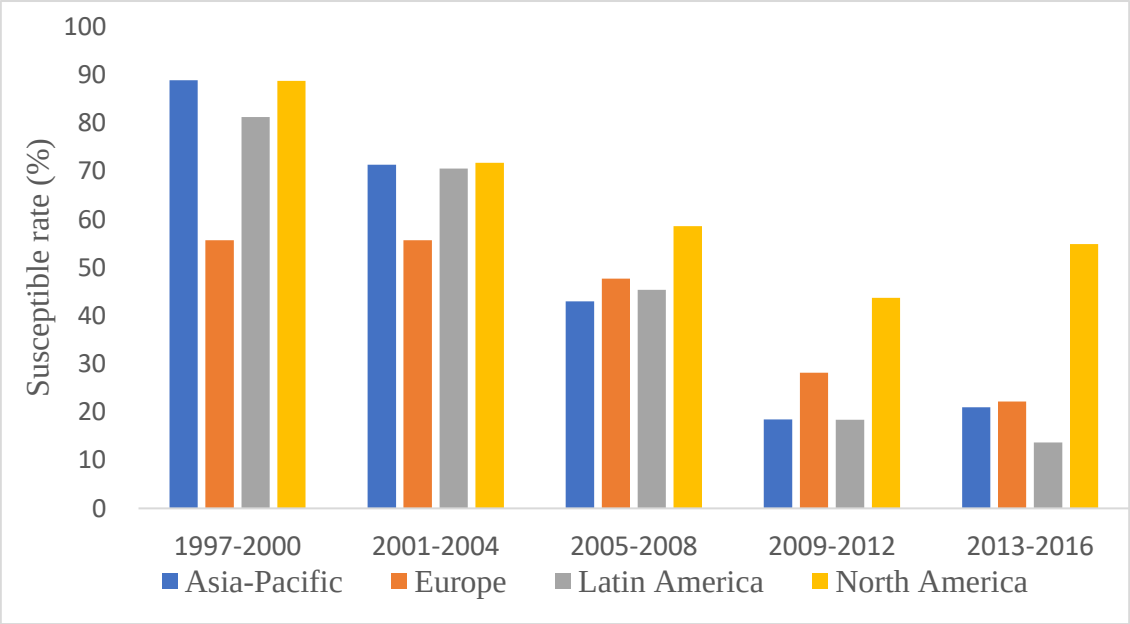

Figure 1-6. The prevalence of *Acinetobacter spp.* isolates susceptible to meropenem in the SENTRY surveillance program from 1997 to 2016 (Gales et al., 2019).

In the US, 75.9% and 75.4% resistance rates were reported in patients with central line-associated BSI and VAP caused by *Acinetobacter spp.* in long-term acute-care hospitals (Weiner et al., 2016). Although the resistance rate of *Acinetobacter spp.* was relatively lower in general wards than that in long-term acute-care hospitals, it was

still as high as 33.1% in patients with central line-associated BSI (Weiner et al., 2016). In China, the issue of CRAB is also challenging. According to data from the CHINET, the resistance rate to carbapenems in *A. baumannii* has already increased from 31% in 2005 to 70.7% in 2017 (Hu et al., 2018a). Moreover, in Henan province, the carbapenem resistance in *A. baumannii* has been reported to exceed 80% in 2016 (Hu et al., 2018a). Despite the fact that the Chinese government has implemented many stringent policies (e.g. The Chinese national action plan to combat antimicrobial resistance, national campaign to promote rational use of antibiotics, and national antimicrobial stewardship program) trying to control the increasing rate of carbapenem resistance in GNB, the rate of carbapenem resistance in *A. baumannii* was still considerably high, as reported at 72.3% in the latest published annual report of the CHNET for 2021 (Hu et al., 2021). The tread of carbapenem-resistance in *A. baumannii* reported in the CHINET from 2010 to 2015 has been summarized in Figure 1-5.

Apart from *K. pneumoniae* and *A. baumannii*, *S. maltophilia* has also emerged as an important opportunistic pathogen globally (Brooke, 2012). It is naturally resistant to carbapenems and mainly causes nosocomial infections, including HAP, VAP, and BSI, and rarely also causes other types of infections (Brooke, 2012, Looney et al., 2009). According to data from the SENTRY program (1997-2006) (Gales et al., 2019), in which 6467 *S. maltophilia* isolates were collected, 55.8% of the strains were isolated from hospitalized patients with pneumonia, 33.8% from the bloodstream, 7.8% from skin and skin structure, 1.2% from the urinary tract, 1.0% from intra-abdominal and the remaining 0.4% from other sites. In Europe, *S. maltophilia* was always one of the ten most frequently isolated pathogens in patients with ICU-acquired pneumonia between 2012 and 2017 (ECDC, 2019). The prevalence rate was 3.6% in 2012 and increased to 4.5% in 2017. The highest rate of *S. maltophilia* infection in ICU-acquired pneumonia was reported in Belgium, at 8.5%, followed by Spain (5.6%) and France (5.7%) in the 2017 annual report (ECDC, 2019). A recent study using data from the 2010-2015 US Premier Healthcare

Database indicated that *S .maltophilia was the* most common CRGNB causing BSI in the US, accounted for 30.3% of all carbapenem-resistant pathogens (Cai et al., 2020). In China, *S. maltophilia* accounted for 2.8% of all clinical isolates submitted to the CHINET surveillance program in 2020, which ranked ninth among the top 20 most frequently isolated clinical pathogens (Hu et al., 2021). However, due to the lack of systematic surveillance regarding infections caused by *S. maltophilia*, it is difficult to estimate the accurate burden of such infections in China.

1.3.2 MECHANISMS OF CARBAPENEM RESISTANCE

The acquisition of genes encoding carbapenemases is the leading mechanism for *Enterobacteriaceae* to develop carbapenem resistance (Ruppé et al., 2015). According to the Ambler classification system, there are four types of β-lactamases, among which classes A, B and D could mediate carbapenem resistance in GNB (Ambler, 1980, Bush and Jacoby, 2010). *Klebsiella pneumoniae* carbapenemase (KPC) belongs to the class A group and is the most common carbapenemase in CRE in some regions, like the US and China (Han et al., 2020, Hansen, 2021). Enzymes in class B, also named metallo-β-lactamases (MBL), have potent hydrolysing activity against carbapenems, including New Delhi metallo-β-lactamase (NDM), imipenemase metallo-β-lactamase (IMP) and Verona integron-encoded metallo-β-lactamase (VIM) (Abboud et al., 2016). Oxacillin carbapenemases (OXA) belong to the class D group, and OXA-48, one of the members in this group, is frequently involved in mediating carbapenem resistance in *Enterobacteriaceae* (Hansen, 2021, Nordmann et al., 2011, Pitout et al., 2019). The classification of clinically important β-lactamases, their corresponding resistance type and available inhibitors are listed in Table 1-2.

Table 1-2. Classification, characteristics, and inhibitors of clinically important β-lactamases mentioned in this thesis.

Molecular class	Typical enzymes	Clinical resistance type	Inhibitors
serine-β-lactamases			
Class A	KPC[a]	carbapenem resistance	avibactam,vaborbactam, relebactam
	L2	cephalosporins resistance	no clinically approved inhibitor
	CTX-M, SHV, TEM	Third-generation cephalosporins resistance	clavulanic acid, tazobactam, avibactam
Class C	AmpC	Third-generation cephalosporins resistance	aztreonam, avibactam, vaborbactam
Class D	OXA-48, OXA-23	carbapenem resistance	avibactam (OXA-48)
metallo-β-lactamases			
Class B	NDM, IMP, VIM	carbapenem resistance	aztreonam
	L1	carbapenem resistance	no clinically approved inhibitors

[a]Abbreviations: AmpC, AmpC type β-lactamase; CTX-M, cefotaxime hydrolysing β-lactamase-Munich; IMP, imipenem metallo-β-lactamase; KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; OXA, oxacillin carbapenemase; SHV, sulfhydryl variant of the TEM enzyme; TEM, Temoneira class A extended-spectrum β-lactamase; VIM, Verona integron-encoded metallo-β-lactamase. The table was adapted from (Bush and Bradford, 2020, Nordmann and Poirel, 2019)

Similar to the epidemiological profile of CRGNB, the distribution of carbapenemases also varies geographically. According to data from the European survey of carbapenemase-producing *Enterobacteriaceae* (EuSCAPE study*)* (Grundmann et al., 2017), among 1203 submitted non-susceptible *K. pneumoniae* isolates, 70.7% (850) were confirmed as carbapenemase-producing strains. The most frequently detected carbapenemase was KPC (31.5%), followed by OXA-48-like (25.8%), NDM (7.7%) and VIM (5.7%). It is interesting to note that among those carbapenem non-susceptible isolates, 29.3% were negative for the four carbapenemases. Unlike in Europe, genes responsible for carbapenem resistance in the US predominantly belonged to the KPC family. Although other resistant genes (like bla_{NDM} and $bla_{OXA\text{-}48\text{-}like}$ genes) have also been detected in *Enterobacteriaceae* with carbapenem resistance, $bla_{KPC\text{-}2}$ and $bla_{KPC\text{-}3}$ were still the primary genes mediating carbapenem resistance in America, accounting for 51% and 41% respectively based on the

recently published CRACKLE-2 study (van Duin et al., 2020). China shares a similar situation to that of America. In a recent survey, which collected CRKP strains from 27 provinces and municipalities in China during 2016-2020, carbapenemases were detected in 95.8% of the CRKP strains, of 84.3% were KPC-2, 10.5% were NDM, and only 0.8% were OXA-48-like enzymes (Zhang et al., 2017). The similar distribution of carbapenemases in *K. pneumoniae* isolates was also demonstrated in a study which collected CRE from 65 hospitals in 25 provinces across China between 2012 and 2016 (Wang et al., 2018). The distribution of carbapenemases in *Enterobacteriaceae* described in this section has been summarized in Figure 1-7.

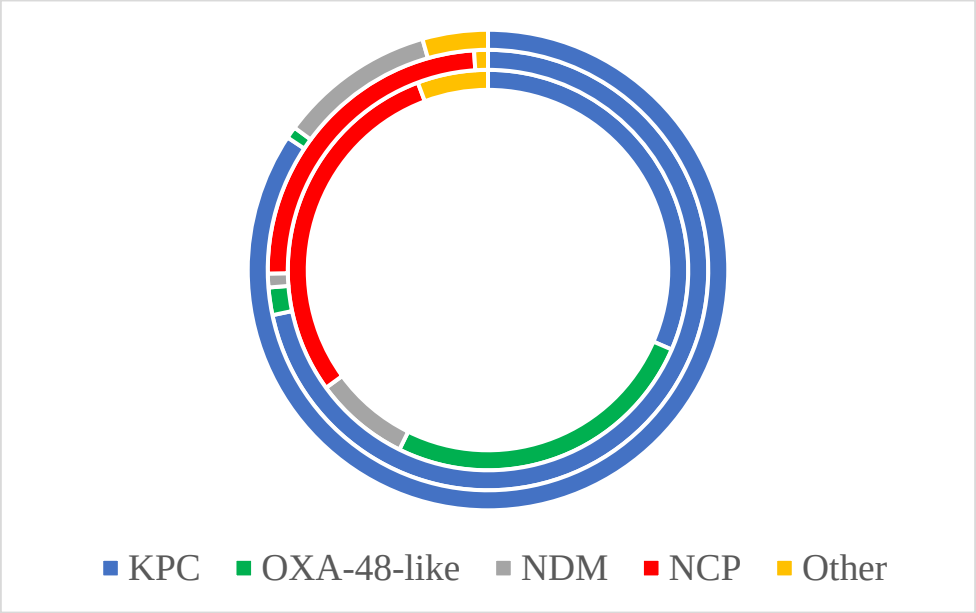

Figure 1-7. The distribution of carbapenemases in *Enterobacteriaceae* reported in Europe (the inner circle, data retrieved from (Grundmann et al., 2017)), the US (the middle circle, data retrieved from (van Duin et al., 2020)) and China (outer circle, data retrieved from (Zhang et al., 2017)). KPC, *Klebsiella pneumoniae* carbapenemase; NCP, non-carbapenemase producing; NDM, New Delhi metallo-β-lactamase; OXA, oxacillin carbapenemase.

Different from *Enterobacteriaceae*, carbapenemases in CRAB mainly belong to the Ambler class D group, the oxacillinase family (Brown and Amyes, 2006, Kostyanev et al., 2021). Although intrinsic *oxaAb* genes (known as $bla_{OXA\text{-}51\text{-}like}$) exist in *A. baumannii*, the evolution of carbapenem resistance is still mainly due to the horizontal acquisition of OXA genes (Hamidian and Nigro, 2019). Because of the

generally weak carbapenem hydrolysing activity of OXAs, *A. baumannii* strains with carbapenem resistance phenotype usually have an insertion sequence (IS) upstream of *bla*$_{OXA}$ genes, which could enhance the expression of *bla*$_{OXA}$ genes by providing a strong promoter (Corvec et al., 2007, Turton et al., 2006). This genomic background is frequently observed in strains with carbapenem resistance mediated by *oxaAb* genes (Hamidian and Nigro, 2019). Among all the acquired *bla*$_{OXA}$ genes, *bla*$_{OXA\text{-}23\text{-}like}$ is the most detected gene in CRAB strains worldwide (Nordmann et al., 2011, Nordmann and Poirel, 2019). According to a recently published study, 228 CRAB recovered from 10 European countries, 67.7% of the isolates harboured *bla*$_{OXA\text{-}23}$, and 30.1% harboured *bla*$_{OXA\text{-}72}$ (Kostyanev et al., 2021). In strains carrying *bla*$_{OXA\text{-}72}$, an IS element was found upstream of the gene and co-located on a small plasmid. In a study conducted in the US from 2013 to 2017, among the included CRAB strains, 73% of them harboured acquired *bla*$_{OXA}$ genes, and most were *bla*$_{OXA\text{-}23}$ (McKay et al., 2022). The prevalence of OXA-23-like carbapenemase is higher in China than in Europe and the US, which was reported as high as 94.3% in clinically collected CRAB strains (Jiang et al., 2019). Apart from the class D carbapenemases, other carbapenemases like MBL, and KPC, were also detected in *A. baumannii* strains. In a study conducted in Southern China, 23.4% of the CRAB contained the *bla*$_{NDM\text{-}1}$ *gene* (Li et al., 2019b). *bla*$_{KPC}$ genes were less frequently detected in CRAB and were usually acquired from *Enterobacteriaceae* through horizontal transformation (Martinez et al., 2016).

Non-enzymatic mechanisms, including porin mutations and overexpression of efflux pumps, also play a role in carbapenem resistance in *A. baumannii* and *K. pneumoniae*. Studies have indicated that the mutation in CarO and YiaD contribute to carbapenem resistance in *A. baumannii* clinical isolates (Zhu et al., 2019, Han et al., 2022). Moreover, frequently higher levels of expression in genes encoding efflux pumps, like *adeB*, *adeR*, *adeS*, *adeJ* and *adeM*, are found in CRAB strains compared to susceptible strains (Zhang et al., 2021b, Hou et al., 2012). Loss or modification of porins and overexpression of efflux pumps was also reported relating to

carbapenem resistance in *K. pneumoniae,* but less than that reported in *A. baumannii* (Tsai et al., 2011, Hamzaoui et al., 2018).

S. maltophilia has intrinsic resistance to β-lactams mediated by chromosomally encoded β-lactamases, called L1 and L2 (Nordmann and Poirel, 2019, Chang et al., 2015). The L1 β-lactamases belong to the Amble class B, which possesses carbapenem hydrolysing activity; in contrast, the L2 β-lactamases are categorized as class A β-lactamases mainly mediating resistance to cephalosporins (Gil-Gil et al., 2020). Both the L1 and L2 enzymes are primarily controlled by the AmpR regulator and induced by β-lactams (Okazaki and Avison, 2008). Apart from AmpR, other elements like AmpD-like protein, AmpN, were also involved in modulating the expression of β-lactamases in *S. maltophilia* (Huang et al., 2010). Moreover, genes encoding efflux pumps might also contribute to some extent to carbapenem resistance in *S. maltophilia*. Studies have observed that the overexpression of *smeGH* and *smeABC* in *S. maltophilia* isolates was associated with higher minimum inhibitory concentration (MIC) of β-lactams, including carbapenems; still, the impact of these efflux pumps might not be as important as that of L1 enzymes in mediating carbapenem resistance in *S. maltophilia,* because the MIC of carbapenems in wild-type strains is already extremely high (mean MIC of 288-488 mg/L) (Chang et al., 2004, Li et al., 2019a).

1.3.3 TREATMENTS AND RESEARCH NEEDS FOR SELECTED CRGNB

To facilitate doctors in choosing antibiotics for infections caused by CRGNB, many clinical guidelines have been published (Sy et al., 2022, Tamma et al., 2021, Tamma et al., 2022, Paul et al., 2022, Tiseo et al., 2022). Recommendations in these guidelines are mainly derived from the epidemiological data of the *in vitro* activity of certain antibiotics and from results of observational retrospective studies. Among those recommended antibiotics, most of the newly approved antibiotics, like meropenem-vaborbactam, imipenem-relebactam and cefiderocol, have not been

marketed in China. Regimens for treating infections caused by CRGNB are usually restricted to tigecycline, polymyxins (B and E; also known as colistin) and ceftazidime-avibactam, with the latter only available in a small proportion of hospitals in China. Therefore, in the following part, I will review therapeutic options for CRGNB focusing on those three antibiotics. The mechanisms of action of these three antibiotics are different. Ceftazidime-avibactam is a β-lactam/β-inhibitor in which ceftazidime inhibits bacterial cell wall synthesis by binding to penicillin-binding protein, and avibactam inactivates β-lactamases, including carbapenemases, then protects ceftazidime from degradation (Shirley, 2018). Tigecycline inhibits protein translation in bacteria by binding to the 30S ribosomal subunit (Yaghoubi et al., 2022). In contrast, the mechanism of action of polymyxins is not clear but is thought to disturb the phospholipids of bacterial cell membranes (Bialvaei and Samadi Kafil, 2015). The potential *in vitro* activity of these three antibiotics against CRGNB with different resistance mechanisms were summarized in Table 1-3.

Table 1-3. *In vitro* activity of available antibiotics against several antimicrobial resistant Gram-negative bacteria.

Antibiotics	**ESBLs**[a]	**CRAB**	**CRE**				**SM**
			KPC	**OXA-48**	**MBL**	**Non-CP**	
Ceftazidime-avibactam	Yes	No	Yes	Yes	No	Uncertain	No
Tigecycline	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Polymyxin B/E	Yes	Yes	Yes	Yes	Yes	Yes	Yes

[a]Abbreviations: CRAB, carbapenem-resistant *Acinetobacter baumannii*; CRE, carbapenem-resistant *Enterobacteriaceae*; ESBL, extended-spectrum β-lactamase; KPC, *Klebsiella pneumoniae carbapenemase*; MBL, metallo-β-lactamase; Non-CP, non-carbapenemase producing; OXA-48, oxacillin carbapenemase-48; SM, *Stenotrophomonas maltophilia*. The table was adapted from (Paul et al., 2022, Tamma et al., 2022).

1.3.3.1 CEFTAZIDIME-AVIBACTAM TREATMENT

Ceftazidime-avibactam is the first recommended antibiotic for treating CRKP by both the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines and the Infectious Diseases Society of America (IDSA) 2022 Guidance (Paul et al., 2022, Tamma et al., 2021), due to its good *in vitro* inhibitory activity against Amble class A (KPC) and Amble class D (OXA-48) carbapenemases, the most distributed carbapenemases in clinical isolates with carbapenem resistance (Zasowski et al., 2015, Dietl et al., 2020, Shirley, 2018). Results from the International Network for Optimal Resistance Monitoring (INFORM) global surveillance program indicated that the overall susceptibility rate to ceftazidime-avibactam among the 1460 meropenem-non-susceptible isolates collected during 2015-2017 was 73%, with Latin America reported the highest susceptibility rate of 87.5%, followed by Europe 76.8% (Spiliopoulou et al., 2020). In the subgroup analyses stratified with different types of resistance mechanisms, 99.8% of carbapenemase-positive but MBL-negative isolates were susceptible to ceftazidime-avibactam, and the susceptibility rate in isolates which were carbapenemase negative was also as high as of 95.9%. In terms of OXA-48, ceftazidime-avibactam also demonstrated good *in vitro* activity, with the susceptibility rate reported as 92.5% of OXA-48 positive strains and 99.2% in OXA-48-positive but MBL-negative strains, respectively. As avibactam could not inhibit the hydrolysing activity of MBLs, almost all MBL-positive clinical isolates demonstrated a ceftazidime-avibactam resistant phenotype (98.9%). In China, the overall susceptibility rate of CRKP to ceftazidime-avibactam was 84.6% according to data from the CHINET 2018 (Yang et al., 2020). Given these good *in vitro* epidemiological data, ceftazidime-avibactam was therefore recommended as the first choice for infections caused by CRKP.

1.3.3.2 TIGECYCLINE TREATMENT

Different from CRKP, CRAB usually demonstrates resistance to ceftazidime-avibactam, further limiting antibiotic choices for the treatment of infections caused

by such a pathogen (Paul et al., 2022, Yang et al., 2020, Tamma et al., 2022). Under this situation, tigecycline and polymyxins turn to be popular therapeutic options, as they both demonstrate strong *in vitro* activity against CRAB and CRKP and are irrelevant to mechanisms of carbapenem resistance. According to data from the INFORM program 2015-2017, the overall susceptibility rate of tigecycline was 78.1% (Spiliopoulou et al., 2020). Regarding isolates producing MBLs, tigecycline also demonstrate good antimicrobial activity, with 71.9% of isolates susceptible to tigecycline (MIC $\leqslant$ 1 mg/L) (Spiliopoulou et al., 2020). In China, the susceptibility rates of tigecycline against CRE were higher than that reported in the INFORM program, with 93.4% susceptible to tigecycline (Yang et al., 2020). In terms of CRAB, tigecycline was still one of the most active agents, with only 3.6% of the isolates resistant to tigecycline (MIC > 2mg/L) (Hu et al., 2021). However, although epidemiological data have demonstrated such promising *in vitro* activities of tigecycline, some questions still need to be addressed when choosing tigecycline as the regimen for CRGNB.

Clinical effectiveness of antibiotics depends not only on *in vitro* activity but also on pharmacokinetic (PK) and pharmacodynamic (PD) profiles. Given the PK/PD profiles of tigecycline, the essential question is what dose is the optimized dosage in treating infections caused by MDR bacteria, especially CRGNB (Xie et al., 2014)? In 2005, tigecycline was approved by the US Food and Drug Administration (FDA) to treat community-acquired pneumonia (CAP), cIAI and complicated skin and soft tissue infections (cSSTI) at its standard-dose (100 mg initial dose followed by 50 mg every 12 hours) (Kaewpoowat and Ostrosky-Zeichner, 2015, Shakil et al., 2008). Due to its good antibacterial activity against MDR bacteria, tigecycline has also been widely used off-label in treating nosocomial infections, including VAP, HAP, and catheter-associated BSI, which are usually involved in bacteria with multidrug-resistance (Kengkla et al., 2018, Xu et al., 2016, Wang et al., 2017, Yahav et al., 2011). However, the efficacy of the standard-dose of tigecycline (SDT) in treating severe infections, especially those caused by MDR bacteria, is still uncertain. In 2008, a

phase 3, open-label, non-comparative study indicated that tigecycline might be a safe and efficacious antimicrobial agent in patients with serious difficult-to-treat infections caused by resistant GNB, as the clinical cure was reported of 72.2% (95% CI 54.8%-85.8%) (Vasilev et al., 2008). Other observational studies with a small sample size also demonstrated similar results indicating that tigecycline might be a promising option for MDR infections (Schafer et al., 2007, Poulakou et al., 2009). Despite these comparable findings, in 2010, the FDA released a black box warning of tigecycline because it was associated with an increased mortality compared to other active antibiotics (FDA, 2010). Contrary to the FDA assessment, a meta-analysis conducted by Tasina et al. in 2011, which included 14 randomized controlled trials (RCT) containing 7,400 patients, did not demonstrate worse clinical outcomes of tigecycline; the results indicated that although the treatment success was lower in patients receiving tigecycline, the difference did not reach an statistical significance (OR 0.87, 95% CI 0.74-1.02) (Tasina et al., 2011). However, in the following year, another meta-analysis by including 13 non-inferiority RCTs with 7434 patients demonstrated different results; in which, tigecycline was associated with increased mortality (RD 0.7%, 95% CI 0.1%-1.2%, p = 0.01) and non-cure rates (RD 2.9%, 95% CI 0.6%-5.2%, p = 0.01) (Prasad et al., 2012). An updated meta-analysis in 2015 also demonstrated worse clinical outcomes in patients receiving tigecycline than those receiving other active antibiotics (Shen et al., 2015). Therefore, it is recommended against using the SDT in treating such resistant infections (Paul et al., 2022). PK/PD research suggested that the lack of efficacy might be due to the inadequate concentrations of tigecycline in the serum and lungs (Leng et al., 2021). When using the high-dose of tigecycline (HDT) (200 mg initial dose followed by 100 mg every 12 hours), concentrations in the serum and lungs were satisfactory; it increased the probability of target attainment of tigecycline from 72.2% to 99.2% at an MIC of 1mg/L, and from 11.3% to 70.8% at an MIC of 2 mg/L (De Pascale et al., 2020). Considering these PK/PD profiles of tigecycline, the high-dose regimen was then proposed in clinical practice. A retrospective study including patients with severe infections mainly caused by CRAB and CRKP has demonstrated that the HDT

regimen might result in better clinical outcomes than the SDT regimen, as it was associated with lower mortality in patients with VAP (De Pascale et al., 2014). However, another study included VAP mainly caused by MDR *A. baumannii*, illustrating that the HDT regimen did not reduce the 28-day mortality compared with the SDT (Chen and Shi, 2018). Therefore, the efficacy of the HDT regimen is still unclear. To further assess the effectiveness of the HDT regimen in treating MDR infections, a meta-analysis synthesizing the current clinical evidence would be highly welcomed.

1.3.3.3 POLYMYXIN COMBINATION THERAPY

Like tigecycline, polymyxins also demonstrates good *in vitro* activity against CRKP and CRAB, with 93.8% of CRKP and 90.1% of CRAB were susceptible to them. However, an important question here is whether the combination therapy with polymyxin would result in better clinical outcomes than the monotherapy in treating infections caused by CRGNB? Due to the suboptimal efficacy of the currently available antibiotics, doctors are predisposed to choose combination therapy aiming to improve the therapeutic efficacy (Piperaki et al., 2019, El-Sayed Ahmed et al., 2020, Savoldi et al., 2021). In a worldwide cross-sectional survey, 86.3% of the respondents acknowledged they preferred prescribing combination therapy for infections caused by CRGNB; among those reported combination regimens, a carbapenem plus polymyxin was the most frequently used regimen, followed by tigecycline plus polymyxin (Papst et al., 2018). Although *in vitro* studies have demonstrated synergistic effects of these combinations, clinical efficacy is still uncertain (Righi et al., 2020, Scudeller et al., 2021). In 2016, a meta-analysis indicated that compared with patients receiving polymyxin monotherapy, the combination therapy with carbapenems was associated with decreased mortality (Zusman et al., 2017). However, such a beneficial effect has not been demonstrated in a subsequent RCT, in which patients receiving the combination therapy did not have a lower rate of clinical failure at 14 days after randomization than those receiving colistin monotherapy (RR 0.93%, 95% CI 0.83-1.03) (Paul et al., 2018).

Therefore, the combination with polymyxin and a carbapenem was ranked as low-certainty evidence by the recent ESCMID clinical guidelines (Paul et al., 2022). Clinical studies regarding the efficacy of tigecycline in combination with polymyxin in treating CRGNB are rarely limited. As of writing, only two studies evaluated its effectiveness in treating CRAB bacteraemia (Amat et al., 2018, Cheng et al., 2015). Compared with colistin-carbapenem combination therapy, colistin-tigecycline therapy did not reduce the crude 14-day mortality and in-hospital mortality and was even associated with excess 14-day mortality in the subgroup of tigecycline MIC greater than 2 mg/L (Cheng et al., 2015). Moreover, the combined use of tigecycline with high-dose colistin was not associated with better clinical outcomes than those receiving high-dose colistin monotherapy (Amat et al., 2018). Different from what has been reported in European countries, tigecycline is much more widely used than polymyxins in China in the treatment of infections due to CRGNB. Therefore, it is necessary to conduct studies assessing whether the adjunctive therapy of polymyxin to tigecycline, especially the high-dose regimen, would result in better clinical outcomes.

1.3.3.4 TREATMENTS FOR *S. MALTOPHILIA*

For *S. maltophilia*, therapeutic options are pretty limited due to its natural and acquired antibiotic resistance, which mediates resistance against a broad array of commonly used antibiotics, like aminoglycosides, β-lactams, macrolides, cephalosporins and carbapenems (Chang et al., 2015). Therefore, clinical therapies are mainly restricted to trimethoprim-sulfamethoxazole and fluoroquinolones because of their good *in vitro* activity. According to the results from the SENTRY antimicrobial surveillance program 2016-2019, the susceptibility rate of *S. maltophilia* to trimethoprim-sulfamethoxazole was 96.4% in western Europe, 94.3% in eastern Europe and 94% in the US (Sader et al., 2021). Although the susceptibility rate of levofloxacin was lower than that of trimethoprim-sulfamethoxazole against *S. maltophilia*, it still demonstrated promising *in vitro* activity, as 83.7% of isolates were reported susceptible in western Europe and

84% in eastern Europe (Sader et al., 2021). From the results of CHINET 2020, the antibiotic-resistant diagram of *S. maltophilia* was similar to that reported in the SENTRY program, in which 92.6% of clinical strains were susceptible to trimethoprim-sulfamethoxazole, and 84.6% were susceptible to levofloxacin (Hu et al., 2021). However, due to the high allergy rate, shortage of intravenous dosage of trimethoprim-sulfamethoxazole, and increasing resistance to fluoroquinolones, alternative antibiotics for treating infections caused by *S. maltophilia* are urgently required. Minocycline and tigecycline might be promising alternatives to trimethoprim-sulfamethoxazole and fluoroquinolones in treating *S. maltophilia* infections as they demonstrate good *in vitro* activity. Among the 7485 clinical isolates of *S. maltophilia* in the 2020 CHINET report, the susceptibility rate to minocycline and tigecycline was 93.3% and 94.6%, respectively (Hu et al., 2021). In the SENTRY surveillance report 2016-2019, 99.2% of *S. maltophilia* strains were susceptible to minocycline in the US, and 100% were susceptible in Europe (Sader et al., 2021). Moreover, clinical studies have already demonstrated comparable or even better effectiveness of minocycline in treating infections caused by *S. maltophilia* compared with other therapeutic options (Junco et al., 2021, Jacobson et al., 2016, Hand et al., 2016). A retrospective study included 45 patients with *S. maltophilia* infections, of whom 22 received trimethoprim-sulfamethoxazole and 23 received minocycline; the results indicated that minocycline therapy was not inferior to trimethoprim-sulfamethoxazole therapy (Hand et al., 2016). A recent study with 284 patients demonstrated similar results, in which the rate of treatment failure and 30-day mortality were similar in patients receiving minocycline, fluoroquinolone or trimethoprim-sulfamethoxazole; after controlling for confounders, receiving minocycline was associated with lower mortality than trimethoprim-sulfamethoxazole (OR 0.2, 95% CI 0.1-0.7) (Junco et al., 2021). Unlike minocycline, although tigecycline also demonstrates good *in vitro* activity against *S. maltophilia* strains, clinical studies assessing its effectiveness in treating *S. maltophilia* infections are rarely limited and all restricted to case series and case reports (Wu and Shao, 2014, Tekçe et al., 2012, Farrar et al., 2020). Therefore, it is

reasonable to conduct a well-designed study evaluating the effectiveness of tigecycline in the treatment of infections caused by *S. maltophilia*, as it has been used in clinical practice for such diseases.

1.4 3GC-R ENTEROBACTERIACEAE

3GC-R *Enterobacteriaceae* is another group of pathogens that is becoming a significant threat (Pitout and Laupland, 2008). This group was listed together with CRGNB as one of the major critical priority pathogens by the WHO due to its increasing prevalence, limited antibiotic choices and relatively high mortality (Tacconelli et al., 2018). Studies have demonstrated that infections caused by these resistant pathogens were more likely to receive delayed appropriate antibiotic therapy, consequently leading to more extended hospital stays, higher costs, and higher mortality rates than their susceptible counterparts (Chen et al., 2020, Joo et al., 2017, Maslikowska et al., 2016, Ndir et al., 2016, Tacconelli et al., 2019). Generally, resistance to 3GCs in these pathogens is mainly mediated by the extended-spectrum β-lactamases (ESBL) or AmpC β-lactamase (Castanheira et al., 2021, Meini et al., 2019, Paterson and Bonomo, 2005). As the prevalence of AmpC β-lactamase in 3GC-R *Enterobacteriaceae* is far lower than that of ESBLs, especially in *E. coli* and *K. pneumoniae* (Meini et al., 2019); therefore, in the following sections, the main focus is the epidemiology, molecular mechanisms and therapeutic options for ESBL-producing *Enterobacteriaceae*.

1.4.1 EPIDEMIOLOGY OF 3GC-R *ENTEROBACTERIACEAE*

Among *Enterobacteriaceae*, *E. coli* and *K. pneumoniae* are the most frequently reported pathogens that cause both community and hospital-acquired infections, including BSI, UTI and respiratory tract infections (Khanfar et al., 2009, Pitout and Laupland, 2008). According to data from the SENTRY surveillance program (1997-2016), *Klebsiella spp.* (11%) and *E. coli* (9.6%) were the third and fourth bacterium among all pathogens that caused pneumonia in Europe (Sader et al., 2019). They

were also the top two isolated Gram-negative pathogens (*E. coli* 27% and *K. pneumoniae* 10.1%) in BSI (Diekema et al., 2019). Resistance to expanded-spectrum cephalosporins in these *Enterobacteriaceae* has been observed with a dramatic increase during the SENTRY surveillance period (Diekema et al., 2019, Sader et al., 2019). In patients with BSI, *E. coli* with the ESBL phenotype was found in around 5% of the cases in 1997-2000, but this increased to more than 20% in 2013-2016. During the same period, resistance to 3GCs in *Klebsiella spp.* grew more than 10% and reached over 30% during 2013-2016 (Diekema et al., 2019) (Figure 1-8).

