Conventional and syndromic molecular diagnostics

as a clinical tool for antibiotic choice in Gram-negative MDR infections

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The products imipenem/relebactam, eravacycline, plazomycin, aztreonam/avibactam, cefepime/taniborbactam, meropenem/nacubactam, cefepime/enmetozabactam, cefepime/tazobactam, sulbactam/durlobactam, cefepime/zidebactam, temocillin are not commercially available in Italy at the time of publication of this text (January 2022)

Enterobacterales

INTRODUCTION

Antibiotic resistance is a **global health threat**. Resistance is present in most Gram-positive and Gram-negative pathogens; however, increasingly extensive patterns of resistance are emerging, making infections and patients difficult to treat.

Antibiotic resistance is a global health threat

Enterobacteria are the most common Gram-negative bacteria in both hospital and community settings, and, in the context of infections due to multidrug-resistant organisms, they have an important impact in terms of mortality and morbidity [1].

Enterobacteria are oxidase-negative, glucose fermenting aerobic and facultative anaerobic Gram-negative bacilli. The recent development of modern massive genome sequencing methods has led to a profound review of the taxonomy of *Enterobacteria*, which are now included in the order of the *Enterobacterales* [2], comprising seven families (*Enterobacteriaceae*, *Erwiniaceae*, *Pectobacteriaceae*, *Yersiniaceae*, *Hafniaceae*, *Morganellaceae*, *Budviciaceae*). The most clinically relevant genera are Escherichia, Klebsiella, Shigella, Citrobacter and Enterobacter of the ***Enterobacteriaceae*** family, Yersinia and Serratia of the ***Yersinaceae*** family along with Proteus, Morganella and Providencia of the ***Morganellaceae*** family.

THE PATHOGENIC POTENTIAL OF *ENTEROBACTERALES*

Some ***Enterobacterales*** species (*Shigella spp.*, *Salmonella enterica*, *Yersinia spp.*), have evolved specific pathogenic mechanisms enabling them to cause invasive infections associated with defined clinical syndromes while others (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter cloacae*, *Serratia marcescens*, *Citrobacter spp.*, *Morganella morganii*) generally behave as opportunistic pathogens, causing infections in patients with predisposing conditions. These conditions may impair normal host defences and often occur in hospitalized subjects or patients undergoing various treatments.

However, among these species, it is also possible to find strains that have acquired peculiar pathogenic mechanisms that make them capable of causing infections associated with specific syndromes. An example is represented by ***Escherichia coli*** strains capable of causing diarrhoea with various mechanisms (production of enterotoxins, invasion of the intestinal mucosa, functional and structural alteration of the intestinal mucosa) [3,4].

Klebsiella pneumoniae (hvKp)

Another example is represented by hypervirulent strains of *Klebsiella pneumoniae (hvKp)*, initially reported in some areas of Southeast Asia. These strains have acquired many virulence factors (siderophore production, some types of capsular polysaccharides, new metabolic pathways, regulators of gene expression) that promote invasive infections. When grown in culture, these strains typically form the frankly mucoid colonies of the hypermucoviscosity-phenotype, which are positive to the ***string test*** (Figures 1 and 2).

Hypervirulent Kp strains are clinically relevant as they **cause invasive infections** with severe sepsis generally spreading from the biliary tract through the bloodstream and originating hepatic abscesses and septic embolisms (pulmonary, cerebral, ocular).

The *hvKp* strains generally belong to some clonal lineages (e.g., ST23, ST65

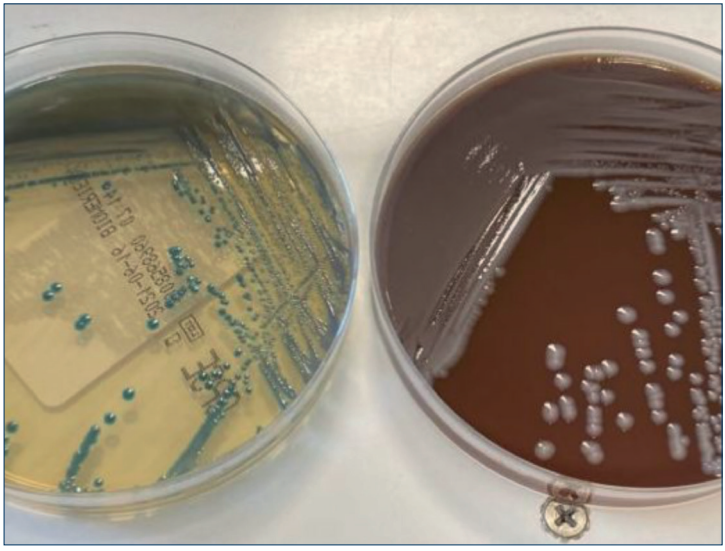


Figure 1. *Klebsiella pneumoniae on chromogenic agar (left) and chocolate agar (right) (Image courtesy of Dr. Tommaso Giani).*

and ST86) [5,6] and often do not exhibit acquired resistance phenotypes. However, the emergence of hvKp strains displaying multi-resistance phenotypes (including resistance to carbapenems) due to the convergence of resistance determinants, has been recently reported [7,8]. This phenomenon is obviously considerably worrying due to the possible clinical and epidemiological implications.

Figure 2. *String test (Image courtesy of Dr. Fabio Arena).*

ANTIBIOTIC RESISTANCE IN *ENTEROBACTERALES*

Enterobacterales are characterized by an intrinsic resistance profile to antibiotics including benzyl-penicillin, glycopeptides, daptomycin, fusidic acid, macrolides, lincosamides, streptogramins, rifampicin and oxazolinidones [9]. For some of them, intrinsic resistance can also extend to other molecules such as β-lactams, tetracyclines, polymyxins, fosfomycin and nitrofurantoin, according to species-specific resistance mechanisms.

For example, *Klebsiella pneumoniae* strains harbour a chromosomal gene encoding SHV-type broad-spectrum β-lactamase conferring intrinsic resistance to ampicillin, amoxicillin, piperacillin and narrow-spectrum cephalosporins.

AmpC type

Another example is provided by bacterial species with inducible chromosomal **AmpC** (e.g., *Enterobacter spp.*, *Citrobacter freundii*). This β-lactamase confers intrinsic resistance to ampicillin, amoxicillin, ampicillin-sulbactam, amoxicillin-clavulanate, narrow-spectrum cephalosporins and cefoxitin and cannot be inhibited by sulbactam or clavulanate [9].

Enterobacterales may also acquire resistance to antibiotics following chromosomal mutations or horizontal gene transfer, which is common in this order of bacteria and often ascribed to plasmid-mediated transmission.

Among the various pathogenic species of *Enterobacterales*, *Klebsiella pneumoniae* outstands for its ability to acquire complex and extensive resistance phenotypes to many antibiotics. The burden of acquired resistance also concerns *Escherichia coli*, the most commonly represented species among clinical isolates.

Among Enterobacterales, the **main mechanism of acquired resistance** to β-lactam antibiotics is the **production of β-lactamases**, enzymes that hydrolize the β-lactam ring causing inactivation of the drug.

β-lactamases enzymes naturally evolved as defence mechanism against na-

tural β-lactams, within the competition processes between microorganisms. Starting from the mid-1900, the use of these molecules in the clinical setting has ever since generated a selective pressure favouring recruitment of novel β-lactamases among the pathogenic *Enterobacterales*, often mediated by transferable plasmids.

β-lactamase production represents the main resistance mechanism among *Enterobacterales*

Acquired β-lactamases began to spread among *Enterobacterales* over the last century mid-60s. They initially comprised broad-spectrum enzymes such as **TEM-1** and **SHV-1**, the latter being encoded by a gene residing on the *Klebsiella pneumoniae* mobilized chromosome.

These enzymes are responsible for both acquired resistance to penicillins (ampicillin, ticarcillin, piperacillin) as well as for resistance to narrow spectrum cephalosporins (cephalothin, cefazolin) in species usually displaying intrinsic sensitivity to these drugs, such as *Escherichia coli*, *Salmonella enterica* and *Proteus mirabilis*.

The subsequent wide use of expanded spectrum cephalosporins (ESC) (e.g., cefotaxime, ceftriaxone, ceftazidime), which resist TEM and SHV broad spectrum β-lactamase activity, has increased selective pressure leading to: i) selection of point mutations encoding for enzymes capable of hydrolysing ESCs too (for example, **TEM-3**, **TEM-10**, **TEM-24**, **TEM-52**; **SHV-5**, S**HV-12**); ii) recruitment of new β-lactamases with activity against ESC (such as **CTX-M**, **PER**, **GES**, **VEB**) [10-13].

The spread of *Enterobacterales* strains harbouring such enzymes, collectively referred to as **extended spectrum β-lactamases (ESBL)**, has taken on a pandemic dimension in a relatively short time, affecting human medicine along with both the veterinary field and the environment.

ESBL production is typically associated with ESC resistance. In the presence of the latter, ESBL production should be suspected though other underlying mechanisms of resistance may concur.

As ESBLs may display preferential activity against different ESCs, it is impor-

tant to assess susceptibility to at least two representatives of this drug family (such as cefotaxime or ceftriaxone, and ceftazidime) in order to identify ESBL producing strains with a high degree of sensitivity.

Infections due to ESBL-producing *Enterobacterales* were mainly treated with carbapenems. However, their use grew consequently as ESBL-producing strains diffused, further increasing the selective pressure in the clinical setting. As a result, enzymes capable of carbapenem degradation, called **carbapenemases**, were selected for and propagated.

For this reason, alternative treatments to carbapenems (**carbapenem-sparing therapies**) for the management of infections sustained by ESBL producing *Enterobacterales* are of great clinical and scientific interest. Combination regimens based on β-lactam and β-lactamase inhibitors (BLICs) are among the most studied alternatives to carbapenems, despite their non-inferiority is still partly controversial [14].

Different types of carbapenemases have emerged, mainly among *Klebsiella pneumoniae*. The most commonly encountered ones are **KPC** and **OXA-48** (serine carbapenemases), as well as **NDM**, **VIM** and **IMP** (metallo-carbapenemases).

Along with their ability to degrade carbapenems, these enzymes are also active against most β-lactams and can therefore confer a very broad resistance phenotype against β-lactams, including penicillins, cephalosporins and carbapenems.

Furthermore, carbapenemases are not inhibited by β-lactam-derived β-lactamase inhibitors (clavulanate, sulbactam and tazobactam) and only some of them are inhibited by the most recent non-β-lactam inhibitors. In particular, KPC-type enzymes are inhibited by avibactam, relebactam and vaborbactam, whereas OXA-48 type enzymes are only inhibited by avibactam. None of the novel commercially available molecules inhibit metallo-β-lactamases (MβLs) with the sole exception of cefiderocol.

Carbapenemase producing enterobacterales (CPE) strains often har-

bour resistance mechanisms against other non-β-lactam antibiotics thus exhibiting extensively resistant (XDR) phenotypes.

Indeed, only a few among older antibiotics preserve some activity against CPE (polymyxins, tigecycline, fosfomycin, some aminoglycosides), while modern β-lactam–β-lactamase inhibitors combinations only protect against serine carbapemases (ceftazidime/avibactam) or KPC type enzymes (imipenem/relebactam and meropenem/vaborbactam).

Only cefiderocol and aztreonam-avibactam (not yet available as such but may be obtained by combining ceftazidime/ avibactam with aztreonam) are active against metallo-enzyme producing CPE.

Acquired resistance against new BLICs (in particular to ceftazidime/avibactam, the oldest available at the moment) has been repeatedly reported and attributed to various mechanisms (enzymatic mutants, enzyme overproduction, permeability defects) [15-18]. This highlights the need to handle these new antibiotics in accordance with strict antibiotic stewardship criteria in order to preserve their efficacy.

Among β-lactamases acquired by *Enterobacterales*, other types of enzymes include AmpC β-lactamases and OXA β-lactamases.

AmpC β-lactamase

AmpC β-lactamases, typically encountered in some Enterobacterales species (see above), can also be plasmid encoded. Similarly to ESBLs, their acquisition is associated with ESC resistance phenotypes that cannot be reversed by conventional β-lactamase inhibitors.

The prevalence of acquired AmpC β-lactamases is overall lower than ESBLs.

OXA β-lactamase

OXA β-lactamases are comprised within molecular class D serine enzymes with some peculiarities in the catalytic mechanism, making them generally resistant or only partially susceptible to β-lactamase inhibitors [19].

Many of them display a narrow profile activity against penicillins and narrow spectrum cephalosporins, and their presence may contribute to a resistan-

ce phenotype to BLIC according to old penicillin.
Some of these enzymes, however, have evolved the ability to hydrolyze ESC (OXA-ESBL) or carbapenems (OXA-carbapenemase) and may therefore contribute to resistance against these drugs.
OXA-ESBLs are relatively rare, while OXA-carbapenemases, such as OXA-48 type, have spread rapidly in some geographic regions, reaching high prevalence among CPEs [20].

DIAGNOSTICS OF *ENTEROBACTERALES* SUSTAINED INFECTIONS

The diagnosis of infections due to *Enterobacterales* represents an important chapter in clinical bacteriology. Indeed, these pathogens are the main cause of nosocomial and community-acquired infections, and the diversity of their antibiotic susceptibility profiles cannot be predicted according to simple to species identification.
To this extent, integrating the use of modern rapid diagnostic technologies with conventional methods within diagnostic-therapeutic algorithms can provide an advantage especially for the management of *Enterobacterales* infections.
The most recent approach to tackle antibiotic resistance and bacterial infections, as a fact, integrates antibiotic stewardship with **diagnostic stewardship**, defined as the use of the right test for the right patient, providing clinically relevant results within the least amount of time.

Impact of initial appropriate empirical therapy on outcome

An **appropriate initial empirical therapy** is unambiguously reported to correlate with a **positive impact on the outcome** of critically ill patients with severe nosocomial infections due to Gram-negative MDR pathogens [21,22].

Timely microbiological diagnosis plays a fundamental role in appropriate management of severe infections. In this context, rapid mi-

crobiology diagnostic technologies (often referred to as **fast microbiology**) currently meet this need to a wide extent.

With the introduction of the most modern rapid microbiological diagnostics, it is possible to quickly obtain information relating to the identification of the pathogen and its sensitivity/resistance profile to antimicrobials.

A scrupulous clinician has the duty of employing fast microbiology especially on patients with a high risk for MDR infections. Multiparametric risk stratification can help clinicians establish where fast microbiology is more cost-effective, within a collaborative frame involving both infectious diseases physicians and microbiologists (Figure 3).

Most modern clinical microbiology rapid diagnostic technologies in are based on detection of specific molecular markers of bacterial pathogens responsible for specific syndromes.

This is achieved through highly automated systems, with a rapid time to re-

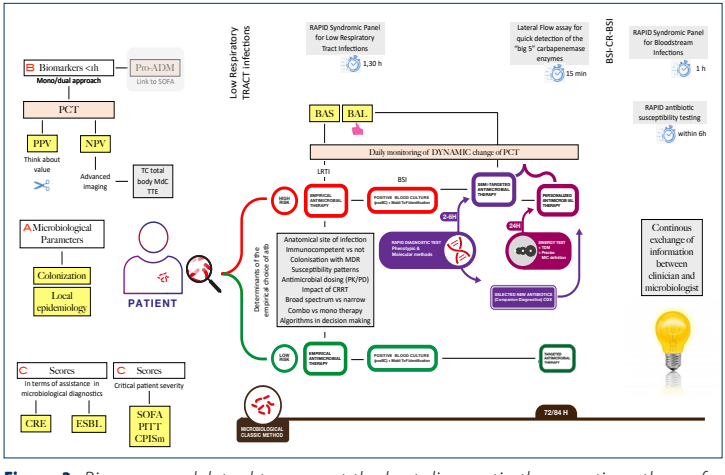


Figure 3. *Bioscore model: tool to support the best diagnostic-therapeutic pathway for the management of the critically ill patient with severe MDR infection. It answers the question "who goes to rapid diagnostics?"*

sult requiring minimal technical staff commitment.
Typical examples include systems based on "syndromic panels", that is tests performed on positive blood cultures when bacteraemia o fungaemia are suspected (Figure 4) or on lower respiratory tract (BAL, BAS, sputum) samples upon suspicion of pneumonia (Figures 4 and 5). Such tests yield results in just about over 1 hour.
Other molecular diagnostic systems are available and can be performed directly on whole blood. In these cases, however, turn-around times are longer (4-5 hours), the number of pathogens and resistance determinants screened is lower and costs are higher. As already mentioned, rapid molecular diagnostics can provide rapid pathogen identification, but also detection of antibiotic resistance profiles relevant to antimicrobial stewardship (such as carbapenemases and ESBL resistances in *Enterobacterales*).
Results from fast microbiology tests allow clinicians to quickly evaluate strain sensitivity to the most important groups of antibiotics for the treatment of *Enterobacterales* (broad spectrum cephalosporins, carbapenems, novel BLICs and cefiderocol).
This approach (also referred to as molecular antibiogram) must consider that **the information provided differs from conventional phenotypic antibiograms** (unreplaceable by molecular technologies for the moment) **and requires careful interpretation**.
Let us practise by analysing test results in Figure 6, depicting a molecular test performed on a positive blood culture. It reports the presence of *Klebsiella pneumoniae* and a **CTX-M** type resistance determinant and the absence of carbapenemase. What could we infer?
- probable resistance to third and fourth generation cephalosporins;
- a probable susceptibility to carbapenems;
- probable susceptibility to novel BLICs comprising new inhibitors, such as ceftazidime/avibactam (CZA/AVI), meropenem/vaborbactam (MEM/VAB), imipenem/relebactam (IMI/REL);

Gram-positive strains	Gram-negative strains
Enterococcus faecalis *Enterococcus faecium* *Listeria monocytogenes* *Staphylococcus* - *Staphylococcus aureus* - *Staphylococcus epidermidis* - *Staphylococcus lugdunensis* *Streptococcus* - *Streptococcus agalactiae* - *Streptococcus pyogenes* - *Streptococcus pneumoniae*	*Acinetobacter calcoaceticus-baumanni complex* *Bacteroides fragilis* *Enterobacterales* - *Enterobacter cloacae complex* - *Escherichia coli* - *Klebsiella aerogenes* - *Klebsiella oxytoca* - *Klebsiella pneumonia group* - *Proteus* - *Salmonella* - *Serratia marcescens* *Haemophilus influenzae* *Neisseria meningitidis* *Pseudomonas aeruginosa* *Stenotrophomonas maltophilia*
Yeasts	**Antibiotic resistance genes**
Candida albicans *Candida auris* *Candida glabrata* *Candida krusei* *Candida parapsilosis* *Candida tropicalis* *Cryptococcus neoformans/ gattii*	Carbapenemase - IMP - KPC - *OXA-48 like* - NDM - VIM Resistance to colistin - *mcr-1* ESBL - CTX-M Resistance to methicillin - *mecA/C* - *mecA/C e MREJ (MRSA)* Resistance to vancomycin - vanA/B

Figure 4. *BioFire® Blood Culture Identification 2 (BCID2) Panel, 43 targets.*

Bacteria (semi quantitative assessment)	**Antibiotic resistance genes**
Acinetobacter calcoaceticus-baumanni complex *Enterobacter cloacae* *Escherichia coli* *Haemophilus influenzae* *Klebsiella aerogenes* *Klebsiella oxytoca* *Klebsiella pneumonia group* *Moraxella catarrhalis* *Proteus spp.* *Pseudomonas aeruginosa* *Serratia marcescens* *Staphylococcus aureus* *Streptococcus agalactiae* *Streptococcus pyogenes* *Streptococcus pneumoniae*	ESBL - CTX-M Carbapenemase - KPC - NDM - *OXA-48 like* - VIM - IMP Resistance to methicillin - *mecA/mecC e MREJ*
Atypical bacteria (qualitative assessment)	**Virus**
Legionella pneumophila *Mycoplasma pneumoniae* *Chlamydia pneumoniae*	Influenza B virus Adenovirus Coronavirus Parainfluenza virus Respiratory syncytial virus Human rhinovirus/entero-virus Human metapneumovirus Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

Figure 5. *BioFire® Pneumonia plus (PNplus) Panel, 34 target.*

- a possible susceptibility to old BLICs comprising old inhibitors such as piperacillin/tazobactam (PIP/TAZ) and ceftolozane/tazobactam (C/T) (Figure 7).

