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Multidrug-Resistant Gram-Negative Infections

What are the Treatment Options?

Helen Giamarellou and *Garyphallia Poulakou*

4th Department of Internal Medicine, Athens University School of Medicine, 'ATTIKON' University Hospital of Athens, Athens, Greece

Abstract

The emergence of multidrug-resistant (MDR) Gram-negative bacilli creates a challenge in the treatment of nosocomial infections. While the pharmaceutical pipeline is waning, two revived old antibacterials (colistin and fosfomycin), a newer one (tigecycline) and an 'improved' member of an existing class (doripenem) are the only therapeutic options left.

The class of polymyxins, known since 1947 and represented mostly by polymyxin B and polymyxin E (colistin), has recently gained a principal role in the treatment of the most problematic MDR Gram-negative pathogens (such as *Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae* and *Stenotrophomonas maltophilia*). Future prospective studies are needed to answer important clinical questions, such as the possible benefit of combination with other antimicrobials versus monotherapy, the efficacy of colistin in neutropenic hosts and the role of inhaled colistin. As new pharmacokinetic data emerge, clarification of the pharmacokinetic/pharmacodynamic (PK/PD) profile of colistin as well as appropriate dosing seems urgent, while development of resistance must be carefully monitored.

Fosfomycin tromethamine, a synthetic salt of fosfomycin discovered in 1969, has regained attention because of its *in vitro* activity against extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae and MDR *P. aeruginosa*. Although in use for decades in oral and parenteral formulations for a variety of infections without significant toxicity, its clinical utility in MDR infections remains to be explored in future studies.

Tigecycline, the first representative of the new class of glycylcyclines, holds promise in infections from MDR *K. pneumoniae* (*K. pneumoniae* carbapenemase [KPC]- and ESBL-producing strains) and Enterobacteriaceae with various mechanisms of resistance. The *in vitro* activity of tigecycline against *A. baumannii* makes it a tempting option, as it is currently the most active compound against MDR strains along with colistin. However, the usual minimum inhibitory concentration values of this pathogen are approximately 2 mg/L and compromise clinical outcomes based on PK/PD issues. Its advantageous penetration into various tissues is useful in infections of the skin and soft tissues as well as intra-abdominal infections (official indications), whereas low serum concentrations compromise its use in bloodstream infections. Therefore, prospective studies with dose escalation are urgently needed,

as well as clarification of its role in nosocomial pneumonia, after poor results in the study of ventilator-associated pneumonia.

Finally, doripenem, the recently licensed member of the carbapenems (without significant spectrum alterations from the ascendant members) seems to possess a lower potential for resistance selection and a more favourable pharmacokinetic profile when given as an extended infusion. The latter strategy could prove helpful in overcoming low level resistance of *A. baumannii* and *P. aeruginosa* strains.

In 2009, infections caused by multidrug-resistant (MDR) bacteria continue to challenge physicians and endanger their patients' lives. MDR microorganisms were recently named as the 'ESKAPE' pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp.) to emphasize that they 'escape' the effects of antibacterial agents.[1-3]

The pharmaceutical pipeline against MDR Gram-negative pathogens is extremely limited. At the same time, data published in 2004 from the US National Nosocomial Infection Surveillance System (NNIS), report resistance rates among *P. aeruginosa* isolates to imipenem and quinolones at 21.1% and 29.5%, respectively; in intensive care unit (ICU) isolates, the respective rates of resistance were even higher (up to 51.6% for ciprofloxacin, 31.4% for piperacillin/tazobactam, 38% for imipenem and 23.6% for ceftazidime).[4] Relevant figures for ICU isolates of *P. aeruginosa* derived from Europe are even worse, as from 1990 through to 1999 resistance to aminoglycosides reached 37–70%, to ceftazidime 57%, to piperacillin/tazobactam 53%, to ciprofloxacin 56% and to imipenem 52%.[5]

MDR *A. baumannii* isolates are becoming one of the most serious therapeutic problems worldwide. Data from the NNIS indicate that from 1986 to 2003, among *Acinetobacter* organisms causing pneumonia in ICUs, resistance to imipenem had increased from 0% to 42% and to ceftazidime from 18% to 68%. The substantial rise in the incidence of *A. baumannii* among nosocomial pathogens has elucidated this micro-organism as an indisputable indicator of poor clinical outcomes.[6,7]

Currently, increasing rates of Enterobacteriaceae harbouring extended-spectrum β-lactamases (ESBLs), including CTX-M, plasmid-mediated AmpC β-lactamases and carbapenemases, represent a new emerging threat. Resistance to third-generation cephalosporins in *K. pneumoniae* strains was reported at 20.6% for the year 2003, with a worrisome 47% increase from 1998 to 2002.[4] Recently, the emergence of *K. pneumoniae*-producing metallo-β-lactamases (MBLs) has been witnessed in certain areas.[8]

Based on our very weak antimicrobial armamentarium, this review is focused on four compounds: colistin, a re-emerging old antibacterial; tigecycline, a genuinely new antibacterial; doripenem, a new antibacterial from an existing class; and fosfomycin, an old antibacterial being revivied. These are currently the only active compounds against MDR Gram-negative microorganisms available.

The PubMed database was searched for relevant literature published between 1970 to March 2009 by using the terms 'colistin', 'polymyxins', 'tigecycline', 'doripenem' and 'fosfomycin'. Abstracts from Interscience Conferences on Antimicrobial Agents and Chemotherapy from 2005 to 2008 were also searched.

1. Polymyxins

The emergence of MDR Gram-negative bacilli (mainly *P. aeruginosa, A. baumannii* and recently *K. pneumoniae*) in parallel with the lack of new antibacterials, led to the revival of polymyxins, which is an old class of cyclic polypeptide antibiotics that was discovered in 1947 from *Bacillus polymyxa*.[9,10] They consist of polymyxins A–E, of which only polymyxin B and polymyxin E (colistin) are currently on the market. Colistin is available in two forms: colistin sulfate (tablets or

syrup for bowel decontamination and powder for topical use) and colistin methanesulfonate (colistimethate sodium; CMS) for parenteral use. In the USA, as well as in Brazil, polymyxin B sulfate is also used parenterally.[11] In terms of potency, polymyxin B sulfate is the most potent, followed by colistin sulfate and CMS.[9-11]

1.1 Mode of Action and Resistance Mechanisms

The target of antimicrobial action of colistin is the bacterial cell membrane, where the polycationic peptide ring interacts with the lipid A of lipopolysaccharides, allowing penetration through the outer membrane by displacing Ca^{2+} and Mg^{2+}. Insertion between the phospholipids of the cytoplasmic membrane leads to loss of membrane integrity and to bacterial cell death.[9,12] Studies with colistin-resistant *P. aeruginosa* have reported alterations at the outer membrane of the cell, such as reduction in cell envelope Mg^{2+} and Ca^{2+} contents, lipid alteration and substitution of protein OprH for magnesium.[13] An efflux pump/potassium system has been also identified in a polymyxin B-resistant *Yersinia* spp.[14] Despite the very slow reported development of resistance, in Greece in 2005, among 98 MDR *K. pneumoniae* strains (ESBL and VIM producers) derived from ICU patients and belonging to six different clones, 16% were found to be resistant to colistin; resistance increased to 37% after 2005.[15,16]

Colistin has potent antiendotoxin activity, but the significance of the latter mechanism in humans is not clear yet. However, the application of a polymyxin B-immobilized fibre column (PMX-F) in septic patients or in septic shock was rather successful.[17]

1.2 Antibacterial Activity

Colistin is active *in vitro* against *Escherichia coli, Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., *Salmonella* spp., *Shigella* spp., *Haemophilus influenzae, Legionella pneumophila, Bordetella pertussis*, MDR *P. aeruginosa* and *Acinetobacter* spp., and *Stenotrophomonas maltophilia* and *Aeromonas* spp. including most of the pan-drug resistant (PDR) strains.[18-20] By applying population analysis profiles, heteroresistance to colistin in MDR *A. baumannii* strains was first demonstrated in 2006.[21] Heteroresistance has been attributed to potentially suboptimal dosing regimens.[21] A significantly higher proportion of cells exhibiting heteroresistance was observed among patients pre-treated with colistin.[22]

Since 2005, the Clinical Laboratory Standards Institute (CLSI) established minimum inhibitory concentration (MIC) breakpoints of ≤2 mg/L for *P. aeruginosa* (susceptibility [S] ≤2 mg/L, resistance [R] ≥8 mg/L) and *Acinetobacter* spp. (S ≤2 mg/L, R ≥4 mg/L),[23] whilst the European breakpoints published by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [S ≤2 mg/L, R ≥4 mg/L] include all Enterobacteriaceae.[24] In terms of disk susceptibility testing, CLSI recommends susceptibility breakpoints only for *P. aeruginosa* (10 μg colistin disk; R ≤10 mm, S ≥11 mm). Guidelines for colistin disk susceptibility testing have also been published by the French Society for Microbiology and the British Society for Antimicrobial Chemotherapy.[25,26]

However, correlation of agar dilution (the gold standard method) with disk diffusion shows that the latter is unreliable for detecting colistin resistance,[27] while the poor agar diffusion characteristics of colistin limit the predictive accurary of the disk diffusion test and consequently values of 12–13 mm should be confirmed with MIC determination by E-test.[28] MICs obtained by the VITEK®-2 system have been also reported to be unreliable.[29] It should be pointed out that the formulation of colistin that must be used in susceptibility tests is colistin sulfate and not CMS.

