Multidrug-resistant Gram-negative bacteria-resistant infections: epidemiology, clinical issues and therapeutic options

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ABSTRACT

In the last decade, we have witnessed a dramatic increase in the number of multidrug resistant Gram-negative (MDRGN) bacterial pathogens, both in Italy and worldwide, with *Enterobacteriacae* (mostly *Klebsiella pneumoniae*), *Pseudomonas aeruginosa* and *Acinetobacter baumannii* being the major threats in clinical practice. Inadequate empirical antimicrobial therapy of severe infections caused by MDR *Enterobacteriacae* has been associated with an increased morbidity and mortality. However, a careful selection of patients who may receive empirical treatment covering MDR *Enterobacteriacae* is important to avoid the overuse of broad-spectrum antibiotics. The aim of this review is to describe the mechanism of resistance, epidemiology, risk factors, clinical issues, and therapeutic options for MDRGN pathogens.

Introduction

In the last decade, we have witnessed a dramatic increase worldwide in the number of multidrug resistant Gram-negative (MDRGN) bacterial pathogens, with *Enterobacteriacae* (mostly *Klebsiella pneumoniae*), *Pseudomonas aeruginosa* and *Acinetobacter baumannii* being the major threats in clinical practice. Due to the resistance to the most common antibiotics prescribed as empiric regimens, MDRGN bugs have been associated with delays in an adequate treatment, leading to significant increases in morbidity and mortality.[1] In addition, the spread of MDRGN pathogens resulted over last years in a vicious circle of an indiscriminate prescription of broad-spectrum antimicrobials and further resistance selection.[2]

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The aim of this review is to describe the mechanism of resistance, epidemiology, risk factors, clinical issues, and therapeutic options of MDRGN pathogens.

Mechanism of resistance of Gram-negative bacteria

MGRGN bacteria are defined as pathogens carrying resistance to one or more antimicrobials from at least three different classes. The most common mechanism of resistance is represented by intrinsic and acquired production of β-lactamases, which can be chromosomal or plasmid mediated. β-lactamases are hydrolytic enzymes able to disrupt the β-lactam ring, thus inactivating different classes of β-lactams.[3,4] The most common enzymes in clinical practice are the extended-spectrum-β-lactamases (ESBLs), which are mostly expressed by *Enterobacteriaceae*. A novel type of class C β-lactamases also showing activity against cefepime and denominated extended spectrum AmpC β-lactamases has been described.[4]

The consequent abuse of carbapenems, representing the first choice for ESBL infections, led to a progressive increase in carbapenem resistance, mainly due to the production of carbapenem-hydrolyzing β-lactamases, or carbapenemases, that usually confer clinical resistance to most β-lactams.[5,6] *K. pneumoniae* carbapenemases (KPCs) are the most relevant enzymes among *Enterobacteriaceae*, and confer resistance to all the β-lactams, including β-lactam/β-lactamase inhibitors combinations. Class B enzymes, named metallo-β-lactamases are expressed by both enterics and *P. aeruginosa* and confer resistance to all β-lactams with the exception of aztreonam. Oxacillinases belong to the class D β-lactamases, and are mostly expressed in *P.*

aeruginosa and *A. baumannii*.[7] Table 1 summarizes the classification of β-lactamases.[3]

Together with the production of β-lactamases, additional mechanisms of carbapenem-resistance are the down-regulation of porins and efflux pumps, the last most common in *P. aeruginosa* and the expression of additional genes harbouring resistance to other antimicrobial classes, such as fluoroquinolones and aminoglycosides, thus narrowing the spectrum of therapeutic options.[8]

Epidemiology of emerging multidrug resistant Gram-negative bacteria in Italy

The burden of MDRGN bacteria has alarmingly increased worldwide over last decade, even with a wide variability between different countries.[9] In Europe, the distribution of antimicrobial resistance is widely jeopardized, but the Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net) showed a generalized increase in antimicrobial resistance particularly in Southern regions.[10]

Focusing on the Italian epidemiological setting, more than 50% of *K. pneumoniae* isolates showed resistance to third-generation cephalosporins, and the large majority of isolates (85-100%) expressed ESBL enzymes. In *Escherichia coli,* a combined resistance to third-generation cephalosporins, fluroquinolones and aminoglycosides was reported in 10-25% of cases.

