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Priorities and Progress in Gram-negative Bacterial Infection Research by the Antibacterial Resistance Leadership Group

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Addressing the treatment and prevention of antibacterial-resistant gram-negative bacterial infections is a priority area of the Antibacterial Resistance Leadership Group (ARLG). The ARLG has conducted a series of observational studies to define the clinical and molecular global epidemiology of carbapenem-resistant and ceftriaxone-resistant Enterobacterales, carbapenem-resistant *Pseudomonas aeruginosa*, and carbapenem-resistant *Acinetobacter baumannii*, with the goal of optimizing the design and execution of interventional studies. One ongoing ARLG study aims to better understand the impact of fluoroquinolone-resistant gram-negative gut bacteria in neutropenic patients, which threatens to undermine the effectiveness of fluoroquinolone prophylaxis in these vulnerable patients. The ARLG has conducted pharmacokinetic studies to inform the optimal dosing of antibiotics that are important in the treatment of drug-resistant gram-negative bacteria, including oral fosfomycin, intravenous minocycline, and a combination of intravenous ceftazidime-avibactam and aztreonam. In addition, randomized clinical trials have assessed the safety and efficacy of step-down oral fosfomycin for complicated urinary tract infections and single-dose intravenous phage therapy for adult patients with cystic fibrosis who are chronically colonized with *P. aeruginosa* in their respiratory tract. Thus, the focus of investigation in the ARLG has evolved from improving understanding of drug-resistant gram-negative bacterial infections to positively affecting clinical care for affected patients through a combination of interventional pharmacokinetic and clinical studies, a focus that will be maintained moving forward.

Keywords. gram-negative; antimicrobial resistance; observational studies; interventional studies; clinical trials.

Antibacterial resistance (AR) in gram-negative bacteria is an increasingly challenging public health issue. Globally, gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*) account for 4 of the top 6 pathogens in terms of estimated global deaths associated with AR [1]. In the United States, >50 000 hospitalized patients are infected annually by carbapenem-resistant *P. aeruginosa* (CRPA), carbapenem-resistant Enterobacterales (CRE), or carbapenem-resistant *A. baumannii* (CRAB), about

8% of whom die from the infection [2]. Extended-spectrum β-lactamase (ESBL)–producing Enterobacterales are now major pathogens in healthcare and community settings, causing approximately 200 000 infections and 9000 deaths annually in the United States.

Worryingly, the incidence of these AR gram-negative infections rose significantly during the coronavirus disease 2019 (COVID-19) pandemic owing to increased antimicrobial use and challenges in following infection prevention procedures [3]. Despite the scope of the problem, innovation in therapy has lagged, with no new class of antimicrobial agents with gram-negative activity having been approved in the last 50 years. In addition, significant limitations exist in our knowledge of the global epidemiology of carbapenem-resistant gram-negatives and how to optimize existing agents to improve the outcomes of infected patients and minimize the emergence of AR. Since its inception, the Antibacterial Resistance Leadership Group (ARLG) has identified, designed, and implemented clinical

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studies that inform the epidemiology, treatment, and prevention of drug-resistant gram-negative bacterial infections and improve outcomes in affected patients (Figure 1). In this article, we review the accomplishments of the ARLG in the area of gram-negative bacterial infections according to each scientific priority (see Table 1 for summaries of studies).

RESEARCH PRIORITY 1: DEFINE THE GLOBAL EPIDEMIOLOGY OF MULTIDRUG-RESISTANT GRAM-NEGATIVE BACTERIAL INFECTIONS TO OPTIMIZE THE DESIGN AND EXECUTION OF INTERVENTIONAL STUDIES

Multidrug-Resistant Organism Network Studies

CRACKLE was initially launched as a multicenter, observational study at hospitals in the Midwest United States to better understand the emerging public health problem of CRE [19]. Under ARLG leadership, CRACKLE evolved into a global network, the Multi-Drug Resistant Organism (MDRO) Network to comprehensively study clinically important gram-negative pathogens and infections from 5 regions (the United States, South/Central America, China, the Middle East, and Australia/Singapore). Four prospective, observational studies of hospitalized patients have been completed to address specific pathogens: CRACKLE-2 for CRE, POP for CRPA, Study Network of *Acinetobacter* as a Carbapenem-Resistant Pathogen (SNAP) for CRAB, and SHREC for ceftriaxone-resistant *E. coli* (see Table 1 for full names of all studies mentioned in the text). MDRO Network studies combine detailed clinical data with in-depth phenotypic and genotypic characterization of isolates. In addition, these studies assess the feasibility of, and build infrastructure for, future interventional trials that will address these key antimicrobial-resistant gram-negative infections. Table 2 summarizes the key features and findings of each study.

