Treatment options for infections caused by carbapenem-resistant Klebsiella pneumoniae isolates

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Treatment options for infections caused by carbapenem-resistant *Klebsiella pneumoniae* isolates

Graduate Thesis

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This graduate thesis was made at the Department of Internal Medicine,
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mentored by assistant professor Luka Bielen, MD, PhD,
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Abbreviations

ADE	Antimicrobial de-escalation
AMR	Antimicrobial resistance
ATP	Adenosine triphosphate
AUC	Area under the curve
BLI	β-Lactamase inhibitor
CAESAR	Central Asian and European Surveillance of Antimicrobial Resistance network
CBA	Colistin base activity
CCRE	Colistin- and/or carbapenem-resistant *Enterobacteriaceae*
CDC	Centres for Disease Control and Prevention
CG	Clonal group
CLSI	Clinical and Laboratory Standards Institute
CMS	Colistin methanesulfonate
COVID-19	Coronavirus disease 2019
CRE	Carbapenem-resistant *Enterobacteriaceae*
CRKP	Carbapenem-resistant *Klebsiella pneumoniae*
CSF	Cerebrospinal fluid
CTX-M	Cefotaximase from Munich
DBO	Diazabicyclooctanones
DCT	Dual carbapenem therapy
DHP	Dehydropeptidase
DNA	Deoxyribonucleic acid
EARS-Net	European Antimicrobial Resistance Surveillance Network
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
EMA	European Medicines Agency
ESBL	Extended-spectrum β-lactamases
ESKAPE	Acronym for: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter spp.*
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EURGen-Net	European Antimicrobial Resistance Genes Surveillance Network
EuSCAPE	European survey of carbapenemase-producing *Enterobacteriaceae*
FDA	Food and Drug Administration
GRS	Giannella risk score
ICE	Integrative conjugative elements
ICU	Intensive care unit

IDSA	Infectious Diseases Society of America
IM	Intramuscular
IMP	Carbapenemase “active for imipenem”
IPC	Infection prevention and control
IU	International units
IV	Intravenous
KP	*Klebsiella pneumoniae*
KPC	Klebsiela pneumoniae carbapenemase
LPS	Lipopolysaccharide
LTCF	Long-term care facilities
MALDI-TOF MS	Matrix-assisted laser desorption ionization–time of flight mass spectrometry
MATE	Multi-antimicrobial extrusion protein
MBL	Metallo-β-lactamases
MDK	Medium duration to kill
MDR	Multi-drug resistant
MGE	Mobile genetic element
MIC	Minimum inhibitory concentration
mRNA	Messenger ribonucleic acid
NDM	New Delhi Metallo-β-Lactamase
OAT	Organic anion transporters
OXA	Oxacillinase
PAE	Postantibiotic effect
PBP	Penicillin-binding proteins
PCR	Polymerase chain reaction
PCT	Procalcitonin
PD	Pharmacodynamic
PDR	Pandrug-resistant
PK	Pharmacokinetic
RRT	Renal replacement therapy
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SHV	Sulfhydryl variant of TEM
SNP	Single-nucleotide polymorphisms
ST	Sequence type
TEM	β-Lactamase named after ‘Temoneira’, a patient
TOTEM	Acronym for the top ten microorganisms
tRNA	Transfer ribonucleic acid
US	United States
UTI	Urinary tract infection
VIM	Verona Integron-encoded Metallo-β-Lactamase
WHO	World Health Organisation
XDR	Extensively drug-resistant

Contents

1. Summary

Treatment options for infections caused by carbapenem-resistant *Klebsiella pneumoniae* isolates

Luka Joakim Csenar

Carbapenem-resistant *Klebsiella pneumoniae* poses a global public health threat. Newly emerging resistances deplete the antibiotic armamentarium and represent a therapeutic challenge even for the most experienced physician. Epidemiological data on carbapenem-resistant *K. pneumoniae* show high mortality rates and growing resistance rates.

Carbapenems are broad-spectrum β-lactam antibiotics being used in complicated and hard to treat infections including those caused by *K. pneumoniae*. The acquisition of various resistance genes enabled *K. pneumoniae* to produce carbapenemases. Distinct carbapenemases provide drug targets for novel β-lactamase inhibitors. However, some *K. pneumoniae* isolates show high adaptability resulting in resistance against new drugs, only leaving salvage therapy as last option.

This graduate thesis provides insight into carbapenem resistance mechanisms in *K. pneumoniae*. Furthermore, rational approaches regarding the possible options for the treatment of infections caused by carbapenem-resistant *K. pneumoniae* isolates are outlined. The therapeutic regimen is mainly based upon the class of carbapenemase being produced by the *K. pneumoniae* strain causing the infection. Screening by molecular microbiological methods enables infection prevention and control, as well as prompt initiation of therapy.

Keywords: *Klebsiella pneumoniae*, Carbapenem-resistance, Monotherapy, Combination therapy, KPC, OXA-48

2. Sažetak

Mogućnosti liječenja infekcija uzrokovanih sojevima *Klebsiella pneumoniae* rezistentnima na karbapeneme

Luka Joakim Csenar

Klebsiella pneumoniae rezistentna na karbapeneme predstavlja veliki globalni javnozdravstveni problem. Pojava novih mehanizama rezistencije dovodi do smanjenja broja učinkovitih antibiotika zbog čega liječenje ovih infekcija predstavlja izazov čak i najiskusnijim kliničarima. Epidemiološki podatci o *K. pneumoniae* rezistentnoj na karbapeneme ukazuju na globalni porast rezistencije i na visoku stopu smrtnosti od infekcija uzrokovanih ovim mikroorganizmom.

Karbapenemi su β-laktamski antibiotici širokog spektra. Koriste se za komplicirane teško lječive infekcije, u koje ubrajamo i one uzrokovane *K. pneumoniae*. Stjecanje različitih gena rezistencije omogućilo je *K. pneumoniae* produkciju karbapenemaza. Različite karbapenemaze predstavljaju mete za liječenje novim inhibitorima β-laktamaze. Međutim, neki izolati *K. pneumoniae* pokazuju visoku sposobnost adaptacije, što dovodi do razvoja rezistencije i na nove lijekove. Time kao posljednja opcija kliničarima preostaju samo antibiotici zadnje linije liječenja.

U ovom se diplomskom radu opisuju mehanizmi rezistencije *K. pneumoniae* na karbapeneme. Raspravlja se o mogućnostima liječenja infekcija uzrokovanih izolatima *K. pneumoniae* rezistentnima na karbapeneme. Terapijska shema prvenstveno se temelji na klasi karbapenemaze koju producira soj *K. pneumoniae* koji je doveo do infekcije. Probir molekularnim mikrobiološkim testiranjem omogućava prevenciju i kontrolu širenja infekcija, kao i rano započinjanje terapije.

Ključne riječi: *Klebsiella pneumoniae*, Rezistencija na karbapeneme, Monoterapija, Kombinirana terapija, KPC, OXA-48

3. Introduction

Fleming, Chain, and Florey were awarded the Nobel Prize for Medicine or Physiology in 1945 for having discovered penicillin and the successful clinical use of the drug (1). This marks the beginning of the antibiotic era. Early reports of antibiotic resistance associated with treatment failures were combatted by the discovery and development of new antimicrobial drugs and classes.

However, a combination of decades-long misuse and overuse of antibiotics, intrinsic and acquired escape mechanisms, evolutionary advances of bacteria, and a stagnating rollout of new antibiotics have led to a significant problem in modern healthcare: antimicrobial resistance (AMR).

The global spread of resistant organisms poses a significant threat to public health (2). An estimated 25000 people annually die of drug-resistant bacteria in the European Union (EU). This causes at least €1.5 billion in health care costs and productivity loss (3). Besides, research and development of new antimicrobials are slow due to lower financial returns, regulatory burden and deflected attention (4).

Klebsiella pneumoniae (KP) is a gram-negative, rod-shaped bacterium belonging to the family of *Enterobacteriaceae* (5). As a part of our gut microbiota it is an opportunistic pathogen. Infections caused by this bacterium can be both community- and hospital-acquired (nosocomial). Up to one-third of all gram-negative infections are caused by KP (6).

KP is contributing to the crisis of AMR by the expression of carbapenemases, rendering available treatment options ineffective. One of the main drivers of worldwide carbapenem resistance is a carbapenemase first discovered in *Klebsiella pneumoniae*: KPC-1 (Klebsiella pneumoniae carbapenemase 1) (7).

Carbapenems are a class of broad-spectrum antibiotics which are used as last-line antibiotics. The increase in AMR is being fuelled by the spread of carbapenem-resistant bacteria and is exhausting the physician's armamentarium in battling these infections. The lack of treatment modalities and slowly accumulating reports of pan-resistant bacteria forebode a possible end of the antibiotic era and underscore the importance of safeguarding current antibiotic options.

Not only KP but also *Enterococcus faecium*, *Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter spp.* contribute to this global issue. Jointly they form the acronym ESKAPE, first coined by Rice in 2008 (8). These pathogens “escape the

lethal action of antibiotics" (8) and are responsible for the greater part of nosocomial infections. These bacteria are also found on the top ten microorganisms list (TOTEM), a global pathogen priority list by the World Health Organization (WHO), which sets priorities for the development of new antibiotics (9). Carbapenem-resistant *Enterobacteriaceae* (CRE) (which includes carbapenem-resistant *Klebsiella pneumoniae*), carbapenem-resistant *Acinetobacter baumannii*, and carbapenem-resistant *Pseudomonas aeruginosa* form the highest ('critical') priority tier of this list (9).

3.1. History

The genus *Klebsiella* is named after the German physician and bacteriologist Edwin Klebs (10). KP was initially known as the causative agent of Friedländer disease, which is pneumonia mainly affecting alcoholics and is characterised by its severity, localisation in the upper lung lobes and expectoration of "currant jelly"-like sputum (11). The bulging fissure sign of upper lobe consolidation on lung radiographs was formerly attributed to KP infections (12).

Community-acquired KP infections include urinary tract infections (UTI) more frequently than cases of pneumonia, contrary to the classical view and its suggestive name of pneumoniae (13).

One remarkable trait of KP is the ability to acquire plasmids with resistance genes. As a result KP was one of the index species for plasmid-encoding ESBLs (extended-spectrum β-lactamases) in the 1980s (14).

The discovery of carbapenemases was closely followed by its spread and worldwide dissemination. Various carbapenemases are now responsible for endemic regions all around the world.

4. Epidemiology

Infections caused by CRKP include bloodstream infections, UTIs, pneumonia, and soft tissue infections, all of which can progress to bacteremia. The mortality rate for CRKP infections ranges around 41%, while oncological and transplanted patient groups have mortality rates that exceed 50% (15).