Carbapenem-resistance represents nowadays the most alarming problem for our country. Among *Enterobacteriaceae*, the major threat comes from *K. pneumoniae*, since in 2014 in Italy approximately 25-50% of isolates were resistant to carbapenems, compared to less than 5% of isolates in 2009. Conversely, in *E. coli* carbapenem-resistant is currently reported in less than 5% of cases, but represents an emerging problem in clinical practice.[11]

P. aeruginosa also represents a worrisome problem. A significant increase in resistance to piperacillin/tazobactam has been observed (around 17% in 2014 in Europe). Moreover, in Italy, approximately 25-50% of *P. aeruginosa* isolates showed resistance to carbapenems and up to 10-50% of strains were classified as MDR. Similar data have been reported in *A. baumannii*, which showed combined resistance to fluoroquinolones, aminoglycosides and carbapenems in up to 50% of cases, with an alarming increase in colistin-resistance, reported in approximately 4% of isolates, mostly in Italy. Table 2 summarizes epidemiology of MDRGN bacteria worldwide and in Italy.

Risk factors, clinical issues and empirical treatment for emerging multidrug resistant Gram-negative infections

Emerging MDRGN pathogens are related both to individual risk factors and to local epidemiological

Table 1. Summary of relevant, emerging broad-spectrum, plasmid-mediated β-lactamases in multidrug-resistant Gram-negative bacteria.

Molecular class	Enzymes	Spectrum	Epidemiology
A	Extended-spectrum β-lactamases (TEM, SHV, CTX-M, others)	Penicillins, cephalosporins (except cefamycins), aztreonam Inhibited by β-lactamase inhibitors	Worldwide spread: USA, Greece, Italy, Israel, China Community and nosocomial infections
	KPC	Penicillins, cephalosporins, aztreonam, carbapenems. Inhibited by β-lactamase inhibitors	Mainly nosocomial outbreaks Frequent in some areas of USA, Greece, Israel, Italy, *etc*.
B	Metallo β-lactamases (VIM, IMP, NDM, others)	Penicillins, cephalosporins and carbapenems Monobactams are susceptible Not inhibited by β-lactamase inhibitors	Worldwide spread: Greece, Italy, Indian subcontinent, Balkans, Middle East Nosocomial outbreaks and endemic situations
C	AmpC type (CMY-2, DHA-1, FOX-1, others)	Penicillins, cephalosporins (except cefepime), and monobactams Not inhibited by β-lactamase inhibitors	Worldwide spread Community and nosocomial infections
D	OXA (OXA-48, OXA-23, others)	Penicillin, aztreonam and carbapenems Not inhibited by β-lactamase inhibitors	Nosocomial outbreak Spread in Middle East, Mediterranean countries (Spain) and Africa

TEM, temoniera (a patient from whom the strain was first isolated in Greece); SHV, sulphydryl variable; CTX-M, cefotaxime hydrolyzing capabilities; KPC, *Klebsiella pneumoniae* carbapenemase; VIM, Verona integron-encoded metallo-β-lactamase; IMP, imipenem-type metallo-β-lactamase; NDM, New Delhi metallo-β-lactamase; CMY-2, cephamycins; DHA-1, Dhahran Hospital; FOX-1, cefoxitin; OXA, oxacillinase. *Modified from Delgado-Valverde* et al., *2013.*[4]

background, including community, long-term care facilities or hospital setting. While ESBL *Enterobacteriaceae* are found in the community and hospital environment, carbapenemase-producing *Enterobacteriacae* (CRE), MDR *P. aeruginosa* and MDR *A. baumannii* are still mainly found in hospitalized patients, although OXA-48 CRE might be isolated in the community.[12] In clinical practice, Intensive Care Unit (ICU) setting has been traditionally considered the tip of the iceberg of hospital MDRGN infections.[13-15] However, beside ICU setting, in recent years the spread of MDRGN pathogens has been increasingly described also in other settings, such as oncologic and hematologic wards[16,17] and long-term-care facilities.[18,19] A bedridden status, presence of indwelling devices, recent hospitalization or contact with health care facilities and recent antibiotic therapy may represent the most important risk factors for the development of emerging MDRGN infections (Table 3).

Thus, the prescription of an early and effective antibiotic regimen is crucial for the management of severe MDRGN infections. The available evidence suggests that the greatest benefit of combination antibiotic therapy stems from the increased likelihood of choosing an effective agent during empiric therapy, rather than exploitation of *in vitro* synergy or the prevention of resistance during definitive treatment.[20] When a MDR gram-negative pathogen is suspected, the early prescription of a broad-spectrum, combination regimen, followed by a prompt de-escalation upon availability of susceptibility tests should be recommended, looking for a balance between the early start of an effective treatment and the risk of resistance selection.

