Insights into Newer Antimicrobial Agents Against Gram-negative Bacteria

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ABSTRACT: Currently, drug resistance, especially against cephalosporins and carbapenems, among gram-negative bacteria is an important challenge, which is further enhanced by the limited availability of drugs against these bugs. There are certain antibiotics (colistin, fosfomycin, temocillin, and rifampicin) that have been revived from the past to tackle the menace of superbugs, including members of *Enterobacteriaceae, Acinetobacter* species, and *Pseudomonas* species. Very few newer antibiotics have been added to the pool of existing drugs. There are still many antibiotics that are passing through various phases of clinical trials. The initiative of Infectious Disease Society of America to develop 10 novel antibiotics against gram-negative bacilli by 2020 is a step to fill the gap of limited availability of drugs. This review aims to provide insights into the current and newer drugs in pipeline for the treatment of gram-negative bacteria and also discusses the major challenging issues for their management.

KEYWORDS: novel antibiotics, gram-negative bacteria, challenges in management, drug resistance, carbapenem resistance

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Introduction

Infections caused by gram-negative bacteria pose an immense challenge due to emerging drug resistance among these pathogens. Multidrug resistance (MDR) and extensively drug resistance (XDR) render even the most effective drugs ineffective.[1] Drug resistance is one of the key threats to human health. Extended-spectrum beta-lactamases (ESBLs), AmpC beta-lactamases, and carbapenemase-producing gram-negative bacteria have emerged as an important therapeutic challenge. The organisms posing most danger have been clubbed together under the term "ESKAPE," ie, *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa*, and *Enterobacter* species, as these have the ability to escape the effect of antimicrobial drugs.[2] The ones leading to increased mortality include carbapenem-resistant *Enterobacteriaceae* (CRE), *P. aeruginosa*, and *A. baumannii*, which have acquired multiple mechanisms of resistance.[3–7] The global emergence of ESBLs in the 1990s led to the widespread use of carbapenems followed by the emergence of pandemic of CRE. The Centers for Disease Control and Prevention has categorized CRE as *urgent* and ESBL-producing gram-negative bacteria as *serious* antibiotic threats in the USA.[8] All the state-of-art developments in organ transplantation, chemotherapy, and surgery stand threatened by the aggressive advent of these gram-negative superbugs. In today's scenario, the list of effective/working antibiotics is shrinking. There seem to be no major breakthrough discoveries similar to penicillin and aminoglycosides in the near future. While in 2000s, the pharmaceutical industries focused on the development of antibacterial agents against MDR gram-positive bacteria such as methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *Enterococcus*, the development of drugs against gram-negative bacteria got neglected.[2,9] In fact, of all the compounds developed for treating gram-positive bacteria, only tigecycline has activity against gram-negative bacteria. Currently, there are certain drugs that have been revived again from their previous usage in 1970s–1980s and subsequent abandonment due to their side effects. To combat the problem of drug resistance in gram-negative bacteria, newer drug combinations as well as newer drugs are in pipeline for development.[4,5] The limited availability of certain drugs in many countries is also a drawback.[10,11] There is an increasing requirement of development of novel antimicrobials for gram-negative bacteria so as to combat the impending menace of pandrug resistance (PDR). In this regard, the Infectious Disease Society of America has taken up a global initiative of 10 × '20 meaning development of 10 new antibiotics against gram-negative bacilli by the year 2020.[12] This review summarizes the important antimicrobial agents currently being used and newer drugs in pipeline for treating gram-negative bacterial infections and some of the therapeutic challenges posed by MDR gram-negative bacteria.

Old but Young

The drugs used in the past, which have been revived and now are used to treat the infections caused by gram-negative bacteria, include colistin, fosfomycin, temocillin, and rifampicin.

Colistin. The drug derived from *Bacillus polymyxa* was banned in 1970s due to its adverse effects in the form of nephrotoxicity and neurotoxicity. Colistin is a

concentration-dependent bactericidal drug.[13] The drug being a cationic peptide interacts and destabilizes the negatively charged bacterial cell membranes, leading to leakage of intracytoplasmic material.[5] The maintenance dose of colistin is recommended after 24 hours, and taking into account the nephrotoxicity caused by this drug, its maximum loading should not be higher than 10 MU.[14,15] Colistin sulfate is available for both topical and oral usage, while colistin methanosulfonate is administered parenterally.[5] About 70% of colistin methanosulfonate is excreted through the kidney, while colistin is reabsorbed and excreted through the nonrenal route.[13]

