AMERICAN SOCIETY FOR MICROBIOLOGY | mBio®

OBSERVATION
Clinical Science and Epidemiology

Colistin Heteroresistance Is Largely Undetected among Carbapenem-Resistant *Enterobacterales* in the United States

Victor I. Band,[a,b] Sarah W. Satola,[c,d,e] Richard D. Smith,[f] David A. Hufnagel,[b,d] Chris Bower,[e] Andrew B. Conley,[g] Lavanya Rishishwar,[g] Suzanne E. Dale,[h] Dwight J. Hardy,[i] Roberto L. Vargas,[j] Ghinwa Dumyati,[k,l] Marion A. Kainer,[m] Erin C. Phipps,[n,o] Rebecca Pierce,[p] Lucy E. Wilson,[q] Matthew Sorensen,[r] Erik Nilsson,[r] I. King Jordan,[g] Eileen M. Burd,[c,d,s] Monica M. Farley,[c,d,e] Jesse T. Jacob,[c,d,e] Robert K. Ernst,[f] David S. Weiss[b,c,d,t]

[a]Department of Microbiology and Immunology, Emory University, Atlanta, Georgia, USA
[b]Emory Vaccine Center, Atlanta, Georgia, USA
[c]Emory Antibiotic Resistance Center, Atlanta, Georgia, USA
[d]Division of Infectious Diseases, Department of Medicine, Emory University School of Medicine, Atlanta, Georgia, USA
[e]Georgia Emerging Infections Program, Atlanta, Georgia, USA
[f]Department of Microbial Pathogenesis, University of Maryland—Baltimore, Baltimore, Maryland, USA
[g]Center for Integrated Genomics, Georgia Institute of Technology, Atlanta, Georgia, USA
[h]ACM Global Laboratories, Rochester, New York, USA
[i]Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, New York, USA
[j]Department of Pathology and Laboratory Medicine, Rochester General Hospital, Rochester, New York, USA
[k]Infectious Diseases Division, University of Rochester Medical Center, Rochester, New York, USA
[l]Center for Community Health and Prevention, University of Rochester Medical Center, Rochester, New York, USA
[m]Tennessee Department of Health, Nashville, Tennessee, USA
[n]University of New Mexico, Albuquerque, New Mexico, USA
[o]New Mexico Emerging Infections Program, Albuquerque, New Mexico, USA
[p]Public Health Division, Oregon Health Authority, Salem, Oregon, USA
[q]Maryland Department of Health and Mental Hygiene, Baltimore, Maryland, USA
[r]Pataigin, LLC, Seattle, Washington, USA
[s]Department of Pathology and Laboratory Medicine, Emory University, Atlanta, Georgia, USA
[t]Research Service, Atlanta VA Medical Center, Decatur, Georgia, USA

ABSTRACT Heteroresistance is a form of antibiotic resistance where a bacterial strain is comprised of a minor resistant subpopulation and a majority susceptible subpopulation. We showed previously that colistin heteroresistance can mediate the failure of colistin therapy in an *in vivo* infection model, even for isolates designated susceptible by clinical diagnostics. We sought to characterize the extent of colistin heteroresistance among the highly drug-resistant carbapenem-resistant *Enterobacterales* (CRE). We screened 408 isolates for colistin heteroresistance. These isolates were collected between 2012 and 2015 in eight U.S. states as part of active surveillance for CRE. Colistin heteroresistance was detected in 10.1% (41/408) of isolates, and it was more common than conventional homogenous resistance (7.1%, 29/408). Most (93.2%, 38/41) of these heteroresistant isolates were classified as colistin susceptible by standard clinical diagnostic testing. The frequency of colistin heteroresistance was greatest in 2015, the last year of the study. This was especially true among *Enterobacter* isolates, of which specific species had the highest rates of heteroresistance. Among *Klebsiella pneumoniae* isolates, which were the majority of isolates tested, there was a closely related cluster of colistin-heteroresistant ST-258 isolates found mostly in Georgia. However, cladistic analysis revealed that, overall, there was significant diversity in the genetic backgrounds of heteroresistant *K. pneumoniae* isolates. These findings suggest that due to being largely undetected in the clinic, colistin heteroresistance among CRE is underappreciated in the United States.