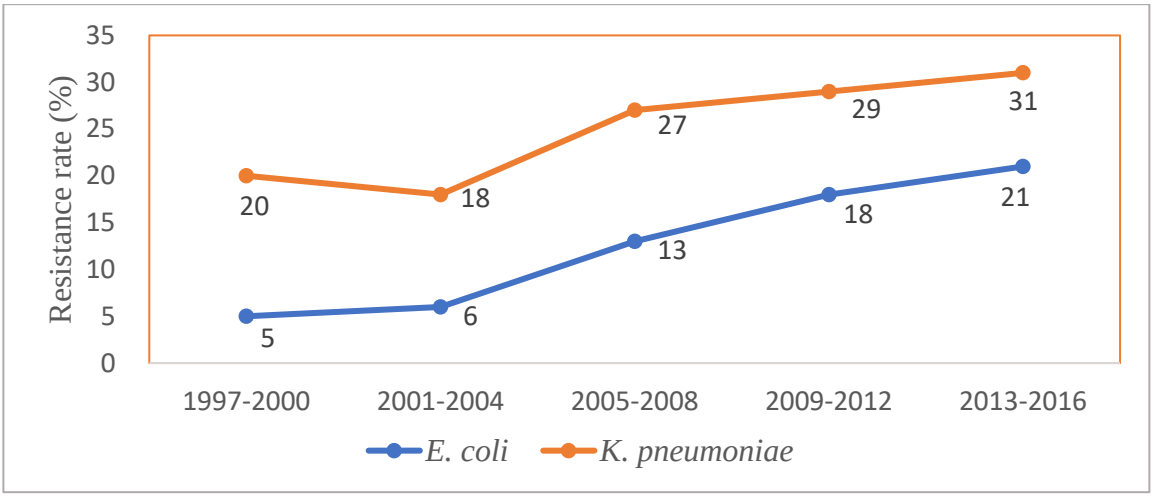

Figure 1-8. The prevalence of third-generation resistant *E. coli* and *K. pneumoniae* isolated from bloodstream infections in the SENTRY surveillance program (1997-2016) (Diekema et al., 2019).

Similar to BSI, pneumonia caused by *Enterobacteriaceae* that were resistant to 3GCs also increased significantly (Sader et al., 2019). During 1997-1998, only around 5% *of E. coli* were non-susceptible to ceftriaxone, but the rate increased fivefold in the following 20 years, reached at 25% in 2015-2016. A subregional analysis showed the highest increase of ESBL-producing *E. coli* (26.9%) was in Latin America, while Europe demonstrated the highest growth in ESBL-producing *K. pneumoniae* (19.5%) during the surveillance period (Castanheira et al., 2019) (Figure 1-9).

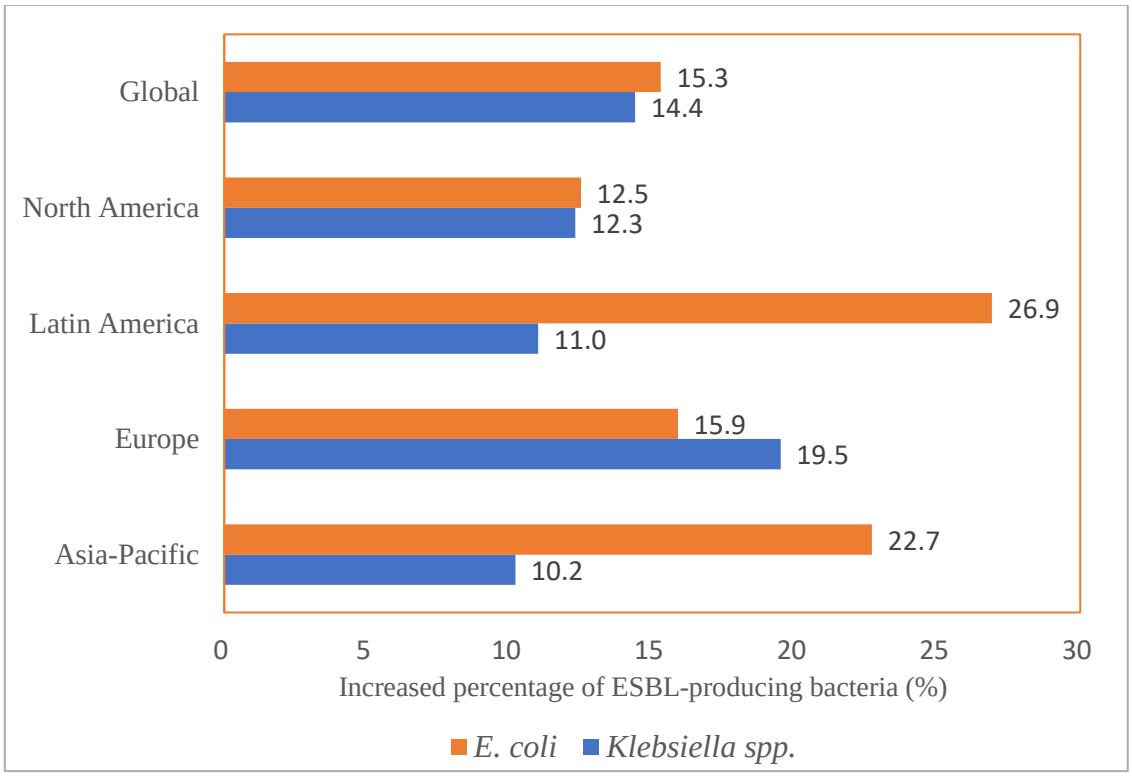

Figure 1-9. The increased percentage of ESBL-producing *E. coli* and *K. pneumoniae* in the SENTRY program from 1997 to 2016 *(Castanheira et al., 2019)*.

A study that collected data from 890 hospitals in the US indicated a 53.3% increase in ESBL-producing *Enterobacteriaceae* infection between 2012 and 2017, from 38 cases to 57 cases per 10,000 hospitalizations (Jernigan et al., 2020). An even more serious situation was detected in China. In 2005, the resistance rate to cefotaxime in *E. coli* was 52.2%, peaked at 63.2% in 2010, then kept stable at around 60% during the following years (2012 to 2017) (Hu et al., 2018a). Like *E. coli*, resistance to cefotaxime in *K. pneumoniae* was also relatively stable and high during the surveillance period, hovering around 50% (Hu et al., 2018a). The prevalence of ceftriaxone (or cefotaxime) resistant *E. coli* and *K. pneumoniae* isolated in the CHINET between 2005 and 2020 has been summarized in Figure 1-10.

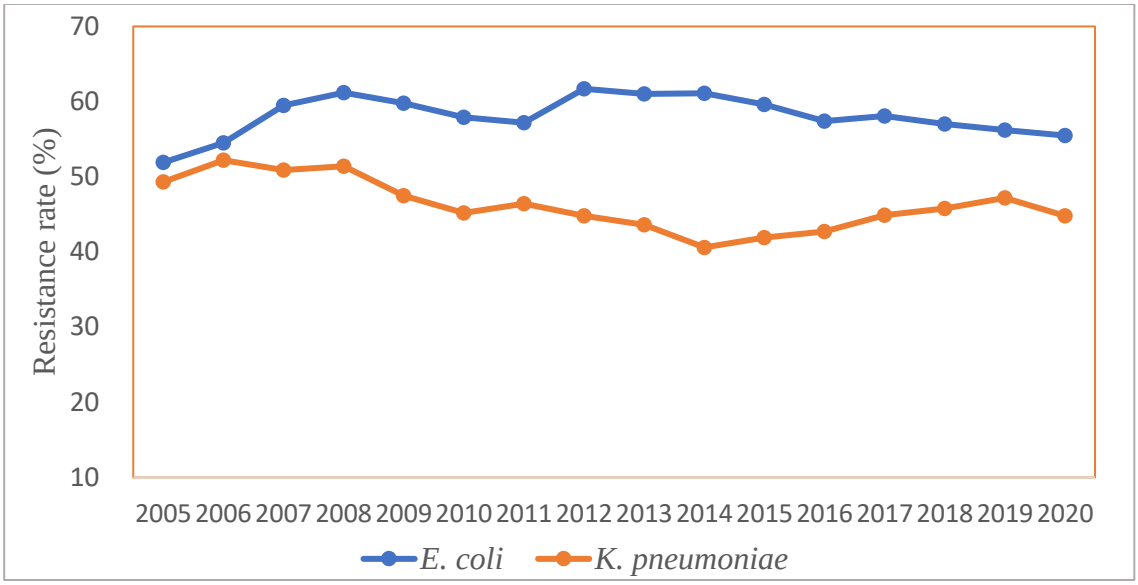

Figure 1-10. The prevalence of ceftriaxone (or cefotaxime) resistant *E. coli* and *K. pneumoniae* isolated in the CHINET between 2005 and 2020 (data from https://www.chinets.com/).

1.4.2 Resistance mechanisms to 3GCs

Resistance to β-lactams in *Enterobacteriaceae* is primarily mediated by β-lactamases, a group of enzymes that can hydrolyse β-lactams (Bush, 2018). Among these enzymes, ESBLs and AmpCs represent the two main groups of β-lactamases responsible for expanded-spectrum cephalosporins resistance (Castanheira et al., 2021, Tamma et al., 2019). Based on the Amble classification, ESBLs belong to the Class A (Ghafourian et al., 2015). They are characterized by the ability to hydrolyse expanded-spectrum cephalosporins (e.g., ceftazidime, ceftriaxone, cefotaxime) and the inhibition by classical β-lactamases inhibitors, especially clavulanate (Castanheira et al., 2021). Although many ESBLs have been discovered in GNB to date, the major types are TEM (Temoneira class A extended-spectrum β-lactamase), SHV (sulfhydryl variant of the TEM enzyme) and CTX-M (cefotaxime hydrolysing β-lactamase-Munich) (Peirano and Pitout, 2019). The classification, characteristics, and available inhibitors of these β-lactamases can be found in Table 1-2.

1.4.2.1 TEM-TYPE B-LACTAMASES

Initially, the TEM-type β-lactamase cannot hydrolyse expanded-spectrum cephalosporins (Bradford, 2001). However, after two amino acid substitutions in specific positions (Gly238Ser and Glu240Lys), the new TEM-variants are able to perform this enzymatic action and bacteria containing them demonstrate an ESBL-phenotype (Raquet et al., 1994). The first variant showed ESBL-activity was TEM-3, and till now, more than 246 variants have been identified (Sougakoff et al., 1988). Like carbapenemases, the distribution of TEM-type ESBLs also varied geographically. In a recent molecular epidemiology study, the most prevalent TEM-type ESBLs in Europe were TEM-29 and TEM-71, while TEM-10 was the most common ESBL in the US (Sepp et al., 2019, Wiener et al., 1999). There have not been such national molecular epidemiology studies in China, so information regarding the prevalence of TEM-type ESBLs is limited. In small-scale molecular epidemiology studies conducted in China, no such genes were detected (Miao et al., 2017, Zhang et al., 2016).

1.4.2.2 SHV-TYPE B-LACTAMASES

SHV-type β-lactamases are highly related to TEM-type β-lactamases, with only a few amino acid substitutions (Paterson and Bonomo, 2005). Similar to the parental TEM, the first identified SHV-1 did not present the ability to hydrolyse expanded-spectrum cephalosporins. The first SHV-type ESBL was SHV-2, isolated from *Klebsiella ozaenae* with a single amino acid substitution (Gly238Ser) from SHV-1 (Huletsky et al., 1993). As of writing, 228 variants of SHV-type β-lactamases have been identified. In the clinic, SHV-5 and SHV-12 are the most prevalent variants found in *Enterobacteriaceae* and are very often found in *K. pneumoniae* (Kazmierczak et al., 2020, Empel et al., 2008, Perilli et al., 2011). A recent Europe molecular study indicated that the prevalence of SHV-type ESBLs in *K. pneumoniae* ranged from 3.1% to 17% (Kazmierczak et al., 2020). Like TEM-type ESBLs, although SHV-type ESBLs are still encountered in clinical isolates with the

ESBL-phenotype, the prevalence of these two types of ESBLs is continually going down.

1.4.2.3 CTX-M-TYPE B-LACTAMASES

Instead, CTX-M-type ESBLs, especially the later identified variants, possessing potent hydrolytic activity against cefotaxime, ceftriaxone, and ceftazidime, have become the most prominent ESBLs in clinical isolates worldwide since the 2000s (Cantón and Coque, 2006, Naseer and Sundsfjord, 2011). According to data from the SENTRY surveillance program, of the 2895 ESBL-positive strains, 92.5% harboured CTX-M-type enzymes, while only 8.6% and 0.69% harboured SHV and TEM-type enzymes, respectively (Castanheira et al., 2019). CTX-M-15 was also the dominant genotype in ESBL-positive *E. coli* (66.2%) and *K. pneumoniae* (50%) in Canada based on results from a 5-year molecular surveillance (Denisuik et al., 2013). The epidemiological profile of ESBLs in China is consistent with the global findings, with CTX-M-type enzyme being the most prevalent ESBLs in *Enterobacteriaceae*. A recent study collected ESBL-positive *K. pneumoniae* from 31 Chinese secondary hospitals indicated that the most prevalent ESBLs were CTX-M-14, CTX-M-15, and CTX-M-3 (Zhang et al., 2016). For *E. coli*, a rate of 100% of CTX-M in ESBL-positive isolates was reported in a tertiary hospital in China (Miao et al., 2017).

1.4.2.4 AMPC B-LACTAMASES

AmpCs belong to the Class C of β-lactamases, which can hydrolyse expanded-spectrum cephalosporins and cannot be inhibited by β-lactamase inhibitors like clavulanate, sulbactam and tazobactam (Jacoby, 2009). Genes encoding these enzymes are either located on the chromosome (cAmpC) or on plasmids (pAmpC) (Meini et al., 2019). Usually, the chromosomal genes are expressed at negligible levels, but when the expression is inducible or de-repressed, it could induce clinically relevant resistance. Studies have demonstrated that the overexpression of cAmpC due to mutations in the promoter or attenuator in *E. coli* could lead to the

3GC-R phenotype (Peter-Getzlaff et al., 2011). Unlike cAmpCs, pAmpCs are usually expressed constitutively and confer resistance to expanded-spectrum β-lactams in *Enterobacteriaceae* (Jacoby, 2009). Despite the fact that more than 20 different AmpCs have been discovered till now, the prevalence of AmpCs in *Enterobacteriaceae* is far lower than that of ESBLs (Giani et al., 2017). A study conducted in a Dutch teaching hospital between 2013-2016 indicated that the prevalence of cAmpC and pAmpC genes in clinical isolates was relatively low, with 0.9% and 1.4%, respectively (den Drijver et al., 2018). Similarly, in a Chinese study, pAmpCs were only detected in 4.3% *K. pneumoniae* and 1.9% *E. coli* isolates (Li et al., 2008). Moreover, the prevalence ratio of ESBLs to AmpCs in 3GC-R *Enterobacteriaceae* was estimated to be around 12 to 1 in an Italian survey (Giani et al., 2017). Therefore, it can be concluded that at this point in time, among 3GC-R *Enterobacteriaceae*, ESBLs are still the principal culprit.

1.4.3 TREATMENTS AND RESEARCH NEEDS FOR 3GC-R *ENTEROBACTERIACEAE*

Carbapenems are the first-choice antibiotics recommended by the latest published guidelines in treating severe infections caused by 3GC-R *Enterobacteriaceae* (Tamma et al., 2021, Paul et al., 2022). Generally, 3GC-R *Enterobacteriaceae* are susceptible to carbapenems unless other resistance mechanisms co-exist, because ESBLs and AmpCs lack the efficiency to hydrolyse this group of antibiotics (Meini et al., 2019, Peirano and Pitout, 2019). According to data from a recent study conducted in the US and Europe during 2016-2018, among those 1252 3GC-R Enterobacterales, the susceptibility rate to meropenem was 100%; although the rate was slightly decreased in bacteria with ESBL-phenotype, it was still as high as 97.6% (Belley et al., 2021). In China, the susceptibility rate to meropenem in ESBL-positive *Enterobacteriaceae* was also high, with 100% reported in ESBL-positive *E. coli* and 94.4% in ESBL-positive *K. pneumoniae*, based on results from the China Antimicrobial Resistance Surveillance Trial 2015-2016 (Zhang et al., 2019). However, the wide use of carbapenems in treating 3GC-R *Enterobacteriaceae* raised

concern about the development of carbapenem resistance in these pathogens. Therefore, finding carbapenem-sparing regimens for infections caused by 3GC-R *Enterobacteriaceae* is urgently required.

Among all the carbapenem-sparing regimens, piperacillin-tazobactam seems to be one of the most promising alternatives in treating infections caused by 3GC-R *Enterobacteriaceae* (Aslan and Akova, 2019, D'Angelo et al., 2016). As ESBLs are the main enzymes mediating resistance to 3GCs and they could be inhibited by classical β-lactamase inhibitors, like clavulanic acid and tazobactam, piperacillin-tazobactam might still be active in these resistant pathogens if no other resistant mechanisms co-exist (Peirano and Pitout, 2019). Actually, i*n vitro* studies have demonstrated that a certain proportion of 3GC-R *Enterobacteriaceae* was susceptible to piperacillin-tazobactam (Zhang et al., 2019, Belley et al., 2021). However, studies assessing the clinical effectiveness of piperacillin-tazobactam in the treatment of 3GC-R *Enterobacteriaceae* demonstrated heterogeneous results (Ko et al., 2018, Henderson et al., 2021, Tamma et al., 2015, Harris et al., 2018, Gutiérrez-Gutiérrez et al., 2016). Among these clinical studies, the INCREMENT study and the MERINO trial are the most impactful studies (Harris et al., 2018, Gutiérrez-Gutiérrez et al., 2016). The INCREMENT study was a multinational preregistered cohort study in which 365 patients with BSI caused by ESBL-producing *Enterobacteriaceae* were included in the empirical therapy cohort and 601 patients in the targeted therapy cohort (Gutiérrez-Gutiérrez et al., 2016). Compared with patients receiving carbapenems, those receiving β-lactam/β-lactamase inhibitor combinations (mainly piperacillin-tazobactam) were not associated with lower clinical cure rates and higher mortality in both cohorts. Then, the authors concluded that if β-lactam/β-lactamase inhibitor combinations were active *in vitro*, they might be as effective as carbapenems in treating BSI due to ESBL-producing *Enterobacteriaceae* (Gutiérrez-Gutiérrez et al., 2016). Interestingly, the later conducted MERINO study, a RCT, demonstrated an opposite result: piperacillin-tazobactam was inferior to meropenem on 30-day mortality in treating BSI due to *E. coli* and *K.*

pneumoniae with ceftriaxone resistance (Harris et al., 2018). As the only randomized trial, its results significantly impacted clinical practice after publication. Guidelines even recommended against using piperacillin-tazobactam in treating such infections based on findings from the MERINO trial (Tamma et al., 2021, Paul et al., 2022). However, a post hoc analysis found that a significant proportion of piperacillin-tazobactam non-susceptible strains were included in the MERINO trial because the non-referred antimicrobial susceptibility testing method was used in participating centres; when these non-susceptible strains were excluded, the inferiority no longer existed (Henderson et al., 2021). Therefore, it is still reasonable to speculate that piperacillin-tazobactam might be a promising alternative to carbapenems in treating 3GC-R *Enterobacteriaceae*, if its activity was validated by the microbroth dilution method, but this still clearly requires further studies. Information about the clinical studies mentioned in this section of the thesis is summarised in Table 1-1.

1.5 RESEARCH QUESTIONS AND STRUCTURE OF THE THESIS

The main aim of this thesis is to explore what strategies are used in China to manage infections caused by MDR-GNB and evaluate the effectiveness of these strategies in treating certain bacterial infections. As nosocomial pneumonia (including HAP and VAP) is frequently caused by MDR-GNB, it is very much suited for studying the management of infections caused by such pathogens. Therefore, in the present thesis, all clinical studies assessing specific therapeutic regimens in treating MDR bacteria were conducted in patients with nosocomial pneumonia.

Question 1: What antibiotic strategies are used in China to manage VAP due to MDR-GNB?

To address this question, a nationwide survey was conducted in China between July 2021 and September 2021 to get an overview of strategies used in China to manage VAP due to MDR bacteria. The results are summarized in **Chapter** 2 and form the

basis of the rest of this thesis, with every chapter addressing one major research question.

Question 2: Does HDT result in better clinical outcomes than the SDT regimen in treating MDR-GNB?

A meta-analysis was conducted to answer this question and reported in **Chapter 3**.

Question 3: Does adding intravenous polymyxin B to HDT improve clinical outcomes in patients with nosocomial pneumonia caused by CRAB and CRKP?

According to results from **Chapter 2**, tigecycline-based therapy was the most prescribed regimen in treating pneumonia caused by CRAB and CRKP in China. Among these tigecycline-based therapies, polymyxin plus tigecycline was one of the most prevalent regimens in such infections. Therefore, a retrospective study was conducted to evaluate the benefit of adding intravenous polymyxin B to HDT in patients with nosocomial pneumonia due to CRAB and CRKP. Results from this study are presented in **Chapter 4**.

Question 4: Could tigecycline be an alternative therapy for VAP due to *S. maltophilia*?

As the emerging CRGNB, alternative therapies for *S. maltophilia* infections are always of great interest. As reported in **Chapter 2**, 17.9% of the participating hospitals used tigecycline as one of the most popular alternative therapies for treating VAP due to *S. maltophilia*. To evaluate the effectiveness of tigecycline in treating *S. maltophilia* infections, a multicentre retrospective study was conducted in three teaching hospitals and the results are reported in **Chapter 5**.

Question 5: Could piperacillin-tazobactam be a potential carbapenem-sparing regimen in treating nosocomial pneumonia due to ESBL-producing *K. pneumoniae*?

To address this question, a retrospective study was conducted in two tertiary teaching hospitals and its findings are described in **Chapter 6**.

Finally, in the **General Discussion**, I have discussed the most striking findings of my work and formulated future recommendations for both clinical practice as well as research in this area.

1.6 STATUS OF MANUSCRIPTS ARISING FROM WORK

Of the studies in this thesis that address the proposed research questions outlined in section 1.5, four have been published in peer-reviewed journals, and the fifth will be submitted soon.

The content regarding strategies in treating CRKP presented in **Chapter 2** has been published in *Journal of Infection*:

Zha L, Li S, Ren Z, Li X, Zhang D, Zou Y, Pan L, Xu Q, Rui Z, Chen S, Yang G, Chen Z, Tefsen B, Guo J. Clinical management of infections caused by carbapenem-resistant Klebsiella pneumoniae in critically ill patients: A nationwide survey of tertiary hospitals in mainland China. J Infect. 2022 Jun;84(6): e108-e110. doi: 10.1016/j.jinf.2022.03.023. Epub 2022 Mar 31. PMID: 35367511.

Work presented in **Chapter 3** has been published in *Advances in Therapy*:

Zha L, Pan L, Guo J, French N, Villanueva EV, Tefsen B. Effectiveness and Safety of High Dose Tigecycline for the Treatment of Severe Infections: A Systematic Review and Meta-Analysis. Adv Ther. 2020 Mar;37(3):1049-1064. doi: 10.1007/s12325-020-01235-y. Epub 2020 Jan 31. PMID: 32006240; PMCID: PMC7223407.

A manuscript arising from the work presented in **Chapter 4** will be submitted soon:

Zha L, Zhang X, Pan L, Liu L, Chen S, Guo J, Tefsen B. Intravenous Polymyxin B As Adjunctive Therapy to High-Dose Tigecycline for the Treatment of Nosocomial

Pneumonia due to Carbapenem-Resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae*.

Work presented in **Chapter 5** has been published in *Infectious Diseases and Therapy*:

Zha L, Zhang D, Pan L, Ren Z, Li X, Zou Y, Li S, Luo S, Yang G, Tefsen B**.** Tigecycline in the Treatment of Ventilator-Associated Pneumonia Due to *Stenotrophomonas maltophilia*: A Multicenter Retrospective Cohort Study. Infect Dis Ther. 2021 Dec;10(4):2415-2429. doi: 10.1007/s40121-021-00516-5. Epub 2021 Aug 10. PMID: 34374953; PMCID: PMC8354101.

Work presented in **Chapter 6** has been published in *Antibiotics*:

Zha L, Li X, Ren Z, Zhang D, Zou Y, Pan L, Li S, Chen S, Tefsen B. Pragmatic Comparison of Piperacillin/Tazobactam versus Carbapenems in Treating Patients with Nosocomial Pneumonia Caused by Extended-Spectrum β-Lactamase-Producing *Klebsiella pneumoniae*. Antibiotics (Basel). 2022 Oct 10;11(10):1384. doi: 10.3390/antibiotics11101384. PMID: 36290042; PMCID: PMC9598608.

2 CHAPTER 2 – CLINICAL MANAGEMENT OF VENTILATOR-ASSOCIATED PNEUMONIA CAUSED BY MULTIDRUG-RESISTANT GRAM-NEGATIVE BACTERIA: A NATIONWIDE SURVEY OF TERTIARY HOSPITALS IN CHINA

2.1 BACKGROUND

VAP caused by MDR-GNB, especially those mediated by ESBLs and carbapenemases, has been increasing significantly worldwide (Rhodes et al., 2018, Barbier et al., 2013, Mohd Sazlly Lim et al., 2019, Gupta et al., 2017). The SENTRY antimicrobial surveillance program indicated that the meropenem susceptibility rate of *A. baumannii* strains recovered from patients with pneumonia decreased from 79.1% in 1997-1998 to 31.6% in 2015-2016, while the rate of meropenem non-susceptible *K. pneumoniae* increased more than 25% during the same period (Sader et al., 2019). The susceptibility to meropenem of *P. aeruginosa*, one of the most prevalent pathogens isolated from patients with pneumonia, was also found to be low, only 66.3% in Europe in 2015-2016, and 74.8% in the US during 2016-2019 (Sader et al., 2019, Sader et al., 2021). The situation in China is similar or even worse than that in

Europe and the US, as data from the 2018 annual report of CHINET indicated that 27.6% of *K. pneumoniae*, 28.8% of *P. aeruginosa*, and 80.7% of *A. baumannii* isolated from lower respiratory tracts were resistant to meropenem (Hu et al., 2019).

Patients with VAP caused by MDR usually have worse clinical outcomes than those infected with their susceptible counterparts, with increased duration of mechanical ventilation, length of hospital stays, hospital cost and mortality (Papazian et al., 2020, Nelson et al., 2017, Tabak et al., 2020). These worse outcomes were largely due to the inappropriate use of antibiotics. Studies have demonstrated well that the appropriate initial antibiotic therapy could mitigate mortality caused by those resistant pathogens (Lodise et al., 2019, Bonine et al., 2019, Zilberberg et al., 2019, Kollef et al., 2008). Additionally, the dose and administration model of antibiotics targeting MDR bacteria could also affect clinical outcomes in these infections, as studies have illustrated that using carbapenems by continuous or extended infusion, initiating colistin with a loading dose, delivering tigecycline in the high-dose regimen decreased the mortality in those patients infected with MDR or CRGNB (Katip et al., 2021, Xia and Jiang, 2020, Falagas et al., 2014, Wang et al., 2022, Taccone et al., 2012, Pascale et al., 2019). However, not all hospitals adhere to these good clinical practices. A survey conducted in 115 large hospitals reported that only 89.2% of participating hospitals used a loading dose of colistin, and only 54.5% of hospitals used tigecycline in high dosage (Papst et al., 2018).

Worryingly, studies have demonstrated that 40-70% of patients with suspected VAP actually did not have VAP. Still, a considerable fraction of them received broad-spectrum antibiotics, potentially exposing them to adverse effects, especially the emergence of antimicrobial resistance (Nussenblatt et al., 2014, Leone et al., 2007, Klompas et al., 2017). Furthermore, with the increase of MDR bacteria causing VAP, doctors are likely to prescribe combination therapy either for empirical or the targeted treatment, despite the lack of high-quality evidence supporting these combination regimens (Carrara et al., 2022, Papst et al., 2018). Therefore,

minimizing unnecessary antibiotic administration in suspected VAP and reducing unnecessary combination therapy in confirmed VAP turns out to be one of the most crucial fundamental actions in antibiotic stewardship (Chiotos et al., 2019, Luyt et al., 2014, Yoshimura et al., 2020).

To help better treating infections caused by MDR-GNB, the IDSA and the ESCMID both updated their guidelines with the latest clinical evidence (Tamma et al., 2021, Paul et al., 2022). However, with a different diagnostic capacity, antibiotic availability, and prescribing culture in China, management of VAP caused by MDR bacteria is likely to be different from that in other countries. Therefore, understanding the current strategies for managing VAP caused by MDR-GNB in China is crucial, as this could help uncover the gap between clinical practices and the latest evidence, further optimizing the design of antibiotic stewardship in such infections. In this chapter, the availability of antibiotics, the capacity of local microbiological laboratories and antibiotic regimens targeting CRGNB and ESBL-producing *Enterobacteriaceae* in 212 tertiary teaching hospitals are reported.

2.2 METHODS

2.2.1 SURVEY DESIGN

To explore the current strategies used for managing VAP in China, we conducted this nationwide internet-based questionnaire survey from July 2021 to September 2021. The questionnaire included four parts, the basic information of hospital (location, type of hospital, ICU beds, number of patients admitted in ICU in 2020, local resistance data, antibiotic and infection control policy), the diagnostic capacity of microbiological laboratories, the availability of antibiotics, and antibiotic strategies (regimens, dosage and administration model) in treating patients with VAP caused by MDR-GNB, including CRAB, CRKP, CRPA, S. *maltophilia,* and ESBL-producing Enterobacterales. The final version of the questionnaire was pre-tested in three

tertiary hospitals in Wuhu, Anhui, to assess whether it can reflect the actual practice in VAP management. The complete questionnaire is attached in the Appendix I.

2.2.2 TARGET HOSPITALS AND SURVEY ADMINISTRATION

The objective of the survey was to reflect the current practice of VAP management in tertiary teaching hospitals in China. Based on the 2021 annual statistical data, there were 1,580 tertiary teaching hospitals in China (NHC, 2021). Therefore, if we want to reflect these hospitals accurately, 310 hospitals are required to participate in this survey, calculated at a 5% margin of error and 95% confidence of level. Moreover, in case there were not enough hospitals participating, we sought to include at least one tertiary hospital in each province of China in the survey. The questionnaire was sent to the director or representative doctor (usually the chief resident doctor) in each target hospital with the help of survey coordinators in different provinces through convenience sampling method. The representatives were asked to fill out the questionnaire to reflect their actual practice in VAP management. If no response was received in due time, we would seek help from another person in the target hospital. For each hospital, only one response was included in the final analysis. Moreover, we defaulted that only a complete questionnaire could be submitted through the online platform to avoid receiving incomplete questionnaires.

2.2.3 STATISTICAL ANALYSIS

The raw data of the survey was extracted from the survey platform and analysed with R software (version 4.1.3). Categorical data was expressed as counts and percentage and the quantitative data was expressed as median with inter-quartile range. When calculating the percentage of each antibiotic regimen, the denominator was the number of total reported regimens instead of the number of the participating hospitals.

2.2.4 ETHICS APPROVAL

The study was approved by the ethics committee of Xi'an Jiaotong-Liverpool University (reference number 19-01-05) (Appendix II).

2.3 RESULTS

2.3.1 CHARACTERISTICS OF INCLUDED HOSPITALS

In total, 212 tertiary hospitals participated in the survey during the study period, of which 56.6% were provincial, and 43.4% were municipal hospitals. Of the participating hospitals, 40.1% were situated in Western China, 35.4% in Central, and 24.5% in Eastern and Northeastern China (Figure 2-1).

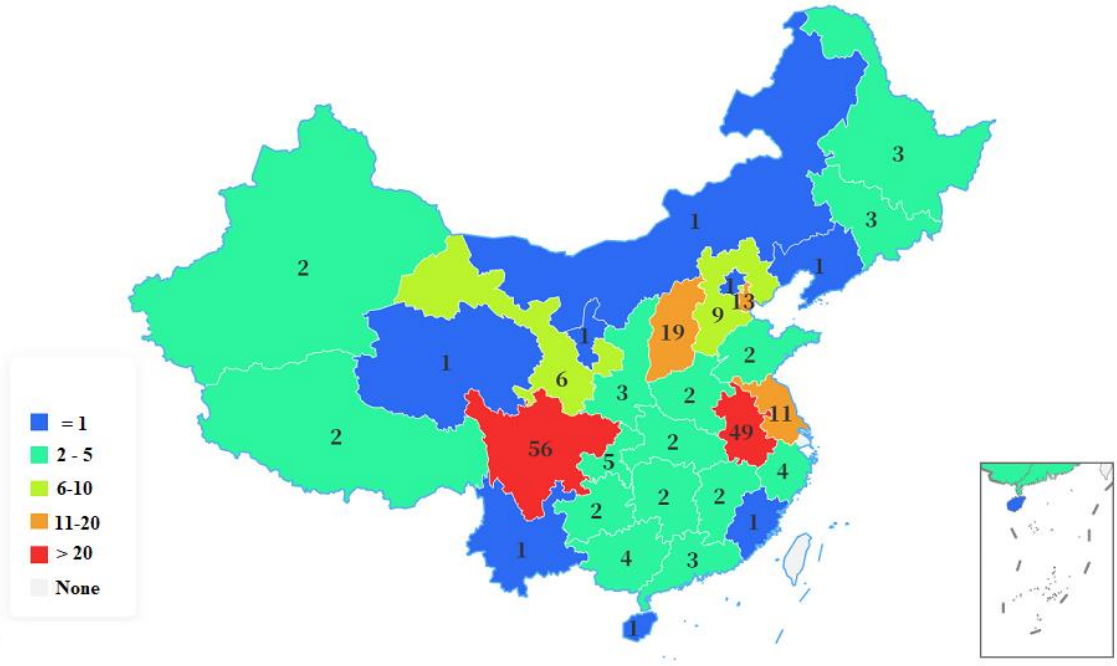

Figure 2-1. Distribution and number of hospitals included in the present study.

The median number of patients admitted to the ICU in 2020 was 800 (IQR 479-1500). Of the participating hospitals, 90.1% had their local antibiotic resistance data guiding antibiotic use, while 13.9% of hospitals had clinical pharmacists monitoring

antibiotic use routinely. The incidence of VAP in 2020 varied in participating hospitals, with the median incidence being 6.2% (IQR 2.8-12.7%). Most respondents (81.1%) reported that the VAP incidence was adopted as a performance indicator in their hospitals (Table 2-1).

Table 2-1. Characteristics of participating hospitals.