CRE (CRACKLE-2)

Patients were enrolled into CRACKLE-2 based on antimicrobial susceptibility testing at participating hospitals using the Centers for Disease Control and Prevention's CRE definition [20]. Carbapenemase genes were present in 59% of CRE isolates from 1040 patients at US hospitals, with 92% of carbapenemase-producing (CP) CRE isolates harboring a *K. pneumoniae* carbapenemase gene [4]. Some isolates without a carbapenemase gene were susceptible to all carbapenems when tested in a central laboratory. However, clinical outcomes did not differ significantly among patients infected with CP-CRE, non-CP-CRE, or isolates that tested carbapenem susceptible in the central laboratory. In an international cohort of 991 patients with carbapenem-resistant *K. pneumoniae*, 90% of carbapenem-resistant *K. pneumoniae* isolates harbored a carbapenemase gene, bla_{KPC} in 91% [5]. Important differences in patient and bacterial characteristics and clinical outcomes between global regions were identified. A bacterial genetic marker that encodes a bacterial lipopolysaccharide, O2 variant 2, was associated with lower mortality rate, highlighting the need for additional research on bacterial biomarker–based risk stratification.

In addition, CRACKLE-2 enrolled 114 patients with CP *E. coli*, of whom 43% had metallo-β-lactamases (MBLs) [6]. Patients infected with MBL-producing *E. coli* had a higher probability of a favorable desirability of outcome ranking (DOOR) and lower 30-day mortality rate than patients infected with *E. coli* that harbored serine carbapenemases.

CRPA (POP)

In the first analysis of 972 patients with CRPA enrolled in POP, the 30-day mortality rate was 18% among infected patients [8]. This rate was highest with bacteremia (30%), but substantial differences in mortality rates were observed among geographic regions. In addition, the prevalence of carbapenemases varied by geographic region: carbapenemase genes were present in 2% of US isolates but in 30%, 32%, 57%, and 69% of isolates from the Middle East, China, Australia/Singapore, and South/Central America, respectively. *K. pneumoniae* carbapenemase 2 and Verona integron-encoded metallo-β-lactamase 2 were the most common carbapenemases. Compared with CRPA isolates without a carbapenemase, CP-CRPA isolates were more likely to exhibit high-level meropenem resistance and less likely to be susceptible to other antipseudomonal agents. Infections caused by CP-CRPA were associated with increased mortality rates compared with those caused by CRPA without a carbapenemase. These findings highlight the global geographic variability in CRPA, with implications for the optimal diagnosis and treatment of CRPA infections in each region and for the design of interventional trials of therapeutics against CRPA.

CRAB (SNAP)

CRAB has emerged as a critical priority antibiotic-resistant pathogen [2]. SNAP enrolled 990 patients with CRAB, and clinical and genome sequencing data are available for 842 of them. Marked geographic differences in clinical presentations were evident. The 30-day mortality rate was 24% for infected patients, and bloodstream infection and higher age-adjusted Charlson comorbidity index were independently associated with the 30-day mortality rate. Clonal group 2 strains predominated in all regions except South/Central America, representing 59%–97% of strains in other regions. These findings highlight differences in CRAB infection types and clinical outcomes across regions and will inform the design and execution of future interventional studies of this increasingly important pathogen.