Citation Band VI, Satola SW, Smith RD, Hufnagel DA, Bower C, Conley AB, Rishishwar L, Dale SE, Hardy DJ, Vargas RL, Dumyati G, Kainer MA, Phipps EC, Pierce R, Wilson LE, Sorensen M, Nilsson E, Jordan IK, Burd EM, Farley MM, Jacob JT, Ernst RK, Weiss DS. 2021. Colistin heteroresistance is largely undetected among carbapenem-resistant *Enterobacterales* in the United States. mBio 12:e02881-20. https://doi.org/10.1128/mBio.02881-20.

Editor Robert A. Bonomo, Louis Stokes Veterans Affairs Medical Center

Address correspondence to David S. Weiss, david.weiss@emory.edu.

Received 13 October 2020
Accepted 1 December 2020
Published 26 January 2021

IMPORTANCE Heteroresistance is an underappreciated phenomenon that may be the cause of some unexplained antibiotic treatment failures. Misclassification of heteroresistant isolates as susceptible may lead to inappropriate therapy. Heteroresistance to colistin was more common than conventional resistance and was overwhelmingly misclassified as susceptibility by clinical diagnostic testing. Higher proportions of colistin heteroresistance observed in certain *Enterobacter* species and clustering among heteroresistant *Klebsiella pneumoniae* strains may inform colistin treatment recommendations. Overall, the rate of colistin nonsusceptibility was more than double the level detected by clinical diagnostics, suggesting that the prevalence of colistin nonsusceptibility among CRE may be higher than currently appreciated in the United States.

KEYWORDS colistin, heteroresistance, CRE, antibiotic resistance, polymyxins, *Enterobacterales*, *Enterobacteriaceae*

Increasing antibiotic resistance has been recognized as a major health threat by the Centers for Disease Control and Prevention (CDC) and World Health Organization, resulting in at least 35,000 deaths and 2.8 million infections annually in the United States (1). To combat infections due to highly resistant bacteria, such as carbapenem-resistant *Enterobacterales* (CRE), that have up to a 40% mortality rate (2), clinicians are increasingly turning to drugs of last resort, including the polymyxin antibiotic colistin (polymyxin E) (3). However, resistance even to last-line drugs is increasing (4–6). Further complicating efforts to combat multidrug-resistant bacteria are instances of treatment failure of strains classified as susceptible to a given antibiotic. Heteroresistance is a form of resistance in which a strain harbors both an antibiotic-resistant subpopulation and a majority population of susceptible cells. We recently demonstrated that colistin heteroresistance can lead to colistin treatment failure in an *in vivo* mouse model of infection with multiple *Enterobacter* and *Klebsiella* clinical isolates (7, 8). Furthermore, colistin heteroresistance may not be detected by traditional clinical testing methods. Failure to identify heteroresistance in the clinical laboratory may lead to treatment failures in serious CRE infections. We performed a retrospective study among multidrug-resistant CRE isolates collected between 2012 and 2015 to determine the frequency of colistin heteroresistance within this collection.

Four hundred eight CRE isolates included in this project were collected as part of the U.S. CDC Emerging Infections Program's Multisite Gram-Negative Surveillance Initiative (MuGSI) (9, 10), an ongoing, active, population- and laboratory-based surveillance system for CRE isolated from urine and sterile sites, such as blood. Isolates were collected from clinical laboratories in metropolitan areas in eight U.S. states and adhered to a strict definition of CRE based on susceptibility testing and species identification (9). All isolates that fit this definition within the surveillance area during the study years of 2012 to 2015 were included in this study. All isolates are summarized in Table S1 in the supplemental material and belonged to 3 genera: *Klebsiella*, *Enterobacter*, and *Escherichia* (Fig. 1a). They originated from various culture sources (Table S1) and displayed resistance to last-line antibiotics, such as aminoglycosides and tigecycline (Table S2).