Characteristics	**n = 212**
Region of included hospitals[a]	
Western	85 (40.1%)
Central	75 (35.4%)
Eastern and Northeastern	52 (24.5%)
Type of hospital	
Provincial	120 (56.6%)
Municipal	92 (43.4%)
ICU beds, Median (IQR)	20 (15-30)
Numbers of patients admitted in ICU in 2020, Median (IQR)	800 (479-1500)
Incidence of VAP in 2020, Median (IQR)	6.2% (2.8-12.7%)
Having local antibiotic resistance data	191 (90.1%)
Having clinical pharmacists guiding antibiotics use	29 (13.9%)
VAP incidence as a performance indicator	172 (81.1%)

[a]The geographical breakdown of the four economic regions in China was based on (Lin et al., 2018); Abbreviations: ICU, intensive care unit; IQR, interquartile range; VAP, ventilator-associated pneumonia.

2.3.2 DIAGNOSTIC METHODS AND TESTING CAPACITY OF MICROBIOLOGICAL LABORATORIES

From patients suspected of having VAP, 59.6% of participating hospitals collected respiratory specimen through endotracheal aspirate, while 40.1% collected it with invasive bronchoalveolar lavage. All the participating hospitals had their local microbiology laboratory, which could perform standard pathogen identification and antibiotic susceptibility testing. Semiquantitative culture method was adopted in

60.8% of the participating hospitals, while 34.4% of the participating hospitals performed quantitative culture. A third (32.5%) of the hospitals reported exact MIC values for carbapenems in CRGNB, and 47.6% identified carbapenemases in those strains. Moreover, a majority of the hospitals did not test susceptibility for polymyxins (69.3%) and tigecycline (78.3%), and 36.8% of hospitals tested susceptibility for ceftazidime-avibactam in CRGNB. Moreover, among these participating hospitals, 37.7% performed *in vitro* combination antimicrobial susceptibility testing for CRGNB (Table 2-2).

Table 2-2. Diagnostic methods for ventilator-associated pneumonia and testing capacity of microbiological laboratories in included hospitals.

Variable	**n = 212**
Diagnostic methods	
Sampling method in suspected VAP[a] patients	
Endotracheal aspirate	126 (59.4%)
Bronchoalveolar lavage	85 (40.1%)
Protected specimen brush	1 (0.5%)
Culture method	
Quantitative	73 (34.4%)
Semiquantitative	129 (60.8%)
Qualitative	10 (4.7%)
Capacity of microbiology laboratory	
Standard etiology testing	212 (100%)
Matrix assisted laser desorption ionization time of flight	86 (40.6%)
Metagenomic next generation sequencing	37 (17.5%)
Reporting MIC value for tested antibiotics	69 (32.5%)
Testing ESBL phenotype in Enterobacteriaceae	193 (91%)
Testing carbapenemases in CR-GNB	101 (47.6%)
Reporting susceptibility of following antibiotics for CRGNB	
Polymyxins	147 (69.3%)
Tigecycline	166 (78.3%)
Ceftazidime-avibactam	78 (36.8%)
Performing combination antimicrobial susceptibility testing for CRGNB	80 (37.7%)

[a]Abbreviations: CRGNB, carbapenem-resistant Gram-negative bacteria; VAP, ventilator-associated pneumonia.

2.3.3 AVAILABILITY OF ANTIBIOTICS IN INCLUDED HOSPITALS

Among the participating hospitals, 76.9% of them routinely offered tigecycline, 35.4% routinely offered polymyxins, and 27.8% and 16% of participating hospitals routinely offered ertapenem and ceftazidime-avibactam, respectively. The availability of trimethoprim-sulfamethoxazole was also low, with 20.8% of hospitals routinely offering this therapy. Slightly more than half (57.1%) of the participating hospitals routinely offered fosfomycin and 45.8% offered tobramycin, while 36.8% routinely offered minocycline (Table 2-3).

Table 2-3. Availability of antibiotics in hospitals included in this study.

Antibiotics	**Routinely offered, n (%)**	**Conditionally offered, n (%)**	**Not offered, n (%)**
Aztreonam	158 (74.5)	24 (11.3)	30 (14.2)
Ceftazidime-avibactam	34 (16)	101 (47.6)	77 (36.3)
Ertapenem	59 (27.8)	28 (13.2)	125 (59)
Fosfomycin	121 (57.1)	34 (16)	57 (26.9)
Imipenem	199 (93.9)	9 (4.2)	4 (1.9)
Meropenem	193 (91.0)	16 (7.5)	3 (1.4)
Minocycline	78 (36.8)	89 (42.0)	45 (21.2)
Polymyxin	75 (35.4)	103 (48.6)	34 (16.0)
Tigecycline	163 (76.9)	45 (21.2)	4 (1.9)
Tobramycin	97 (45.8)	28 (13.2)	87 (41)
Trimethoprim-sulfamethoxazole	44 (20.8)	36 (17.0)	132 (62.3)

2.3.4 ANTIBIOTIC STRATEGIES FOR MDR BACTERIA

Almost all (95.8%) participating hospitals used procalcitonin for guiding antibiotic therapy in patients with suspected VAP and 30.7% of hospitals reported that they had empirically covered CRGNB in more than 50% of VAP cases in 2020. Regarding the target therapy, combination therapy was the most preferred regimens, as 89.6% of hospitals using monotherapy in less than 25% of CRGNB VAP cases (Table 2-4).

Tigecycline-based therapy was the most frequently reported regimen for CRKP and CRAB, among which tigecycline plus cefoperazone-sulbactam was the most preferred combination therapy, followed by tigecycline plus a carbapenem. Ceftazidime-avibactam was the most frequently used monotherapy for CRPA, followed by cefoperazone-sulbactam and polymyxins. The preferred combination regimens for CRPA were cefoperazone-sulbactam plus an aminoglycoside or polymyxin (Table 2-5).

A large majority (72.6%) of hospitals reported using carbapenems by extended infusion, 71.2% of hospitals initiated polymyxins with a loading dose, and 34.9% of hospitals adopted the HDT for VAP caused by CRGNB. Less than half (45.3%) of hospitals used inhaled colistin in more than 50% of CR VAP cases in 2020. The vast majority (94.8%) of participating hospitals used a 10 to 15 days antibiotic course for the treatment of VAP caused by MDR bacteria, while only 5.2% of hospitals adopted the short antibiotic course (Table 2-4).

The main therapy for VAP caused by ESBL-producing *Enterobacteriaceae* was carbapenems, used in 61.8% of hospitals, while 36.8% of hospitals used piperacillin-tazobactam. Fluoroquinolone was the most frequently reported choice (38.2%) for treating *S. maltophilia* VAP, followed by trimethoprim-sulfamethoxazole (21.7%) and tigecycline (17.9%) (Table 2-6).

Table 2-4. Antibiotic strategies for ventilator-associated pneumonia.

Strategies	**n = 212**
Empirical therapy	
Using procalcitonin to guide the initiation of antibiotics in suspected VAP[a] patients	203 (95.8%)
Percentage of suspected VAP patients receiving empirical therapy covering CR-GNB	
< 25%	102 (48.2%)
25% to 50 %	45 (21.2%)
50% to 75%	29 (13.7%)
> 75%	36 (17%)
Target therapy	
Percentage of patients receiving monotherapy therapy for VAP caused by CR-GNB	
< 25%	190 (89.6%)
25% to 50 %	15 (7.1%)
50% to 75%	4 (1.9%)
> 75%	3 (1.4%)
Percentage of patients receiving inhaled colistin for VAP caused by CR-GNB	
< 25%	64 (30.2%)
25% to 50 %	52 (24.5%)
50% to 75%	35 (16.5%)
> 75%	61 (28.8%)
Administration models and dosages of antibiotics for treating VAP caused by CRGNB	
Administration model of carbapenems	
Standard intravenous	51 (24.1%)
Extended infusion (3-4 hours)	154 (72.6%)
Continuous infusion	7 (3.3%)
Tigecycline dose	
High-dose (200 mg followed 100 mg per 12 h)	74 (34.9%)
Standard-dose (100 mg followed 50 mg per 12 h)	138 (65.1%)
Polymyxin	
Using loading dose	151 (71.2%)
Do not using loading dose	61 (28.8%)
Antibiotic course for VAP	
Short course (7 days)	11 (5.2%)
8-15 days	201 (94.8%)

[a]Abbreviations: CRGNB, carbapenem-resistant Gram-negative bacteria; VAP, ventilator-associated pneumonia.

Table 2-5. Top three antibiotic regimens for ventilator-associated pneumonia caused by carbapenem-resistant Gram-negative bacteria.

	Monotherapy	**Double combination therapy**	**Triple combination therapy**
Klebsiella pneumoniae	**N = 81**	**N = 151**	**N = 20**
	TIG[a] 27 (33.3%)	CEF/SUL + TIG 39 (25.8%)	CARB + AMG + TIG 5 (25%)
	CAZ/AVI 22 (27.2%)	CARB + TIG 31 (20.5%)	CARB + TIG +POL 4 (20%)
	POL 12 (14.8%)	POL + TIG 14 (9.3%)	CEF/SUL + POL + TIG 4 (20%)
Pseudomonas aeruginosa	**N = 72**	**N = 103**	**N = 14**
	CAZ/AVI 26 (36.1%)	CEF/SUL + AMG 24 (23.3%)	CARB + AMG + POL 4 (28.6%)
	CEF/SUL 19 (26.4%)	CEF/SUL + POL 17 (16.5%)	CEF/SUL + AMG + FLQ 3 (21.4%)
	POL 16 (22.2%)	CARB + POL 14 (13.6%)	CEF/SUL + POL + AMG (FLQ) 2 (14.3%)
Acinetobacter baumannii	**N = 73**	**N = 163**	**N = 23**
	TIG 29 (39.7%)	CEF/SUL +TIG 76 (46.6%)	CARB + CEF/SUL + TIG 6 (26.1%)
	CEF/SUL 28 (38.4%)	CARB + TIG 14 (8.6%)	CEF/SUL + POL +TIG 5 (21.7%)
	POL 7 (9.6%)	CEF/SUL + AMG 11 (6.7%)	CEF/SUL + TIG +AMG 4 (17.4%)

[a]Abbreviations: AMG: aminoglycoside; CAZ/AVI: ceftazidime/avibactam; CARB, carbapenems, CEF/SUL, cefoperazone/sulbactam, FLQ, fluoroquinolone, POL, polymyxin; TIG, tigecycline.

Table 2-6. Antibiotic regimens for VAP caused by ESBL-producing *Enterobacteriaceae* and *Stenotrophomonas maltophilia.*

	n = 212
ESBL-producing *Enterobacteriaceae*	
Carbapenems	131 (61.8%)
Piperacillin-tazobactam	78 (36.8%)
Others	3 (1.4%)
Stenotrophomonas maltophilia	
Trimethoprim-sulfamethoxazole	46 (21.7%)
Fluoroquinolone	81 (38.2%)
Tigecycline	38 (17.9%)
Colistin	8 (3.8%)
Minocycline	21 (9.9%)
Others	18 (8.5%)

2.4 DISCUSSION

The aim of this study was to explore the current strategies used for managing VAP in China, especially those infected with MDR-GNB. To the best of our knowledge, this is the first nationwide survey conducted in China with data gathered from every province. Therefore, results from the study provide a comprehensive overview of the current clinical practice for managing VAP caused by MDR bacteria throughout China.

In the present study, 94.8% of the participating hospitals reported using the longer antibiotic course to treat VAP caused by MDR-GNB, albeit the shorter antibiotic course was recommended by clinical guidelines (Kalil et al., 2016, Torres et al., 2017, Shi et al., 2019). Evidence supporting the short antibiotic course in VAP is derived from randomized controlled trials (RCT) that mainly included patients with VAP caused by susceptible pathogens rather than MDR bacteria (Pugh et al., 2015, Dimopoulos et al., 2013). Therefore, the lack of direct evidence diminished the confidence of doctors to implement the short course of antibiotics for such infection; instead, procalcitonin plus clinical criteria were widely used to guide antibiotic duration in China. Studies have demonstrated that using procalcitonin to determine antibiotic therapy in patients with VAP did reduce antibiotic consumption, but all exceeded the recommended 7-days course (Bouadma et al., 2010, Stolz et al., 2009, M et al., 2021, Beye et al., 2019). Therefore, the procalcitonin-guided antibiotic use should be avoided in treating VAP. However, without solid evidence directly supporting the efficacy and safety of the short antibiotic course in patients with VAP caused by MDR pathogens, it is challenging for global doctors to implement it, as the fear that the shorter courses may lead to inadequate control of infection is the primary driver of excess antibiotic therapy in critically ill patients (Dyar et al., 2016, Hellyer et al., 2020, Broom et al., 2016). Therefore, RCTs comparing the shorter with the longer antibiotic course in patients with VAP caused by MDR-GNB should be prioritized.

Apart from shortening antibiotic duration, another important aspect of antibiotic stewardship is early exclusion of VAP in clinically suspected patients, which would subsequently avoid unnecessary antibiotic exposure in these patients (Yoshimura et al., 2020). In the present survey, 95.8% of hospitals used procalcitonin to guide the initiation of antibiotics in suspected VAP patients. However, the use of this intervention should be with caution, as procalcitonin is ineffective for the diagnosis or exclusion of VAP (Kyriazopoulou and Giamarellos-Bourboulis, 2022). A study has demonstrated that by using 0.5 ng/ml as the threshold of procalcitonin, the sensitivity for diagnosing VAP was 72%, while the specificity was only 24% (Corbacho Re et al., 2019). Unlike procalcitonin, quantitative culture through bronchoscopic sampling might reduce the antibiotic exposure in patients with VAP and is recommended by the latest European guideline (Torres et al., 2017). However, although the accessibility to bronchoscopy was as high as 91.9% in the participating hospitals, less than half of the hospitals (40.1%) used bronchoscopic sampling and only 34.4% of them performed quantitative culture. The rate was similar to that reported in a UK national survey, leaving considerable rooms for improvement (Browne et al., 2014). It was encouraging to notice that 90.1% of hospitals participated in the present survey had their own local antibiotic resistant data guiding the empirical therapy, which is higher than a survey conducted in Europe (Lanckohr et al., 2021), but there is still room for improvement.

Regarding target therapy, we found there are three important causes that might hinder compliance with the latest guidelines by the participating hospitals in treating VAP caused by MDR pathogens: unavailability of recommended antibiotics; incomprehensive testing of resistance in strains that caused the infections and inappropriate dosing due to the lack of monitoring from clinical pharmacists.

First, I will discuss the unavailability of the recommended first-line antibiotics. Even being the only marketed new antibiotic therapy for treating CRGNB infections in China, the availability of ceftazidime-avibactam was still relatively low, as only 16%

of the participating hospitals routinely offered it. Under such circumstances, doctors would rely on using other available antibiotics as alternatives, like tigecycline. As demonstrated in the present study, tigecycline has been widely used for VAP caused by CRKP and CRAB, although recommendations are against using it for these indications (Tamma et al., 2021, Paul et al., 2022). A similar situation was reported in a 2018 study had conducted a survey among large hospitals in France, Greece, Israel, Italy, Kosovo, Slovenia, Spain and the US; it demonstrated that tigecycline and polymyxins were the most prescribed antibiotics for CRGNB because of the lack of newly approved antibiotics in these countries at that time (Papst et al., 2018). Like CR pathogens, with the lack of availability of intravenous trimethoprim-sulfamethoxazole, tigecycline, minocycline and colistin were widely used in treating *S. maltophilia* VAP, despite the lack of evidence supporting their efficacy (Tamma et al., 2022). Therefore, each hospital should improve its antibiotic supply chain to ensure that the availability of antibiotics matches clinical requirements.

The second cause identified is incomprehensive testing for antibiotic resistance of strains that caused the infections in clinical microbiology laboratories. According to the latest guideline, carbapenem based combination therapy was only suggested for CR pathogens with meropenem MIC < 8 mg/L (Paul et al., 2022). However, only 32.5% of the participating hospitals in this study measured exact MIC values for carbapenems in CR strains. Since carbapenem-based therapy was one of the most frequently reported regimens for CRGNB infections in China, we recommend measuring meropenem MIC for all CR strains, which should subsequently lead to the reduction of unnecessary use of carbapenems. Moreover, although the vast majority (84.8%) of CRKP strains in China produce KPC (Liu et al., 2022), which renders them susceptible to ceftazidime-avibactam, it is still better to identify carbapenemases and test *in vitro* susceptibility for ceftazidime-avibactam in all CRKP strains, as *K. pneumoniae* pathogens resistant to both ceftazidime-avibactam and carbapenems are emerging (Zhang et al., 2020). However, in the present study, only 47.6% of the participating hospitals identified carbapenemases and 36.8% tested the

susceptibility of ceftazidime-avibactam in CRGNB strains. If recommendations mentioned above are not included in the standard test regimen in clinical microbiology laboratories, it will be impossible to avoid misusing ceftazidime-avibactam in those resistant strains, especially when access to ceftazidime-avibactam becomes easier. Therefore, building a comprehensive and scandalized microbiological laboratory in each hospital is necessary to support the rational use of antibiotics for MDR pathogens.

As the third cause of not complying with the latest guidelines might be the low percentage of clinical pharmacists monitoring antibiotics use routinely in the participating hospitals. Several ways of antibiotic misuse, in the form of inappropriate dosing and administration model of antibiotics are occurring. As suggested by the guidelines, using tigecycline in the high-dose regimen and carbapenems with an extended infusion is recommended, because it has been demonstrated to produce better clinical outcomes than comparators (Tamma et al., 2021, Paul et al., 2022). However, only 34.9% of hospitals in the present study adopted the high-dose regimen of tigecycline, and 24.1% of hospitals still did not use carbapenems with extended or continuous infusion. Given that the low percentage (13.9%) of participating hospitals have clinical pharmacists monitoring antibiotic use routinely, it is reasonable to suggest hospitals establish a well-trained clinical team of pharmacists to guide the appropriate use of antibiotics, which could help minimize inappropriate antibiotic use (Subedi et al., 2020, Sakeena et al., 2018).

There are two major limitations of this survey. First, although 212 hospitals participated in our research, all were restricted to tertiary hospitals, and around half of them are from two single provinces (Anhui and Sichuan); therefore, the results demonstrated here might not reflect the situation in all medical care facilities of China. Moreover, the number of included hospitals was lower than the calculated sample size, which might introduce bias in the interpretation of the results. Second, antibiotic regimens were reported by representative doctors in each hospital, which

might reflect personal opinions to some extent rather than the actual strategies used for MDR bacteria in the participating hospitals. Despite these limitations, this is the only survey that has data from every province of China, thus findings from the present study could help optimize the clinical management of VAP and better design antibiotic stewardship program in critically ill patients in China, especially those infected with MDR or CR bacteria.

2.5 CONCLUSIONS

Procalcitonin guided antibiotic use is widely adopted in patients with VAP in China, which usually resulted in using a longer antibiotic course than recommended. Although bronchoscopy is accessible in most participating hospitals, quantitative culture through bronchoscopic sampling was only implemented in a small proportion of hospitals. With the low availability rate of the first recommended antibiotics, treatments for VAP caused by CRGNB primarily relied on traditional antibiotic-based combination therapy. Moreover, incomprehensive testing capacity and the lack of well-trained pharmacists closely monitoring antibiotic use led to the misuse of certain antibiotics. Future antibiotic stewardship programs should target these shortcoming factors, which could help reduce antibiotic use and improve the quality of care by administrating antibiotics in the correct dose and optimal modality.

3 CHAPTER 3 – EFFECTIVENESS AND SAFETY OF HIGH-DOSE TIGECYCLINE FOR THE TREATMENT OF SEVERE INFECTIONS: A SYSTEMATIC REVIEW AND META-ANALYSIS

From the results of the survey in **Chapter 2**, tigecycline-based therapy was the most prevalent regimen in China to treat infections caused by CRAB and CRKP. Among those participating hospitals, 138 (65.1%) used SDT, while the other 74 (34.9%) adopted the HDT regimen. This chapter evaluated whether the HDT regimen would lead to better clinical outcomes than the SDT or other alternative regimens through systematic review and meta-analysis.

3.1 BACKGROUND

Severe infections, especially those caused by MDR bacteria, are associated with increased mortality, length of hospital stay and cost (Arthur et al., 2015, Thabit et al., 2015, Mauldin et al., 2010). MDR bacterial infections are responsible for more than 30% of hospital-acquired infections, with even higher rates in critically ill, cancer and immunosuppressed patients (Peleg and Hooper, 2010, Hawkey, 2015).

Resistance to carbapenems, initially considered potent broad-spectrum antibiotics used to treat these infections (Hawkey et al., 2018), has increased significantly within the last decade because of the prevalence of carbapenemases among these pathogens (Bedenić et al., 2014, Tal-Jasper et al., 2016, Munoz-Price et al., 2013, Duin and Doi, 2017). Moreover, infections caused by extensively drug resistant (XDR) or pan-drug resistant (PDR) organisms have emerged and spread all over the world (Horcajada et al., 2019, Kaye and Pogue, 2015, Nowak et al., 2017, Pérez et al., 2019). Under such situations, traditional drugs like colistin, tigecycline, fosfomycin, clindamycin and cotrimoxazole are being deployed as the last resort in clinical practice for infections caused by MDR bacteria (Cassir et al., 2014, Falagas and Kopterides, 2007).

Tigecycline was the first glycylcline approved by the US FDA to treat cSSTI, cIAI, and CAP (Kaewpoowat and Ostrosky-Zeichner, 2015, Livermore, 2005, Pankey, 2005). Due to its broad spectrum antibacterial activity, particularly against GNB which are resistant to other antibiotics, it has been widely used off-label in VAP, HAP and BSI caused by MDR pathogens, especially CRGNB (Kuti et al., 2019, Wang et al., 2017, Xu et al., 2016).

The efficacy of SDT (100 mg initial dose, followed by 50 mg twice per day) in clinic is controversial. Previous studies had indicated that tigecycline was not better than other antimicrobial agents and might be associated with increased mortality (Tasina et al., 2011, Shen et al., 2015, Prasad et al., 2012). PK/PD research suggested that this lack of efficacy may be due to its suboptimal concentrations in both serum and pulmonary epithelial lining fluid (Giamarellou and Poulakou, 2011). Therefore, a regimen of HDT (200 mg initial dose, followed by 100 mg twice per day) has been used in clinical practice. In Chapter 2, 34.9% of hospitals in China adopted the high-dose regimen in treating such infections. A systematic review in 2014 attempted to evaluate the effectiveness of HDT for the treatment of severe infections, but it could not draw conclusions regarding the efficacy of HDT due to limited clinical evidence

(Falagas et al., 2014). With the accumulation of new studies, we aimed to reassess the effectiveness and safety of HDT for the treatment of severe infections.

3.2 METHODS

3.2.1 PROTOCOL AND GUIDELINE

The full protocol of the systematic review and meta-analysis was registered in PROSPERO (https://www.crd.york.ac.uk/prospero/) as CRD42019129283. The systematic review adhered to the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Hutton et al., 2015).

3.2.2 LITERATURE SEARCH

We performed an extensive search of PubMed, Web of Science, Embase, MEDLINE, and the Cochrane Library using the terms “tigecycline”, “dose” and “dosage” up to February 20, 2019. In order to identify completed but unpublished or ongoing studies, ClinicalTrails.gov was also searched. The reference lists of identified reports were hand-searched for relevant studies.

3.2.3 STUDY SELECTION

The relevant studies were examined by me and another reviewer (L.P.) independently. Eligible studies compared the efficacy of HDT with SDT or other non-tigecycline antibiotics regimens in the treatment of severe infections regardless of pathogens. Single-arm studies, repetitive studies, case report, reviews, studies with limited or uncertain information and those using tigecycline in other dosage (i.e., not the defined high-dose) were excluded, as were animal, PK/PD, and *in vitro* studies. No restrictions were placed on the characteristics of participants, lengths of follow-up, antibiotics used in combination with HDT, and antibiotic regimens in the control

group. Any disagreements were resolved through discussion with a third assessor (J.G.).

3.2.4 DATA EXTRACTION

The following information was extracted from each study: first author's name and year of publication, study design, patient characteristics (age, infection sites, and score of the severity of diseases), type of microorganism, concomitant antibiotics and antimicrobial agents used in the control groups, outcomes (all-cause mortality, clinical cure and microbiology eradication rate) and reported clinical adverse events.

3.2.5 QUALITY ASSESSMENT

The quality of the included non-randomized studies was evaluated using the modified Newcastle-Ottawa scale (NOS) (Peterson et al., 2011). Studies with NOS scores < 3 were considered as poor quality and excluded from this review. The risk of bias of included non-randomized studies were assessed using the ROBINS-I tool (Risk Of Bias In Non-randomized Studies of Interventions) (Sterne et al., 2016). The risk of bias of the single RCT included in this review was assessed with the Cochrane Collaboration's tool for assessing the risk of bias (Higgins et al., 2011).

3.2.6 DEFINITIONS AND OUTCOMES

The outcome of primary interest of the review is all-cause mortality. Secondary outcomes include the clinical cure rate, microbiological eradication rate and adverse events (diarrhoea, nausea, vomiting, renal impact, hepatic injury, and haematological injury). Clinical cure was defined as complete resolution or improvement from the symptoms and signs of infection. Microbiological eradication was defined as sterile culture or absence of the original pathogen in sequential culture after antibiotics treatment. Due to lack of standard definitions of adverse events, the criteria as reported in each study was used. HDT was defined as using tigecycline 100 mg twice per day after a 200 mg loading dose, whereas SDT was

defined as using 50 mg twice per day after a 100 mg loading dose. HAP, VAP, BSI, cIAI and cSSTI were defined using criteria reported in each study. Mixed infection was defined as the presence of at least two types of infection in patients (i.e., patients diagnosed as cIAI, BSI and HAP were all included in one study).

3.2.7 STATISTICAL ANALYSIS

The review was performed using Review Manager 5.3 (Cochrane Collaboration, Oxford, UK). Statistical heterogeneity was assessed by the I^2 test, and $I^2 > 50\%$ was defined as substantial heterogeneity (JP et al., 2003). In the presence of substantial heterogeneity, a random-effects model was used. Otherwise, a fixed-effects model was calculated. Pooled odds ratios (OR) and 95% confidence intervals (CI) were calculated using the Mantel-Haenszel method, in which the ORs were calculated through a weighted average. The sequential monitoring boundary and required information size (RIS) were constructed and calculated with the software Trial Sequential Analysis (http://www.ctu.dk/tsa/) (Thorlund et al., 2010). Publication bias was evaluated with funnel plots and the Egger regression-based test implemented in Stata version 14 (StataCorp, College Station, Texas). A two-tailed $p < 0.05$ was considered statistically significant.

3.3 RESULTS

3.3.1 INCLUDED STUDIES AND CHARACTERISTICS

Overall, 591 studies were identified from five databases, and one was identified through reference lists. After application of eligibility criteria, 10 studies were included in the systematic review and meta-analysis (Figure 3-1). Among the included studies, 8 were retrospective observational studies (Balandin Moreno et al., 2014, Chen and Shi, 2018, De Pascale et al., 2014, Geng et al., 2018, Ibrahim et al., 2018, Maseda et al., 2015, Vardakas et al., 2015b, Wu et al., 2016), 1 was a prospective observational study (Di Carlo et al., 2013), and 1 was an RCT (Ramirez et al., 2013). A total of 593 patients were enrolled, with the majority (88.4%) being

admitted to ICU with severe infections (these patients had an Acute physiology and chronic health evaluation II (APACHE II) score more than 15).

The main pathogens were CRGNB, especially CRKP. The indications for using tigecycline were nosocomial pneumonia (HAP and VAP), BSI, cIAI and cSSTI. Seven studies (Balandin Moreno et al., 2014, De Pascale et al., 2014, Di Carlo et al., 2013, Geng et al., 2018, Ibrahim et al., 2018, Vardakas et al., 2015b, Wu et al., 2016) evaluated the sensitivity of pathogens to tigecycline, and the susceptibility rate ranged from 79.5% to 100%. The most commonly used antibiotics in the control group was SDT; only two studies (Maseda et al., 2015, Ramirez et al., 2013) assessed non-tigecycline treatments. The characteristics of the studies included in this review are shown in Table 3-1.

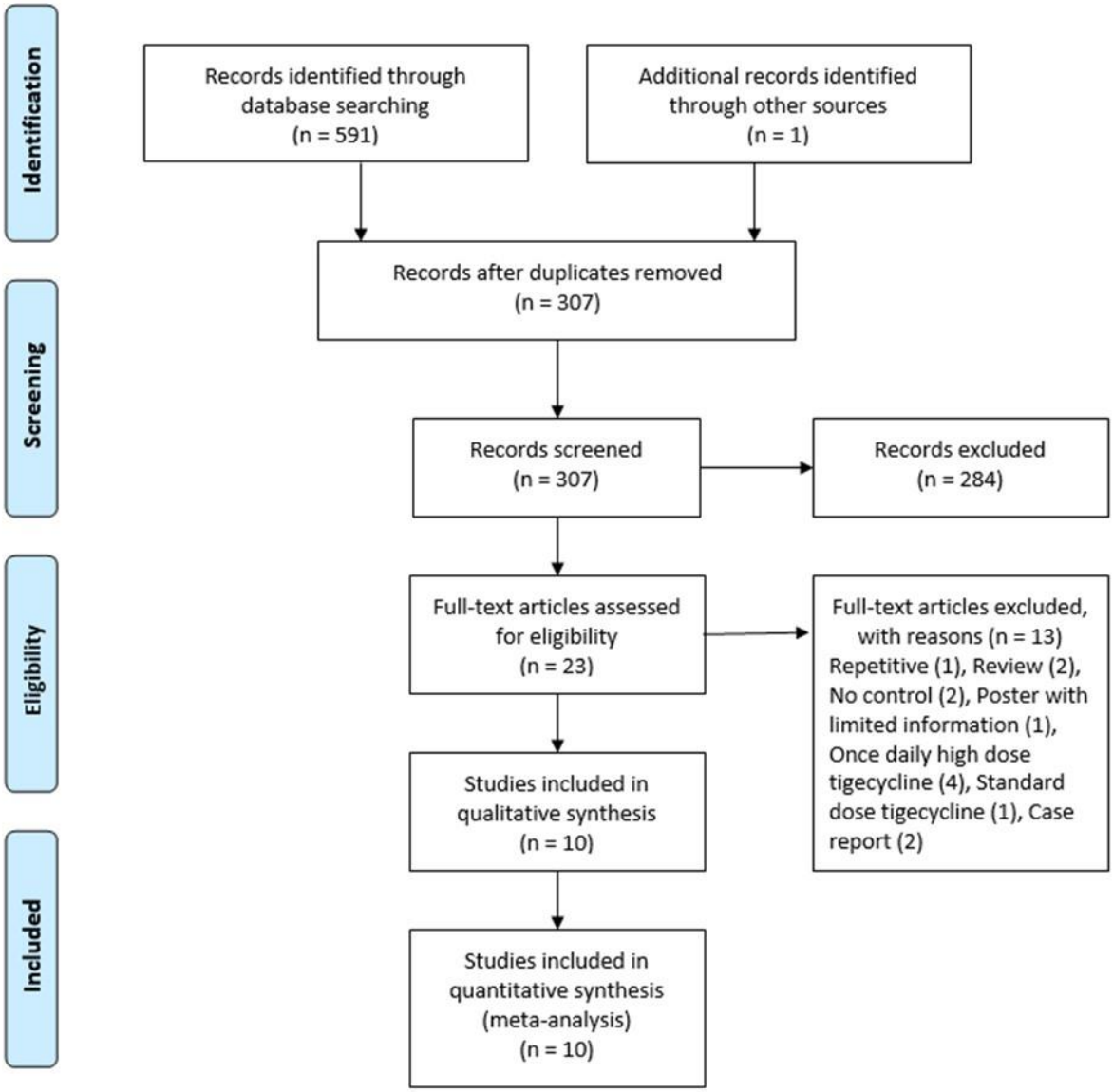

Figure 3-1. Flow chart indicating the process of literature search and review for effectiveness and safety of high-dose tigecycline for the treatment of severe infections based on eligible criteria.

Table 3-1. Characteristics of included studies in the systematic review and meta-analysis.

Reference	Study design, period, country	Characteristics HDT/Control	Type of infection	Causative pathogens	Mortality assessed	Sample size	Concomitant Antibiotics in HDT group	Antibiotics used in control group	Sensitivity to TGC
Chen, 2018	SC[a], RS, 2013-2015, CHN	NCU patients, APACHE II 18.4±4.7/19.6±5.8, Age 58.3±17.5/64.6±19.7	VAP	MDR (AB, KP), Other GNB	28-days	69/54	CEF-SUL/PTZ/IPM/MEM	SDT+CEF-SUL/PTZ/IPM/MEM	NR
De Pascale, 2014	SC, RS,2009-2012, ITA	ICU patients, SOFA 7.4±2.7/7.8±3.2, Age 60.7±12.5/64.5±16.9	VAP	GNB (AB, KP), CR (94%)	ICU	33/30	87.9% concomitant antibiotics	SDT+80% concomitant antibiotics	All MIC≤2 mg/ml
Di Carlo, 2013	SC, PS, 2011-2012, ITA	ICU patients, APACHE II 23.4±1.7, Age 56.6±15	BSI	CRKP	ICU	12/18	COL	SDT +COL	100%
Geng, 2018	SC, RS, 2014-2016, CHN	ICU patients, APACHE II 20.7±9.4 / 20.2±6.0, Age 65.1±14.3/61.8±13.9	BSI	CRKP	Hospital	23/17	CARB or BLI or AMG	SDT+CARB or BLI or AMG	79.5%
Ibrahim, 2018	SC, RS, 2013-2014, EGY	ICU patients, SOFA 9.5, Age 57.5	cSSTI, cIAI, CAP	AB, KP, EC, ETB, ETC, SA, SP	ICU	35/33	No	SDT	100%
Maseda, 2015	MC, RS, 2012-2013, ESP	SICU patients, SOFA 7.0±3.3/5.5±3.7, Age 65.7±7.3/65.7±16.3	cIAI	PM	28-days	54/67	PTZ/antifungals	CARB	NR
Moreno, 2014	SC, RS, 2009-2011, ESP	ICU patients, APACHE II 19.7±8.2/21.8±3.1, Age 56.4±15.8/51.5±7.5	PNA, UTI, cIAI , BSI, MEN	CRKP	30-days	10/6	COL/ CARB / CFX/PTZ	SDT+COL/CARB/CFX/AMK	100%

To be continued.

Table 3-1. Continued.