Another example depicting a molecular antibiogram reports the presence of a KPC-type carbapenemase in the absence of other carbapene-

CTX-M	**Detected**
KPC	Not detected
VIM	Not detected
IMP	Not detected
NDM	Not detected
OXA-48	Not detected

Figure 6. *CTX-M producing Klebsiella pneumoniae; molecular antibiogram.*

Antibiotics	MIC mg/L
Amoxicillin/ Clav. acid	?
PIP/TAZ	?
Ceftriaxone	R
Ceftazidime	R
Cefepime	R
Imipenem	S
Meropenem	S
CZA/AVI	S
C/T	S
IMI/REL	S
MEM/VAB	S
FDC	S

Figure 7. *CTX-M producing K. pneumoniae; on the left hypothetical molecular antibiogram, on the right definitive antibiogram which will be available after 48 hours.*

Antibiotics	MIC mg/L
Amikacin	≤4 S
Amoxicillin/ Clav. acid	32 R
Ceftazidime	>64 R
Cefotaxime	>64 R
Ciprofloxacin	1 R
Colistin	≤ 0.5 S
Ertapenem	≤ 0.5 S
Gentamycin	≤ 1 S
Meropenem	≤ 0.25 S
PIP/TAZ	> 128 R
Trimethoprim/ avibactam	> 8/152 R
CZA/AVI	≤1 S
C/T	1 S
Cefepime	> 16 R

mases along with the presence of CTX-M-type ESBLs (Figure 8).

What can we infer from the results of this test?

- a probable resistance to all old β-lactams, including carbapenems;
- a probable susceptibility to novel BLICs such as ceftazidime/

CTX-M	**Detected**
KPC	**Detected**
VIM	Not detected
IMP	Not detected
NDM	Not detected
OXA-48	Not detected

Figure 8. *KPC-producing K. pneumoniae; molecular antibiogram.*

Antibiotics	MIC mg/L
Amoxicillin/clavulanic acid	R
PIP/TAZ	R
Ceftriaxone	R
Ceftazidime	R
Cefepime	R
Ertapenem	R
Imipenem	R
Meropenem	R
Fosfomycin	?
Amikacin	?
Gentamycin	?
Ciprofloxacin	?
Tigecycline	?
Colistin	?
CZA/AVI	S
MEM/VAB	S
IMI/REL	S
FDC	S

Antibiotics	MIC mg/L
Amoxicillin/clavulanic acid	>64 R
PIP/TAZ	>128 R
Ceftriaxone	>4 R
Ceftazidime	>128 R
Cefepime	>32 R
Ertapenem	>1 R
Imipenem	>16 R
Meropenem	>64 R
Fosfomycin	>128 R
Amikacin	>16 R
Gentamycin	1 S
Ciprofloxacin	>4 R
Tigecycline	0.5 S
Colistin	
CZA/AVI	4 S

Figure 9. *KPC-producing K. pneumoniae; on the left hypothetical molecular antibiogram, on the right definitive antibiogram which will be available after 48 hours.*

avibactam, meropenem/vaborbactam, imipenem/relebactam and to cefiderocol;
- no information, on the other hand, can be deduced regarding sensitivity or resistance to other molecules (Figure 9).

Finally, a third example of a molecular antibiogram reports the presence of an NDM-type resistance determinant in a positive blood culture yielding *Klebsiella pneumoniae* (Figure 10).

CTX	Not detected
KPC	Not detected
VIM	Not detected
IMP	Not detected
NDM	**Detected**
OXA-48	Not detected

Figure 10. *NDM producing K. pneumoniae NDM; molecular antibiogram.*

What can be hypothesized from this result?
- a probable resistance to all β-lactams, including carbapenems and new BLICs (CZA/AVI, IMI/REL, MEM/VAB);
- a probable sensitivity to cefiderocol and to aztreonam in combination with avibactam (Figure 11).

The detection of different resistance mechanisms underlying meropenem resistance by means of molecular diagnostics has improved the use of the most recently approved antibiotics displaying activity against CPE (Figure 12).

TREATMENT OF *ENTEROBACTERALES* INFECTIONS

A comprehensive description of therapeutic options for the treatment of *Enterobacterales* infections ought not ignore resistance patterns. Indeed, the underlying enzymatic mechanism of resistance determines the choice of the antibiotic. For this reason, discussions concerning treatment regimens will focus individually on either ESBL-, AmpC-, carbapenemase- or MβL- producing strains. A small section focusing on cefiderocol will conclude the chapter.

Antibiotics	MIC mg/L
Amoxicillin/ Clav. acid	R
PIP/TAZ	R
Ceftriaxone	R
Ceftazidime	R
Cefepime	R
Ertapenem	R
Imipenem	R
Meropenem	R
Fosfomycin	?
Amikacin	?
Gentamycin	?
Ciprofloxacin	?
Tigecycline	?
Colistin	?
CZA/AVI	R
MEM/VAB	R
IMI/REL	R

Antibiotics	MIC mg/L
Amoxicillina/Clav. acid	>64 R
PIP/TAZ	>128 R
Ceftriaxone	>4 R
Ceftazidime	>128 R
Cefepime	>32 R
Ertapenem	>1 R
Imipenem	>16 R
Meropenem	>64 R
Fosfomycin	>128 R
Amikacin	>16 R
Gentamycin	>8 R
Ciprofloxacin	>4 R
Tigecycline	0.5 S
Colistin	1 S
CZA/AVI	>8 R

Figure 11. *NDM producing K. pneumoniae; the hypothetical molecular antibiogram is reported to the left. The definitive antibiogram, available after 48 hours, is reported to the right.*

Resistance determinants	CZA	M/V	C/T	I/R	ATM/ AVI	FEP/ TANI	FEP/ ZIDE	MEM/ NACU	FDC
KPC	+	+	-	+	+	+	+	+	+
OXA-48	+	-	-	-	+	+	+	+	+
VIM	-	-	-	-	+	+	+	+	+
IMP	-	-	-	-	+	-	?	+	+
NDM	-	-	-	-	+	+	+	+/-	+

Figure 12. *Spectrum of activity of novel anti-CPE antibiotics.*

EXTENDED-SPECTRUM β-LACTAMASE (ESBL)

Several meta-analyses have compared piperacillin/tazobactam and carbapenems for the treatment of ESBL-producing Enterobacterales sustained infections, both as empirical and targeted therapy. None of the studies reported significant carbapenem superiority [23-27].

Along this line, the BICAR study confirmed this same finding in a cohort of neutropenic patients: both multivariate analysis and propensity score matching indicated that treatment with BLICs was not associated with a worse outcome compared to carbapenem regimens [28]. A lot of attention has been focused on the precise role of piperacillin/tazobactam MICs on outcome. According to Delgado-Valverde, piperacillin-tazobactam (PIP/TAZ) in the presence of very low real MICs, or near its breakpoint (16 mg/L), retains its effectiveness, which is completely lost in the event of higher MICs [29]. Of note, the EUCAST breakpoint was recently decreased to 8 mg/L [30]. This led to the use of piperacillin/tazobactam for the treatment of ESBL-producing Gram-negative infections in the presence of MIC values ≤8 mg/L, assessed by broth-dilution (reference method according to EUCAST).

Therefore, treatment of ESBL infections in body sites that do not represent a challenge in terms of β-lactams penetration (**cUTIs: complicated Urinary Tract Infections, cIAIs: complicated Intra-Abdominal Infections, BSIs: Blood Stream Infections**) may be safely based on piperacillin/tazobactam regimens provided that PIP/TAZ MICs ≤8 mg/L. The appropriate dosage is 4.5 g q6h, administered as continuous infusion, preceded by an adequate loading dose.

There is solid evidence supporting continuous infusion of PIP/TAZ in critically ill patients [31]. Furthermore, a correct loading dose of hydrophilic antimicrobials in sepsis (increased volume of distribution-Vd) should be at least 1.5 times higher than the normal dose [32]. Hence, administration of PIP/TAZ in the critically ill should include a loading dose of 6.75 g followed by 16 g q24h administered as continuous infusion.

On the other hand, due to the possible variability of PIP/TAZ efficacy against ESBL-producing strains (especially in difficult to reach sites such as the lungs), carbapenems are still considered the therapeutic "*gold standard*" in this clinical setting.

The MERINO study [33] is currently the only randomized controlled trial (RCT) evaluating the efficacy of piperacillin/tazobactam vs meropenem on 30-day mortality of patients with Blood Stream Infections (BSI) due to *Escherichia coli* or *Klebsiella pneumoniae* resistant to ceftriaxone. The trial showed superiority of carbapenem therapy over the comparator (12.3% mortality at 30 days in the piperacillin/tazobactam group vs only 3.7% of those treated with meropenem).

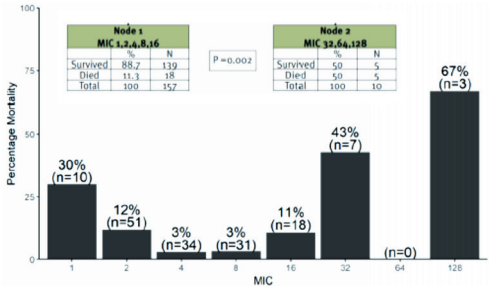
However, several criticisms moved to the MERINO study design have prompted the set-up of further studies. The MERINO 2 study, a pragmatic RCT, is of great interest and will most likely put an end to the carbapenem vs BLIC diatribe for the treatment of ESBL infections. MERINO 3 compares meropenem with ceftolozane/tazobactam, considered as a more appropriate **carbapenem sparing** option [34]. Of notice, the "defeated" BLIC in the MERINO study is PIP/TAZ and not the new BLICs such as ceftolozane/tazobactam (C/T) and ceftazidime/avibactam (CZA/AVI).

EUCAST recently re-assessed PIP/TAZ MICs by means of broth-dilution for all *Escherichia coli* and *Klebsiella pneumoniae* strains isolates from the MERINO study, confirming that mortality varied significantly only for PIP/TAZ MICs> 8 mg/L. This data provided the rationale for modifying breakpoints for PIP/TAZ, alongside stressing the importance of precise MIC values (Figure 13).

Ceftolozane/tazobactam (C/T) displays more advantageous characteristics compared to PIP/TAZ both in vitro and in the clinical setting as suggested by clinical studies [35-38]. C/T is the first-choice therapy among non-carbapenem drugs for the treatment of ESBL.

Despite its great potency against all ESBL-producing strains, ceftazidime/avibactam (CZA/AVI) (100% sensitivity CZA vs 91, 6% of C/T) [39], should

Figure 1: 30-day mortality by reference broth microdilution MIC of isolates of *E. coli* and *K. pneumoniae* from the MERINO trial [Henderson et al., 2019]



MIC distribution and ECOFF

Piperacillin distributions

MIC	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	ECOFF
E. coli	0	0	0	12	30	67	507	6314	8421	1445	479	924	1454	1225	1250	2185	1593	8
K. pneumoniae	0	0	0	0	0	2	21	199	509	1567	1024	422	226	166	279	435	219	8
P. aeruginosa	0	0	0	0	9	4	45	340	1095	3429	1446	884	331	237	199	245	192	16

Piperacillin-tazobactam distributions

MIC	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	ECOFF
E. coli	8	6	9	47	105	221	2978	16376	20910	5495	1905	1233	801	526	752	187	89	8
K pneumoniae	2	0	3	11	24	46	444	2539	7785	5041	1802	1076	560	397	1280	336	209	8
P. aeruginosa	5	1	0	4	23	37	453	886	3147	10479	5692	3595	1879	1506	3135	863		16

Notes:
Wild types and ECOFFs for piperacillin-tazobactam reflect those of piperacillin alone, which has the greater capacity to detect strains without phenotypically-detectable acquired resistance mechanisms.
Yellow highlighting is used to denote the mode of the wild type

Figure 13. *EUCAST Piperacillin-tazobactam Breakpoints for Enterobacterales* [30].

be reserved for selected cases: infections due to ESBL-producing *Proteus mirabilis*, where C/T could lose some of its effectiveness; all cases of infection sustained by serine-carbapenemase producing *Enterobacterales*, where CZA/AVI represents the only truly effective currently available therapeutic option.

A recently published Italian multicentric, retrospective study (CEFTABUSE II) [40], conducted on 153 patients presenting with severe infections due to ESBL-producing *Enterobacterales* (27.5% presenting with septic shock) confirmed C/T based regimens as valid options for both empirical and targeted therapy. Clinical success in the CEFTABUSE II study was reported in 100% of patients undergoing empirical treatment with C/T, in 83.8% of patients on targeted therapy and in 66.7% of patients where C/T was used as **rescue therapy**. An increased risk of treatment failure was reported upon admini-

stration of standard C/T dosing regimens in septic patients undergoing CRRT. Further evidence to support C/T for the treatment of severe ESBL-producing Enterobacterales is provided by the ASPECT-NP study results, where C/T proved as effective as meropenem for the treatment of ventilator-associated pneumonia (VAP) due to *Klebsiella pneumoniae* and ESBL-producing *Escherichia coli* (67% vs 67% and 83% vs 86%, respectively) [41].

Treatment of ESBL-producing *Enterobacterales* could soon benefit from a couple novel molecules and BLICs currently under development.

Cefepime is of uttermost importance in the context of **carbapenem sparing regimens**, as it lacks activity against anaerobic organisms. As such, it offers protection against potential **collateral damage** [42] induced by carbapenems [43]. Cefepime has been combined with new β-lactamase inhibitors (zidebactam, taniborbactam, enmetazobactam) along with the well-known tazobactam [42]. Of these combinations, cefepime/enmetazobactam and cefepime/tazobactam appear to have the greatest anti-ESBL potential as **carbapenem sparing** regimens, reserving the other two options for carbapenemase-producing strains.

Enmetazobactam is a new ESBL inhibitor; similarly to tazobactam, it is a penicillanic acid sulfone with increased ability to penetrate the bacterial cell and enhanced activity. Alike tazobactam, it inhibits CTX-M, TEM, SHV and some other class A β-lactamases. The proposed dosage for cefepime/enmetazobactam from ongoing clinical trials is 2.5 g q8h administered as 2-hours extended infusion.

Cefepime/tazobactam has demonstrated efficacy in pre-clinical studies. In a newly published paper, Lasko *et al.* tested high-dose cefepime/tazobactam (WCK 4282) against serine β-lactamase-producing *Enterobacterales* isolates in a neutropenic mouse model of lung infection. They reported a cell density reduction >1 log10 on all ESBL producing strains, further supporting the potential use of this new BLIC in this clinical setting [44].

Temocillin is another available option as carbapenem sparing regimens

for the treatment of ESBL. It is a 6-alpha methoxy derivative of ticarcillin, stable to hydrolysis by many class A (ESBL, KPC) and class C (**AmpC**) serine β-lactamases. The recommended dose for temocillin is 2g every 8 hours. However, the molecule is currently not available in European countries.

Plazomicin, a semi-synthetic aminoglycoside, has demonstrated activity against MDR *Enterobacterales* strains (ESBL, AmpC and carbapenemase producers, including MβLs) [45]. Plazomicin displays excellent pulmonary penetration, and could serve as ideal partner for C/T or CZA/AVI for the treatment of respiratory syndromes, such as VAP [46]. Plazomicin is currently approved as 1 mg / kg twice a day regimen for the treatment of cIAI. However, this indication will likely be extended also to lower respiratory tract infections [46].

Eravacycline, a synthetic fluorocycline, offers several advantages over tigecycline. It demonstrated *in vitro* activity against both Gram-positive cocci and Gram-negative bacilli (2 to 8 times greater compared to tigecycline), including MRSA, vancomycin resistant enterococcus (VRE), *Enterobacterales* (ESBL, KPC and OXA) as well as against MDR *Acinetobacter baumannii* (four times more potent than tigecycline). Eravacycline concentrates in ELF and macrophages, whereby concentrations may reach 6- and 50-times plasma levels in ELF and macrophages respectively [47].

AmpC

In some *Enterobacterales* species, AmpC enzymes are encoded by inducible chromosomal genes (ESCPM group, acronym for *Enterobacter cloacae complex*, *Enterobacter aerogenes*, *Serratia marcescens*, *Citrobacter freundii*, *Providencia stuartii* and *Morganella morganii*). They are responsible for about 15-20% resistance to third generation cephalosporins [48].

AmpC are stable to hydrolysis by older generation β-lactamase inhibitors and cephamycins; consequently, **AmpC-producing strains are resistant to cefoxitin and synergy with clavulanic acid is not observed unlike in ESBL producing strains**.

Distinguishing AmpC-producing strains from ESBL-producing strains is clinically very important as they both require different therapeutic approaches. In these cases, molecular biology may provide support as dedicated tests are often used for research only. On the other hand, results from phenotypic antibiograms allow for differentiation between AmpC and ESBL production (Figure 14).

Third generation cephalosporins are AmpC substrates but not inducers and generally preserve *in vitro* activity against inducible AmpC-producing strains. However, therapy with third generation cephalosporins can select for resistant mutants with constitutive AmpC expression. For this reason, despite *in vitro* susceptibility, **the use of third generation cephalosporins against inducible AmpC-producing species is not recommended**. PIP/TAZ is also not a preferential option in this setting (Figure 15 - AmpC antibiogram). Within the ESCPM group, En*terobacter cloacae* group easily select for mutants with de-repressed AmpC. Therefore, third generation cephalosporins should always be avoided for the treatment of infections sustained by this group of organisms. Furthermore, the microbiology laboratory might decide to exclude these molecules for the production of susceptibility testing reports, with the exception of uncomplicated urinary infections [49].

ENZYME	CZA	CTX	CRO	CPD	FOX	FEP	TZP	IMI MEM	CLA/ CZA*
ESBL	V	V	V	R	S	V	V	S	+
AmpC**	R	R	R	R	R	S	R	S	-
CTX-M	V	R	R	R	S	V	V	S	+/-

CZA: *ceftazidime*, **CTX**: *cefotaxime*, **CRO**: *ceftriaxone*, **CPD**: *cefpodoxime*, **FOX**: *cefoxitin*, **FEP**: *cefepime*, **ATM**: *aztreonam*, **PIP/TAZ**: *piperacillin/tazobactam*, **IMI**: *imipenem*, **MEM**: *meropenem*, **CLA/ CZA**: *clavulanate / ceftazidime synergism*, **V**: *variabile S/R.*
**synergism, not necessarily susceptibility, ** *non-wild-type depressed strain profile.*

Figure 14. *ESBL, AmpC and CTX-M resistance phenotypes in Enterobacterales.*

Other species belonging to the ESCPM group have a lower ability to select for de-repressed mutants. Some authors advice treatment according to MICs, implying antibiotic selection according to MIC values reported on the phenotypic antibiogram. This may be performed provided the patient is not critical, a good source control is achieved and the antibiotic is administered at high dose and, when indicated, by means of continuous infusion [49]. More recently, AmpC-type enzymes encoded by transferable plasmids have also emerged in *Proteus mirabilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella enteritidis* [50]. The AmpC-type resistance mediated by plasmids is generally constitutive and the interpretation of the *sensitivity pattern* is often easy.