To detect heteroresistance to colistin, all isolates were tested via the population analysis profile (PAP) method. This consists of plating overnight cultures of each isolate onto solid Muller-Hinton (MH) agar with or without colistin concentrations of 0.5, 1, 2, 4, 16, 32, and 100 μg/ml. Surviving colonies were enumerated and used to detect colistin-resistant subpopulations characteristic of heteroresistance. Overall, PAP revealed a proportion of 10.1% (41/408 isolates) colistin heteroresistance (Fig. 1a), which was found among all genera tested. Compared to the proportion of isolates demonstrating heteroresistance, there was a lower proportion of isolates demonstrating "conventional" colistin resistance (7.1%, 29/408), in which all cells within the population exhibited a resistant phenotype. Additionally, the proportion of isolates with colistin

A

	Heteroresistant/ Total (%)	p value[a]
***Enterobacter* sp.**	16/74 (21.6%)	0.0005
E. cloacae	7/61 (11.5%)	0.6882
E. asburiae	4/5 (80.0%)	0.0011
E. kobei	3/6 (50.0%)	0.0067
E. ludwigii	2/2 (100.0%)	0.0137
Minority *Enterobacter* species[b]	9/13 (69.2%)	< 0.0001
***Klebsiella* sp.[c]**	24/286 (8.4%)	0.0914
***Escherichia* sp.[d]**	1/48 (2.1%)	0.0839

[a] p value for % colistin heteroresistance in each category, by odds ratio
[b] Includes *E. asburiae, E. kobei,* and *E. ludwigii*
[c] Includes *K. pneumoniae, K. aerogenes* and *K. oxytoca*
[d] Includes *E. coli* and *E. albertii*

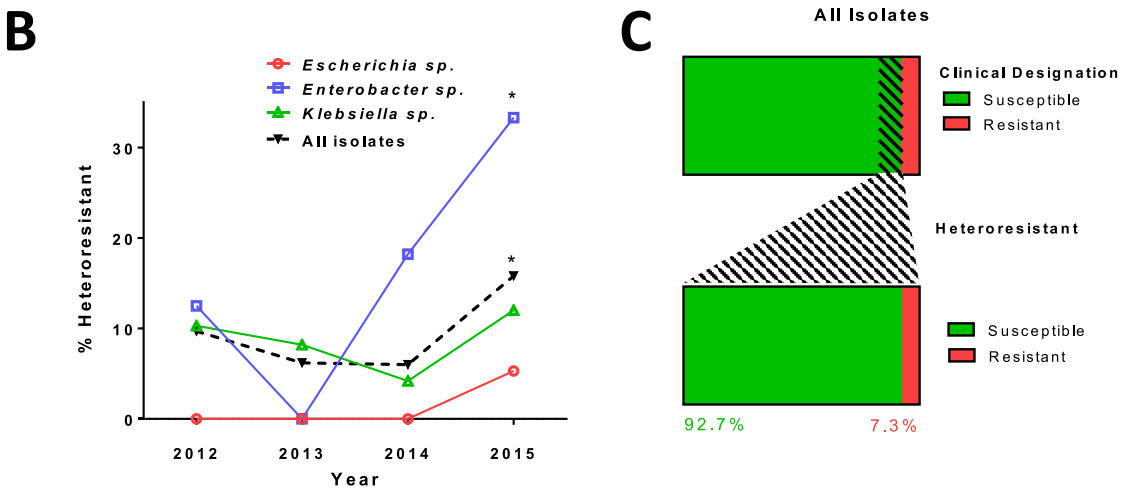

FIG 1 Rate of colistin heteroresistance by genus, year, and clinical detection. (A) Table of colistin heteroresistance incidence by the indicated genus, species, and *P* value for the odds ratio in each category. (B) Incidence of total colistin-heteroresistant isolates as a percentage in each year from 2012 to 2015. *, $P < 0.05$ for the positive linear trend from 2012 to 2015 (Cochran-Armitage trend test). (C) Proportion of isolates designated colistin susceptible (green) or colistin resistant (red) by standard clinical susceptibility testing. The portion with hatched lines indicates isolates that were heteroresistant by population analysis profile. The right margin represents the clinical designations of all colistin-heteroresistant isolates.