Klebsiella pneumoniae shows evolutionary and geographical differences with varying clinical features. Hypervirulent *Klebsiella pneumoniae* (hvKP) is a distinct entity which should be differentiated from 'classical' KP (cKP).

While CRKP represents a high-risk type of cKP and is mainly responsible for nosocomial spread, hvKP is causing invasive disseminating infections across communities, mainly in the Asian-Pacific region (16). The ability of hvKP to spread through blood and to cause invasive

infections is enabled by the production of bacterial capsular polysaccharides which form a thick capsule layer allowing complement evasion and the escape of neutrophil phagocytosis (17).
Despite the invasive nature of hvKP infections, including pyogenic liver abscesses, meningitis, osteomyelitis, and endophthalmitis, most infections have been susceptible to antibiotics (18). Compared to CRKP, which often causes infections in elderly patients with comorbidities, hvKP frequently affects young, healthy individuals (19). Characteristic virulence factors for hvKP are hypermucoviscous phenotype (positive "string test"), its association with K1/K2 capsular antigens and the expression of various siderophores (11,20). Isolates of hvKP that carry specific virulence plasmids can cause fatal outbreaks (21).
Convergence of high-risk cKP and hvKP can lead to a significantly worsened clinical outcome. Gene transfer via plasmids enables the spread of resistance genes and virulence factors. Two separate KP populations quite possibly coalesce into one, exacerbating this public threat (22). Convergent variants have already been reported globally, showing the necessity for advanced monitoring variants in high-risk regions, in order to take measures for the purpose of infection control and prevention (23).

4.1. Risk factors

CRKP infections usually are preceded by colonisation. However, colonisation with CRKP is, by definition, asymptomatic. The time window between colonisation and infection allows to recognise the potential pathogen which causes the infection. Vulnerable patient groups can be identified via screening in due time.
Patients with the following risk factors are at risk of CRKP colonisation and infection (24–27):

- Long duration of hospital stay
- Mechanical ventilation
- Urinary catheterisation
- Central vascular access
- Enteral feeding
- Blood transfusions
- Dialysis
- Prior antimicrobial use
- Underlying conditions that result in an increased exposure to invasive devices and procedures (e.g. renal failure, hepatic failure, hematologic cancer)
- Metastatic malignancy

- Heart disease
- Immunosuppression
- Complex thoracic pathology
- Complex intra-abdominal pathology

Considering these risk factors, a higher screening efficiency is being reached, resulting in more accurate data.

Cano et al found in a prospective study that patients colonised with KPC-positive KP in their rectal swabs at admission have a lower risk of infection and death in contrast to patients colonised after admission (28). They showed that the risk of infection was higher among those patients who got colonised after admission as compared with those colonised at the admission. This could mean that the gut microbiota plays an important role for the priming of the immune system and preventing infection. The authors revealed a time window of increased infection risk, during which the priming of the immune system could possibly occur. However, there is still missing information, on how colonisation influences the outcome of such infections.

4.2. Surveillance of antimicrobial resistance

There are multiple efforts by health agencies to monitor current developments in AMR. The European Centre for Disease Prevention and Control (ECDC) is collecting data via European Antimicrobial Resistance Surveillance Network (EARS-Net) (29). This network is collecting AMR data from all EU member states as well as from the EEA (European Economic Area) states Iceland and Norway regarding multi-resistant bacteria, including all ESKAPE organisms. The rest of the European countries are being monitored by the Central Asian and European Surveillance of Antimicrobial Resistance network (CAESAR), a joint project of the WHO and other public health institutions (30).

In the United States (US), the Centres for Disease Control and Prevention (CDC) are monitoring AMR via the CDC's Antibiotic Resistance Laboratory Networks (31).

Looking into these networks' collected data one is faced with restraints. Each network has its way of data collection with differences in participating laboratories, differences in cut-off values defined by EUCAST (European Committee on Antimicrobial Susceptibility Testing) and CLSI (Clinical and Laboratory Standards Institute), different isolate inclusion criteria and variances in the quality of laboratory equipment (29).

Nevertheless, data from the EARS-Net from 2016-2020 show both, an increase in total invasive KP infections (samples from blood and cerebrospinal fluid) and an increase in the fraction of carbapenem-resistant isolates (imipenem/meropenem resistance). We notice a rise in invasive

KP infections from 8.4% in 2016 to 10.0% in 2020 in the entire EU/EEA region (29). Resistance rates across European countries vary highly between 0.0% and 66.3%, with lower resistance rates in the north of Europe and higher rates in the south and east (29). Table 1 presents the European countries having reported resistance rates above 10% in 2020.

Table 1. Percentages of carbapenem-resistant K. pneumoniae isolates causing invasive infections in European countries with the highest incidence (29)

Country	Number of tested isolates	Carbapenem-resistant isolates N / (%)
Greece	726	481 / (66.3)
Romania	474	229 / (48.3)
Italy	8293	2447 / (29.5)
Bulgaria	249	70 / (28.1)
Cyprus	172	34 / (19.8)
Croatia	267	51 / (19.1)
Portugal	2780	322 / (11.6)

Preliminary data on AMR in the US provided by the CDC shows a decrease in CRE cases from 2017-2018 (32). This downward trend turned around in 2019 and continued to rise in 2020 when there was a 35% increase in hospital-onset cases of CRE compared to 2018 (32). CDC possibly implicates a connection between the change in antibiotic use during the COVID-19 pandemic and increased rates of AMR across the majority of monitored bacteria (32). The COVID-19 pandemic increased the workload on healthcare workers and augmented mental strain (33). Related to the lack of implementation of antibiotic stewardship programmes this brought about a misuse of antibiotics (34). Clinical discrimination between pneumonia caused by SARS-CoV-2 and bacterial community-acquired is complex. At the beginning of the COVID-19 pandemic only 8% of patients admitted with COVID-19 had a bacterial co-infection, while 72% received antibiotics (35). Additionally, resources needed for the tracking of AMR were shifted in favour of COVID-19 diagnosis and its tracking (36).

Increasing difficulty in the identification of all carbapenem-resistant bacteria due to intrinsic differences between various carbapenemases and other mechanisms is infringed by phenotypic testing methods. Newly emerging OXA-48 carbapenemases have a weak ability to hydrolyse carbapenems and unlike other carbapenemases they cannot hydrolyse cephalosporins making them especially hard to identify.

Clinical testing methods and epidemiological surveillance methods must be extended in order to closely follow the spread of various carbapenemases. Thereby molecular testing methods such as whole genome analyses should be utilised. Starting with the European survey of carbapenemase-producing *Enterobacteriaceae* (EuSCAPE) in 2014, the ECDC conducted a survey in 2019 on colistin- and/or carbapenem-resistant *Enterobacteriaceae* (CCRE survey). These surveys complement the EARS-Net's data with whole-genome sequencing within the European Antimicrobial Resistance Genes Surveillance Network (EURGen-Net). This network, being in an early phase will help to understand the distribution and spread of resistance genes. In the long run it will be possible to identify and prevent the spread of high-risk bacterial isolates. (37)

4.3. Spreading of resistant microbes

The varying prevalence of CRKP among European countries is attributed to several factors. Countries with the highest CRKP infection rates coincide with the highest AMR rates overall (29). This correlation may be due to the inconsistent implementation of antimicrobial stewardship and infection prevention and control (IPC) measures.

The spreading of CRKP is marked by hospital outbreaks (38–47). The dynamics of how these resistant bacteria spread, can be traced back by whole genome sequencing and differences in single-nucleotide polymorphisms (SNP). The number of SNPs gives information on the geographic and evolutionary progress of KP isolates. Data collected via the EuSCAPE showed that within-hospital spread is the most common way of CRKP spreading, followed by inter-hospital transmission within the same country (48). Genetic sequencing also enables epidemiological investigations of high-risk clones. For instance, the spread of the high-risk clone sequence type ST258 can be traced with these methods from Greece back to the US, while ST512 emerged in Israel, from where it spread to Italy (48). These spreading events are likely attributable to travel. ST258 spread worldwide and typically carries KPC-2 and KPC-3 (43,49). Advanced surveillance has clear benefits due to the possibility of understanding the origins of outbreaks. At the same time public access to genomic data enables researchers to achieve further insight into the evolution of high-risk clones and unveil possible targets for epidemiologic interventions.

The nosocomial spread remains the main driving force for the dissemination of CRKP.

4.4. Clinical importance

Various newly emerging carbapenemases which limit available treatment options in associated with diagnostic difficulties lead to high mortality rates making *Klebsiella pneumoniae* infections a clinical challenge. A multimodal approach is needed to recognize, treat, and prevent these infections successfully. Physicians are faced with poly-morbid patients as well. This makes it harder to combat these infections and prevent future ones.

OXA-48-like carbapenemases has spread unnoticed and has been recognised as a clinical threat recently. Low minimum inhibitory concentrations (MIC) of KP with OXA-48 expression may be classified as susceptible to carbapenems. In this case isolates are associated with a high treatment-failure rate (50). Molecular testing can help in recognising OXA-48-producing KP isolates. Being overshadowed by the spread of other carbapenemases connected with its deceptive susceptibility to broad-spectrum cephalosporins, OXA-48 was able to spread silently across the globe as a "phantom menace" (51).

Screening methods for at-risk patients can guide antibiotic therapy and enable cohorts of patients when preventive measures such as contact precautions can be applied to limit spread. Guidelines for IPC measures and their implementation with computer assistance and staff education can lead to a 16-fold reduction of CRKP cases, as demonstrated in an Israeli hospital (52). Screening criteria need to be predefined to make the detection of multidrug-resistant (MDR) bacteria easier. Staff education with easy-to-follow instructions can help increasing staff compliance and give rise to higher chances of success in combating AMR.

5. Carbapenem Resistance

It is a widely accepted view that the use of antibiotics in modern medicine is causing widespread AMR. While new studies show that the emergence of specific resistances might have predated the antibiotic era (53), thus challenging this paradigm, the overuse and misuse of antibiotics certainly accelerate the issue of AMR. Genomic investigations help to get a better understanding of the evolution and spread of AMR genes. Not only human factors but also animals contribute to AMR and their spread. The One Health approach is an interdisciplinary, multisectoral approach promoted among others by the WHO, ECDC and CDC (54–56). It promotes the integration and expansion of AMR monitoring in animals, humans, and the environment. Targeting and monitoring antimicrobial consumption in the agricultural sector and the health care sector, especially long-term care facilities (LTCFs), the One Health approach aims to reduce AMR and improve health outcomes in humans, animals and the

environment. In combination with incentivising the development of new treatment options and vaccines, the One Health approach aims toward international cooperation and regulation (55).