Ceftriaxone-Resistant *E. coli* (SHREC)

The incidence of ceftriaxone-resistant *E. coli* has increased owing to increasing numbers of isolates that produce ESBLs [2].
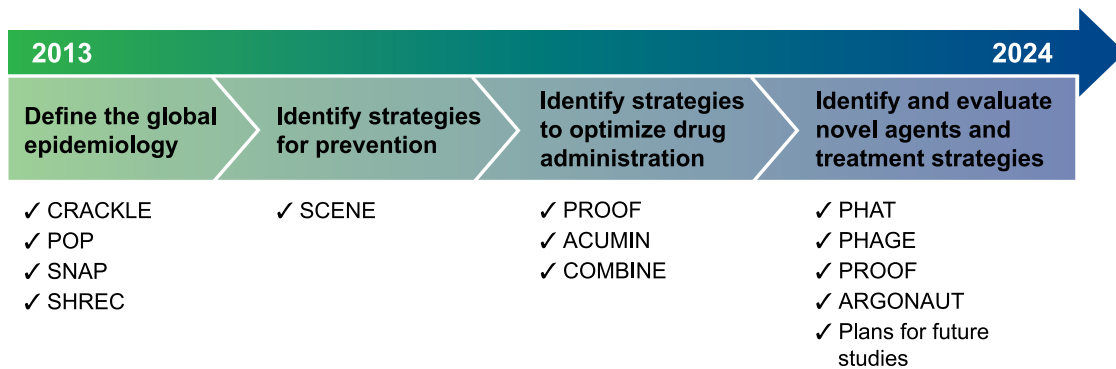
Figure 1. Antibacterial Resistance Leadership Group (ARLG) clinical studies of drug-resistant gram-negative bacterial infections. Abbreviations: ACUMIN, Acute Care Unit Minocycline; ARGONAUT, ARLG Reference Group for the Testing of Novel Therapeutics; COMBINE, Ceftazidime-Avibactam in Combination With Aztreonam; CRACKLE, Consortium on Resistance Against Carbapenems in *Klebsiella* and Other Enterobacterales; PHAGE, Study of the Safety and Microbiologic Activity of Bacteriophages; PHAT, Phages That Target MDR Bacteria; POP, Prospective Observational *Pseudomonas* Study; PROOF, Pharmacokinetics, Pharmacodynamics and Safety/Tolerability of Two Dosing Regimens of Oral Fosfomycin Tromethamine in Healthy Adult Participants; SCENE, Screening for Colonization with Resistant Enterobacterales in Neutropenic Patients with Hematologic Malignancies; SHREC, Study of Highly Resistant *Escherichia coli*; SNAP, Study Network of *Acinetobacter* as a Carbapenem-Resistant Pathogen.

SHREC compared the clinical outcomes of 300 patients with bloodstream infections due to ceftriaxone-resistant (n = 150) and ceftriaxone-susceptible *E. coli* (n = 150) [10]. Patients infected with ceftriaxone-resistant *E. coli* were more likely than those infected with ceftriaxone-susceptible *E. coli* to have a high acuity of illness and longer delays until receipt of active antibiotic therapy (12 vs 1 hour, respectively). Unadjusted DOOR analyses indicated a significantly higher probability of a worse clinical outcome in the ceftriaxone-resistant group. In addition, these patients had a longer median postinfection length of stay (8 vs 6 days, respectively) and were more likely to be admitted to a long-term care facility (22% vs 12%). However, no differences were observed in the DOOR probability or 30-day mortality rate between groups after adjustments for confounding. Thus, patients with ceftriaxone-resistant *E. coli* bloodstream infection generally had worse clinical outcomes than those with ceftriaxone-susceptible *E. coli* bloodstream infection, with differences driven primarily by host factors.

RESEARCH PRIORITY 2: EVALUATE NOVEL STRATEGIES TO PREVENT MULTIDRUG-RESISTANT GRAM-NEGATIVE INFECTIONS IN IMMUNOCOMPROMISED HOSTS

Screening for Colonization With Fluoroquinolone-Resistant Enterobacterales in Neutropenic Patients (SCENE)

Patients with hematologic cancers and hematopoietic cell transplant (HCT) recipients who receive chemotherapy frequently experience severe neutropenia and gastrointestinal mucositis, placing them at high risk for life-threatening bacteremia from Enterobacterales [21]. Fluoroquinolone (FQ) prophylaxis is recommended to prevent bacterial infections in patients expected to have prolonged neutropenia [22].