The drug is active against ESBL- and carbapenemase-producing *Enterobacteriaceae* (CPE), *P. aeruginosa*, and *A. baumannii*, the most worrisome pathogens. However, certain organisms such as tribe *Proteae*, *Burkholderia* species, and *Serratia marcescens* are intrinsically resistant to the drug.[5] The drug has shown synergistic results with daptomycin and vancomycin against *A. baumannii*.[16] The combination therapy against carbapenem-resistant *K. pneumoniae* with drugs such as tigecycline and gentamicin has shown superiority over colistin monotherapy.[3] Inhaled or aerosolized colistin has been found to be a useful alternative for the treatment of ventilator-associated pneumonia (VAP) and tracheobronchitis and has the advantage of lesser toxicity.[17] Intrathecal and intraventricular administration of colistin has a successful outcome in ventriculitis and meningitis cases caused by resistant *A. baumannii*.[18] Due to the inadvertent use of colistin, bugs have developed mechanisms to escape the drug effects, especially carbapenemase-producing *K. pneumoniae* (CPKP).[6,19] The use of colistin for >14 days is significantly associated with resistance development.[20] Among the adverse effects, nephrotoxicity remains the most common although reversible by renal replacement therapy and usually associated with the duration of therapy.[21,22] Neurotoxicity as reported earlier is a rare entity nowadays.[21]

Fosfomycin. It is a phosphonic compound discovered in 1969 in Spain.[23] It works by inhibiting cell wall biosynthesis by inactivating phosphoenolpyruvate transferase enzyme.[24] Many countries have approved the oral administration of fosfomycin tromethamine for treating urinary tract infections (UTIs) caused by *Escherichia coli* and *E. faecalis*, whereas fosfomycin disodium is given parenterally.[25] The drug is excreted by glomerular filtration. It is neither metabolized nor protein bound and has a low molecular weight, thereby achieving good penetration and concentration in tissues.[26] Hemodialysis removes the drug completely; therefore, the drug is readministered after the procedure is over.[26,27]

It is a broad-spectrum bactericidal drug acting both on gram-positive and gram-negative bacteria.[23,24] It has shown good activity against both ESBL and CPE and >90% MDR *P. aeruginosa*.[28–32] However, *A. baumannii* is intrinsically resistant to the drug.[28] The data regarding its activity against XDR pathogens are scarce and require clinical investigations. The drug resistance to fosfomycin monotherapy has been demonstrated both in vitro and in vivo, which may be due to chromosomal mutation in transport system or enzymatic modification. Its use as a single agent is usually restricted in critically ill patients. The side effects of the drug are very few, the most common being hypokalemia.

Temocillin. The drug was available in the UK market in 1980s and was manufactured by Beecham Pharmaceuticals. It is derived from ticarcillin. Eumedica relaunched the drug in UK, Belgium, and Luxembourg. The drug is mainly excreted by the kidney.[33]

Temocillin acts on ESBL- and AmpC-producing *Enterobacteriaceae* but is ineffective in treating *Pseudomonas*, *Acinetobacter*, and anaerobic bacteria.[34] The organisms possessing oxacillinase (OXA)-48, IMP, NDM, and VIM do not show susceptibility to temocillin.[35] Further clinical studies are necessary for evaluation of the drug. Presently, temocillin is licensed in UK and Belgium for treating UTI, sepsis, and respiratory infections caused by ESBL- and AmpC-producing *Enterobacteriaceae* as an alternative to carbapenems.[36]

Rifampicin. Rifampicin is one of the earliest antibiotics currently prescribed as a first-line treatment for tuberculosis. However, in this era of drug resistance, the combination of rifampin with colistin and meropenem/doripenem has demonstrated synergistic effects against MDR *Pseudomonas* spp., *Acinetobacter* spp., and CPE.[37] Before its use in routine, the clinical efficacy and survival rate needs to be evaluated as data on the combinations are limited to a few uncontrolled studies.[38]

New Alternatives

Tigecycline. It is a bacteriostatic drug derived from minocycline and approved by the US Food and Drug Administration (FDA) for complicated intra-abdominal infections (cIAIs) and skin-soft tissue infections and community-acquired (CA) pneumonia.[5,39,40] It is available only for parenteral administration and excreted in bile. It possesses a large volume of distribution and gets concentrated well in bile, gall bladder, colon, and neutrophils, while low levels are found in blood, epithelial lining fluid, and urinary tract.[41,42]

Tigecycline efficiently tackles ESBL-producing *Enterobacteriaceae*, CPKP, and *A. baumannii* (both MDR and XDR), while tribe *Proteae* and *Pseudomonas* are intrinsically resistant pathogens.[40,43,44] The clinical studies evaluating the efficacy of tigecycline usually depict the results of its combination with other drugs, thereby masking the real effect of the drug.[3,45–47] Combination with colistin, meropenem, or aminoglycoside has shown low failure rates in infections caused by XDR-CPKP. However, its excessive use has led to increasing resistance, especially in CPKP.[48] The common side effects include nausea, vomiting, and diarrhea. Its use has also showed controversy as the death rate is shown to be higher with this drug as compared with other antibiotics, although the results were not statistically significant.[49,50]