Reference	Study design, period, country	Characteristics HDT/Control	Type of infection	Causative pathogens	Mortality assessed	Sample size	Concomitant Antibiotics in HDT group	Antibiotics used in control group	Sensitivity to TGC
Ramirez, 2013	MC, RCT, 2008-2011,	APACHEII, 74.3%≤15/67.7%≤15, Age 61.5±16.1/64.9±15.3	HAP (VAP 40.6%)	GNB, SA, SP	21-days	35/34	CEF and TOB or AMK	IMI + VAN and TOB or AMK	NR
Vardakas, 2015	SC, RS, GRC	ICU patients, APACHEII 16.3±7 Age 65.8±13.5	BSI, LRTI, UTI, cIAI, cSSTI	CRKP	Hospital	26/6	COL/AMG/CRAB	SDT+COL/AMG/CARB	96.8%
Wu, 2016	SC, RS, 2013-2015, CHN	RICU patients, APACHEII 15-19 (IQR), SOFA 3.5±1.1, Age 74.6±9.4	HAP (VAP)	CR- GNB (CRAB, CRKP)	ICU	20/11	CEF-SUL/ PTZ/ CRAB	SDT+CEF-SUL/PTZ/CARB	96.8%

[a]Abbreviations: AB, *Acinetobacter baumannii*; AMG, aminoglycoside; AMK, amikacin; APACHE-II, Acute physiology and chronic health evaluation-II; BLI, β-lactamase inhibitor; BSI, bloodstream infection; CAP, community-acquired pneumonia; CARB, carbapenems; CEF-SUL, Cefoperazone-sulbactam; CFX, ciprofloxacin; CHN, China; ITA, Italy; cIAIc, complicated intra-abdominal infections; COL, colistin; CR, carbapenem resistant; CRAB, carbapenem resistant *Acinetobacter baumannii*; CRKP, carbapenem-resistant *klebsiella pneumoniae*. cSSTIc, complicated skin and soft tissue infections; EC, *Escherichia coli*; ESP, Spain; ETB, *Enterobacter*; ETC, *Enterococcus*; GNB, Gram-negative bacteria; GRC, Greece; HAP, hospital-acquired pneumonia; HDT, high-dose tigecycline; ICU, Intensive care unit; IPM, imipenem; IQR, interquartile range; KP, *Klebsiella pneumoniae*; LRTI, lower respiratory tract infection; MC, multiple centres; MDR, multidrug-resistant; MEM, meropenem; MEN, meningitis; MIC, minimum inhibitory concentration; NCU, neurological care unit; NR, not report; PM, polymicrobial; PNA, pneumonia; PS, prospective study; PTZ, piperacillin-tazobactam; RICU, respiratory intensive care unit; RS, retrospective study; SA, *Staphylococcus aureus*; SC, single centre; SDT, standard-dose tigecycline; SOFA, Sequential organ failure assessment; SP, *Streptococcus*; TGC, tigecycline; TOB, tobramycin; UTI, urinary tract infection; VAN, vancomycin; VAP, ventilator-associated pneumonia.

3.3.2 ASSESSMENT OF BIAS

Most of the included non-randomized studies had serious or critical risks of bias due to the nature of the design of observational studies (Table 3-2). When studies with critical risk or no information were excluded, the all-cause mortality in the HDT group was still lower than that in the control group without obvious heterogeneity (OR 0.32, 95% CI 0.20-0.50, I^2 = 0%, p < 0.001). The included RCT was assessed as unclear risk of bias because of unclear information in the selection bias domain, although other domains were at low risk.

Table 3-2. Assessment of the risk of bias for included non-randomised studies.

	Confounding	**Selection bias**	**Classification bias of interventions**	**Deviations from intended interventions**	**Bias due to missing data**	**Measurement bias**	**Report bias**	**Overall**
Chen et al. 2018	Serious	Serious	Serious	Moderate	Low	Low	Moderate	Serious
De Pascale et al. 2014	Serious	Serious	Serious	NI	Low	Low	Moderate	Serious
Di Carlo et al. 2013	Serious	Serious	Serious	Moderate	Low	Low	Moderate	Serious
Geng et al. 2018	Serious	Serious	Serious	NI	Low	Low	Moderate	Serious
Ibrahim et al. 2018	Serious	Serious	Serious	NI	Low	Low	Moderate	Serious
Maseda et al. 2015	Critical	Critical	Serious	Serious	Low	Low	Moderate	Critical
Moreno et al. 2014	Serious	Serious	Serious	Serious	Low	Low	Moderate	Serious
Vardakas et al. 2015	NI	NI	Serious	NI	Low	Low	Moderate	NI
Wu et al. 2016	Serious	Serious	Serious	Serious	Low	Low	Moderate	Serious

The funnel plot of all-cause mortality of the included studies is shown in Figure 3-2. The Egger regression-based test gave $p = 0.303$, which means no obvious publication bias was detected.

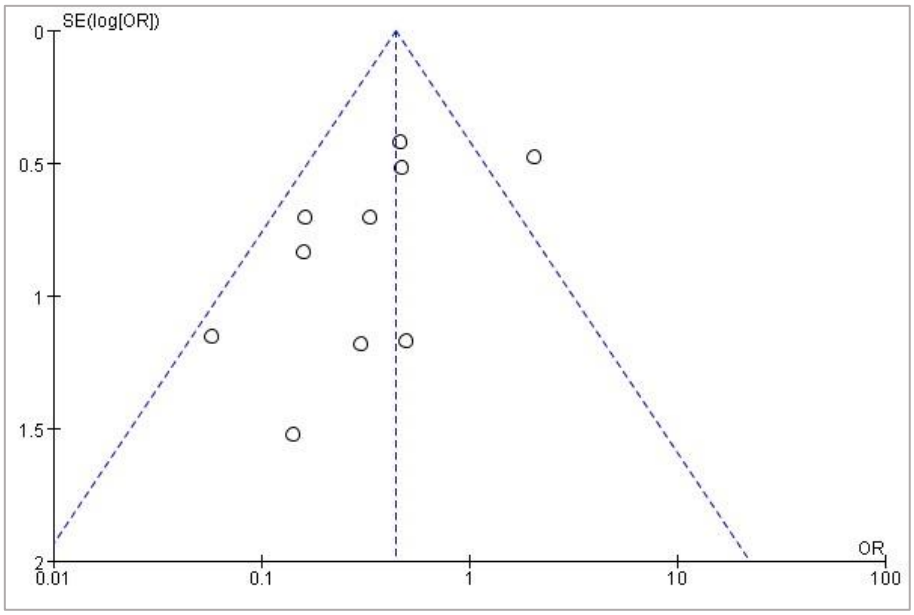

Figure 3-2. Funnel plot of all-cause mortality in high-dose tigecycline (HDT) regimens compared with controls

3.3.3 ALL-CAUSE MORTALITY

The pooled all-cause mortality was 31.4%. Compared with the control group, mortality in the HDT group was statistically lower (OR 0.44, 95% CI 0.30-0.66, I^2 = 49%, $p < 0.001$). Further analysis indicated that the major pathogens were *Enterococcus faecium* and *Enterococcus faecalis* in Maseda et al. (Maseda et al., 2015), whereas in other studies, the main pathogens were GNB. When Maseda et al. was excluded, statistical heterogeneity was eliminated (OR 0.30, 95% CI 0.19-0.48, I^2 = 0%, $p < 0.001$).

All subgroups (HAP (including VAP)) (Chen and Shi, 2018, De Pascale et al., 2014, Ramirez et al., 2013, Wu et al., 2016), BSI (Di Carlo et al., 2013, Geng et al., 2018), and mixed infections (Balandin Moreno et al., 2014, Ibrahim et al., 2018, Vardakas et

al., 2015b)) except cIAI (Maseda et al., 2015) showed a favourable outcome in the HDT group. In cIAI no statistical differences between HDT and control were seen (OR 2.04, 95% CI 0.80-5.23, p = 0.140). The impact of carbapenem resistance on mortality showed that HDT containing regimens reduced mortality in CRGNB (OR 0.20, 95% CI 0.09-0.45, I^2 = 0%, p = 0.001) (Figure 3-3) (Balandin Moreno et al., 2014, Di Carlo et al., 2013, Geng et al., 2018, Vardakas et al., 2015b, Wu et al., 2016).

3.3.4 CLINICAL CURE AND MICROBIOLOGICAL ERADICATION RATE

Four studies (Chen and Shi, 2018, De Pascale et al., 2014, Ramirez et al., 2013, Wu et al., 2016) with 286 patients evaluated the clinical cure rate. Patients given HDT had a higher clinical cure rate compared to controls (OR 3.43, 95% CI 2.09-5.63, I^2 = 0%, p < 0.001). In the seven studies (Balandin Moreno et al., 2014, Chen and Shi, 2018, De Pascale et al., 2014, Geng et al., 2018, Ibrahim et al., 2018, Ramirez et al., 2013, Wu et al., 2016) and 344 patients assessing the microbiological eradication rate, a pooled result favouring the HDT group was found (OR 2.25, 95% CI 1.44-3.50, I^2 = 30%, p < 0.001) (Figure 3-4). However, the pooled result of the microbiological eradication rate did not reach statistical significance when bacteria were resistant to carbapenem (OR 1.07, 95% CI 0.44-2.60, I^2 = 32%, p = 0.870) (Figure 3-4) (Balandin Moreno et al., 2014, Geng et al., 2018, Wu et al., 2016).

3.3.5 ADVERSE EVENTS

Five studies (Chen and Shi, 2018, De Pascale et al., 2014, Geng et al., 2018, Ibrahim et al., 2018, Ramirez et al., 2013) documented 197 adverse events, including diarrhoea (n=36), nausea (n=18), vomiting (n=14), renal injury (n=21), hepatic injury (n=66), and haematological injury (n=42). There were no statistical differences in the distributions of adverse events in the two groups (Figure 3-5).

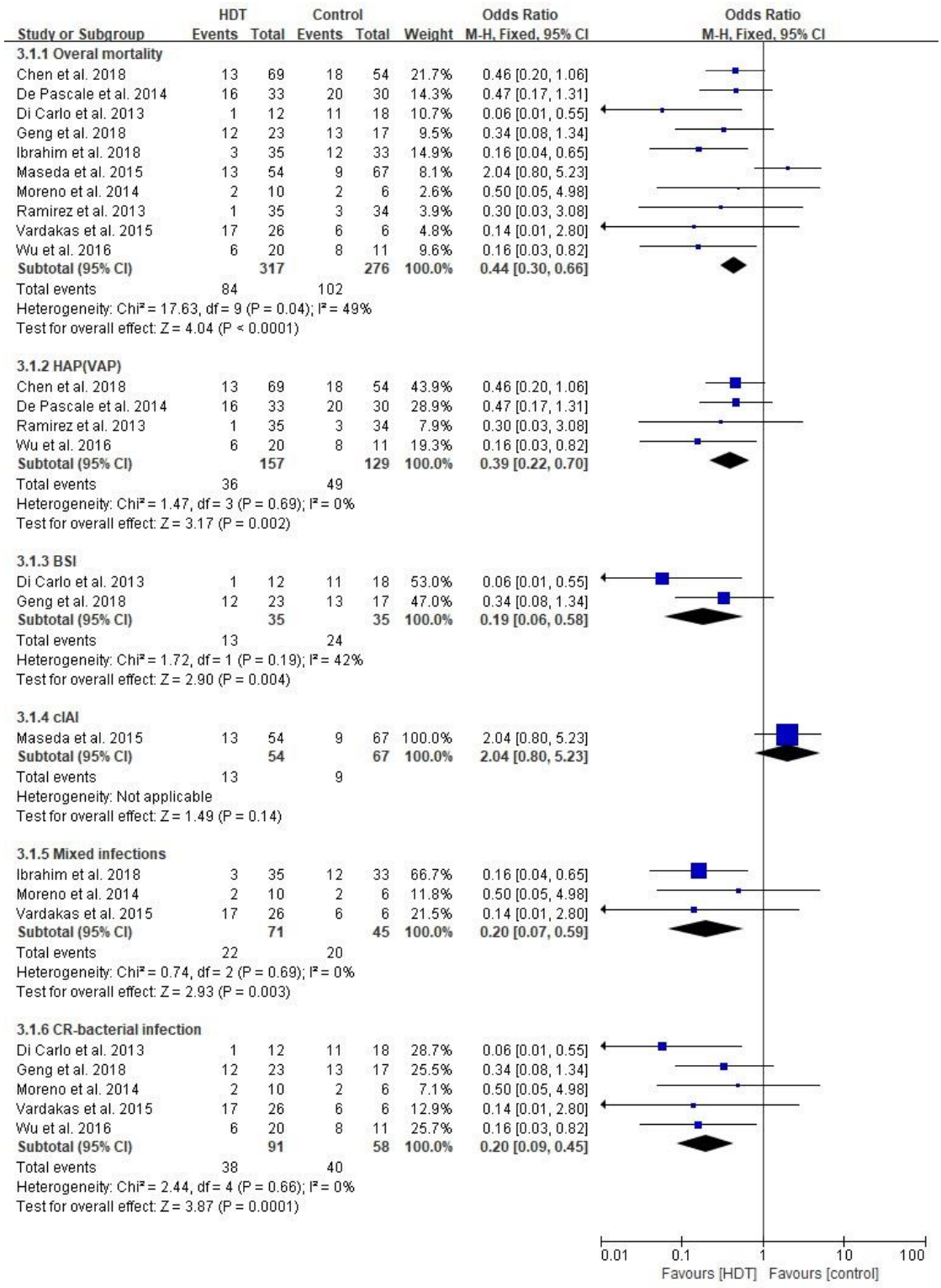

Figure 3-3. All-cause mortality of the high-dose tigecycline (HDT) regimens compared with controls. HAP, hospital acquired pneumonia; VAP, ventilator associated pneumonia; BSI, bloodstream infection: cIAI, complicated intra-abdominal infections; CR, carbapenem resistant.

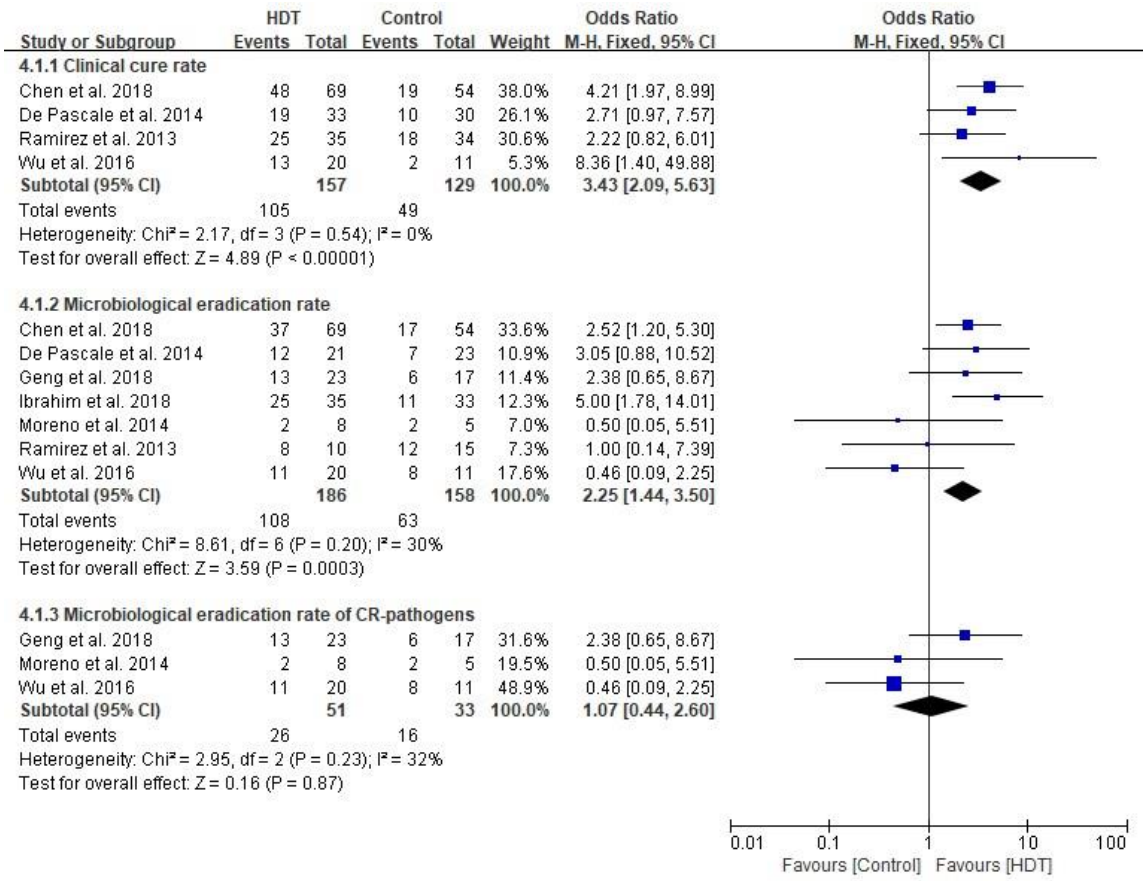

Figure 3-4. Clinical cure rate and microbiological eradication rate of high-dose tigecycline (HDT) regimens compared with controls. CR, carbapenem resistant.

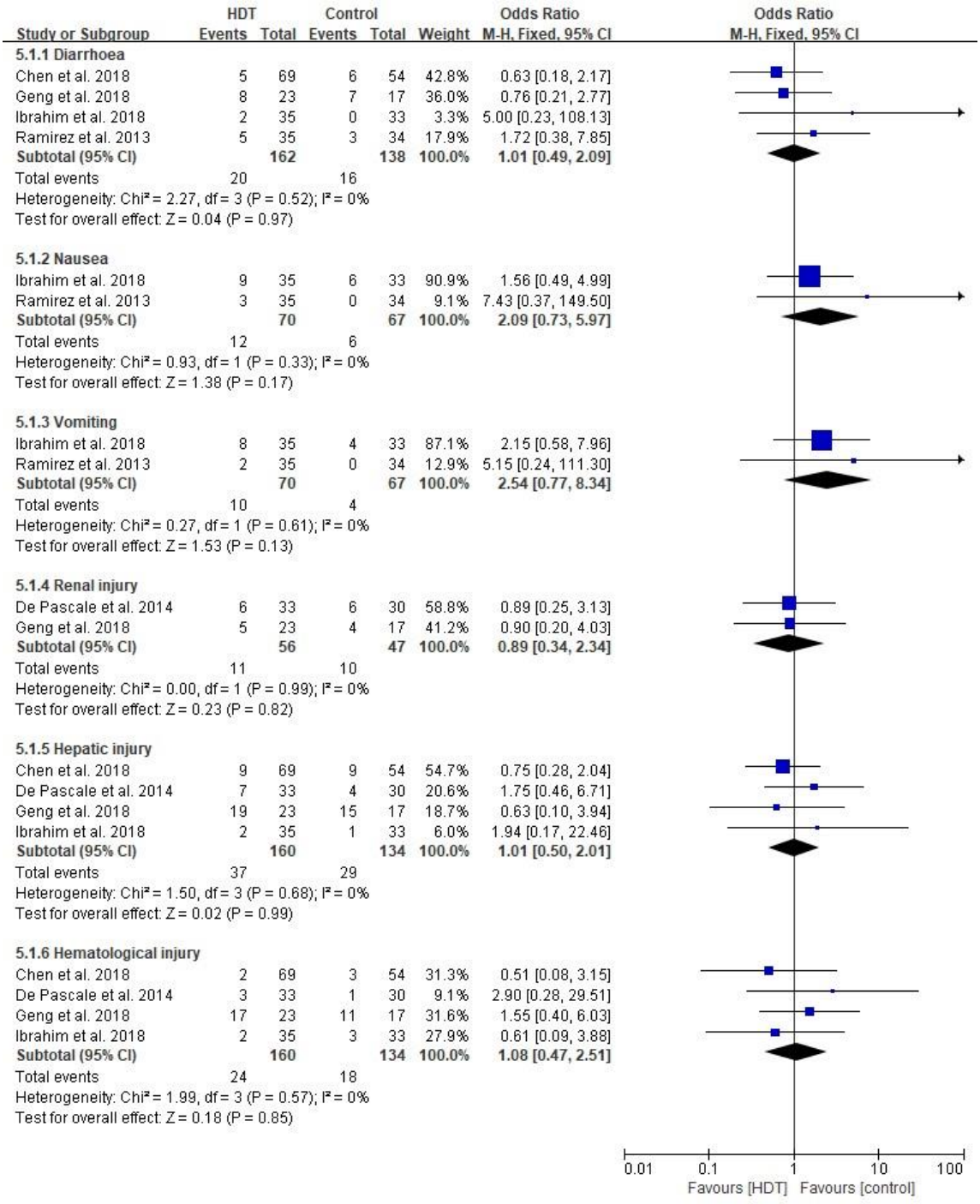

Figure 3-5. Adverse events of the high-dose tigecycline (HDT) regimens compared with controls.

3.3.6 RELIABILITY AND CONCLUSIVENESS OF THE PRIMARY OUTCOME

To determine the RIS for overall mortality, the control event rate was assumed to be 42% (calculated in this meta-analysis), the relative risk reduction was defined as 12% (estimated from this meta-analysis) with 80% power and a two-sided α error of 5%. At least 1,586 patients were required to get a reliable treatment effect analysis. In this review, there were 593 patients enrolled for the analysis of all-cause mortality. Although the pooled sample size was less than the RIS, the cumulative curve (Z-curve) crossed the sequential monitoring boundary indicating that the result in our meta-analysis is reliable and conclusive (Figure 3-6).

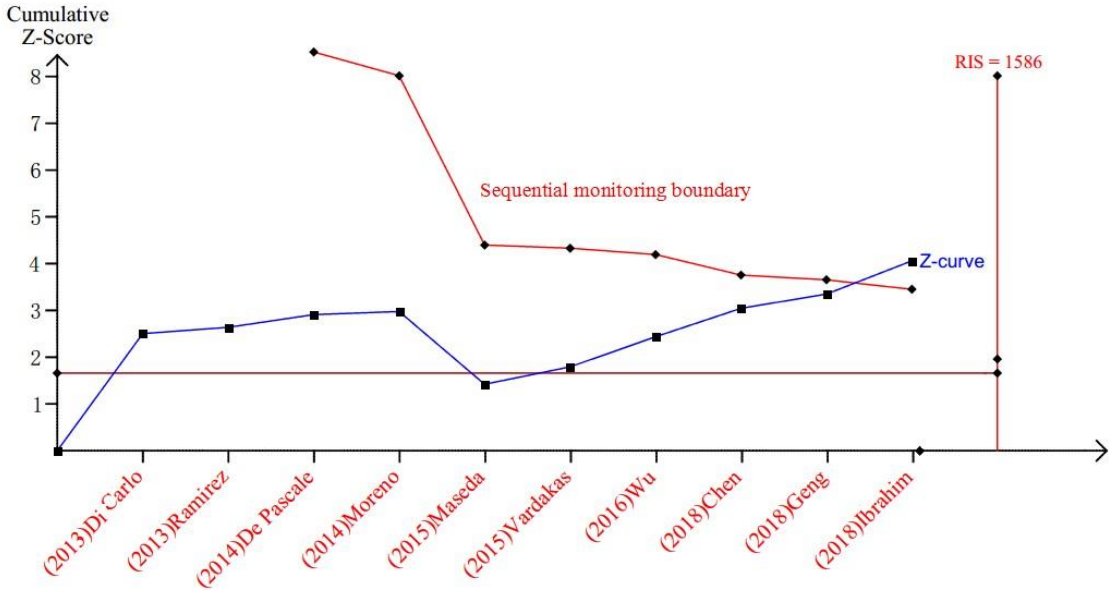

Figure 3-6. Cumulative meta-analysis assessing the effect of high-dose tigecycline on all-cause mortality of severe infections. The sequential monitoring boundary, which assumes a 42% control event rate and a 12% relative risk reduction with 80% power and two-sided α of 5%, has been crossed, indicating that the cumulative evidence is conclusive.

3.4 DISCUSSION

Tigecycline is widely used for difficult-to-treat infections because of its broad spectrum of antimicrobial ability and low rate of resistance. Studies illustrated that tigecycline had good activity against MDR pathogens, included methicillin-resistant *Staphylococcus aureus*, *A. baumannii*, *K. pneumoniae*, vancomycin-resistant *Enterococcus*, *Clostridium difficile* and other *Enterobacteriaceae* (Draghi et al., 2008, Rizek et al., 2015, Livermore, 2005, Hawkey et al., 2018). However, studies evaluating the efficacy of SDT in the treatment of severe infections raised concern about its effectiveness. A meta-analysis of 14 RCTs of around 7400 patients showed that tigecycline treatment is no better than the control antibiotics (Tasina et al., 2011). Two other studies concluded that tigecycline increased mortality and adverse events (Shen et al., 2015, Yahav et al., 2011). Ni et al. (Ni et al., 2016) reported that in terms of CR *Enterobacteriaceae* infections, SDT had similar overall mortality, clinical response and microbiological eradication rates when compared with other antibiotics, but that the HDT group decreased the mortality rate compared with the SDT regimen (OR 0.08, 95% CI 0.013-0.080, p = 0.006).

In our meta-analysis, 10 studies with 593 patients indicated that treatment with HDT decreased overall mortality while improving both the clinical cure and microbiological eradication rates. Subgroup analysis of the type of infection illustrated that all subgroups except cIAI showed favourable results under HDT. In the cIAI group, the lack of effect of HDT could be explained by the severity of infection in patients enrolled in the HDT group compared to the control group (i.e., patients had significantly higher Sequential Organ Failure Assessment [SOFA] and Simplified Acute Physiology Score II [SAPS II], and a higher percentage of patients required mechanical ventilation, renal replacement therapy and presented septic shock) (Maseda et al., 2015). Since there are no other studies focusing on cIAI included in our meta-analysis, the true effect of HDT in cIAI cannot be concluded with certainty.

In the subgroup analysis of infections caused by CR pathogens, the microbiological eradication rate did not show any statistical significance in the HDT group compared with SDT treatment. Epidemiological studies have shown that CRGNB usually present resistances against other antibiotics (Vardakas et al., 2015a, Tal-Jasper et al., 2016, Duin and Doi, 2017). In our meta-analysis, all the included pathogens for CR subgroup analysis were MDR *K. pneumoniae* and MDR *A. baumannii*, and the antibiotics used were those with high resistant rates in CRKP and CRAB. Tigecycline being a tetracycline-derived bacteriostatic agent, without the synergistic effect of other active bactericides, the clearance of those pathogens would be slow (Livermore, 2005). The combination of these reasons may explain the lack of difference in the microbiological eradication rate between HDT and the control group in the CR-subgroup.

Our analysis of the impact of carbapenem resistance on mortality showed that the HDT group experienced better outcomes. Previous studies have reported that CRGNB infections had higher mortality rates because of the proportion of inappropriate antibiotics therapy was higher (Siwakoti et al., 2018, Liu et al., 2015b, Morata et al., 2012). In our meta-analysis, the patients in almost all studies (excepting Geng et al. (Geng et al., 2018)) showed around 100% susceptibility to tigecycline. Therefore, although the microbiological eradication rate did not show any statistical differences, the mortality rate is still lower in the HDT group of the CR-infections subgroup.

Previous studies reported higher rates of adverse events in the SDT group when compared with non-tigecycline regimens (Shen et al., 2015). In our meta-analysis, eight out of 10 studies used SDT as controls. The lack of any statistically significant differences in our results suggests that HDT is tolerable and as safe as SDT, although it has higher adverse events rate when compared with non-tigecycline regimens reported in one included study (Ramirez et al., 2013).

The adverse events analysis of our review failed to assess the rate of development of tigecycline resistance in the HDT and SDT group due to limited information of the included studies. However, the results in this review showed that the microbiological eradication rate was lower in the SDT group compared with the HDT group. The lower microbiological eradication rate suggests that there were more pathogens exposed to a suboptimal concentration of tigecycline and hence would possibly select for more antimicrobial resistant bacteria. A similar result was also found in a review paper, which illustrated that the microbiological eradication rate was lower in the SDT group compared with other non-tigecycline antibiotics in the treatments of pneumonia caused by MDR *A. baumannii* (Mei et al., 2019). Therefore, the selection of SDT-containing regimens as the clinical choice should be reconsidered because it might increase the probability of emergence of XDR or PDR pathogens. Nevertheless, the real effect of tigecycline dose on the selection of antimicrobial resistance should be further studied.

There are several limitations in our study. First, all the included studies are limited to observational studies of small sample sizes and one RCT. Although the accumulated sample size crossed the sequential monitoring boundary, the real efficacy of HDT would only be concluded through a well-designed, properly-powered RCT, due to the nature of unavoidable confounders and bias in observational studies. Second, apart from the only RCT and one observational study, all the other studies included in the review had the control group utilizing SDT regimens, which limited the conclusion to comparisons between HDT and SDT, rather than comparisons between HDT and other commonly used non-tigecycline antibiotics. Third, although the calculated heterogeneity values between studies are low, the variations of interventions (concomitant antibiotics, time to start therapy, inappropriate antibiotics therapy rate), outcomes measurement (time to assess mortality, definition of clinical response, time to evaluate microbiological eradication rate, etc.) may affect the interpretation of the results.

3.5 CONCLUSIONS

The systematic review and meta-analysis suggest that HDT treatment has better outcomes in the treatment of severe infections when compared with SDT and other non-tigecycline containing regimens. The HDT regimen is associated with lower mortality rate, higher clinical cure, and microbiological eradication rate, while having similar adverse events rates compared with controls. We recommend using HDT if a tigecycline containing regimen is the clinical choice for severe infections, especially those infected with MDR bacteria. However, due to the high risks of bias of the included studies, well-designed, properly-powered RCTs are warranted to confirm the effectiveness and safety of HDT compared with SDT and other commonly used antibiotics.

4 CHAPTER 4 – INTRAVENOUS POLYMYXIN B AS ADJUNCTIVE THERAPY TO HIGH-DOSE TIGECYCLINE FOR THE TREATMENT OF NOSOCOMIAL PNEUMONIA DUE TO CARBAPENEM-RESISTANT *ACINETOBACTER BAUMANNII* AND *KLEBSIELLA PNEUMONIAE*

The increased effectiveness of HDT over SDT in treating severe infections, including CRGNB infections, has been demonstrated in Chapter 3. Given tigecycline is a bacteriostatic agent, it is usually used in combination with other antibiotics, especially when treating infections caused by CRGNB. In China, tigecycline plus polymyxin is the third most prevalent regimen in treating such infections as reported in Chapter 2. However, whether adding intravenous polymyxin B to HDT regimen would improve clinical outcomes in patients with CRGNB is still unclear. In this chapter, the effectiveness of the combination of intravenous polymyxin B and HDT in treating nosocomial pneumonia caused by CRAB and CRKP is assessed.

4.1 BACKGROUND

CRGNB, including CRAB, CRPA, and CRE (including *K. pneumoniae*, *E. coli*, and *Enterobacter* spp.) have been prioritized by the WHO as a critical group of pathogens that requires new antibiotics due to their increasing prevalence and extremely limited therapeutic options (Tacconelli et al., 2018). Data from the EARS-Net 2017 indicated that in the top ten pathogens causing ICU acquired pneumonia, carbapenem resistance rate in *Acinetobacter spp.* and *Klebsiella spp*. was as high as 64% and 15%, respectively (ECDC, 2018). The disease burden of infections caused by these resistant pathogens has also increased significantly. According to the results from a population-level modelling analysis using data from the EARS-Net, the proportion of the disability-adjusted life-years (DALYs) due to CRGNB increased from 18% in 2007 to 28% in 2015, while the DALYs due to CRKP doubled during the same period (from 4.3% to 8.79%) (Cassini et al., 2019).

Mortality in patients with pneumonia, one of the most common diseases resulting from infection by CR pathogens is worryingly high. A recent study including 690 critically ill patients with nosocomial pneumonia caused by CRGNB reported in-hospital mortality of 46.1% (Chen et al., 2022). Although the mortality was slightly lower in a multicentre study conducted in 18 hospitals in the US, carbapenem resistance still contributed to 27% of excess hospital mortality in patients with pneumonia (Hauck et al., 2016). The excess mortality could be partially attributed to inappropriate antibiotic therapy. A study indicated that patients with nosocomial pneumonia caused by CRGNB were more likely to receive inappropriate antibiotic therapy than their susceptible counterparts (25.8% vs 10%) (Zilberberg et al., 2019). Moreover, with limited antibiotic choices, targeted therapies for such infections are usually restricted to suboptimal agents, potentially leading to worse clinical outcomes as well.

Although there are new antibiotics marketed in recent years, the availability of these drugs in certain regions is still insufficient. As presented in chapter 2, among the 212 participating hospitals, only 16% of hospitals routinely offered the newly marketed β-lactam/β-lactamase inhibitor ceftazidime-avibactam (Zha et al., 2022). It comes as no surprise that traditional antibiotics, like tigecycline and polymyxins, turn to be the mainstream therapy for infections caused by CRGNB (Doi, 2019, El-Sayed Ahmed et al., 2020). Although tigecycline and polymyxins present good *in vitro* activity against CR bacteria, the clinical efficacy of these antibiotics is still unsatisfactory (Paul et al., 2022, Tamma et al., 2021). Compared with ceftazidime-avibactam, polymyxin E (colistin) therapy resulted in higher mortality and lower clinical response in a study with patients with CRE infections (van Duin et al., 2018). Likewise, other studies have demonstrated that tigecycline-containing treatment was associated with increased mortality compared to other regimens (Liang et al., 2018, Lou et al., 2022). In patients with severe infections and when ceftazidime-avibactam is lacking, using two active antibiotics together to increase effectiveness is widely accepted.

Due to the good *in vitro* synergistic effect of tigecycline and polymyxins (Barth et al., 2015, Cai et al., 2016), combination therapy with these two drugs has become one of the most preferred regimens in treating infections caused by CRAB and CRKP (Papst et al., 2018). In the latest Chinese clinical guidelines, polymyxin plus tigecycline is also recommended as one of the first choices in treating HAP and VAP caused by CRKP and CRAB; therefore, it turns out to be one of the most popular regimens in clinical practice in China (Shi et al., 2019). As shown in chapter 2, even with the limited availability of polymyxins in some hospitals, tigecycline plus polymyxin was still the third most frequent therapeutic choice in China for CRGNB infections, after tigecycline plus cefoperazone-sulbactam and tigecycline plus carbapenem (Zha et al., 2022). Despite the wide use of this combination, clinical studies of its efficacy are still limited. As of writing, only three studies assessed its effectiveness in treating infections (bacteraemia and intra-abdominal infection) due to CRAB, and none of

them demonstrated any benefit (Amat et al., 2018, Cheng et al., 2015, Chusri et al., 2019). Moreover, in patients with the minimum inhibitory concentration (MIC) of tigecycline against *A. baumannii* greater than 2 mg/L, the combination of tigecycline and colistin was even associated with an increased 14-day mortality in comparison with colistin-carbapenem therapy (hazard ratio 6.93, 95% CI, 1.61-29.78, $p = 0.009$) (Cheng et al., 2015).

