Review

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Review

Colistin: Lights and Shadows of an Older Antibiotic

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Abstract: The emergence of antimicrobial resistance have represented a serious threats for public health and infections due to multidrug-resistant (MDR) microorganisms represent one of the most important causes of death worldwide. The renew of old antimicrobials, such as colistin, have been proposed as valuable therapeutic alternative to the emergence of the MDR microorganisms. Although colistin is well known to presents several adverse toxic effect, its usage in clinical practice have been reconsidered due to the broad spectrum of activity to gram-negative (GN) bacteria and to the important role of "last resort" agent against MDR-GN. Despite the revolutionary prospective of treatment of this old antimicrobial molecule, many questions remain open regarding the emergence of novel phenotypic traits of resistance and the optimal usage of the colistin in clinical practice. In the last years, several forward steps have been done in the understanding of resistance determinants, clinical usage and pharmacological dosage of this molecule, however, different points regarding the role of colistin in clinical practice and the optimal pharmacokinetic/pharmacodynamic targets are not well defined yet. In this review, we summarize the mode of action, the emerging resistance determinants and its optimal administration in the treatment of difficult-to-treat infections due to MDR Gram-negative bacteria.

Keywords: lipopeptide; antimicrobials; antimicrobial resistance;

1. Introduction

Antibiotic resistance represents a serious public health and it is associated to to million of deaths annually [1]. Since the discovery of first antimicrobial molecules, the emergence of novel traits of resistance to antimicrobials have been observed concomitantly [2]. It's well known that antimicrobial resistance have been associated with their misuse and overuse in different field of applications (humans, animals and plants). Indeed, the presence of antimicrobial rich environments create a favourable conditions that allow the selection of resistant subpopulations in opposition to sensitive microorganisms [3].

With the diffusion and rapid increase of antimicrobial-resistance, the development of microorganisms resistant to multiple antimicrobial classes of compounds have been observed subsequently [4]. The emergence of multi-drug resistant (MDR) microorganisms posed different limitations to the clinicians by reducing the available antimicrobial armamentarium. In the last years, the diffusion of MDR strains have been considered an urgent threat especially among gram-negative bacteria that requires a prompted response. To overcome these limitations, several strategies have been adopted including new schemes of treatment by combining antimicrobial molecules with no activity alone and the development of novel antimicrobial molecules [5,6]. At the same time, the revival of older antibiotics considered as last resort drug have posed new prospective in treatment of difficult-to-treat (DTR) infections due to MDR strains [5].

Colistin, also known as polymyxin E, is an old antimicrobial molecule that it was discovered in the middle of 19th century in Japan from a culture of *Paenibacillus polymyxa* subspecies [7]. Colistin is a cyclic oligopeptides antimicrobials belonging to the class of polycationic antibiotic and it's active

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against most Gram-negative bacteria by binding to the lipopolysaccharide (LPS) of the outer cell membranes by electrostatic interaction. The linkage between colistin and outer membrane create a disorganization of the outer membrane structure thus resulting in an alteration of the outer membrane and consequently intracellular contents release and bacterial death [7].

In the last years, renew of older antibiotics such as colistin have created new prospective in treatment of DTR infections [6,8]. However, the emergence of new traits of resistance to this drug and the adverse toxic effects to mammalian cells have mitigated its use in clinical practice [6,7]

In this review we discuss the principle of the mode of action, the emerging traits related to the resistance and the use of colistin in clinical practice from a pharmacological and clinical point of views.

2. Mechanisms of Action, Antibacterial Activity and Adverse Effects

2.1. Structure and Mode of Action

Colistin is an amphiphilic lipopeptide antibiotic discovered in 1947 by Koyama [9,10], produced by *Paenibacillus polymyxa subspecies colistinus*. Colistin, also called polymixyn E, is a member of polymyxin family of antibiotics. Since 1952, the first formulation in clinical use is a solution for intravenous administration and shown its bactericidal function against many Gram-negative bacteria, but not against Gram-positive, anaerobic bacteria or mycoplasmas. Due to potent antibacterial activity against Gram-negative bacteria colistin was initially considered a "miraculous molecule". However, since 1970's the use in clinical practice was mitigated due to severe its severe adverse effects [11]. The original molecule has been modified to reduce principally the nephrotoxicity effect and actually, two forms of colistin are clinically available for human treatment: colistin sulfate and colistin methanesulfonate, also called colistimethate sodium. Differences between these compounds are related to their use and toxicity. In particular, colistin sulfate is an active compound administered topically and orally, while colistimethate, is used in formulations administered by parenteral and nebulization routes.