Double-carbapenem regimen. Combination of two carbapenems was successfully evaluated in three patients by Bulik and Nicolau in CPKP strains.[51,52] The protocol implied included administration of 1 g ertapenem 24 hourly followed 1 hour later by meropenem (2 g) every 8 hours in an infusion

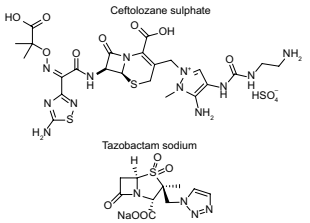
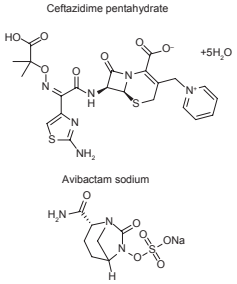
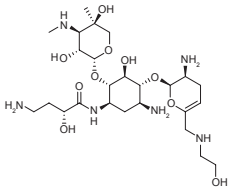
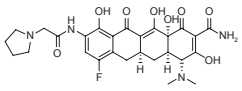
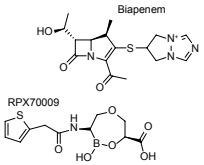
that is to be carried out over 3 hours duration. The same regimen tested subsequently in 21 patients with carbapenem-resistant *K. pneumoniae* isolation showed 80.7% clinical success and 96% microbiological cure.[53,54] However, the double-carbapenem regimen requires further clinical assessment in a larger number of patients.

Novel Drugs

Among the newer drugs in pipeline, two drugs have been approved by the FDA, namely, ceftolozane/tazobactam (Zerbaxa) and ceftazidime/avibactam (Avycaz). The remaining drugs are in various phases of trials (Table 1). Both these drugs referred to as *superheroes of gram-negative bacteria* mark the first combination of cephalosporins with beta-lactamase inhibitors. Both of them are bactericidal drugs (time-dependent killing) that inhibit bacterial cell wall synthesis by binding to penicillin-binding proteins. The major difference between the two drugs is the spectrum of bactericidal action. Zebraxa acts on gram-negative bacteria, some anaerobes, and some gram-positive bacteria and does not act on any carbapenemases, while Avycaz has action only against gram-negative bacteria (*Enterobacteriaceae* and *P. aeruginosa*) and is the first cephalosporin having action against carbapenemases.[55–61]

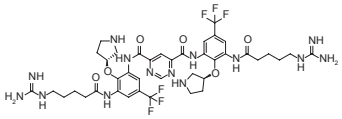
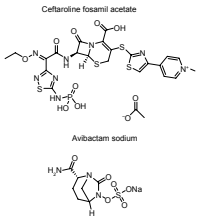
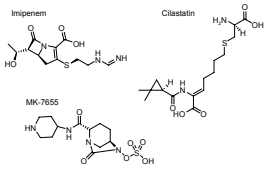
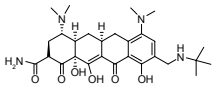
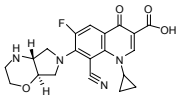
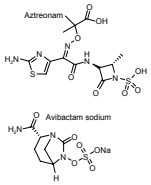
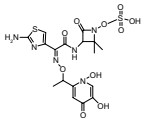
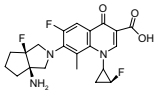
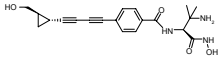
Ceftolozane/tazobactam (Zerbaxa). Zerbaxa is a combination product of a novel cephalosporin and a beta-lactamase inhibitor. It is approved by the FDA for treating complicated urinary tract infections (cUTIs), including acute pyelonephritis caused by *Enterobacter cloacae, E. coli, Klebsiella*

Table 1. Novel drugs in pipeline for gram-negative bacteria.

	DRUG	CLASS	COMPANY	TRIAL	INDICATIONS
1	Ceftolozane/Tazobactam (Zerbaxa) 	Cephalosporin/ Beta lactamase inhibitor combination	Cubist pharmaceuticals (owned by Merck)	Approved Dec 19, 2014	cUTI, cIAI, AP, possible—HAP/VAP
2	Ceftazidime/Avibactam (Avycaz) 	Cephalosporin/ Beta lactamase inhibitor combination	AstraZeneca/Actavis	Approved Feb 25, 2015	cUTI, cIAI, AP, possible—HAP/VAP
3	Plazomicin 	Aminoglycoside	Achaogen	Phase 3	cUTI, CRBSI, HAP/VAP, cIAI
4	Eravacycline 	Tetracycline	Tetraphase pharmaceuticals	Phase 3	cUTI, cIAI, HAP
5	Carbavance (RPX7009 + meropenem) 	Meropenem + novel boronic beta lactamase inhibitor	Rempex parmaceuticals	Phase 3	cUTI, cIAI, AP, HAP/VAP, febrile neutropenia, bactremia