However, clinicians should be aware that the *in vitro* susceptibility of the pathogen to the prescribed antimicrobial regimen is not the only point to be considered. The optimization of antimicrobial doses and ways of administration in order to achieve and maintain optimal plasmatic and/or tissue concentrations according with patient's characteristics (age, renal function, obesity) and source of infection are adjunctive crucial elements to be taken into consideration.[21,22] A recent study conducted in several ICU worldwide showed that antimicrobials are routinely underdosed, especially β-lactams. Probably a paradigm change to more personalized antibiotic dosing may be necessary to improve outcomes for these most seriously ill patients.[23] The dosages of the different antimicrobial agents are listed in Table 4. An algorithm for the prescription of and adequate empiric treatment for each MDR gram-negative pathogen is proposed in Figure 1.

Targeted treatment

Extended-spectrum-β-lactamases-producing *Enterobacteriaceae*

The main dilemma in infections caused by ESBL-producing *Enterobacteriaceae* is the best use of carbapenems. In order to avoid the threat of CRE, carbapenem-saving strategies should be considered for ESBL infections and β-lactam/β-lactamase inhibitor (BLBLIs) combinations, such as piperacillin-tazobactam, have been recommended as an alternative to carbapenems for ESBLs.[24]

Carbapenems are still considered the empirical treatment of choice against serious infections (severely ill patients and/or those with septic shock) caused by ESBL-producing bacteria.[2] The empirical administration of ertapenem for suspected ESBL *Enterobacteriacae* is not recommended, since some concerns are rising regarding the isolated *in vitro* resistance to this drug and the need for further data on severely ill patients.[25,26]

However, according to recent data, BLBLIs, if active *in vitro*, appear to be as effective as carbapenems for empirical therapy of bloodstream infections due to ESLB-*Enterobacteriaceae*, regardless of the source and specific species, if used at appropriate doses.[27] Therefore, high dosage with loading dose and semi-continuous administration of BLBLIs, supported by therapeutic drug monitoring should be preferred for clinically stable patients.[2,23]

Among other β-lactams, there is still poor information about the efficacy of active cephalosporins and

Table 2. Epidemiology of emerging gram-negative bacteria in USA and Europe.

Enzyme	USA (%)	North-Centre Europe* (%)	Italy-Greece (%)
ESBL *Escherichia coli*	14	5-25	10-50
ESBL *Klebsiella pnemoniae*	23	1-25	>50
CRE-KPC	11	1-5	25-50
MDR *Pseudomonas aeruginosa*	13	1-25	25-50
MDR *Acinetobacter*	63	1-10	>50

*North Centre Europe include all European country except Italy and Greece. ESBL, extended-spectrum-β-lactamase; CRE, carbapenemase-producing *Enterobacteriacae*; KPC, *Klebsiella pneumoniae* carbapenemase; MDR, multidrug resistant.

is likely to result in treatment failure (even with *in vitro* susceptibility). However, cephalosporin-β-lactamase inhibitors, namely ceftolozane-tazobactam and ceftazidime-avibactam, are novel agents that appear to have greater activity against ESBL-producing organisms.[28]

Key point

In conclusion, in our opinion the use of carbapenems may be reserved for patients with severe infections caused by ESBL-producing bacteria, and de-escalation therapy should always be performed if feasible (Table 2). The best alternative to carbapenems for the treatment of ESBL infections is represented by BLBLIs, which can be used in patients with bloodstream infection (BSI) caused by ESBL, especially with lower piperacillin/tazobactam minimum inhibitory concentration (MIC) (*e.g.*, ≤16/4 μg/mL).[29,30] The use of β-lactams should be maximized by a pharmacokinetic/pharmacodynamics (PK/PD) point of view with the administration of high dosages and prolonged infusion strategies maximizing the time above the MIC (t>MIC). A loading dose followed by maintenance doses with extended or continuous infusion is recommended (Table 2).[29] Although there is less clinical experience to recommend the use of other antibiotics (tigecycline, aminoglycosides, fosfomycin, fluoroquinolones or trimethoprim/sulfamethoxazole), alternative therapeutic approaches could be considered for ESBL-producing *Enteobacteriacae* based on the susceptibility test results.