heteroresistance was highest in the most recent year of the study (2015, 15.8%, 24/152, $P = 0.0039$, odds ratio = 2.636, 95% confidence interval [CI] = 1.366 to 5.087), and proportions were significantly higher than in all previous years (9.8% [4/41] in 2012, 6.2% [5/81] in 2013, 6.0% [8/134] in 2014) (Fig. 1b). Although only four sites collected isolates during all 4 years, including only those three sites, the rate of colistin heteroresistance in 2015 was still significantly higher than in prior years (17/118, 14.4%, $P = 0.0135$, odds ratio = 2.665, 95% CI = 1.225 to 5.799).

Of the 41 colistin-heteroresistant isolates detected by PAP, only 7.3% (3/41) were classified as colistin resistant by broth microdilution (BMD), while the vast majority (92.7%, 38/41) were classified as colistin susceptible (Fig. 1c). Overall, BMD classified 32 of the 408 CRE isolates as colistin nonsusceptible, a 7.2% rate of nonsusceptibility (including 3 heteroresistant isolates and 29 resistant isolates). However, testing by PAP revealed that the proportion of colistin-nonsusceptible isolates was actually 17.1% (70/

408, 41 heteroresistant and 29 resistant isolates), which is more than double the rate detected by standard clinical testing (Table S3).

Enterobacter spp. displayed the highest proportion of colistin heteroresistance (21.6%, 16/74, $P = 0.0005$, odds ratio = 3.410, 95% CI = 1.709 to 6.758), followed by *Klebsiella* spp. (8.4%, 24/286) and *Escherichia* (2.1%, 1/47). Among *Enterobacter*, the proportion of colistin heteroresistance was significantly higher in 2015 (33.3%, 11/33, $P = 0.0338$, odds ratio = 3.600, 95% CI = 1.103 to 11.748) than in prior years (12.2%, 5/41 from 2012 to 2014) (Fig. 1b). To further examine the rate of colistin heteroresistance among *Enterobacter* isolates in the collection, we stratified by species (as determined by the clinical laboratory). *Enterobacter* isolates included *E. asburiae*, *E. cloacae*, *E. kobei*, and *E. ludwigii*. Of these, *E. cloacae* (82.4%, 61/74) was the most common (Fig. 1a). The less-common species (designated here as "minority *Enterobacter* species"), *E. kobei* (8.1%, 6/74), *E. asburiae* (6.8%, 5/74), and *E. ludwigii* (2.7%, 2/74), accounted for the remaining *Enterobacter* isolates. The rate of colistin heteroresistance was highest among these minority *Enterobacter* species, with heteroresistance observed in 69.2% (9/13, $P < 0.0001$, odds ratio = 17.357, 95% CI = 4.209 to 71.576) of these isolates, compared to 11.5% (7/61) of *E. cloacae* isolates (Fig. 1a). Potentially in agreement with these findings, a previous report described variation in colistin heteroresistance rates among isolates from different *Enterobacter cloacae* complex genomic clusters (11). At least one colistin-heteroresistant *Enterobacter* isolate was identified from each of the eight study sites, indicating that *Enterobacter* isolates exhibiting this resistance phenotype were present in a wide distribution of geographic sites within the United States.