(*Continued*)

Table 1. (*Continued*)

	DRUG	CLASS	COMPANY	TRIAL	INDICATIONS
6	Brilacidin 	Peptide defense protein mimetic	Cellceutix	Phase 3	ABSSI
7	Ceftaroline/Avibactam 	Cephalosporin/ Beta lactamase inhibitor combination	AstraZeneca/Actavis	Phase 2	Bacterial infections
8	Imipenem/Cilastatin + MK-7655 (Relebactam) 	Carbapenem/Beta lactamase inhibitor combination	Merck	Phase 2	cUTI, cIAI, HAP/VAP, AP
9	S-649266 (structure not disclosed yet)	Cephalosporin	Shionogi	Phase 2	cUTI
10	Omadacycline 	Tetracycline	Paratek pharmaceuticals	Phase 2	CAP, ABSSI, cUTI
11	POL7080 (RG 7929) (structure not disclosed yet)	Macrocycle (protein epitope mimetic) LptD inhibitor	Polyphor (roche licensee)	Phase 2	VAP (*P. aeruginosa*), LRTI
12	Finafloxacin 	Fluoroquinolone	MerLion pharmaceuticals	Phase 2	cUTI, AP, cIAI, ABSSI
13	Aztreonam/Avibactam 	Monobactam/Beta lactamase inhibitor combination	AstraZeneca/Actavis	Phase 1	Bacterial infections
14	BAL30072 	Monosulfactam	Basilea pharmaceuticals	Phase 1	MDR GN bacterial infections
15	DS-8587 	Fluoroquinolone	Daiichi-Sankyo	Phase 1	Enteric bacteria, Acinetobacter spp.
16	ACHN-975 	LPS synthesis inhibitor	Achaogen	Phase 1	Enterobacteriaceae, *P. aeruginosa*
17	CB-618 (structure not disclosed yet)	Beta lactamase inhibitor	Cubist	Phase 1	MDR GN bacteria

oxytoca, *K. pneumoniae*, *Proteus mirabilis*, *P. aeruginosa*, *Bacteroides fragilis*, *Streptococcus anginosus*, *Streptococcus constellatus*, and *Streptococcus salivarius*, which was established in a trial including 1068 individuals where it was compared with levofloxacin.[55–57] For the management of cIAIs caused by *E. coli, K. pneumoniae, P. mirabilis, and P. aeruginosa*, its administration is recommended in combination with metronidazole rather than meropenem according to a clinical trial consisting of 979 patients.[58,59] The drug showed good in vitro activity against members of *Enterobacteriaceae* producing ESBLs and other beta-lactamases such as TEM, SHV, CTX-M, and OXA. It did not demonstrate activity against serine group of carbapenamases, ie, KPC and metallo-beta-lactamases. The recommended dosage of the drug for >18 years adult (CrCl > 50 mL/minute) is 1.5 g iv (1 g/0.5 g) 8 hourly followed by intravenous infusion over 1 hour for 7 days in cUTI and 4–14 days in cIAIs.[60] The dose needs to be adjusted in patients with renal inefficiency as ceftolozane and tazobactam metabolite M1 are eliminated through the kidney. The most common side effects associated with the drug include nausea, diarrhea, headache, and fever.

Ceftazidime/avibactam (Avycaz). Avycaz is a combination of cephalosporin and new nonbeta-lactam beta-lactamase inhibitor, which was approved for the treatment of cIAI in combination with metronidazole caused by *E. coli, K. pneumoniae, P. mirabilis, Providencia stuartii, E. cloacae, K. oxytoca,* and *P. aeruginosa* and cUTI, including pyelonephritis, caused by *E. coli, K. pneumoniae, Citrobacter koseri, Enterobacter aerogenes, E. cloacae, Citrobacter freundii, Proteus* spp., and *P aeruginosa*.[61,62] The addition of avibactam (a novel nonbeta-lactam beta-lactamase inhibitor) to ceftazidime protects it from TEM, SHV, CTX-M, KPC, AmpC, and some OXA-producing bacteria. It has limited activity in case of metallo-beta-lactamases. The recommended dosage of the drug for >18 years adult (CrCl > 50 mL/minute) is 2.5 g iv (2 g/0.5 g) 8 hourly followed by intravenous infusion over 2 hours for 7 days in cUTI and 4–14 days in cIAIs. Both the components of the drug are excreted through the kidney, thereby demanding dose adjustment in renal insufficiency cases.[62] The commonly observed adverse effects include nausea, vomiting, and constipation. As the safety and efficacy data are limited, the drug is restricted for use only in patients with no alternative drug left for administration.