Carbapenemase resistant *Enterobacteriacae*

Treatment recommendations in CRE infections are mostly based on the accumulating clinical experience from KPC and should be based on several aspects. Combination treatment containing two or three active drugs has shown significant advantages over monotherapy in terms of survival for KPC infections.[31-33]

The role of carbapenems in infections caused by KPC is still debated. Among the different combinations, high dose carbapenem regimens have been associated with better outcome in previous reports.[31,34] Tumbarello *et al*. reported that survival rates for combination regimens that included meropenem were 87% at meropenem MICs <4, 75% at MICs of 8 mg/L and 65% at MICs >16 mg/L, which was better than the overall survival rate (58%) reported in the study.[31] Daikos *et al*. also found that patients with KPC infections who received carbapenem-containing combination regimens had significantly lower mortality rates compared with patients who received non-carbapenem-containing regimens (12 *vs* 41%; P=0.006), especially in cases where the MIC of the infecting isolate was <4 mg/L.[33]

In keeping with these results, a recent study by Tumbarello *et al.* supported the use of carbapenems

Table 3. Risk factors for gram-negative bacteria.

Baseline characteristics	Previous antibiotic therapy	Epidemiological background	Previous colonization	Indwelling devices
Age >70	Previous use of aminopenicillins	Previous hospital admission	Gut colonisation ESBL	Urinary catheter
Diabetes mellitus	Previous use of cephalosporins	Prolonged hospitalization	Gut colonisation CRE	Gastrostomy or jejunostomy
Charlson index ≥3	Previous use of fluoroquinolones	Transfer from another healthcare facility	Colonization with MRSA	Nasogastric tube
Recurrent or obstructive UTIs	Previous use of carbapenems	Current or prior ICU admission	Colonization with *Acinetobacter*	CVC
Use of corticosteroids	Previous use of aminoglycosides	Local epidemiology, outbreak	Endotracheal colonization with	Mechanical ventilation
Immunosuppression		Travel from high endemic area	*Pseudomonas aeruginosa*	Hemodialysis
Trauma				
Malignancy				
Organ transplantation				
COPD				
Neutropenia				
Recent surgery				

ESBL, extended spectrum β-lactamase; CRE, carbapenemase resistant *Enterobacteriacae*; MRSA, methicillin-resistant *Staphylococcus aureus*; UTIs, urinary tract infections; CVC, central venous catheter; ICU, Intensive Care Unit; COPD; chronic obstructive pulmonary disease.

for the treatment of KPC, but with some fundamental conditions, such as low carbapenem MIC for the infecting organism (≤8 mg/L), optimal PK/PD exposure to carbapenem, and combination with another active compound.[32]

Clinical experience with therapeutic drug monitoring (TDM) of β-lactams for KPC remains scarce, but it is most likely to be beneficial.[35] For carbapenems, as for other time-dependent agents, the maintenance of concentrations (C_{trough}) above MIC for about 40%-50% of the time between dosing interval represents the target for bactericidal activity. In KPC related infections, however, there is the need to maintain a C_{trough} level above the MIC for the entire dosing interval.[36] Pharmacokinetic data have found that high-dosed, prolonged (continuous or extended) infusion of meropenem could achieve adequate exposures (40% C_{trough} Time >MIC) in 100, 75 and 40% of septic patients infected with KPC-Kp isolates with MICs of 4, 8 and 16 mg/L, respectively.[37-40]

Antibiotics that permeabilize the bacterial cell membrane (*e.g.*, polymyxins), interfere with cell wall synthesis (*e.g.*, fosfomycin), or inhibit protein synthesis (*e.g.*, aminoglycosides or tigecycline) may decrease the MIC sufficiently so that it is exceeded when a carbapenem is co-administered. Therefore, combination therapy should be strongly considered.[41,42] Colistin is considered a highly active *in vitro* agent against KPC.[43] Clinical use of tigecycline for MDR infections has been heterogeneous, but seem to be effective and safe in the treatment of CRE as part of a combination regimen especially when administered at higher doses.[44] Aminoglycoside-containing regimens (particularly gentamicin) and fosfomycin have also been associated with favourable outcomes and should be encouraged particularly in view of increasing rates of colistin resistance.[2,33]

In addition, the double-carbapenem regimen (ertapenem plus high-dose meropenem or doripenem) has shown to enhance efficacy over either agent alone in previous *in vitro* and *in vivo* studies and has been recently considered a possible therapeutic strategy in KPC with high carbapenem MIC or colistin resistance.[45-47] The proposed rationale is that ertapenem has a higher affinity to the KPC enzyme, therefore acting as a suicide substrate and allowing the second carbapenem to be protected from the KCP carbapenemase.[45-47] Controlled clinical data, however, are needed to determine the efficacy of this treatment.