Antibiotics	MIC mg/L
Amikacin	S ≤ 2
Piperacillin/ tazobactam	S ≤ 4
Cefepime	S ≤ 1
Cefotaxime	S ≤ 1
Ceftriaxone	S ≤ 1
Ceftazidime	S ≤ 1
Ciprofloxacin	S ≤ 0.25
Imipenem	S ≤ 0.25
Meropenem	S ≤ 0.25

Figure 15. *BAL: Enterobacter cloacae complex > 100 thousand UFC/ml. E cloacae harbors inducible chromosomal AmpC.*

Cefepime, a fourth-generation cephalosporin, unlike other cephalosporins, is much less affected by AmpC hydrolysis, thus representing an excellent alternative for the treatment of these infections. Indeed, cefepime MICs remain low and often within the susceptibility range (≤1 mg/L) as opposed to other cephalosporins.

BLICs based on new β-lactamase inhibitors (avibactam and vaborbactam) represent a carbapenem sparing resource when cefepime fails. Lee *et al.* retrospectively analysed over 300 cases of *Enterobacter cloacae* BSI and found that **cefepime is not inferior to carbapenems with the exception of susceptible-dose-dependent (SDD) strains**.

In this study, difference in 30 day-mortality was not statistically significant

Cefepime is comparable to carbapenems

(26.4% in the cefepime group vs 22.2% in the carbapenem group, p = 0.7) [51].

A meta-analysis by Harris *et al.* conducted on seven observational studies found no significant differences in mortality between BLIC (essentially piperacillin/tazobactam and cefepime) and carbapenem based regimens administered as either empirical and targeted therapy (OR 0.87; 95% CI: 0.32-2.36 and OR 0.48; 95% CI: 0.14-1.60, respectively) [52]. However, currently available data, albeit deriving only from observational studies, favours cefepime over piperacillin/tazobactam [49].

A recent retrospective cohort study by Tan *et al.* including 241 patients with bacteraemia due to ESCPM group found no statistically significant differences in 30-day mortality between the two treatment arms comparing empirical treatment with either PIP/TAZ (aOR 0.29; CI 95%: 0.07-1.27) or cefepime (aOR 0.65; 95% CI: 0.12-3.55) versus meropenem regimens [53].

Cefepime, however, should be used with caution on strains with reduced sensitivity; carbapenems should undoubtedly be preferred in these cases. Ceftazidime/avibactam demonstrated greater efficacy as opposed to C/T for the treatment of AmpC-producing *Enterobacterales*. Isler *et al.* recently performed a meta-analysis on five randomized controlled trials (272 patients in total) comparing ceftazidime/avibactam (246 patients) to carbapenems (271 patients) for the treatment of ESBL and AmpC-producing *Enterobacterales* infections. Reported clinical response at TOC (test of cure) was 91% in the CZA/AVI arm and 89% in the carbapenem arm for ESBL producers (RR 1.02; 95% CI: 0.97-1.08; p = 0.45; I2 = 0%.). However, when AmpC producers were considered, clinical response at TOC in the CZA/AVI arm was 80% (32/40) vs 88% (37/42) in the carbapenem arm (0.91; 95% CI: 0.76-1.10; p = 0.35; I2 = 0%.) No data is available on microbiological response and mortality. The authors conclude that CZA/AVI may represent a valid option for the treatment of ESBL-producing *Enterobacterales sustained infections*, but

Carbapenem better than cefepime on reduced susceptibility strains

no definitive recommendations can be made on the role of CZA/AVI for the treatment of AmpC producers [54].

Avibactam inhibits class C β-lactamases by means of direct interaction of its sulfonate groups with the Asn^{346} amino acid residue belonging to AmpC cephalosporinase. In *Citrobacter freundii*, the substitution of Asn^{346} by $N^{346}Y$ correlated with the acquisition of AmpC resistance against CZA/AVI. This plasmid-mediated mechanism of resistance attributed to the substitution of Asn^{346} to $N^{346}Y$, has also been reported in both *Enterobacter cloacae* and in *Pseudomonas* strains. Compain *et al.* suggest that loss of hydrogen interactions between Asn^{346} and avibactam could explain the mechanism of resistance to CZA/AVI in AmpC-producing bacterial strains [55]. These particular mutants are referred to as **extended-spectrum AmpCs (ESAC)** and have been described after prolonged exposure to cefepime. They display resistance to fourth generation cephalosporins, avibactam and reduced susceptibility to cefiderocol [56, 57].

ESAC

CARBAPENEMASES

The underlying cause of antibiotic resistance in *Enterobacterales* may be ascribed to several mechanisms: reduction of membrane permeability (by alteration of porin channels and / or over-expression of efflux pumps), overproduction of ESBL or AmpC-type β-lactamases, production of carbapenemases, capable of efficient carbapenem hydrolysis.

Carbapenemase production is the most relevant mechanism of resistance both at clinical and epidemiological level. Expression of carbapenemases must be suspected upon meropenem MICs > 0.125 mg/L (despite the EUCAST clinical breakpoint is set to a higher value) and confirmed by the use of phenotypic or genotypic tests. The therapeutic approach to carbapenem-resistant *Enterobacterales* (CRE) infections has evolved in recent years thanks to the introduction of new and highly efficacious antibiotics targeting these pathogens.

Treatment options for KPC

Several carbapenemases have been reported in *Enterobacterales*: class A serine-carbapenemase (KPC and the less common IMI, SME, FRI and GES), class D serine-carbapenemase (OXA-48 like), along with MβLs (VIM, NDM and the rarer IMP, GIM and KHM). In the past, carbapenemase characterization was of epidemiological interest. Nowadays, however, it has acquired considerable clinical relevance as different antibiotics with anti-CPE spectrums are also characterized by enzyme-specific profiles.

Ceftazidime/avibactam has replaced colistin-based regimens as therapeutic backbone for the treatment of infections stained by KPC-producing strains [58].

In a prospective study involving patients with KPC-kp (KPC producing *Klebsiella pneumoniae*) BSI, Shields et al. demonstrated that CZA/AVI was superior in terms of efficacy, mortality, and clinical cure at 30 days, compared to any other option [59]. The use of CZA/AVI as a standalone molecule or in combination regimens is however still a matter of debate. An Italian retrospective case-control study assessed the use of CAZ-AVI as compassionate use therapy in 104 patients with KPC-kp BSI from. Study results confirmed that 30-day mortality was significantly lower in the CZA/AVI treated group (36.5% vs 55.7%; p = 0.005). Of notice, CZA/AVI was used in combination with other drugs in 78% of cases (20% of cases with carbapenems) [60].

Karaiskos *et al.* recently published a prospective observational multicentre study including 140 KPC- and 7 OXA-48-producing strains treated with CZA/AVI alone or in combination. An 18.3% 28-day mortality was observed in the monotherapy treated arm (46.3% of cases) versus 40.8% 28-day mortality in the combo therapy group (53.7% of cases) (p = 0.005) [61]. A meta-analysis by Onorato *et al.* demonstrated that efficacy of CZA/AVI monotherapy for the treatment of CRE infections was comparable to combination regimens [62].

Nonetheless, several reports warn on potential dangers associated to CZA/AVI monotherapy. Indeed, the use of CZA/AVI alone has been related to the selection of resistant strains through different mechanisms: porin deficien-

cy (mutations in OmpK36), over-expression of efflux pumps or KPC enzyme mutations, often associated with functional alterations of the enzyme. Here are a couple of examples: D179Y and 165EL166 determine loss of activity on carbapenems, piperacillin/tazobactam and aztreonam; T243M causes loss of activity on carbapenems and piperacillin/tazobactam; V240G reduces activity on meropenem [16-18, 63].

In a recent paper, Bianco *et al.* observed *in vivo* selection of two subpopulations of ***Klebsiella pneumoniae*** harbouring a KPC-2 variant displaying significantly increased MICs following prolonged exposure to CZA/AVI. The strains harboured a deletion in the **D242-GT-243 position** (bla_{KPC-14}) and KPC-33, a blaKPC-2 variant, featuring a **D179Y** mutation (bla_{KPC-33}). The latter is characterized by loss of carbapenemase activity and increased affinity to ceftazidime, preventing avibactam's binding and inhibition of the enzymatic activity [64].

Currently, in clinical practice, CZA/AVI is often included in combination therapy regimens, especially for the treatment of lower respiratory tract infections, in order to protect its effectiveness.

CZA/AVI is most frequently combined with meropenem, as it may guarantee activity against CZA/AVI-resistant KPC mutants harbouring the D179Y mutation [17], gentamycin (currently the least employed), and fosfomycin.

Fosfomycin is a concentration-dependent antibiotic exhibiting time-dependent pharmacological features, thus justifying its high dose administration at very short intervals or continuous/prolonged infusion regimens. The recommended dose in critically ill patients is 6g q6h (Figure 16). The time-dependent pharmacokinetic (PK) driver reduces the risk of rapid resistance induction to fosfomycin [65]. Over 60% of KPC-kp strains in Italy are susceptible to fosfomycin.

Shields *et al.* observed *in vitro* antagonistic effect between colistin and CZA/AVI in 46% of the KPC-kp strains tested, which would suggest against the use of this association [66]. The approval of meropenem/vaborbactam and

imipenem/relebactam will significantly increase the number of available options against KPC-producing strains.

Meropenem/vaborbactam (MEM/VAB) is the most potent association in terms of activity against KPC-kp strains. Vaborbactam is a novel boronic acid derivative and non-β-lactam β-lactamase inhibitor, and acts by protecting meropenem from hydrolysis [67]. In addition, meropenem/vaborbactam appears to have a lower propensity to induce emergence of resistance during treatment as opposed to CZA/AVI, especially in susceptible isolates with MICs ≤ 4/8 mg/L [68].

Antibiotics	MIC mg/L
Amoxicillin/Clav. acid	>64 R
PIP/TAZ	>256 R
Ceftazidime	>64 R
Ertapenem	>2 R
Imipenem	0.5 S
Meropenem	2 S
CZA/AVI	>16 R
Amikacin	32 R
Gentamicin	1 S
Tigecycline	0.5 S
Colistin	0.5 S
Ciprofloxacin	>2 R

Figure 16. *Antibiogram of Klebsiella pneumoniae D179Y (blaKPC-33).*

However, mutations capable of inducing resistance also to MEM/VAB have already been described in two main membrane porins, ***OmpK35*** and ***OmpK36*** [69]. More specifically, Dulyayangkul *et al.* reported a mutation in kvrA, a transcriptional repressor gene, which determines down-regulation of the ***OmpK35*** and ***Ompk36*** porin channels and a reduced susceptibility to meropenem/vaborbactam in KPC-producing strains of ***Klebsiella pneumoniae*** [70]. ***OmpK36*** plays a major role in the passage of meropenem/vaborbactam across the bacterial wall. The sequential or combined use of CZA/AVI and MEM/VAB could induce resistance to both through the following steps: ***OmpK36*** mutation; ramR mutation; acquisition of OXA-232 and KPC-3-D179Y plasmids. Therefore, the combined or sequential therapeutic option is currently not to be pursued.

The pOXA-232 plasmid encoding for the OXA-232 carbapenemase induces ramR mutation thus causing an overproduction of AcrAB-TolC (important membrane efflux pump) and a reduced expression of porin ***OmpK35*** [71].

Compared to CZA/AVI, imipenem/relebactam (IMI/REL), another combination consisting of a protected carbapenem and a potent KPC-2 inhibitor [72], displays greater binding stability with the target enzyme and improved ***epithelial lining fluid (ELF)*** penetration. In particular, ELF penetration for each molecule is as follows: 20/25% for CZA/AVI, 65/79% for MEM/VAB and 55% for IMI/REL [73].

The main mechanism of resistance to IMI/REL is represented by altered bacterial membrane permeability ascribed to ***OmpK36*** mutations. **The approved dosage scheme is 2g q8h administered as extended infusion (3h) for MEM/VAB, and 1.25g q6h in 30 minutes for IMI/REL.**

Ceftazidime/avibactam is considered as the "gold standard" for the treatment of OXA-48 carbapenemase producing strains. As of today, there are yet no reports concerning resistance when CZA/AVI is administered as monotherapy [74], since avibactam retains a high level of activity against OXA-48 [75]. OXA-48 enzymes cause high level resistance to penicillins and carbapenems, though hydrolysis of the latter occurs at a slower rate [76]. Different phenotypic OXA-48 variants are known [20]; some have greater affinity for carbapenems (OXA-162, OXA-181), others show preferential activity against oximino-cephalosporins, such as ceftazidime (ESBL-like OXA such as OXA-163 and OXA-405). The carbapenem-hydrolizing activity of OXA-48 is stronger against imipenem and ertapenem compared to meropenem.

Treatment options for OXA

Hrabak *et al.* argue that the weaker and inconsistent activity of OXA-48 against carbapenems is responsible for a troublesome detection of this specific mechanism of resistance [77]. OXA-48 is not susceptible to old β-lactamase inhibitors (clavulanate, sulbactam, tazobactam), with the only exception of OXA-163.

In addition, OXA-48-producing strains, are often co-carriers of additional β-lactamases such as MβLs and ESBLs [78] (Figure 17).

METALLO-β-LACTAMASE (MβL)

Metallo-β-lactamases (MβLs) are not susceptible to BLICs, as avibactam, relebactam and vaborbactam do not display any inhibitory activity against this class of enzymes. Treatment of infections caused by MβL producing organisms has historically included colistin-based regimens. More recently, aztreonam in combination with ceftazidime/avibactam [79] and **cefiderocol** have been employed, with efficacies of **100% and 64% against IMP/VIM and NDM** [80] **producers respectively**. In addition, new molecules are under investigation.

Aztreonam is resistant to hydrolysis by Gram-negative MβLs, though it is readily inactivated by class A and class C β-lactamases, often co-expressed by the same strains. Avibactam inhibits ESBLs and AmpCs, offering protection to aztreonam (AZT) [81]. The optimized AZT+CZA administration scheme would therefore stand as follows: CZA 2.5g q8h and AZT 8g q24h both administered by continuous infusion [82]. Aztreonam/avibactam alone, is still in its final stages of development.

Niu *et al.* reported a 128-fold MIC reduction in MβL-producing *Klebsiella pneumoniae* upon combina-

Antibiotics	MIC mg/L
Amoxicillin/Clav. acid	>128 R
Piperacillin/tazobactam	>128 R
Cefotaxime	32 R
Ceftazidime	32 R
Cefepime	64 R
ESBL	+
Imipenem	2 S
Meropenem	1 S
Ertapenem	>32 R
Ciprofloxacin	>32 R
Amikacin	2 S
Gentamicin	1 S

Figure 17. *OXA-48 and ESBL-producing Klebsiella pneumoniae broncho-aspirate samples (BAS).*

tion of avibactam with aztreonam yielding MIC50 and MIC90 of 0,25 and 1 mg/L respectively. Within the same study, the authors alarmingly reported development of in-vitro resistance to this new combination. An ST 101 lineage Klebsiella pneumoniae strain harbouring NDM-1, OXA-48, CTX-M-15, CYM-16 showed resistance to aztreonam/avibactam with a 16-fold MIC increase. Genome sequencing revealed aminoacid substitution in the Tyr-150Ser and Asn346His positions of the CYM-16 gene, responsible for the newly acquired resistance pattern [83].

The association of AZT+MEM/VAB has shown similar activity to AZT+CZA in MβLs-CRE strains lacking OXA enzyme production [84].

Two diazabicyclooctane, nacubactam and zidebactam, in combination with meropenem and cefepime respectively, are currently under investigation. Along with serine *β-lactamase* inhibition, navubactam and zidebactam also show intrinsic antibacterial activity as PBP2 inhibitors, providing synergistic effect with β-lactams targeting PBP3. Cefepime/zidebactam and meropenem/nacubactam are effective against over 75% of MβLs producing CREs [85].

However, taniborbactam seems to be the most promising molecule. The association of taniborbactam with cefepime is currently undergoing development (VNRX-5133). Taniborbactam is a bicyclic boronate with displayed activity against class A, B, C and D β-lactamases. Taniborbactam covalently binds serine β-lactamases enzyme occupying the active site of the enzyme for a prolonged time before slowly dissociating. With MβLs, on the other hand, it behaves according to competitive inhibition [86]. Taniborbactam is effective against most B1 MβLs (VIM and NDM), but weak against IMP-producers, which to date are still uncommon (0.4% of CRE, 3.4% of MβLs-producers) [87].

CEFIDEROCOL

Cefiderocol: the Trojan horse strategy

Cefiderocol is a new generation cephalosporin and the first of a new group of antibiotics that behave as siderophore (iron carrier) with a **trojan horse-like mechanism** (Figure 18).

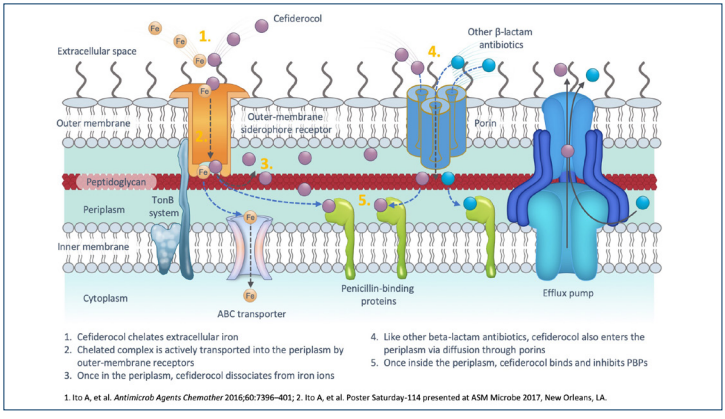


Figure 18. *Mechanism of action of cefiderocol.*

Cefiderocol chelates iron ions using its catechol group; iron is a key co-factor of bacterial enzymes, and it is imported as cefiderocol- Fe^{3+} complex through active transport. The protein complex TonB, ExbB, ExbD is responsible for generating the energy required for active transport, while ***TBDT (TonB dependent transporter)*** actively moves the iron-siderophore complex into the periplasmatic space. Within the periplasm, cefiderocol dissociates from iron which gets further moved to the cytoplasm where it is oxidized to Fe^{+2} and stored or included into enzymes as co-factor. Cefiderocol binds PBP3 of Gram-negative bacteria. The unique active transport mechanism allows cefiderocol to overcome most of the previously discussed resistance mechanism [88].

Cefiderocol is active against most Gram-negative bacteria (*Enterobacterales* and non-fermenting species) harbouring class A, B, C and D β-lactamase. In the SIDERO-WT-2014 study, cefiderocol was tested against 1,272 strains of carbapenem-resistant *Enterobacterales*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* demonstrating elevated susceptibility rates in the presence of a variety of resistance mechanisms. Sensitivity was ob-

served in 100% of OXA58-producers (MIC_{90} 1 µg/ml), KPC-producers (MIC_{90} 2 µg/ml), OXA48-like-producers (MIC_{90} 1 µg/ ml), in 97.2% of OXA23-producers (MIC_{90} 1 µg/ml), 95.2% of OXA24-producers (MIC_{90} 1 µg/ ml), 91.7% of GES-producers (MIC_{90} 4 µg/ml). Sensitivity against MβLs showed wider ranges (MIC ≤4 µg/ml in 97.7% of strains) spanning from 100% in IMP- (MIC 1-2 µg/ml) and VIM-producers (MIC_{90} 2 µg/ml), to 64.3% in NDM-producers (MIC_{90} 8 µg/ml) [80]. Mushtaq *et al.* evaluated cefiderocol's MICs against 305 *Enterobacterales* isolates tested in iron-depleted Mueller-Hinton broth. They found that concentrations of 2 and 4 mg/L of cefiderocol inhibited 78.7% and 92.1% of all strains respectively. Efficacy against carbapenem-resistant isolates ranged between 80% and 100% with the exception of NDM-producers (41% inhibited by 2 mg/L and 71% inhibited by 4 mg/L) and isolates co-expressing ESBL and porin loss (61.5% at 2 mg/L and 88.5% at 4 mg/L) [89]. Interestingly, ceftazidime/avibactam resistant KPC-producing strains may retain susceptibility to cefiderocol [90] as shown by the antibiogram reported in Figure 19.