5.1. Definition of resistance

CLSI and EUCAST in the US and Europe respectively, provide clinical guidance for resistance and subsequent therapeutic success.

The outcome of an antimicrobial treatment regimen depends on three factors (57):

- MIC (the minimum concentration needed to inhibit bacterial growth)
- exposure (factor, dependent on the free unbound fraction of the drug, the dose and its pharmacokinetic properties – represented by the area under the curve: fAUC)
- efficacy (effect of a drug that depends on the fAUC/MIC ratio)

Assuming standard dosing regimens, these factors are combined with Monte Carlo simulations to derive MIC breakpoint values (57).

These breakpoint values classify microorganisms into susceptible, intermediate, and resistant groups (58). While susceptible microorganisms have a high chance of treatment success, resistant microorganisms have a high chance of treatment failure. The intermediate testing result has an uncertain therapeutic effect which may be modified by choosing an alternative dose regimen that has increased tissue concentration in the area of infection. Intermediate results also represent an area of uncertainty which may be caused by technical factors and should not lead to misinterpretation (58).

Characterisation of resistance is based on susceptibility testing. Definitions for bacteria resistant to multiple classes of antimicrobials are available. EUCAST, CLSI and the United States Food and Drug Administration (FDA) released guidance on standard definitions for acquired resistance (59). According to expert proposal bacteria are defined as multi-drug resistant (MDR), extensively drug-resistant (XDR) or pandrug-resistant (PDR), as shown in Table 2.

Table 2. Categories of bacterial resistance phenotype as proposed by Magiorakos et al. 2012 (59)
* Non-susceptibility includes resistant and intermediately susceptible isolates based on susceptibility testing.

MDR	Non-susceptible* to at least one agent in ≥ 3 antimicrobial categories, excluding intrinsic resistance
XDR	Non-susceptible* to at least one agent in all but ≤ 2 antimicrobial categories, excluding intrinsic resistance
PDR	Non-susceptible* to all antimicrobial agents, excluding intrinsic resistance

5.2. Mechanisms of resistance

The development of resistance is the evolutionary path of bacteria in order to survive. The mechanisms can be intrinsic, acquired or adaptive and represent an evolutionary advantage, even if some mechanisms come at a cost of fitness.

Resistance genes encode the resistance to antimicrobial drugs, and the entirety of these genes makes up the bacterial resistome. We know four principal ways of how resistance functions:

- Decreased uptake of the drug
- Inactivation of the drug
- Modification of the drug target
- Active efflux of the drug

These mechanisms are either intrinsic, acquired or adaptive. Intrinsic resistance mechanisms of various bacterial species differ in their respective structure or the expression of specific resistance genes (e.g., Mycoplasma does not have a cell wall and is resistant to all antibiotics that target the cell-wall synthesis) (60).

5.2.1. Decreased drug uptake

So-called porins enable the uptake of drugs in bacteria. Mutations and loss of porins can change the selectivity for substrates or altogether prevent their entry. Changes in the amount of expressed porins on the cell wall will also decrease the drug concentration within the organism, increasing resistance (61). Resistance of CRKP is also driven by the modification of the porins ompK35 and ompK36 (22). Besides carbapenem resistance, mutations of these porins are also relevant for decreased susceptibility of extended-spectrum cephalosporins and newer combination agents such as ceftazidime/avibactam (62,63).

5.2.2. Inactivation of the drug

Inactivation of antimicrobial agents can be achieved by degrading the drug altogether or by the enzymatic attachment of a chemical group to the drug, rendering it ineffective. This attachment of chemical groups is facilitated via transferases that mainly acetylate and phosphorylate antimicrobials (61).

The most widespread group of enzymes responsible for degrading drugs are β-lactamases. Carbapenemases that are mostly responsible for carbapenem resistance belong to the group of β-lactamases, which will be discussed in more detail below.

5.2.3. Modification of the drug target

Antimicrobial drugs can target several bacterial structures and pathways. Modifications to these targets may cause resistance.

β-Lactam antibiotics target penicillin-binding proteins (PBPs) which enable transglycosylation and transpeptidation, steps required for the biosynthesis of peptidoglycan, making up the principal structure of bacterial cell walls (64). Inhibition of cell wall synthesis eventually leads to bacterial cell death. Modifications in the number and structure of PBPs lead to ineffective drug binding.

Colistin, an antimicrobial agent used in CRKP infections binds to lipid A moieties of the outer membrane of gram-negative bacteria where it acts as a detergent, disrupting the cell wall integrity (65). Colistin resistance is mediated by mutations of mgrB and crrB genes leading to up-regulatory changes involving two-component systems PmrAB and PhoPQ eventually resulting in the addition of a cationic product (LAra4N) into lipid A decreasing the binding-ability of colistin (66,67).

5.2.4. Active efflux pumps

Bacteria can use efflux pumps to transport antibiotics back out of the cell, decreasing the intracellular concentration of the drug. Energy for these pumps to work is generated due to the help of ATP or the electrochemical potential across the bacterial cell membrane (68).

Multidrug efflux pumps *oqxAB* and *acrAB* in KP are associated with resistance to tigecycline, fosfomycin, carbapenems, fluoroquinolones, and nitrofurantoin (22). Regulatory genes involved in the overexpression of efflux pumps and subsequent resistance include *ramA, marA, soxS, rarA* (69).

5.3. Spread of resistance

Antibiotic use exerts constant selective pressure on bacteria. KP's proficiency in acquiring and spreading of its resistome plays a significant role in its evolutionary success. The spread of genes can be accomplished via vertical or horizontal pathways. Bacteria reproduce asexually via binary fission, where genetic material from the parent cell is split between the two daughter cells. The genetic transfer during this process is known as vertical gene transfer.

Horizontal gene transfer, on the other hand, occurs between two unrelated cells. Transformation (uptake of exogenous free DNA material), transduction (transfer mediated via bacteriophages), and conjugation (mediated by plasmids and integrative conjugative elements (ICE); requires cell-to-cell contact and specific transfer genes) are all forms of horizontal gene

transfer (70). A plasmid is a mobile genetic element (MGE) that allows the spread of various genetic materials, including virulence and antimicrobial resistance genes. Plasmids are circular double-stranded self-replicating molecules and are separate entities. They do not partake in any essential cellular pathway (70).

Several different plasmids have been identified as the carriers of resistance genes, including β-lactamases.

KP sequence type 258 (ST258) is one of the prototypes for the worldwide spread of CRKP. Chen et al. have shown that ST258 is a hybrid strain of ST11 and ST442 having acquired ICEKp258.2 and replaced its *cps* region associated with capsular polysaccharide biosynthesis (49). ICEKp258.2 is an MGE containing two genes encoding for a type IV pilus being able to acquire new genetic material via conjugation and increase the adherence to surfaces; and a type III restriction-modification system which increases the selection of external DNA acquisition (49). The switch in the *cps* region of ST258 shows how KP is able to change capsular polysaccharides modifying antigen presentation and deflect immune responses (49). Recombination events as in ST258 indicated an epidemiological success.

Other clonal groups show similar success, even though the drivers are not entirely understood. Clonal group CG147, mainly represented by ST147, was first reported in Hungary in 2008, where it caused six nosocomial outbreaks (71). Initially reported as ciprofloxacin-resistant, the CTX-M-15-producing KP strain spread worldwide and acquired carbapenem-resistance (72). This rapidly spreading clone presents with XDR and PDR isolates worldwide as it acquired myriad AMR genes. ST147 also shows capsular antigen switching, which appears to be a common denominator of successful clonal groups (72). The acquisition of a multitude of plasmids led to the evolutionary triumph of ST147, culminating in PDR.

Currently it is not known how many lineages of KP exist, how often they recombine, neither the source of its extensive AMR gene pool has been revealed yet (73).

5.4. β-lactamases

The expression of β-lactamases represents the most common resistance mechanism in gram-negative bacteria (74). They are enzymes that hydrolyse the β-lactam-ring, rendering them ineffective. β-lactamases are classified according to their functionality or their molecular structure. Once the first amino acid sequences became available, Richard Penry Ambler in 1980 proposed to classify β-lactams according to structural differences into class A and class B (75). The classification system according to Ambler was adopted and soon after extended with classes C and D (76). Functional classification of β-lactamases is grounded on Bush-

Jacoby scheme (77). Focusing mainly on carbapenemases we will use the molecular classification by Ambler. While the active sites of classes A, C, and D use serine, class B uses zinc ions. Class B β-lactamases are therefore defined as metallo-β-lactamases (MBL).

5.4.1. Class A

Class A encompasses serine β-lactamases including various penicillinases, cephalosporinases and carbapenemases. Important representatives of this group are TEM (named after 'Temoneira', a patient from Athens, Greece), SHV (sulfhydryl variant of TEM), CTX-M (cefotaximase from Munich) and KPC (78). TEM and SHV are narrow-spectrum β-lactamases that can hydrolyse older cephalosporins. Newer extended-spectrum oxyimino-cephalosporins (cefotaxime, ceftazidime) are used to treat bacterial infections expressing TEM and SHV. Since oxyamino-cephalosporins have a 'bulkier' molecular side chain, the β-lactam ring cannot be hydrolysed by the active site of these β-lactamases (79). However, mutations leading to steric changes which widen the active site, enable the hydrolysis of this bulkier oxyimino-cephalosporins like ceftazidime (79). These mutations allowed TEM to exhibit ESBL activity. CTX-M is a native ESBL that is even more efficient in hydrolysing extended-spectrum cephalosporins. While TEM ESBLs effectively hydrolyse ceftazidime, CTX-M mainly hydrolyses cefotaxime (79). Variants of β-lactamases having unique and extended features are assigned to new numbers (more than 200 variants of TEM are known) (79).

In 1996 the discovery of KPC-1in a North Carolina hospital marked a striking turning point in AMR history since KPC became the worldwide dominant carbapenemase (7).

The carbapenemase KPC significantly differs from other non-carbapenemase enzymes where the β-lactam ring of carbapenems does not have to deeply enter the catalytic cleft of the active site of the enzyme, allowing the β-lactam ring of carbapenems to be more easily degraded (80). Minimal changes in the amino acid sequences of these enzymes lead to allosteric changes that aid in substrate specificity, widening their spectrum of degradation. Research on the structure of various β-lactamases brought forward the development of β-lactamase inhibitors. This led to the availability of a new group of inhibitors, diazabicyclooctanones (DBOs) (78). The first approved DBO was avibactam. It is a non-β-lactam β-lactamase inhibitor that can reversibly acetylate serine β-lactamases, including KPC. These new agents restore the function of carbapenems and cephalosporins. However, resistance to these new drug combinations have been reported (78).