Colistin's basic structure consists of a core region formed by a hydrophobic portion, a cyclic heptapeptide linked by a tripeptide bridge, to a hydrophilic part, a fatty acid. The colistin molecule is positively charged due to the presence of five diaminobutyric acid residues linked to the core [12]. The prodrug form differs from this structure for the presence of methan-sulfonates linked to the diaminobutyric acids (**Figure 1**) [13].

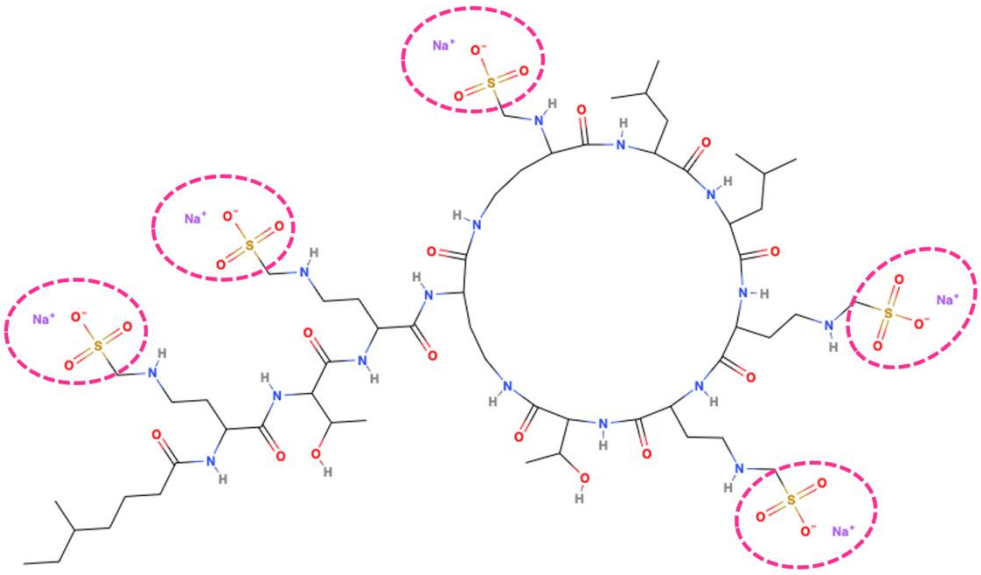

Figure 1. *Colistindrug structure.* 2D representation of colistin methanesulfonate molecule highlighted the five methanesulfonate groups (inside the purple dotted circles) responsible for the difference

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between active compound and its prodrug form. This 2D representation was performed with MolView v2.4 online tool (https://molview.org/).

It is noteworthy that the five diaminobutyric acid residues, which confer the positive charge to the molecule, play a determining role in the drug's antibacterial effect, generally described with the Shai-Matsuzaki-Huang (SMH) model [14–16].

Colistin acts by competition and displacement of Ca^{2+} and Mg^{2+} from the negatively charged sulfate portion of the lipid A in the lipopolysaccharide molecule (LPS) of Gram-negative bacteria. This ionic dislocation by colistin seems necessary for forming pore-like structures [17–19]. The loss of ions binding and their substitution with colistin molecules alters the tertiary structure of LPS, creating the possibility for colistin itself to insert its own portion of fatty acids into the membrane, definitively compromising the permeability of the outer membrane. In addition, colistin acyl fat inserted in the bilayer alters the inner membrane stability, leading to bacterial membrane disruption and a bactericidal effect [20]. Colistin also plays a key role in preventing endotoxin-induced shock through its binding to lipide A portion [21]. This drug acts both at the surface and intracellular levels, in particular, altering vesicle-vesicle contact of bacterial cells. In brief, colistin crosses the membrane and causes the fusion of the inner leaflet of the outer membrane and the outer leaflet of the cytoplasmic membrane, disrupting the cytoplasmic bilayer, altering the osmotic balance and leading to cell death [22,23]. The antibacterial action of colistin was also reported at the molecular level, where it can induce oxidative stress and, consequently, DNA, protein, and lipid damage in bacteria through ROS production and could inhibit essential enzymes involved in the respiratory chain, such as the NADH-quinone oxidoreductase [24], leading to cell death.