In contrast, *Klebsiella* isolates that were heteroresistant to colistin were found in only 5 states, with the majority (66.7%, 16/24) originating in the state of Georgia. Additionally, the proportion of heteroresistance in *Klebsiella* isolates was significantly higher in Georgia (15.0%, 16/107, $P = 0.0034$, odds ratio = 3.758, 95% CI = 1.550 to 9.115) than the proportion among isolates from all other states combined (4.5%, 8/179) (Table S4). The predominant *Klebsiella* species was *K. pneumoniae*, making up 90.9% of isolates, while other species included *K. aerogenes* and *K. oxytoca*. The higher rate of colistin heteroresistance among *Klebsiella* isolates in Georgia led us to consider whether this might be due to the presence of a predominant strain. To address this, we used cladistic analysis to determine the genetic relatedness of the colistin-heteroresistant *K. pneumoniae* isolates based on their whole-genome sequences (Fig. 2). This analysis revealed that there was a cluster of 15 closely related isolates within a 0.05 *P* distance (Fig. 2a, blue box) that were all sequence type 258 (ST-258) and contained similar antibiotic resistance genes. This genetic branch included isolates from all 4 years of the study, which did not cluster together temporally. The cluster of isolates consisted of 14 from Georgia and one from Minnesota, indicating that there was a highly related cluster of colistin-heteroresistant *Klebsiella* isolates present in Georgia. Among all the *K. pneumoniae* strains in the study, however, there was no association between whole-genome sequence and colistin susceptibility status (Fig. 2b). Taken together, the data indicate that colistin heteroresistance is a widespread phenomenon and that the presence of a cluster of heteroresistant *K. pneumoniae* isolates in Georgia may explain the high rate of colistin heteroresistance in this state.

Previous studies have shown that colistin heteroresistance can be mediated by PhoPQ/PmrAB-dependent cationic sugar modifications to the lipid A component of lipopolysaccharide (LPS) (12–15), resulting in an increased charge of the Gram-negative bacterial outer membrane and reduced susceptibility to colistin, a cationic antimicrobial peptide. Lipid A was analyzed using a new extraction method termed fast lipid analysis technique (FLAT) (16), as well as the previously used El Hamidi et al. method (17), on a representative sample of 12 colistin-heteroresistant isolates from this study using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry in the negative-ion mode (Table S5). After culture in the presence of colistin, which enriches for the resistant subpopulation, we observed an aminoarabinose ($\Delta m/z$ 131 mass shift)