5.4.2. Class B

Class B metallo-β-lactamases harbour zinc ions in their active site. MBLs have a remarkable spectrum of activity degrading penicillins, cephalosporins, and carbapenems (78). They do not show activity against monobactams (81). Clinically the most relevant MBLs are VIM, NDM and IMP.

VIM (Verona Integron-encoded Metallo-β-Lactamase) was discovered in February 1997, isolated from a patient with a *Pseudomonas aeruginosa* infection at an ICU in Verona, Italy (82). In 2008 in a New Delhi hospital, in a patient with a UTI caused by *Klebsiella pneumoniae*, NDM (New Delhi Metallo-β-Lactamase) was detected (83).

The carbapenemase IMP-1 ("active for imipenem" (84)), was found in Japan in 1991 (85).

While novel β-lactamase inhibitors show promising inhibition of serine β-lactamases, they are not useful inhibitors for MBLs while emerging colistin and tigecycline resistances are further limiting therapeutic options (86). Differences between MBL subgroups further slow the development of such inhibitors. 'Taniborbactam' a cyclic boronate derivate acts as an MBL inhibitor and is currently in clinical phase 3 trials (87). Other in-vitro and animal studies with indole-2-carboxylates that imitate β-lactam binding show promising results in MDR and XDR gram-negative infection models and a wide spectrum of MBL inhibition (86).

5.4.3. Class C

Class C is a further structural group of serine β-lactamases. The most common representatives of this class are AmpC β-lactamases (named after the *ampC* gene). AmpC β-lactamases can be either plasmid or chromosomally mediated. It is assumed that plasmid-encoded AmpC enzymes are derived from chromosomal genes (88). AmpC β-lactamases present in *Klebsiella pneumoniae* are plasmid mediated (89). Class C β-lactamases do not show penicillinase or carbapenemase activity (76). They are the structurally largest serine β-lactamases, which mainly show activity against cephalosporins (76). Some of these β-lactamases show ESBL-activity too. When β-lactamases like AmpC without carbapenemase activity occur associated with other resistance mechanisms (e.g. permeability defects), they display lower susceptibility to carbapenems. In the absence of additional resistance mechanisms, the true carbapenemases (Class A, B, D) are the main drivers of nonsusceptibility to carbapenems (90). As class C β-lactamases do not show carbapenemase activity, the treatment of these infections is in line with carbapenemase-negative treatment options (see figure 1). Therefore, treatment options for

infections caused by CRKP isolates that express class C β-lactamases will not be mentioned in detail.

5.4.4. Class D

Class D β-lactamases also known as oxacillinases (OXAs) were originally described as having a higher affinity for hydrolysing oxacillin contrary to benzylpenicillin, hence the name. By 2019 over 750 different types of OXAs have been identified (74). In addition to the amino acid serine in the active site, OXAs exhibit a carbamylated lysine, achieving more efficient hydrolysis of its substrate (74). OXAs have great diversity, reaching from narrow- to broad-spectrum and carbapenem-hydrolysing abilities. However, OXA-48-like carbapenemases only hydrolyse carbapenems and cannot hydrolyse cephalosporins (91). The most important representative of carbapenem-hydrolysing class D β-lactamases is OXA-48. It was first described in a patient from Istanbul, Turkey, in September 2001 with a *Klebsiella pneumoniae* UTI (92).

The composite transposon Tn1999 with two copies of the insertion sequence IS1999 is the main driver of the international spread of the gene $bla_{\text{OXA-48}}$. IS1999 can increase the expression of OXA-48 increasing its in-vitro resistance. Tn1999 is most commonly associated with IncL/M plasmids. (74)

OXA-48 producers often show additional resistance mechanisms to overcome the shortcomings of not hydrolysing extended-spectrum cephalosporins. These include the production of ESBLs and porin modifications to achieve high levels of resistance (74).

The second most common derivative of OXA-48-like β-lactamases is OXA-181. It was first reported in India in 2006. (74)

In many instances clinical *K. pneumoniae* isolates harbour various concomitantly present resistance mechanisms, including simultaneous production of β-lactamases of different classes.

5.5. Tolerance, Persistence, and Biofilm

Resistance is the most familiar term describing the ability of bacteria to survive antimicrobial exposure. While resistance can be quantified with levels of MIC, mechanisms other than resistance as well enable bacteria to survive exposure to antibiotics, but unfortunately cannot be quantified that easily.

Tolerance is the ability of a bacterial population to transiently survive antimicrobial exposure what would otherwise be lethal. This is realised via slowing down the respective cellular

machinery, subsequently decreasing the impact of antibiotics which usually exploit the mechanisms needed for reproduction (β-lactams rely on bacterial cell wall production) (93). While the MIC of bacteria provide information about the fact at which level no more net growth of bacteria occur, tolerance can be quantitively described with the help of the medium duration to kill (MDK). This is to be measured in addition to MIC (93). Tolerant bacteria may survive longer compared with the length of an antibiotic course, so that treatment eventually fails.

Persistence is the heterogeneous ability of a bacterial subpopulation to survive antibiotic exposure. A bimodal time-kill curve of a bacterial population can be observed in the presence of the same antibiotic (94). So-called persisters belong to a subpopulation that normally accounts for less than 1% of the entire population (93). Failure of the host to clear remaining persisters can lead to treatment failure and regrowth of the bacteria. When an antibiotic is reintroduced, these regrown bacteria show the same bimodal time-kill curve (93). This heterogeneity shows how bacteria can benefit from different specialised phenotypes within the same population (95).
Besides, the survival benefits, tolerance and persistence also promote evolution. As mutations supporting both tolerance and resistance would be lost by the eradication of the bacterial population, the survival of these bacteria additionally means that newly acquired mutations are carried on (96). Recognition of tolerance and adequate therapeutic choice can thus prevent resistance.

Biofilm is a matrix comprised of polysaccharides, proteins and extracellular DNA enwrapping heterogeneous bacterial communities (97). The consistency of biofilms protects bacteria from host defence. It enables its attachment to surfaces on which it is nearly impossible to remove them and which may serve as a bacterial reservoir (61). Classical definitions of resistance and tolerance cannot be applied to biofilms, although classical resistance mechanisms also contribute to biofilm resistance (97). The proximity of cells within biofilms is likely to facilitate horizontal gene transfer (61). The complexity of biofilms is reflected by hard-to-treat infections that often afflict medical implants like prostheses, catheters, and heart valves (97).

The exact mechanisms responsible for the above-mentioned phenomena are beyond the scope of this graduate thesis. However, they add another layer of complexity to the issue of AMR. Physicians need to be aware of these circumstances, because they may account for a complex treatment and perhaps lead to treatment failures.

6. Drugs

In this section, we will focus on a selection of the drugs which are currently approved and used in the treatment of CRKP in Europe. The following list of drugs is not exhaustive but represents a basic framework for comprehending the treatment of this sort of infections.

6.1. β-lactams

β-lactams are bactericidal antibiotics working according to a similar mechanism of action. They bind to PBPs, a bacterial protein needed for transpeptidation in the last step of building the peptidoglycan cell wall of bacteria. They activate autolytic enzymes leading to lesions in the cell wall. They have time-dependent killing. This means that their effectiveness depends on the time the free (unbound) drug concentration exceeds the MIC level. Structural alterations while retaining the active β-lactam ring introduced new classes of β-lactam antibiotics such as cephalosporins and carbapenems. Classes are distinguished by their different spectrum of activity and resistance to β-lactamases. Adding β-lactamase inhibitors can help retain antimicrobial function in bacteria having developed resistance. Carbapenems have the broadest spectrum of activity in this group of antibiotics. (98)

6.1.1. Carbapenems

Imipenem is the first known carbapenem and acts like the other β-lactam antibiotics in binding to PBPs and thus inhibiting cell wall synthesis. Meropenem and ertapenem are newer carbapenems. They have a wide spectrum of activity against gram-positive and gram-negative bacteria and a good resistance against β-lactamases, such as ESBLs. However, they can be degraded by carbapenemases. Different molecular structures between carbapenems account for slightly different spectra of activity and resistance to degradation. Ertapenem shows less resistance against degradation by β-lactamases compared with imipenem and meropenem. (99–102)

All three mentioned carbapenems have low oral bioavailability and have to be administered intravenously (IV). Ertapenem can also be given intramuscularly (IM). Meropenem, Imipenem, and Ertapenem show protein binding of 2%, 20%, and 95% respectively. Imipenem is degraded by the enzyme dehydropeptidase-1 (DHP-1) being expressed in the kidney. DHP-1 is inhibited via the reversible competitive inhibitor cilastatin and is added in the clinical application of imipenem. Meropenem and ertapenem are not susceptible to degradation by means of DHP-1. (100–102)

Carbapenems are principally eliminated by the kidneys, mainly in unchanged forms. Seventy percent of the excreted imipenem (when combined with cilastatin) and meropenem are unchanged. Eighty percent of ertapenem is excreted via urine, of which 38% is unchanged, while 37% is in the form of inactive metabolites. Due to the elimination pathway, in patients with renal function impairment, the dose of carbapenems must be adjusted. Carbapenems are removed through haemodialysis. Ertapenem has the longest elimination half-life of 4 hours. Imipenem and meropenem have shorter elimination half-lives of around 1 hour. Carbapenems in general show very good distribution into various tissues. However, only meropenem reaches clinically relevant concentration in the cerebrospinal fluid (CSF). (100–102)

Carbapenems have well-known safety profiles. The most notable adverse reactions shared among carbapenems are:

- associated with the gastrointestinal tract: nausea, vomiting, diarrhoea, abdominal pain
- reactions to local administration: phlebitis/thrombophlebitis, pain at the injection site, erythema
- skin rash, pruritus, urticaria
- headache, seizures, hepatic enzymes elevation, hepatic failure. (100–102)

Special caution must be taken in patients with brain lesions, neurological disease, and a history of seizures. Carbapenems can lower the seizure threshold, especially when used in higher dosages or in patients with renal impairment. Imipenem has the highest seizure potential. When co-administered, imipenem lowers serum concentrations of valproic acid, increasing seizure risk in these patients. (100–102)

6.1.2. Monobactams

Unlike other β-lactams, monobactams only have a monocyclic β-lactam ring (103).