2.2. Adverse Effects

Colistin treatment was dismissed in clinical use principally due to its nephrotoxicity effect, which is lower for the prodrug form. Adverse effects were principally due to its re-absorption by proximal tubule cells through an endocytotic process, mediated by megalin, and through a facilitative transport by two transporters located in the apical cell membrane, the human peptide transporter 2 (PEPT2) and the carnitine/organic cation transporter 2 (OCTN2) [25,26]. Intracellular accumulation of colistin induces mitochondrial and endoplasmic reticulum stress with consequent toxic cellular effects [27]. This mechanism leads to cellular lysis and acute tubular necrosis [28,29]. The incidence of colistin-induced acute kidney injury varies between 12.7 and 70% in intensive cure units patients [30–33]. A recent study by Kilic and colleagues demonstrated that the nephrotoxicity effect depends proportionally on the duration of treatment and is related to older patients [28,34].

Due to the high lipid content of neuronal cells, colistin could also exert its action in these cells, and some patients (with an incidence of about 7% [34]) experienced neurological adverse effects, such as paresthesia, seizures, confusion, ataxia, and visual disturbances [35]. The mechanism by which colistin induces these effects is a non-competitive presynaptic myoneuronal blockade of acetylcholine releasee [36]. Adverse effects on neuronal cells could be reverted by discontinuing the therapy.

2.3. In Vitro Antimicrobial Activity

In vitro activity of colistin was tested with success on *Acinetobacter baumannii*, a large part of *Enterobacteriaceae* and *Pseudomonas aeruginosa* [37]. In particular, for 106 non-duplicate isolates of *A. baumannii* was reported a minimum inhibiting concentration of 0.5 ug/mL for MIC_{50} and of 1.0 ug/mL for MIC_{90}, in monotherapy [37]

Walkty et al. [38] analyzed the colistin antibacterial activity on 3,480 isolates of Gram-negative bacilli from patients recruited during 2 years in 12 hospitals in Canada (CANWARD Study). In this study authors reported a MIC_{90} value ≤2 μg/ml against a several clinically relevant gram-negative bacilli, such as *Escherichia coli* (1,732 isolates), *Klebsiella spp.* (515 isolates), *Enterobacter spp., A. baumannii, and P. aeruginosa* (561 isolates), including all 76 MDR *P. aeruginosa* isolates tested in CANWARD Study.

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A cross-sectional and descriptive study conducted on 52 MDR *P. aeruginosa* isolates, collected from urine, pus specimens and respiratory tract, reported a MIC_{50} value of 1.0μg/mL and a MIC_{90} of 3.0μg/mL [39].

in the last years, several studies reported the activity colistin in combination other antimicrobial molecules. In particular, colistin, in combination with meropenem or tigecycline shown synergistic activity against colistin-resistant KPC-producing *K. pneumoniae* [40]. Kheshti and coworkers [41] on a study based on an in vitro checkerboard assay, reported a good synergistic activity of colistin treatment in combination with ciprofloxacin, levofloxacin (5%, the lowest level), imipenem, meropenem, ampicillin–sulbactan molecules and higher synergism in combination with rifampin (55%) tested on 20 isolated of *A. baumanii*.

A recent study [42], conducted on 219 *K. pneumoniae* isolates demonstrated the high synergy of minocycline and colistin on colistin-resistant and minocycline-intermediate or - resistant *K. pneumoniae*. This drug combination acts by disrupting the outer membrane (by colistin) without affecting the cytoplasmic membrane, allowing the entrance and accumulation at intracellular level of minocycline.