However, FQ resistance has become increasingly common, and many FQ-resistant Enterobacterales (FQRE) that cause breakthrough infection also produce ESBLs that confer resistance to therapies for fever and neutropenia [23].

SCENE is an observational study to determine whether screening for FQRE colonization before neutropenia can identify patients who are at high risk of gram-negative bacteremia despite FQ prophylaxis. A single-center study found that 31% of HCT recipients colonized with FQRE before transplantation developed gram-negative bacteremia during posttransplant neutropenia, compared with only 1% of noncolonized patients [24]. SCENE expands on these findings by enrolling 820 patients who have acute leukemia or are undergoing HCT at 10 oncology centers. Participants receive FQ prophylaxis per the standard of care, are screened for colonization with FQRE before neutropenia, and are followed up for episodes of bacteremia. The incidence of gram-negative bacteremia during neutropenia will be compared between FQRE-colonized and noncolonized patients, and bloodstream isolates will be compared with colonizing strains to determine whether participants develop bacteremia from their colonizing strain.

RESEARCH PRIORITY 3: IDENTIFY STRATEGIES TO OPTIMIZE THE ADMINISTRATION OF ANTIBIOTICS FOR THE TREATMENT OF GRAM-NEGATIVE INFECTIONS

Pharmacokinetics, Pharmacodynamics, and Safety/Tolerability of Oral Fosfomycin (PROOF)

E. coli is the most common urinary pathogen and has increasingly become resistant to oral therapies, such as FQs, trimethoprim-sulfamethoxazole, and oral β-lactam agents. Thus, there is a critical need for alternative oral antibiotics to treat complicated urinary tract infections (cUTIs) [2].

Table 1. Research Priorities for the Gram-negative Committee in the Antibacterial Resistance Leadership Group

Research Priority/Study Title	Study Description	Status
1. Define the global epidemiology of multidrug-resistant gram-negative bacterial infections to optimize the design and execution of interventional studies		
Multi-Drug Resistant Organism (MDRO) Network	Observational cohort studies of hospitalized patients with resistant gram-negative bacteria	…
CRACKLE-2 (Consortium on Resistance Against Carbapenems in *Klebsiella* and Other *Enterobacterales*)	International cohort of patients with carbapenem-resistant Enterobacterales	Analysis partially completed [4–7]
POP (Prospective Observational *Pseudomonas* Study)	International cohort of patients with carbapenem-resistant *Pseudomonas aeruginosa*	Analysis partially completed [8]
SNAP (Study Network of *Acinetobacter* as a Carbapenem-Resistant Pathogen)	International cohort of patients with carbapenem-resistant *Acinetobacter baumannii*	Analysis partially completed [9]
SHREC (Study of Highly Resistant *Escherichia coli*)	Domestic cohort of patients with ceftriaxone-resistant and ceftriaxone-susceptible *E. coli* bloodstream infections	Analysis partially completed [10]
2. Evaluate novel strategies to prevent multidrug-resistant gram-negative bacterial infections in immunocompromised hosts		
SCENE (Screening for Colonization with Resistant Enterobacterales in Neutropenic Patients with Hematologic Malignancies)	Multicenter cohort study to assess the prevalence and clinical impact of colonization with fluoroquinolone-resistant Enterobacterales in neutropenic patients with hematologic cancers	Ongoing
3. Identify strategies to optimize the administration of antibiotics for the treatment of gram-negative bacterial infections		
PROOF (Pharmacokinetics, Pharmacodynamics and Safety/Tolerability of Two Dosing Regimens of Oral Fosfomycin Tromethamine in Healthy Adult Participants)	Phase 1 clinical trial to evaluate the pharmacokinetics, pharmacodynamics, and safety/tolerability of different dosing regimens of oral fosfomycin	Completed [11, 12]
FOCUS (The Fosfomycin Oral for Complicated Urinary Syndromes Study)	Randomized clinical trial to assess the safety and efficacy of fosfomycin versus levofloxacin oral step-down therapy for complicated urinary tract infections	Data analysis underway
ACUMIN (Acute Care Unit Minocycline)	Phase 4 clinical trial to evaluate the pharmacokinetics of intravenous minocycline in critically ill patients	Completed [13]
COMBINE (Ceftazidime-Avibactam in Combination with Aztreonam)	Phase 1 clinical trial to evaluate the safety and pharmacokinetics of ceftazidime-avibactam in combination with aztreonam	Completed [14, 15]
4. Identify and evaluate novel antimicrobial agents or treatment strategies for multidrug-resistant gram-negative bacterial infections		
ARGONAUT III, IV, and V (ARLG Reference Group for the Testing of Novel Therapeutics)	Characterization of the in vitro activity of novel agents against genetically defined clinical isolates of carbapenem-resistant gram-negative bacteria	Analysis partially completed
PHAT (Phages That Target MDR Bacteria)	Isolation and characterization of lytic bacteriophages that target *P. aeruginosa* and *Enterobacter* species	Completed [16, 17]
PHAGE (Study of the Safety and Microbiologic Activity of Bacteriophages)	Phase 1b/2 randomized, placebo-controlled trial of the safety and microbiologic activity of a single intravenous dose of bacteriophage therapy in patients with cystic fibrosis who are colonized with *P. aeruginosa*	Ongoing [18]