Drugs in Trials

Plazomicin. The drug belongs to the aminoglycoside group of antibiotics commonly referred as next-generation aminoglycoside and was developed from sisomycin. Its structure possesses two substitutions at position 1 (hydroxyl-aminobutyric acid) and 6 (hydroxyethyl), which makes it resistant to inactivation by aminoglycoside-modifying enzymes. However, it is not active against those bacteria that have ribosomal methyltransferases as a mechanism of drug resistance. It acts by inhibiting protein synthesis and has activity against both gram-negative and gram-positive bacteria. Synergistic activity of this drug has been demonstrated against *P. aeruginosa* when combined with cefepime, imipenem, doripenem, and piperacillin tazobactam and against MRSA, VISA, and VRSA when combined with daptomycin or ceftobipirole.[63] This drug is currently in Phase 3 trial, which is aimed to demonstrate its superiority to colistin including a factor of mortality at 28 days for treating bloodstream infections or nosocomial pneumonia caused by CRE.

Newer Fluoroquinolones

The newer flouroquinolones under development for the treatment of infections caused by gram-negative bacteria include avarofloxacin and nemonoxacin.[64,65] The activity of nemonoxacin against gram-positive bacteria (including MRSA and MDR *Streptococcus pneumoniae*) is better than ciprofloxacin, levofloxacin, or moxifloxacin.[66] Other newer agents with intracellular activity against gram-negative bacteria include delafloxacin (Phase 3 trial) and finafloxacin (Phase 2 trial).[67,68] Another similar compound, DS-8587 (Phase 2 trial), has activity against both gram-negative (including *A. baumannii*) and gram-positive bacteria.[68] An oxoquinolizine compound, GC-072 (preclinical phase), possesses good efficacy against *A. baumannii* and *Burkholderia pseudomallei*.[69,70] A pyrrolopyrimidine inhibitor that acts on GyrB and ParE and other compounds have been developed, which have broad-spectrum activity against *P. aeruginosa, A. baumannii*, and *E. coli*, which have the advantage of lack of cross-resistance.[71,72]

Tetracyclines

Eravacycline. Eravacycline (Phase 3 trial) is a tetracycline compound with modified C-7 and C-9 positions, which has shown effective treatment of infections caused by MDR gram-negative, gram-positive, and anaerobic organisms.[73] The drug's activity is not hindered by efflux pumps and ribosomal protection mechanism of drug resistance.[74] It has been shown to be superior to tigecycline in activity against MDR *Acinetobacter* spp. and ESBL-producing *Enterobacteriaceae*.[73] This drug is also being evaluated for oral formulation for ease of intravenous to oral transition.

Omadacycline. Omadacycline belongs to 9-aminomethyl class of tetracyclines, which has entered into Phase 3 clinical trials for demonstration of its broad-spectrum activity against gram-positive, gram-negative, anaerobic, and atypical pathogens causing acute bacterial skin and skin structure infections, CA bacterial pneumonia, and UTI.[75,76] It is being evaluated in both oral and parenteral formulations. It is active against CA MRSA, β-hemolytic streptococci, penicillin-resistant *S. pneumoniae*, *Haemophilus influenzae*, and *Legionella*. Omadacycline works even against those bacteria that are resistant to other tetracyclines, methicillin, vancomycin, erythromycin, and ciprofloxacin.[75,76]

Cephalosporins

The extended-spectrum cephalosporins and monocyclic beta-lactams are active against gram-negative pathogens, including

P. aeruginosa. Newer combinations with beta-lactam inhibitors and monobactams are under evaluation with the aim of developing broad-spectrum novel antimicrobials. A compound BAL30072 (Phase 1 trial), which is a monosulfactam, is shown to be active against many gram-negative bacteria, including those producing MBLs and KPC, and possesses synergistic effect with carbapenems.[77] Landman et al have shown a ≥4-fold decrease in the BAL30072 MIC_{90} for both *A. baumannii* and *K. pneumoniae* when combined with meropenem.[78] It enters the bacteria through iron transport systems and porins. The drug consists of a siderophore moiety, which confers activity against *A. baumannii*. Other two candidates, namely, S-649266 and S200, are in early developmental stages possessing activity against gram-negative bacteria producing ESBLs and carbapenemases.[79] There are other cephalosporins being evaluated in combination with avibactam such as ceftaroline fosamil and aztreonam (Table 1). These combinations are considered as broad-spectrum antimicrobials covering bacteria producing beta-lactamases of class A and C.[80,81] Aztreonam has the advantage of activity against *Pseudomonas*, while ceftaroline minimally affects it.[82] Combination of imipenem and meropenem with new beta-lactamase inhibitors such as MK-7655 and RPX7009, respectively, are being evaluated for infections caused by gram-negative bacteria and *Pseudomonas*.[83] AIC499 is another compound in the preclinical stage which in combination with a beta-lactamase inhibitor has shown good activity against gram-negative pathogens, including MDR strains, *P. aeruginosa*, and *Acinetobacter*.[84]