The *in vitro* interaction of CMS with rifampicin has been evaluated against PDR *P. aeruginosa* strains, including colistin. Synergy depending on exposure time was reported in 11.8–41.7% of strains.[30] Synergy has also been reported with colistin and rifampicin in MDR *A. baumannii* strains producing OXA-58 carbapenemase.[31] Recent reports have described synergistic *in vitro* results after the combination of colistin with minocycline on imepenem-resistant *A. baumannii* clinical isolates,[32] as well as after the combination

of meropenem with colistin against *P. aeruginosa* and *A. baumannii* strains.[33]

1.3 Pharmacokinetics and Pharmacodynamics

To avoid confusion, it should be mentioned that CMS 2.4 mg contains 1 mg of colistin base, whereas colistin pure base has a potency of 30 000 IU/mg and CMS 12 500 IU/mg.

There are two formulations of polymixin B available. For adults and children aged >2 years with normal renal function, polymyxin B is given as follows: (i) intravenous, 15 000–25 000 IU/kg (2.5–5.0 mg/kg) daily in two divided doses; (ii) intramuscular, 25 000–30 000 IU/kg (2.5–3.0 mg/kg) daily in four or six divided doses; and (iii) intrathecal/intraventricular, 50 000 IU (5.0 mg) once daily for 3–4 days, then 50 000 IU (5.0 mg) once every other day for at least 2 weeks after cultures of cerebrospinal fluid become negative and/or glucose normal.[11]

Because of the lack of accurate pharmacokinetic and pharmacodynamic information, the optimal dosage of colistin is unclear. Regarding polymyxin B sulfate pharmacokinetics, there is scanty information available derived from noncontemporary studies. A decade ago it was reported that after a 50 mg intravenous dose, peak concentrations of 18 μg/mL are achieved in 2 hours with a serum half-life of 6 hours.[11] In impaired renal function, the modification of dosing schedules should be as follows: (i) for creatinine clearance 30–80 mL/min, a dosage of 2.5 mg/kg/day; (ii) for creatinine clearance <30 mL/min, a loading dose of 2.5 mg/kg on day 1 and thereafter 1.0–1.5 mg/kg given every 2–3 days; and (iii) in anuric patients, a loading dose of 2.5 mg/kg on day 1 and thereafter 1.0 mg/kg given every 5–7 days.[11] For parenteral administration in patients with normal renal function, CMS is given in the US at a dose of 2.5–5.0 mg/kg/day (31 250–62 500 IU/kg) divided into two to four equal doses (colistin 1 mg equals 12 500 IU). In the UK, CMS is given at a dose of 4–6 mg/kg/day (50 000–75 000 IU/kg) in three divided doses for adults and children with bodyweights of ≤60 kg and at a dose of 80–160 mg (1–2 million IU) every 8 hours for body weights of >60 kg.[9,12] However, the Greek experience has proved that a higher dose of 3 million IU every 8 hours can be safely administered.[9]

Older pharmacokinetic data are based on microbiological assays and are inaccurate because of erroneous diffusion in agar and instability of CMS in aqueous media.[34] Two more recent studies employed more accurate high-performance liquid chromatography methods to measure colistin and its prodrug CMS in patients with cystic fibrosis (CF).[35,36] In these studies, colistin was studied after 2 days of treatment and half-lives were determined to be 4.2 and 3.4 hours, respectively. However, it is not known whether data from patients with CF can be extrapolated to the critically ill patient, because in CF patients, drugs such as β-lactams and aminoglycosides typically display shorter half-lives.[37]

In a recent study that described colistin pharmacokinetics in 14 critically ill patients after at least 2 days of CMS administration (3 million IU, 3 times daily), the mean maximum concentration (C_{max}) was 2.93 mg/L and occurred 15 minutes after the end of the 30-minute infusion with a half-life of 7.1 hours.[38] However, CMS was not measured and pharmacokinetic analysis of concentrations after the first dose was not performed. From another recent study in 18 ICU critically ill patients where colistin concentrations were measured in plasma by a novel rapid chromatography–tandem mass spectrometry method,[39] the half-lives of CMS disposition were 2.3 hours and for colistin 14.4 hours.[40] The predicted C_{max} was 0.60 mg/L after the first dose of 3 million IU CMS and 2.3 mg/L following repeated administration of 3 million IU every 8 hours. The latter results indicate that as a rule, colistin concentrations remain below the MIC breakpoints (2 mg/L) after the first few doses of the currently used dosing regimen, signifying a major delay in appropriate treatment. The latter event is of major importance in the septic ICU patient, in whom early (within the first hour of septic shock) and appropriate antimicrobial therapy is directly related to survival.[41] Even at steady-state, plasma concentrations as measured in the latter study were below MIC breakpoints

for Enterobacteriaceae and *P. aeruginosa,* the most frequent MDR pathogens in critically ill patients. These observations are a matter of concern as they may also indicate suboptimal efficacy in infections caused by pathogens with borderline susceptibilities, as well as selection of resistant strains. Consequently, a re-evaluation of CMS dose appears to be necessary. Predictions from modelling indicate that without a loading dose, it takes 2–3 days before the steady-state concentration of colistin is obtained. A loading dose of 9 million IU CMS and a maintenance dose of 4.5 million IU CMS every 12 hours would result in the same average steady-state concentration of colistin as the current dosing schedule, but would achieve the target faster.[41]

Concerns over renal toxicity with the administration of high doses have been expressed based on animal experiments, but not confirmed in humans. In rats, after 7 days of intravenous administration in regimens mimicking twice- and once-daily dosing of a clinically relevant dose in humans, histological examination revealed more severe renal lesions with the regimen corresponding to once-daily dosing, which indicates that the potential for renal toxicity may be greater with extended-interval dosing.[42]

The intrathecal and intraventricular doses of CMS are equal to 125 000 IU/day. By the inhalation route, the recommended dosage ranges from 500 000 IU every 12 hours to 2 million IU every 8 hours.[9,12] As far as colistin clearance is concerned, it appears that it is mainly excreted by non-renal routes.[43] The lack of correlation between colistin kinetics and renal function raises the issue of colistin dosing in critically ill patients with renal failure. In renal dysfunction, the CMS dosage adjustment recommended by the manufacturers is as follows: for serum creatinine levels of 1.3–1.5, 1.6–2.5 or ≥2.6 mg/dL, the suggested dosage of colistin for intravenous administration is 160 mg (2 million IU) every 12, 24 or 36 hours, respectively. During hemodialysis the recommended dose is 80 mg (2 million IU) after each session,[9] whereas recent data in critically ill patients receiving continuous venovenous hemodiafiltration and CMS in reduced doses indicate that colistin C_{max} may well be <1 mg/L (Giamarellou H., unpublished data). Therefore, in the latter situation, it seems that CMS should be given at the same dosage schedule as for normal renal function, otherwise subtherapeutic levels of CMS will further compromise patient outcome. Other pharmacokinetic parameters of CMS are depicted in table I.