Abbreviations: ARLG, Antibacterial Resistance Leadership Group; MDR, multidrug-resistant.

Fosfomycin tromethamine is an old oral agent that remains active against most *E. coli* strains, including ESBL-producing *E. coli*. It is approved in the United States as a single-dose treatment of uncomplicated urinary tract infection, but the pharmacokinetic-pharmacodynamic (PK/PD) profiles and safety of multiple-dose regimens for cUTI are unknown. The PROOF study randomized 18 healthy adult participants to receive oral fosfomycin 3 g every other day for 3 doses or 3 g daily for 7 days, followed by a crossover period when participants received the opposite regimen [11]. Systemic plasma PK parameters on days 1 and 5 and cumulative urinary excretion of fosfomycin were similar after every-other-day or daily dosing. The most common fosfomycin-related treatment-emergent adverse events were gastrointestinal. Daily dosing of fosfomycin was associated with significantly more diarrhea than the every-other-day regimen (diarrhea-free days, 61% [daily] vs 77% [every other day]; $P < .001$).

In addition, PROOF evaluated the ex vivo urinary bactericidal activity and PD profiles of fosfomycin against strains of *E. coli*, *K. pneumoniae*, and *Proteus mirabilis* [12]. Urinary antibacterial activity of fosfomycin was similar for the daily and every-other-day dosing regimens. Fosfomycin had reliable urinary bactericidal activity against *E. coli*, but not against the other organisms.

Fosfomycin for Complicated Urinary Tract Infection (FOCUS)

FOCUS was a multicenter, randomized, open-label pragmatic superiority trial that evaluated the efficacy of oral fosfomycin versus oral levofloxacin in cUTIs, including pyelonephritis. The trial compared 2 strategies for initial or step-down oral therapy for cUTI without bacteremia after 0–48 hours of parenteral antibiotic therapy. Participants received 3 g of fosfomycin or 750 mg (or dose adjusted for kidney function) of levofloxacin daily for 5–7 days. Clinical and microbiological cures were assessed at the end of therapy and test of cure (approximately 21 days from the start of antibiotics). The study used a unique pragmatic design, Comparing Personalized Antibiotic Strategies (COMPASS), valuable in the AR setting

Table 2. Summary of Key Features and Findings of the Multi-Drug Resistant Organism (MDRO) Network Studies