Aztreonam was first approved in 1986 by the FDA and is currently the only available monobactam approved by the EMA in Europe. Like other β-lactams it binds to PBPs, inhibiting cell wall synthesis. Aztreonam has a high affinity for PBP3 in gram-negative rods and does not show any affinity for gram-positive or anaerobic PBPs. Aztreonam can be hydrolysed by β-lactamases other than MBLs. (87)

Due to poor gastrointestinal absorption aztreonam should be administered via IV or IM routes. Nebulized application is also approved. Around 77% of aztreonam is bound to proteins and has wide tissue distribution including the CSF. Hepatic metabolism plays a minor role. Elimination is mainly by the kidney, with 60-70% of the drug excreted unchanged. Elimination half-life after parenteral administration of the drug is 1.7 hours. When nebulised, elimination half-life

is prolongated to 2.1 hours. In patients with reduced kidney function the dose should be adjusted. Aztreonam is cleared via haemodialysis. (104)
Adverse effects of aztreonam include:

- increased hepatic enzymes (more in paediatric than adult population)
- gastrointestinal symptoms (vomiting, nausea, diarrhoea, abdominal pain)
- skin reactions (urticaria, pruritus, erythema)
- local injection reaction (phlebitis/thrombophlebitis, discomfort and swelling
- fever. (104)

6.2. Novel β-lactams and β-lactamase inhibitors

The discovery of the first β-lactamase inhibitor (BLI) clavulanic acid was in 1972, sulbactam and tazobactam followed (105). This first generation BLIs have a structure similar to β-lactams and act as suicide inhibitors. They get degraded by the β-lactamases but remain bound to the active site, rendering them ineffective (106).
Avibactam is the first approved drug of the second generation BLIs by the EMA. Its approval in 2016 broke a 30-year stagnation in the development of new BLIs (105,107). BLIs can restore antimicrobial activity of drugs against which bacteria developed resistance. This new generation of drugs does not have structure similar to β-lactams. Thus, the second generation BLIs are also called non-β-lactam β-lactamase inhibitors. They mostly function as reversible inhibitors. Currently available novel BLI belong to the group of diazobicyclooctanes and boronic acid derivates. So far, there are no available BLI inhibiting MBLs. There are, however, clinical trials underway for agents that also target class B β-lactamases.
Another way of combating carbapenem-resistant microbes is the development of new drugs being refractory to the hydrolysing activity of carbapenemases. In 2020 for example the drug cefiderocol was approved (106,108).

6.2.1. Ceftazidime/Avibactam

As a third-generation cephalosporin, ceftazidime inhibits cell wall synthesis through the binding to PBPs. Avibactam is a novel BLI with the potential of inhibiting a wide spectrum of β-lactamases including class A, C and D carbapenemases, making it the only currently available BLI that inhibits OXA-48. (87)
Ceftazidime/avibactam is approved for the treatment of complicated intra-abdominal infections, complicated UTIs, hospital-acquired and ventilator-associated pneumonias as well as bacteremia associated with these infections (107).

This combination preparation is intended for IV use. Ceftazidime shows protein binding of around 10% and is distributed into most tissues including the CSF. Five to eight percent of avibactam is protein bound. Its volume of distribution is 22 L. Neither ceftazidime nor avibactam are metabolised in the liver. Elimination is achieved via the kidney where up to 90% of ceftazidime and 85% of avibactam are excreted unchanged. Both drugs are substrates for renal organic anion transporters (OAT) 1 and 3. Elimination depends on renal function. Dose adjustment is needed in impaired renal function. Both drugs are removed via haemodialysis. They have similar half-lives of around 2 hours, depending on renal function. (109)

Common adverse reactions include gastrointestinal upset that can manifest as nausea, diarrhoe, vomiting, and abdominal pain. Other common side effects include fever, headache, chest pain, elevated liver enzymes, cough, insomnia and local reactions at the infusion site. (109)

Special care has to be taken in patients with renal impairment, as higher concentrations of ceftazidime/avibactam may lead to neurotoxicity, encephalopathy, and present with seizures and myoclonic episodes. (109)

As cephalosporins can rarely cause hypothrombinemia, special care is advised for patients with coagulopathies and vitamin K deficiencies, due to elevation of bleeding risk. (109)

6.2.2. Meropenem/Vaborbactam

Vaborbactam is a non-suicidal BLI and is active against class A carbapenemases, such as KPC (87). In combination with meropenem, it is approved for the treatment of complicated UTIs, complicated intra-abdominal infections, hospital-acquired and ventilator-associated pneumonia as well as bacteremia associated with these infections. (110)

Meropenem and vaborbactam are administered intravenously. Vaborbactam shows protein binding of 33% and has a volume of distribution of 18.6 litres. It is not metabolised by the liver. Vaborbactam is eliminated by the kidney. Seventy five to ninety five percent of vaborbactam is excreted in its unchanged form. The dose of meropenem/vaborbactam must be adjusted in patients with decreased renal function. Haemodialysis more effectively clears meropenem in comparison with vaborbactam. Owing to this the exposure to vaborbactam is higher in patients with end-stage renal disease requiring dialysis. The adverse reaction profile does not change with the addition of vaborbactam. (111)

6.2.3. Imipenem/Cilastatin/Relebactam

Relebactam is a non-suicidal BLI. It may restore the activity of imipenem caused by serine carbapenemases. Relebactam shows effectiveness against class A β-lactamases including KPC, TEM, SHV and CTX-M. (87)

The drug combination of imipenem/cilastatin/relebactam is approved for the treatment of hospital-acquired and ventilator-associated pneumonia, bacteremia as a cause of pneumonia and for the treatment of other aerobic gram-negative infections, when no other treatment option is available. (112)

Imipenem/cilastatin/relebactam is administered intravenously. Relebactam binds 22% to proteins and has a volume of distribution of 19 litres. Metabolism of relebactam is minimal. Ninety percent of the drug is excreted unchanged in urine. The half-life of relebactam is 1.2 hours. Relebactam is a substrate of OAT3 and 4 and multi-antimicrobial extrusion protein (MATE) 1 and 2K. (113)

Dose adjustment of imipenem/cilastatin/relebactam is necessary for patients with impaired renal function. All three components of this preparation are cleared by haemodialysis.

Common adverse reactions overlap with imipenem/cilastatin with the addition of hypertension. Caveats of imipenem/cilastatin are also valid for this combination preparation with relebactam.

6.2.4. Cefiderocol

Cefiderocol is a new drug approved by EMA in 2020 for treating complicated UTIs and hospital-acquired or ventilator-associated pneumonia when other treatment options might fail (108).

It is a β-lactam antibiotic but has a unique mechanism for entering the periplasmic space of bacteria. Cefiderocol consists of a siderophore and cephalosporin core. The siderophore binds iron which is then transported to the periplasmic space via bacterial ferric ion transporters. When reaching the periplasmic space, the iron dissociates, and cefiderocol binds to PBP3, inhibiting cell wall synthesis. Due to its unique mechanism of cell entry it is called a ‘Trojan horse’. We find that reflected in the trade name ‘Fetroja’ in the US and ‘Fetcroja’ in Europe (‘Fe’ is the symbol for iron in the periodic table and ‘Troja’ is referring to the Trojan horse) (87).

Administration of cefiderocol is intravenous. The protein binding ranges between 40-60% with a volume of distribution of 18 litres. Metabolism of cefiderocol is minimal. The drug is eliminated mostly unchanged (90%) via the kidney in the urine. In patients with impaired renal

function, the dose of cefiderocol must be adjusted. Cefiderocol can be cleared with haemodialysis. (114)

Common adverse reactions have been reported and include diarrhoea, vomiting, constipation, skin rash, candidiasis, cough, elevated liver enzymes and local reactions at the infusion site as well. (114)

The phase 3 trial CREDIBLE-CR showed an increased all-cause mortality with cefiderocol compared with best available therapy. The difference in all-cause mortality was highest in bloodstream infections and nosocomial pneumonia where *Acinetobacter spp.* were the causative pathogen. (115)

6.3. Aminoglycosides

6.3.1. Amikacin

Amikacin belongs to the group of aminoglycoside antibiotics. It inhibits the 30S ribosomal subunit and impairs mRNA binding to the ribosome. This results in abnormal proteins losing their function and eventually leading to cellular dysfunction. Aminoglycosides are bactericidal drugs with a concentration dependent effect. Their efficacy relates to the ratio of peak serum concentration to MIC. Due to its narrow therapeutic index and risk of nephrotoxicity, neurotoxicity and ototoxicity monitoring through serum concentrations is recommended. Synergistic effects can be achieved when combined with β-lactams. Aminoglycosides exhibit an postantibiotic effect (PAE) of around 2-7h in aerobic gram-negative bacteria. (116,117)

Due to their very poor oral bioavailability aminoglycosides must be administered parenterally. Amikacin can be administered IV, IM as well as applied in nebulised form for inhalation. When administered IV it should be infused within 30-60 minutes to achieve adequate tissue concentrations. It distributes to the extracellular fluid with a volume of distribution of around 0.25 L/kg. As the volume of distribution roughly represents the extracellular space, patients with more extracellular fluid may have lower peak plasma concentrations. Binding to proteins is around 0-11%. (116,117)

Amikacin is mainly eliminated via glomerular filtration. Plasma elimination half-life is around 2 hours and depends on renal function. Reabsorption of amikacin in the proximal tubule may be the basis of its nephrotoxicity. Close monitoring of renal function and serum concentrations are recommended. In patients with renal impairment the time interval between amikacin administration and/or the amikacin dose should be adjusted. Dose adjustments may also be necessary according to the type of infection, renal function, and serum concentrations of the

drug. In overweight patients, dosing should be based on a modified body weight. Amikacin is removed via haemodialysis. (116,117)

Adverse reactions include:

- Nephrotoxicity: especially with prolonged amikacin use (more than seven to ten days) or with concomitantly administered nephrotoxic drugs, renal tubular necrosis or fibrosis as well as renal tubular acidosis are possible. Due to the drug accumulation in tubular cells, nephrotoxicity can ensue even after treatment has been discontinued (especially if the amikacin administration has been prolonged). The nephrotoxic effects are generally reversible after the amikacin is discontinued. Increases in creatinine, as well as signs of renal irritation such as white and red blood cells in urine, proteinuria or hyposthenuria are possible too. The nephrotoxic effects can be potentiated through concomitant application of drugs such as colistin, β-lactams or other nephrotoxic agents. (116–118)
- Ototoxicity: risk for this side effect is increased with higher exposure to the drug, as well as in patients with renal impairment (due to decreased clearance). Initial high frequency hearing loss can progress to complete deafness. Ototoxicity is commonly irreversible and may occur even after treatment having been discontinued. The risk for ototoxicity is increased when co-administered with loop-diuretics or other ototoxic drugs. (116,117)
- Neurotoxicity: Convulsions, encephalopathy, tetany, peripheral neuropathy and several other neurological symptoms have been reported. Neuromuscular blockade leading to paralysis and respiratory insufficiency requiring mechanical ventilation especially in patients receiving anaesthetics or massive blood transfusions (citrate-anticoagulated) have been described. Patients with neuromuscular disease may present with aggravated symptoms when treated with amikacin. (116,117)