Both polymyxin B and colistin express their bactericidal activity as concentration-dependent agents, with the latter effect related to the area under the concentration-time curve (AUC)/MIC ratio that is the pharmacodynamic parameter most closely linked to killing.[36,44] The post-antibiotic effect (PAE) of colistin was studied on 19 MDR isolates of *A. baumannii* with the viable count method. The mean PAE of 1×MIC and 4×MIC concentrations of colistin on the tested isolates were 3.90 and 4.48 hours, respectively, indicating that increased dosing intervals may retain activity.[45] However, other investigators reported on a minor or negative PAE, signalling that extended intervals in dosing may be problematic, particularly for colistin-heteroresistant strains of *A. baumannii* and *K. pneumoniae*.[46,47] Supporting the latter findings were the results of an *in vitro* pharmacodynamic model where an 8-hourly regimen of CMS, when compared with 12- or 24-hourly regimens, appeared to be most effective at minimizing emergence of resistance.[48]

1.4 Clinical Experience

From 2003 to 2007, 175 non-CF patients who mostly had pneumonia were given intravenous polymyxin B therapeutically for infections due to MDR Gram-negative bacteria with clinical response rates ranging from 47.3% to 95% and

Table I. Pharmacokinetic parameters of colistin methanesulfonate (CMS) and colistin in critically ill patients[40]

Parameter	CMS (RSE %)	Colistin (RSE %)
$t_{½}$ (h)	2.3	14.4
C_{max} predicted (mg/L)		
first dose		0.60
steady state		2.3
CL (L/h)	13.7 (10)	9.09 (19)
Vd (L)	28.9 (22)	189 (12)

CL = total body clearance; **C_{max}** = maximum concentration; **RSE** = relative standard error; **$t_{½}$** = half life; **Vd** = volume of distribution.

mortality rates from 20% to 48%.[11] However, all studies were retrospective and polymyxin B was usually given in combination with other antibacterials, obscuring the precise evaluation of the results.

In total, from 1999 until August 2005, in seven retrospective studies involving almost 300 patients without CF, among whom most represented ICU patients with ventilator-associated pneumonia (VAP), intravenous CMS was given at a dose of 1–3 million IU every 8 hours for 12–22 days.[49-55] In almost all patients, at a rate close to 50%, either MDR *P. aeruginosa* or MDR *A. baumannii* were isolated in relevant cultures. Irrespective of the susceptibility patterns of the isolated pathogens, as a rule, CMS was given in combination with other antibacterials (mostly with a carbapenem). Clinical cure rates ranged between 57% and 73%, with mortality rates of 20–61.9%. Clinical efficacy in nosocomial pneumonia exceeding 50% was comparable to previously reported rates of outcome with piperacillin/tazobactam, imipenem/cilastatin and ciprofloxacin.

In 2007, two retrospective monotherapy studies with CMS were published. In the first study, no difference in mortality rates (51.6% vs 45.1%) was observed between 31 patients with VAP caused by isolates susceptible only to colistin who were treated with CMS monotherapy and 30 patients with VAP caused by carbapenem-susceptible strains who were treated with imipenem/cilastatin or meropenem as monotherapy.[56] Patients who received appropriate empirical therapy had significantly better outcome compared to those who received inappropriate empirical therapy (mortality 36.6% vs 70%, respectively; $p = 0.014$). Appropriate antimicrobial therapy was administered more commonly to patients with carbapenem-susceptible rather than colistin-susceptible pneumonia, and this probably contributed to a lower mortality rate in this group. It was concluded that VAP episodes susceptible only to colistin can be treated effectively using CMS, whereas the carbapenem-resistance pattern of pathogens should be suspected in patients with previous VAP or prior antibacterial use for >10 days preceding the current VAP episode.

In the largest retrospective matched case-control study thus far to assess the efficacy of monotherapy with CMS, the latter was compared with imipenem in VAP caused by isolates susceptible only to colistin ($n = 60$) and carbapenem-susceptible ($n = 60$) *A. baumannii* (51.6% vs 61.7%) or *P. aeruginosa* (48.4% vs 38.3%).[57] A favourable clinical response was observed in 75% of patients without difference in the time to resolution of infectious parameters between the two groups.

The effectiveness of CMS was also studied retrospectively in 95 cancer patients diagnosed with infections caused by MDR *P. aeruginosa*. Patients were either treated with colistin ($n = 31$) or with at least one active antipseudomonal agent such as a β-lactam or fluoroquinolone (control group, $n = 64$).[58] In 13 patients, colistin was given in combination with other antipseudomonal agents; in 18 patients it was given as monotherapy. Among patients from the colistin-only and control group, 45% and 37%, respectively, were neutropenic. In patients treated with colistin monotherapy compared with those in the control group, higher clinical and microbiological responses were observed (52% vs 31% and 48% vs 41%, respectively), relapse rates were compatable (10% vs 11%) and infection-related mortality was higher (26% vs 17%). However, none of the differences in endpoints reached statistical significance. No difference in the incidence of nephrotoxicity (23% vs 22%) was observed. Multiple logistic regression analysis showed that patients treated with colistin were 2.9-fold more likely than patients in the control group to experience a clinical response to therapy ($p = 0.026$). However, a major limitation of the study is the lack of evaluation of the time to initiate adequate therapy.

There are also three small studies published in which the effectiveness of colistin monotherapy (28 patients) did not appear to be inferior to that of colistin in combination with a carbapenem, rifampicin or an aminoglycoside (74 patients) in the therapy of MDR-Gram-negative infections, with successful results in 75% versus 66.2%.[59] The results of the combination colistin plus rifampicin in ten patients with carbapenem-resistant *A. baumannii* VAP, suggested that the combination was

safe and effective with seven of ten patients having favourable clinical outcome and six of ten patients being microbiologically cured.[60] Of interest is also the therapeutic approach for 28 ICU patients with PDR Gram-negative infections in whom antibacterials that were ineffective *in vitro* proved life saving, especially in combination regimens containing colistin, with successful results for 14 patients.[61]

The efficacy of aerosolized colistin in the non-CF patient is currently a matter of concern, since experience is still limited and retrospective. A small retrospective study of 21 patients has been published where the therapeutic efficacy of aerosolized CMS in combination with other parenteral antibacterials is reported.[62] Patients were treated for nosocomial pneumonia or VAP caused by MDR strains of *P. aeruginosa* or *A. baumannii*. Eighteen (85.7%) of 21 patients responded favourably to nebulized colistin therapy; both favourable clinical and microbiological outcomes were observed in 12 of 21 patients (57.1%), whereas favourable microbiological outcome only was observed in another 6 patients. Of 18 patients with a favourable microbiological outcome, 11 (61.1%) had documented eradication of the MDR pathogen, and eradication was presumed in the remaining 7 patients. Although solid data from prospective studies are lacking, in line with many experts in the field[63,64] we consider that aerosolized colistin should be thought of as being adjunctive to intravenous therapy in patients with VAP due to MDR Gram-negative bacteria that are susceptible to colistin. However, a specific vibrating nebulizer is required.[65] The US FDA has not yet approved the use of nebulized antibacterials in the treatment of VAP. Prospective evaluation and careful monitoring for adverse effects, resistance development and superinfections is required, whereas the efficacy of inhaled monotherapy is still unclear.[65]

Of particular interest are the cumulative results reported in the treatment of MDR *A. baumannii* CNS infections in 14 patients: five with meningitis, two with ventriculitis and five with both meningitis and ventriculitis (the exact type of CNS infection was not reported for two patients). CMS was given at a dosage of 2.5–5.0 mg/kg/day intravenously in seven patients, 3.5–10 mg intrathecally every 12 or 24 hours in two patients and 5–20 mg/day intraventricularly in three patients, and in two patients it was given both intravenously and intrathecally, for 15–63 days.[66] Of the 14 patients, 13 were cured with CNS sterilization in 1–6 days (median 4.5 days). Finally, the experience with CMS in critically ill children, as well as in 326 children without CF who had a variety of infections and were mostly included in studies published before 1977, has suggested that systemic colistin is an effective and acceptably safe option in children without CF who have MDR Gram-negative infections.[67,68]

The reported studies share common drawbacks as they are mostly small and retrospective, and are without definite designed protocols. In most situations, other antibacterials, irrespective of the susceptibilities of the isolated pathogens, were given simultaneously with CMS, which confounded its therapeutic efficacy. Moreover, variable dosing of colistin and treatment durations were applied, and resistance development during and at the end of the studies was not monitored.