2.4. Antimcirobial Susceptibility Testing

The chemical structure of colistin and its cationic charge, make difficult the use of classical susceptibility test, like E-tests and disc diffusion. To overcome this limitation and to provide a pharmacological alternative to the numerous multi-resistant bacterial species, classical diagnostic protocols have been modified allowing the measurement of colistin susceptibility. Broth microdilution, the gold standard for colistin susceptibility test, is modified using a cation-adjusted Muller-Hinton broth without adding surfactant [43,44]. Another method approved by CLSI only for *Enterobactarales* and *Pseudomonas spp*, is a broth disc elution modified by Simner and colleagues [45]. The test, renamed as Colistin Broth Disc Elution (CBDE), is easier than the Broth microdilution and is based on analysis of the efficacy of a graded concentration of colistin (of 1,2,4 ug/mL) obtained from colistin disc elution in 10 mL of cation adjusted Muller-Hinton Broth, tested on a 0,5 Mc Farland of bacteria. EUCAST colistin breakpoints table, version 14.0 reports the following cut-off value for the detection of phenotypic resistance: MIC of 2 mg/L for Enterobacterales and for Acinetobacter spp., and MIC of 4 mg/L for P. aeruginosa.

3. Mechanisms of Colistin Resistance

A variety of mechanisms may be involved in the acquisition of colistin resistance in Gram-negative bacteria, and they can be summarized into four groups: modification of LPS structure by chromosomal mutations (i), modification of LPS structure by acquisition of plasmids (ii), loss of LPS structure (iii), overexpression of efflux pumps (iiii).

3.1. Modification of LPS Structure by Chromosomal Mutations

Reduction of negative charge of lipid A of LPS lead to loss of electrostatic interaction with colistin and consequently to resistance [46]. Many genes and operons are involved in LPS modifications: 1. pmrC and pmrE genes and the pmrHFIJKLM operon, which promote addition of phosphoethanolamine (PEtn) and/or 4-amino-4-deoxy-L-arabinose (L-Ara4N) to lipidA. 2. regulatory two-component systems such as PmrAB, PhoPQ, and crrAB, 3. mgrB negative regulator gene.

Addition of L-Ara4N and/or PEtn to lipidA changes the negative charge of the cell membrane by neutralizing the negatively charged phospholipids [47–50]. In detail, the addition of PEtn to the 1′- or 4′-phosphate group of lipid A is carried out by PmrC, a putative membrane protein with phosphoethanolamine transferase activity encoded by pmrABC operon [11,51–54]. The synthesis of L-Ara4N from uridine diphosphate glucuronic and its addition to lipid A is promoted by pmrHIJKLM operon (also called *arnBCADTEF*) and PmrE activity [51]. PmrB is a cytoplasmic membrane-bound which activates PmrA by phosphorylation, and PmrA in turns activates regulation of the pmrABC and pmrHFIJKLM operons and the pmrE gene. Subsequently, these operons and

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genes lead to LPS modification by adding PEtn and L-Ara4N to lipid A [55]. Although the L-Ara4N modification of LPS has been described as a common mechanism of colistin resistance among Gram-negative bacteria (*Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella enterica*, and *Pseudomonas aeruginosa*), it does not occur in *Acinetobacter baumannii* because lacks all the genes required for L-Ara4N biosynthesis [51]. Alternatively, addition of galactosamine to the 1′-phosphate position of lipid A, following activation of the sensor kinase PmrB, has been associated with moderate levels of colistin resistance in *A. baumannii* [52].

Mutation of pmrA/pmrB results in upregulation of the pmrABC and pmrFHIJKLM operons and pmrE gene which lead to PEtN modification of lipid A, and in turn, results in colistin resistance. Several mutations have been reported in many Gram-negative bacteria, such as *Salmonella enterica* [56,57], *K. pneumoniae* [58–60], *A. baumannii* [61–63], *P. aeruginosa* [64,65], and *E. coli* [57,66,67].