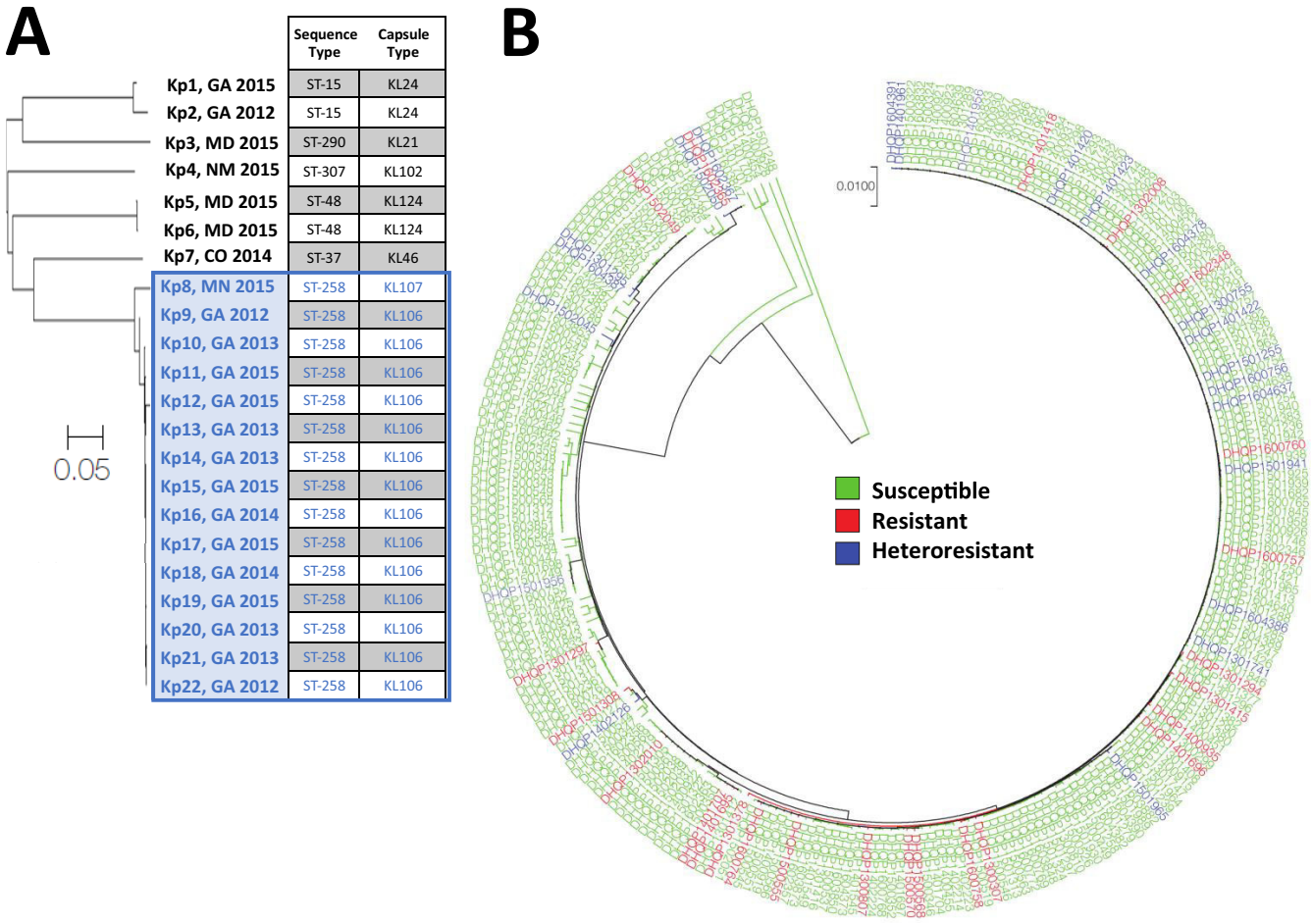

FIG 2 Colistin heteroresistance in *K. pneumoniae* occurs in genetically diverse isolates and forms a cluster of related isolates in Georgia. (A) Cladistic analysis of colistin-heteroresistant *K. pneumoniae* isolates by whole-genome sequence, with the corresponding sequence type and capsule type for each isolate. A closely related cluster of ST-258 isolates is highlighted in blue. (B) Cladistic analysis of all *K. pneumoniae* isolates in the study.

modification to the terminal phosphate in all heteroresistant isolates evaluated, as well as an additional phosphoethanolamine ($\Delta m/z$ 123 mass shift) modification in the *Escherichia coli* isolate analyzed (Fig. S1). These data further confirm that specific lipid A modifications are strongly associated with colistin heteroresistance, in agreement with previous studies (7, 18). Interestingly, MALDI-TOF mass spectrometry was able to detect lipid A modification in 9 out of 12 heteroresistant isolates even when the cultures were not enriched for resistant cells by growth in colistin (Table S5). These data indicate that MALDI-TOF approaches, which will be investigated further in future studies, may have diagnostic utility in detecting colistin heteroresistance which was missed by other diagnostic techniques.