1.5 Toxicity and Adverse Reactions

The most common and important adverse effects of colistin are nephrotoxicity and neurotoxicity. Early experience with CMS revealed 20.2% nephrotoxicity that was attributed mainly to acute tubular necrosis.[69] In contrast to older information, recent data indicate that nephrotoxicity in ICU patients after CMS administration ranges from 0% to 36%.[9,12] Safety data from 19 courses of prolonged intravenous CMS administration (mean duration 43.4 days and mean daily dose 4.4 million IU) showed that the median creatinine value increased only by 0.25 mg/dL, which returned to close to baseline at the end of therapy.[70] The reported discrepancies could be attributed to the improvement in supportive care offered to seriously ill patients, the possible avoidance of coadministering other nephrotoxic drugs, different formulations of colistin lacking sulfate impurities and the different definitions of nephrotoxicity. On the other hand, nephrotoxicity in the recently reported studies

where polymyxin B was administered ranged from 0% to 14%.[11]

The incidence of neurotoxicity in earlier studies of colistin reached approximately 7%.[70,71] Facial paresthesias, dizziness, weakness, vertigo, visual disturbances, confusion, ataxia, and neuromuscular blockade leading to respiratory failure and apnoea have been reported. Only one study included prospective electrophysiological testing among 12 colistin recipients with evidence of neuromuscular junction blockade; findings consistent with critical polyneuropathy were seen in six of the tested patients.[51,71] Intraventricular high-dose administration may also cause convulsions. It seems that both nephro- and neurotoxicity are dose dependent and reversible.[9,12] Bronchoconstriction has been reported with aerosolized CMS, which is an adverse effect that can be controlled by the inhalation of β-adrenoceptor agonists before CMS administration.[9]

1.6 Resistance Development

Only three recent studies have investigated the possibility of resistance development with colistin. In a New York hospital after 4 years of increasing purchases of polymyxin B, a 5% resistance rate had developed in *P. aeruginosa* isolates to colistin during and at the end of therapy.[72] In a Greek ICU, the emergence of colonization in 37% of patients with colistin-resistant *K. pneumonia* in bronchial and bowel floras is of concern.[73] The simultaneous occurrence of various infections with colistin-resistant Gram-negative bacteria and breakthrough bacteraemias with *Proteus* and *Serratia* spp. that are intrinsically resistant to colistin in patients receiving treatment with colistin for >12 days, is certainly worrying.[74] On the other hand, the emergence of *K. pneumoniae* strains producing MBLs in Greek ICUs since 2001 and recently *K. pneumoniae* carbapenemase (KPC) enzymes, rendering *K. pneumoniae* strains PDR with the exception of colistin, resulted in excessive empirical use of colistin. This led to a cluster of multiclonal PDR *Klebsiella* strains implicated in bacteraemias, VAP and soft tissue infections, mostly in patients who had prolonged administration of colistin (median 27 days).[17] Horizontal transmission of PDR *Klebsiella* through caregivers' hands was also proved by repetitive extragenic palindromic-polymerase chain reaction.[73] The analysis of risk factors after a Greek ICU outbreak with PDR *P. aeruginosa* causing VAP revealed that the sole independent predictors were the administration of colistin for ≥13 days or the combined use of colistin with a carbapenem for >20 days.[75] The outbreak resolved after a reduction in the days of therapy with colistin plus reinforcement of infection control measures. Additionally, in a recent matched case-control study, in the multivariable model the use of colistin for >14 days was identified as the only independent risk factor ($p = 0.002$).[76]

1.7 Summary

It is evident that future studies with colistin necessitate (i) large prospective, possibly comparative, trials in MDR infections of ICU patients under well-designed protocols and reliable susceptibility testing; (ii) further pharmacokinetic/pharmacodynamic (PK/PD) exploitation; (iii) clarification *in vivo* of the possible benefits of co-administering colistin with other antimicrobials, such as the carbapenems and rifampicin; (iv) evaluation of nebulized colistin as single VAP therapy versus combination with parenteral colistin; (v) better monitoring and elucidation of resistance mechanisms; and (vi) larger experience in the febrile neutropenic host. There is no doubt that we must explore ways for maintaining the activity and usefulness of colistin. Colistin is not an ICU panacea to be prescribed casually but only under certain strict indications, as in ICU infections with PDR pathogens susceptible only to colistin or empirically in ICU nosocomial sepsis of late onset in settings with high prevalence of MDR isolates. Even then, de-escalation should be prompt whenever culture results permit replacement with another antibacterial.

2. Tigecycline

Tigecycline is a newer semisynthetic glycylcycline. Tigecycline was approved in 2005 by the US

FDA and in 2006 by the European Medicines Agency (EMEA) for the treatment of complicated skin and skin structure infections and complicated intra-abdominal infections in adults.[77] On 20 March 2009, the FDA approved tigecycline for the treatment of community-acquired pneumonia (CAP) caused by *Streptococcus pneumoniae* (penicillin-susceptible isolates), including cases with concurrent bacteraemia, *H. influenzae* (β-lactamase negative isolates), and *L. pneumophila*.[78] However, the EMEA has raised concerns about the evidence available from clinical studies in patients with CAP; therefore the manufacturing company has withdrawn the application for this indication in the European market.[79,80] It represents a modified minocycline and holds promise as monotherapy for patients with serious polymicrobial infections.[81] Tigecycline is not currently approved for infections caused by MDR *A. baumannii*; however, off-label use in that indication is increasing globally because of the appealing *in vitro* spectrum of tigecycline and the lack of other active antimicrobial agents against this pathogen.

2.1 Mode of Action

Tigecycline is a bacteriostatic agent that acts through inhibition of the bacterial protein translation by binding to the 30S ribosomal subunit and blocking the entry of amino-acyl to RNA molecules into the A site of the ribosome. It has the ability to overcome many known mechanisms of resistance that inactivated the older teracyclines, such as the active efflux and the ribosome protection mechanisms.[81] Although by definition a static drug, a possible bactericidal activity has been demonstrated and needs further exploration.[82]

2.2 Antibacterial Activity

Tigecycline is active *in vitro* against a wide range of aerobic and anaerobic bacteria, including meticillin-resistant *S. aureus*, meticillin-resistant *S. epidermidis*, *S. pneumoniae* (penicillin-intermediate and -resistant included), vancomycin-resistant *Enterococcus* and several anaerobic species (including *Bacteroides fragilis*).[83,84]

Its Gram-negative spectrum includes MDR *A. baumannii*, ESBL-producing Enterobacteriaceae, KPC- and VIM-producing *K. pneumoniae* and *S. maltophilia* strains.[15,85,86] Early reports have shown MIC values for Enterobacteriaceae ranging from 0.06 μg/mL to 2 μg/mL, with no difference among ESBL-producing and -non-producing strains.[85,86] However, an increase in tigecycline resistance among *Enterobacter* and *Klebsiella* spp. was documented from 2001 to 2006 in many parts of the world.[87,88] The *in vitro* activity of tigecycline against imipenem-susceptible and imipenem-resistant *A. baumannii* ranges from 2 to 8 μg/mL among various studies.[85] According to TEST (Tigecycline Evaluations Surveillance Trial) data from the US during 2004–2005, tigecycline had the lowest MIC_{90} (MIC for 90% of islates) against *A. baumannii* (1 mg/L),[89,90] and data from TEST 2005–2007 indicate that the tigecycline MIC_{90} for *A. baumannii* remained stable (≤2 mg/L).[91] Areas with increasing resistance of *A. baumannii* reported tigecycline MIC_{50} (MIC for 50% of isolates) and MIC_{90} of 2 mg/L and 4 mg/L, respectively; depending on the resistance cut-off (2 mg/L or 4 mg/L), 80.9% or 93.1% of the isolates are considered to be susceptible, respectively.[92] Tigecycline has no activity against *P. aeruginosa*, with 90% of strains having MICs >4 mg/L.[81]

In a recent review of *in vitro* studies, susceptibility rates for MDR *Klebsiella* spp. were 91.2% for 2627 isolates by the FDA criteria and 72.3% for 1504 isolates by the EUCAST criteria (92.3% for 2030 and 72.3% for 1284 ESBL-producing isolates, by the FDA and EUCAST criteria, respectively).[93]

Tigecycline was 100% active against a total of 104 carbapenemase (serine-β-lactamase- and MBL)-producing strains of the Enterobacteriaceae family collected from 2000 to 2005. The most frequent carbapenemase was KPC-2 or -3 (73 strains), followed by VIM-1, IMP-1, SME-2 and NMC-A. Tigecycline, with MIC_{90} values at 1 mg/L, appears to be more potent *in vitro* than the polymyxins (88.1% susceptibility) against these strains.[94] In our institution, among 34 KPC-producing *K. pneumoniae* strains, the percent susceptibility of the most active drugs was as follows: gentamicin 90%, colistin 79.3% and tigecycline 58.8% by FDA breakpoints

(Giamarellou H., unpublished data). Tigecycline is intrinsically vulnerable to the chromosomal-encoded multidrug efflux pumps of *Pseudomonas* spp., *Proteus* spp., *Providencia* spp. and *Morganella morganii*, against which the compound has poor activity.[95]

All existing breakpoints of resistance for tigecycline against Enterobacteriaceae and *A. baumannii* are summarized in table II. The CLSI has not yet issued breakpoints.[96-100] It is evident that there is an urgent need for harmonization across all relevant societies.