Trascription of pmrFHIJKLM operon is also activated by PhoPQ regulatory two-component system. PhoQ is a sensor kinase that promote espression of the regulator protein PhoQ, which promote pmrFHIJKLM operon transcription via phosphorylation. Furthermore, PhoP indirectly activates pmrA through the PmrD connector protein, which subsequently activates the transcription of the pmrHFIJKLM operon. This then leads to synthesizes and transfer of PEtn to lipid A [11,49,50]. Mutation of the phoP/Q genes that led to acquired colistin resistance has been identified in *K. pneumoniae* and *E. coli* [68–70]. Higher polymyxin MICs have been observed in PhoQ-deficient *P. aeruginosa* mutants when additional alterations affected other regulatory two-component systems (CprRS and ColRS) [71].

More evidence has accumulated on the role played by *mgrB*, a gene encoding a small regulatory transmembrane protein, MgrB, that exerts negative feedback on the kinase activity of PhoQ [72]. Inactivation of mgrB leads to the ‘activation of a phosphorylation cascade involving at chain PhoQ, PhoP, PmrD and/or PmrAB and finally triggering the expression of the pmrHIJKLM operon resulting in LPS modification. Mutations of mgrB including points mutations, deletion, nonsense, and insertion sequences (IS*5*-like, IS*1F*, IS*Kpn14*, IS*Kpn13*, IS*10R*) represents the most common mechanism of colistin resistance in clinical *K. pneumoniae* isolates [69,73–77]. The wide range of resistance level showed by Gram-negative strains harbouring mutations in the genes *pmrAB, phoPQ*, or *mgrB* suggests a role for other genetic loci. Mutations in the CrrAB two-component system has been associated with increased level of colistin resistance in strains of *K. pneumoniae* [74,78]. Mutation/inactivation of the crrB gene led to activation of the pmrHFIJKLM operon and the pmrC and pmrE genes through overexpression of the pmrAB operon [77,78]. Furthermore, various of PEtn-coding genes, such as eptA (pmrC), eptB (pagC), and eptC (cptA), are able to add PEtn to LPS and can be involved in colistin resistance [79]. Overespression of eptA has been associated with colistin resistance in *A. baumannii* [61,80]. Gerson et al. showed that mutations in the eptA gene (R127L and ISAba1 insertion) was associated with overexpression of EptA and colistin resistance in *A. baumannii* [61].

3.2. Loss of LPS Structure

The complete loss of lipid A or LPS core leading to colistin resistance has been observed in *A. baumannii*. Analysis of laboratory-induced colistin-resistant *A. baumannii* showed that high level of resistance to colistin was caused by the inactivation of LPS biosynthesis genes *lpxA*, *lpxC*, *lpxD* and *lpsB [81].* Various nucleotide substitutions, deletions, and insertions that cause frameshifts or result in truncated proteins have been reported from in vitro mutants and clinical isolates [81–84]. Moreover, disruption of lpxC and lpxD by insertion of IS elements, was described in colistin-resistant *A. baumannii* isolates [81–86]. Although LPS loss is an effective mechanism of colistin resistance, it has significant fitness costs and this explains why such mutants are rarely encountered in the clinical setting [87].

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3.3. Plasmid-Mediated Colistin Resistance

Since the first report of the *mcr* gene encoding for phosphoethanolamine transferase (*mcr-1*) in *E. coli* in China in 2015 [88], several reports worldwide have demonstrated the presence of mcr-1 and additional 9 families (mcr-2 to mcr-10) with more than 100 overall variants in different Gram-negative species distributed worldwide [11,49–53,89–94]. MCR is a member of the PETN enzyme family, and its activity results in the modification of lipid A by PETN addition. The enzyme has a domain inserted in the inner membrane and a periplasmic C-terminal sulfatase catalytic domain.