Peptidomimetics

The molecules are similar to host defense proteins and act in a bactericidal manner in a similar way as healthy host immune response tackles bacteria, thereby minimizing the chances of development of drug resistance. Brilacidin and POL7080 are the two drugs in this category. POL7080 acts specifically on LPS of *P. aeruginosa*.[84]

Miscellaneous Newer Antimicrobial Techniques Against Gram-negative Bacteria

Bacteriophages. Bacteriophage (virus infecting bacteria) therapy is an evolving branch of medical management. Studies have shown a success rate ranging from 75% to 100% in suppurative bacterial infections caused by antibiotic-resistant *E. coli*, *Klebsiella* species, and *Pseudomonas* species.[85] Phages have the advantage of high specificity for their host without any notable adverse effects or probability of emergence of resistance.[86] Phages were historically in use in Europe for the treatment of bacterial infections such as osteomyelitis, skin/wound infections, UTI, and ear infection.[86] The research on the subject restarted when antimicrobial resistance issues were rising. Many human clinical trials have shown promising results of phage therapy as an alternative for treating bacterial infections.[87] However, ongoing and future research would be helpful in combating the menace of drug resistance. Even the combination of phages and antimicrobials (phage–antibiotic synergy) is an evolving approach demonstrating higher antibacterial effects in many studies.[87]

Bacteriocins. Bacteriocins are proteins secreted by bacteria in response to challenges of nutrient starvation or interbacterial competition. Colicins are one of these bacteriocins produced by *E. coli*. Colicin-like bacteriocins are also produced by other gram-negative bacteria (S-type pyocins by *P. aeruginosa*). The use of these bacteriocins has been explored in the treatment of chronic bacterial infections, especially those in whom biofilm formation is common, such as catheter-associated infection or lung infection in cystic fibrosis patients.[88] Trautner et al has shown that precoating of catheters with colicin-producing *E. coli* K12 prevents colonization of colicin-susceptible *E. coli* clinical strains.[89] Similarly, pyocins have been investigated for antibiofilm properties by Saeidi et al, where they formulated pyocin-producing strains of *E. coli* in response to the presence of *P. aeruginosa*.[90]

Utilization of Natural Products

The antimicrobial compounds derived from natural products have gained the interest of researchers, where products such as actinonin, pleuromutilin, ramoplanin, and tiacumicin B are undergoing analysis. Other compounds that are undergoing research include arylomycin, GE23077, mannopeptimycin, muraymycin, nocathiacin, and ECO-0501.[91]

Other Therapeutic Targets Undergoing Research

Quorum-sensing inhibitors. They have been found in many products such as chamomile, carrot, garlic, and algae. They may play a role in inhibiting cross-talk among bacteria, especially in biofilms.[92,93]

Lectin inhibitors. They inhibit the outer membrane proteins called lectins, which play a role in biofilm formation. They have shown a significant reduction in bacterial colony-forming units in cystic fibrosis patients.[94]

Endolysins. Though endolysins produced by bacteriophages are more active against gram-positive bacteria, their role in gram-negative bacteria may be achieved by combination with an agent facilitating the penetration of the outer membrane.[95]

Immunotherapy. This approach is still under development. It aims to play a synergistic role in combination with antibiotics by prior stimulation of the immune system.[96]

Insights into some Challenging Issues in the Management of Infections by Gram-negative Superbugs

Gram-negative bacteria have not only specific mechanisms such as production of ESBLs and carbapenemases but also nonspecific mechanisms such as porins and efflux pumps, which can augment the resistance caused by the specific mechanisms. *P. aeruginosa* can reduce the expression of outer

membrane porins to limit the entry of imipenem in the periplasmic space. Efflux pumps are protein transporters that can extrude antibiotics from inside of cells to the outer environment. The RND family of efflux pumps is the most common and clinically important for gram-negative bacteria. Interim standard consensus definitions for acquired resistance have been proposed by Magiorakos et al for MDR, XDR, and PDR bacteria.[1] An isolate is defined as MDR gram-negative bacteria if it is not susceptible to at least one agent in at least three antimicrobial categories, which are potentially active against the respective bacteria. An isolate is XDR, if it is nonsusceptible to at least one agent in all but two or less than two antimicrobial categories, which are potentially active against the respective bacteria. Finally, PDR is defined as nonsusceptibility to all agents in all antimicrobial categories potentially active for this isolate.[1] Although these definitions for MDR and XDR per say do not require resistance to carbapenems, the carbapenem-resistant phenotype is usually present in MDR and particularly for XDR isolates. XDR and PDR gram-negative bacteria are the major therapeutic challenges. Resistance to carbapenems in CRE, *P. aeruginosa*, and *A. baumannii* is invariably associated with resistance to several other classes of antibiotics, as carbapenemase-encoding genes are located on plasmids, transposons, and integrons.