Antibiotics	MIC mg/L
Amoxicillin/Clav. acid	>64 R
PIP/TAZ	>128 R
Cefotaxime	>64 R
Ceftazidime	>64 R
Cefepime	>64 R
Ertapenem	>32 R
Imipenem	>16 R
Meropenem	>32 R
Amikacin	>64 R
CZA/AVI	>8 R
MEM/VAB	>16 R
Gentamicin	>16 R
Levofloxacin	>8 R
Tigecycline	0.5 S
Colistin	>16 R
Cefiderocol	0.5 S

Figura 19. *Phenotypic antibiogram of KPC-overexpressing Klebsiella pneumoniae, resistant to CZA/AVI and MEM/VAB, but retaining susceptibility to cefiderocol (courtesy of Dr. Tommaso Giani).*

Cefiderocol inhibits biofilm-producing Gram-negative strains, pos-

Cefiderocol: inhibitory activity against MDR Gram-negative strains

sibly related to the role played by iron in biofilm formation among *Enterobacterales* of the *Serratia*, *Escherichia* and *Klebsiella genera* [91,92]. The iron import system is indeed up-regulated upon biofilm production, therefore providing the ideal setting for cefiderocol to exert its bactericidal potential [93].

Pybus *et al.* have reported consistently lower cefiderocol MIC_{90} compared to other antibiotics (ceftolozane/tazobactam, ceftazidime/avibactam, piperacillin/tazobactam, ceftazidime, tobramycin, imipenem, clarithromycin) in Gram-negative MDR isolates. In particular, cefiderocol showed superior activity in biofilm formation reduction in Pseudomonas strains as opposed to comparators (93% vs 49-82%, with a reduction of 82% in ceftolozane/tazobactam). In *Klebsiella pneumoniae*, *Stenotrophomonas maltophilia* and *Burkholderia cepacia*, cefiderocol's activity in reducing biofilm production ranged between 83% and 91%.

Cefiderocol: powerful anti-biofil activity

Cefiderocol's Summary of Product Characteristics is the first to contain detailed information on optimized dose adjustment, especially in the case of particular PK/PD scenarios. Cefiderocol should be administered as an extended infusion (2g q8h over three hours) to ensure optimal exposure in the interval between doses and decrease the risk of resistance emergence. In case of Augmented Renal Clearance (CrCl≥120 ml/min), such as in critical patients with sepsis, the dose should be increased to 8g/24h (**2g q6h 3hr infusion**). Dose-adjustments for renal function is required only for CrCl<15 mL/min or intermittent haemodialysis (0,75g q12h) [94-96].

Kawaguchi *et al.* [97] recently published results from a pharmacokinetic population model analysis based on 3,427 determinations of cefiderocol plasma levels in 91 volunteers and 425 patients with pneumonia, BSI/sepsis and cUTI. They reported a 90% probability of therapeutic target attainment with registered dosage regimens (100% of the time over MIC – fT>MIC) with MICs ≤ 4 mg/L. This held true for all sites of infection regardless of renal function except for patients with BSI/sepsis and normal renal function, whereby the

probability of target attainment was 85%. Moreover, Kawaguchi's study suggests that **site of infection and blood albumin concentration do not affect cefiderocol kinetics**. As a fact, albumin levels above or below 2.8 g/dl had no impact on cefiderocol's C_{max} and daily AUC at steady state were similar in both groups of patients [84] [97].

Also, **ventilation does not affect cefiderocol's plasma levels** as opposed to ceftazidime, whereby a 50% Vd reduction is reported to occur in ventilated patients [98].

Cefiderocol, similarly to carbapenem-BLI combinations, **has a good ELF** (epithelial lining fluid) **penetration** as reported by Katsube *et al.* [99]. They demonstrated that approved dosing schemes of cefiderocol ensure ELF target attainment of 100% fT>MIC, with MICs ≤ 4 mg/L regardless of renal function. In conclusion, we propose a couple decisional algorithms that include fast microbiology investigations, to guide antibiotic selection for the treatment of severe syndromes caused by ESBL, KPC and MβL producing strains (Figures 20-22)

Cefiderocol: good ELF penetration

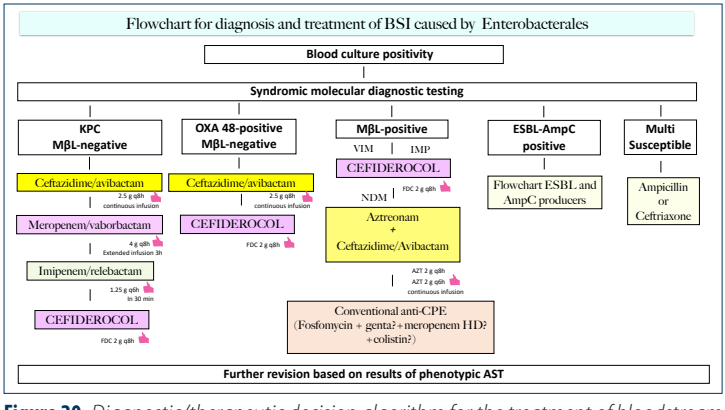


Figure 20. *Diagnostic/therapeutic decision algorithm for the treatment of bloodstream infections due to KPC, OXA-48 and MβL-producing Enterobacterales.*

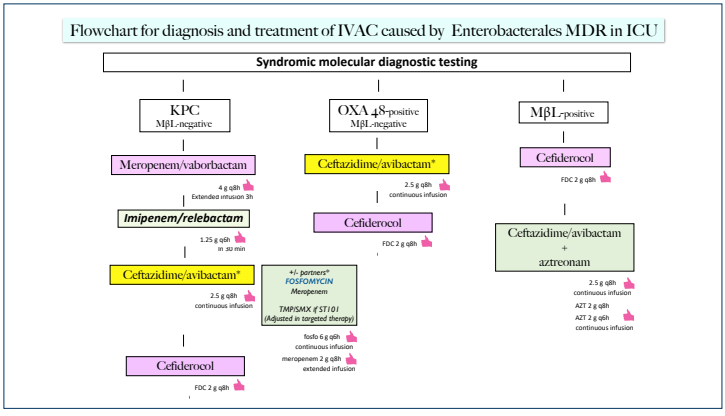


Figure 21. *Diagnostic/therapeutic decision algorithm for the treatment of ventilator-associated pneumonia due to KPC, OXA-48 and MβL-producing Enterobacterales.*

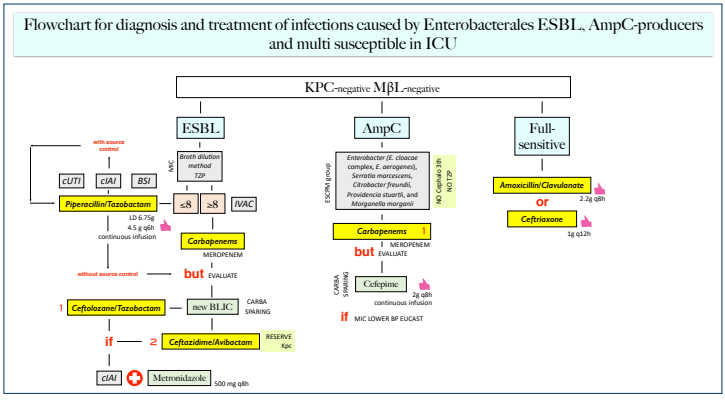


Figure 22. *Diagnostic/therapeutic decision algorithm for the treatment of ESBL, AmpC-producing and multi-susceptible Enterobacterales sustained infections.*

Pseudomonas aeruginosa

Pseudomonas aeruginosa is considered among the most dangerous nosocomial pathogens, especially due to the development of multi-drug resistance (MDR) and pan-drug resistance (PDR). *Pseudomonas aeruginosa* often causes infections in critically ill and in immunosuppressed patients. In patients with febrile neutropenia, *Pseudomonas aeruginosa* represents the leading cause of death in case of bacteremia.

Pseudomonas aeruginosa easily and rapidly acquires resistance to antibiotics and can spread these determinants further [100]. Moreover, spreading of hypervirulent clones represents the underlying cause of most nosocomial epidemics. MDR strains have increased in recent years and detection of 15-30% of resistant isolates are not uncommon in some geographic areas [101]. According to EARSNET, 5.5% of *Pseudomonas aeruginosa* strains are resistant to all 5 antibiotics undergoing periodic monitoring, whereas 13% of strains are resistant to at least 3 antimicrobial agents [102]. Table 1 reports resistance of *Pseudomonas aeruginosa* to five different classes of antibiotics in Italy between 2015 and 2019.

The main resistance mechanisms of *Pseudomonas aeruginosa* include reduced membrane permeability and of β-lactams inactivation.

Membrane impermeabilization is achieved via porin mutation and/or downregulation as well as by over-expression of efflux pumps. Porin channels enable diffusion of hydrophilic substances through the outer membrane of Gram-negative bacteria. In the presence of porins, β-lactams cross the membrane into the periplasmic

Main resistance mechanisms of *Pseudomonas aeruginosa*

SPECIES	ANTIBIOTIC RESISTANCE	2015	
		N	%
Pseudomonas aeruginosa	Resistance to PIP/TAZ	1,074	28.7
	Resistance to CZA	1,068	21.7
	Resistance to carbapenems (IMI/MER)	1,082	22.8
	Resistance to fluoroquinolones (CPX/LEV)	1,080	24.6
	Resistance to aminoglycosides	1,050	17.2
	Combined resistance to ≥3 groups of antimicrobials (PIP/TAZ, CZA, carbapenems, FQ, aminoglycosides)	1,082	19.8

Table 1. *Prevalence of Pseudomonas aeruginosa resistance in Italy from 2015 to 2019.*

space where they bind and inhibit the bacteria penicillin-binding proteins (PBPs). Efflux pumps, on the other hand, are complex protein pumps that actively expel substances from the bacterial cytoplasm to the extracellular environment.

Porin OprD is a substrate specific channel and main entry point for carbapenems. Its inactivation is either due to mutations or insertions in specific genes, as well as to down-regulation of genes due to ORF mutations. Such mutations lead to resistance to both meropenem and imipenem. Resistance to imipenem in over 20% of cases is due to inactivation of this porin.

Pseudomonas aeruginosa harbors 4 efflux pumps which are associated to mutations causing hyperactivation.

MexAB-OprM is detected in 10-30% of MDR *Pseudomonas aeruginosa* strains. This efflux pump exerts its activity on FQs and causes low level resistance to many β-lactams including ceftazidime/avibactam (CZA/AVI) and meropenem. MexAB-OprM associated with porin OprD inactivation is the

2016		2017		2018		2019	
N	%	N	%	N	%	N	%
1,146	29.8	1,309	23.2	2,938	23.9	3,768	24.1
1,160	23.0	1,332	20.0	2,974	19.9	3,798	19.1
1,206	23.3	1,433	19.6	3,014	15.8	3,793	13.7
1,166	24.7	1,390	25.1	2,994	22.9	3,874	21.7
1,203	19.1	1,428	18.0	2,983	12.8	3,859	11.4
1,205	19.8	1,434	17.2	3,006	14.9	3,882	13.1

most frequent cause of resistance to meropenem [103]. On the other hand, imipenem (IMI), imipenem/relebactam (IMI/REL) and ceftolozane/tazobactam (C/T) escape its effect and may not be considered substrates [104]. C/T shows significantly lower rates of resistance compared to CZA/AVI in isolates of ***Pseudomonas aeruginosa*** with reduced oprD porins and increased MexB expression [105].

The MexXY pump is present in 10-30% of *Pseudomonas aeruginosa* strains; FQ and cefepime are both substrates. MexXY pumps represent the intrinsic resistance mechanism against aminoglycosides [106].

Two other efflux pumps are rarer, such as MexCD-OprJ (present in 5% of strains) targeting FQ and cefepime and MexEF-OprN (present in 5% of strains) which expels FQ and sometimes imipenem, especially in case of OprD inhibition.

Efflux pumps overexpression, unlike β-lactamases or DNA gyrase mutations for fluoroquinolones, rarely produce MIC increases. However, in association

with other mechanisms of resistance, they may contribute to antibiotic inefficacy. Indeed, Gomis-Font *et al.* recently reported in vitro selection of resistance to imipenem/relebactam due to MexAB-OprM efflux pumps, in association with inhibition of porin OprD and PBP1 mutations [107].

Cefiderocol is not affected by the activity of either efflux pumps or porin mutations as it overcomes reduced membrane permeability by using active iron transport channels to access the periplasmic space [108].

Resistance to β-lactams in *Pseudomonas aeruginosa* mainly occurs via production of beta-lactamase and PBP mutations. Penicillin-binding proteins are the molecular target of β-lactams antibiotics. Covalent binding of β-lactams with PBPs inhibits the latter and prevents termination of peptidoglycan resulting in bacterial cell death. Mutations leading to PBP target site modification results in β-lactam resistance. On the other hand, β-lactamase are bacterial enzymes that hydrolyze β-lactams. AmpC is the most commonly encountered beta-lactamase in Pseudomonas.

AmpC overproduction is the most frequently detected mechanism of resistance in ***Pseudomonas aeruginosa***. It is due to inactivating mutations of AmpD gene and PBP4 mutations. AmpC production can be further increased by AmpR mutations (transcription factor involved in regulating AmpC transcription). The AmpR R154H mutation is associated with the ST175 XDR strain epidemic [48, 109] (Figure 23). Aminopenicillins and cephalosporins (especially cefoxitin) are strong AmpC inducers leading to overexpression of this enzyme caused by mutations in genes encoding for regulatory molecules. Amp C overexpression accounts for resistance to many β-lactams except for cefepime, ceftolozane/tazobactam and imipenem. **Meropenem resists hydrolysis by AmpC along with the new beta-lactamase inhibitors (BLICs)** (avibactam, vaborbactam, relebactam) and cefiderocol, whose MIC is generally not affected by AmpC. Ceftolozane also evades ***Pseudomonas aeruginosa*** AmpC hydrolysis. Other clinically relevant chromosomal β-lactamases are OXA-50/Pox B.

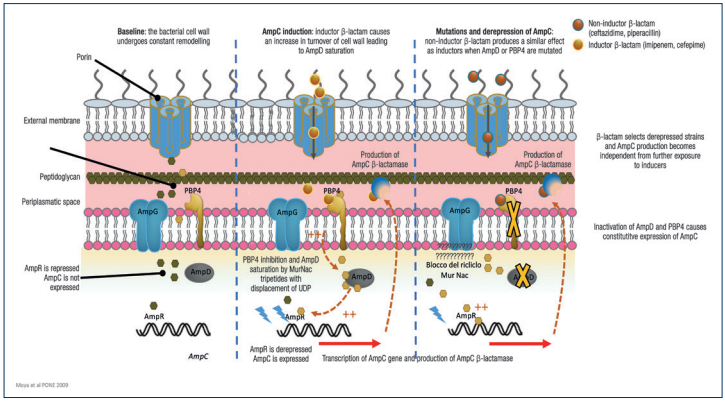


Figure 23. *Mechanism of inhibition and induction of the AmpC enzyme.*

Along with hyperexpression, **AmpC mutations** leading to both ceftolozane/tazobactam, and ceftazidime/avibactam resistance have been reported.

AmpC mutation and resistance to CZA and C/T

Hundreds of mutants have been identified and may be of clinical relevance when they yield detectable phenotypical changes. Table 2 enlists the **"Pseudomonas derived cephalosporinase" (PDC)**, mutant AmpC variants causing β-lactam resistance, including cefepime, C/T, CZA/AVI. MICs of PIP/TAZ, ceftazidime, cefepime and C/T and susceptibility category based on EUCAST Clinical Breakpoints (CBs) are reported for each variant. MICs of Cloxacillin (AmpC phenotypic inhibitor) are reported in brackets. If the MIC value does not drop by 3 dilutions, it implies that the enzyme has lost its ability of being inhibited by cloxacillin (phenotypic test for AmpC, genotypic test commercially unavailable). As reported in the table, some strains may exhibit intermediate susceptibility to piperacillin/tazobactam along with resistance to C/T. Moreover, the information conveyed by MIC values for ceftazidime and cefepime may suggest the presence of an underlying mutant, as well as AmpC inhibition by cloxacillin. Effects of other β-lactamase inhi-

PDC	MUTATIONS	PIP/TAZ MIC	CEFTAZIDIME MIC	CEFEPIME MIC	C/T MIC
50	T79A, **V213A**	32(4) R	64(2) R	8(1) I	4(0,5) S
74	T79A, **G216R**	8(4) I	>64(32) R	8(4) I	8(4) R
78	**R100H, G216R**	32(16) R	>64(>64) R	16(16) R	16(16) R
86	1GD, T79A, V179L, **E221K**	16(16) I	>64(>64) R	16(8) R	>64 (64) R
90	1GD, T79A, V179L, **DT290-DM292**	16(4) I	>64(8) R	>64 (16) R	4(1) S

Table 2. *Pseudomonas derived cephalosporinase (PDC).*

bitors and other β-lactams may also be of interest. **Penicillin binding protein modifications may also confer resistance to β-lactams**. Penicillin Binding Proteins (PBPs) are classified according to their molecular weight as either high or low molecular weight. High molecular weight PBPs include PBP1a/1b, PBP2, PBP3 and are considered essential PBPs for the bacterial life cycle as they are involved in the final stages of peptidoglycan synthesis. Indeed, inactivation of PBP1a/1b causes bacterial lysis and death in *Escherichia coli*, whereas PBP2 confers rod morphology. Inactivation of PBP3 causes filamentation of the bacterium while PBP 3 mutations also confer resistance to β-lactams such as **ceftazidime, cefepime, piperacillin/tazobactam, ceftolozane/tazobactam, ceftazidime/avibactam, and meropenem**.

Pseudomonas aeruginosa low molecular weight PBPs (PBP4, PBP5 and PBP7) have been studied for their role in determining resistance to β-lactams. As a fact, clinical isolates harboring PBP4 mutations (PA3047) have been correlated to increased β-lactam resistance due to the induction of chromosomal AmpC, while the role played by PBP5 (PA3999) is yet unknown. Table 3 reports the I_{50} concentrations for different β-lactams as a function of their binding to the different PBPs in ***Pseudomonas aeruginosa***.

The different activity exerted by cephalosporins against *Pseudomonas aeruginosa* may be guessed according to their capacity to inhibit PBPs which is inversely proportional to the effective concentration. As a result, ceftobiprole and ceftolozane have the greatest activity on PBP4, while displaying no inhibition of PBP5/6. Carbapenems are the most potent β-lactams according to the PBP binding capacity, including PBP4 for imipenem.

The vast variety of resistance mechanisms exerted by *Pseudomonas* and the equally diverse phenotypes can result in insidious interpretation of antibiograms. The following considerations might facilitate the task.