Patients need to be closely monitored by means of amikacin serum concentrations, blood urea nitrogen and creatinine, audiometry. (116,117)

6.4. Fosfomycin

Fosfomycin was first available in Europe in 1988. It mimics the structure of phosphoenolpyruvate and irreversibly inhibits uridine-diphosphate-N-acetylglucosamine enolpyruvyl transferase (MurA) which is needed in one of the first steps of peptidoglycan synthesis, the main component of the bacterial cell wall. This inhibition causes the accumulation of the peptidoglycan precursors and eventually leads to cell lysis. (119,120)

There are two forms of fosfomycin in clinical usage: oral and parenteral. The oral form is approved as a single-dose treatment of cystitis. Fosfomycin tromethamine is administered orally with a bioavailability of about 33-53%, in fasted state. It has a mean half-life of around 4 hours. Fosfomycin disodium is administered intravenously and has a serum half-life of 2 hours, provided normal renal function. The drug does not bind to plasma proteins and has good tissue distribution. Fosfomycin is not metabolised and is excreted in both urine and feces. (119,120)

Adverse reactions mainly include gastrointestinal complaints such as nausea, abdominal pain, dyspepsia, abnormal stool, flatulence, and vomiting. Other side effects concern hypokalemia, headache, dizziness, rhinitis, pharyngitis, back pain and myalgias. (119,120)

Although fosfomycin has a safe and established side effect profile, the intravenous preparation is rich in sodium, which can lead to salt-overload, especially in patients receiving higher dosages. A 24g dose of the IV fosfomycin preparation contains 8g of sodium. In vulnerable groups of patients such as those with chronic heart failure, chronic kidney disease, and liver cirrhosis, this can lead to fluid overload. (119,120)

Fosfomycin has gained a lot of interest in recent years mainly due to its good in vitro activity against many MDR and XDR gram-negative pathogens in spite of decades of clinical use. Moreover, it shows synergistic effects with many other antibiotics, including carbapenems, which is useful in treating infections caused by resistant microbes. There is some remaining ambiguity about the use of fosfomycin in these complex infections, as there is a lack of relevant clinical data regarding the treatment of severe infections caused by MDR and XDR gram-negative pathogens, as well as a discrepancy between the MIC cut-off values between CLSI and EUCAST. Recommended dosages range (of parenteral form) between 8-12g/day for gram-positive infections and 16-24g/day when used in gram-negative infections. Clinical data for prolonged intravenous fosfomycin application for the treatment of infections caused by MDR and XDR bacteria is needed. (119,120)

6.5. Tigecycline

Tigecycline is a glycycline antibiotic, a derivative of tetracycline. As tigecycline is not affected by resistance mechanisms to tetracyclines, it often retains in vitro activity when resistance to other tetracyclines has been demonstrated. It is approved by the EMA for complicated soft tissue infections (excluding diabetic foot infections) and complicated intra-abdominal infections, in case that other treatment options are not applicable. (121,122)

Tigecycline is bacteriostatic and acts by binding to the A site of the 30S subunit of ribosomes, inhibiting tRNA binding and polypeptide elongation (123).

Tigecycline is only administered intravenously and shows in vitro protein binding of around 80%. The volume of distribution reaches between 500-700 L, indicating extensive tissue distribution. Metabolism of tigecycline does not exceed 20%. Two thirds of tigecycline are eliminated on the biliary/faecal route and one third is excreted in urine. Elimination half-life after several doses is around 42 hours with high variations between patients. In moderate and severe liver disease (Child Pugh B and C) clearance is reduced by 25% and 55% respectively. Half-life of tigecycline is increased in Child Pugh B and C by 23% and 43% respectively. Renal impairment does not significantly change pharmacokinetics of the drug. It is not removed by haemodialysis. (123)

When compared to other antibiotics, tigecycline is associated with an increased all-cause mortality, the causes of which are not known. Acute pancreatitis, liver injury with a cholestatic pattern and superinfections are possible adverse reactions, which occur during tigecycline treatment. Provided that a nosocomial pneumonia presents as a superinfection, tigecycline therapy should be re-evaluated as a new infection focus (other than an infection of approved indications) supports the initiation of an alternative antimicrobial therapy. (123,124)

6.6. Polymyxins

Polymyxins available since the 1960s are an older group of antibiotics. They include polymyxin B and colistin (also known as polymyxin E). In Europe only colistin is approved for clinical use. Availability of antibiotics with better side effect profiles decreased the use of polymyxins until the emergence of multi-drug-resistance led to a return of polymyxins. Concerns have been raised that the product information needs an updating, especially including more explicit dosing-recommendations, since polymyxins first got marketed before rigorous drug approval policies were established. Polymyxins should only be used in patients with susceptible infections and in combination with other antibiotics, when other treatment options are limited. (125)

6.6.1. Colistin

The target of colistin is the bacterial cell wall. Colistin is a cationic polypeptide that interferes with the anionic lipopolysaccharide (LPS) molecules on the outer membrane of aerobic gram-negative bacteria. This leads to increased permeability, the loss of cellular contents and cell

death. Other mechanisms of action such as targeting intracellular components, such as ribosomes, have been described too. (126)

Pharmacodynamic and pharmacokinetic parameters correlate best with a time-dependent killing. Colistin is administered as colistin methanesulfonate (CMS) and is the inactive prodrug. Conversion rates are dependent on various factors having not been completely characterised. Unclarity about the dosing partially stems from differences in dosing conventions. Colistin dosages can be expressed as CMS in international units (IU), milligrams of CMS, or colistin base activity (CBA). The EMA recommends to express dosages in IU of CMS. Conversion tables should be available in every product information to prevent possible medication errors. (125,126)

Administration of colistin may be intravenous, intrathecaly or by inhalation (as nebulised solution). Colistin concentrations in pulmonary epithelial lining fluid are much higher after aerosolised CMS administration compared to IV administration. Due to a slow conversion of CMS to colistin, peak plasma concentration is reached with a significant delay. Uncertainty about factors influencing conversion rates influence the volume of distribution as well. In healthy people the volume of distribution is close to the extracellular volume. Conversion rates vary in critically ill patients as well as in patients with renal impairment leading to very heterogenous plasma concentrations among various patient groups. Moderate protein binding is dose dependent. Higher concentrations lead to a lower protein-bound fraction. (126)

The elimination of CMS is dependent on creatinine clearance. A decrease in renal function is increasing the concentration of CMS and subsequently increases the conversion rate of CMS to colistin. In healthy subjects the conversion rate is around 30%, whereas in patients with impaired renal function it can increase to 60-70%. While CMS is excreted in urine, colistin is reabsorbed in the kidney and undergoes non-renal elimination. Colistin is accumulated or metabolised in the kidney and presumably not in the liver. The exact means of its elimination remain unspecified. As colistin is nephrotoxic, renal accumulation is concerning. Its half-life increases in critically ill patients to 14 hours compared to 3-4 hours in healthy individuals. Creatinine clearance is a major predictor for both CMS and colistin concentrations in serum. Both are removed by haemodialysis. (126)

The main adverse reaction is nephrotoxicity, reported in up to 45% of patients. Therapy duration shorter than 14 days as well as lower serum concentrations can mitigate kidney injury. While a loading dose of colistin should be administered, international consensus guidelines for the optimal use of polymyxins recommend that target steady-state plasma concentrations for maintenance should average around 2 mg/L. Thereby the incidence and severity of acute

kidney injury should be reduced. However, at this plasma concentration pulmonary tissue distribution is inadequate. (126,127)

Neurotoxicity is reported as usually mild and resolves after discontinuation of colistin albeit less common. Due to the nature of complex infections in critically ill patients adverse reactions to colistin may be underreported. (126)

Clinicians need to be aware of the risks and benefits, when choosing a polymyxin based treatment regimen.

7. Treatment of Infections

The treatment of infections caused by CRKP are to be based on the clinical features of infection, and antimicrobial susceptibility testing. Guidance released by the Infectious Diseases Society of America (IDSA) in 2022 provide helpful principles for the treatment of CRE-infections (122). Part of the difficulty in treating these infections is the lack of clinical data physicians can rely upon. Especially for XDR and PDR infections very limited data regarding therapeutic options are available, so that decision-making is complicating. Mechanisms of resistance vary significantly between different countries and regions. The prevalence of carbapenemases shows heterogenous distribution. While MBLs are more common in Asia, OXA-48-like carbapenemases are predominately found in the Mediterranean Basin. On the other hand KPCs can be found worldwide. Carbapenemase-negative isolates which often demonstrate porin mutations and overexpression of efflux pumps in connection with other β-lactamases have heterogenous distribution as well. (128)

7.1. Treatment rationale

Initial testing of a CRKP isolate should be directed towards the identification of a carbapenemase. The presence of a carbapenemases should point the clinician towards the treatment including novel β-lactamase inhibitors. Tamma et al showed that patients with carbapenemase-producing CRE infections have four time higher odds for deadly outcomes compared with non-carbapenemase-producing CRE highlighting the need for early recognition of these resistance-mechanisms and timely appropriate intervention (129).

Molecular testing methods are recommended by the IDSA guidance as carbapenemase-producers can still show meropenem susceptibility, depending on the testing method. When carbapenemase-producers are treated only with meropenem (in case the isolate shows susceptibility), treatment failure rates are increased. (122,130)

In case that carbapenemase-producers are recognised, classification of the type of carbapenemase further directs antimicrobial therapy. Currently available novel BLIs show limited activity against class B and class D β-lactamases restricting treatment options for these infections (see table 3).

Table 3. Effectivity of selected antimicrobials against microbiological targets. Modified according to Principe et al. 2022 (87) and Giamarellou et al. 2022 (131).

'+' indicates activity against this class of β-lactamase while

'-' indicates no activity.

* Currently this drug combination can only be administered in form of ceftazidime/avibactam & aztreonam.

Drug	Class A (ESBL, KPC)	Class B (NDM, VIM, IMP)	Class C (AmpC)	Class D (OXA-48)
Ceftazidime/avibactam	+	-	+	+
Imipenem/cilastatin/relebactam	+	-	+	-
Meropenem/vaborbactam	+	-	+	-
Cefiderocol	+	+	+	+
Aztreonam	-	+	-	-
Aztreonam/avibactam*	+	+	+	+

Comparing early appropriate treatment with delayed appropriate treatment there is a significant increase in mortality, when appropriate treatment is given >24 hours after culture collection, especially in cases of bloodstream infections (132). Bloodstream infections remain a clinical challenge independent of the causative microbe. Kumar et al demonstrated that effective antimicrobial therapy within the first hour optimises outcomes in septic shock while mortality increases by every additional hour of delayed treatment (133).