Tigecycline used in these studies was all at its approved standard-dose (100 mg loading dose following 50 mg per 12 hours) rather than the recommended high-dose (200 mg loading dose following 100 mg per 12 hours) (Amat et al., 2018, Cheng et al., 2015, Chusri et al., 2019). PK studies have demonstrated that using the SDT resulted in suboptimal concentration in blood (0.72 mg/L) and lung (0.34 mg/L), which is insufficient in controlling infections caused by those carbapenem-resistant pathogens, given MICs of the contemporary clinical isolates (Barbour et al., 2009, Conte et al., 2005, Leng et al., 2021). Moreover, the synergistic effect of colistin and tigecycline is dose dependent. When colistin was combined with a high concentration of tigecycline, the bactericidal effect increased, while the bactericidal effect was attenuated when combined with the low concentration of tigecycline (Sato et al., 2021). Taken together, the use of SDT in the combination regimen might be a probable reason for the no differences between colistin and colistin-tigecycline combination therapy in the aforementioned studies.

However, it is still unclear whether using HDT could offset the disadvantages mentioned above. Compared with the SDT, the high-dose regimen increased the probability of target attainment at MICs of 1 and 2 mg/L from 72% to 99% and 11% to 71%, respectively (Ni et al., 2018). Moreover, favourable clinical outcomes of HDT in treating severe infections have been demonstrated in observational studies (De Pascale et al., 2014, Zha et al., 2020). In this chapter we present clinical outcomes in patients with pneumonia due to CRKP and CRAB after polymyxin B and HDT combination therapy.

4.2 METHODS

4.2.1 STUDY DESIGN

A retrospective cohort study was conducted in the West China Hospital, Sichuan University. Patients admitted to the intensive care unit between July 2019 and December 2021 with the diagnosis of nosocomial pneumonia were reviewed. The ethics committee of Xi'an Jiao tong-Liverpool university (reference number 19-01-05) (Appendix II) and the institutional review board of the West China Hospital approved the study, and the patient's consent was obtained from their family member or authorized person (reference number 2019-843).

4.2.2 DEFINITION AND DIAGNOSIS OF PNEUMONIA

The diagnosis of nosocomial pneumonia, including HAP and VAP, is made based on the 2016 clinical practice guidelines by the IDSA (Kalil et al., 2016). In brief, patients with a new or progressive infiltrate, consolidation, cavitation, or pleural effusion on their chest radiographs along with two or more of the following criteria were considered pneumonia: fever (> 38 ℃) or hypothermia (< 36 ℃), leucocytosis (> 12 × 10^{12}/L) or leukopenia (< 4 × 10^{12}/L), newly onset or worsening cough with purulent sputum or aspirate, and deteriorated oxygenation that required increment of oxygen or ventilation support.

VAP is defined as pneumonia developed more than 48 hours after intubation. HAP was pneumonia not incubating at the time of hospital admission but occurred 48 hours or more after the admission. Pathogens responsible for the corresponding nosocomial pneumonia were determined by the quantitative or semi-quantitative culture of specimens from bronchoalveolar lavage, endotracheal aspirate, or sputum, collected within 48 hours before or after the onset of pneumonia (Spalding et al., 2017). All endotracheal aspirate and sputum samples were subjected to microscopic analysis; only specimens with more than 25 neutrophils and less than ten squamous

epithelial cells per low-power field were considered qualified specimens for culture (Liu et al., 2019). The threshold of quantitative culture for a positive bronchoalveolar lavage and endotracheal aspirate is 10^4 CFU/ml and 10^5 CFU/ml, respectively; the threshold for the semi-quantitative culture of sputum is at least moderate growth on plates (JRS, 2009).

4.2.3 MICROBIOLOGICAL TESTS

The identification of pathogens was performed with the Vitek 2 system (bioMérieux) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (bioMérieux). Antimicrobial susceptibility testing was performed with the microdilution method and interpreted according to the breakpoint recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (EUCAST, 2022). Pathogens with the MIC of meropenem ≥ 8 mg/L were defined as carbapenem-resistance, and those with the MIC of tigecycline and polymyxin B ≤ 2 mg/L were considered susceptible to tigecycline and polymyxins, respectively.

4.2.4 PARTICIPANTS AND ANTIMICROBIAL THERAPY

Patients aged over 18 years with the diagnosis of nosocomial pneumonia caused by CRKP and CRAB receiving either tigecycline or tigecycline-polymyxin B as their targeted therapy within 3 days after the report of the responsible pathogen were eligible for inclusion in the present study. Tigecycline used in the present study was at its high-dose regimen (200 mg loading dose following 100 mg per 12 hours). Polymyxin B was administrated with a loading dose of 1,000,000 IU following 750,000 IU per 12 hours. In patients with renal impairment, the maintenance dose of polymyxin B was adjusted to 500,000 IU per 12 hours. Combination therapy with carbapenems, aminoglycosides, fluoroquinolones, and classical β-lactam/β-lactamase inhibitors (piperacillin-tazobactam, cefoperazone-sulbactam) was allowed, while the combination with ceftazidime-avibactam or minocycline was excluded in the present study. Only patients who received polymyxin B for more

than 50% of the total tigecycline treatment time were treated as the combination therapy. Polymicrobial infections were permitted if the concomitant pathogens were susceptible to tigecycline. Patients meeting the following criteria were excluded: patients received the targeted therapy less than 48 hours, including patients who died and those who received the change of antibiotics within 48 hours; pathogens had the MIC of tigecycline or polymyxin B > 2 mg/L; concomitantly had other site infections during the pneumonia course, like intra-abdominal infection, central nervous system infection, central catheter infections, UTI and wound infections; co-infected with *P. aeruginosa* or *S. maltophilia*; co-infected with Gram-positive bacteria or fungi. In case of patients with multiple nosocomial pneumonia, only the first admission was included.

4.2.5 CLINICAL OUTCOMES AND DEFINITIONS

The primary interest of the study was the 14-day all-cause mortality after the onset of pneumonia. The secondary outcomes were the 14-day clinical cure, microbiological cure, and nephrotoxicity occurring during the targeted antibiotic course. Clinical cure was defined as the complete resolution of symptoms and signs due to pneumonia or such improvement of patients that antibiotics were stopped within 14 days after the onset of pneumonia. The microbiological cure was defined as the absence of responsible pathogens recovered from the following cultures of specimens from the sputum, endotracheal aspirate or the bronchoalveolar lavage within 14 days after the initiation of the targeted therapy. Nephrotoxicity was defined as an increment of serum creatinine of 0.5 mg/dl from the baseline to at least two consecutive measurements during the antibiotic course after receiving 2 or more days of the targeted therapy (Cheng et al., 2015). For patients who died within 14 days, the follow-up time point of the assessment was set to the date of death.

4.2.6 DATA EXTRACTION

The following information was collected from the patient's medical record: age, gender, pre-existing medical conditions, type of pneumonia, responsible pathogens, antimicrobial regimens, duration of the targeted antibiotic therapy, whether having septic shock, whether receiving vasopressors, the SOFA score, the 14-day mortality, clinical and microbiology cure within 14 days after the onset of pneumonia, and nephrotoxicity. Moreover, the age-adjusted *Charlson* comorbidity index score was calculated based on information from the medical records.

4.2.7 STATISTICAL ANALYSIS

Categorical variables were summarized as counts and percentages. The differences in categorical variables between patients in the tigecycline group and the tigecycline-polymyxin B group were analysed with the Chi-squared test or Fisher's exact test ($n < 10$ events). Continuous variables were expressed as median and interquartile ranges and compared with the Mann-Whitney *U* test.

The propensity score matching was applied to identify a cohort with similar baseline characteristics in the two groups. The propensity score was calculated using the multivariable logistic regression model, with receiving the combination therapy as the dependent variable and *a priori* decided variables (the age-adjusted *Charlson* comorbidity index score, inappropriate initial antibiotic therapy, pathogen, polymicrobial pneumonia and the SOFA score) as covariates. The matched cohort was created with the 1:1 matching protocol through a greedy-matching algorithm, with a calliper of 0.2 of the standard deviation of the logit of the propensity score. Standardized mean differences of baseline variables were calculated to assess the balance in the matched cohort. OR and 95% CI for clinical outcomes were calculated by adjusting the SOFA score, polymicrobial infection, inappropriate initial antibiotic therapy, and the age-adjusted *Charlson* comorbidity index score in the matched cohort. The same analyses were also conducted in the original cohort as the

sensitivity analysis. The discrimination of the multivariable logistic regression model of the primary outcome was assessed with the area under the receiver operating characteristic curve (AUC).

Subgroup analyses of the primary outcome were also conducted in the matched cohort. Patients were stratified with age (≤ 65 or > 65 years), pneumonia type (HAP or VAP), pathogen (*A. baumannii or K. pneumoniae*), and initial empirical antibiotic therapy (appropriate or inappropriate). Moreover, we also conducted sensitivity analyses in patients without polymicrobial pneumonia and septic shock. The OR and 95% CI of the primary outcome in each subgroup were calculated in the univariable logistic regression model by defining patients receiving tigecycline as the reference. All reported *p* values were two-sided, and the $p < 0.05$ was considered statistically significant. All statistical analyses were performed with R software version 3.6.2 (R Foundation for Statistical Computing).

4.3 RESULTS

4.3.1 STUDY COHORT

There were 314 patients with nosocomial pneumonia due to CRAB and CRKP identified from medical records, among whom 152 met the exclusion criteria. Of the remaining 162 patients in the original study cohort, 68 (42%) received the combination therapy, and 94 (58%) received tigecycline therapy (Figure 4-1).

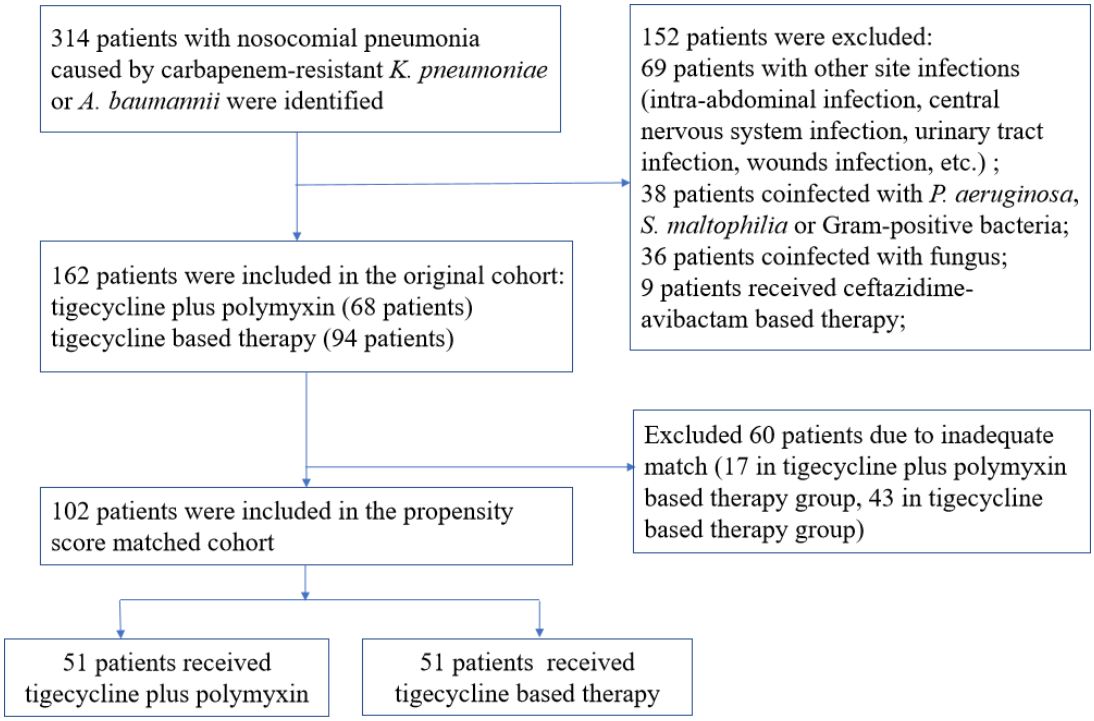

Figure 4-1. Flowchart of the inclusion and matching process.

There were imbalances in the prespecified baseline variables between the two groups in the original cohort, like the inappropriate initial antibiotic therapy, responsible pathogen, polymicrobial pneumonia and the SOFA score, which have been demonstrated well to be associated with clinical outcomes in patients with severe pneumonia. After propensity score matching, 51 patients receiving the combination therapy were matched with 51 patients receiving tigecycline (Figure 4-1). The absolute standardized mean differences of the prespecified variables were then less than 0.1 in the matched cohort, indicating acceptable minor differences between the two groups (Figure 4-2).

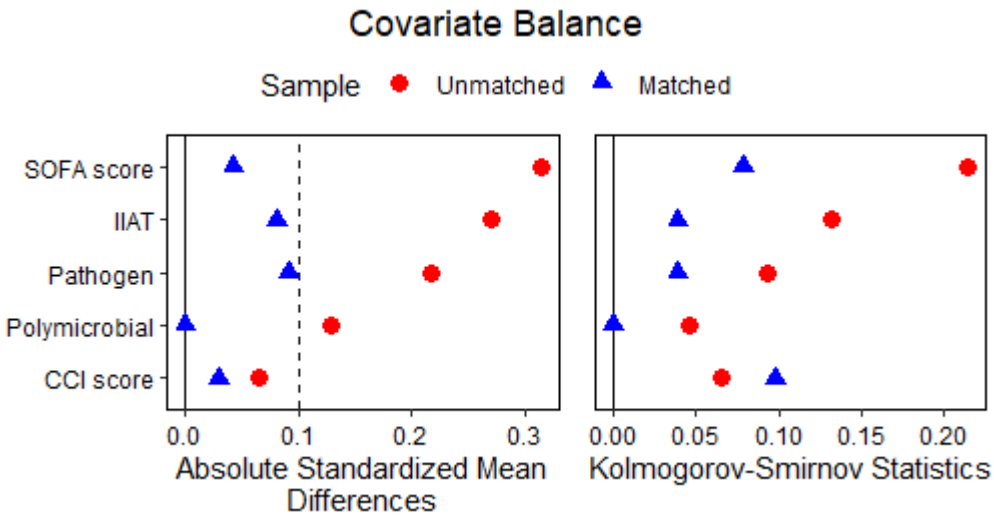

Figure 4-2. The covariate balance in the two groups before and after the propensity score matching. Red dots and blue triangles indicated the absolute standardized mean differences (left) and the Kolmogorov-Smirnov statistics (right) of the covariate in the original and the matched cohort, respectively. SOFA, Sequential Organ Failure Assessment, IIAT, Inappropriate Initial Antibiotic Therapy, CCI score, the age-adjusted Charlson comorbidity index.

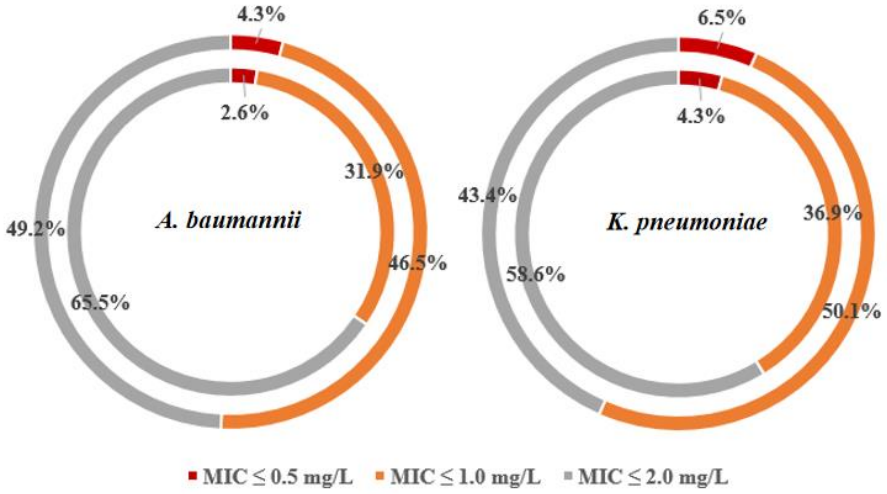

Figure 4-3. The distribution of minimum inhibitory concentration values of tigecycline and polymyxin B in *A. baumannii* and *K. pneumoniae* strains included in the study. The outer cycle represents the distribution of tigecycline MICs, while the inner cycle is the distribution of polymyxin B MICs. MIC, minimum inhibitory concentration.

4.3.2 CHARACTERISTICS OF PATIENTS IN THE MATCHED COHORT

The median age of patients in the matched cohort was 60 (IQR 51.25-76) years, and 69.6% were male. Most patients reported comorbidities, with an age-adjusted *Charlson* comorbidity index score of 4 (IQR 2-6). 63 (61.8%) patients were diagnosed with VAP, and 49 (38.2%) were diagnosed with HAP. Among the included patients, 70.6% (72) were infected with *A. baumannii*, 29.6% (30) were with *K. pneumoniae*, and 9.8% (10) of them were polymicrobial pneumonia. The MICs of tigecycline and polymyxin B in *A. baumannii* and *K. pneumoniae* strains are shown in Figure 4-3. At the onset of pneumonia, patients were critically ill, with a SOFA score of 9 (IQR 7.25-12). 87.3% (89) of patients developed septic shock during the pneumonia course, and the median time of using vasopressors was 10 (IQR 6-18) days (Table 4-1).

Table 4-1. Characteristics of patients with nosocomial pneumonia caused by carbapenem-resistant *A. baumannii* or *K. pneumoniae*.

	Original cohort			**Propensity score-matched cohort**			
Variable	**Tigecycline-polymyxin B, n = 68, 42%**	**Tigecycline, n = 94, 58%**	***p***	**Tigecycline-polymyxin B, n = 51, 50%**	**Tigecycline, n = 51, 50%**	***p***	**Standardized Mean Differences**
Age, years, median [IQR]	63 [52, 74.25]	63 [51, 75.75]	0.764	67 [54, 76.5]	56 [49, 75.5]	0.235	0.238
Male Gender, n (%)	48 (70.6)	64 (68.1)	0.867	36 (70.6)	35 (68.6)	1.000	0.043
Preexisting Medical Conditions, n (%)							
Hypertension	26 (38.2)	44 (46.8)	0.354	20 (39.2)	21 (41.2)	1.000	0.04
Diabetes Mellitus	16 (23.5)	24 (25.5)	0.915	14 (27.5)	11 (21.6)	0.645	0.137
Chronic Heart Disease	16 (23.5)	14 (14.9)	0.233	13 (25.5)	7 (13.7)	0.212	0.3
Chronic Kidney Disease	4 (5.9)	3 (3.2)	0.660	3 (5.9)	3 (5.9)	1.000	<0.001
Chronic Liver Disease	5 (7.4)	8 (8.5)	1.000	2 (3.9)	6 (11.8)	0.269	0.295
Malignancy	10 (14.7)	17 (18.1)	0.722	8 (15.7)	11 (21.6)	0.611	0.152
History of Surgery	29 (42.6)	38 (40.4)	0.903	20 (39.2)	20 (39.2)	1.000	<0.001
***Charlson* Comorbidity Index, median (IQR)**	3 [1, 4]	3 [1, 5]	0.506	4 [2, 6]	4 [2, 6]	0.935	0.027
Type of Pneumonia, n (%)			0.730			0.222	0.285
Hospital-acquired Pneumonia	28 (41.2)	35 (37.2)		23 (45.1)	16 (31.4)		
Ventilator-associated Pneumonia	40 (58.8)	59 (62.8)		28 (54.9)	35 (68.6)		
Pathogen, n (%)			0.26			0.828	0.086
A. baumannii	45 (66.2)	71 (75.5)		35 (68.6)	37 (72.5)		
K. pneumoniae	23 (33.8)	23 (24.5)		16 (31.4)	14 (27.5)		

Table continued on next page.

Table 4-1. Continued.

	Original cohort			**Propensity score-matched cohort**			
Variable	**Tigecycline-polymyxin B, n = 68, 42%**	**Tigecycline, n = 94, 58%**	***p***	**Tigecycline-polymyxin B, n = 51, 50%**	**Tigecycline, n = 51, 50%**	***p***	**Standardized Mean Differences**
Polymicrobial Pneumonia, n (%)	7 (10.3)	14 (14.9)	0.533	5 (9.8)	5 (9.8)	1.000	<0.001
Concomitant Antibiotics, n (%)			0.23			0.253	0.651
Aminoglycoside	0 (0.0)	2 (2.4)		0 (0.0)	2 (4.3)		
Fluoroquinolone	1 (1.8)	2 (2.4)		0 (0.0)	0 (0.0)		
Carbapenems	27 (48.2)	21 (25.6)		21 (53.8)	15 (32.6)		
Piperacillin-Tazobactam	0 (0.0)	4 (4.9)		0 (0.0)	2 (4.3)		
Cefoperazone-Sulbactam	24 (42.9)	50 (61.0)		16 (41.0)	25 (54.3)		
Carbapenem plus Sulbactam	4 (7.1)	2 (2.4)		2 (5.1)	2 (4.4)		
Carbapenem plus Moxifloxacin	0 (0.0)	1 (1.2)		0 (0.0)	0 (0.0)		
Inappropriate Initial Antibiotic Therapy, n (%)	33 (48.5)	58 (61.7)	0.132	26 (51.0)	28 (54.9)	0.843	0.079
Duration of antibiotic therapy, days, median (IQR)	16 [10, 25]	14 [10, 21]	0.378	16 [10, 25]	15 [10, 25.5]	0.730	0.011
Septic Shock, n (%)	60 (88.2)	70 (74.5)	0.049	46 (90.2)	43 (84.3)	0.553	0.177
Duration of Vasopressors, days, median (IQR)	12 [7, 21.25]	8 [4, 15]	0.03	12.5 [8, 21.75]	9 [5, 15]	0.063	0.286
SOFA score, median [IQR]	10 [7, 12]	8 [5, 11]	0.018	10 [7.5, 12]	9 [7.5, 12.5]	0.944	0.043

Less than half of patients (47%, 48) received appropriate initial antibiotic treatment. For the targeted therapy, 83.3% (85) of patients received concomitant antibiotics in addition to tigecycline or tigecycline-polymyxin B, of which carbapenems and cefoperazone-sulbactam were the most frequently used. The duration of targeted antibiotic therapy was relatively long in the matched cohort, with a median time of 15 (IQR 10-25) days.

4.3.3 CLINICAL OUTCOMES

The overall 14-day mortality, clinical cure, and microbiological cure in the matched cohort were 24.5% (25/102), 53.9% (55/102), and 27.7% (28/102), respectively (Table 4-2). Nephrotoxicity developed in 50 (49%) patients during the targeted antibiotic course. In the multivariable logistic regression analysis, patients receiving the combination therapy was not associated with better clinical outcomes than those receiving tigecycline, with comparable 14-day mortality (adjusted OR 0.72, 95% CI 0.27-1.83, p = 0.486), 14-day clinical cure (adjusted OR 1.09, 95% CI 0.48-2.54, p = 0.823), 14-day microbiological cure (adjusted OR 0.96, 95% CI 0.39-2.35, p = 0.928). Moreover, the adjunctive therapy of polymyxin B to HDT did not increase the rate of nephrotoxicity (adjusted OR 0.85, 95% CI 0.36-1.99, p = 0.712). Similar results were also shown in the original study cohort.

Table 4-2. Clinical outcomes in patients with nosocomial pneumonia caused by carbapenem-resistant *A. baumannii* and *K. pneumoniae*.

	Patients included in analysis, No./Total No. (%)		
Clinical outcomes	**Tigecycline-polymyxin B**	**Tigecycline**	**Odds ratio[a] (95% CI), *p* Tigecycline as reference**
Overall analysis			
14-day mortality	14/68 (20.6%)	23/94 (24.5%)	0.73 (0.32-1.62), 0.449
Clinical cure	39/68 (57.4%)	57/94 (60.6%)	0.95 (0.47-1.92), 0.895
Microbiological cure	19/68 (28.4%)	32/94 (34.0%)	0.84 (0.39-1.72), 0.627
Nephrotoxicity rate	31/68 (45.6%)	37/94 (39.4%)	0.91 (0.44-1.85), 0.792
Matched cohort			
14-day mortality	11/51 (21.6%)	14/51 (27.5%)	0.72 (0.27-1.83), 0.486
Clinical cure	28/51 (54.9%)	27/51 (52.9%)	1.09 (0.48-2.54), 0.823
Microbiological cure	13/51 (25.5%)	15/51 (29.4%)	0.96 (0.39-2.35), 0.928
Nephrotoxicity rate	24/51 (47.1%)	26/51 (51.0%)	0.85 (0.36-1.99), 0.712

[a]The odds ratios were calculated by adjusting for the SOFA score, polymicrobial infection, inappropriate initial antibiotic therapy and the age-adjusted *Charlson* comorbidity index score. Area under the receiver operating characteristic curve for the multivariable logistic model of the primary outcome was 0.73 (95% CI, 0.63-0.83) in the original cohort and 0.71 (95% CI, 0.58-0.83) in the propensity score matched cohort, respectively. SOFA, Sequential Organ Failure Assessment; CI, confidence interval.

4.3.4 SUBGROUP AND SENSITIVITY ANALYSES

Among the predefined subgroup analyses, patients receiving the combination therapy tended to have a lower 14-day mortality in patients with VAP, but this did not reach statistical significance (OR 0.25, 95% CI 0.04-1.11, p = 0.09). In other subgroups stratified with age and the responsible pathogen, results were similar to the overall analyses. As polymicrobial infection and inappropriate initial antibiotic therapy might impact patients' outcomes, sensitivity analysis by excluding these patients did not change the trend of the results. Moreover, when only including patients with septic shock, the combination therapy was still not associated with decreased 14-day mortality (Table 4-3).

Table 4-3. Subgroup and sensitivity analyses of the primary outcome in patients with nosocomial pneumonia caused by carbapenem-resistant *A. baumannii* and *K. pneumoniae*.

Subgroup analysis[a]	**OR (95% CI)**	***p***
Age		
≤ 65	1.22 (0.21-7.21)	0.812
> 65	0.40 (0.12-1.31)	0.137
Pneumonia type		
HAP	1.15 (0.31-4.47)	0.832
VAP	0.25 (0.04-1.11)	0.09
Pathogen		
A. baumannii	0.43 (0.13-1.28)	0.139
K. pneumoniae	2.72 (0.48-21.9)	0.283
Initial empirical antibiotic therapy		
Appropriate	0.39 (0.07-1.69)	0.221
Inappropriate	1.11 (0.34-3.62)	0.859
Excluded polymicrobial pneumonia	0.56 (0.21-1.44)	0.231
Excluded patients without septic shock	0.65 (0.25-1.65)	0.366

[a]The subgroup and sensitivity analyses was conducted in the propensity score weighted cohort, unadjusted OR of the 14-day mortality in each subgroup was calculated by defining patients receiving tigecycline as the reference group. OR, odds ratio; CI, confidence interval; HAP, Hospital-acquired Pneumonia; VAP, Ventilator-associated Pneumonia.

4.4 DISCUSSION

As mentioned in the introduction, although the combination of tigecycline and polymyxins has been widely used, clinical evidence supporting the effectiveness of this combination is rarely limited. In some case report studies, the success of using this combination in treating infections caused by CRAB was observed (Guo et al., 2018, Li et al., 2022b). In contrast, case-control or cohort studies did not support such benefit of this combination when compared with polymyxin alone (Amat et al., 2018, Cheng et al., 2015, Chusri et al., 2019). In China, tigecycline-based therapy is the mainstream regimen for treating infections caused by CRGNB (Zha et al., 2022); therefore, it is imperative to know whether adding polymyxin B to tigecycline could result in better clinical outcomes than tigecycline-based therapy. However, such comparative studies are extremely limited. Through a comprehensive literature

review, only one retrospective study was published recently, in which the effectiveness of tigecycline-polymyxin B combination in the treatment of hospital-acquired pneumonia caused by CRE or CRAB was assessed in comparison with tigecycline-based therapy or polymyxin B-based therapy (Chang et al., 2022). In this study, the authors found that the combination of polymyxin B and tigecycline was not superior to appropriate polymyxin B-based therapy and tigecycline-based therapy (HR 0.50, 95% CI 0.31-0.81, p = 0.004, HR 0.77, 95% CI 0.53-1.12, p = 0.169, respectively). Such no-benefit might be partially attributed to the low concentration of tigecycline, as stated by the authors. Therefore, we reported results from our present study to answer whether the HDT regime would contribute to better clinical outcomes in this combination. To the best of our knowledge, this is the first study assessing the effectiveness of the adjunctive therapy of polymyxin B to HDT in treating nosocomial pneumonia caused by CRAB and CRKP. The results indicate that the combination therapy was not associated with better clinical outcomes when compared with the HDT therapy. These findings are in line with previous studies that the combination therapy with antibiotics demonstrating *in vitro* synergistic effects might not be superior to the monotherapy in treating infections due to CRGNB (Paul et al., 2018, Durante-Mangoni et al., 2013, Nutman et al., 2020).

Evidence supporting the combination of tigecycline and polymyxins in treating CRGNB infections was mainly derived from *in vitro* studies (Paul et al., 2022, Tamma et al., 2021, Tsuji et al., 2019). In these studies, tigecycline and polymyxin B (or colistin) were usually used at a high concentration; several times higher than the MICs of the tested pathogens (Scheetz et al., 2007, Petersen et al., 2006, Urban et al., 2010). When polymyxins were used at their approved dosage in clinical practice, the concentration would be much lower than that was used in those *in vitro* studies (Landersdorfer et al., 2018). Combining the clinically achievable concentration of polymyxin B with tigecycline did not demonstrate any synergistic activity against

CRAB *in vitro* (Hagihara et al., 2014). Therefore, it is not surprising to observe inconsistent results between *in vitro* and clinical studies.

It is of note that the tigecycline used in the present study was at its high-dose regimen. As demonstrated in *in vitro* studies, using the high-dose regimen resulted in better PK/PD parameters of tigecycline in plasma and the lung (De Pascale et al., 2020, Ni et al., 2018). Clinical studies have also demonstrated promising outcomes in patients who received the HDT. A study including critically ill patients with MDR bacterial infections indicated that the HDT regimen was the only independent predictor of clinical cure in VAP patients (De Pascale et al., 2014). As demonstrated in the meta-analysis in chapter 3, the HDT regimen was associated with decreased mortality in patients with HAP and VAP (OR 0.39, 95% CI 0.22-0.70, p = 0.002) (Zha et al., 2020). In the present study, the mortality was much lower (24.5%) than reported in other studies (46.1% to 57.1%) (Chen et al., 2022, Tuon et al., 2017), further supporting the promising effectiveness of the high-dose regimen.

Furthermore, the specific PK/PD profile of polymyxins in the lung might also contribute to the non-benefit of the combination therapy in the present study. PK studies have indicated that even when using the highest tolerable dose of polymyxins, the concentration of polymyxins in the lung is likely to be below optimal for combating infected strains unless MICs of these pathogens are well below the breakpoint (Cheah et al., 2015, Landersdorfer et al., 2018). Therefore, a higher-dose of polymyxin is required to achieve sufficient antibiotic attainment in such infections. However, the opposite situation in clinical practice is that doctors are likely to prescribe polymyxins at a dosage lower than the recommended to avoid dose-dependent side effects rather than using a higher-dose (Wertheim et al., 2013). As also demonstrated in the present study, polymyxin B was used at a fixed dosage of 750,000 IU per 12 hours, lower than the 12,500 to 15,500 IU/kg per hours recommended in clinical guidelines (Tsuji et al., 2019), which might further diminish the benefit of the combination therapy in treating pneumonia due to CRGNB.

To overcome these PK disadvantages of polymyxins in the lung, the inhalation of polymyxins was therefore recommended (Tsuji et al., 2019, Kalil et al., 2016). Compared with intravenous colistin, inhaled colistin resulted in higher concentrations in lung tissues and the epithelial lining fluid. In mechanically ventilated critically ill patients, the inhalation of 80 mg colistimethate sodium every 8 hours achieved sufficient concentrations of colistin up to 4 hours (median 6.7 and 3.9 mg/L at 1 and 4 hours after the inhalation), which were several times higher than the MIC breakpoint for *A. baumannii* and *K. pneumoniae* (Athanassa et al., 2012). When aerosolized a higher dose of colistin (2 million IU of colistimethate sodium), the epithelial lining fluid concentrations were even higher (9.53-1137 mg/L) (Boisson et al., 2014). Therefore, the adjunctive therapy with aerosolized colistin might lead to better clinical outcomes. Indeed, clinical studies have demonstrated that compared with intravenous polymyxin alone, intravenous plus aerosolized polymyxin was associated with better clinical outcomes in patients with pneumonia due to MDR pathogens (Liu et al., 2015a, Valachis et al., 2015). However, whether the adjunctive therapy of aerosolized colistin (or polymyxin B) to tigecycline could result in better results is still unclear. A recent retrospective study indicated that the adjunctive therapy of nebulized colistin to conventional intravenous antibiotics (36.5% were tigecycline) resulted in lower 14-day mortality than those patients who did not receive the nebulized colistin (Feng et al., 2021). Moreover, compared with patients receiving placebo aerosols, patients receiving nebulized colistin experienced favourable microbiological outcomes in a RCT (Rattanaumpawan et al., 2010). Therefore, it is rational to hypothesize that the adjunctive therapy of aerosolized polymyxin B (or colistin) to intravenous HDT might function better than the HDT alone in treating nosocomial pneumonia caused by CRGNB. However, as no clinical evidence directly supports it, further studies are warranted.

Another reason for clinicians to apply combination therapies is trying to lower the rate of development of antimicrobial-resistant pathogens (Ahmed et al., 2014). In clinical practices, the evolution of tigecycline resistance in MDR-GNB was reported

during tigecycline monotherapy (Anthony et al., 2008, Du et al., 2018). As the mechanism of action of tigecycline and polymyxins are different, the resistance mechanisms are different as well and results in efflux pump upregulation and lipopolysaccharide modification, respectively (Cheong et al., 2019, Zheng et al., 2018, Chiu et al., 2017). This means the selection of resistant strains might be minimized by using the combination therapy. Indeed, the results of an *in vitro* study showed that by applying the combination of tigecycline and colistin no strains lost their susceptibility to tigecycline; while by contrast, the MICs of tigecycline increased 4 to 32-fold when using tigecycline monotherapy (Cai et al., 2017). However, concentrations of antibiotics used in the previous study were higher than the clinically achievable concentration with the current dosage regimen. When using the clinically achievable concentration of tigecycline and colistin in an *in vitro* study, the occurrence of antibiotic resistance was not prevented (Ni et al., 2013). Moreover, the clinical evolution of resistance to tigecycline during the combination therapy with tigecycline and polymyxin B has also been reported in one patient (Jin et al., 2021). In the present study, tigecycline resistance emerged in both groups. However, because the duration of antibiotic therapy and subsequent sampling time points varied significantly in each patient, we cannot draw any conclusions regarding the efficacy of the combination therapy in curbing the emergence of tigecycline resistance. Nevertheless, the results from the present study highlight the possibility of isolating tigecycline-resistant strains even when using the combination therapy, which subsequently might lead to treatment failure.