Clinical breakpoints set by CLSI and EUCAST change over time. Indeed, EU-

	PBP1A	PBP1B	PBP2	PBP3	PBP4	PBP5/6	MIC µ/ml
Ceftriaxone	0.2	ND	ND	ND	**ND**	ND	ND
Ceftobiprole	0.1	0.5	3	0.1	**0.2**	>32	1
Ceftazidime	0.2	5	>32	0.1	**2**	>32	1
Cefepime	0.1	2	8	0.1	**0.3**	>32	2
Imipenem	0.5	0.5	0.1	0.1	**0.01**	2	1
Aztreonam	2	2	16	0.03	**16**	>16	4
Ceftolozane	0.12	0.89	1.59	0.04	**0.21**	>2	0.5
Avibactam	>13	>13	1.1	1.8	**11**	>13	>128

Tabella 3. *I_{50} concentrations of β-lactams.*

CAST revised its *Pseudomonas aeruginosa* breakpoints over the past years as clarified in table 4 [110].

Available molecular antibiograms performed on *Pseudomonas aeruginosa* provide information exclusively on the most common genes encoding for carbapenemases. Absence of β-lactam resistance mechanisms cannot be inferred solely upon negative molecular test results (Figure 24).

ANTIBIOTIC	SUSCEPTIBILITY AS DETERMINED BY PREVIOUS CB (mg/L)	RESISTANCE AS DETERMINED BY PREVIOUS CB (mg/L)	SUSCEPTIBILITY AS DETERMINED BY CURRENT CB (mg/L)	RESISTANCE AS DETERMINED BY CURRENT CB (mg/L)
Piperacillin/ tazobactam	≤16	>16	≤0.001	>16
Cefepime	≤8	>8	≤0.001	>8
Ceftazidime	≤8	>8	≤0.001	>8
Ceftazidime/ avibactam	≤8	>8	≤8	>8
Ceftolozane/ tazobactam	≤4	>4	≤4	>4
Imipenem	≤4	>4	≤0.001	>4
Imipenem/ relebactam	ND	ND	≤2	>2
Meropenem	≤2	>8	≤2	>2
Meropenem/ vaborbactam	ND	ND	≤8	>8
Aztreonam	≤16	>16	≤0.001	>8
Cefiderocol	ND	ND	≤2	>2

Table 4. *EUCAST clinical breakpoint for Pseudomonas aeruginosa.*

A negative molecular test likely implies susceptibility to C/T; however, a phenotypic test remains mandatory in such cases.

Resistance to carbapenems can be detected by means of phenotypic assays despite a negative molecular antibiogram result as carbapenem resistance in *Pseudomonas aeruginosa* is mainly due to non-enzymatic mechanisms.

CTX-M	Not detected
KPC	Not detected
VIM	Not detected
IMP	Not detected
NDM	Not detected
OXA-48	Not detected

Figure 24. *Pseudomonas aeruginosa, molecular antibiogram.*

Intermediate resistance to PIP/TAZ can coexist with resistance to C/T. A few examples of possible phenotypic susceptibility patterns are shown in Figure 25 (A-E), ranging from total susceptibility to all available anti-pseudomonal molecules to full resistance to carbapenems.

ANTIBIOTIC	STRAIN 1	MIC (mg/L)
Amikacin	S	≤4
Cefepime	S	2
Ceftazidime	S	2
Ciprofloxacin	S	0.5
Gentamycin	S	0.5
Imipenem	S	≤1
Meropenem	S	0.5
Piperacillin/ tazobactam	S	8/4
Ceftolozane/ tazobactam	S	≤1/4

Figure 25A.

ANTIBIOTIC	STRAIN 1	MIC (mg/L)
Amikacin	S	4
Ceftazidime	S	2
Ciprofloxacin	S	0.12
Gentamycin	S	≤1
Meropenem	I	4
Piperacillin/ tazobactam	S	≤4

Figure 25B.

ANTIBIOTIC	STRAIN 1	MIC (mg/L)
Amikacin	S	≤4
Ceftazidime	R	16
Ciprofloxacin	R	8
Gentamycin	S	2
Meropenem	S	2
Piperacillin/ tazobactam	R	32/4

Figure 25C.

ANTIBIOTIC	MIC mg/L
Amikacin	<4 S
Aztreonam	16 I
Ciprofloxacin	>16 R
Ceftazidime	>32 R
Colistin	≤1 S
Gentamycin	16 R
Meropenem	>32 R
Piperacillin/ tazobactam	≤1/4 S
Ceftolozane/ tazobactam	≤1/4 S

Figure 25E.

(A-E. Possibile phenotypic antibiograms of Pseudomonas aeruginosa related to antibiogram depicted in Figure 24).

ANTIBIOTIC	STRAIN 1	MIC (mg/L)
Amikacin	S	4
Ceftazidime	S	2
Ciprofloxacin	R	≥4
Gentamycin	R	≥16
Meropenem	I	4
Piperacillin/ tazobactam	S	≤4

Figure 25D.

HORIZONTAL TRANSMISSION OF RESISTANCE

Pseudomonas aeruginosa is capable of acquiring resistance determinants by means of transmission of mobile agents, including ESBLs and carbapenemases, consequently leading to resistance to β-lactams, especially upon acquisition of metallo-enzymes [111]. The percentage of isolation ranges from 1% to 50% according to regional contexts and detection skills.

Among ESBLs, the most frequently detected enzymes are PER, VEB and GES types whereas β-lactam-

ases typically encountered in *Enterobacterales* such as TEM, SHV and CTX-M are rarely found.

Among the carbapenemases, metallo-β-enzime (MβLs) such as VIM and IMP are most frequently expressed, while KPC and GES are rarely reported [112]. Of notice, the latter are inhibited by avibactam [113].

CEFTOLOZANE/TAZOBACTAM (C/T)

Ceftolozane binds and inhibits most *Pseudomonas aeruginosa* PBPs while resisting to hydrolysis by AmpC (both wild type and mutant forms). Conversely, tazobactam protects ceftolozane from ESBLs, though the latter are rarely harbored by *Pseudomonas aeruginosa*. The association, however, does not offer protection against carbapenemases.

Pivotal studies conducted for ceftolozane/tazobactam (C/T) involved low percentages of Pseudomonas aeruginosa strains that were implicated in infections. This is true for both the study conducted by Solomkin *et al.* [35] where Pseudomonas aeruginosa strains represented 72 out 806 cases of cIAI and the study by Wagenlehner *et al.* [36], where 12 out of 226 bacterial isolates causing cUTI were Pseudomonas strains.

Use of ceftolozane/tazobactam for the treatment of *Pseudomonas aeruginosa* infections has been reported in some retrospective studies. Caston *et al.* described treatment of 20 cases of *Pseudomonas aeruginosa* MDR infections with C/T for the following syndromes: 12 septic shocks, 6 pneumonias, 1 otomastoiditis and 1 CLABSI (central line associated blood stream infection). Among these, 75% showed clinical improvement, 73% microbiological eradication and an overall 25% mortality [114]. Haidar *et al.* treated 21 *Pseudomonas aeruginosa* MDR infections, most of which were represented by pneumonias. Overall, 15/21 patients reported clinical success, whereas 4 of the 6 patients showing clinical failure died. A total of 20/21 patients had undergone previous treatment with an anti-pseudomonal drug and clinical failure was correlated to a worse SAPS II score [115]. Munita *et al.* reported tre-

atment of 35 carbapenem-resistant *Pseudomonas aeruginosa* infections, with pneumonia being the prevalent syndrome (51%). Clinical success was achieved in 74% of cases with an in-hospital mortality of 23%. Ninety-one percent of patients had been previously treated with other anti-pseudomonal antibiotics. Four patients with strains showing MIC> 4 mg/L for C/T resulted in clinical failure [116]. The report by Gallagher et al. described 205 *Pseudomonas aeruginosa* MDR infections, most of which were pneumonia cases (59%). Both clinical and microbiological success were observed in 74% and 71% of cases respectively, while 30-day in hospital mortality was reported in 19% of cases overall. Prompt administration of C/T (within 4 days) was a predictor of clinical success [117].

Bassetti *et al.* published data on 101 patients with MDR *Pseudomonas aeruginosa* sustained infections, 32% of which were pneumonia cases while 21% were BSIs. Clinical success was achieved in 83% of cases, while predictors of failure were sepsis and CVVH. A C/T resistant strain was detected in 3% of cases during treatment [118].

The prospective study involving patients with nosocomial pneumonia caused by Gram-negative pathogens was recently published (ASPECT- NP Study).

Hospital-acquired pneumonia may be further classified as ventilator-associated pneumonia (VAP), hospital-acquired pneumonia requiring ventilation (vHAP), and hospital-acquired pneumonia (HAP). VAP is defined as pneumonia occurring in intubated patients undergoing mechanical ventilation and may be further classified as either "early VAP" (pneumonia occurring within the first 5 days from intubation) whereby the underlying pathogens are similar to those involved in community acquired pneumonia and "late VAP" (pneumonia occurring after 5 days from intubation) frequently caused by multi-drug resistant (MDR) pathogens [119-121].

C/T double dose for pneumonia

The ASPECT- NP trial aims to compare C/T administered as IV double dose (**3 g every 8 hours, infused over one hour**) versus

meropenem (MEM) (1 g every 8 hours, infused over one hour) for the treatment of VAP and ventilated HAP sustained by Gram-negative bacteria with demonstrated susceptibility to the study drugs [122].

The 3g dose employed for C/T was chosen according to results from previous studies conducted on healthy volunteers whereby ELF concentrations of C/T persisted above 8 mg/L for 40% of time between administrations (dosing interval) and for 50% of the dosing interval for thresholds= 4 mg/L [123]. The latter thresholds are above the clinical breakpoint set for C/T for both *Enterobacteria* (≤ 4mg/L) and *Pseudomonas aeruginosa* (≤ 8 mg/L). Some guidelines recommend administering meropenem as extended infusion for the treatment of ventilated pneumonia [123].

However, the ASPECT-NP study was designed before the publication of such recommendations. Furthermore, all pathogens of interest displayed low MICs to meropenem: hence, the pharmacological target for the drug could be easily attained with a 1-hour meropenem infusion regimen. The primary endpoint was 28-day mortality, which may generally range from 18% to 27% for VAP and vHAP. Although 28-day mortality as primary endpoint is the preferred choice in many studies for evaluating antibiotic therapy in severe infections, some object that mortality at day-28 may be biased by the underlying conditions of the patient as well as by comorbidities rather than serve as indicator of outcome of the infection itself. Mortality at day 14 ranges between 6-19% in VAPs and 6-24% in vHAP, with values often below 10% in pivotal studies [124], hence 28-day mortality seemed a fair tradeoff for an achievable cohort size.

The secondary endpoints of the ASPECT-NP were clinical response at test of cure (TOC), clinical response at follow-up, clinical response according to isolate, 28-day mortality according to pathogen.

Overall, 361 patients were enrolled in the C/T arm and 359 in the MEM arm for a total of 720 patients. The cohort mainly consisted of patients with severe disease; 42% of the C/T arm and 46% of the patients randomized to the

MEM arm were receiving concomitant therapy with vasopressors. The mean duration of ventilation was 5 days. Concomitant administration of other antibiotics with activity against Gram-negative pathogens was allowed within the first 72 hours of antibiotic therapy. Aminoglycosides were allowed in study centers with prevalence of carbapenem-resistant *Pseudomonas aeruginosa* infections above 15%. Amikacin was administered in most cases, and a second drug was co-administered in 28% of patients in the C/T arm and in 31% in the MEM arm.

Pathogens were identified in 511 cases, of which 264 in the C/T arm and 247 in the MEM arm. *Enterobacteriaceae* represented 74% (380) of isolates, while 25% (128) were *Pseudomonas aeruginosa*; together they accounted for 99% of strains. Consequently, the study may be considered as being focused on patients affected by ventilated nosocomial pneumonia sustained by *Enterobacteria* and *Pseudomonas*.

Among *Pseudomonas aeruginosa* strains, resistance to C/T (MIC ≥ 8 mg/L) reached 3% versus 12% resistance against Meropenem. **In Italy, reported resistance to C/T in Pseudomonas aeruginosa strains is approximately 10%, mainly due to production of MβLs** [125].

Indeed, analysis of the primary endpoint results revealed that the 28-day mortality in the C/T arm was 24%, compared to 25% in the MEM arm, **confirming non-inferiority of the C/T treatment for the primary endpoint**. Furthermore, when patients were stratified according to either diagnosis of vHAP or failure of previous antibiotic therapy for the same pneumonia episode, **mortality rates were significantly lower for the C/T arm as opposed to the MEM treated arm**. This difference is clinically relevant, although the study was not designed to demonstrate superiority; hence these values require further validation.

C/T in vHAP

Setting aside statistical relevance, these data are of great significance. Patients with vHAP are generally frail and highly burdened by co-morbidities.

Nosocomial pneumonia in this population is characterized by severity and higher mortality and it is often associated with MDR germs. VAPs, on the other hand, are more frequent among younger patients, whose reactivity to infection and chances of survival are greater. In such unfavorable settings, C/T has demonstrated non-inferiority (and possibly superiority) to carbapenems.

The greater efficacy of C/T even in pre-treated patients is also of importance. In clinical practice, many clinicians turn to carbapenems as rescue-therapy once convinced of antibiotic failure in VAP. **This study demonstrates that C/T may be considered as an alternative option to MEM for salvage therapy**.

Microbiological eradication with clinical cure shows similar percentages across the two arms of the study. When *Pseudomonas aeruginosa* is considered, differences in eradication favor C/T (by 12%) when the *in vitro* susceptibility differences were 9%. As reported in Table 5, a discrepancy between susceptibility to C/T and clinical efficacy is observed in *Enterobacteria* (especially when ESBL producers are considered), which is similar and even higher than that of MEM, although only 1% resistance to the latter was reported among these strains.

In conclusion, ceftolozane/tazobactam administered at double the registered dose, is a suitable carbapenem sparing antibiotic regimen for the treatment of both VAP and vHAP caused by *Pseudomonas aeruginosa*.

	% RESISTANCE TO C/T	MICROBIOLOGICAL ERADICATION C/T	% RESISTANCE TO MEM	MICROBIOLOGICAL ERADICATION MEM
Pseudomonas aeruginosa	3%	75%	12%	63%

Table 5. *Resistance and microbiological eradication in Pseudomonas aeruginosa infections in ASPECT-NP.*

CEFTAZIDIME/AVIBACTAM (CZA/AVI)

Ceftazidime/avibactam (CZA/AVI) is active against AmpC and ESBL producing strains of *Pseudomonas aeruginosa* as well as against strains harboring class A carbapenemase such as GES. In vitro studies reported activity ranging between 66% and 86% against MDR *Pseudomonas aeruginosa* strains [123]. *In vitro* data reported a weaker activity of CZA/AVI against *Pseudomonas aeruginosa* compared to *Enterobacterales*. Clinical trials involving CZA/AVI are scarce. A randomized study by Carmeli et al. included 21 patients with UTI and IAI sustained by *Pseudomonas aeruginosa*. In UTIs, favorable clinical outcome was observed in 86% of cases and microbiological eradication in 79% of cases [127]. A Spanish study reported 9 patients with XDR infections who were treated with CZA/AVI. Clinical cure was reported in 50% of cases. Most failures occurred in pneumonia [128]. Similar data were recently reported by Tumbarello *et al.* for the treatment of Enterobacterales [129]. A report assessing efficacy of CZA/AVI for the treatment of hospital-acquired pneumonia detected CZA/AVI resistance in 9 strains of *Pseudomonas aeruginosa* out of 355 total strains identified in the study [130]. **Compared to C/T, CZA/AVI displays lower anti-pseudomonal efficacy** mainly due to a lower ability of the molecule to evade effects of efflux pumps. To this extent, Wi *et al.* found significantly lower C/T resistance compared to CZA/AVI when 42 carbapenem-resistant non-carbapenemase-producing isolates of *Pseudomonas aeruginosa* were tested. This behavior could be explained by a better performance of C/T on strains showing decreased oprD and increased MexB expression [105].

CEFIDEROCOL (FDC)

The novel cephalosporin cefiderocol employs the iron siderophore uptake mechanism to cross the outer membrane of Gram-negative pathogens and enter the bacterial periplasmic space (hence limiting its activity exclusively against Gram-negative bacteria). This transport route makes it insensitive to

resistance mechanisms based on membrane permeability such as porin deficiencies or overexpression of efflux pumps.

Effect of porins and efflux pumps on cefiderocol

Once in the periplasmic space, cefiderocol resists hydrolysis by beta-lactamases including AmpC, serine carbapenemases as well as by MβL carbapenemases [131-133]. AmpC mutation or over-expression do not increase cefiderocol MICs, a phenomenon that is instead observed with cefepime (stable to activity of non-mutant AmpC) and ceftazidime.

The CREDIBLE-CR study aimed to assess efficacy and safety of cefiderocol versus best available therapy (BAT) for the treatment of patients with carbapenem-resistant Gram-negative infections. The trial included 12 patients (15%) with *Pseudomonas aeruginosa* sustained infections in the cefiderocol arm (6 pneumonias, 2 BSIs and 4 UTI) and 10 patients (26%) in the BAT arm (5 pneumonias, 3 BSIs and 2 UTIs). *Pseudomonas aeruginosa* strains had MIC90 = 2 mg/L (range 0.12–4mg/L) in the cefiderocol arm and MIC90 =2 mg/L (range 0.06–4mg/L) in the BAT arm. Mortality rates in patients with Pseudomonas aeruginosa infections treated with cefiderocol were higher compared to the BAT arm, though clinical cure was similar across groups. The study reported a mortality imbalance in the cefiderocol treated arm. However, upon exclusion of deaths occurring before day 4 from enrollment and after day 28 days from analysis, the mortality rates appear to re-equilibrate. Subgroup analysis by pathogen showed higher clinical cure rate of cefiderocol over BAT in *Enterobacterales* and similar clinical efficacy non-fermenting Gram-negatives. **The overall clinical success for Pseudomonas aeruginosa was 58% (7/12) for the cefiderocol group versus 50% for the BAT group (5/10)**.

Cefiderocol in *Pseudomonas aeruginosa*: the CREDIBLE study

A MIC increase was observed in three patients with ***Pseudomonas aeruginosa*** infections during treatment with cefiderocol. The initial MIC increased by 4 dilutions during treatment from 0.12 mg/L to 2 mg/L and from 0.5 mg/L to 2 mg/L in two infections respectively at day 22 and day16 from treatment start (a MIC=2 mg/L is still within the susceptibility range). In one

case, a MIC increased from 0.12 mg/L to 16mg/L after only 3 days of treatment was reported [134]. The study by Portsmouth *et al.* assessed the efficacy and safety of cefiderocol administered as 2 g infusion every 8 hours versus imipenem 1 g infusion every 8 hours for the treatment of complicated urinary tract infections [135]. Cefiderocol demonstrated non-inferiority to the comparator treatment. ***Pseudomonas aeruginosa*** isolates represented 7% (18/252) in the cefiderocol arm and 4.2% (5/119) in the imipenem arm. Susceptibility of ***Pseudomonas aeruginosa*** strains to cefiderocol can be summarized by MIC_{50}= 0.06 mg/L, MIC_{90}= 0.25 mg/L (range=0.004-2mg/L) with no resistant strains isolated. MIC_{90} for imipenem was greater than 8 mg/L, indicating presence of MDR strains. Clinical success for Pseudomonas aeruginosa infections was 10/18 in the cefiderocol arm compared to 1/5 in the imipenem arm [135]. The APEKS-NP study has demonstrated the non-inferiority of cefiderocol to meropenem for the treatment of nosocomial pneumonia due to Gram-negative pathogens. Cefiderocol was administered as a 2 g infusion over 3 hours every 8 hours and meropenem as 2 g extended infusion every 8 hour. Pseudomonas aeruginosa represented the second most frequent pathogen with 24 isolates (17%) in the cefiderocol arm, and 24 isolates (16%) in the meropenem arm. Clinical success was achieved in 67% (16/24) of pneumonia cases sustained by *Pseudomonas aeruginosa* in the cefiderocol arm compared with 71% (17/24) in the meropenem arm. Microbiological eradication was achieved in 38% (9/24) of pneumonia cases sustained by *Pseudomonas aeruginosa*, compared to 46% (11/24) in the meropenem arm. The observed differences were not statistically significant [94].