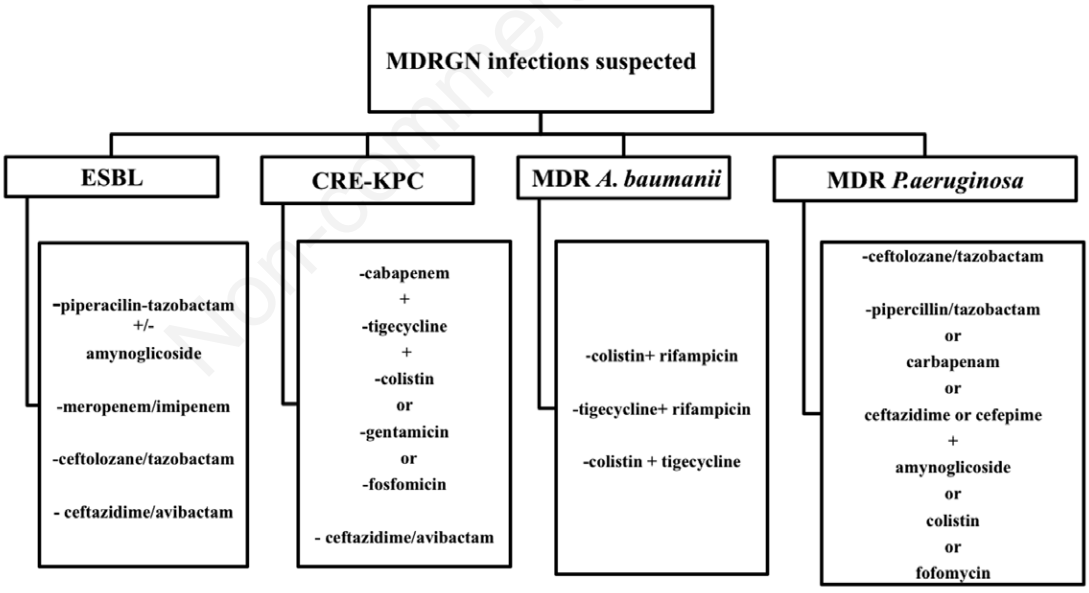

Figure 1. Empirical treatment for suspected multidrug-resistant Gram-negative infections. Choice of antibiotic should be based on suspected site of infection and local penetration. Colistin and aminoglycosides (amikacin and tobramicin) represent good options in respiratory tract infections, however due to the lack of adequate lung penetration inhaled formulations should be preferred (colistin 2 MUI every 8 h or *tobramycin 300 mg every 12 h* or *amikacin 500 mg every 12 h*). Intravenous aminoglycosides and colistin should be preferred mainly in bloodstream infections. Intravenous fosfomycin is an option for the treatment of urinary tract infections. Inhaled antibiotic can be associated to empirical intravenous therapy for suspected respiratory tract infections. Metronidazole 500 mg every 8 h should be associated to ceftolozane/tazobactam or ceftazidim/avibactam for suspected intraabdominal infection. ESBL, extended-spectrum β-lactamase; CRE-KPC, carbapenemase resistant *Enterobacteriacae - Klebsiella pneumoniae* carbapenemase; MDR, multidrug resistant; *A. baumannii*, *Acinetobacter baumannii*; *P. aeruginosa*, *Pseudomonas aeruginosa*.

Table 4. Recommended doses of antimicrobials for empirical treatment of multidrug resistant infections in patients with normal renal function.

Antibiotic	Loading dose	Daily dose	Observations
Ceftazidime	2 g in 1 h	6 g every 24 h	CI is recommended
Cefepime	2 g in 1 h	6 g every 24 h	CI is recommended
Piperacillin-tazobactam	4.5 g in 1 h	16/2 g every 24 h	CI is recommended
Meropenem	1 g in 1 h	4 g every 24 h*	Extended infusion is recommended: 1 g every 6 h in 6 h
Imipenem	0.5 g or 1 g in 1 h	2-3 g every 24 h	Extended infusion is recommended: 0.5 g every 6 h or 1 g every 8 h in 2 h High doses are associated with seizures
Ertapenem	0.5 g in 1 h	2 g every 24 h	Extended infusion is recommended: 0.5 g every 6 h
Sulbactam	Not required	9-12 g/day (in 3 or 4 doses)	4-h infusion is recommended
Levofloxacin	Not required	750 mg every 24 h	
Ciprofloxacin	Not required	400 mg every 8 h	
Colistin	9 MU in 1 h	4.5 MU every 12 h	High doses are associated with renal toxicity
Tigecycline	200 mg in 1 h	100 mg every 12 h	For other sources including pneumonia and primary bloodstream infection: consider combination with another antimicrobial
Rifampicin	Not required	600 mg/day or 600 mg/12 h	Always in combination therapy
Fosfomycin	Not required	24 g/day (in 4 doses)	Always in combination therapy
Amikacin	Not required	15-20 mg/kg/day every 24 h	High doses are associated with renal toxicity
Gentamicin	Not required	3-5 mg/kg/day every 24 h	High doses are associated with renal toxicity
Ceftolozane/tazobactam	1.5-3 g in 1 h	1.5-3 g every 8 h	Extended infusion is recommended: 1.5-3 g every 8 h in 3 h
Ceftazidime-avibactam	2.5 g in 1 h	2.5 g every 8 h	Extended infusion is recommended: 2.5 g every 8 h in 3 h