In 2018, Partridge et al. proposed a nomenclature for *mcr* genes. Several variants have been identified, especially for MCR-3 and followed by MCR-1 [95]. MCR-1 and MCR-2 share 81% identity at the amino acid sequence level. Sequence identity suggests that these two variants originated from *Moraxella* spp [89], as mcr-3, mcr-4 and mcr-7 from *Aeromonas* spp. and *Shewanella frigidimarina*, respectively[90–93]

The *mcr*-1 variant can be connected to various types of plasmids, including IncHI2, IncI2, IncX4, IncP, IncX, and IncFIP. The *mcr-2* gene was detected on an IncX4 plasmid. The presence of insertion sequence (IS*Apl1*, IS*1595)* on the genetic environment of *mcr* genes explains the possibility to integrate on bacterial chromosomes [94].

Plasmid-mediated colistin resistance represents the mechanism of greatest concern because of the ease of intra- and inter-species spread. Despite most of MCR-harboring microorganisms belong to the Enterobacterales order, such as *E.coli*, *Salmonella* spp., and *K. pneumoniae*, several reports showed the presence of *mcr*-genes in non-fermenting Gram-negative species such as *P. aeruginosa* and *A. baumannii* complex [96–110]. The *mcr-1* gene is the most commonly detected in *P. aeruginosa* both in clinical [96–99] and animal setting [100–103], and followed by *mcr-5* [104,105]. In *A. baumannii complex*, the *mcr-1* and *mcr-4.3* are the major variants observed in clinical isolates from Asia and Europe [98,99,106–109]. Other *mcr* genes found in *A. baumannii* include *mcr-2* and *mcr-3* [110].

3.4. Overexpression of Efflux Pumps

The role of efflux pump in colistin resistance is suggested by few studies. Efflux pumps, such as the KpnEF and AcrAB have been reported in *Enterobactericeae*. The ΔKpnEF mutants showed increased susceptibility to various cationic antimicrobial peptides such as colistin [111]. On the other hand, AcrAB is a part of the AcrAB–TolC complex and its overespression has been observed in colistin-resistant *E.coli*, *K. pneumoniae* and *Salmonella* strains [112–114].

The contribution of EmrAB efflux system to colistin resistance in *A. baumannii* was shown by in vitro experiments with the ΔemrB mutant [115]. Moreover, the upregulation of genes encoding protein components of efflux pumps (adeI, adeC, emrB, mexB, and macAB) was also observed in colistin-resistant *A. baumannii* strains [83].

The overexpression of the efflux pumps MexXY (RND family) under exposure to ribosome-targeting antibiotics was found to correlate with increased level of colistin resistance in *P. aeruginosa* [116]. However, the heterogeneity of MexXY expression observed in clinical isolates of *P. aeruginosa* showing variable levels of colistin resistance suggested that contribution of the efflux pumps to colistin resistance might also be related to other specific genetic backgrounds [117].

Further evidence for the role of efflux pumps in colistin resistance is the suppression of resistance by efflux pump inhibitor (EPI), cyanide-3-chlorophenylhydrazone (CCCP) in *A. baumannii*, *P. aeruginosa*, *K. pneumoniae*, and *S. maltophilia* [118]. However, a possible explanation is that CCCP-mediated depolarization of the electrochemical gradient may restore the negative charge of the outer membrane and lead to increased susceptibility to colistin [48,118]. Furthermore, various studies suggested a complex regulatory relationship between the efflux pumps and their transcriptional regulators and LPS synthesis, transport, and modification [48].

4. Pharmacokinetic/Pharmacodynamic Features

According to several preclinical evidence, the free area under the concentration-to-time curve to minimum inhibitory concentration ratio (*f*AUC/MIC) was defined as the best