There are several limitations in the present study. First, the nature of the retrospective study with the small sample size diminished the statistical power. In the comparative effectiveness analysis, although we applied the propensity score matching by incorporating covariates that were believed to be associated with clinical outcomes, it is unlikely to consider all possible confounders, especially with a limited sample size. Second, most patients received concomitant antibiotics in the present study, like carbapenems, sulbactam, fluoroquinolone, and aminoglycosides,

complicating the interpretation of the results because these concomitant antibiotics were demonstrated to have *in vitro* synergistic effects with tigecycline and polymyxin B. Third, although we only included patients receiving tigecycline or polymyxin B within three days after the release of the result of the antimicrobial susceptibility testing, polymyxin B was usually administrated as the adjunctive therapy to tigecycline 2 to 3 days later. This delay could also impair the true effectiveness of polymyxin B in treating CRGNB pneumonia. Moreover, the underdosed polymyxin B used in the present study could also be contributing to the non-beneficial effect of the combination therapy. Finally, the degree of bacteraemia in the two groups was unknown due to the low rate of blood culture. As tigecycline and polymyxin B demonstrate suboptimal serum concentrations, the lack of such information may underestimate the efficacy of the regimen in treating pneumonia, especially in a cohort with a high proportion of patients (87.3%) having septic shock.

4.5 CONCLUSIONS

In conclusion, compared with HDT therapy, the combination of polymyxin B and HDT did not improve clinical outcomes in patients with nosocomial pneumonia due to CRAB and CRKP. As polymyxin B used in the present study was underdosed, whether the combination with an adequate dose of polymyxin could result in better clinical outcomes is still unknown and worth further clinical trials. At the current stage, adding polymyxin B with the dosage recommended by the package insert to the HDT regimen should be done with caution.

5 CHAPTER 5 – TIGECYCLINE IN THE TREATMENT OF VENTILATOR-ASSOCIATED PNEUMONIA DUE TO *STENOTROPHOMONAS MALTOPHILIA*

Although trimethoprim-sulfamethoxazole is the first-line antibiotic for treating *S. maltophilia* infections, the lack of intravenous form of such antibiotic in China force doctors to choose alternative antibiotics for infections caused by *S. maltophilia*. According to results from Chapter 2, apart from fluoroquinolones, tigecycline is the most widely used antimicrobial agent in treating such infections, despite no solid clinical evidence supporting it. This chapter assessed the effectiveness of SDT in treating VAP caused by *S. maltophilia*.

5.1 BACKGROUND

S. maltophilia, previously *Pseudomonas maltophilia* or *Xanthomonas maltophilia*, has emerged as an important hospital-acquired pathogen in critically ill patients, causing pneumonia, BSI, and less frequently, cSSTI, as well as UTI (Brooke, 2012, Looney et al., 2009). It has been reported as one of the top 10 pathogens responsible for ICU-acquired pneumonia in European countries, accounting for 0.4% to 8.7% of all HAP (ECDC, 2018). Although the incidence of HAP caused by *S. maltophilia* is

relatively low, the corresponding mortality was around 50% (Guerci et al., 2019), and even reported as high as 77% in some populations (Paez et al., 2008).

Therapeutic options for *S. maltophilia* are often limited due to its extensive intrinsic or acquired resistance to antibiotics commonly used in nosocomial infections, including cephalosporins, β-lactam/β-lactamase inhibitors, aminoglycosides, and carbapenems (Adegoke et al., 2017, Chang et al., 2015). Trimethoprim-sulfamethoxazole is therefore considered the first choice because of its potent *in vitro* activity against 90% of all clinical isolates (Falagas et al., 2008). However, the high rate of allergy, side-effects, and intravenous drug shortage, limit its clinical use. Another popular alternative for the treatment of *S. maltophilia* infections in clinical practice is fluoroquinolones, because of their good *in vitro* activity, convenient availability and relatively lower rate of side-effects (Falagas et al., 2008, Chang et al., 2015). The clinical effectiveness of fluoroquinolones has been assessed in a meta-analysis that indicated fluoroquinolones are an effective alternative to trimethoprim-sulfamethoxazole in the treatment of bacteraemia and pneumonia caused by *S. maltophilia* (Ko et al., 2019).

However, resistance to fluoroquinolones has seen an alarming trend in *S. maltophilia* (Matson et al., 2019, Wu et al., 2012). Global surveillance demonstrated a decreased susceptibility of *S. maltophilia* to levofloxacin, from 83.4% during 2003-2008 to 77.3% in 2011 (Farrell et al., 2010, Sader et al., 2014), and even lower susceptibility rate was reported in Chinese surveys (Lu et al., 2012, Hu et al., 2018b). Therefore, other alternative antibiotics are urgently required.

Tigecycline is one of the new tetracyclines with broad-spectrum antibacterial activity and has been widely used in the treatment of GNB with multidrug resistance, like *Acinetobacter baumannii*, and *Klebsiella pneumoniae*, as shown in chapter 2 (Zha et al., 2020, Hawkey et al., 2018). It also presents good activity against *S. maltophilia*. A study testing tigecycline against a worldwide collection of clinical *S. maltophilia*

strains, reported a susceptibility rate of 95.5% (Sader et al., 2013), and the SENTRY antimicrobial surveillance program conducted in the US, Europe and the Mediterranean region during 2009–2012 demonstrated a similar susceptibility rate (96%) (Sader et al., 2014). Moreover, tigecycline also displayed good activity against *S. maltophilia* that is resistant to levofloxacin and/or trimethoprim-sulfamethoxazole (Biagi et al., 2020).

Clinical studies assessing the effectiveness of tigecycline in the treatment of *S. maltophilia* infections are limited. Apart from one study with a small sample size comparing trimethoprim-sulfamethoxazole with tigecycline in patients with all kinds of nosocomial infection (Tekçe et al., 2012), only two case reports reported the potential role of tigecycline in the treatment of *S. maltophilia* infections (Wu and Shao, 2014, Farrar et al., 2020). As tigecycline and fluoroquinolones are frequently used in China as shown in chapter 2 (38.2% of the participating hospitals used fluoroquinolones, while 17.9% used tigecycline as the target therapy in treating *S. maltophilia* VAP), we conducted this study to evaluate the effectiveness of tigecycline in treating VAP caused by *S. maltophilia* in comparison with fluoroquinolones.

5.2 METHODS

5.2.1 STUDY DESIGN

This is a retrospective, observational cohort study conducted in three tertiary teaching hospitals in Wuhu, Anhui, China, The First People's Hospital of Wuhu, The Second People's Hospital of Wuhu, and The First Affiliated Hospital of Wannan Medical College. The medical records of patients with the diagnosis of VAP were reviewed from January 2017 to December 2020. The study was approved by the ethics committee of Xi'an Jiao tong-Liverpool university (reference number 19-01-05) (Appendix II) and the institutional review board in each participating hospital.

5.2.2 *S. MALTOPHILIA* VAP

VAP is diagnosed according to the 2016 clinical practice guidelines by the IDSA (Kalil et al., 2016). Patients with a new or progressive lung infiltrate after 48 hours of tracheal intubation and manifesting one of the following criteria were considered as VAP: temperature > 38°C or < 36°C; leukocyte count > 12×10^{12}/L or < 4×10^{12}/L; purulent endotracheal aspirate. Pathogens responsible for the episode of VAP was determined with quantitative culture of samples collected within 48 hours before or after the onset of VAP (endotracheal aspirate ≥ 10^5 CFU/ml or bronchoalveolar lavage ≥ 10^4 CFU/ml) (Bouadma et al., 2015, Magill et al., 2013). *S. maltophilia* VAP was diagnosed when *S. maltophilia* was recovered at the concertation reaching the threshold of the corresponding specimens, irrespective of monomicrobial or polymicrobial infection. The identification of microorganism and susceptibility test of antibiotics were performed with the Vitek 2 system (bioMérieux) and interpreted according to the Clinical and Laboratory Standards Institute criteria (CLSI) (CLSI, 2017).

5.2.3 PARTICIPANTS

Patients (age > 18 years) with the diagnosis of VAP caused by *S. maltophilia* receiving either fluoroquinolones (levofloxacin or moxifloxacin) or tigecycline as the definitive therapy for more than 48 hours were eligible. Levofloxacin was administrated as 500 mg twice daily, moxifloxacin was administrated as 400 mg once daily, and 50 mg tigecycline was used twice per day following a 100 mg loading dose. Dosage adjustments according to renal function were acceptable. Patients meeting the following criteria during their VAP course were excluded: received both fluoroquinolones and tigecycline concomitantly or sequentially; concomitant pathogen was susceptible to neither of the antimicrobial agents in the definitive therapy regimens; use of inappropriate antibiotics as the definitive therapy for *S. maltophilia* ≥ 48 hours (defined as antibiotics used for which *S. maltophilia* strains were not susceptible based on antimicrobial susceptibility testing results (Lodise et

al., 2019)). In cases where patients experienced more than one episode of *S. maltophilia* VAP, only the first episode was included.

5.2.4 OUTCOMES AND DEFINITIONS

The primary endpoint of the study was to assess the rate of clinical cure. Secondary endpoints investigated were 28-day mortality and microbiological cure. Clinical cure was defined as complete resolution of all signs and symptoms of pneumonia at 14 days after the initial given dose of target antibiotics (fluoroquinolones or tigecycline) (De Pascale et al., 2014). Whenever patients died or were discharged within 14 days after the inclusion, the clinical cure was assessed by the end of antibiotic therapy. The microbiological cure was defined as the absence of *S. maltophilia* in the culture of specimens collected within 2 days before or after the follow up time point, the 14th day after the initial given dose of target antibiotics (Cisneros et al., 2019, Magill et al., 2013). Patients who died or were discharged within 14 days were excluded from the microbiological cure analysis.

5.2.5 DATA EXTRACTION

Data extracted from the medical records included age, gender, the reason for ICU admission, comorbidities, Charlson comorbidity index score (Frenkel et al., 2014), severity of disease at the time of *S. maltophilia* VAP onset (APACHE II (Niewiński et al., 2014)), duration of mechanical ventilation before the onset of *S. maltophilia* VAP, concomitant isolated bacteria, antibiotics, duration of antibiotic therapy targeting *S. maltophilia,* microbiological results, clinical cure, and 28-day mortality. For patients discharged from hospitals earlier than 28 days after the onset of *S. maltophilia* VAP, information on 28-day mortality was obtained from their one-month follow up records.

5.2.6 STATISTICAL ANALYSIS

Continuous variables were summarized as median and interquartile range. Categorical variables were described as counts and percentages. The differences between patients receiving tigecycline or fluoroquinolones were analysed with Fisher's exact test for categorical variables and Mann-Whitney *U* test for continuous variables.

To analyse the clinical outcomes between tigecycline and fluoroquinolones in the treatment of VAP caused by *S. maltophilia*, an inverse probability of treatment weighting (IPTW) univariable logistic regression model was performed (Allan et al., 2020). The propensity score was estimated by using a non-parsimonious multivariable logistic regression model, with receiving tigecycline as the dependent variable and baseline characteristics in the two groups with a standardized mean difference of more than 0.2 as covariates (Austin and Stuart, 2015). The included covariates were the severity of disease (APACHE II score), underlying comorbidities (malignancy, chronic liver failure, chronic heart failure, coagulation disorder), and polymicrobial infection.

Moreover, a conventional multivariable regression model was run as a sensitivity analysis (Steyerberg et al., 1999), by adjusting for variables determined *a priori* that might be the risk factors for mortality in *S. maltophilia* pneumonia: age, malignancy, polymicrobial infection, definitive antibiotic therapy, and APACHE II score. Sensitivity analysis was also performed by excluding patients co-infected with *Pseudomonas aeruginosa* as this pathogen is naturally resistant to tigecycline (French, 2008). Moreover, to remove the impact of appropriate initial antibiotic therapy on the assessment of effectiveness of antibiotic therapy, a sensitivity analysis by excluding patients receiving appropriate empiric antibiotic therapy was also conducted.

OR and 95% CI was reported. Two-tailed $p < 0.05$ was considered statistically significant. All the statistical analyses were performed with R software version 3.6.2 (R Foundation for Statistical Computing).

5.3 RESULTS

5.3.1 PATIENT'S CHARACTERISTICS

Of the total of 142 patients with VAP caused by *S. maltophilia* meeting the inclusion criteria, 60 were excluded according to the exclusion criteria. The remaining 82 patients were included in the final analysis, among which 46 patients were treated with tigecycline, while the other 36 patients received fluoroquinolones levofloxacin or moxifloxacin (Figure 5-1).

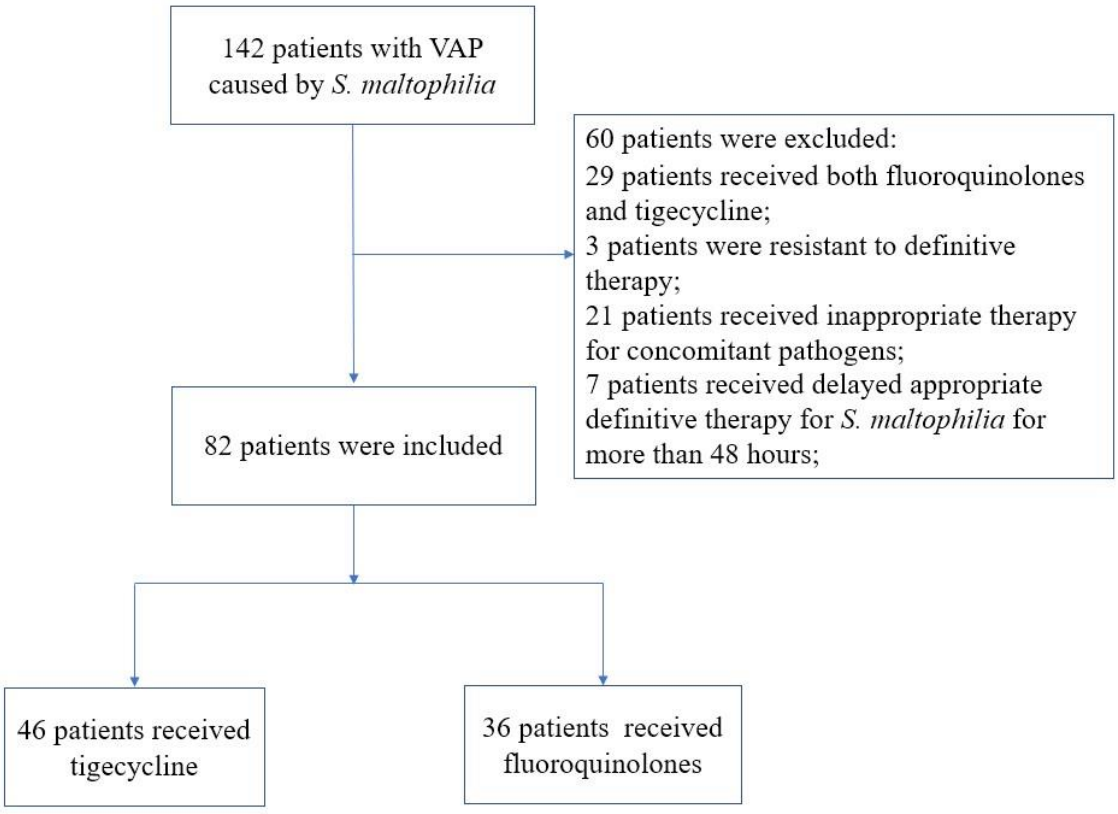

Figure 5-1. Flowchart of study inclusion process.

Patients with VAP caused by *S. maltophilia* were relatively old, with the median age of 76 years (IQR 64.25-85), and had both serious acute and chronic diseases, with an APACHE II score of 21 (IQR 16.25-24), and a Charlson index comorbidity score of 5 (IQR 4-6). Most patients had at least one comorbidity, and hypertension was the most frequently reported comorbidity (45/82, 54.9%). A chronic underlying respiratory disease was present in 11 patients (13.4%). Reasons for patients to be admitted in ICU were respiratory failure, stroke, sepsis, brain trauma, scheduled surgery and trauma.

VAP caused by *S. maltophilia* occurred late, with the median duration from intubation to VAP onset of 15 days (IQR 9-33.75). A majority of patients (58/82, 70.7%) with *S. maltophilia* VAP were polymicrobial, with *A. baumannii* (26/58, 44.8%) as the most commonly co-isolated bacterium, followed by *Enterobacteriaceae* (22/58, 37.9%) and *P. aeruginosa* (10/58, 17.2%). All patients with *S. maltophilia* VAP had received combination therapy at the onset of VAP, by combining with either carbapenems (60/82, 73.2%) or β-lactam/β-lactamase inhibitors (22/82, 26.8%). The duration of antibiotic therapy targeting *S. maltophilia* VAP was 9 days (IQR 5.25-13). In terms of appropriate initial antibiotic therapy, only 14.6% (12/82) of the patients received effective antimicrobial therapy and they were all in the fluoroquinolones therapy group.

Compared with patients receiving fluoroquinolones, patients receiving tigecycline were more likely to have a higher APACHE II score, Charlson comorbidity index score and longer time between the start of mechanical ventilation and the diagnosis of *S. maltophilia* VAP, albeit not statistically significant. Moreover, there were more patients receiving carbapenems in the tigecycline group, while more β-lactam/β-lactamase inhibitors were used in patients receiving fluoroquinolones (40/46, 87% vs. 20/36, 55.6%, $p = 0.003$) (Table 5-1).

Table 5-1. Characteristics of patients with ventilator-associated pneumonia due to *S. maltophilia* receiving tigecycline or fluoroquinolones.

Variables	**All n = 82**	**Tigecycline n = 46**	**Fluoroquinolones n = 36**	***p***
Age (year), median (IQR[a])	76 [64.25, 85]	76 [65, 85]	75 [62.5, 84.75]	0.650
Male gender n (%)	65 (79.3)	38 (82.6)	27 (75)	0.569
Reasons for ICU admission n (%)				0.134
Respiratory failure	42 (52.1)	25 (54.3)	17 (47.2)	
Stroke	17 (20.7)	8 (17.4)	9 (25)	
Sepsis	8 (9.8)	6 (13)	2 (5.6)	
Brain trauma	7 (8.5)	2 (4.3)	5 (13.9)	
Scheduled surgery	6 (7.3)	5 (10.9)	1 (2.8)	
Trauma	2 (2.4)	0 (0)	2 (5.6)	
Comorbidities n (%)				
Hypertension	45 (54.9)	25 (54.3)	20 (55.6)	1.000
Coronary heart disease	31 (37.8)	18 (39.1)	13 (36.1)	0.960
Chronic heart failure	17 (20.7)	12 (26.1)	5 (13.9)	0.281
Chronic respiratory disease	11 (13.4)	6 (13)	5 (13.9)	1.000
Chronic kidney disease	7 (8.5)	5 (10.9)	2 (5.6)	0.648
Chronic liver disease	10 (12.2)	7 (15.2)	3 (8.3)	0.545
Malignancy	10 (12.2)	8 (17.4)	2 (5.6)	0.199
Diabetes mellitus	15 (18.3)	8 (17.4)	7 (19.4)	1.000
Coagulation disorder	10 (12.2)	8 (17.4)	2 (5.6)	0.199
Charlson comorbidity score, median (IQR)	5 [4, 6]	5 [4, 6]	4 [4, 6]	0.123
APACHE II score, median (IQR)	21 [16.25, 24]	21.5 [17, 25]	19.5 [15.75, 23]	0.195
Time from mechanical ventilation to *S. maltophilia* VAP (day), median (IQR)	15 [9, 34]	18.5 [9.75, 35]	14.5 [7, 26.25]	0.328
Polymicrobial infection	58 (70.7)	35 (76.1)	23 (63.9)	0.387
A. baumannii	26 (44.8)	18 (51.4)	8 (34.8)	
P. aeruginosa	10 (17.2)	6 (17.1)	4 (17.4)	
Enterobacteriaceae	22 (37.9)	11 (31.4)	11 (47.8)	
Concomitant antibiotics, n (%)				0.003
Carbapenems	60 (73.2)	40 (87)	20 (55.6)	
β-lactam/β-lactamase inhibitors	22 (26.8)	6 (13)	16 (44.4)	
Duration antibiotic therapy targeting *S. maltophilia*	9 [5.25, 13]	9 [5, 13]	9 [7, 13.25]	0.533
Appropriate initial antibiotic therapy n (%)	12 (14.6)	0 (0)	12 (33.3)	< 0.001

[a]Abbreviations: APACHE II score, acute physiology and chronic health evaluation II score, ICU, intensive care unit; IQR, interquartile range; VAP, ventilator-associated pneumonia.

5.3.2 MICROBIOLOGICAL PROFILE

Results of antimicrobial susceptibility testing were included for *S. maltophilia* isolates from 129 patients. The formal lab report of antimicrobial susceptibility testing of the remainder 13 patients cannot be found in the database, but part of the information (microorganism, quantitative culture result, whether susceptible to the prescribed antibiotics) was extracted from chart notes in their medical records. Therefore, the lack of formal testing reports in these patients did not influence the inclusion process and the final analysis. Tigecycline (98.4%, 127/129) presented the highest susceptibility rate against *S. maltophilia*, followed by trimethoprim-sulfamethoxazole (82.9%, 107/129) and levofloxacin (80.6%, 104/129), while susceptibility rate was lower in ticarcillin-clavulanate and ceftazidime, 75.2% (97/129) and 61.2% (79/129), respectively. No information regarding the susceptibility of *S. maltophilia* to carbapenems and β-lactam/β-lactamase inhibitors was reported.

5.3.3 CLINICAL OUTCOMES

Compared with patients receiving fluoroquinolones, patients receiving tigecycline resulted in a lower clinical cure (15/46, 32.6% vs. 23/36, 63.9%, p = 0.009) and microbiological cure (10/35, 28.6% vs. 13/22, 59.1%, p = 0.045). There was no statistical difference in 28-day mortality between patients receiving tigecycline and fluoroquinolones (22/46, 47.8% vs. 10/36, 27.8%, p = 0.105), although the trend is in favour of fluoroquinolones therapy (Table 5-2). In the IPTW univariable regression model, tigecycline therapy was associated with a reduced clinical cure (0.78, 95% CI 0.64-0.98, p = 0.030), while it was not associated with an increased 28-day mortality (1.19, 95% CI 0.97-1.46, p = 0.096). Similar results were also shown in the multivariable logistic regression model that was run as the sensitivity analysis (Table 5-3 and Table 5-4). Sensitivity analysis by excluding patients co-infected with *P. aeruginosa* did not change the trend of any of these results. When patients receiving appropriate empiric antibiotic therapy were excluded, tigecycline therapy

still resulted in a lower clinical cure (15/46, 32.6% vs. 15/24, 62.5%, $p = 0.032$) than patients receiving fluoroquinolones (Table 5-2).

Table 5-2. Clinical outcomes of patients with ventilator-associated pneumonia due to *S. maltophilia* receiving tigecycline or fluoroquinolones.

Outcomes	**All**	**Tigecycline**	**Fluoroquinolones**	***p***
Overall analysis				
Clinical cure (n = 82)	38 (46.3%)	15 (32.6%)	23 (63.9%)	0.009
Microbiological cure (n = 57)	23 (40.4%)	10 (28.6%)	13 (59.1%)	0.045
28-day mortality (n = 82)	32 (39%)	22 (47.8%)	10 (27.8%)	0.105
Sensitivity analysis by excluding *P. aeruginosa* co-infection				
Clinical cure (n = 72)	35 (48.6%)	14 (35%)	21 (65.6%)	0.019
28-day mortality (n = 72)	30 (41.7%)	21 (52.5%)	9 (28.1%)	0.065
Sensitivity analysis by excluding appropriate initial antibiotic therapy				
Clinical cure (n = 70)	30 (42.9%)	15 (32.6%)	15 (62.5%)	0.032
28-day mortality (n = 70)	27 (38.6%)	22 (47.8%)	5 (20.8%)	0.052

Table 5-3. Analysis of clinical cure and 28-day mortality in patients with ventilator-associated pneumonia due to *S. maltophilia* receiving tigecycline or fluoroquinolones.

Analysis (tigecycline vs. fluoroquinolones)	**Odds ratio (95% CI[a])**	***p***
Clinical Cure		
Crude unadjusted analysis	0.27 (0.10-0.67)	0.006
Multivariable logistic regression	0.32 (0.12-0.86)	0.026
IPTW model	0.78 (0.64-0.98)	0.030
28-day Mortality		
Crude unadjusted analysis	2.38 (0.96-6.23)	0.070
Multivariable logistic regression	1.64 (0.58-4.77)	0.355
IPTW model	1.19 (0.97-1.46)	0.096

[a]Abbreviations: CI, confidence interval; IPTW, inverse probability treatment of weighting.

Table 5-4. Logistic regression analysis of factors associated with clinical cure in patients with ventilator-associated pneumonia caused by *S. maltophilia*.

Variable	**Multivariable analysis**		
	Odds Ratio	**95% CI**[a]	***p***
Age	0.96	0.92, 0.99	0.033
Malignancy	0.06	0.02, 0.45	0.019
APACHE II score	0.87	0.78, 0.96	0.008
Tigecycline therapy	0.32	0.12, 0.86	0.026

[a]Abbreviations: APACHE II score, acute physiology and chronic health evaluation II score; CI, confidence interval.

5.4 DISCUSSION

Tigecycline is a promising alternative to trimethoprim-sulfamethoxazole in the treatment of *S. maltophilia* infections due to its potent antimicrobial activity (Farrell et al., 2010). Although resistance to tigecycline has been reported as an increasing trend, the susceptibility rate in global surveillance reports is still high (90.6% inhibited at ≤ 2 mg/L) (Pfaller et al., 2018, Zhao et al., 2018). Therefore, it was recommended as one of the antibacterial choices by the Chinese expert consensus statement to treat *S. maltophilia* infections since 2013 (Zhou et al., 2013), especially for those critically ill patients or those concomitantly infected with other GNB, like *A. baumannii* and *K. pneumoniae*, as these pathogens are usually presenting high rate of resistance to other commonly used antibiotics in VAP (Kalil et al., 2016). In this study, patients with a higher APACHE II score and Charlson comorbidity index score were more likely to receive tigecycline and the combination therapy with carbapenems, indicating that clinicians are prone to prescribe tigecycline and combination therapy with other high-level antibiotics to those patients with greater severity of underlying diseases (Hand et al., 2016, Papst et al., 2018). Besides, co-isolated with MDR or carbapenem-resistant bacteria was another factor that led doctors to use tigecycline in our study, as there were few choices for such infection.

In the present study, a lower rate of clinical cure and microbiological cure were reported in patients receiving tigecycline when compared with patients receiving fluoroquinolones in the treatment of VAP caused by *S. maltophilia*. A reasonable explanation for the lower success rate of tigecycline in the treatment might be that the dose of tigecycline used in the study was not optimal, as a pharmacokinetic and pharmacodynamic (PK/PD) study has demonstrated that the SDT (50 mg twice per day following a 100 mg loading dose) resulted in suboptimal concentration of tigecycline in the lung (Giamarellou and Poulakou, 2011). Moreover, as demonstrated in chapter 3, clinical evidence also opposed the use of SDT in treatment of severe infections caused by other pathogens than *S. maltophilia*, as it was associated with a poorer clinical outcome when compared with the HDT or other comparators (Zha et al., 2020). A study performed on 476 clinical isolates of *S. maltophilia* from a global collection revealed that 1 mg/L tigecycline was required to inhibit the growth of 50% of those isolates, and 2 mg/mL, to inhibit the growth of 90% of those isolates (Pfaller et al., 2018). Combining this finding with a recent PK study of HDT (100 mg twice per day following a 100 mg loading dose) in critically ill patients with severe infections caused by different organisms that indicated that only 40.6% and 28.1% of patients reached a concentration of tigecycline ≥ 1 mg/L and 2 mg/L, respectively (De Pascale et al., 2020), it seems improbable to expect a superior clinical outcome for HDT therapy. Therefore, whether HDT could result in better clinical outcomes than SDT or other alternatives in the treatment of *S. maltophilia* pneumonia needs further scrutiny. On the other hand, it should be noted that the levofloxacin used in the present study (500 mg twice daily) was higher than that recommended (750 mg once daily) in clinical guideline (Kalil et al., 2016), although which has also been used in European countries in this way (Cisneros et al., 2019). Studies indicated 500 mg twice daily of levofloxacin was comparable to 400 mg once daily moxifloxacin in PK/PD profile, while were superior to levofloxacin 500 mg and 750 mg once daily in patients with severe lower respiratory tract infections (Kontou et al., 2013). However, whether this superiority in PK/PD contributed to the better clinical outcomes in patients receiving fluoroquinolone is

still uncertain, as there were no patients using levofloxacin 750 mg once daily in the present study.

As it is well known that tigecycline is largely used as the salvage therapy for pathogens that are difficult to treat, it is rarely prescribed as an empirical therapy for patients with suspected VAP (Hawkey et al., 2018, Yaghoubi et al., 2022). By contrast, fluoroquinolones are commonly recommended as empiric therapy for VAP (Kalil et al., 2016). Combined with the nature of extensive drug resistance of *S. maltophilia* against most of the commonly used antibiotics in the empiric therapy of VAP (Chang et al., 2015), a certain proportion of patients treated with tigecycline would have experienced the delayed appropriate initial antibiotic therapy, when compared with patients receiving fluoroquinolones. In the present study, patients in the tigecycline group all received tigecycline after the result of antimicrobial susceptibility testing was known and only 14.6% of patients received appropriate initial antibiotic therapy, and were all in the fluoroquinolones group. Therefore, it is reasonable to speculate the lower clinical cure in tigecycline therapy might due to the delay in adequate antibiotic therapy, as this has been well established in other MDR bacterial infections (Gutiérrez-Gutiérrez et al., 2017, Kohler et al., 2017, Park et al., 2012). However, when patients receiving appropriate empiric antibiotic therapy were excluded, the results did not change as well, indicating that inappropriate empiric antibiotic therapy cannot fully explain the lack of efficacy in tigecycline group. Apart from the delayed use of appropriate antibiotics, another factor that might diminish the effectiveness of tigecycline is the relatively short course of antibiotic therapy, as the nature of tigecycline is a bacteriostatic agent (French, 2008). Although guidelines now all recommend using a short course of antibiotic therapy for VAP, the duration for non-fermenting GNB seems not sufficient (Kalil et al., 2016, Zilahi et al., 2016). A RCT compared a 7-day antibiotic therapy with a 10-day course indicated that patients with VAP caused by *P. aeruginosa* had a significantly higher 28-day mortality in the 7-day course (Kollef et al., 2012). Other studies also suggested that a longer course of antibiotic therapy is required to

successfully treat VAP caused by MDR-GNB (Florescu et al., 2012, Kollef et al., 2008). Therefore, a longer course of tigecycline therapy for *S. maltophilia* VAP determined by integrating biomarkers and clinical assessment might be rational but still need more evidence.

When interpreting the therapeutic effect of tigecycline in the treatment of *S. maltophilia* VAP, an important factor that needs to be considered is polymicrobial infection, as this was reportedly accounting for 54.4% to 73.3% of *S. maltophilia* pneumonia in several studies (Guerci et al., 2019, Hand et al., 2016, Saied et al., 2020, Shah et al., 2019). In the present study, the similar result was also demonstrated, as 70.7% of patients were documented with polymicrobial infection. The pathogens co-isolated with *S. maltophilia* were consistent with these studies, with *A. baumannii*, *Enterobacteriaceae* and *P. aeruginosa* as the most frequently isolated. Studies assessing the efficacy of tigecycline in the treatment of other infections have shown that tigecycline was associated with poorer clinical outcomes compared with other antibiotics (Prasad et al., 2012, Shen et al., 2015, Tasina et al., 2011). Importantly, *P. aeruginosa* is naturally resistant against tigecycline (French, 2008). Taken these together, it is better not to recommend the use of tigecycline in polymicrobial *S. maltophilia* VAP, as the role of co-isolated pathogens in clinical course is still unclear, in spite of studies that indicated no association between mortality and polymicrobial infection (Guerci et al., 2019, Hand et al., 2016, Saied et al., 2020). Paradoxically, the indication of using tigecycline to treat *S. maltophilia* VAP in the present study is usually the co-isolation of MDR or CR bacteria. Before 2019, the management strategies for these pathogens in the participated centres were restricted to tigecycline, double carbapenems, prolonged infusion of carbapenem or high-dose of sulbactam, as polymyxins, minocycline, fosfomycin or other new antibiotics were not available at that time. Although we have implemented the IPTW model in the statistical analysis, try eliminating the indication bias. Still, with the small sample size and nature of the retrospective study, we cannot incorporate all factors in the

final analysis, especially the various resistant profile of the co-isolated bacteria, which might ultimately influence the interpretation of the results.

It is interesting to note that even with a lower rate of clinical and microbiological cure in patients receiving tigecycline, the 28-day mortality was not statistically different from that of patients receiving fluoroquinolones, although the absolute risk difference was as high as 20%. One possible explanation for this observation could be that the severity of underlying disease might contribute more to death than *S. maltophilia* VAP itself (Bekaert et al., 2011, Falagas et al., 2009), as patients developing *S. maltophilia* VAP shared common features with patients at high risk of death, like prolonged in-hospital stay and mechanical ventilation, the higher score of severity of underlying disease (Colpan et al., 2005, Hanes et al., 2002, Li et al., 2016, Paez et al., 2008, Saied et al., 2020). Consistent results were also reported in other studies, indicating that the mortality of *S. maltophilia* pneumonia was independently associated with the severity of diseases (SOFA score) rather than factors related to antibiotic therapy, like a specific antimicrobial agent, appropriate initial therapy or whether being part of combination therapy (Guerci et al., 2019, Hand et al., 2016, Saied et al., 2020, Shah et al., 2019). Moreover, the small sample size should also be taken into consideration when interpreting the no statistical difference in 28-day mortality, as the small sample in present study might be not sufficiently powered to detect a difference between the groups and turned to be falsely negative and then leading to a type II error (Button et al., 2013).