Study	Isolates	Setting and Years	No. of Patients	Carbapenemase Prevalence	30-d Mortality Rate in Infected Patients	Summary of Key Findings
CRACKLE-2 [4–6]	CRE (all body sites)	49 US hospitals; 2016–2017	1040	59% (92% of CP-CRE had KPC)	Overall: 24% (107/449)	Clinical outcomes not significantly different between CP-CRE, non-CP-CRE and isolates not confirmed to be carbapenem-resistant; CG 258 *K. pneumoniae* is the most common CP-CRE, but CG 307 *K. pneumoniae* is emerging
	CRKP (all body sites)	71 Hospitals in South America, Australia, China, Lebanon, Singapore, and the US; 2017–2018	991	90% (91% of CP-CRE had KPC); Australia, Lebanon, and Singapore: 75%; China: 98%; South America: 75%; US: 88%	Overall: 19% (93/502); BSI: 34% (44/130)	The 30-d mortality rate is lower in China (12%) than in South America (28%) or the US (23%), but adjusted DOOR outcomes are similar; O2 variant 2 O genetic locus is associated with survival
	CP *Escherichia coli* (all body sites)	26 Hospitals in Australia, China, Colombia, Lebanon, Singapore, and the US; 2017–2018	114	57% Serine carbapenemase, 43% MBLs (96% of MBLs had NDM)	Overall: 16% (18/114)	MBL-producing *E. coli* is most common in China; 30-d mortality rate is lower with MBL-producing *E. coli* (6%) vs other CP *E. coli* (23%)
POP [8]	CRPA (bloodstream, respiratory, urine, and wound isolates)	44 Hospitals in South/ Central America, Australia, China, Lebanon, Saudi Arabia, Singapore, and the US; 2018–2019	972	22% (49% of CP-CRPA had KPC-2% and 36% had VIM-2); Australia and Singapore: 57%; China: 32%; Middle East: 30%; South/Central America: 69%; US: 2%	Overall: 18% (105/581); BSI: 30% (21/69); pneumonia: 19% (69/358)	CP-CRPA is rare in the US (2% of isolates) but common elsewhere (30%–69% of isolates); CP-CRPA infections: increased 30-d mortality rate compared with non-CP-CRPA infections (22% vs 12%)
SNAP	CRAB (all sites)	46 Hospitals in South/ Central America, Australia, China, Lebanon, Saudi Arabia, Singapore, and the US; 2017–2019	842	91% (88% of CP-CRAB had OXA-23); Australia and Singapore: 100%; China: 99%; Middle East: 100%; South/Central America: 92%; US: 83%	Overall: 24% (128/536); BSI: 42%	Infection types and mortality rates varied substantially across regions; CG2 was the most common CRAB lineage except in South/Central America
SHREC [10]	*E. coli* (bloodstream isolates)	14 US hospitals; 2020–2021	300	NA (150 CRO-resistant, 150 CRO-susceptible)	CRO-resistant: 13% (20/150); CRO-susceptible: 8% (12/150)	Patients with CRO-resistant *E. coli* BSI had increased mortality rates and worse outcomes in unadjusted but not adjusted analyses

Abbreviations: BSI, bloodstream infection; CG, clonal group; CP, carbapenemase-producing; CRAB, carbapenem-resistant *Acinetobacter baumannii*; CRACKLE-2, Consortium on Resistance Against Carbapenems in *Klebsiella* and Other *Enterobacterales*; CRE, carbapenem-resistant Enterobacterales; CRKP, carbapenem-resistant *Klebsiella pneumoniae*; CRO, ceftriaxone; CRPA, carbapenem-resistant *Pseudomonas aeruginosa*; DOOR, desirability of outcome ranking; KPC, *Klebsiella pneumoniae* carbapenemase; MBL, metallo-β-lactamase; NA, not applicable; NDM, New Delhi metallo-β-lactamase; POP, Prospective Observational *Pseudomonas* Study; SHREC, Study of Highly Resistant *Escherichia coli*; SNAP, Study Network of *Acinetobacter* as a Carbapenem-Resistant Pathogen; VIM-2, Verona integron-encoded metallo-β-lactamase 2.

because the management of patients with cUTI is dynamic. Treatment was tailored based on AR patterns and participants' ability to tolerate the prescribed antibiotic. This approach was different from conventional cUTI trials that compare drugs rather than treatment strategies. Analysis of the trial data is ongoing.

Minocycline Pharmacokinetics in Critically Ill Patients (ACUMIN)
ACUMIN was a phase IV PK study that evaluated intravenous minocycline concentrations in critically ill patients [13]. Approved for intravenous use by the US Food and Drug Administration (FDA) in 2015, minocycline is increasingly used to treat infections caused by multidrug-resistant (MDR) *A. baumannii* [25]. Before this study, little was known about

the PK profile of intravenous minocycline, because prior PK data were only from uninfected participants who were not critically ill [26]. Furthermore, it was unclear whether intravenous minocycline required dosage adjustments for renal impairment or whether FDA-approved dosages in critically ill patients were sufficient to reliably achieve PK/PD exposure targets associated with the killing of *A. baumannii* [27, 28].