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pharmacokinetic/pharmacodynamic (PK/PD) target for colistin efficacy in infections caused by *P. aeruginosa* and *A. baumannii* [119]. In a neutropenic murine thigh and lung infection model against three *P. aeruginosa* strains, Dudhani et al. [120] found that the *f*AUC/MIC ratio was the best PK/PD index correlating with colistin efficacy both in thigh (R^2=0.87) and lung infection model (R^2=0.89). The colistin *f*AUC/MIC targets required to achieve 1-log and 2-log kill against the three strains were 15.6 to 22.8 and 27.6 to 36.1, respectively, in the thigh infection model, whereas a *f*AUC/MIC ratio ranging from 12.2 to 16.7 and from 36.9 to 45.9 was found in the lung infection model for achieving 1-log and 2-log kill [120]. In a neutropenic murine thigh and lung infection model against three *A. baumannii* strains (of which two were colistin heteroresistant), Dudhani et al. [121] reported that the *f*AUC/MIC ratio was the best PK/PD index correlating with colistin efficacy both in thigh (R^2=0.90) and lung infection model (R^2=0.80). The colistin *f*AUC/MIC targets required to achieve stasis and 1-log kill against the three strains were 1.89–7.41 and 6.98–13.6 in the thigh infection model, respectively, and 1.57–6.52 and 8.18–42.1, respectively, in the lung infection model [121]. Notably, these colistin PK/PD targets against *P. aeruginosa* and *A. baumannii* were consistent with those retrieved in a recent murine thigh and lung infection model [122]. Indeed, the *f*AUC/MIC ratio was confirmed as the best PK/PD target for predicting colistin efficacy, being desired *f*AUC/MIC ratios for achieving 2-log kill against *Pseudomonas aeruginosa* and *A. baumannii* strains of 7.4–13.7 and 7.4–17.6, respectively [122]. It should be noticed that these PK/PD targets could be attained only in two *P. aeruginosa* strains and in one *A. baumannii* strain in the lung infection model even at the highest colistin dose tolerated [122].

In *Enterobacterales*, an in vitro model investigated the best PK/PD target of colistin efficacy against three *K. pneumoniae* strains exhibiting MIC values of 0.5, 1, and 4 mg/L, respectively [123]. The *f*AUC/MIC ratio emerged as the best PK/PD target for colistin efficacy, being an *f*AUC/MIC ≥25 more predictive for a bactericidal effect [123]. Notably, this PK/PD target may be attained at standard colistin dose of 9 MU in 100%, 5-70%, and 0% of *K. pneumoniae* isolates showing an MIC value of 0.5, 1, and 2 mg/L, respectively [123]. These findings may suggest on the one hand the need for revising current colistin clinical breakpoint against *Enterobacterales*, and on the other hand the potential relevance of implementing a therapeutic drug monitoring (TDM)-guided approach for personalizing colistin dosage.

It should be noticed that evidence investigating the relationship between optimal PK/PD target attainment for colistin retrieved in preclinical studies and clinical outcome are currently limited. A prospective observational study investigated the relationship between PK/PD target attainment of colistin and microbiological/clinical outcome in nine patients affected by multidrug-resistant (MDR) Gram-negative infections (eight caused by *A. baumannii* and one by *K. pneumoniae*) [124]. After the fifth colistin dose of 2 MU, the $AUC_{0\text{-}8}$/MIC ranged from 35.5 to 126. Although no significant relationship between AUC/MIC ratio and microbiological/clinical cure was found, a positive trend was observed at logistic regression (p=0.28) [124]. A prospective observational study including 33 patients affected by urinary tract infections and/or pyelonephritis caused by extremely drug-resistant *P. aeruginosa* reported no significant difference in *f*AUC/MIC ratio between cases exhibiting favourable clinical outcome and those with clinical failure (21.5 vs. 47.4; p=0.85) or in proportion of attainment of an AUC/MIC ratio ≥60 mg/L (32.3% vs. 50.0%; p=0.99) [125]. At multivariate analysis, average steady-state colistin concentration showed a trend towards statistical significance for acute kidney injury occurrence at the multivariate analysis (OR 4.36; 95%CI 0.86-20.0; p=0.07 (Sorlí et al., 2019).

Studies assessing colistin penetration in different sites of infection are reported in **Table 1**. Currently, data are available only for lung, central nervous system (CNS), and eye (Table 1). Specifically, a prospective observational study investigating epithelial lining fluid (ELF) penetration of intravenous colistin administered at a dosage of 2 MU every 8 hours in 13 critically ill patients affected by ventilator-associated pneumonia reported undetectable colistin concentrations in ELF [126].

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Table 1. Colistin penetration and assessment of PK/PD target attainment in different sites of infection.