Several *in vitro* studies have addressed the question of potential synergy between tigecycline and other antimicrobials, with an indifferent response in the majority of combinations and very rare antagonism. A number of synergisms that might be interesting for further evaluation were observed when tigecycline was combined with rifampicin against *Enterobacter* spp. and with amikacin against *Enterobacter* spp., *K. pneumoniae*, *Proteus* spp. and *S. maltophilia*. In two time-kill studies, the exposure of a highly tigecycline-resistant isolate to tigecycline resulted in enhanced susceptibility to amikacin and synergistic bactericidal activities of the two drugs, whereas the combination of tigecycline with cotrimoxazole was synergistic *in vitro* for *Serratia marcescens*, *Enterobacter cloacae*, *Proteus* spp. and *S. maltophilia*.[101,102]

Time-kill assays have demonstrated a bactericidal synergistic effect of tigecycline plus amikacin against *A. baumannii* and *Proteus vulgaris*, and with tigecycline plus colistin against a MBL (VIM-1)- and ESBL (SHV-12)-producing *K. pneumoniae*.[101,103-105] Paradoxically, *in vitro* synergy with tigecycline plus ampicillin/sulbactam or piperacillin/tazobactam has been achieved against *P. aeruginosa*. A murine model of *P. aeruginosa* pneumonia has also shown that tigecycline in combination with gentamicin significantly reduced bacterial densities in lung tissue compared with either drug alone.[106]

A time-kill study of tigecycline alone and in combination with other antimicrobials (carbapenems, polymyxin B, amikacin, ciprofloxacin, rifampicin, etc.) against carbapenem-intermediate or -resistant *A. baumannii*, demonstrated indifference for tigecycline in combination with the antimicrobials tested.[107] Similarly, all 19 *A. baumannii* isolates studied by a modified E-test method indicated indifference between polymyxin and tigecycline.[108] Thirty-five isolates with colistin MIC values of 0.12–4 μg/mL were tested for synergy between colistin and tigecycline with the checkerboard method; the result was again indifference.[109]

2.3 Pharmacokinetics and Pharmacodynamics

Tigecycline is available as an intravenous formulation and is administered at a 50 mg dose as a 1-hour infusion every 12 hours, after an initial

Table II. Breakpoints of susceptibility of tigecycline against Enterobacteriaceae and *Acinetobacter baumannii*

Bacteria	MIC (mg/L)			Inhibition zone (mm)			Implementing committee or proposing authors	Reference
	S	I	R	S	I	R		
Enterobacteriaceae[a]	≤2	4	≥8	≥19	15–18	≤14	FDA	96
	≤1	2	>2				EUCAST	97
	≤1	2	>2	≥24	20–23	≤19	BSAC	98
A. baumannii							FDA[b]	96
	IE	IE	IE				EUCAST	97
	≤1	2	>2	≥24	20–23	≤19	BSAC	98
	≤2	4	≥8	≥16	13–15	≤12	Jones et al.	99

a *Proteus* spp., *Morganella morganii* and *Providencia* spp. are considered inherently non-susceptible to tigecycline.

b Tigecycline is not US FDA-approved for infections caused by *A. baumannii*.

BSAC = British Society for Antimicrobial Chemotherapy; **EUCAST** = European Committee on Antimicrobial Susceptibility Testing; **I** = intermediate resistant; **IE** = insufficient evidence for the implementation of breakpoints; **MIC** = minimal inhibitory concentration; **S** = susceptible; **R** = resistant.

Table III. Main plasma pharmacokinetic parameters of tigecycline[110,111]

Plasma parameter	Pharmacological studies		Efficacy studies
	100 mg	50 mg	50 mg
C_{max} (mg/L)			
30-min infusion	1.45 ± 0.32	0.87 ± 0.23	0.80 ± 0.46
60-min infusion	0.90 ± 0.27	0.63 ± 0.10	0.49 ± 0.28
C_{min} (mg/L)	NA	0.13 ± 0.08	0.16 ± 0.09
CL (L/h)	21.8 ± 8.9	23.8 ± 7.8	19.9 ± 8.1
$t_{½}$ (h)	27.1 ± 14.3	42.4 ± 35.3	NA
AUC_{24} (mg • h/L)	NA	4.70 ± 1.70	5.85 ± 2.48
AUC_{∞} (mg • h/L)	5.19 ± 1.86	NA	NA
Fraction unbound (%)	13–29	13–20	NA
Vd (L)	568 ± 244	639 ± 307	NA

AUC_t = area under the concentration-time curve to time *t*; **CL** = total body clearance; **C_{max}** = maximum concentration; **C_{min}** = minimum concentration; **NA** = not available; **$t_{½}$** = half-life; **Vd** = volume of distribution.

loading dose of 100 mg. Pharmacokinetics are unaffected by age, sex or race. Tolerability is better if the compound is given after a meal. In patients with severe hepatic insufficiency (Child-Pugh class C), a 50% reduction of the maintenance dose is recommended following the loading dose of 100 mg. Pharmacokinetics are not changed in severe renal failure or haemodialysis. After a dose of 50 mg, tigecycline exhibits linear pharmacokinetics, with C_{max} at 0.62 ± 0.09 μg/mL, a half-life of 37 ± 12 hours and a protein binding capacity at 78%. The drug is primarily excreted in the bile, and does not affect the cytochrome P450 enzyme family. The principal pharmacokinetic properties of tigecycline are presented in tables III and VI.[110-112]

Dose escalation of tigecycline is supported by new PK/PD studies. In a recent study, pharmacokinetic parameters were obtained through blood and bronchoalveolar lavage sampling in three mechanically ventilated critically ill patients on the fifth day of tigecycline administration at 1, 4 and 12 hours after drug infusion.[113] Tigecycline concentrations were calculated in the epithelial lining fluid (ELF) and alveolar cells, which are considered to best depict the extracellular and intracellular 'drug-pathogen' interaction, respectively. Whereas plasma parameters did not deviate from current published evidence, at all three sampling times tigecycline concentrations in ELF were very low (maximum value attained was 0.02 ± 0.01 mg/L at 4 hours after tigecycline infusion) compared with previous studies in volunteers.[114] In this study, the penetration of tigecycline into the intracellular compartment was comparable to that achieved in plasma. However, a very low ELF : plasma concentration ratio indicates a suboptimal penetration into the extracellular lung compartment; the maximum value attained (0.18 ± 0.09 mg/L) at 12 hours post-infusion is insufficient to eradicate Gram-negative extracellular bacteria such as *E. coli* (MIC_{90} 0.25 mg/L) or *K. pneumoniae* (MIC_{90} 1.0 mg/L).

In another study, *in vivo* bactericidal activity of tigecycline against various *A. baumannii* isolates (MIC 0.25–1.0 mg/L) was assessed in a murine neutropenic pneumonia model.[115] The PK/PD parameters of (free) *f*AUC/MIC and effective exposure value EI_{80} and EI_{50} (exposure value required to produce 80% and 50% of maximal effect, respectively) were used. By both parameters, considerably more drug exposure was required to produce antibacterial effects in *Acinetobacter* than in Enterobacteriaceae.[116] According to the authors, and taking into account the difficulties in extrapolating this model into human pneumonia, available pharmacokinetic data from infected humans (AUC 6.37 mg • h/L) indicate that tigecycline dosages of up to 200 mg/day are probably required to provide adequate exposure for the treatment of *A. baumannii* pneumonia.[115,117]

Table IV. Pharmacokinetic parameters of tigecycline in various body tissues[111]

Body tissue	Concentration (mg/L or mg/kg) after single 100 mg/dose	
	4 h	12 h
Bile	309 ± 420	148 ± 155
Gall bladder	6.6 ± 6.6	7.3 ± 7.9
Colon	0.55 ± 0.34	1.3 ± 2.4
Lung	0.76 ± 0.67	0.38 ± 0.26
Bone	0.07 ± 0.04	0.12 ± 0.13
Synovial fluid	0.12 ± 0.06	0.09 ± 0.05
Cerebrospinal fluid	0.015 ± 0.003	0.025 ± 0.005

2.4 Clinical Experience

The efficacy of tigecycline has been evaluated in two pairs of phase III clinical trials, where tigecycline as monotherapy was non-inferior to the comparators in the treatment of complicated skin and soft structure infections (cSSSI) and complicated intra-abdominal infections (cIAIs).[118,119] In the first pair study of patients with complicated cSSSI, tigecycline was compared with vancomycin plus aztreonam. Clinical response was 86.5% with tigecycline versus 88.6% with vancomycin plus aztreonam. Non-inferiority criteria were met in all four phase III trials.