ACUMIN found no relationship between minocycline clearance and creatinine clearance, indicating that the dosage should not be adjusted for renal impairment. In addition, the study found that dose adjustments are not required for weight, sex, or albumin concentrations. The PK/PD target attainment analyses suggested that even the highest FDA-approved intravenous minocycline dosage (200 mg every 12 hours) confers a

suboptimal probability of target attainment in plasma for a substantial proportion of critically ill patients with *A. baumannii* infections. These analyses suggest that combination antibiotic therapy should be considered in patients with *Acinetobacter* infections when intravenous minocycline is used and that the highest FDA-approved dosage of 200 mg every 12 hours should be used to improve the probability of target attainment. These data cast uncertainty on the appropriateness of the current FDA and the Clinical and Laboratory Standards Institute minocycline susceptibility breakpoint of ≤4 µg/mL.

Ceftazidime-Avibactam in Combination With Aztreonam (COMBINE)

Treatment of infections caused by MBL-producing gram-negative bacteria is challenging because MBLs hydrolyze all β-lactams except aztreonam and are not inhibited by available β-lactamase inhibitors [29]. Although MBLs do not directly hydrolyze aztreonam, these bacteria are frequently resistant to aztreonam because they produce other β-lactamases that hydrolyze aztreonam. Since avibactam inhibits most β-lactamases except MBLs, the combination of ceftazidime-avibactam and aztreonam effectively kills MBL-producing bacteria in vitro [30]. COMBINE was a phase 1 clinical trial to assess the safety and PK of this combination in healthy volunteers. Participants received ceftazidime-avibactam or aztreonam by continuous or intermittent infusion or ceftazidime-avibactam combined with aztreonam by intermittent infusion for 7 days [14].

Overall, 19 (40%) of 48 participants had alanine aminotransferase/aspartate aminotransferase (ALT/AST) elevations, and 2 participants who received 8 g daily aztreonam by continuous infusion experienced severe ALT/AST elevations. All participants with ALT/AST elevations were asymptomatic, with no other findings suggestive of liver injury, and the addition of ceftazidime-avibactam to aztreonam did not increase the risk of ALT/AST elevation. In the population PK analyses, administration of the combination reduced aztreonam clearance by 16% but had a negligible effect on ceftazidime clearance [15]. These results suggest that the combination of ceftazidime-avibactam and aztreonam is safe when administered as 2-hour intermittent infusions, but close monitoring of liver function is prudent when using high-dose aztreonam by continuous infusion.

RESEARCH PRIORITY 4: IDENTIFY AND EVALUATE NOVEL ANTIMICROBIAL AGENTS OR TREATMENT STRATEGIES FOR MDR GRAM-NEGATIVE BACTERIAL INFECTIONS

In Vitro Activity of New Agents vs Carbapenem-Resistant Gram-negatives (ARGONAUT)

The development of novel treatments for carbapenem-resistant gram-negative pathogens remains a critical priority, and a comprehensive understanding of the spectrum of activity of new antimicrobial agents is essential. To address this need, ARGONAUT studies examined the in vitro activity of novel therapeutics against genetically characterized carbapenem-resistant gram-negative isolates. ARGONAUT-III, IV, and V examined the susceptibility of *K. pneumoniae* and *P. aeruginosa* isolates to cefepime-taniborbactam and ceftibuten–VNRX-5236. Taniborbactam is a novel boronic acid transition state inhibitor that inhibits both serine carbapenemases and MBLs [31]. Partnered with cefepime, this combination had positive results in a phase 3 clinical trial for cUTIs. VNRX-5236 is another boronic acid transition state inhibitor that inhibits ESBLs and serine carbapenemases and is being developed as an orally bioavailable prodrug, VNRX-7145 [32]. The combination of VNRX-7145 with ceftibuten (an oral cephalosporin) is currently in phase 1 clinical trials.