CI, continuous infusion. The loading dose should be administered in all patients including those with renal dysfunction. *For *Klebsiella pneumoniae* carbapenemase meropenem 2 g every 8 h (extended infusion in 6 h) is recommended.

Key point

In our opinion, in KPC with MIC ≤8-16 mg/L meropenem should be administered at high doses and prolonged infusion in combination regimens (with two antibiotics with *in vitro* activity), according to TDM. When the MIC is higher than 16 mg/L carbapenem excluding combination, therapy should be performed considering *in vitro* activity of antimicrobials. Double-carbapenem regimen is a possible therapeutic strategy in KPC with colistin resistance or high carbapenem MIC (meropenem MIC >8-16 μg/Ml) (Table 5).

Multidrug resistant *Acinetobacter*

Adequate therapy of severe infections caused by *A. baumannii* is crucial in terms of survival.[48] However, the inherent and acquired resistance of *A. baumannii* limits the number of antimicrobial options, and makes the selection of an appropriate antimicrobial regimen extremely difficult. Traditionally, carbapenems (except ertapenem) have been considered as the drug of choice for the treatment of *A. baumannii* infections, in areas with high rates of susceptibility.[49] In those areas with high rates of carbapenem-resistant *A. baumannii*, carbapenems should not be used, at least in monotherapy. Other therapeutic options include sulbactam, aminoglycosides, polymyxins and tigecycline.[50] Optimal dosing of sulbactam is unclear and there is concern about the fact that *in vitro* activity does not necessarily predict clinical outcomes.[51]

Colistin and tigecycline, although active *in vitro*, are limited *in vivo* by suboptimal PK characteristics and by the emergence of resistance during therapy.[52] In strains susceptible to colistin and demonstrating a low MIC for sulbactam (≤4 mg/L), the use of sulbactam may be preferable in the directed therapy based on its better safety profile and to preserve colistin. Tigecycline may be a suitable alternative in the directed therapy for infections of the approved indications caused by MDR *A. baumannii* if the MIC to this agent is ≤1 mg/L and the isolate is resistant to other agents. However polymyxins have shown the greatest level of *in vitro* activity against *A. baumannii* and are the most commonly used agents for *Acinetobacter* isolates resistant to first-line agents; various combination regimens have been considered, mostly with colistin serving as backbone.[53]

In regards to colistin plus rifampin, a large prospective study performed in Italy comparing the use of colistin monotherapy *versus* colistin plus rifampicin in patients with severe infections due to MDR *Acinetobacter*. Combination therapy was associated with higher microbiological eradication without an impact on mortality.[54]

The combination of colistin with a carbapenem for the treatment of MDR *A. baumannii* infections has been analysed only in retrospective studies suggesting that colistin-carbapenem combinations may result in improved clinical responses and survival compared to other regimens and may also limit the emergence of colistin resistance.[55]

The largest comparative study analyzed 250 patients with XDR-Ab BSIs matching colistin monotherapy to combination therapy with colistin + meropenem, col-

Table 5. Drugs recently approved by Food and Drug Administration or in clinical development with activity against multidrug resistant *Enterobacteriaceae*.