Choices for ESBL-producing *K. pneumoniae* include carbapenems, which are the drugs of choice. However, if in vitro susceptibility to beta-lactam/beta-lactamase inhibitors and fluoroquinolones is present, these can be useful treatment options. The role of fourth-generation cephalosporins is controversial. For carbapenemase-producing *K. pneumoniae* (KPC), choices are limited and include colistin or polymyxin B and tigecycline. Usage of tigecycline is generally restricted to intra-abdominal infections. The combination of polymyxins with another antibiotic can overcome the potential for therapeutic failure due to the amplification or emergence of heteroresistant subpopulations, which is a shortcoming of polymyxin monotherapy.[97–100] Heteroresistance is the presence of resistant bacterial subpopulation at the initiation of therapy. Heteroresistance should be differentiated from tolerance/adaptive resistance, which refers to a reversible change of bacterial resistance in response to antibiotic therapy. Adaptive resistance has been described both for polymyxins and aminoglycosides in *P. aeruginosa* and can be minimized by longer dosing intervals (ie, 24 hours) for aminoglycosides.[101,102] However, in the case of polymyxins, it is not clear whether once daily dosing of polymyxins will minimize the emergence of resistance.

Most of the in vitro and animal studies on antibiotic combinations against CRE have been conducted on CPKP, and fewer studies are available for carbapenem-resistant *E. coli*. The most beneficial combinations include polymyxin plus a carbapenem demonstrated in in vitro and in murine infection models.[103–107] Colistin plus imipenem combination showed synergistic killing against colistin-susceptible, metallo-β-lactamase-producing *K. pneumoniae* isolates but was less promising against colistin-resistant *K. pneumoniae*.[107] A study using polymyxin B plus doripenem in CPKP and carbapenem-resistant *E. coli* showed >3.5 $\log_{10}$ killing at 24 hours for four of the five tested *E. coli* strains. However, triple drug combination of polymyxin B, doripenem, and rifampicin was required for *K. pneumoniae* strains to achieve at least 2.7 $\log_{10}$ killing at 24 hours.[108] In another study, fosfomycin combined with meropenem demonstrated synergistic killing in 65% of the 17 tested KPC-producing *K. pneumoniae* strains.[107] In vitro checkerboard data have suggested synergistic killing when colistin is combined with rifampicin against KPC-producing *K. pneumoniae*.[109] Overall, though polymyxin plus carbapenem combinations appear to be most promising based on the available preclinical data, many more studies are required for CRE to optimize the combination therapies.

Finding suitable antimicrobial treatment options for MDR *A. baumannii* and *P. aeruginosa* can be really daunting, and many times, physicians have to resort to the usage of combinations though the data for efficacy of their usage may be lacking. The cornerstone drugs in these combinations include polymyxins and tigecycline. The adjuvant drugs include carbapenems, tigecycline, fosfomycin, aminoglycosides, and rifampicin. Randomized control trials are not available for many of these combinations. However, observational studies and preclinical data in animal experiments suggest that combinations of antibiotics may be better than single-agent therapy against carbapenem-resistant gram-negative bacteria such as *P. aeruginosa, A. baumannii*, and other *Enterobacteriaceae*. The choice of the antibiotic combination to be used will vary according to the organism, its susceptibility profile, the underlying illness in the patient, and the site of the infection. The main objective of combination therapy is to optimize the use of cornerstone drug. The adjuvant will be chosen on the basis of its activity/probable activity, favorable PK/PD profile, and the toxicity profile. However, it is possible to have an adjuvant compound that is completely inactive in monotherapy and still highly beneficial in a rationally designed combination regimen. Figure 1 shows the advantages of using combination therapy. In vitro and animal infection models against carbapenem-resistant *A. baumannii* have demonstrated a good level of synergy of a combination of polymyxin with rifampicin or a carbapenem.[108] In the dynamic hollow fiber infection model and the murine thigh infection model, the colistin plus rifampicin combination not only provided substantial killing but also minimized the emergence of resistance.[103,110,111] Similarly, other studies have shown suggested synergistic killing for carbapenem plus sulbactam combinations and for rifampicin in combination with imipenem or sulbactam.[110] A few studies are also available for other combinations such as colistin plus tigecycline or minocycline.[110] Overall, polymyxin plus rifampicin or a carbapenem/two or three drug combinations containing a carbapenem, rifampicin, or sulbactam are promising.