In a subsequent comparative, double-blind phase III study, tigecycline at standard dose in combination with ceftazidime and an aminoglycoside was compared with imipenem/cilastatin in combination with vancomycin with or without an aminoglycoside in the treatment of nosocomial pneumonia. A total of 945 patients were randomized. The clinically evaluable population did not meet non-inferiority criteria (tigecycline 67.9% and imipenem/cilastatin 78.2%; absolute difference −10.4, 95% CI −17.8, −3.0; p = 0.120). This was due to the group with VAP, for which tigecycline did not establish non-inferiority in either population (clinically evaluable tigecycline cure rate 47.9% and imipenem/cilastatin 70.1%; absolute difference −22.2, 95% CI −39.5, −4.9; p = 0.762; clinical modified intent-to-treat (c-mITT) population tigecycline cure rate 46.5% and imipenem/cilastatin 57.8%; absolute difference −11.3, 95% CI −24.6, 2.0; p = 0.326). However, in patients with nosocomial pneumonia (without VAP), non-inferiority criteria were met in both populations (clinically evaluable tigecycline cure rate 75.4% and imipenem/cilastatin 81.3%; absolute difference −5.9, 95% CI −14.8, 3.0; p < 0.001; c-mITT population tigecycline cure rate 69.3% and imipenem/cilastatin 71.2%; absolute difference −1.9, 95% CI −9.4, 5.6; p = 0.022).[120]

From January 2007 to April 2007 nine studies related to MDR Gram-negative infections were published or became available online[121-129] (table V). Most of the studies are retrospective and non-comparative, with low numbers of monotherapies; therefore, the elucidation of the true role of tigecycline in the outcomes is difficult. The indications for which tigecycline was administered was very heterogenous, compromising in some studies of the evaluation of an adverse outcome (i.e. tracheobronchitis, urinary tract infections, bone and joint infections). The MIC of the targeted pathogen against tigecycline is not universally available, so results cannot be stratified according to the size of the MIC. Finally, definitions of MDR were very different across the studies.

In order to bypass some of these methodological problems, we performed a retrospective study in three tertiary Greek hospitals.[128] Among patients treated with tigecycline, 45 adult patients (35 in ICU) met strictly defined criteria for infections with MDR Gram-negative pathogens and were subsequently analyzed. They received tigecycline at standard dose for 28 *A. baumannii* and 23 *K. pneumoniae* infections with an MDR or PDR profile: 21 VAP and healthcare-associated pneumonia (HCAP), 10 bloodstream infections (BSIs) and 14 surgical infections. Tigecycline was administered either as monotherapy (22 patients) or as presumed active monotherapy (23 patients). In the latter group, all coadministered antimicrobials were resistant *in vitro* against the targeted pathogen(s), or had been clinically and microbiologically failing after at least five days of therapy (in most patients the companion agent was colistin).

Successful clinical response rates of 90.5% and 80% were recorded for VAP/HCAP and BSI, respectively, with an overall successful clinical response of 80%. No difference was recorded between the monotherapy group and the presumed active monotherapy group (81.8% vs 78.3%). A successful clinical response of 85% was recorded for 20 patients in septic shock, with all 6 patients treated with tigecycline monotherapy documenting clinical success. Tigecycline MIC values among isolates of *A. baumannii* ranged from 1 to 8 mg/L, whereas those of *K. pneumoniae* ranged from 0.5 to 3 mg/L. Bacteraemic strains (all but two were *K. pneumoniae*) had tigecycline MIC values of 0.5–3 mg/L. Cumulative successful microbiological outcomes were lower than clinical success rates, and were certainly compromised by

Table V. Clinical studies of tigecycline (TIG) in the treatment of multidrug resistant (MDR) Gram-negative infections

Study[a]	Type of infection (no.)	No. of pts	Pathogens and resistance characteristics	Coadministered antibacterials (no. of pts)	Treatment outcomes
Schafer et al.[121]	VAP (19), VAP with BSI (3), BSI (3)	25 critically ill pts (60% surgical)	*Acinetobacter baumannii*: MDR 13/20 (65%) TIG-susceptible, 6/20 (30%) TIG-intermediate, 1/20 (5%) TIG-resistant	TIG monotherapy (5), IMP (9), IMP + nebulized COL (3), IMP + IV COL (1), nebulized COL (6), IV COL (1)	Resolution 21/25 (84%); recurrence in 3 pts with VAP; microbial eradication in 12/15 (80%); emergence of resistance in 1 pt
Anthony et al.[122]	VAP (3), VAP with empyema (3), HAP (2), tracheobronchitis (2), mediastinitis with secondary BSI (1), BSI (1), endovascular (1), UTI (2), cellulitis (1), diabetic ulcer with osteomyelitis (1), pelvic abscess (1)	18 (19 infection courses)	10 *A. baumannii*: MDR 4/9 (44%) TIG-susceptible, 5/9 (56%) TIG-intermediate, 6 *Klebsiella pneumoniae* (4 ESBL + 1 ESBL + KPC), 2 *Enterobacter cloacae,* 1 *Escherichia coli* (KPC)	TIG monotherapy (9), CEF (1), AMK + COL (1), inhaled COL (1), TOB (1), inhaled TOB (2), GEN (1), LEV (1), MER + IV COL (1)	Positive clinical response 5/10 (50%), uncertain in 1/10 (10%); survival 6/10 (60%), all deaths related to infection with TIG-intermediate pathogens; microbiological response in 3/4 (75%); emergence of resistance in 1 pt
Swoboda et al.[123] [b]	Severe sepsis and septic shock cIAI (30), HAP (12), cIAI + HAP (10), BSI (2), UTI (2), SSTI (6), bone and joint (1), unknown (2)	70	Gram-positive (58), *Stenotrophomonas maltophilia* (13), *A. baumannii* (1), *E. cloacae* (1), *Burkholderia cepacia* (1)	TIG monotherapy (1), CAR (29), FQL (5), 3rd-generation CEP (3), COL (3), AMK (2), TOB (1), VAN (1)	30% ICU mortality; outcomes of Gram-negative infections cannot be separated
Vasilev et al.[124] [c]	cSSTI (24), cIAI (5), HAP (5), CAP (1), BSI (1)	34 (microbiologi-cally evaluable population)	*A. baumannii* (17), *K. pneumonia* (6, 1 susceptible only to TIG), *E. coli* (9), *Enterobacter* spp. (4), no strict MDR definition used (included ESBL or single-class resistance, or *A. baumannii)*	TIG monotherapy (34)	72.2% clinical cure rate, 66.7%, microbiological cure; *A. baumannii* infections 82.4% clinical and 64.7% microbiological cure rates
Gallagher and Rouse[125]	HAP (15), BSI (6, 2 of them with HAP), UTI (3), wound infection (3), tracheobronchitis (1), IAI (1)	28 (29 courses)	29 *A. baumannii* isolates, only 11 isolates tested against TIG (MIC 3–8 mg/L), 8 tested against COL (all susceptible)	TIG monotherapy (12), IMP (6), COL (6), PTZ (3), AMS (2), AMK (1)	28% positive clinical outcome, 44% microbiological eradication; 62% (18 pts) negative outcome, 68% mortality (19 pts, attributable in 15/19 pts); clinical and microbiological outcomes significantly associated