IMIPENEM/RELEBACTAM (IMI/REL)

The imipenem/cilastatin + relebactam (IMI/REL) combination has been approved for the treatment of hospital-acquired pneumonia (HAP and VAP), cUTI and cIAI as well as for treatment of infections caused by Gram-negative pathogens in adult patients with limited treatment options.

Relebactam is a novel diazabicyclooctane β-lactamase inhibitor which confers protection to imipenem from hydrolysis by Classes A (e.g. KPC) and C (e.g. AmpC) enzymes including *Pseudomonas aeruginosa* AmpC cephalosporinases (PDC). It does not offer any protection against Class B β-lactamases (NDM, VIM and IMP) and Class D (OXA). Neither imipenem nor relebactam are substrates of *Pseudomonas aeruginosa* efflux pumps.

Susceptibility to IMI/REL was assessed in the SMART study, a surveillance study conducted in 2015-2016 in several countries across the world, including USA, Europe and China. Susceptibility to **imipenem/relebactam (IMI/REL)** in *Pseudomonas aeruginosa* was reported in 90.8% of all strains and 70.7% of MDR isolates. Relebactam restored imipenem susceptibility to 70.3% (2,656/3,776) of imipenem-non-susceptible isolates (MIC> 32mg/L) [136].

A study describing a collection of 1,445 of *Pseudomonas aeruginosa* clinical isolates reported IMI/REL $MIC_{90/50}$ = 0.5/1 mg/L respectively, which were 4 and 16 times lower than those observed for IMI alone. Against IMI non susceptible strains, IMI/REL showed 80.5% susceptibility.

Overall, 37/1445 isolates had MICs> 8mg/L (**considered as IMI/REL resistant**). All resistant strains were carbapenemase producers, distributed as follows: 26 VIMs (3 VIM-1, 11 VIM-2, and 12 VIM-20), 4 IMP (1 IMP-1, 2 IMP-8, and 1 IMP-33), and 7 GES-5.

Resistance to IMI/REL in *Pseudomonas aeruginosa*

A couple of strains showing intermediate susceptibility to IMI/REL harbored VIM-2 while the others overexpressed MexXY and AmpC (due PBP4 mutation), as well as PBP2 and 3 mutations. **The IMI/REL combination retains activity also against ESBL-producing strains of *Pseudomonas aeruginosa* (4 PER-1, 2 GES-1, 1 OXA-15) resistant to both C/T and CZA/AVI**. In particular, 39/78 (50%) C/T-resistant strains and 51/84 (60.7%) CZA/AVI-resistant (non-carbapenemase producing) strains remained susceptible IMI/REL [137].

Mushtaq *et al.* analyzed a collection of ESBL- and carbapenemase-producing *Pseudomonas aeruginosa* clinical isolates from UK hospitals. They found the

following underlying mechanisms of resistance; a) ESBLs: VEB (n = 97), PER (n = 9), GES ESBL (n = 7, all harboring GES-1 and GES-7, 3 with GES-9 and two GES26), SHV (n = 2, both harboring SHV-5 and SHV-12) and CTX-M-15 (n = 1); b) Carbapenemases: GES -5 (n = 37), OXA48-like (n = 4, one with OXA-181), MBL (n = 11, of which 5 NDM, 5 VIM and one harboring both types) and 2 KPCs. The study aimed to assess the entity of enhancement of susceptibility to IMI offered by REL protection in the presence of both loss of OprD and resistance mediated by Class A Carbapenemases. In VEB producing strains, a 4-fold or 8-fold reduction in MIC values was observed though MIC values remained above EUCAST breakpoints in many strains. This suggests that enhancement of IMI susceptibility is due to a concomitant inhibition of AmpC by REL and not to REL interaction with ESBLs. Among carbapenemase producers, GES -5 isolates were the most prevalent with IMI MICs ranging between 64–128 mg/L. Only a one dilution reduction in MICs was observed adding REL, while good activity on KPC producers was retained. C/T showed no activity on GES-5 (MICs were generally between 8-16 mg/L) [138].

Mechanisms of resistance to IMI/REL include production of β-lactamases not susceptible to inhibition by REL such as MβLs and OXAs. In the SMART study collection, of the 29 *Pseudomonas aeruginosa* strains displaying non susceptibility to IMI/REL, 14% were found to be MβL producers as well as one GES producer [139].

Resistance to IMI/REL in *Pseudomonas aeruginosa*

Other mechanisms leading to onset of resistance are impaired membrane permeability and overexpression of efflux pumps.

Pharmacokinetics of IMI and REL are complementary with steady state C_{max} values of 88.9 μM and 58.5 μM and a zero to 24 h AUC of 500 μM·h and 390.5 μM·h respectively (following administration of 30 min multiple infusions of 500/500 mg imipenem/cilastatin + 250 mg relebactam every 6 h in patients with bacterial infections). Plasma protein binding is approximately 20%, 40% and 22% for imipenem, cilastatin and relebactam, respectively. The half-life of relebactam is similar to imipenem, supporting its co-administration [140].

IMI/REL has a plasma/ELF ratio of approximately 50% [141].

IMI/REL mainly undergoes renal excretion (≥ 63%, 77% and> 90% of the intact administered dose of imipenem, cilastatin and relebactam can be recovered from urine). As a result, dose adjustments in patients with renal impairment are required according to renal function. IMI/REL is a substrate of the OAT3, OAT4, MATE1 and MATE2K transporters, although it has no impact when co-administered with probenecid, an inhibitor of OAT3 [140].

Co-administration with valproic acid, divalprox sodium or with ganciclovir is not recommended due to interactions.

Two phase 2 studies were conducted to assess both efficacy and safety of IMI/REL (**NCT01506271** and **NCT01505634**) [142,143]. They were multicentric, double-blind, randomized, non-inferiority study on adult hospitalized patients requiring intra-venous (IV) antibiotic treatment for cIAI and cUTI/acute pyelonephritis respectively. Patients were randomized according to the same 1:1:1 scheme into three groups: relebactam 250 mg or 125 mg or placebo, all combined with imipenem/cilastatin every 6 h for 4–14 days. Relebactam-containing schemes showed non-inferior to imipenem/cilastatin alone in both studies with an overall rate of adverse events of 9-14%, with no difference between intervention and placebo groups.

The phase 3 RESTORE-IMI 1 trial was a multicentric, randomized, double-blind controlled study aimed at comparing both efficacy and safety of IMI / REL with the combination scheme of IMI+colistin (COL) for the treatment of infections (HAP/VAP, cIAI and cUTI confirmed by culture) in hospitalized patients sustained by IMI-resistant pathogens. Randomization was performed according to a 2:1 scheme: IMI/REL IV (500mg/250 mg, or based on renal function) every 6 hours, or COL (300 mg loading dose, followed by up to 150 mg based on renal function) IV every 12 hours + IMI IV (500 mg, or based on renal function) every 6 hours. The primary efficacy endpoint was clinical response at day 28 in the modified Intention to Treat (mITT) population. Response was defined according to syndrome and as follows:

- HAP/VAP, 28-day all-cause mortality;
- cIAI, clinical response on day 28;
- cUTI, composite outcome of microbiological eradication and clinical recovery at Early Follow up Visit.

Overall, the study enrolled 31 patients in the IMI/REL arm and 16 in the COL + IMI arm, with a similar response across both arms (71% vs 70%, for IMI/REL and IMI + COL, respectively). Adverse events were lower in the IMI/REL arm as opposed to the control arm (16% vs 31%), including adverse events leading to nephrotoxicity (10% vs 56%) as expected [144].

A favorable response was observed in patients with *Pseudomonas aeruginosa*-sustained infections, accounting for 13/16 patients (81%) in the IMI/REL arm and 5/8 (63%) in the IMI + COL arm, respectively. Of the 16 *Pseudomonas aeruginosa* isolates from the IMI/REL group, 7 isolates were from cUTI (PDC, CTX-M, TEM and SHV producers), 1 from cIAI (PDC producer), 8 were from HAP/VAP (PDC and TEM producers). In the IMI + COL treated arm, 2 isolates were collected from cIAI (PDC producers), 3 from HAP/VAP (PDC producers) and 5 from cUTI (CTX-M, TEM, SHV, OXA and/or PDC producers).

Another phase 3 study, the RESTORE IMI 2, was also conducted as a randomized, double-blind controlled trial to assess efficacy of IMI REL in adult patients with HAP/VAP. Patients were randomized 1:1 to the following treatment schemes: IMI/REL 500 mg/500 mg/250 mg IV administration or piperacillin/tazobactam (PIP/TAZ) 4 g/500 mg, IV every 6 hours (based on renal function) for 7–14 days. Linezolid was empirically administered to all patients (600 mg every 12 hours) unless presence of MRSA was excluded. The established primary endpoint was 28 all-cause mortality determined in the MITT population. Overall, 537 patients were randomized, of which 266 to the IMI/REL treatment arm and 269 to the PIP/TAZ arm. IMI/REL demonstrated non-inferiority versus treatment with PIP/TAZ for the established primary endpoint of 28-day all-cause mortality (15.9% mortality in the IMI/REL arm versus 21.3% in the PIP/TAZ arm) [145].

AZTREONAM/AVIBACTAM (AZT/AVI)

The aztreonam/avibactam (AZT/AVI) combination is active on MBL-producing Gram-negative bacteria. This is due to resistance of AZT to hydrolysis by Class B enzymes, alongside the protection provided by AVI against Class A (ESBL) and C (AmpC), or D enzymes.

A study by Karlowsky *et al.* evaluated 11,842 *Pseudomonas aeruginosa* clinical isolates collected worldwide between 2012-2015. MIC_{90} for AZT/AVI and AZT were both 32 mg/L. When MBL-producing isolates of *Pseudomonas aeruginosa* were considered, aztreonam-avibactam MIC_{90} (32 mg/L) were 1 or 2 - dilutions lower than those observed for aztreonam alone (64 mg/L), with similar MIC distributions for AZT/AVI and AZT in all MBL producing *Pseudomonas aeruginosa* isolates [146]. **Combination of AZT + CZA/AVI did not show synergy on IMP-producing strains of *Pseudomonas aeruginosa*** [147]. Though promising for the treatment of infections sustained by MβL- and serine- β-lactamase producing *Enterobacterales*, the **AZT/AVI combination** (that is, combining AZT with CZA/AVI) **has not provided alike efficacy in *Pseudomonas aeruginosa* strains**, due to various resistance mechanisms harbored by the pathogen (such as efflux pumps, porin mutations and PBP3 mutations) that make the addition of avibactam ineffective.

AZT/AVI and *Pseudomonas aeruginosa*

The types of β-lactamases and the frequency of expression is also different in *Pseudomonas aeruginosa*, leaving colistin as the last resort treatment of these pathogens [148].

Real life cases describing treatment with the CZA/AVI and AZT combination are available in the literature, although therapeutic schemes are substantially different from the ongoing trials [149,150]. Both cases involve MBL-producing strains (confirmed by either time kill curves or by E-test).

AZT/AVI combination is still undergoing clinical development, with a phase 3 study currently ongoing (recruitment deadline expected for 2022).

The Phase 1 population kinetics study laid the foundation for the dosing

scheme adopted in the Phase 2 trial (REJUVENATE) [151], aimed at selecting the dosing scheme for the ongoing Phase 3 study [152].

The REJUVENATE study was designed to evaluate safety, pharmacokinetics (PK) and tolerability of AZT/AVI for the treatment of hospitalized adult patients with cIAI sustained by Gram-negative pathogens.

The study comprised 3 cohorts of patients (40 patients enrolled) treated with 3 different AZT/AVI regimens, based on renal function, for 5-14 days:

- Cohort 1, patients with CLCR clearance> 50: 1 loading dose of AZT/AVI 500/137 mg IV administered over 30 min, maintenance dose of AZT/AVI of 1500/410 mg over 3 hr, q6hr (started immediately after the loading dose) IV infusion over 30 min, + metronidazole 500mg 1hr IV, q8hr
- Cohort 2 and 3, patients with CLCR clearance> 50: 1 loading dose of AZT/AVI 500/167 mg IV administered over 30 min, maintenance 1500/500 mg 3 hr, q6hr (first administered immediately after loading dose) + metronidazole 500mg 1hr IV, q8hr
- Cohort 2 and 3, patients with clearance CLCR 31-50: 1 loading dose AZT/AVI 500/167 mg IV administered over 30 min, a second EXTENDED loading dose with AZT/AVI 1500/500 mg administered over 3 hr and a subsequent maintenance dose 750/250 mg, administered over 3 hrs q6hr (the first dose administered 3 hr after the extended loading dose) + metronidazole 500mg 1hr IV, q8hr

Plasma concentrations of AZT and AVI were similar both before infusion and at 6 hours, with end-infusion C_{max} values for AZT and AVI of 62.5mg/L and 11.6mg/L in cohort 1, and 55.4mg/L and 12.1mg/L in cohort 2 and 3 respectively. Steady state was achieved on day 4. PK data were similar between groups except in cohorts 2 and 3 where AVI showed higher AUC_{0-6} values (as expected due to augmented dosage). The study therefore confirmed dosage schemes of cohorts 2 and 3, subsequently employed in the pivotal Phase 3 study (loading dose of 500/167 mg administered in 30 min and maintenance dose of 1500/500 mg administered in 3 hr, q6h in patients wi-

th CLCR> 50 mL / min).

Overall, 68.8% and 67.6% of patients in cohorts 1 and 2 + 3 respectively experienced adverse events (AE). The most common AE according to the MeDRA definition [Medical Dictionary for Regulatory Activities preferred Term] was liver enzyme elevation, mainly reported as asymptomatic and reversible. However, a greater number of diarrhea cases were observed, also relatively to SmPC reports (not attributable to C. difficile infection). Serious adverse events (9 patients, 26.5%) were observed in cohorts 1 and 2 + 3, relatively to hepatic (3 patients) and renal (1 patient) function. Two recorded deaths were not attributed to the study drug.

Clinical cure assessed at End of Treatment was recorded in 67.6% and 73.9% of patients in the MITT (modified intention to treat - all enrolled patients who were administered the drug) and mMITT (microbiologically evaluable modified Intention to treat-ITT population with a diagnosis of cIAI and ≥1 intra-abdominal pathogen isolated at baseline) respectively, with a 60% clinical cure at Test of Cure (TOC, day 25) (58.8% in the MITT population and 60.9% in the mMITT population).

Most isolates consisted of Enterobacterales, with only 1 *Pseudomonas aeruginosa* isolate (MIC for AZT/AVI = 0.25 mg/L). Among mMITT patients, 23/34 (67.6%) presented with infections caused by *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca*. None of the strains were MBL or ESBL producers.

The study concludes that the safety profile of AZT/AVI is in line with AZT monotherapy, with a favorable risk/benefit profile, pending the definitive evaluation of efficacy and safety deriving from the ongoing Phase 3 study.

The ongoing NCT03580044 study is a prospective, randomized multicenter open label study for the evaluation of efficacy, safety and tolerability of AZT/AVI combination compared to the Best Available Therapy for the treatment of patients with cIAI, nosocomial pneumonia (NP) including HAP, and VAP, cUTI or BSI infections, sustained by MBL producing Gram-negative pathogens.

The study expects to randomize 60 participants, with a 2:1 randomization. Enrollment will allow for no more than 75% of cUTIs over total cases.
The trial will employ the following therapeutic schemes: one loading dose AZT/AVI 500/167 mg IV or 675 mg /225 mg AZT/AVI administered over 30 min, maintenance 1500/500 mg 3 hr, q6hr (the first administered immediately after loading dose) IV in 30 min or 675 mg AZT and 225 mg AVI in 3hr. After a gap of 3 or 5 hr, subjects will receive a maintenance dose of 1500 mg of AZT + 500 mg AVI every 6hr or 750 AZT + 250 AVI every 6 hr or 650 mg AZT + 225 AVI every 8 hr. Subjects with cIAI will also receive adjunct IV metronidazole 500 mg q8hr administered over a 60 min infusion.
Efficacy, safety and tolerability of AZT/AVI will also be assessed by an additional planned Phase 3 (NCT03329092, REVISIT) prospective, randomized, multicentric, blinded open label (central assessor blinded) study comparing AZT/AVI ± metronidazole (metronidazole-MTZ for cIAI only) to a combination of meropenem ± colistin (MEM ± COL, subject or clinician's discretion based on local clinical practice) for the treatment of severe infections caused by MBL-producing MDR Gram-negative bacteria in adult patients (> 18 years) with limited treatment options. The interventional study will involve enrollment of 375 participants at 158 sites, with completion expected by end of 2022. The established dosage of AZT/AVI varies according to renal function. MEM and COL comparators will be administered according to renal function or according to susceptibility of the isolate: upon suspicion of MEM resistance, MEM will be administered as a 2g infusion over 80 min q8h instead of 1 g in 30 min q8h.
The primary efficacy endpoint will be the proportion of patients with clinical cure in the ITT and CE populations at Test of Cure (TOC) at days 28 +/- 3.
Table 6 summarizes indications, mechanism of action and mechanism or resistance of the new antibiotics active against Pseudomonas aeruginosa.
In conclusion, we hereby propose a decision algorithm for diagnosis and treatment of MDR Pseudomonas aeruginosa (Figure 26)

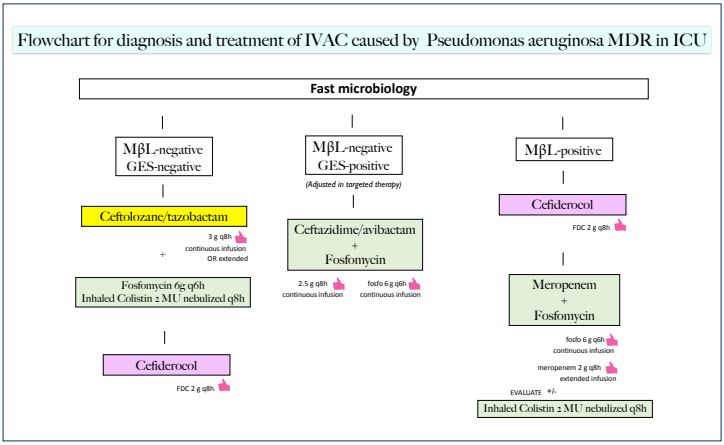


Figure 26. *Decision algorithm for diagnosis and treatment of VAP caused by Pseudomonas aeruginosa MDR.*