Drug name	ESBL	CRE	MDR *P. aeruginosa*	MDR *Acinetobacter*
Cephalosporin				
S-649266	Yes	KPC and NDM-1	Yes	Yes
Cephalosporin + β-lactamase inhibitor				
Ceftolozane-tazobactam	Yes	NO	Yes	No
Ceftazidime-avibactam	Yes	KPCs and OXA-48 (not active against MBLs)	Yes	
Ceftaroline fosamil-avibactam	Yes	KPCs and OXA-48 (not active against MBLs)	No	No
Monobactam + novel β-lactamase inhibitor				
Aztreonam-avibactam	Yes	MBLs such as NDM	Yes	No
Carbapenem + novel β-lactamase inhibitor				
Meropenem/vaborbactam	Yes	KPCs	No*	No°
Imipenem/cilastatin-relebactam	Yes	KPCs and OXA-48 (not active against MBLs)	No*	No°
Aminoglycoside				
Plazomicin	Yes	Most KPCs (not active against many NDMs)	No*	No
Tetracycline				
Eravacycline	Yes	KPCs	No	Yes

ESBL, extended-spectrum β-lactamases; CRE, carbapenemase resistant *Enterobacteriacae*; MDR, multidrug resistant; *P. aeruginosa*, *Pseudomonas aeruginosa*; KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo-β-lactamase; OXA, oxacillinase; MBL, metallo-β-lactamase. *Active against *P. aeruginosa*, but not MDR *P. aeruginosa*; °active against *Acinetobacter baumannii* but not against MDR *A. baumannii*.

istin + sulbactam and colistin plus other agents.[56] Outcome in terms of 14-day survival, eradication rate and in-hospital mortality was significantly better among patients receiving combination therapy, although no differences between the 3 different combination regimens were found. Colistin-glycopeptide and colistin-fosfomycin combination has shown to have synergy activity against MDR *A. baumannii*.[57,58]

Key point

In conclusion, no definitive recommendation can be made with regard to combination treatment or monotherapy for MDR *Acinetobacter* infections. Moreover, one potential benefit of combination therapy is the prevention of the emergence of resistance under therapy (especially for colistin and tigecycline).[59] The combination of sulbactam or a polymyxin with a second agent (tigecycline, rifampicin, or fosfomycin) may be considered for clinical failures or for infections caused by an isolate with MIC in the upper limit of susceptibility.

Multidrug resistant *Pseudomonas aeruginosa*

Adequate initial antibiotic treatment is crucial for the management of severe *P. aeruginosa* infections.[60,61] Recent studies showed that nowadays the prescription of an empiric monotherapy is frequently inadequate in *P. aeruginosa* infections.[62] Thus, when *P. aeruginosa* is suspected, due to the high rates of antimicrobial resistance, the empiric prescription of a combination therapy should be nowadays encouraged. Several factors, including source of infections and local epidemiology, should be considered in the choice of the optimal empiric combination regimen. The ideal approach consists in the prescription of an antipseudomonal beta-lactam (piperacillin/tazobactam, ceftazidime, or a carbapenem) plus and adjunctive second-agent.[63]

A large meta-analysis compared the effectiveness and safety of β-lactam combined with aminoglycoside or fluoroquinolone for the treatment of *P. aeruginosa* infection compared with β-lactam monotherapy.[64] The evidence suggests that combo regimen does not result in survival benefit; sub-analyses that included patients with bacteraemia, severe or milder infections did not show any benefit with combination therapy. According to these results, inappropriate antibiotic treatment was confirmed to be the main predictor for mortality; therefore, empirical combination therapy should improve outcome since it increases the chance of adequacy.

In an epidemiological setting characterised by the high prevalence of strains of *P. aeruginosa* resistant to fluoroquinolones, the combination of a beta-lactam and an aminoglycoside is probably safer. Once the susceptibility of *P. aeruginosa* has been attested, switching to monotherapy or de-escalating is considered to be safe and effective. De-escalation and prompt discontinuation of antibiotic therapy may lessen the risk of drug-related adverse events, reduce antibiotic pressure on bacterial ecology and finally diminish the emergence of resistant pathogens.[65]

The emergence of multidrug-resistant *P. aeruginosa* strains and the lack of new antimicrobials in the drug development pipeline have renewed the interest in old antibiotics, which had been abandoned for many years because of their excessive toxicity or poor handling. Among these, colistin, which is a cationic and multicomponent lipopeptide belonging to the class of polymyxins has been widely re-entered in clinical use.