Continued next page

Table V. Contd

Study[a]	Type of infection (no.)	No. of pts	Pathogens and resistance characteristics	Coadministered antibacterials (no. of pts)	Treatment outcomes
Curcio et al.[126] [d]	VAP (6 with BSI)	75	*A.baumannii*, 44/73 IMP-susceptible, 29/73 only COL and TIG susceptible	29 pts received other antibacterials >48 h earlier; 22 pts received no other antibacterials or <48 h earlier (37% received concomitant antipseudomonal treatment)	69.86% clinical success; success in 2/6 bacteraemic infections; 33% crude mortality
Gordon and Wareham[127]	IAI (2), HAP (8), IAI + HAP (1), IAI + VAP (3), BSI (2), BSI + other (7), bone, joint and soft tissue (10), intracranial (1)	34	*A. baumannii* (19), polymicrobial with *A. baumannii* (15), all CAR-resistant, 18/19 COL and TIG susceptible (<2 mg/L)	TIG monotherapy (12), MER (1), IMP (1), nebulized COL (1), COL (1), AMK (4), TOB (1), MET (1), VAN (2), CLA + COT (1), AMK + CIP + MET (1)	68% positive clinical outcome, 29.4% microbiological eradication, 56% positive results in bacteraemia, overall mortality 41%, 26.4% attributable mortality, 3 breakthrough BSI (1 documented resistance)
Poulakou et al.[128]	VAP/HCAP (21, 2 with BSI), BSI (10), SI (14)	45	*A. baumannii* (26 MDR, 2 PDR), all CAR-resistant, TIG MIC 1–8 mg/L, bacteraemia 2 and 3 mg/L; *K. pneumoniae* (20 MDR, 3 PDR), TIG MIC 1–3 mg/L, bacteraemia 0.5–2 mg/L; Enterobacteriaceae (3)	TIG monotherapy (22), presumed active monotherapy (23: COL 8, COL + MER 6, CIP 1, MER 1, COL + AMK 3, AZT 1, COL + LIN 1, MER + MET 1, COL + VAN 1)	Clinical success: VAP 90%, BSI 80%, SI 64.3%; monotherapy group 81.8%, combined therapy group 78.3%; 4 breakthrough Gram-negative bacteraemia (1 documented emergence of resistance), 10 superinfections from micro-organisms inherently resistant to TIG
Maltezou et al.[129]	HAP (62% of cases), SSI (19%), BSI (9.5%), UTI (4.7%), peritonitis (4.7%)	22, data available for 16 pts	KPC-2-producing *K. pneumoniae*	COL + TIG (7), COL + GEN (3), COL + TIG + GEN (2), TIG + GEN (1), GEN (1)	Clinical failure 22.2% of 18 pts available for assessment; microbiological failure 87.5% of 8 pts available for assessment, repeatedly positive cultures

a Studies are based on retrospective case series unless otherwise indicated.

b Retrospective study.

c Phase III, open-label, prospective non-comparative study.

d Prospective non-comparative study.

AMK = amikacin; **AMS** = ampicillin/sulbactam; **AZT** = aztreonam; **BSI** = bloodstream infection; **CAP** = community-acquired pneumonia; **CAR** = carbapenem; **CEF** = cefepime; **CEP** = cephalosporin; **cIAI** = complicated intraabdominal infection; **CIP** = ciprofloxacin; **CLA** = clarithromycin; **COL** = colistin; **COT** = cotrimoxazole (sulfamethoxazole/trimethoprim); **cSSTI** = complicated skin and soft tissue infection; **ESBL** = extended-spectrum β-lactamase; **FQL** = fluoroquinolone; **GEN** = gentamicin; **HAP** = hospital-aquired pneumonia; **HCAP** = healthcare-associated pneumonia; **IAI** = intraabdominal infection; **ICU** = intensive care unit; **IMP** = imipenem; **IV** = intravenous; **KPC** = *K. pneumoniae* carbapenemase; **LEV** = levofloxacin; **LIN** = linezolid; **MDR** = multidrug-resistant; **MER** = meropenem; **MET** = metronidazole; **MIC** = minimal inhibitory concentration; **PDR** = pan-drug resistant; **pt(s)** = patient(s); **PTZ** = piperacillin/tazobactam; **SI** = surgical infection; **SSI** = surgical site infection; **SSTI** = skin and soft tissue infection; **TOB** = tobramycin; **UTI** = urinary tract infection; **VAN** = vancomycin; **VAP** = ventilator-associated pneumonia.

13 episodes of superinfections and breakthrough infections among 10 patients.[128] One episode represented the development of resistance of the targeted pathogen, whereas in three other bacteraemic episodes the pathogens had tigecycline MIC values within the range of susceptibility.

Finally, experience with tigecycline has been reported during a nosocomial outbreak of KPC-2-producing *K. pneumoniae* from Greece[129] (table V). The strains were susceptible to colistin and tigecycline, and occasionally to gentamicin. Among 16 patients for whom therapeutic data were available, 14 (87.5%) were treated with a combination of colistin and/or tigecycline and/or gentamicin. Clinical failure was noted in 22.2% of 18 patients available for assessment and microbiological failure in 87.5% of 8 patients available for assessment of microbiological outcome. Despite the clinical response, some patients had repeatedly positive cultures on treatment. Scarce evidence from case reports have demonstrated favourable clinical outcomes in seven patients with MDR *A. baumannii* infections and a patient with mediastinitis due to PDR *K. pneumoniae*.[130,131] In a recent extensive review of clinical studies related to infections from carbapenem-resistant or ESBL-producing or MDR Enterobacteriaceae, 69.7% of the 33 reported patients treated with tigecycline achieved resolution of infection.[93]

2.5 Toxicity and Adverse Reactions

Safety analysis of the phase II study comparing tigecycline with vancomycin plus aztreonam in the treatment of cSSSI, revealed a significantly higher number of patients in the tigecycline group reporting mild to moderate gastrointestinal adverse events, including nausea (34.5%), vomiting (19.6%), anorexia (3.4%) and diarrhoea (8.5%).[118] In the other phase II study comparing tigecycline with imipenem/cilastatin in the treatment of cIAIs, gastrointestinal disturbances (nausea 24.4% and vomiting 19.2%), although significantly more frequent in the tigecycline arm than the comparator arm, were not as common as in the cSSSI study.[119] A decrease in fibrinogen levels that could not be attributed to any other event or medication was observed in two patients (one developed epistaxis) and probably another three in our department; monitoring of fibrinogen levels is now performed in every patient receiving tigecycline for more than 1 week in our institution (Giamarellou H., unpublished data and Poulakou et al.[128]).

2.6 Resistance Development

Low concentrations attained in the serum are probably the driving force for the development of resistance while on treatment, particularly when the MIC of the targeted pathogen exceeds the C_{max} of the drug, which is almost the rule for all targeted *A. baumannii* strains.[128,132] Five cases of development of resistance by the targeted pathogen have been reported, either as case series or case reports.[121,122,128,132,133] Moreover, breakthrough bacteraemic episodes from Gram-negative pathogens have been reported, and this is despite *in vitro* susceptibility to tigecycline and in some of them below the 'C_{max} threshold'.[127,128] Interestingly, in our cohort of 45 patients treated with tigecycline for MDR or PDR infections (see section 2.4), ten episodes of superinfections (nine bacteraemia and one urinary tract infection [UTI]) with pathogens inherently resistant to tigecycline were observed (i.e. *Proteus* sp., *Providencia* sp., *P. aeruginosa*, etc.). Most occurred within the first 10 days of tigecycline treatment; the monotherapy group was statistically more affected than the combined treatment (mostly colistin) group (31.8% vs 13%; $p < 0.001$).[128]

The genetic basis of development of resistance has been investigated with molecular studies. Constitutional overproduction of MarA, a transcriptional activator, and AcrAB, an resistance-nodulation-division (RND)-type efflux pump, resulted in decreased susceptibility to tigecycline in clinical isolates of *E. coli* from phase III studies,[134] whereas in *E. cloacae* and *K. pneumoniae*, decreased susceptibility to tigecycline is the result of RamA-mediated overexpression of the AcrAB efflux pump. The AdeABC multidrug efflux pump is associated with decreased susceptibility to tigecycline in *A. calcoaceticus-A. baumannii* complex.[135-139]

2.7 Summary

Tigecycline appears to be an extremely useful addition to our currently limited armamentarium against MDR pathogens. However, clinicians should adopt a cautious approach in off-label use of the drug because of the currently limited evidence (i.e. *A. baumannii* infections). Since *P. aeruginosa,* as well as *Proteus* spp. and *Morganella* spp., are not included in the antibacterial spectrum of tigecycline, the use of the drug as monotherapy is recommended only for patients with documented non-pseudomonal infections or warrants the addition of an antipseudomonal agent to empirical regimens in patients with risk factors for pseudomonal infections. In settings with MDR epidemiology, particularly in institutions with KPC predominance, aminoglycosides or colistin appear to be the most attractive companions for tigecycline.