CRKP infections represent a special clinical challenge due to high mortality rates. For MDR, XDR and PDR infections to be treated effectively, special drug selection based on time-consuming molecular testing methods are necessary. Additionally, the use of broad-spectrum antibiotics should be limited in order to decrease the further spread of antimicrobial resistance and improve patient safety.

Identification of risk factors can be useful for the support of screening strategies and the recognition of at-risk patients as well. Screening for CRKP (and other high-risk CRE) aids in early recognition and characterisation of the pathogen which may cause subsequent infection. Early identification of the pathogen enables early effective therapy.

Travel history (especially when including hospital-visits) of patients to endemic regions as well as previous infections/antibiotic use should play an important role in the clinical decision-making process (122).

Giannella et al conducted a study focusing on infection following CRKP colonisation. They showed that colonisation has a 7.8% risk for bloodstream infections and that multiple factors predict bloodstream infections (especially multisite colonization) (134). They utilized these factors develop a risk score. The Giannella risk score (GRS, see table 4) was validated in a prospective observational cohort study of 94 patients by Cano et al and showed useful in stratifying patients into low-risk (GRS < 7) and high-risk (GRS ≥ 7) groups regarding the progression of colonisation to infection (this study included only KPC-type carbapenemases) (135).

Table 4. Giannella risk score (GRS). Adapted from Giannella et al. 2014 (134)

Risk factor	Score
ICU admission	2
Abdominal surgical procedures	3
Chemotherapy/radiotherapy	4
Colonisation site (excluding stool)	5 (per individual site)

7.2. Choosing a treatment regimen

Current guidance by the IDSA recommend that carbapenemase testing should be performed by every microbiological laboratory to improve treatment decisions. In case that carbapenemase production is confirmed, the use of carbapenems without addition of a novel BLI is not recommended. (122)

7.2.1. Treatment of infections caused by KPC-producing isolates

CRKP infections with class A carbapenemase (KPC) producers should be treated with ceftazidime/avibactam, meropenem/vaborbactam or imipenem/cilastatin/relebactam. Since there are limited studies comparing these three drugs, there is no support for using one drug over another. However, the emergence of resistance seems to be higher with ceftazidime/avibactam, which favours meropenem/vaborbactam over ceftazidime/avibactam and imipenem/cilastatin/relebactam for KPC-producing CRKP infections. (122)

In addition a dual carbapenem therapy (DCT) including ertapenem in combination with meropenem was described for treating KPC-producing CRKP (136). The rationale behind this

combination is the fact that ertapenem with its high affinity for carbapenemases acts as a suicide inhibitor of carbapenemases, inhibiting hydrolysis of other carbapenems (128,136). DCT regimens are not mentioned in current recommendations but may be useful in cases when a cost-effective option is needed, or availability of novel drugs is limited (128).

7.2.2. Treatment of infections caused by MBL-producing isolates

For infections caused by CRKP producing class B carbapenemases (NDM, VIM, IMP) the preferred treatment option consists of ceftazidime/avibactam in combination with the monobactam aztreonam. MBLs cannot degrade monobactams, while avibactam inhibits other β-lactamases which may degrade aztreonam. The combination of aztreonam and avibactam has shown to be effective against MBLs (137). As avibactam is currently available only in combination with ceftazidime, the only way to administer aztreonam and avibactam is in combination with ceftazidime. A new preparation containing solely aztreonam/avibactam is currently in phase 3 trials (87).

Another option for the treatment of MBLs is cefiderocol. The IDSA expert panel recommends that cefiderocol should be reserved for infections caused by MBL-producing bacteria (122). Cefiderocol has an important role in the treatment of carbapenem-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and other glucose nonfermenting bacteria, such as *Stenotrophomonas maltophilia* (138). Currently there is no available data on clinical outcomes comparing the combination of aztreonam and ceftazidime/avibactam with cefiderocol.

7.2.3. Treatment of infections caused by OXA-48-producing isolates

CRKP isolates expressing OXA-48 like carbapenemases should be primarily treated with ceftazidime/avibactam in case of in vitro susceptibility. Carbapenems without the addition of novel BLIs, irrespective of susceptibility testing should not be used for OXA-48 producers (50). Meropenem/vaborbactam and imipenem/cilastatin/relebactam are not active against OXA-48 like carbapenemases and should not be used either (122).

Alternatively, cefiderocol may be used (122). While clinical data is still lacking, case reports of compassionate use of cefiderocol showed successful results (139). The increased all-cause mortality of cefiderocol and the lack of clinical data should be considered when choosing the respective drug.

The optimal treatment of OXA-48-producing isolates remains unclear. In case the isolate is non-susceptibile to ceftazidime/avibactam and cefiderocol, combination therapy regimens

which show better outcomes than monotherapy should be used. Combination therapy is to be guided by antimicrobial susceptibility testing. (50)

7.2.4. Treatment of CRKP-infections expressing multiple resistance mechanisms

Even though the above-mentioned therapeutic options are used in settings of serious hard-to-treat infection they represent an ideal case scenario. The recent emergence of the β-lactamase VEB-25, associated with the resistance of ceftazidime/avibactam, represents another major setback (128). Concurrent resistances to last line drugs are associated with high mortality rates and mostly affect critically ill patients. There is a lack of clinical evidence and guidance for the treatment of these infections. An individualised approach according to in vitro antimicrobial susceptibility testing accompanied by pharmacokinetic considerations has to be applied. Combination therapy exploiting synergism between antibiotics is appropriate. (140)

7.2.5. Monotherapy and combination therapy

The availability of novel β-lactam/β-lactamase-inhibitors caused a paradigm shift in the treatment of CRKP isolates. When susceptibility is demonstrated, according to IDSA guidance these novel drugs should be used in monotherapy. They show the same efficacy with fewer adverse reactions and decreased mortality rates when compared to combination therapy based on polymyxins or aminoglycosides. (122,141–149)

Before the introduction of these novel drugs, polymyxin based combination therapies were the mainstay for treating CRKP. Colistin was most commonly used together with amikacin, tigecycline, or carbapenems (150).

Currently, combination therapy is eligible for salvage therapy for XDR and PDR infections when isolates do not show susceptibility for novel β-lactam/β-lactamase-inhibitors (131). A retrospective study of 115 episodes of PDR *Klebsiella pneumoniae* infections showed that the administration of at least three antimicrobials shows better patient outcomes in contrast to using only one or two agents (151). The same study does not report any significant superiority to any of the investigated combination therapies. Colistin is widely used as an adjunct due to its synergistic activity, even if colistin-resistance is demonstrated (152).

Dual carbapenem therapy represents another form of combination therapy. A systematic review and meta-analysis reported a lower mortality rate with DCT when compared with other regimens (153). However, this study did not include comparisons with novel β-lactam/β-lactamase inhibitors and the majority of reported carbapenemases were KPC. Ertapenem is

considered to have a high sensitivity for KPC carbapenemases which supports the use of DCT for KPC-producers (153,154).

Combination therapy focuses on antibiotics with multifaceted effects for the purpose of lowering respective MICs. The use of combination regimens is appropriate in treating heteroresistant *Klebsiella pneumoniae*.

7.2.6. Rapid diagnostic tests

The need for rapid carbapenemase detection methods is demonstrated by the fact that it has additional therapeutic implications dictating drug choice than in contrast to antibiotic susceptibility testing alone. Several modes of carbapenemase recognition and differentiation are available. However, each method comes with its own limitation. The time a method requires to provide a result is a matter of concern. Beyond that, cost-effectiveness is a further factor.

Molecular and phenotypic testing methods are available. While molecular testing methods are based on recognising genes, phenotypic testing is based on chemical reactions inducing colour shifts or changes of inhibition zones. Most assays require prior culturing of bacterial specimens. Some tests can be used directly on blood cultures, allowing faster results. (155,156)

Polymerase chain reaction (PCR) based methods specifically target sequences of carbapenemases. Allele variants of carbapenemases can be accessed from various databases. Provided that the sequence is already known, this rapid, yet costly method allows unequivocal recognition of various carbapenemases, even if several of them are expressed within one isolate. (157)

PCR-based methods directly making use of clinical specimens have the potential of providing a clinically relevant result within a few hours. However, they show low positive predictive values, limiting reasonable application (155).

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is, contrary to its name, a simple method that combines the advantages of both phenotypic and molecular testing. It is able to characterise and differentiate various carbapenemases within 60 minutes with a sensitivity of 93.9% and a specificity of 100%. The detection of OXA-48-like carbapenemases is with a percentage of 88.4 slightly poorer. The weak hydrolytic activity of OXA-48 demonstrates a common intricacy, which makes a great number of phenotypic tests fail. MALDI-TOF MS is an easy testing method overcoming the drawbacks of subjective visual interpretation. This is an issue in phenotypic testing methods. Additionally, MADLI-TOF MS comes with the benefit of lower cost while providing reliable results. (155,158,159)

7.3. Therapy optimisation

7.3.1. Antimicrobial de-escalation

While a uniform definition of antimicrobial de-escalation (ADE) is still lacking, the rationale behind ADE is to streamline antimicrobial therapy to limit the unnecessary use of broad-spectrum antibiotics, adverse reactions and AMR. This is achieved by discontinuation of drugs that are not effective (e.g. vancomycin in the case of gram-negative infection) and choosing the antimicrobial treatment option with the narrowest spectrum which still retains in vitro activity against the causative microbe. (160,161)

Empirical therapy is initiated in accordance with the infection focus, patient history, and local resistances. Its aim is to provide prompt appropriate therapy, which covers the most probable pathogens. However, appropriate empirical therapy is possible only in case of overshooting the initial unknown microbial target in favour of early treatment response. A treatment plan should therefore include ADE as early as possible. The result of the antimicrobial therapy should be a balance between appropriate antimicrobial coverage and limiting antibiotic exposure to curtail AMR. Additionally, a reduction of antimicrobials has the benefits of decreased side effects and lower cost (162,163).

Screening of patients, furthermore, provides useful clinical and microbiological information for initiation of early appropriate antimicrobial therapy. The GRS is able to assist in recognizing patients at risk and guide clinical decisions. (135)

Gonzales et al have demonstrated that antibiotic de-escalation does not show any change in mortality or length of ICU stay, and that an interdisciplinary approach for antimicrobial de-escalation including infectious disease specialists, microbiologists, and clinical pharmacists is possible without influencing the prognosis or MDR bacteria acquisition (164).

Two randomised clinical trials showed a higher rate of reinfection without changes in mortality rates in ventilator associated pneumonia and sepsis when ADE strategies were used (165,166).