Site of infection	Study design	Number of patients	Setting	Dose	Absolute tissue concentrations	Absolute plasmatic concentrations	Penetration rate ($AUC_{tissue}/AUC_{plasma}$)	PK/PD target attainment	Ref.
Lung	Prospective observational	13	ICU VAP	2 MU q8h IV	Undetectable	C_{min} 1.03±0.69 mg/L AUC/MIC ratio 17.3±9.3 (for MIC=2 mg/L)	0.00	Suboptimal in ELF	[126]
CSF	Prospective observational	5	ICU	2-3 MU q8h IV	C_{min} 0.47 mg/L AUC 0.53 mg*h/L	C_{min} 9.26 mg/L AUC 10.4 mg*h/L	0.05	Optimal PK/PD target attainment only for *P. aeruginosa* and *A. baumannii* strains exhibiting MIC values up to 0.06 mg/L	[127]
Ocular	Preclinical rabbit uveitis model	20	Uveitis induced after endotoxin injection	5 mg/kg IV	*Aqueous humor* 0.62±0.07 (at 0.5h) 0.45±0.05 (at 3h) 0.38±0.08 (at 6h) *Vitreous humor* 0.02±0.01 (at 3h)	9.84±2.0 (at 0.5h) 0.93±0.07 (at 3h) 0.24±0.08 (at 6h)	0.07 (aqueous humor at 0.5h) 0.48 (aqueous humor at 3h) 1.58 (aqueous humor at 6h) 0.02 (vitreous humor at 3h)	Not assessable	[128]

AUC: area under concentration-to-time curve; C_{min}: trough concentrations; CSF: cerebrospinal fluid; ELF: epithelial lining fluid; ICU: intensive care unit; IQR: interquartile range; IV: intravenous; MIC: minimum inhibitory concentration; PK/PD: pharmacokinetic/pharmacodynamic; VAP: ventilator-associated pneumonia.

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A prospective observational study including five critically ill patients assessed colistin penetration in cerebrospinal fluid (CSF) administered intravenously at a dosage of 2-3 MU every 8 hours [127]. Colistin CSF-to-plasma ratio was 0.05, with absolute concentrations retrieved in CSF allowing to attain optimal PK/PD target only against P. aeruginosa and A. baumannii strains showing an MIC value up to 0.06 mg/L [127]. In regard to ocular penetration, only a preclinical animal model currently assessed this issue in twenty rabbits receiving intravenous colistin at a dosage of 5 mg/kg [128]. Overall, absolute colistin concentrations were extremely low in aqueous humor and undetectable in vitreous humor in most of included cases [128].

Overall, these findings may strongly support the implementation of alternative agents in case of deep-seated infections, according to the limited penetration colistin penetration rate in lung and CSF and the failure in attaining optimal PK/PD targets. Notably, these findings may be expected according to the physicochemical and PK features of colistin, namely hydrophilic properties, large molecular weight, and limited volume of distribution [129].

5. Conclusions

In the last years, the renewed of older antimicrobial molecules have revolutionized the treatment of infections due to MDR-GN microorganisms. At the same time, novel approaches including the therapeutic drug monitoring (TDM) for personalizing antimicrobial dosage of the different antimicrobial molecules and new therapeutic schemes of treatment by combining antibiotics with limited antimicrobial activity have revolutionized the treatment of infections due to MDR pathogens. In this context, the clinical usage of colistin alone and in combination with other antimicrobials with scarce and/or limited antimicrobial activity have recently reinvented its role in clinical practice. Also, considering the limited antimicrobial options against these pathogens, colistin was defined as the "last-hope resource" for the treatment of DTR infections especially among critical-ill patients.

On the other side, the adverse toxic effects and the limited tissue penetrations in different anatomical districts prompted to mitigate its role in clinical setting by limiting its use. In addition, the widespread of colistin resistant strains poses a serious limitation in the sue of this molecule especially to the light of the new antimicrobial molecules recently developed with high bactericidal activity against MDR microorganisms (i.e., cefiderocol, ceftazidime/avibactam, meropenem/vaborbactam, etc.).

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