There are several limitations in this study. First, we aimed to compare the effectiveness between tigecycline and fluoroquinolones in the treatment of VAP infected with *S. maltophilia*, but were unable to remove the impact of concomitant antibiotics on clinical outcomes, especially those β-lactam/β-lactamase inhibitors as they were reported having around 50% of susceptibility rate against *S. maltophilia* isolates in China (Hu et al., 2018b). As a result, it is difficult to link clinical outcomes to one specific antibiotic. Additionally, in the present study, the effectiveness of

tigecycline was compared with fluoroquinolones instead of the first recommended trimethoprim-sulfamethoxazole, which might limit the clinical value of the study, as trimethoprim-sulfamethoxazole is still one the most popular regimens in treating such infections. Second, 70.7% of patients included in this study were with polymicrobial infection. Although patients concomitantly infected with a pathogen that was not susceptible to neither antibiotic in the combination regimen were excluded, the various virulence and resistance of pathogens in the individual level still complicate the interpretation of the effectiveness of specific antibiotic in *S. maltophilia* VAP. Moreover, with the meagre rate of blood culture implemented in the study cohort and different methods applied for collecting respiratory secretions, we failed to incorporate bacteraemia and bacterial load in the final analysis. Third, because there were no patients receiving tigecycline as the empirical therapy for *S. maltophilia* VAP, we could not analyse the effectiveness of appropriate empiric therapy with tigecycline in these patients. Finally, although we tried to address the confounding by using the IPTW model, and adjusted for variables through multivariable logistic regression, the small sample size prevents us from adjusting all variables, especially those unmeasured confounding factors that exist as the nature of the multicentre observational retrospective study, like the heterogeneity in clinical practice, medical human resources, and experience of medical staff among these centres, and hence diminished the power of statistics. In spite of these limitations, this is the only study specifically focused on the assessment of clinical effectiveness of tigecycline in the treatment of VAP due to *S. maltophilia*.

5.5 CONCLUSIONS

In the present study, therapy with SDT in patients with VAP caused by *S. maltophilia* resulted in a significantly lower clinical and microbiological cure rate than fluoroquinolones; therefore, using SDT to treat *S. maltophilia* VAP should be done with caution.

6 CHAPTER 6 – EFFECTIVENESS OF PIPERACILLIN-TAZOBACTAM IN THE TREATMENT OF NOSOCOMIAL PNEUMONIA CAUSED BY EXTENDED-SPECTRUM B-LACTAMASE PRODUCING *KLEBSIELLA PNEUMONIAE*

Although carbapenems are recommended as the first-line antibiotic by clinical guidelines to treat infections caused by 3GC-R *Enterobacteriaceae*, carbapenem-sparing regimens are still of great interest. According to data from Chapter 2, 36.8% of the participating hospitals in China used piperacillin-tazobactam to treat pneumonia caused by ESBL-producing *Enterobacteriaceae*. As of writing, no studies assessed the effectiveness of piperacillin-tazobactam in the treatment of pneumonia due to ESBL-producing *Enterobacteriaceae*. In this chapter, I report results from a retrospective study that evaluated piperacillin-tazobactam's efficacy in nosocomial pneumonia caused by ESBL-producing *K. pneumoniae* compared with carbapenems.

6.1 BACKGROUND

Infections caused by 3GC-R *Enterobacteriaceae* mediated mainly by the expression of ESBLs, have increased significantly, thereby posing great challenges to clinicians by restricting the choice of antimicrobial agents (Castanheira et al., 2021, Pitout and Laupland, 2008). Data from the report of EARS-Net in 2019 indicated that 15.1% of *E. coli* and 31.7% of *K. pneumoniae* were resistant to 3GCs (ECDC, 2020), while the rate was reported as high as 61.3% for *E. coli* and 60.3% for *K. pneumoniae* in the Chinese surveillance in 2018 (Yang et al., 2020). The clinical impact of ESBL-producing *Enterobacteriaceae* has been well studied, as these pathogens cause worse clinical outcomes when compared with their non-ESBL-producing counterparts (Ling et al., 2021). A recent meta-analysis including 84 studies with 22030 patients reported increased attributable mortality by a factor of 1.75 (95% CI, 1.45-2.11) in BSI caused by ESBL-producing *Enterobacteriaceae* (Shamsrizi et al., 2020).

Carbapenems are typically the first-choice antibiotics recommended by clinical guidelines to treat infections caused by ESBL-producing *Enterobacteriaceae* (Tamma et al., 2021, Kalil et al., 2016). They withstand hydrolysis by ESBL enzymes well and therefore present good activity against those pathogens (Bassetti et al., 2020, Peirano and Pitout, 2019, Gutiérrez-Gutiérrez and Rodríguez-Baño, 2019). A recent study that collected 7168 clinical isolates of *Enterobacteriaceae* from patients in the US and Europe between 2016-2018, demonstrated a high susceptibility rate to meropenem at 97.6% in those isolates with the ESBL genotype (Belley et al., 2021). A similar good *in vitro* activity was also described in a Chinese surveillance study, with the susceptibility rate to imipenem of ESBL-producing *E. coli* at 99.7% and of ESBL-producing *K. pneumoniae* at 98%, respectively (Cui et al., 2016). However, the increased use of carbapenems has led to the emergence of carbapenem resistance, which put added burden to public health (Gandra and Burnham, 2020, Zhang et al.,

2018). Therefore, it is necessary and urgent to find an effective carbapenem-sparing therapy for infections caused by ESBL-producing *Enterobacteriaceae*.

Classic β-lactam/β-lactamase inhibitor combinations, like amoxicillin-clavulanate, ampicillin-sulbactam, cefoperazone-sulbactam, and piperacillin-tazobactam, usually demonstrate good *in vitro* activity against ESBL-producing *Enterobacteriaceae* when these pathogens do not possess other antimicrobial resistant mechanism(s) (Belley et al., 2021, Peirano and Pitout, 2019). Among the available classic β-lactam/β-lactamase inhibitor combinations, piperacillin-tazobactam is one of the most interesting carbapenem-sparing therapies against infections caused by ESBL-producing *Enterobacteriaceae* (Bassetti et al., 2020, Aslan and Akova, 2019). A recent antimicrobial resistance surveillance study indicated that susceptibility to piperacillin-tazobactam of *Enterobacteriaceae* with the ESBL genotype was as high as 71.4%, compared to only 18% and 11.9% for amoxicillin-clavulanate and amoxicillin-sulbactam, respectively (Belley et al., 2021). Clinical studies have demonstrated comparable effectiveness of piperacillin-tazobactam and carbapenems in the treatment of UTIs (Zhang et al., 2021a, Sharara et al., 2020, Yoon et al., 2017). However, the efficacy of piperacillin-tazobactam in treating bacteraemia caused by ESBL-producing *Enterobacteriaceae* is still uncertain, as some studies indicated comparable effectiveness (Gutiérrez-Gutiérrez et al., 2016, Henderson et al., 2021, John et al., 2019, Ko et al., 2018, Nasir et al., 2019), while others demonstrated inferiority (Tamma et al., 2015, Ofer-Friedman et al., 2015, Harris et al., 2018).

Evidence supporting the use of β-lactam/β-lactamase inhibitor combinations in the treatment of BSI due to ESBL-producers is primarily based on the INCREMENT study, a large sample size, multinational, preregistered cohort study, in which the 30-day mortality did not show any statistical differences between β-lactam/β-lactamase inhibitor combinations (amoxicillin-clavulanate and piperacillin-tazobactam) and carbapenems (ertapenem, meropenem, imipenem, and doripenem) in both empiric

and definitive therapy cohorts (Gutiérrez-Gutiérrez et al., 2016). Moreover, there were no differences detected between amoxicillin-clavulanate and piperacillin-tazobactam versus carbapenems in the subgroup analysis, indicating support for the use of the β-lactam/β-lactamase inhibitor combination to minimize carbapenem use (Gutiérrez-Gutiérrez et al., 2016). Similar results were subsequently published in two meta-analysis studies (Son et al., 2018, Sfeir et al., 2018).

By contrast, the MERINO trial, which compared piperacillin-tazobactam with meropenem in the treatment of BSI caused by ceftriaxone-resistant *E. coli* or *K. pneumoniae* did not support the use of piperacillin-tazobactam (Harris et al., 2018). The 30-day mortality in patients receiving piperacillin-tazobactam was statistically higher than those receiving meropenem (12.3% vs 3.7%) (Harris et al., 2018). However, a post hoc analysis by the same group of authors, done by re-performing the antimicrobial susceptibility testing with the referred broth microdilution methods did not support the inferiority of piperacillin-tazobactam (Henderson et al., 2021). It found there were a significant proportion of piperacillin-tazobactam non-susceptible strains included in the MERINO trial and when those non-susceptible strains were excluded, the between group difference in 30-day mortality was reduced to 5% (95% CI, -1% to 10%) (Henderson et al., 2021). Therefore, it can be concluded that piperacillin-tazobactam is as effective as meropenem in treating bacteraemia that caused by piperacillin-tazobactam susceptible ESBL-producers.

Because there have not been any clinical studies dedicated to therapeutic options for pneumonia caused by ESBL-producing *Enterobacteriaceae*, guidelines (Tamma et al., 2021, Kalil et al., 2016) against using piperacillin-tazobactam in such cases has been largely based on the original findings by Harris et al. (Harris et al., 2018) on the MERINO trial. However, when considering the aforementioned factors of the MERINO trial described two years later by Henderson et al. (Henderson et al., 2021), it is reasonable to hypothesize that piperacillin-tazobactam might be an effective alternative to carbapenems in treating pneumonia caused by ESBL-producers if

susceptibility is established. Therefore, we conducted this retrospective cohort study to test this hypothesis by including nosocomial pneumonia patients caused by ESBL-producing *K. pneumoniae* either receiving carbapenems or piperacillin-tazobactam.

6.2 METHODS

6.2.1 STUDY DESIGN

This is a retrospective, observational cohort study conducted in two tertiary teaching hospitals in Wuhu, Anhui, China, The First Affiliated Hospital of Wannan Medical College, and The Second People's Hospital of Wuhu, which in total have around 5,000 beds for inpatients. The medical records of patients diagnosed with nosocomial pneumonia caused by ESBL-producing *K. pneumoniae* were reviewed from January 2018 to July 2021. The study was approved by the ethics committee of Xi'an Jiaotong-Liverpool University (reference number 19-01-05) (Appendix II) and the institutional review board in each participating hospital, and the informed consent was waived because of the nature of the retrospective study.

6.2.2 ESBL-PRODUCING *K. PNEUMONIAE* NOSOCOMIAL PNEUMONIA

Nosocomial pneumonia (including HAP, and VAP) is diagnosed according to the 2016 clinical practice guideline by the IDSA (Kalil et al., 2016). The diagnosis of pneumonia is made based on a newly developed or progressive lung infiltration or consolidation on chest radiographs plus two or more of the following criteria: new onset or worsening cough; temperature > 38°C or < 36°C; leukocyte count > 12×10^{12}/L or < 4×10^{12}/L; purulent sputum or endotracheal aspirate; hypoxemia or worsening oxygenation that required increment of ventilation support. HAP is defined as pneumonia occurring ≥ 48 hours after hospitalization, and VAP is defined as pneumonia occurring in patients receiving invasive mechanical ventilation ≥ 48 hours. Pathogens responsible for the episode of nosocomial pneumonia were determined by semiquantitative culture of qualified respiratory specimens (Magill

et al., 2013). Microorganism identification and ESBL-phenotype determination were performed with the Vitek 2 system (bioMérieux). Susceptibility to piperacillin-tazobactam was measured with the microdilution method and determined according to the breakpoint recommended by EUCAST (MIC > 8 mg/L) (EUCAST, 2022). In brief, the Vitek 2 ESBL test is a rapid detection tool with good specificity (99.7%) and sensitivity (98.1%), which is based on simultaneous assessment of the inhibitory effects of cefepime, cefotaxime, and ceftazidime, alone and in the presence of clavulanic acid, similar to that was recommended by the CLSI (Spanu et al., 2006, CLSI, 2017).

6.2.3 PARTICIPANTS

Patients aged > 18 years with the diagnosis of nosocomial pneumonia caused by ESBL-producing *K. pneumoniae* receiving either piperacillin-tazobactam or carbapenems (either imipenem or meropenem in this study) within 24 hours from the onset of pneumonia and for at least the subsequent 72 hours were eligible. Piperacillin-tazobactam was administrated as 4.5 g every 6 hours by extended infusion. Imipenem or meropenem was administrated as 1 g every 8 hours intravenously without an extended infusion. Dosage adjustments were made based on renal function. Patients meeting the following criteria were excluded: received both carbapenems and piperacillin-tazobactam during the pneumonia course; combined with other antibiotics; pneumonia was polymicrobial; ESBL-producing *K. pneumoniae* was non-susceptible to piperacillin-tazobactam (defined as MIC > 8 mg/L according to EUCAST breakpoint (EUCAST, 2022)) or carbapenems (defined as MIC of imipenem > 1 mg/L); patients with concurrent infections other than pneumonia that required other antimicrobials in addition to piperacillin-tazobactam or carbapenems, like intra-abdominal infections, etc. Only the first episode was included in this study in cases where patients experienced more than one episode of nosocomial pneumonia caused by ESBL-producing *K. pneumoniae*.

6.2.4 OUTCOMES AND DEFINITIONS

The primary endpoint was 28-day all-cause mortality after the onset of nosocomial pneumonia. Secondary outcomes were clinical cure and microbiological cure. Clinical cure was defined as complete resolution of all signs and symptoms of pneumonia or such improvement of patients that antibiotics were stopped at day 14 after the onset of pneumonia. Microbiological cure was defined as the absence of ESBL-producing *K. pneumoniae* in the culture of specimens collected within two days before or after the visit time point, day 14 after the onset of pneumonia. Patients who died or were discharged within 14 days were excluded from the microbiological cure analysis.

6.2.5 DATA EXTRACTION

Data were collected from medical records, and included patients' demographics (age, gender), reasons for hospitalization, pre-existing medical conditions, severity of disease at the time of nosocomial pneumonia onset (APACHE II score (Niewiński et al., 2014)), type of pneumonia (HAP, VAP), duration of antibiotic therapy for pneumonia, clinical and microbiological outcomes, and the 28-day mortality. For patients discharged from the hospital earlier than 28 days after the onset of nosocomial pneumonia, information on 28-day mortality was obtained from the one-month follow up records.

6.2.6 STATISTICAL ANALYSIS

Continuous variables were summarized as median and interquartile ranges. Categorical variables were described as count and percentage. The differences between patients receiving piperacillin-tazobactam or carbapenems were analysed with Chi-squared test or Fisher's exact test ($n < 10$ events) for categorical variables and Mann-Whitney U test for continuous variables.

To balance baseline differences in the two groups, an IPTW was performed. The propensity score was estimated using a non-parsimonious multivariable logistic regression model, with receiving piperacillin-tazobactam as the dependent variable and the baseline characteristics in the two groups with a standardized mean difference of more than 0.2 and those pre-existing medical conditions (decided *a priori*) as covariates. The final variables were gender, shock, APACHE II score, immunocompromised status, chronic kidney disease, chronic liver disease, chronic heart disease, chronic respiratory disease, malignancy, cerebrovascular disease, and diabetes mellitus. Weights were stabilized to reduce the influence of extreme weights if needed. Characteristics in the inverse probability of treatment weighted cohort were considered balanced if the standardized mean difference values were less than 0.1. OR and 95% CI for the 28-day mortality and clinical cure were then estimated using the weighted cohort adjusting for age, type of pneumonia and APACHE II score. The OR and 95% CI for the microbiological cure were calculated in the microbiologically evaluable population by multivariable regression adjusting for the same covariates.

Subgroup analyses of the primary outcome were also performed by stratifying patients with age (> 65 years, or ≤ 65 years), APACHE II score (> 15, or ≤15), and type of pneumonia (HAP, or VAP). The OR and 95% CI in each subgroup were estimated by adjusting for age and APACHE II score in the multivariable regression analysis. Two-tailed $p < 0.05$ was considered statistically significant. All the statistical analyses were performed with R software version 3.6.2 (R Foundation for Statistical Computing).

6.3 RESULTS

6.3.1 PATIENTS' CHARACTERISTICS AND MICROBIOLOGICAL PROFILE

There had been 326 patients diagnosed with nosocomial pneumonia caused by ESBL-producing *K. pneumoniae* meeting the inclusion criteria during the study period, 190 of whom met the exclusion criteria. The remaining 136 patients were included in the final analysis, with 64 patients being treated with piperacillin-tazobactam, while the other 72 patients were treated with carbapenems (58 patients with meropenem and 14 patients with imipenem) (Figure 6-1).

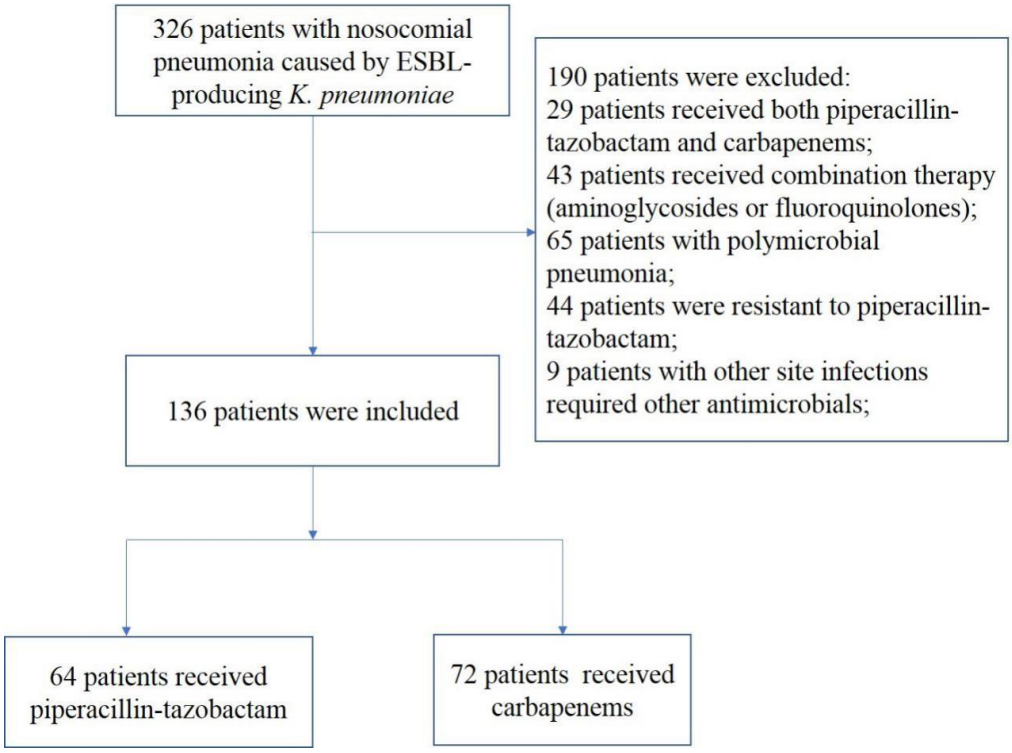

Figure 6-1. Flowchart of the study inclusion process.

The median age of the included patients was 68 years (IQR 55-76), and 108 (79.4%) were male. Most patients had at least one comorbidity, with cerebrovascular disease (57, 41.9%) and hypertension (54, 39.7%) being the most reported. Moreover, 36

(26.5%) patients had a history of malignancy, and 14 (10.3%) patients were immunocompromised. The median APACHE II score was 14 (IQR 11-19), without a statistical significance between patients receiving piperacillin-tazobactam or carbapenems. Reasons for hospitalization included both internal and surgical diseases, among which stroke, respiratory failure and scheduled surgery were the most documented. VAP was the diagnosis for 43 (31.6%) patients in the cohort and 93 (68.4%) patients were diagnosed with HAP. The duration of the target antibiotic therapy in the whole cohort was 8 days (IQR 5-12.25) and did not differ between patients receiving piperacillin-tazobactam or carbapenems. Other baseline characteristics were similar between the two groups (Table 6-1).

57.4% (78/136) of patients infected by *K. pneumoniae* strains with a piperacillin-tazobactam MIC of ≤ 4 mg/L, and 42.6% (58/136) ≤ 8 mg/L. More patients with an MIC ≤ 4 mg/L received piperacillin-tazobactam therapy (76.6% vs 40.3%, $p < 0.01$). The antimicrobial susceptibility testing result of certain antibiotics against ESBL-producing *K. pneumoniae* included in the present study is shown in Figure 6-2.

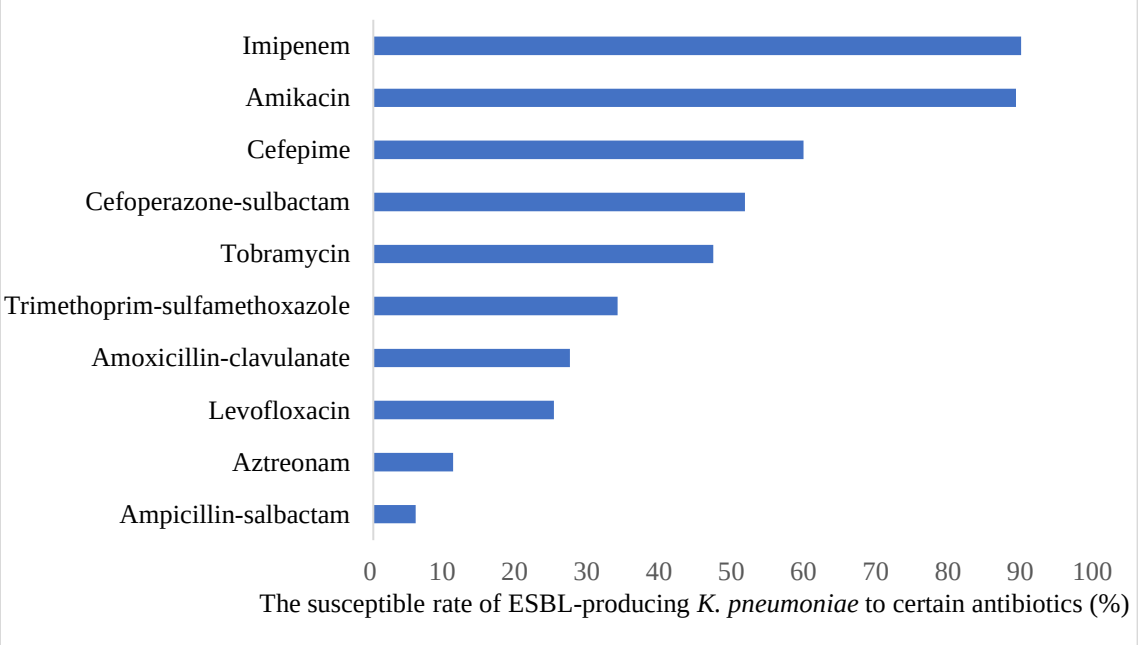

Figure 6-2. Antimicrobial susceptibility testing results of ESBL-producing *K. pneumoniae* to certain antibiotics.

Table 6-1. Characteristics of patients with nosocomial pneumonia caused by ESBL-producing *K. pneumoniae*.

	Original cohort			**Inverse probability of treatment weighted cohort**			
Variable	**Piperacillin-Tazobactam, n = 64, 47%**	**Carbapenems, n = 72, 53%**	***p***	**Piperacillin-Tazobactam, 50.5%**	**Carbapenems, 49.5%**	***p***	**Standardized Mean Differences**
Age, years, median [IQR]	68 [56, 75]	68 [54, 76]	0.929	69 [56, 75]	68 [53, 77]	0.911	0.006
Male Gender, n (%)	53 (82.8)	55 (76.4)	0.476	77.6	78.7	0.884	0.028
Reasons for Admission, n (%)			0.051			0.181	
Traumatic Brain Injury	0 (0.0)	10 (13.9)		0.0	12.0		
Coronary Heart Disease	2 (3.1)	7 (9.7)		6.1	7.8		
Cancer Therapy	9 (14.1)	5 (6.9)		10.2	10.6		
Respiratory Failure	17 (26.5)	1 (13.9)		26.3	12.3		
Renal Failure	2 (3.1)	3 (4.2)		3.2	3.8		
Sepsis	1 (1.6)	2 (2.8)		1.7	2.0		
Stroke	15 (23.4)	20 (27.8)		22.9	29.8		
Scheduled Surgery	14 (21.9)	12 (16.7)		22.5	18.1		
Trauma	4 (6.2)	3 (4.2)		7.2	3.6		
Preexisting Medical Conditions, n (%)							
Immunocompromised Status	9 (14.1)	5 (6.9)	0.280	10.2	10.6	0.942	0.013
Hypertension	24 (37.5)	30 (41.7)	0.749	37.6	41.9	0.631	0.088
Cerebrovascular Disease	26 (40.6)	31 (43.1)	0.910	43.1	42.4	0.936	0.015

To be continued.

Table 6-1. Continued.

	Original cohort			Inverse probability of treatment weighted cohort			
Variable	**Piperacillin-Tazobactam, n = 64, 47%**	**Carbapenems, n = 72, 53%**	***p***	**Piperacillin-Tazobactam, 50.5%**	**Carbapenems, 49.5%**	***p***	**Standardized Mean Differences**
Diabetes Mellitus	12 (18.8)	11 (15.3)	0.757	17.1	16.6	0.938	0.014
Malignancy	22 (34.4)	14 (19.4)	0.076	25.4	25.5	0.985	0.003
Chronic Respiratory Disease	8 (12.5)	4 (5.6)	0.262	8.8	8.0	0.879	0.028
Chronic Kidney Disease	6 (9.4)	13 (18.1)	0.226	14.3	14.3	0.989	0.003
Chronic Liver Disease	6 (9.4)	6 (8.3)	1.000	8.5	6.4	0.617	0.081
Chronic Heart Disease	7 (10.9)	16 (22.2)	0.128	17.6	17.1	0.948	0.013
Shock	5 (7.8)	10 (13.9)	0.393	10.2	10.6	0.938	0.015
APACHE II score, median [IQR]	13 [10, 17]	15 [11, 20]	0.071	14 [11, 19]	14 [10, 19]	0.772	0.044
Type of Pneumonia, n (%)			0.312			0.793	0.049
Ventilator-Associated Pneumonia	17 (26.6)	26 (36.1)		34.2	31.9		
Hospital-Acquired Pneumonia	47 (73.4)	46 (63.9)		65.8	68.1		
Antibiotic duration, day	9 [6, 13]	7 [5, 10.25]	0.160	9 [6, 13]	7 [5, 10.25]	0.073	0.274

6.3.2 INVERSE PROBABILITY OF TREATMENT WEIGHTED COHORT

As there were variables with a standardized mean difference of more than 0.2, we conducted the IPTW using the propensity score to balance the baseline covariates between patients receiving piperacillin-tazobactam and carbapenems. After weighting, the absolute standardized mean differences of variables of interest were lower than 0.1, indicating a similar distribution of observed covariates in the two groups (Figure 6-3).

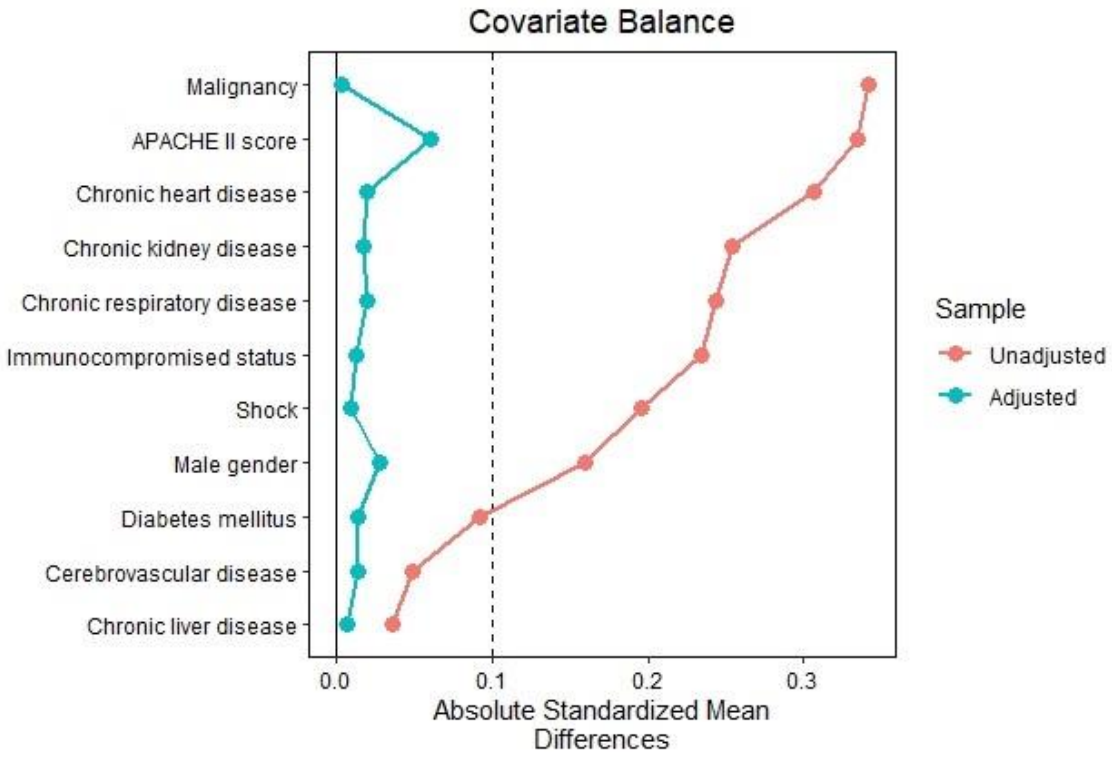

Figure 6-3. Standardized mean differences (SMD) between patients receiving piperacillin-tazobactam and carbapenems. The red dots represent the SMD in the whole unweighted cohort; the blue dots represent the SMD in the weighted cohort after the inverse probability of treatment weighting.

6.3.3 OUTCOMES

There were 26 (19.1%) patients who died within 28-days from the onset of pneumonia in the whole population; 11 (17.2%) patients in the piperacillin-

tazobactam group and 15 (20.8%) patients in the carbapenem group. Receiving piperacillin-tazobactam was not associated with higher 28-day mortality than receiving carbapenems (OR, 0.82, 95% CI, 0.23-2.87, p = 0.748), estimated in the IPTW cohort adjusting for age, APACHE II score and type of pneumonia. The overall clinical cure rate was 59.6% (81/136) at day 14, without any statistical differences between the two groups (62.5% (40/64) vs 56.9% (41/72), respectively; OR, 0.94, 95% CI, 0.38-2.35, p = 0.894). For determining microbiological cure, 44 patients were included in the final analysis; of those, 57.9% (11/19) in the piperacillin-tazobactam group and 64% (16/25) in the carbapenem group were microbiologically cured on the follow-up visit (OR, 1.10, 95% CI, 0.53-2.30, p = 0.798) (Figure 6-4).

	Patients included in analysis, No./Total No. (%)		
Clinical outcomes	Piperacillin-Tazobactam	Carbapenems	Odds ratio (95% CI), *p* Carbapenems as reference
28-day mortality	11/64 (17.2%)	15/72 (20.8%)	0.82 (0.23-2.87), 0.748
Clinical cure	40/64 (62.5%)	41/72 (56.9%)	0.94 (0.38-2.35), 0.894
Microbiological cure	11/19 (57.9%)	16/25 (64.0%)	1.10 (0.53-2.30), 0.798

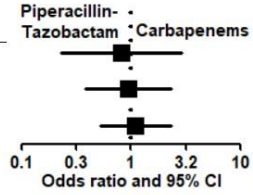

Figure 6-4. Clinical outcomes in patients with nosocomial pneumonia caused by ESBL-producing *K. pneumoniae*. In the weighted cohort, the 28-day mortality was 17.4% vs 18.5%, and the clinical cure was 63.8% vs 62.2% in patients receiving piperacillin-tazobactam or carbapenems, respectively. The odds ratio and 95% confidence intervals for the 28-day mortality and clinical cure were calculated in the weighted cohort adjusting for age, APACHE II score, and type of pneumonia. The OR and 95% CI for the microbiological cure was estimated in the microbiologically evaluable cohort.

Moreover, subgroup analyses by stratifying patients with age (> 65 years, or ≤ 65 years), APACHE II score (> 15, or ≤15), and type of pneumonia (HAP, or VAP) did not indicate any statistical differences in the 28-day mortality between patients receiving piperacillin-tazobactam and those receiving carbapenems (Figure 6-5).

Subgroup analysis	Patients included in analysis, No./Total No. (%)		Odds ratio (95% CI), *p*
	Piperacillin-Tazobactam	Carbapenems	Carbapenems as reference
Age			
≤ 65	6/27 (22.2%)	4/34 (11.8%)	2.78 (0.64-4.02), 0.383
> 65	5/37 (13.5%)	11/38 (28.9%)	0.37 (0.10-1.25), 0.122
APACHE II score			
≤ 15	6/42 (14.3%)	5/37 (13.5%)	0.90 (0.24-3.53), 0.871
> 15	5/22 (22.7%)	10/35 (28.5%)	0.79 (0.21-2.78), 0.717
Pneumonia type			
HAP	9/47 (19.1%)	7/46 (15.2%)	1.44 (0.48-4.59), 0.520
VAP	2/17 (11.8%)	8/26 (30.8%)	0.36 (0.05-2.00), 0.272

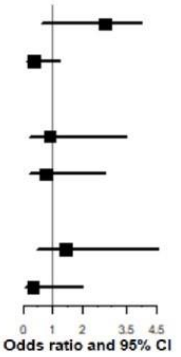

Figure 6-5. Subgroup analysis of the primary outcome in patients with nosocomial pneumonia caused by ESBL-producing *K. pneumoniae*. The odds ratio and 95% confidence intervals for the 28-day mortality in each subgroup were estimated using carbapenems as the reference and adjusted for age and APACHE II score. HAP, hospital-acquired pneumonia; VAP, ventilator-associated pneumonia; APACHE II score, acute physiology and chronic health evaluation II score.

6.4 DISCUSSION

Although many studies have assessed the effectiveness of piperacillin-tazobactam in treating various infections caused by ESBL-producing *Enterobacteriaceae*, there have not been any published studies specifically evaluating its efficacy in treating pneumonia caused by these pathogens. In the present study, we assessed the effectiveness of piperacillin-tazobactam in treating patients with nosocomial pneumonia due to ESBL-producing *K. pneumoniae* in comparison with carbapenems. The results suggest that piperacillin-tazobactam might be an effective alternative to carbapenems in treating such infections, as it resulted in similar 28-day mortality, 14-day clinical and microbiological cure. Clearly, our results differ from some studies that negatively concluded on the effectiveness of piperacillin-tazobactam in the treatment of bacteraemia due to ESBL-producing *Enterobacteriaceae* (or ceftriaxone-resistant bacteria), which has been summarized and discussed in reviews (Rodríguez-Baño et al., 2021, Paterson and Isler, 2021, Karaiskos and

Giamarellou, 2020, Schuetz et al., 2018, Rodríguez-Baño et al., 2018). Next, two important factors that might contribute to the different outcomes of such studies, the MIC breakpoints of piperacillin-tazobactam and the dosing/administration model used, will be discussed.