According to data published in a prospective cohort study in 2010, due to lower efficacy and tolerability of colistin compared to beta-lactam antibiotics, its use should be restricted to the treatment of infections caused by *P. aeruginosa* resistant to other more active antibiotics.[66]

Key point

In conclusion, defining the best strategy for empirical treatment of patients at risk of MDR-*P. aeruginosa* infection will need further studies. On choosing a combination regimen, clinicians have to balance the risk of a greater toxicity with the emergence of antimicrobial resistance. Although fluoroquinolones have optimal tissue penetration, synergy with beta-lactams and low toxicity, resistances have been steadily increasing. Therefore, the combination of a β-lactam and an aminoglycoside seems to be safer in the empirical treatment. We recommend initiating empirical therapy with two antipseudomonal agents during the first 3-5 days, while waiting for microbiological results of cultures. If *P. aeruginosa* is isolated, the combination therapy can be de-escalated to monotherapy on the basis of the specific susceptibility pattern of the pathogen. Colistin should be restricted to the treatment of pathogens resistant to other more active molecules. Recently approved antimicrobial compounds including new cephalosporins or novel β-lactamase inhibitors have considerable potential in this setting.

Inhaled antibiotic therapy for multidrug resistant Gram-negative bacteria

Inhaled antibiotic therapy is conceptually appealing because of the toxicity and the unsatisfactory PK behavior of both colistin and aminoglycosides in the lung. Published studies suggest that aerosolized antibiotic therapy can achieve sputum and airway concentrations 100-fold greater than the MIC of most bacteria, including many MDR strains, although concentrations in lung tissue or epithelial lining fluid are often lower.[67]

Table 6. Hospital infection control measures for multidrug-resistant Gram-negative bacteria.

Bundled interventions
Standard hand hygiene
Early identification and contact precautions for colonized and infected patients
Active surveillance and isolation (until culture results are available) for previously colonized or infected with MDRGN patients recently treated at endemic institutions asymptomatic carriers during outbreaks
Cohort nursing staff
Antimicrobial stewardship policies
Education programmes
Cleaning of environmental surfaces
Decolonization of patients (chlorhexidine gluconate baths)
Gut decolonization

MDRGN, multidrug-resistant Gram-negative.

New antibiotics

Novel antimicrobials that could provide clinical efficacy towards MDR Gram-negative pathogens are urgently needed. Food and Drug Administration recently approved two novel combination antibiotics, ceftolozane-tazobactam and ceftazidime-avibactam, which has recently been approved for the treatment of complicated intra-abdominal infections (in combination with metronidazole) and complicated urinary tract infections. Both these combination agents have been shown *in vitro* activity against selected resistant Gram-negative pathogens, including *Enterobacteriaceae* and *P. aeruginosa*.[68]

The antipseudomonal activity of ceftolozane results from its ability to evade multiple resistance mechanisms, including efflux pumps, reduced uptake through porin channels, and modification of penicillin-binding proteins.[69] The development of resistance to ceftolozane tazobactam among strains of *P. aeruginosa* has been reported to be slower than resistance to other antipseudomonal agents, and it remained active against mutants resistant to ceftazidime, ciprofloxacin, and meropenem.[70] Spectrum of activity of ceftolozane/tazobactam includes difficult-to-treat Gram-negative pathogens, including ESBL strains.[28,71-73]

Another new therapeutic option is represented by ceftazidime/avibactam, where the new β-lactam inhibitor agent avibactam improves the activity of ceftazidime against MDR *P. aeruginosa*.[74] Notably, ceftazidime/avibactam has demonstrated consistent activity against KPC-Kp.[28,75] (Table 5). Other inhibitor combinations that have at least completed phase 1 clinical trials are ceftaroline fosamil/avibactam, imipenem/relebactam, meropenem/RPX7009.

Conclusions

For the next years, while waiting for new therapeutic options, it is essential that the last remaining antimicrobials be safeguarded through rational selection and improved infection control. Antibiotic stewardship programs should be developed and implemented by local and international interventions.

It should translate into the implementation of specific guidelines, targeted at education to optimize choice, dosage, and duration of antibiotics in order to improve outcomes and reduce the development of resistance. Moreover, as unmet medical needs call for the use of existing antibiotics in no approved indications or higher-than-approved dosages, regulations will need to be flexible and be updated following the evidence coming from nonrandomized trials.

Infection control protocols are another essential component for reducing the transmission of resistance. The key components of a successful hospital bundle for MDRGN are yet to be determined but have been successful at reducing nosocomial infection rates and controlling outbreaks. Most include improved hand hygiene compliance, contact precaution, control interventions cohort, staff education, and environmental disinfection[76-80] (Table 6). Other infection prevention strategies such as decolonization of patients by the use of daily chlorhexidine bathing have also demonstrated a reduction in the acquisition of MDR Gram-negative bacteria.[78] In addition, selective decontamination strategies have shown to be safe and possibly effective during therapy, but success at decolonization may favour the emergence of resistant strains and long-term effects are unclear.[81]

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