Low tigecycline serum C_{max} may compromise the ability of the drug to treat intravascular infections or infections with concomitant bacteraemia, particularly when the causative organism possesses an MIC near the C_{max}. The development of resistance while on treatment is also a concern, particularly with pathogens with borderline MICs. For the latter reason (low C_{max}), exact and early determination of the MIC of the pathogen is required. Moreover, MICs of the most problematic MDR pathogens should be routinely monitored in each institution and resistance rates regularly communicated to the clinicians. This strategy, while helpful in the optimization of empirical regimens, will contribute to a prompt 'fine tuning' of the treatment. It is evident that in settings with high rates of antimicrobial resistance, documentation of the pathogen is imperative.

Superinfections from micro-organisms with intrinsic resistance to tigecycline (i.e. *Proteus* spp., *Pseudomonas* spp., etc.) should also be taken into account when a patient receiving tigecycline develops new fever as a result of selection of these micro-organisms. Dose-escalation clinical trials are urgently required in order to obtain better pharmacokinetic values and overcome the MIC of the problem pathogens in crucial compartments, such as the blood and ELF, while preventing the development of resistance.

3. Other Options

3.1 Doripenem

Doripenem, the most recently FDA-approved carbapenem, possesses an antimicrobial spectrum similar to meropenem against Gram-negative pathogens and similar to imipenem against Gram-positive pathogens, while retaining 2- to 4-fold lower MICs against *P. aeruginosa* compared with meropenem.[140,141] A potential advantage of this new carbapenem over the existing members of the class is its lower potential for the selection of resistance *in vitro*.[142-144] The combination of doripenem with an aminoglycoside in the treatment of infections caused by *P. aeruginosa* with elevated carbapenem MIC values may be associated with a lower risk of selecting further resistance.[145] However, its potential to develop *in vivo* resistance should be studied prospectively. Interestingly, doripenem retains activity against strains displaying OprD-mediated resistance to imipenem and also against many problematic strains with various resistant mechanisms. Doripenem might be a good option against strains with isolated resistance to imipenem (probably due to the OprD mechanism). In the case of MBL-mediated resistance to meropenem and imipenem, the antibacterial activity of doripenem is also expected to be affected.[142] However, in our experience, VIM-producing strains are the most common and they inactivate all carbapenems.

Preliminary data indicate that PK/PD applications, by means of prolonged infusion and supported by the relative stability of the infusate in ambient conditions, may help in the treatment of strains with borderline MICs, thus overcoming low-level resistance.[146,147] Extended infusion (over 4 hours) enhanced efficacy against selected strains of *P. aeruginosa* with MICs of 4 μg/mL.[148] Exploiting PK/PD evidence, in a large, phase III study of 531 patients with VAP, a 4-hour intravenous infusion of doripenem (500 mg) was clinically efficacious and therapeutically non-inferior to imipenem/cilastatin, with a clinical cure rate of 68.3% (doripenem) and 64.2% (imipenem/cilastatin). In patients infected with *P. aeruginosa*, the clinical cure rate of

doripenem was 80.0% and of imipenem/cilastatin 42.9% (p-value not significant), whereas the microbiological cure was 65.0% (doripenem) and 37.5% (imipenem/cilastatin).[149] In addition to the low adverse event potential, doripenem also has a favourable safety profile.[140]

3.2 Fosfomycin

Fosfomycin tromethamine is a soluble salt of fosfomycin, with improved bioavailability over fosfomycin. It is licensed as single-dose treatment for uncomplicated UTIs caused by *E. coli* and *E. faecalis*. Fosfomycin disodium is the available formulation for intravenous or intramuscular administration.[150] Fosfomycin inactivates the enzyme pyruvyl-transferase, which is required for the synthesis of the bacterial cell wall peptidoglycan. Fosfomycin is a bactericidal antibacterial against a broad spectrum of Gram-positive and Gram-negative bacteria possessing a low potential for cross resistance with other classes of antimicrobials.[151] Toxicity rates are reported to be very low, including gastrointestinal disturbances (nausea, vomiting, diarrhoea), transient elevation of transaminases and local irritation at the site of intravenous injection.[152,153]

Early *in vitro* data indicating an increased risk for development of resistance have led to the adoption of combination regimens in clinical practice, particularly in non-UTIs. However, synergy studies with various antibacterial combinations have failed to demonstrate a consistent synergistic effect, except for the combination with β-lactams against *P. aeruginosa*.[154-156]

ESBL-producing *E. coli* strains (n = 290) and *K. pneumoniae* (n = 138) isolated during 2000–2005 from UTIs in Spain, were tested against fosfomycin by disk diffusion test, agar dilution and broth microdilution techniques. By the agar dilution method, 97.4% of the tested strains (100% of *E. coli* strains) were susceptible to fosfomycin with MICs <64 mg/L.[157]

Another *in vitro* study has tested 45 strains of MDR *P. aeruginosa,* 40 of which were inhibited by microdilution method at concentrations ≤64 mg/L.[158] In a recent *in vitro* study, fosfomycin was tested against 30 *K. pneumoniae* with an MBL plus ESBL resistance pattern, 30 *P. aeruginosa* with an ESBL pattern and 30 MDR *A. baumannii* strains. Because of the lack of a universal breakpoint of resistance, the threshold of >64 mg/L was considered as resistance. *K. pneumoniae* strains exhibited the greatest susceptibility with an MIC_{50} of 16 mg/L and MIC_{90} of 32 mg/L. *P. aeruginosa* strains showed non-negligible susceptibility, with an MIC_{50} of 32 mg/L and MIC_{90} of 128 mg/L, whereas only one *A. baumannii* strain was regarded as susceptible. Since penetration of fosfomycin is reported to be sufficient in various body compartments, particularly during inflammation, peak plasma concentrations are anticipated to exceed the MICs of the tested MDR pathogens after a maximum dosage of 8 g intravenously every 8 hours.[159] Therefore, a new option for those problematic pathogens emerges, especially for UTIs.[160,161] However, caution is needed before *in vitro* data are translated into clinical practice.

Clinical data with fosfomycin for MDR infections are very scarce. In a prospective study of 416 transplant patients with infections due to MDR pathogens (88.5% of which were Gram-negative), fosfomycin was administerd in 1% of the patients; however, no further data on successful treatment and outcome are reported.[162] In a recently published review of the 64 existing (mostly retrospective) fosfomycin studies, among 1529 patients, 81.1% were successfully treated. The major pathogens treated were *P. aeruginosa*, *S. aureus* and *S. epidermidis*, *Enterobacter* spp. and *Klebsiella* spp. However, no data were available regarding an MDR profile. Clinical indications included post-operative infections, meningitis, endophthalmitis, endocarditis, pulmonary infections particularly among CF patients, septicaemia, osteomyelitis and arthritis. Fosfomycin was co-administered with aminoglycosides, cephalosporins or penicillins.[163]

It is evident that experience with fosfomycin against pathogens with a strict definition of multidrug resistance is still limited. Certainly, the growing problem of 'untreatable' pathogens warrants prospective and comparative studies with fosfomycin in order to clarify its future role in the antimicrobial armamentarium. In parallel,

antimicrobial testing issues and susceptibility breakpoints need to be urgently readdressed.

4. Conclusions

As the pharmaceutical pipeline for antibacterials against MDR Gram-negative micro-organisms remains disappointing, clinicians have to spare all currently available antibacterials with residual activity to 'buy time'. Strict adherence to antibacterial stewardship programmes seems mandatory in order to preserve currently available agents. Wise exploitation of PK/PD properties, based on well-designed clinical studies, is urgently needed. The implementation of rigorous infection-control practices has become of the utmost importance in terms of preventing the horizontal spread of antimicrobial resistance and subsequent need for more antibacterials. Finally, the ethical responsibility of all leading medical and public health societies to streamline the pharmaceutical industry to scale up antibmicrobial research is currently the most challenging task, before the 21st century becomes 'the post-antibiotic era'.

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Correspondence: Prof. *Helen Giamarellou*, Professor of Internal Medicine and Infectious Diseases, 4th Department of Internal Medicine, Athens University School of Medicine, 'ATTIKON' University Hospital of Athens, 1 Rimini Street, Haidari 12462, Athens, Greece.
E-mail: hgiama@ath.forthnet.gr