Which breakpoint of MIC for piperacillin-tazobactam is used to interpret the results of antimicrobial susceptibility testing against ESBL-producers greatly matters. The susceptible breakpoint of piperacillin-tazobactam in EUCAST is ≤ 8 mg/L, while it is ≤ 16 mg/L in CLSI, respectively (EUCAST, 2022, CLSI, 2017). Accordingly, those ESBL-producers with an MIC between 8 to 16 mg/L had been included in studies using the CLSI breakpoint while they had been excluded in those using the EUCAST breakpoint. The inclusion of these patients with technical uncertainty (MIC 8 to 16 mg/L) and subsequently treated with piperacillin-tazobactam would have probably affected clinical outcomes. A PK/PD study indicated that the success rate of piperacillin-tazobactam (4 g every 6 hours) achieving the target against ESBL-producers was 99% when the MIC of ESBL-producers was ≤ 8 mg/L, while the success rate decreased to 57% when the MIC reached 16 mg/L (MacGowan, 2008). Clinical studies also suggested that the MIC to piperacillin-tazobactam in ESBL-producers was negatively associated with clinical outcomes. In a retrospective study that included patients with bacteraemia due to ESBL-producing *E. coli*, the mortality rate was 4.5% in patients infected with strains that had an MIC to piperacillin-tazobactam ≤ 4.5 mg/L, while mortality was significantly increased to 23% in those infected by strains with an MIC ≥ 8 mg/L (Rodríguez-Baño et al., 2012). The post hoc analysis of the MERINO trial demonstrated a similar trend (Henderson et al., 2021). In patients with bacteraemia caused by ceftriaxone-resistant *E. coli* or *K. pneumoniae* that did not source from UTI, the mortality rate in patients receiving piperacillin-tazobactam was 27.3% when the MIC was ≤ 8 mg/L, but mounting to 71.4% when the MIC exceeded 8 mg/L (Henderson et al., 2021). Moreover, a retrospective study including patients with bacteraemia caused by cefotaxime non-susceptible *E. coli* and *K. pneumoniae* with the MIC to piperacillin-tazobactam ≤ 8 mg/L (70.7%

patients infected by strains with an MIC of ≤ 4 mg/L, 29.3% patients by strains with an MIC of ≤ 8 mg/L) indicated comparable outcomes between piperacillin-tazobactam and carbapenems (Harris et al., 2015). By contrast, another study comprising patients with bacteraemia due to ESBL-producing bacteria with a higher MIC distribution (all ≤ 16 mg/L, 39% ≤ 4 mg/L, 46% ≤ 8 mg/L, and 14% ≤ 16 mg/L) demonstrated a worse clinical outcome in patients empirically receiving piperacillin-tazobactam (Tamma et al., 2015). Together with the results found in our study, where the EUCAST cut-off was used, it is reasonable to recommend restricting piperacillin-tazobactam use to those ESBL-producers with an MIC of ≤ 8 mg/L.

A second factor that affects clinical outcomes is the dosing and administration model of piperacillin-tazobactam. Since piperacillin-tazobactam is a time-dependent antibiotic combination, the antimicrobial activity depends on the percentage of the dosing interval that the free drug concentration is maintained above the MIC of the target pathogen (fT_{MIC}) (Lodise et al., 2004). PK/PD studies indicated that, compared with intermittent administration of 4 g of piperacillin-tazobactam every 8 hours, those using 4.5 g of piperacillin-tazobactam every 6 hours or by continuous infusion had higher success rates of achieving the probability of attainment for 50% and 100% fT_{MIC} (Andersen et al., 2018, Guet-Revillet et al., 2017). A systematic review and meta-analysis comparing the prolonged infusion of piperacillin-tazobactam with intermittent infusion in severely ill patients indicated that the prolonged infusion was associated with 1.46 times lower odds of mortality (95% CI, 1.20-1.77) (Yusuf et al., 2014). Moreover, a retrospective study including patients with bacteraemia due to ESBL-producers receiving different doses of piperacillin-tazobactam illustrated an opposite result (Tamma et al., 2015). In the subgroup analysis, patients receiving 4.5 g of piperacillin-tazobactam every 6 hours did not show any difference in mortality compared with those receiving carbapenems; in contrast, the adjusted death rate was 1.92 times higher for patients receiving piperacillin-tazobactam than those using carbapenems in the whole population, as there were 61% of patients receiving 3.375 g of piperacillin-tazobactam every 6

hours (Tamma et al., 2015). Therefore, the dose and administration model of piperacillin-tazobactam is essential to maintain favourable outcomes. Although the post hoc analysis of the MERINO study supports that intermittent infusion of piperacillin-tazobactam every 6 hours was as effective as carbapenems when the pathogens were truly susceptible to it (Henderson et al., 2021), considering the inoculum effect in the lung (Lenhard and Bulman, 2019), we still recommend using piperacillin-tazobactam 4.5 g every 6 hours by extended infusion (3 to 4 hours) or continuous infusion for pneumonia as was done in our study.

Despite the promising results of piperacillin-tazobactam demonstrated in this study, using this combination to treat ESBL-producing infections still needs to be assessed with great caution. The inaccuracy in piperacillin-tazobactam susceptibility determined by automatic systems in clinical practice is the primary concern (Henderson and Humphries, 2020), as was demonstrated by the *post hoc* analysis of the MERINO trial (Henderson et al., 2021). A considerable proportion of isolates were, in fact, not susceptible to piperacillin-tazobactam by broth microdilution but were categorized as piperacillin-tazobactam susceptible by using automatic methods, which subsequently led to the failure of piperacillin-tazobactam therapy (Harris et al., 2018, Henderson et al., 2021). The inaccurate susceptibility of piperacillin-tazobactam in these ESBL-producing pathogens was due to the coharbouring OXA-1 (Henderson and Humphries, 2020). Studies have illustrated that pathogens coharbouring OXA-1 and ESBL may show false susceptibility to piperacillin-tazobactam when measured with the Vitek 2 automatic system (Livermore et al., 2019), or strip-gradient test (Etest) (Henderson and Humphries, 2020). Nevertheless, it is still possible to get rid of this disadvantage in clinical practice. Isolates coharbouring OXA-1 and ESBL that were associated with elevated piperacillin-tazobactam MICs (Henderson and Humphries, 2020), typically had the MIC of piperacillin-tazobactam at 8 to 16 mg/L (Livermore et al., 2019), the area of technical uncertainty, a concept introduced by the EUCAST to account for the challenge of test variability (EUCAST, 2022). Therefore, restricting the use of

piperacillin-tazobactam to isolates with an MIC < 8 mg/L, the lower breakpoint of piperacillin-tazobactam suggested by EUCAST, makes it less likely to include clinically relevant OXA-1 strains in piperacillin-tazobactam therapy.

Apart from the coharbouring OXA-1, the coexistence of AmpCs in ESBL-producing bacteria is another concern when using piperacillin-tazobactam. Studies reported a significant proportion of *Enterobacteriaceae* coharboured both AmpC and ESBL (Henderson et al., 2021, Conen et al., 2015). AmpCs typically cause resistance against tazobactam, thereby also diminishing the efficacy of piperacillin in such pathogens (Meini et al., 2019). As AmpCs are not routinely tested in clinical practice, this might add uncertainty to clinical decisions of using piperacillin-tazobactam in ESBL-producing bacterial infections. However, from the perspective of clinicians, it is very less likely to use piperacillin-tazobactam in isolates coharbouring both ESBL and clinically relevant AmpCs when the choice of antibiotics was made based on the results of antimicrobial susceptibility testing. *Enterobacterales* with plasmid-mediated *ampC* or derepressed chromosomal *ampC* usually express AmpC at a clinically relevant level and cause resistance against piperacillin-tazobactam (Arena et al., 2013, Meini et al., 2019); in contrast, those strains harbouring chromosomal *ampC* but still exhibiting susceptibility to piperacillin-tazobactam generally express the AmpC at a very low level and they usually do not cause a clinical issue, like the chromosomal *ampC* in many *E. coli* strains (Peter-Getzlaff et al., 2011, Meini et al., 2019). Moreover, both piperacillin and tazobactam are weak inducers of AmpC enzymes (Kohlmann et al., 2018). Clinical studies have already demonstrated that piperacillin-tazobactam resulted in similar clinical outcomes as carbapenems in treating infections caused by ESBL-producing pathogens that expressed AmpC but are still susceptible to piperacillin-tazobactam (Cheng et al., 2017, Stewart et al., 2021). Taken together, when considering using piperacillin-tazobactam to treat ESBL-producing infections, the accurate phenotypical susceptibility needs to be determined on top of the genomic background of the clinical isolates.

There are several limitations in the present study. First, it is a small sample size, retrospective study, bias and confounders might still affect the final analysis despite implementing the propensity score weighting. Second, the ESBL-production was determined phenotypically by the automatic Vitek 2 system instead of by the referred methods (CLSI, 2017). Although studies indicated an excellent sensitivity and specificity of the Vitek 2 system (Spanu et al., 2006, Robin et al., 2008), it still might include false-positive isolates in this study hence skewing the results favouring piperacillin-tazobactam. Third, the distribution of ESBL enzymes vary geographically (Jean and Hsueh, 2017, Peirano and Pitout, 2019); so, without knowing the genomic background, the results in this study might not be generalizable. Fourth, all patients included in this study displayed mild to moderate pneumonia, and only 11% (15/136) of patients had an APACHE II score > 15. Thus, it is unclear how well piperacillin-tazobactam would function in severe patients. Fifth, with a meagre rate of blood culture implemented in the study cohort, we did not incorporate the impact of bacteraemia in the final analysis. Having this analysis would have strengthened the interpretation of clinical results, as it is known that bacteraemia is an independent risk factor for mortality in patients with pneumonia (Hwang and Handigund, 2020). Sixth, we only included patients receiving piperacillin-tazobactam or carbapenems starting from the onset of pneumonia; those using piperacillin-tazobactam only for definitive therapy were excluded. Therefore, we were unable to draw any conclusions regarding the efficacy of piperacillin-tazobactam definitive therapy in nosocomial pneumonia due to ESBL-producers.

6.5 CONCLUSIONS

Piperacillin-tazobactam might be an effective alternative to carbapenems in treating nosocomial pneumonia due to ESBL-producing *K. pneumoniae*. It was not associated with worse clinical outcomes compared with carbapenems. When considering using piperacillin-tazobactam to treat pneumonia caused by ESBL-producing *K. pneumoniae*, we recommend restricting its use by extended or continuous infusion

of 4.5 g every 6 hours to enhance its PK/PD profile. In light of the limitations in the present study, appropriately powered and well-designed RCTs are required to confirm these findings.

7 CHAPTER 7 – SUMMARIZING DISCUSSION AND FUTURE DIRECTIONS

7.1 SUMMARIZING DISCUSSION

The present thesis was started from a nationwide survey (**Chapter 2**), in which a better understanding of currently used strategies for managing nosocomial pneumonia due to MDR-GNB in China was obtained. The results indicated that with different availability of antibiotics, prescription cultures and antibiotic stewardship implemented in individual hospitals, therapeutic regimens varied considerably from each institution. These findings highlighted the importance of antimicrobial stewardship studies on the treatment of MDR-GNB, especially those studies focusing on using alternative antibiotics in the correct dose and optimal modality. Based on this, many research lines could be envisioned to develop guidelines to aid clinical-decision making eventually, but the scope for this thesis was limited to a few of those. In **Chapters 3-5** of this thesis the focus was on investigating the efficacy of tigecycline monotherapy at a different dosage or combination with polymyxin B in the treatment of pneumonia due to CRGNB. **Chapter 6** dealt with beta-lactam/beta-lactamase inhibitor cocktails in treating pneumonia due to ESBL-producing pathogens.

As demonstrated in **Chapter 2**, with the limited availability of ceftazidime-avibactam in China, therapies for MDR bacteria, especially CRGNB, are largely dependent on tigecycline-based therapy. However, despite the wide use, the dosage

of tigecycline in these hospitals is still inconsistent, with some hospitals using SDT, while others prescribed the high-dose regime. Notably, there was no evidence-based medicine on this topic at this thesis's initiation. Therefore, in **Chapter 3**, I conducted a systematic review and meta-analysis to assess the effectiveness of HDT in treating severe infections, especially those caused by MDR-GNB, compared mainly with the SDT. Findings from this evidence-based medicine study supported our hypothesis that the HDT could improve the prognosis of patients that infected by MDR bacteria, including CRGNB. Results from this study have been cited and recommended in the latest IDSA guidelines.

Given the better effectiveness of HDT was shown in **Chapter 3**, I further conducted a retrospective study in **Chapter 4** to assess whether adding polymyxin B to HDT would bring extra benefit for patients with pneumonia caused by CRKP and CRAB. Interestingly, although the combination of tigecycline and polymyxin was the third most prevalent combination regimen in treating pneumonia due to CRKP and CRAB in China (demonstrated in **Chapter 2)**, results from this study did not support the use of this combination in treating such infections, as it did not reduce the 14-day mortality when compared with HDT therapy. Therefore, taking findings from **Chapter 3** and **Chapter 4** together, it can be concluded that the HDT is an effective therapy in treating CRGNB; meanwhile, adding polymyxin B to the HDT regimen is unnecessary, as this combination did not improve patient's prognosis.

Apart from treating CRKP and CRAB, tigecycline was also widely used as an alternative therapy for *S. maltophilia* pneumonia. According to results from the survey in **Chapter 2**, 17.9% of the participating hospitals in China used tigecycline to treat pneumonia due to *S. maltophilia*. Studies assessing the effectiveness of tigecycline in treating such infections are rarely limited. Therefore, in **Chapter 5**, I conducted a multicentre retrospective study to evaluate the effectiveness of tigecycline in treating *S. maltophilia* pneumonia compared with fluoroquinolones. The results indicated that therapy with SDT was associated with worse clinical

outcomes than fluoroquinolone. With these results, it is rational to recommend against using the SDT in treating *S. maltophilia* pneumonia. However, whether HDT would lead to comparable or better clinical outcomes than fluoroquinolone is still unknown. Considering the promising results of HDT demonstrated in **Chapter 3**, further clinical studies, including both observational studies and RCTs regarding the efficacy of HDT in treating pneumonia due to *S. maltophilia*, are highly welcomed.

Chapte**r 6** was derived from findings in **Chapter 2**, in which 36.8% of the participating hospitals used piperacillin-tazobactam as the targeted therapy for VAP due to ESBL-positive *Enterobacteriaceae*. As there was no clinical evidence assessing the efficacy of piperacillin-tazobactam in treating such infections, a retrospective study was conducted in this chapter. The main conclusion from this work was that piperacillin-tazobactam could be a carbapenem-sparing regimen in treating nosocomial pneumonia due to ESBL-producing *K. pneumoniae*, as the 28-day mortality, 14-day clinical and microbiological cure were similar between the two groups.

To sum up, therapies for clinically important pathogens varied considerably in Chinese hospitals, with some therapeutic regimens having been demonstrated as an inappropriate therapy for such infections. Findings from this thesis could be used as evidence to support the choice of antibiotic therapy in treating nosocomial pneumonia caused by these pathogens. In detail, for treating CRKP and CRAB, HDT is recommended when tigecycline is the clinical choice; combining polymyxin B with HDT should be avoided as this combination does not improve clinical outcomes. Moreover, SDT should not be used as the alternative therapy in treating *S. maltophilia* pneumonia, while piperacillin-tazobactam could be used as a carbapenem-sparing regimen in treating pneumonia caused by ESBL-producing *K. pneumoniae*.

7.2 FUTURE DIRECTIONS AND RECOMMENDATIONS

In this thesis, I referred to evidence-based medicine and clinical epidemiology research methods to assess the effectiveness of different therapeutic regimens in treating nosocomial pneumonia caused by MDR-GNB. The primary focuses were those antibiotic regimens reported in the nationwide survey conducted in **Chapter 2**. Although the pre-proposed five research questions have been addressed and part of the contents raised from this thesis have already been published in peer-reviewed journals, new questions arising from these studies still ask for further research.

7.2.1 RCTS ARE NEEDED AND GENOMIC BACKGROUND OF BACTERIAL STRAINS SHOULD BE INCLUDED

Several limitations of the studies described in this thesis have already been highlighted in the separate chapters and those should be addressed to improve future studies. One major limitation is the observational study design, in which confounders and biases are inevitable. Although some sophisticated statistical methods were implemented to minimize confounding bias, e.g., multivariable regression model, propensity score matching, inverse probability of treatment weighting, it is impossible to get rid of all confounders, especially with the limited sample size and unmeasured confounders in these studies. To overcome this, well-designed RCTs focusing on specific therapeutic regimens need to be executed.

Another major limitation is the lack of genomic background of the included resistant strains, which restricts the generalizability of the conclusions in these studies, as bacteria with the same resistant phenotype could have different resistant mechanisms and virulent factors, hence show distinct responses to the same antibiotic regimens, and might lead to different clinical outcomes. Therefore, in future studies, it is recommended to incorporate the genomic background into the

final analysis, especially those already known resistant mechanisms, which could help promote conventional antibiotic therapy to precision medicine.

Apart from the limitation that should be addressed in future studies, in the following paragraphs, I will argue for specific recommendations addressing the possible therapeutic regimens for pathogens described in **Chapters 3-6**.

7.2.2 Assess the effectiveness of once-daily HDT regimen for CRGNB infections

In **Chapter 3**, the HDT regimen was demonstrated to be associated with better clinical outcomes than the standard-dose regimen or other comparators. In this study, tigecycline in both groups was used twice daily. However, from the PK/PD perspective, using tigecycline twice daily might not be the optimal administration model. The elimination half-life of tigecycline was 27 hours when used in a single dose and 42 hours after multiple administrations (Barbour et al., 2009). Additionally, tigecycline demonstrated a linear kinetic model. When used tigecycline 50 mg twice daily as recommended by the package insert, the peak serum concentration was 0.75 mg/ml, and when administrated this 100 mg tigecycline once daily, although the total dose was the same, the peak serum concentration increased to 1.5 mg/ml; moreover, the once-daily HDT (200 mg once daily as the maintenance dose instead of 100 mg twice daily) resulted in an even higher serum concentration of 3 mg/ml (Cunha et al., 2018). Given these parameters, it is rational to hypothesize that the once-daily HDT might be better than twice daily regimen. However, apart from two case series studies (Baron et al., 2018, Hughes et al., 2019), no other clinical studies assessing the effectiveness of the once-daily HDT regimen in treating infections caused by CRGNB are known. Clearly, it is far from drawing any conclusions but a worthwhile objective for further clinical studies, ideally RCTs.

7.2.3 Determine efficacy of ceftazidime-avibactam based therapy in treating CRKP within the context of carbapenem resistance and explore the usefulness of tigecycline and polymyxins in the treatment of MBL-producing *K. pneumoniae*

Moreover, although the promising efficacy of the HDT was shown in the present thesis, recent studies have illustrated that ceftazidime-avibactam was associated with better clinical outcomes than tigecycline and polymyxins in treating CRKP (Shi et al., 2021, Zheng et al., 2022, Fang et al., 2021). In 2021, a retrospective study including 105 patients with severe nosocomial pneumonia caused by CRKP indicated that ceftazidime-avibactam therapy was associated with a higher clinical cure (OR 3.4, 95% CI 1.3-8.9) and microbiological cure (OR 7.8, 95% CI 2.7-22.3) than that of tigecycline therapy (Shi et al., 2021). However, as there was no information on the dose of tigecycline used in that study, whether ceftazidime-avibactam could result in better clinical outcomes than HDT is still uncertain. Additionally, in another retrospective study which aimed to compare the effectiveness of ceftazidime-avibactam and polymyxin B in treating CRKP infections, the subgroup analysis indicated that the combination of ceftazidime-avibactam and tigecycline reduced the 30-day mortality (HR 0.27, 95% CI 0.08-0.92) (Zheng et al., 2022). As avibactam cannot inhibit the activity of MBLs in CRKP (Li et al., 2022a), whether the combination of ceftazidime-avibactam and tigecycline is a safer and more efficacious regimen in treating such infections is unclear, especially when there is no information regarding the mechanisms of carbapenem resistance. Therefore, in the future, it is highly recommended to conduct RCTs assessing the efficacy of ceftazidime-avibactam based therapy in treating CRKP within the context of mechanisms of carbapenem resistance, especially to explore the potential role of tigecycline and polymyxins in the treatment of MBL-producing *K. pneumoniae*.

7.2.4 POTENTIAL OF ADDING AEROSOLIZED POLYMYXIN TO TIGECYCLINE AS THERAPY TO TREAT CRGNB-PNEUMONIA

In **Chapter 4**, the results indicated that adding intravenous polymyxin B as the adjunctive therapy to HDT did not improve the patient's clinical outcomes in treating nosocomial pneumonia caused by CRKP and CRAB. This study used polymyxin B at the dose instructed by its package insert, which was lower than that recommended by clinical guidelines. Therefore, the non-benefit of this combination was speculated partially due to the underdosed polymyxin B. However, a recently published study indicated that even using the recommended dose of polymyxin B plus tigecycline also did not result in lower mortality than those patients receiving polymyxin B or tigecycline (Chang et al., 2022). Moreover, PK/PD studies have indicated that even when using the highest tolerable dose of polymyxins, the concentration of polymyxins in the lung is likely to be below optimal for infecting strains unless MICs of these pathogens are well below the breakpoint (Cheah et al., 2015, Landersdorfer et al., 2018). Under such circumstances, doctors attempted to use the inhaled antibiotic therapy for pneumonia caused by these resistant pathogens, as it could achieve a concentration many times higher than that achieved with intravenous administration. Although there were no RCTs directly supported the efficacy of aerosolized polymyxin in treating pneumonia due to CRGNB, small sample size studies have demonstrated the benefit of the inhalation of colistin in treating such infections (Feng et al., 2021, Rattanaumpawan et al., 2010). Therefore, adding aerosolized polymyxin to tigecycline might be one of the potential therapeutic regimens in treating pneumonia due to CRGNB and is worth further RCTs.

7.2.5 MOXALACTAM AND AMIKACIN AS POTENTIAL THERAPIES AGAINST ESBL-PRODUCING *ENTEROBACTERIACEAE*

In **Chapter 6**, the effectiveness of piperacillin-tazobactam in treating nosocomial pneumonia due to ESBL-positive *K. pneumoniae* was assessed. The results

demonstrated that if the true sensitivity of piperacillin-tazobactam was established by a reliable method, it could act as an effective carbapenem-sparing regimen in treating such infections. Apart from piperacillin-tazobactam, moxalactam, a kind of cephamycin, might also be a potential regimen in treating ESBL-producing *Enterobacteriaceae* due to its good *in vitro* activity. Based on data from the 10-year Chinese surveillance, moxalactam demonstrated an excellent antimicrobial activity similar to that of carbapenems against ESBL-positive *Enterobacteriaceae*, with 95.1% of the ESBL-positive *K. pneumoniae* and 97.8% of the ESBL-positive *E. coli* susceptible to it (Cui et al., 2016). In an *in vitro* PK/PD model, moxalactam also presented a potent bactericidal effect against ESBL-producing strains (Huang et al., 2018). When moxalactam was used at its approved dosage (1 g every 6 hours), the cumulative fraction of response could exceed 90% against ESBL-producing *E. coli* (Huang et al., 2019). The same dosage can also achieve an 85% probability of target attainment of 70% *f*T > MIC in ESBL-producing *K. pneumoniae* (Ito et al., 2014). Moreover, clinical studies have demonstrated that cephamycins could be used as the carbapenem-sparing regimen in treating BSI caused by ESBL-producing *Enterobacteriaceae*, as they were not associated with a higher rate of mortality when compared with carbapenems (Fukuchi et al., 2016, Yang et al., 2012, Lee et al., 2006). However, as of writing, there have been no reports of clinical studies assessing the efficacy of moxalactam in treating ESBL-producing *Enterobacteriaceae*. Therefore, whether moxalactam could be an alternative in treating it is still unknown. Because moxalactam is already being used in some hospitals to treat such infections (personal communication), preliminary retrospective studies in China assessing its role in treating ESBL-producing *Enterobacteriaceae* could be conducted first.

Another potential therapeutic option for ESBL-producing *Enterobacteriaceae* is amikacin. *In vitro* studies have shown that amikacin still presented good antimicrobial activity against ESBL-producing *Enterobacteriaceae* if no other resistant mechanisms co-existed. In China, among the 1339 clinically isolated ESBL-

positive *K. pneumoniae* strains, the susceptibility rate to amikacin was 77.2%; in those 2883 ESBL-positive *E. coli* strains, the rate was as high as 90.8% (Cui et al., 2016). In South Korea, a similar susceptibility rate was also shown in a study with 291 ESBL-producing *E. coli* isolates, in which 88.3% of strains isolated from the bloodstream were susceptible to amikacin (Cha et al., 2015). Taking the advantageous PK/PD profile of amikacin, which is cleared through the renal route, leads to a high concentration in urine (Kato et al., 2017, Krause et al., 2016), it was therefore recommended by clinical guidelines to treat UTI caused by ESBL-producing *Enterobacteriaceae* (Paul et al., 2022, Tamma et al., 2021). However, in terms of patients with pneumonia, a PK/PD study has indicated that the normal dosage of amikacin (15 mg/kg/day) was unlikely to achieve an optimal PK/PD target in the lung (Kato et al., 2017). Given the nephrotoxicity of amikacin, it is difficult to fulfil the PK/PD target by increasing the dosage of amikacin in clinical practice. Under such circumstances, the inhalation of amikacin might be a practical method, as it could reach a concentration in the epithelial lining fluid 10-100 times higher than the reference MIC (Niederman et al., 2012, Luyt et al., 2009). However, the INHALE study, one of the most impactful RCTs assessing the efficacy of inhaled amikacin in patients with VAP, did not support the routine use of inhaled amikacin in such infections as it did not improve patient outcomes (Niederman et al., 2020). In light of results from the INHALE study, the latest updated IDSA guidelines recommended against using inhaled antibiotics for pneumonia due to MDR-GNB (Tamma et al., 2021). However, the trial was criticized for including 47% of pathogens that were not MDR bacteria (Rouby et al., 2020). Although the results did not change even by only including MDR pathogens, it is still reasonable to speculate that the inhalation of amikacin might be beneficial to pneumonia caused by MDR pathogens, as a further analysis conducted by me with data from the study showed that the eradication rate of important clinical pathogens (*P. aeruginosa*, *A. baumannii*, *K. pneumoniae*, and *E. coli*) was higher in patients receiving amikacin inhalation than that in the placebo group (68.7% VS 58.3%, $p = 0.03$). Considering the limited carbapenem-sparing choices for pneumonia due to ESBL-

producing *Enterobacteriaceae* in clinical practice, it is still worth conducting RCTs to assess the role of inhalation of amikacin in treating such infections.

7.3 CONCLUDING REMARKS

AMR is still one of the biggest challenges for doctors working in the field of infectious diseases. It will persist for a long time in the foreseeable future due to the selection of resistant pathogens through misuse and overuse of antibiotics, poor antimicrobial stewardship system, and drying pipeline of new antibiotics. Although new antibiotics have been approved for treating infections caused by these resistant pathogens during these years, the availability of these new antibiotics in some countries lagged far behind the urgent requirements raised by the high rate of AMR. Under such circumstances, as demonstrated in the survey of the present thesis, antibiotics like tigecycline and colistin are still widely used as the first line choice despite their uncertain effectiveness in treating infections caused by CRGNB. Additionally, aiming to reduce the rate of the emergence of carbapenem resistance in ESBL-producing *Enterobacteriaceae*, carbapenem-sparing regimens are popularly prescribed in clinical settings, though no solid evidence exists. To fill gaps between current clinical practices and evidence-based medicine, the five studies in the present thesis have addressed questions about the treatment of MDR-GNB infections that have not been solved in clinical guidelines. Although there are more new questions raised than that have been solved, findings from the present thesis contribute considerably to our understanding of the treatment of MDR infections, as part of the contents presented in this thesis have already been cited and recommended in the latest updated clinical guidelines.

In the future, with more availability of new antibiotics, more prudent use of antibiotics through education and good antibiotic stewardship system, more rigorous interventions in infection prevention and control, and more closely coordinated actions in the one-health approach, the rates of AMR in all infectious

bacteria could be alleviated to some extent. At the current stage, as a clinical researcher in infectious diseases, the urgent task is continuing to find out more effective regimens and alternative therapies for MDR infections within the currently available antibiotics. I am optimistic to believe that with efforts from the whole society globally, we could turn the “era of antimicrobial resistance” back into one where antibiotics are effective enough.

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9 APPENDIX

9.1 Appendix I: QUESTIONNAIRE OF THE SURVEY

Survey of clinical management of ventilator-associated pneumonia caused by multidrug resistant Gram-negative bacteria (Translated from the original Chinese version).

The survey contains four parts for you to fill, basic information of your hospital, information of the microbiology laboratory, antibiotic availability and strategies for VAP caused by multidrug-resistant Gram-negative bacteria. It is estimated that it will take 90 minutes for you to fill out the questionnaire.

Basic information:

Name of Hospital: _____

1. Type of your hospital:

   Secondary hospital.

   Tertiary Hospital.

   University Teaching Hospital.

2. Beds in ICU:

3. Number of patients admitted in ICU（data of 2020）:

4. Does your hospital have local antibiotic resistance data? Y/N
5. Does your hospital have clinical pharmacists monitoring antibiotic use routinely? Y/N
6. Does the incidence of VAP as a performance indicator in your hospital? Y/N

Information of Microbiology laboratory and diagnostic methods for VAP:

7. Could your hospital perform bedside bronchoscopy in ICU? Y/N
8. Which is the most chosen sampling method in suspected VAP patients?

   Endotracheal aspirate

   Bronchoalveolar lavage

   Protected specimen brush
9. Which culture method was performed in your hospital?

   Quantitative

   Semiquantitative

   Qualitative
10. The infrastructure of the local lab (tick if your hospital has):

    Standard aetiology testing machine (e.g., Vertik 2)

    Matrix-assisted laser desorption ionization-time of flight

    Metagenomic next-generation sequencing
11. Reporting MIC values for tested antibiotics? Y/N
12. Testing ESBL phenotype in *Enterobacteriaceae*? Y/N
13. Testing carbapenemases in CRGNB? Y/N
14. Reporting susceptibility of following antibiotics for CRKP (please tick):

    Tigecycline; polymyxins; Fosfomycin; minocycline; rifampicin; Sulfonamides; ceftazidime/avibactam; Aztreonam; Tobramycin; Ceftolozane-tazobactam; Eravacycline; Meropenem-vaborbactam; Imipenem-Cilastatin-Relebactam; Plazomicin

15. Performing combination antimicrobial susceptibility testing for CRKP? Y/N

16. Antibiotic availability in the hospital：

Antibiotics	Routinely offer	Conditionally offer	Not offer
Doripenem			
Ertapenem			
imipenem			
Meropenem			
Meropenem-vaborbactam			
Imipenem-Cilastatin-Relebactam			
ceftazidime/avibactam			
Ceftolozane-tazobactam			
Aztreonam			
Gentamicin			
Tobramycin			
Fosfomycin (IV)			
Eravacycline			
Plazomicin			
Tigecycline			
polymyxins			
minocycline			
Trimethoprim-Sulfonamides (IV)			
Trimethoprim-Sulfonamides (Oral)			
rifampicin			
Vancomycin			
Teicoplanin			
Linezolid			

Therapies and strategies for VAP：

17. Using procalcitonin to guide the initiation of antibiotics in suspected VAP patients? Y/N

18. Percentage of patients receiving empirical monotherapy for carbapenem-resistant VAP, referring to data of 2020：

    1) < 25%； 2) 25% to 50 %； 3) 50% to 75%; 4) > 75%；

19. Percentage of patients receiving target monotherapy for carbapenem-resistant VAP, referring to data of 2020：

1) < 25%； 2) 25% to 50 %； 3) 50% to 75%; 4) > 75%；

20. Percentage of patients receiving inhaled colistin for VAP caused by CR-GNB:

1) < 25%； 2) 25% to 50 %； 3) 50% to 75%; 4) > 75%

21. Dose of tigecycline in clinical practice (please choose)：

Standard dose (100 mg followed 50 mg per 12 hours)

High dose (200 mg followed 100 mg per 12 hours)

22. Whether initiate polymyxins with a loading dose (please choose)：

With a loading dose Without a loading dose

23. Administration model of carbapenems in treating carbapenem-resistant infections (please choose)：

Standard intravenous; Extended infusion (3-4 hours); Continuous infusion

24. Target therapy for ventilator-associated pneumonia caused by CRKP (Please fill it with data of 2020):

The most frequent monotherapy：

The most frequent double combination therapy：

The most frequent triple combination therapy：

25. Target therapy for ventilator-associated pneumonia caused by CRAB

The most frequent monotherapy：

The most frequent double combination therapy：

The most frequent triple combination therapy：

26. Target therapy for ventilator-associated pneumonia caused by CRPA:

The most frequent monotherapy：

The most frequent double combination therapy：

The most frequent triple combination therapy：

27. Target therapy for ventilator-associated pneumonia caused by ESBL-producing *Enterobacteriaceae* (susceptible to piperacillin-tazobactam)?

    Carbapenems.

    Piperacillin-tazobactam.

    other

28. Target therapy for ventilator-associated pneumonia caused by *Stenotrophomonas maltophilia?*

    Trimethoprim-sulfamethoxazole

    Fluoroquinolone

    Tigecycline

    Colistin

    Minocycline

    Others

9.2 APPENDIX II: ETHICS APPROVAL FOR THE THESIS

Xi'an Jiaotong-Liverpool University
西交利物浦大學

P156D Xi'an Jiaotong-Liverpool University
111 Ren'ai Road, Dushu Lake Higher Education Town SIP
Suzhou 215123,
P.R. China.

13 November 2019

Dear Lei Zha,

Proposal Number 19-01-05

Title:

The clinical epidemiology and management of Gram-negative infections in critically ill patients.

Your application for University Ethics Committee (UEC) approval has been reviewed and approved by the UEC's action.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to, and approved by, the UEC prior to the implementation of any changes. You are required to report to the UEC as soon as possible (or within 5 working days) any issues regarding the occurrence of adverse events, such as risks or harms, involving study participants.

Sincerely,

Professor Igea Troiani
Chair, University Ethics Committee

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