Several observational studies showed improved outcomes in mortality when ADE is used. The discrepancy of findings which may be due to a heterogeneity of clinical and statistical data among various studies highlights the need for improved study designs and a uniform definition of ADE. (160,161)

Several studies investigated the use of procalcitonin (PCT) to guide length of antibiotic treatment. Results showed favorable effects if PCT was used to guide the duration of antibiotic therapy, as duration of antimicrobial exposure was reduced without compromising patient outcome. (167–170)

7.3.2. Pharmacokinetic and pharmacodynamic considerations

Antibiotics are first tested in vitro, then on animals. Tolerability and clinical efficacy studies on healthy humans follow, if administration is assumed safe. Dosing regimens are defined according to the studies performed on healthy individuals. (171)

Several factors changing pharmacokinetic properties are different in critically ill patients. Capillary leakage mediated by an increased release of inflammatory cytokines during SIRS (systemic inflammatory response syndrome) leads to an extravasation of fluids and proteins influencing volumes of distribution. Fluid therapy further exacerbates this effect and leads to a dilution of antibiotics in intra- and extravascular spaces. (172)

Patients with reduced renal function or the need of renal replacement therapy (RRT) have changed drug clearance, requiring adjustment of their treatment regimen. While guidance on dosage adjustment in case of reduced renal function and RRT is available, there is a lack of guidance for patients with augmented renal clearance who require higher or more frequent dosing. Therapeutic drug monitoring can help in the optimisation of dosing regimens, when available. (173,174)

The DALI study compared pharmacokinetic and pharmacodynamic (PK/PD) targets of β-lactams in critically ill patients. It showed that in 20% of patients conservative PK/PD targets of 50% of T_{FREE} > MIC (time of free drug concentration that lies above MIC) could not be met. Critically ill patients have pharmacokinetic variations, which influence treatment success. Therefore there is no dose regimen that fits all patients. (171)

Prolonged and continuous drug infusions are a way to reach PK/PD targets with drugs showing time-dependent killing (e.g., β-lactams). It is worth emphasising that prolonged and continuous drug infusions cannot compensate for incorrect drug-choice, insufficient dosing, or other unfavourable treatment regimen characteristics. When choosing for extended-infusions, product information about the stability of the agent at various temperatures has to be considered. (163)

In contrast to drugs that have time-dependent killing, those whose efficacy depends upon high peak concentration require shorter administration time (e.g., amikacin).

Due to adverse reactions and narrow therapeutic indices some drugs should be used in limited dosages and monitored closely (e.g. colistin, amikacin). In order to achieve higher local tissue concentrations, while limiting adverse reactions, local administration can be considered (e.g. inhalatory drugs).

International consensus guidelines for the optimal use of polymyxins recommend the use of adjunct CMS aerosol in patients with suspected or confirmed XDR gram-negative hospital-acquired or ventilator-associated pneumonia. It has been demonstrated that aerosolised CMS achieves higher colistin concentration in pulmonary epithelial lining fluid than intravenously administered CMS. Clinical data suggest that aerosolised CMS is associated with decreased nephrotoxicity while providing similar efficacy. (127)

Jung et al in a systematic review demonstrated that the combination of IV + inhalatory CMS is superior to IV CMS monotherapy in both patient survival and cure in critically ill patients infected with *Acinetobacter baumannii* (175).

However, taken as a whole, the recommendation for inhalatory colistin use is based on very low-quality evidence (127).

7.4. Summary

Figure 1 provides a summary of possible treatment options for carbapenem-resistant *Klebsiella pneumoniae* isolates.

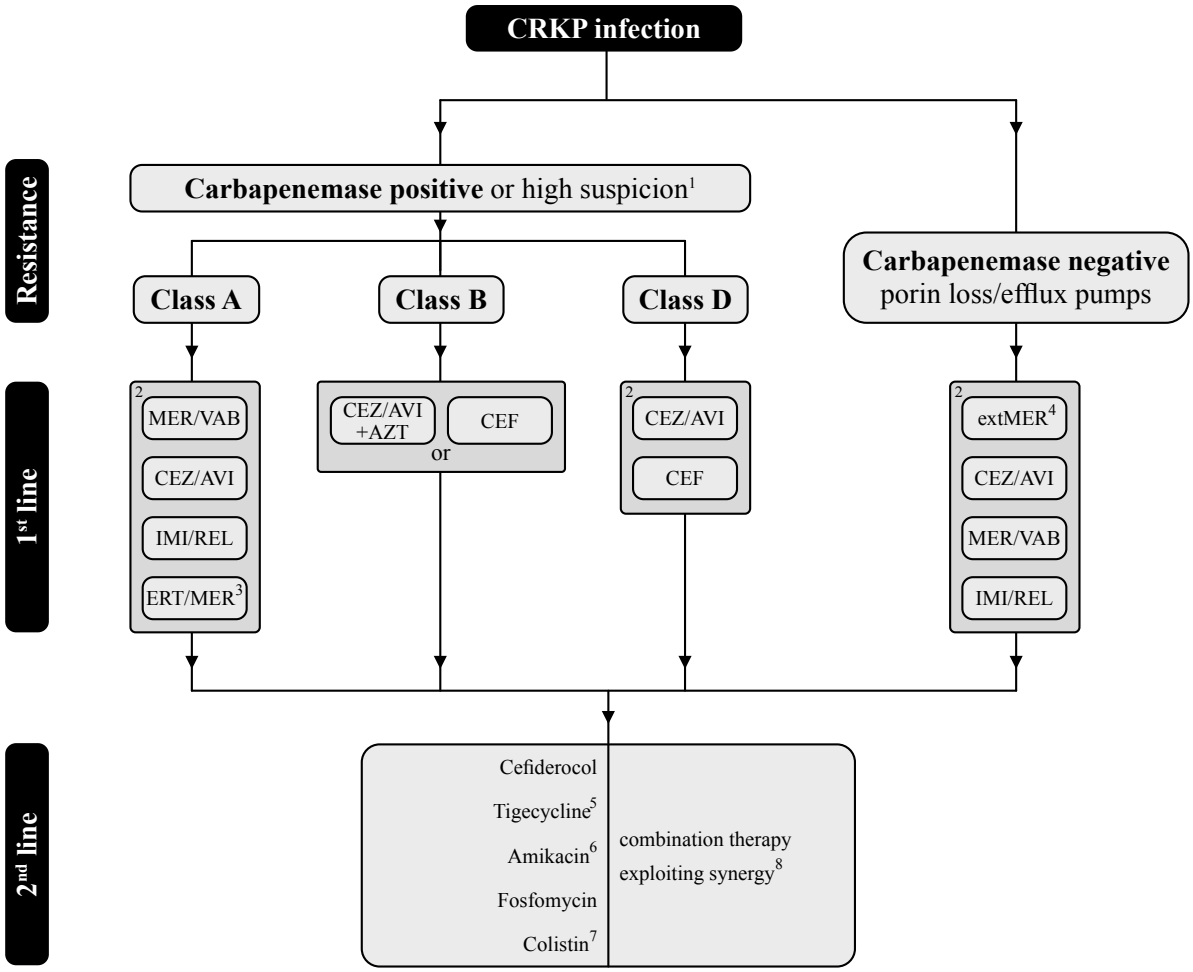

Figure 1. Algorithm for the treatment of CRKP infections. Adapted from Karakonstantis et al. 2020 (128), Tamma et al. 2022 (122), Fritzenwanker et al. 2018 (140). MER/VAB – meropenem/vaborbactam, CEZ/AVI – ceftazidime/avibactam, IMI/REL – imipenem/cilastatin/relebactam, ERT/MER – ertapenem and meropenem, CEF – cefiderocol, extMER – extended-infusion meropenem.

[1] High suspicion for carbapenemase-positive KP infection should be raised when the patient was hospitalised in a country with high prevalence of carbapenemase-producers or in patients who have had past positive screening results.

[2] If susceptible, the drugs are listed order of preference.

[3] Dual carbapenem therapy can be used when novel treatment options are either not available or affordable.

[4] Extended-infusion of meropenem is the preferred option in infections that are resistant to ertapenem but still show susceptibility to meropenem.

[5] Tigecycline should not be used in bloodstream or urinary tract infections.

[6] Amikacin is particularly useful for urinary tract infections.

[7] Regular monitoring of kidney-function is recommended. In nosocomial pneumonia the addition of nebulised CMS is strongly recommended. The IDSA AMR-guidance panel (122) does not recommend the routine use of colistin-based regimens when β-lactam regimens still show susceptibility.

[8] No single combination therapy is preffered over the others

8. Conclusion

The optimal treatment of carbapenem-resistant *Klebsiella pneumoniae* infections should be based on the early characterisation of the resistance mechanism. Screening by molecular microbiological methods allows early identification of carbapenemase-producers and needs to be used whenever possible. However, molecular testing methods are still not widely available. The need for expansion of these methods is highlighted by being necessary for both clinical decision-making and epidemiological surveillance.

Monotherapy with novel β-lactam/β-lactamase inhibitor combination drugs is the preferred treatment option whenever susceptibility is demonstrated. Judicious use of these valuable drugs can prolong their effectiveness and use in clinical settings.

Combination therapy is recommended when other treatment options are not feasible. Studies providing useful information on combination treatment regimens are lacking. When used, a particular combination of antibiotics should be chosen based on the results of antimicrobial susceptibility testing and relevant clinical features of infection.

Current recommendations are based on in-vitro studies, case reports, retrospective observations and drug safety/efficacy data which inadequately represent critically ill patients. Special pharmacokinetic and pharmacodynamic considerations are necessary to achieve the optimal treatment for patients infected with CRKP-isolates.

While safeguarding newly available drugs might slow the evolution leading to new antimicrobial resistance mechanisms, it is only a question of time when physicians are going to be empty-handed again. Therefore, further microbiological and clinical studies tackling the problem of infections caused by CRKP isolates are needed.

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10. Biography

Luka Joakim Csenar was born on 4th of February in Oberpullendorf, Austria. After attending 4 years of a bilingual elementary school in Oslip/Uzlop, he went to grammar school in 2005 in Eisenstadt (BG/BRG/BORG Kurzwiese Eisenstadt). The Burgenlandcroat attended the subject Croatian language in lower grades. The advanced levels of high school focused on natural sciences. The Austrian finished grammar school with the Matura (the prerequisite for higher education in Austria) in 2013. Csenar served his civil service in 2014 working as a paramedic at the Austrian Red Cross. Initially he studied Astronomy and Biology at the University of Vienna. However, returned to fulfil his initial wish of studying medicine in Zagreb, Croatia, at the University of Zagreb, School of Medicine from 2016-2022.