MOLECULE	INDICATION ACCORDING TO EMA	INHIBITOR ACTIVITY	ELF PENETRATION	SUSCEPTIBILITY TO EFFLUX PUMPS	SUSCEPTIBILITY TO MUTATIONS/ DELETIONS OF PORINS
C/T	**Syndromic** (pneumonia, cIAI, cUTI and pyelonephritis) [153]	**Class A** (limited spectrum), ESBLs, some class C. T protects C against ESBL producing *Enterobacterales* and anaerobes [154]	50% C, 62% T VAP, similar in healthy subjects (61%, 63%) [153]	Not a substrate of MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexXY[154,156]	Not a substrate of OprD [154]
IMI/REL	**Pathogen-specific, syndromic** (pneumonia, BSI due to pneumonia)[159]	**Some Class A** (including ESBLs and KPC), **Some class C** (including AmpC and PDC)[159]	54% REL, 55% IMI (healthy subjects)[160]	Resistant to MexAB/ OprM, can be susceptible to MexXY6, other Mex in association with OprD[126]	Susceptible to OprD[159]
CZA/AVI	**Pathogen-specific, syndromic** (cIAI, cUTI and pyelonephritis, pneumonia, bacteremia)[164]	**Some class A, Class D, Class C** [164] Variable activity on ESBL but greater than C/T [126]	52% CZA, 42% AVI (healthy volunteers) [165]	Some MexAB/ OprM (more susceptible than C/T)[126]	Some porins (determines low level R)[166]
AZT (AVI)	**Trials ongoing** For cIAI and treatment of severe infections due to MBL producing Gram-negative bacteria[170]	AZT: **Class B Class A**, some **Class D**, **Class C** [164] **Variable activity on ESBL**[126]	AZT: 36-80% ELF[171]	Susceptible to MexAB/Opr M[172]	Susceptible to mutant forms of OprD[172]
FDC	Pathogen-specific[173]	Classes A, B, C, D[174]	24% healthy vol, 34% VAP patients[175]	No significant impact[176]	Not a substrate[176]

Table 6. *Indication, mechanism of action, resistance mechanism of new antibiotics.*

PBP BINDING/ BINDING TO PBP4 AND AMPC INDUCTION	SAFETY	RESISTANCE MECHANISMS	CROSS RESISTANCE
Strongly binds all relevant PBPs[113] PBP4: binding 15X less than IMI, 4X stronger than CZA, not relevant for induction of AmpC[156]	–	**Class B and some class A** (*K. pneumoniae producing* KPC, VEB, PER, GES), **class D** AmpC mutants (PDC) (cross res CZA/AVI) PBP3 mutants	***mutS*** hypermutant with overexpressed or mutated **AmpC**, increased S to IMI and C/T[157] **Ωloop AmpC mutation** resistance to CZA/AVI but not IMI[137]. **Cross resistance with** CZA, FEP and PIP/TAZ and CZA/AVI (only better on some GES-5) on OXA type, ESBL and PDC producers[158]
Binds DacB (PBP4) induces AmpC[159]	DDI, convulsions[159]	**Class B and D β-lactamases**, PDC overexpression and porin mutations, Some GES[159] Target PBP mutations	**AmpC producers with** Ω loop mutations (CZA/AVI and C/T)[137], **induction of MeXY. MexD and OprD** (cefoxitin) like IMI[161], R determinants such as OXA-48, GES and cell permeability[162,163]
Binds PBP3 (CZA), PBP2 and PBP3 (AVI)[168] Binding of AVI to PBP4 induces AmpC[169]	-	**Class B**, AmpC mutants, efflux pumps and porins some VEB[167]	**OXA and AmpC-PDC mutants** (resistance to C/T and CZA/AVI)[126] Ω loop mutants (C/T)[166], **Cross resistance with** CZA, FEP and PIP/TAZ and C/T upon resistance from OXA type, ESBL and PDC producers[167]
Binding preferentially to PBP3[146]	-	AmpC mutants and efflux pumps/porins. AVI does not offer greater benefit[172]	Overexpression of efflux pumps and reduced permeability (susceptibility similar to MEM), Ωloop mutants. Classes A and C forms that may not be inhibited by AVI and PBP3 mutants[172]
Preferentially binds PBP3[176], no induction of AmpC[131]	Mortality inbalance in CREDIBLE-CR[173]	Iron transporters and siderophoresi[176], AmpC mutants [177]	

Acinetobacter spp.

Acinetobacter are Gram-negative, aerobic, catalase-positive, and oxidase-negative coccobacilli. *Acinetobacter* is characterized by the lack of unique phenotypic microbiological features; this has made recognition and classification of this genus quite cumbersome in the recent past. *Acinetobacter* initially underwent the following classification: non-pigmented (*Achromobacter*), non-motile (*Acinetobacter*), non-fermenting, not capable of reducing nitrates (*anitratus*) leading to a final classification of Moraxella (oxidase positive) and *Acinetobacter* (oxidase negative).

Acinetobacter calcoaceticus-baumannii (Acb) complex

To date, over 50 species of *Acinetobacter* have been identified, the majority of which are considered non-pathogenic. The **Acinetobacter calcoaceticus-baumannii (Acb) complex** cluster is the most clinically relevant.

The cluster includes *A. baumannii*, *A. nosocomialis*, *A. pittii*, *A. seifertii* and *A. dijkshoorniae* along with *A. calcoaceticus*, the only non-pathogenic species. Despite belonging to the same complex and being phenotypically indistinguishable, they differ by their invasive abilities and different degrees of virulence. *Acinetobacter baumannii* is indeed by far the most clinically relevant.

As identification is challenging, the *Acinetobacter calcoaceticus-baumannii* (Acb) complex is often confused with *Acinetobacter lwoffii* and A. *radioresistant*, usually considered as skin colonizers and may seldom be the underlying cause of infection among immuno-compromised patients. *Acinetobacter calcoaceticus* and *Acinetobacter johnsonii*, on the other hand, are considered environmental *Acinetobacter spp*.

Acinetobacter is typically considered a nosocomial pathogen causing care-related infections in critically ill patients [178]. Globally, 2% of healthcare-related infections are attributable to *A. baumannii* and in most cases, such strains show greater resistance rates compared to other Gram-negative pathogens. However, carbapenem resistance rates are generally high: carbapenem resistance is reported in over 75% of clinical isolates from Italy while over 70% of *A. baumannii* isolates in Latin America display MDR features [179]. Mortality rates in patients affected by *Acinetobacter* infection vary greatly w across species, ranging from 37% mortality rates for *A. baumannii* bacteremia to 16% and 14% for *A. nosocomialis* and *A. pittii* respectively [180].

Acinetobacter baumannii most commonly causes lung infections, resulting in either hospital-acquired and ventilator-associated pneumonias. These syndromes are associated with increased length of hospitalization and mortality upon infection with Acinetobacter [181] and mortality from pneumonia increases with concomitant bacteremia [178]. However, **isolation of Acinetobacter baumannii from respiratory tract material is not sufficient to establish its causal relationship with the infection**. Indeed, distinction between infection and colonization remains challenging. Microscopy examination of bronchoalveolar lavage samples showing both Gram-negative coccobacilli and inflammatory cells could help clinicians establish the correct diagnosis. More rarely *Acinetobacter baumannii* can cause urinary and abdominal infections, including surgical wound infections.

Acinetobacter baumannii can cause serious community-acquired infections. It is considered as typical war wound pathogen, to the extent that it was also renamed ***Iraqibacter*** back in the 1990's [182], as well as in trauma-related wounds occurred during natural disasters. Moreover, *Acinetobacter baumannii* has been reported as cause of community-acquired pneumonia in foundry workers and community-acquired meningitis. Possibly, community-acquired infections could be caused by strains with peculiar virulence factors.

The ability to persist for long periods in hostile environments and its resistance to disinfectants has allowed *Acinetobacter* to spread in nosocomial environments. Some *Acinetobacter baumannii* strains can survive in the absence of water up to over one hundred days. This peculiar feature is due to yet unknown multifactorial determinants. Perhaps, membrane lipids composition or the ability to form capsules and/ or biofilms may play an important role.

Genome protection in *Acinetobacter*

On the same line, ***Acinetobacter baumannii* is also capable of protecting its genome from damage caused by rehydration**.

The protective effect is exerted by activation of the **RecA** proteins, a DNA recombination repair system. Indeed, *Acinetobacter's* acquisition of rifampicin resistance has been ascribed to this phenomenon. Supposedly, the repetitive insult of dehydration-rehydration might generate strains displaying the multi drug resistance (MDR) phenotype.

Resistance in nosocomial environments is also ascribed to its ability to endure oxidative stress caused by **ROS**. Expression of **KatG catalases**, capable of neutralizing and deactivating reactive oxygen species, along with pumps such as **AceI**, confer resistance to disinfectants. As a result, chlorhexidine may become ineffective. In addition, alcohol use has been associated with promotion of virulence factors in *Acinetobacter baumannii*. Physiological levels of alcohol can prevent phagocytosis of *Acinetobacter baumannii* strains. As a result, alcohol abuse is considered a risk factor associated with *Acinetobacter baumannii* community -acquired infection [179].

ROS resistance is also exemplified by the ability of *Acinetobacter* to evade host immunity. Animal models suggest that neutrophils are the first line of defense against *Acinetobacter baumannii* in lungs. *Acinetobacter baumannii* rapidly attracts neutrophils, which in turn attempt pathogen eradication by producing an oxidative burst and **NETs** (Neutrophil Extracellular Traps). *Acinetobacter baumannii*, however, inhibits **NETs**

formation despite the presence of neutrophil activators (various cytokines and LPS, etc.) using them as transporters (*in vitro*), while its ability to detoxify H2O2 reactive forms through **KatG and KatE catalases** reduce the impact of oxidative stress [183].

"Persist and resist" strategy in Acinetobacter baumanni

As of today, no genetic or phenotypic determinants have been ascribed to a particular feature in order to predict virulence of *Acinetobacter baumannii strains*. Rather, *Acinetobacter baumannii* likely survives by activation of a variety of mechanisms enabling it to exquisitely respond to external stimuli. For this reason, Harding et al. have described the survival strategy of this bacteria as "**persist and resist**"[179].

BIOFILM

Acinetobacter baumannii can form biofilm virtually on any type of clinically relevant surface, from wounds to abiotic materials or devices, endotracheal tubes, polycarbonates, and stainless steel, significantly contributing to devices-related and nosocomial infections. The ability to create biofilm structures is attributable to the **BfmRS** two-component regulation system [184], which regulates the expression of the **type I chaperone-usher pilus system**, termed pili Csu [185]. The **pili Csu system**, encoded by a six-segment operon, csuA/BABCDE, along with biofilm-associated proteins (Bap), plays a crucial role in the formation and maintenance of biofilms on abiotic surfaces. In addition, a second two-component system, the **GacSA**, controls the expression of the pili CSU system, thus indirectly contributing to biofilm formation [186]. Other mechanisms involved in biofilm formation include membrane proteins such as **OmpA**, also involved in antibiotic and antibodies resistance [183].

Biofilm production may be triggered by different external stimuli, including antibiotics. For example, sub-inhibitory levels of TMP/SMX completely repress the pili Csu system, thereby promoting planktonic bacterial forms [183].

RESISTANCE MECHANISMS

Acinetobacter spp. harbor Ambler class C chromosomal cephalosporinases, termed **Acinetobacter-derived cephalosporinases (ADC)** [187], capable of hydrolyzing penicillins along with first to third generation cephalosporins, including ceftriaxone, ceftazidime, and cefotaxime, while both cefepime and carbapenems retain activity against these strains.

Acinetobacter-derived cephalosporinases (ADC)

Importantly, the expression of ADC -(alike AmpC) is inducible upon exposure to β-lactams [188]. The presence of plasmids encoding for ESBL genes also confer resistance to cefepime. In time, ***Acinetobacter* species also acquired oxacillinases, which conferred resistance to carbapenems**. The **blaOXA-51** chromosomal gene is intrinsic, while other oxacillinases are acquired via plasmids such as the OXA 23, 24 group (33 and 40 alike), 58, 143 and 235 [189,190]. **Acinetobacter rarely harbors metalloenzymes, typically encountered in isolates from the Far East: VIM and IMP are more frequently reported whereas NDM-1 and 2 are rarer**.

Among resistance mechanisms involving membrane permeability, membrane proteins such as **CarO**, whose expression is induced by the presence of carbapenems (such as imipenem), contribute to resistance generation. CarO proteins interact directly with **OXA-23** enzymes; consequently, upon entry in the periplasm, molecules such as imipenem are immediately and rapidly hydrolyzed by the adjacent β-lactamase. In addition, **OprD** and its orthologs are involved in iron transport as well as antibiotic molecules such as fosfomycin and meropenem. Mutations of such proteins are known to confer resistance to carbapenems [183].

Resistance to polymyxins, in *Acinetobacter spp*. is quite rare and depends on mutations of membrane lipo-polysaccharides causing changes in electrical charge of cell membrane components. This comes, however, at a fitness cost for the bacterium, explicative of the limited global spread. Heteroresistance to colistin is nevertheless a cause of concern, due to scarcity of

therapeutic options in the event of overt resistance. The synergistic combination of colistin and rifampicin, previously adopted for the treatment of colistin-resistant *Serratia spp.* has been readily transposed for treating colistin-resistant *Acinetobacter* [191].

Several multi-drug resistant (MDR) ***Acinetobacter baumannii*** outbreaks have been reported over the years, all strains deriving from clones later grouped according to 3 Sequence Types named **International Clones type I, II and III (CC1, CC2, CC3)** [192]. They are all characterized by the presence of different resistance determinants and by rapid clonal expansion at international level.

Considering the heavy burden of *Acinetobacter's* innate and acquired resistance mechanisms, the absence of any pattern of resistance as reported in a molecular antibiogram (Figure 27), could be misleading when choosing the appropriate empiric antibiotic treatment. This is especially true in specific epidemiological settings, such as in Italy, where colistin-only susceptible strains are common (Figure 28).

CTX	Not detected
KPC	Not detected
VIM	Not detected
IMP	Not detected
NDM	Not detected
OXA-48	Not detected

Figure 27. *MDR Acinetobacter baumannii molecular antibiogram.*

ANTIBIOTICS	MIC mg/L
Imipenem	>8 R
Meropenem	>8 R
Amikacin	>16 R
Gentamycin	>4 R
Trimethoprim/ sulfam	>4 R
Levofloxacin	>2 R
Colistin	1 S

Figure 28. *Phenotypic antibiogram showing Acinetobacter baumannii Carba R XDR/COS (Colistin Only Susceptible) - OXA-23.*

THERAPY

Treatment strategies of severe ***Acinetobacter baumannii*** infections have relied on the use of β-lactams

due to their bactericidal activity. The acquisition of resistance to cefepime and carbapenems, however, lead to the use of molecules with less bactericidal capacity, greater toxicity, and worse pharmacokinetic features.
Few therapeutic options have preserved *in vitro* activity against this microorganism. **No novel BLIC is active against Acinetobacter baumannii strains. Eravacycline** has demonstrated *in vitro* activity against *Acinetobacter baumannii*[193]. Regrettably, the molecule did not reach predefined non-inferiority in clinical trials. **Among commercially available options, only colistin and the recently approved cephalosporin, cefiderocol, have shown significant activity**.

CEFIDEROCOL

Cefiderocol, a novel siderophore cephalosporin, is currently the only β-lactam with displayed activity against *Acinetobacter*. *In vitro* data from surveillance studies conducted on a collection of over 20,000 isolates from different infection sites between 2014-2018 reported susceptibility rates for cefiderocol in *Acinetobacter baumannii* strains displaying susceptibility or resistance to carbapenems of 94.9% and 90.7% respectively. Colistin susceptibility rates in carbapenem susceptible or resistant strains were 97, 6% and 84.1% respectively [194]. The **SIDERO** surveillance study [80] conducted on 236 carbapenem non-susceptible strains (MICs> 8 mg / L) reported MIC ranges between 0.015 and > 64 mg/L for cefiderocol and ≤0.25 and> 8 mg/L for colistin [195], with susceptibility rates of 94.9% and 93.6% for cefiderocol and colistin respectively. Susceptibility rates above 90% are reported for OXA-23 and OXA-24 like producers. **All strains yielding cefiderocol MICs> 16mg/L were PER enzyme producers**.
Over the years 2014-2016, cefiderocol MIC90 values for *Acinetobacter baumannii* strains underwent very little variability, ranging from 1 mg/L to 4 mg/L [196], although MIC increases have been reported during therapy, especially upon monotherapy administration [134,197].

Registration studies demonstrated efficacy and safety of cefiderocol for the treatment of severe infections sustained by MDR Gram-negative bacteria, including *Acinetobacter baumannii*. Cefiderocol reached predefined non-inferiority in **clinical trials** [94,134], despite a mortality imbalance reported in the CREDIBLE-CR study, especially when Acinetobacter-sustained lower respiratory tract infections were considered [134].

Clinical efficacy of cefiderocol in CREDIBLE-CR and APEKS-NP

In the **APEKS-NP** study [94] (cefiderocol 2g TID versus high dose meropenem-HD 2g TID for the treatment of HAP/VAP/HCAP caused by Gram-negative bacteria), 16% of patients in both arms were affected by *Acinetobacter baumannii* infections (23 and 24 patients in the FDC and MEM arms respectively). In the FDC arm, 8 infections were classified as HAP, 12 as VAP and 3 as HCAP compared to 11 HAPs, 10 VAPs and 3 HCAPs in the MEM arm. Within the modified Intention to Treat (mITT) population, 52% (12/23) of patients in the cefiderocol arm with *Acinetobacter baumannii* sustained infections achieved clinical cure at Test of Cure (7 days ± 2 days from the end of treatment) versus 58% (14/24) of meropenem-treated patients, with 39% (9/23) and 33% (8/24) microbiological eradication rates for cefiderocol and high dose meropenem respectively. In patients with *Acinetobacter baumannii* infections, mortality rate (primary efficacy endpoint) was 32% (7/22) compared to 25% (6/24) in the MEM arm.

The **CREDIBLE-CR** study for the assessment of efficacy and safety of cefiderocol versus Best Available Therapy (BAT; up to 3 antibiotics with activity against Gram-negative pathogens administered intravenously) was designed to enroll patients with infections caused by Gram- negative carbapenem resistant (CR) pathogens. The study included patients with infections caused by CR *Acinetobacter baumannii*, in the cefiderocol and BAT arms respectively (46% (37/87) vs 45% (17/40) overall, 65% (26/40) vs 53% (10/19) in the HAP group, 44% (10/23) vs 50% (7/14) in the bloodstream infections and sepsis group, 6% (1/17) vs none in the complicated urinary tract in-

fections group). The primary endpoint was clinical cure at the Test of Cure (7 days ± 2 days from the end of treatment). Within the subgroup of patients with Acinetobacter spp. infections, 41% (16/39) and 53% (9/17) demonstrated clinical cure at TOC, while 26% (10/39) and 29% (5/17) demonstrated microbiological eradication in the cefiderocol and BAT arms respectively.

Mortality at day 28 was 38% (16/42) in the cefiderocol arm and 18% (3/17) in the BAT arm. **Mortality in the cefiderocol group was not attributed to specific factors other than a disproportion of patients with shock (26% in the cefiderocol group versus 6% in the BAT group) and a greater proportion of patients admitted to the ICU (81% cefiderocol group versus 47% BAT group)**.

Only 2 patients with *Acinetobacter spp.* infection reported pathogen MICs> 2mg/L. Of these, one patient presenting with a strain with MIC= 4mg/L (OXA-23 and NDM producer) achieved clinical cure with favorable outcome and survival at day 28, whereas in the other case, the *Acinetobacter* strain showed MIC≥ 16mg/L (OXA-23 like producer) and clinical failure and death occurred. **The expression of multiple resistance determinants in *Acinetobacter baumannii* including PER and NDM β-lactamases contribute to cefiderocol MIC increase *in vitro***.

β-lactamases PER and NDM in *Acinetobacter*

In addition, *in vivo* MIC increases are possibly determined by the expression of ADC enzymes. Nevertheless, a synergistic action can be observed when associating cefiderocol with other molecules, including sulbactam and avibactam [198] as shown by the addition of avibactam, which restored and enhanced the activity of cefiderocol in PER-producing strains with cefiderocol MIC> 8 mg/L [209] [199].