Most studies against the current *P. aeruginosa* isolates with different resistance phenotypes have used polymyxin-based

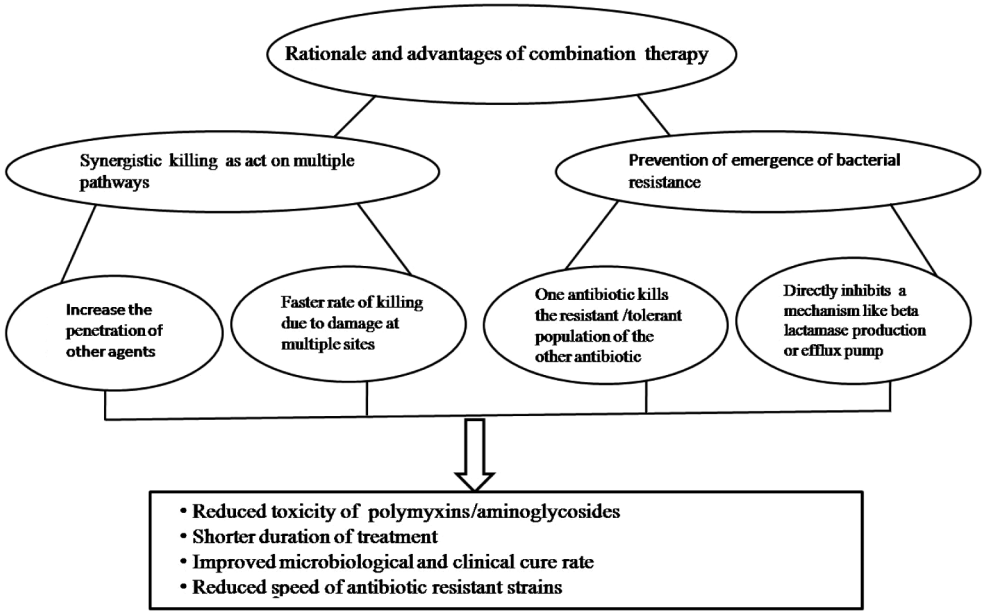


Figure 1. Rationale and advantages of combination therapy.

combinations. In in vitro dynamic flow models and murine infection models, the combination of colistin and carbapenems (doripenem and imipenem) was found to cause the most extensive and synergistic killing in these bacteria.[103,112] This synergy was demonstrated at the clinically relevant concentrations of polymyxin and carbapenem. There is a strong evidence of colistin plus doripenem combination in preventing the emergence of resistance and obtaining substantial killing of a very high inoculum of a colistin-resistant organism.[110] The combination of three drugs, ie, polymyxin B, doripenem, and rifampicin, showed bactericidal activity against all five carbapenem-resistant *P. aeruginosa* strains in the static time–kill studies.[108] Similarly, polymyxin B in combination with supraphysiological concentrations of meropenem or amikacin showed synergy against XDR *P. aeruginosa*.[113] Combination of meropenem with tobramycin or levofloxacin was also shown to achieve fast and substantial killing and minimized resistance against *P. aeruginosa* strain, which overexpressed MexAB-OprM efflux pump. This efflux pump is clinically the most important pump as it extrudes almost all β-lactam antibiotics except imipenem.[110]

Inhalation Therapy

Inhalation has been used as an adjuvant to systemic therapy in VAP caused by carbapenem-resistant gram-negative bacteria. Colistin methanesulfonate sodium is the main agent used. The rationale of the therapy is to reach higher drug concentrations at the site of the infection to reduce systemic toxicity. Though many case series have reported good response rates, most studies lacked a control group.[114–116] In two randomized control trials, no benefit in mortality was observed.[117–119] However, most studies found higher rates of microbiological eradication with adjunctive aerosolized colistin as compared with parenteral colistin alone. Therefore, inhalation therapy cannot substitute an adjuvant parenteral drug in combination and thus cannot be routinely recommended in the treatment of VAP caused by carbapenem-resistant gram-negative bacteria. However, it might be considered in patients who do not tolerate systemic polymyxins. Indeed, it might have implications in the control of dissemination of these organisms, but it still requires further investigations.

Conclusions

The global emergence of carbapenem-resistant gram-negative bacteria threatens an end to the antibiotic era and to all the advances in surgery, transplantation, and chemotherapy. In addition to the urgent need for newer antibiotics, we also need to protect the efficacy of the current last resort ones. Ingenious and judicious approaches are required to tackle these MDR, XDR, and PDR organisms. Some of these approaches include antibiotic stewardship, strict adherence to infection control practices, early and appropriate empiric therapy, and rational use of combinations. A better understanding of the pharmacokinetic/pharmacodynamic properties of the various combination drugs is also required to achieve better therapeutic outcomes.

Author Contributions

Conceived and designed the experiments: NT. Analyzed the data: NT. Wrote the first draft of the manuscript: NT and HK. Contributed to the writing of the manuscript: NT and HK. Agreed with manuscript results and conclusions: NT and HK. Jointly developed the structure and arguments

for the paper: NT and HK. Made critical revisions and approved the final version: NT. All the authors reviewed and approved the final manuscript.

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