ARGONAUT-III and IV evaluated the in vitro activity of cefepime-taniborbactam and cefibuten–VNRX-5236, respectively, against 200 CP *K. pneumoniae* isolates from CRACKLE. The addition of taniborbactam increased cefepime susceptibility (minimum inhibitory concentration, ≤8 µg/mL) from 13.5% to 99.0%, a susceptibility percentage higher than that of meropenem-vaborbactam (95.5%) or ceftazidime-avibactam (98.0%) [33]. Using a provisional ceftibuten susceptibility breakpoint of ≤1 µg/mL, the addition of VNRX-5236 increased ceftibuten susceptibility from 4.5% to 92.5%, highlighting the potential for the combination to be an effective oral agent for carbapenem-resistant *K. pneumoniae* infections.

ARGONAUT-V evaluated the in vitro activity of cefepime-taniborbactam against 197 *P. aeruginosa* isolates previously characterized [34]. Taniborbactam increased cefepime susceptibility (minimum inhibitory concentration, ≤8 µg/mL) from 67.0% to 82.7%, edging out ceftazidime-avibactam (79.7%) and ceftolozane-tazobactam (77.7%).

Isolation and Characterization of Bacteriophages Against MDR Gram-negatives (PHAT)

Phages (viruses of bacteria) were historically used to treat bacterial infections before the availability of antibiotics and have gained renewed interest as potential therapies for AR infections [35]. Phages are attractive alternatives to broad-spectrum antibacterials because they are specific for target pathogens, leaving other members of the microbiome intact, and their effect is self-limited because they replicate only in the presence of a susceptible bacterial host. In the PHAT study, phages that target *P. aeruginosa* and *Enterobacter* spp. were isolated, characterized, and assembled into species-specific libraries [16, 17]. These libraries were then made available for screening against clinical isolates, and several of these phages were used to treat patients with antibiotic-resistant infections under expanded access and compassionate use protocols.

Phage Therapy for Patients With Cystic Fibrosis Colonized With *Pseudomonas Aeruginosa* (PHAGE)

Patients with cystic fibrosis (CF) are prone to lower respiratory tract infections caused by MDR gram-negative pathogens, such

as *P. aeruginosa*. These infections are increasingly challenging to treat because resident bacteria become increasingly resistant and are protected in biofilms, further compromising antibiotic effectiveness [36]. Thus, novel treatment approaches are needed for MDR organisms in persons with CF. Bacteriophages are particularly attractive therapies for gram-negative bacteria in patients with CF because they penetrate biofilms, avoid host tissue damage, and are synergistic with antibiotics [37].

PHAGE is a phase 1b/2 multicenter, randomized, placebo-controlled, clinical trial investigating the safety and efficacy of a single dose of intravenous phage therapy in adult volunteers with CF who have chronic respiratory colonization with *P. aeruginosa* [18]. The intravenous phage consists of a mixture of 4 antipseudomonal phages selected based on their broad range of activity against *P. aeruginosa* isolates. The primary study outcomes include the safety and microbiological activity of intravenous phage therapy. This trial will provide important insights into the safety and efficacy of phage therapy and establish a foundation for future larger, multidose phage trials.

ARLG FUTURE DIRECTIONS AND UNMET NEEDS IN GRAM-NEGATIVE BACTERIAL INFECTIONS

The overarching priority of the ARLG is to improve the diagnosis and treatment of infections caused by antimicrobial-resistant bacteria. For gram-negatives, our effort has focused mainly on understanding and improving therapy for ESBL-producing and carbapenem-resistant bacteria. The ARLG has designed and implemented natural history, PK, and interventional studies to address relevant knowledge gaps for the treatment of these organisms. Future directions will be guided by a survey conducted by the ARLG in collaboration with the Infectious Diseases Society of America, in which the top outstanding scientific questions endorsed by the infectious diseases community were "Are there safe and effective carbapenem-sparing regimens for ESBL-positive bloodstream infections?" and "What is the role of combination therapy versus monotherapy for the treatment of drug-resistant gram-negatives?" The ARLG will continue to work on identifying study opportunities that are feasible and will help answer these important study questions to improve care for patients affected by these pathogens.

Notes

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