Several **real-life experiences reporting use of cefiderocol** in the context of Compassionate Use Programmes and early access are publicly available (Table 7).

AUTHOR	RESISTANCE DETERMINANT	DIAGNOSIS	COMBINATION	*OUTCOME*
Trecarichi *et al.* [200]	-	VAP/BSI	-	Success
Zingg *et al.* [201]	OXA-23 + OXA-58 OXA-23 OXA-40+NDM	Osteomyelitis	FDC+COL+ DAPTO+FLUCO FDC+COL+CZA/AVI FDC+COL	Success
Dagher *et al.* [202]	-	Osteomyelitis	FDC+DAPTO+VANCO	Success
Falcone *et al.* [203]	-	5X BSI 2X VAP	- 1X FDC+FOS	Success ⅘ Success ½
Bavaro *et al.* [204]	-	5X BSI e shock 2X BSI 2 VAP (+BSI) 1 hepatic abscess	FDC+FOS/COL/ MEM/TGC FDC+ COL/FOS FDC+ TGC+COL/FOS FDC+ COL/FOS + DAPTO	Success (death due to other causes)
Oliva *et al.* [205]	XDR PDR XDR	VAP BSI Spondilodiscitis	-	Success

Table 7. *Cefiderocol in vivo experience in compassionate use programs.*

SULBACTAM

None of the novel β-lactamase inhibitors (avibactam, vaborbactam and relebactam) have demonstrated activity against *Acinetobacter* oxacillinases (avibactam only inhibits OXA-48 in *Enterobacterales*), nor against metallo-enzymes; as a result, the new BL/BLI combinations do not display any activity against *Acinetobacter*. Conversely, sulbactam, an old suicidal β-lactamase inhibitor, is active against the bacterium. Its activity, initially ascribed to inhi-

bition of oxacillinases, was later attributed to a different mechanism related to its affinity and acylation of *Acinetobacter* PBPs and, consequently, its bactericidal activity. Indeed, sulbactam inhibits both PBP1a and PBP3 - but not PBP2 - and resistance to sulbactam is rare [206]. The few resistant strains have been shown to harbor PBP3 mutations which are, however, burdened by fitness loss. **The association of sulbactam with fosfomycin (FOS/SUL) is currently of great interest**, despite Acinetobacter's genetic resistance to fosfomycin, mediated by efflux pumps. Lim et al. tested synergism of FOS/SUL on 50 isolates of carbapenem resistant *Acinetobacter baumannii* (CRAB), using the checkerboard method. A synergistic effect was observed in 74% of cases and no cases of antagonism were reported. A 4- to 8-fold drop was observed for MIC_{50}s and MIC_{90}s with the FOS/SUL combination compared to monotherapy. Reduction of MIC values, along with PTA (probability target attainment) yielding a 2-log10 reduction of the bacterial load, potentially places this therapy among the most effective at our disposal for the treatment of severe CRAB infections [207].

Sulbactam is susceptible to hydrolysis by a great variety of enzymes, including OXA-23, TEM and ADC [208]. The addition of avibactam to sulbactam (relebactam to a lesser extent) restores the efficacy of sulbactam by reducing MICs by over 2-fold dilution in 89% of cases in 187 MDR *Acinetobacter* isolates tested, as shown by Pasteran *et al.* [209]. Avibactam can interfere or by-pass the bacterium's **BfmRS**-mediated protection against β-lactam-induced toxicity, or it may act as a hypothetical mediator for a hypersensitivity to sulbactam by interacting with **advA** [210], a crucial protein involved in *Acinetobacter's* cell division.

Meropenem and cefiderocol may serve as possible therapeutic companions for sulbactam. *Acinetobacter* PBP2 is strongly inhibited by meropenem whereas sulbactam preferentially inhibits PBP1a and -3. The association in therefore potentially capable of inhibiting all three main *Acinetobacter* PBPs [211]. On the other hand, cefiderocol mainly inhibits Acineto-

bacter's PBP3 [131]; combining FDC with SUL may therefore mitigate the risk of resistance emergence. Indeed, *in vitro* data suggest synergistic activity against strains with elevated MICs [212].

According to results from a meta-analysis performed by Jung *et al.*, sulbactam proved to be the most effective option on mortality outcomes for the treatment of MDR *Acinetobacter baumannii* pneumonia [213]. The study suggested that high dose sulbactam (SUL) (9 g/day or even higher regimens) co-administered with intravenous colistin in association with inhaled colistin (IV COL + IH COL) was superior to single agent colistin regimens in terms of both survival and clinical cure (SUL P = 98-1%, IV COL + IH COL P = 99.9%) [213]. Along this line, a recently published study by Liu et al., which assessed data through NMA (network meta-analysis) including both direct and indirect evidence, further supported the **use of high dose sulbactam (> 6 g/day) in combination with other molecules such as colistin and/or tigecycline** for an effective treatment of severe MDR and XDR *Acinetobacter baumannii* infections [214]. A bulk of evidence supports sulbactam continuous infusion regimens in order to increase its efficacy and enhance its possible association with polymyxins [191,215]. The combination sulbactam/durlobactam (SUL/DUR) is generating interest as an appealing future treatment option. Durlobactam is a novel serine β-lactamases inhibitor, capable of restoring sulbactam's activity against resistant *Acinetobacter baumannii* strains. Seifert *et al.* evaluated the susceptibility rates to various antimicrobials including sulbactam/durlobactam among 246 AB-CR strains. The study results highlighted the excellent activity of the combination, which is comparable to colistin and superior to amikacin, minocycline and sulbactam alone ($MIC_{50/90}$: 1/4 and 2/4 mg/L (SUL/DUR), 0.5 and 1 mg/L (colistin), 256 and <512 mg/L (amikacin), 2 and 16 mg/L (minocycline), 16 and 64 mg/L (sulbactam) [216]. Resistance to SUL/DUR is currently rare and, when present, it is due to expression of metallo-β-lactamases (such as NDM-1) or PBP3 mutations, sulbactam's main target [217].

High dose sulbactam in combination therapy

COLISTIN

For decades, colistin therapy constituted the preferred backbone for the treatment of severe *Acinetobacter* infections. Validated intravenous dosing of colistin in critically ill patients is 9 MU loading-dose administered over three hours followed by 4.5 MU infused over 3 hours every 12 hours [218].

Intrathecal colistin dose

Intrathecal colistin administration at a dose of 125,000 IU (10 mg) once daily is considered as the treatment of choice for *Acinetobacter baumannii* meningitis and ventriculitis. A literature review by Karaiskos *et al.* reported a therapeutic success rate of 89%, suggesting safety and efficacy of this administration route [219]. The study by Chusri *et al.* reinforced this data, reporting a significant reduction in the mortality of patients affected by post-surgical meningo-ventriculitis due to *Acinetobacter baumannii*, when treated with intrathecal or intraventricular (ITH / IVT) colistin as opposed to intravenous colistin alone (mortality at 14 days 24% vs 38%, at 30 days 29% vs 56%, in-hospital mortality 29% vs 56%) [220].

Undeniably, due to poor ELF kinetics and penetration, inhaled therapy is the elective route for colistin administration for managing *Acinetobacter baumannii* infections involving the lower respiratory tract (VAP), provided the use of effective devices (vibrating mesh nebulizers). Zheng *et al.* performed a multivariate analysis on 183 patients affected by *Acinetobacter baumannii* pneumonia and undergoing colistin treatment for at least 7 days. The study results indicated inhaled colistin (IH) as the only independent predictor for 30-day survival, clinical response, and microbiological eradication. Conversely, intravenous colistin appeared as an independent predictor of clinical failure. Furthermore, nephrotoxicity differed significantly between the two methods of administration (37.5% vs 6.1%, P = 0.001 for inhalation therapy) [221].

Colistin has been administered in combination therapy regimens with rifampicin, sulbactam and/or tetracyclines. However, a litera-

ture analysis revealed that none of the colistin-based combination therapies achieved conclusive results in terms of efficacy. In a study by Durante-Mangoni *et al.*, 210 hospitalized ICU patients with severe XDR *Acinetobacter baumannii* infections were randomized (1: 1) to colistin combination or monotherapy. Results indicated that addition of rifampicin to colistin regimens did not reduce 30-day mortality rates nor length of hospitalization. Nevertheless, a significant increase in microbiological eradication in the colistin / rifampicin group was observed [222]. It is worth noticing that in this study colistin was administered as a 2 MU every 8 hours regimen without loading dose. A prospective study comparing colistin versus colistin/meropenem in severe nosocomial infections (BSI, VAP, HAP, cUTI) caused by carbapenem-resistant Gram-negative pathogens (77% of cases presenting with *Acinetobacter baumannii*) did not demonstrate superiority of combination treatment over monotherapy [223]. These data differ from retrospective observations concerning *Acinetobacter baumannii* bacteremia, whereby combination therapy is superior to monotherapy [224]. This may lead to speculation over the real role of *Acinetobacter baumannii* as the underlying causative pathogen in respiratory infections and consequently to the role of antibiotic therapy in such contexts.

TETRACYCLINS

Tigecycline has demonstrated *in vitro* activity against *Acinetobacter baumannii*. Available clinical data, is however, mostly retrospective and mainly describe therapeutic regimens including **tigecycline in combination with other molecules**. Reports of MIC increases during therapy and low drug concentrations reached at conventional dosages are the major negative drawbacks for tigecycline (TGC) use in the treatment of *Acinetobacter baumannii* infections, as reported by Shao Hua *et al.* [225]. Yang *et al.* recently assessed TCG levels by means of HPLC-MS / MS in 186 plasma samples from 67 patients with severe MDR *Acinetobacter baumannii* infections. Results in-

dicated that a high dose regimen of TGC (100 mg maintenance every 12 hours preceded by loading doses of 200 mg) achieves good clinical responses in terms of efficacy [226]. The clinical efficacy of TCG, a time-dependent antibiotic with a long post-antibiotic effect (PAE), is reached only upon AUC0-24 (*f*AUC0-24) / MIC ratios greater than 0.9. At standard dosages, this ratio is never achieved. This is particularly true for some compartments such as the bloodstream.

Additionally, MIC determination by means of broth dilution techniques is mandatory in order to avoid underestimation of susceptibility to tigecycline. Yang et al. reported TGC susceptibility rates in *Acinetobacter baumannii* (AB) isolates according to different assessment methods: 65.67% by means of broth dilution, 5.97% with agar and 0.75% by disk diffusion methods.

Minocycline, available as intravenous formulation in some countries, may represent another valid treatment option. Beganovic *et al.* used a pharmacodynamic model to simulate the impact of minocycline both at standard dose (200 mg loading dose + 100 mg q12h) and at increased doses (700 mg loading dose + 350 mg q12h), along with colistin (2.5 mg/kg q12h), sulbactam (9 g/ 4 h) and meropenem (6 g/24h in extended infusion) on CRAB isolates. Only high dose minocycline administered as triple combination therapy with continuous infusion sulbactam and polymyxins produced the most significant killing effect [227].

The AYE efflux pump TetA (G) harbored by *Acinetobacter baumannii* confers resistance to a variety of tetracyclines, with the exception of tigecycline. Expression of TetA gene (G) is regulated by the TetR repressor (AbTetR); tigecycline binds the repressor but is not transported by the TetA (G) efflux pump [226] [228].

In conclusion, we hereby propose a decision algorithm for the diagnosis and treatment of MDR/XDR *Acinetobacter baumannii* infections in the critically ill patient (Figure 29).

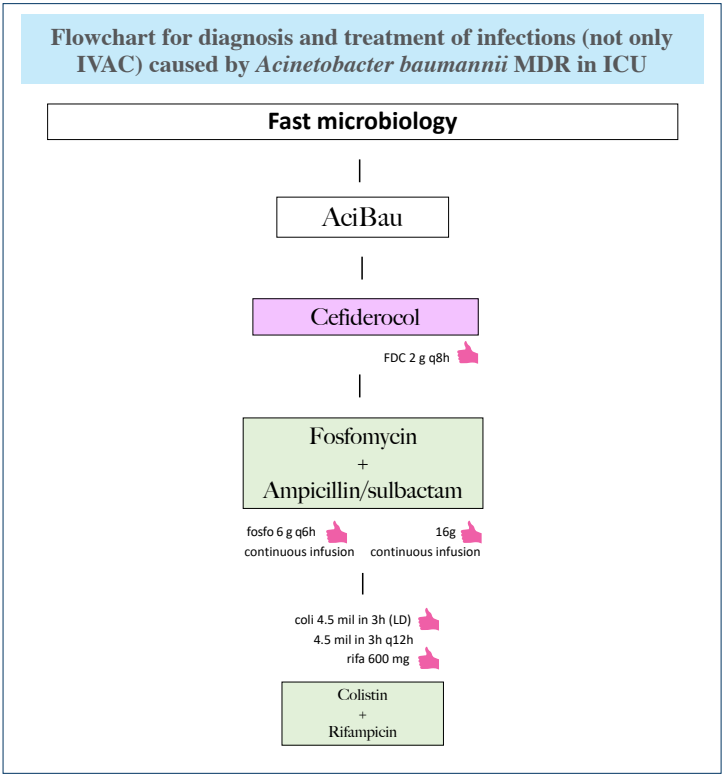


Figure 29. *Diagnostic and therapeutic decisional algorithm for the treatment of MDR/XDR Acinetobacter baumannii infections in critically ill patients.*

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GRAM-NEGATIVE BACTERIA

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Bacteroides fragilis
Enterobacterales
- *Enterobacter cloacae* complex
- *Escherichia coli*
- *Klebsiella aerogenes*
- *Klebsiella oxytoca*
- *Klebsiella pneumoniae* group
- *Proteus*
- *Salmonella*
- *Serratia marcescens*

Haemophilus influenzae
Neisseria meningitidis
Pseudomonas aeruginosa
Stenotrophomonas maltophilia

GRAM-POSITIVE BACTERIA

Enterococcus faecalis
Enterococcus faecium
Listeria monocytogenes
Staphylococcus
- *Staphylococcus aureus*
- *Staphylococcus epidermidis*
- *Staphylococcus lugdunensis*

Streptococcus
- *Streptococcus agalactiae*
- *Streptococcus pneumoniae*
- *Streptococcus pyogenes*

YEAST

Candida albicans
Candida auris
Candida glabrata
Candida krusei
Candida parapsilosis
Candida tropicalis
Cryptococcus neoformans/gattii

ANTIMICROBIAL RESISTANCE GENES

Carbapenemases
IMP
KPC
OXA-48-like
NDM
VIM

Colistin Resistance
mcr-1

ESBL
CTX-M

Methicillin Resistance
mecA/C
mecA/C and MREJ (MRSA)

Vancomycin Resistance
vanA/B

Panel Specifications

Sample Type: Positive blood culture	**Hands-on Time:** Approximately 2 minutes
Sample Volume: 0.2 mL	**Performance:** 99% sensitivity and 99.8% specificity[7]
Storage Conditions: All kit components stored at room temperature (15-25 °C)	

Part Number

BioFire BCID2 Panel Reagent Kit (30 Pouches): RFIT-ASY-0147

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BACTERIA
(Semi-quantitative)
Acinetobacter calcoaceticus-baumannii complex
Enterobacter cloacae complex
Escherichia coli
Haemophilus influenzae
Klebsiella aerogenes
Klebsiella oxytoca
Klebsiella pneumoniae group
Moraxella catarrhalis
Proteus spp.
Pseudomonas aeruginosa
Serratia marcescens
Staphylococcus aureus
Streptococcus agalactiae
Streptococcus pneumoniae
Streptococcus pyogenes

ATYPICAL BACTERIA
(Qualitative)
Chlamydia pneumoniae
Legionella pneumophila
Mycoplasma pneumoniae

VIRUSES
Adenovirus
Coronavirus
Human Metapneumovirus
Human Rhinovirus/Enterovirus
Influenza A
Influenza B
Middle East Respiratory Syndrome Coronavirus (MERS-CoV)
Parainfluenza Virus
Respiratory Syncytial Virus

ANTIMICROBIAL RESISTANCE GENES
Carbapenemases
IMP
KPC
NDM
OXA-48-like
VIM

ESBL
CTX-M

Methicillin Resistance
mecA/C and MREJ (MRSA)

Panel Specifications

Sample Type: Sputum (including endotracheal aspirate) and bronchoalveolar lavage (BAL) (including mini-BAL)
Hands-on Time: Approximately 2 minutes
Performance: BAL-like–96.2% sensitivity and 98.3% specificity, sputum-like–96.3% sensitivity and 97.2% specificity[1]
Storage Conditions: All kit components stored at room temperature (15–25 ˚C)

Part Numbers

BioFire Pneumonia *plus* Panel Reagent Kit (30 Pouches): RFIT-ASY-0143

Product availability varies by country.
Consult your bioMérieux representative.

CE 2797 | **US FDA-Cleared**

Instruction for use and manuals available on: ***https://www.biofiredx.com/support/documents/#toggle-id-3.***

BIOMÉRIEUX BIOFIRE®

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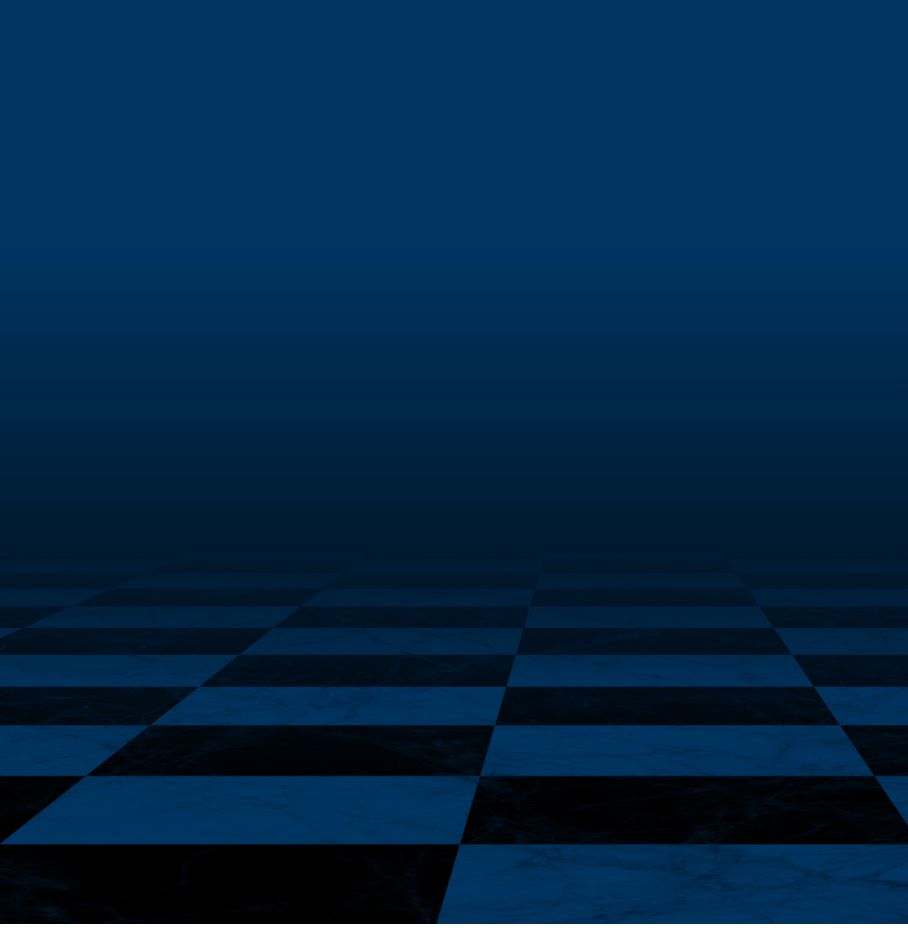

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