This is the first multisite surveillance study for colistin heteroresistance among CRE in the United States. Treatment of highly antibiotic-resistant CRE relies on last-line drugs, including colistin, and the high rate of colistin heteroresistance may threaten the effective use of this antibiotic. The proportion of heteroresistance to colistin exceeded the proportion of "conventional" homogenous resistance, which, taken together, indicates that colistin nonsusceptibility is much more common than previously appreciated. Additionally, the vast majority of colistin-heteroresistant isolates were designated susceptible by standard clinical testing. The inability to detect colistin heteroresistance in the majority of heteroresistant isolates may lead to inappropriate treatment with colistin and might be a significant cause of unexplained antibiotic treatment failure. Detection of heteroresistance in these isolates was possible

using the PAP method, which is both labor- and time-intensive, making it an infeasible diagnostic method to employ in a clinical setting. Improvements in susceptibility testing are pivotal to improving clinical detection of heteroresistance, as observed using MALDI-TOF mass spectrometry in this study. Overall, this study shows that colistin heteroresistance is an underrecognized phenomenon among CRE in the United States.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

TEXT S1, DOCX file, 0.02 MB.

FIG S1, PDF file, 0.1 MB.

TABLE S1, PDF file, 0.1 MB.

TABLE S2, PDF file, 0.1 MB.

TABLE S3, PDF file, 0.1 MB.

TABLE S4, PDF file, 0.1 MB.

TABLE S5, PDF file, 0.1 MB.

ACKNOWLEDGMENTS

We acknowledge all of the submitting clinical laboratories and the state and local public health laboratories that provided isolates and assisted with this study, including Wendy M. Bamberg, Sarah J. Janelle (Colorado Department of Public Health and Environment), Kristin Shaw, Medora Witwer, Kelly Pung (Minnesota Department of Health), Emily B. Hancock (New Mexico), and the Tennessee State Public Health Laboratory. We also thank the CDC's Emerging Infections Program and MuGSI staff, including Sandra Bulens, Isaac See, Shelley Magill, and Maria Karlsson, for clinical isolates and insightful contributions.

This work was supported by the National Institutes of Health (grants RO1 AI141883 and RO1 AI1418661) and the Department of Veteran's Affairs (grant BX002788). D.S.W. is supported by a Burroughs Wellcome Fund Investigators in the Pathogenesis of Infectious Disease award. The Multi-Site Gram-Negative Surveillance Initiative is funded by the U.S. Centers for Disease Control and Prevention.

REFERENCES

1. Centers for Disease Control and Prevention. 2019. Antibiotic resistance threats in the United States. Centers for Disease Control and Prevention, Atlanta, GA. https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf
2. Patel G, Huprikar S, Factor SH, Jenkins SG, Calfee DP. 2008. Outcomes of carbapenem-resistant Klebsiella pneumoniae infection and the impact of antimicrobial and adjunctive therapies. Infect Control Hosp Epidemiol 29:1099–1106. https://doi.org/10.1086/592412.
3. Morrill HJ, Pogue JM, Kaye KS, LaPlante KL. 2015. Treatment options for carbapenem-resistant Enterobacteriaceae infections. Open Forum Infect Dis 2:ofv050. https://doi.org/10.1093/ofid/ofv050.
4. Halaby T, Kucukkose E, Janssen AB, Rogers MRC, Doorduijn DJ, van der Zanden AGM, Al Naiemi N, Vandenbroucke-Grauls CMJE, van Schaik W. 2016. Genomic characterization of colistin heteroresistance in Klebsiella pneumoniae during a nosocomial outbreak. Antimicrob Agents Chemother 60:6837–6843. https://doi.org/10.1128/AAC.01344-16.
5. Halaby T, Al Naiemi N, Kluytmans J, van der Palen J, Vandenbroucke-Grauls CM. 2013. Emergence of colistin resistance in Enterobacteriaceae after the introduction of selective digestive tract decontamination in an intensive care unit. Antimicrob Agents Chemother 57:3224–3229. https://doi.org/10.1128/AAC.02634-12.
6. Antoniadou A, Kontopidou F, Poulakou G, Koratzanis E, Galani I, Papadomichelakis E, Kopterides P, Souli M, Armaganidis A, Giamarellou H. 2007. Colistin-resistant isolates of Klebsiella pneumoniae emerging in intensive care unit patients: first report of a multiclonal cluster. J Antimicrob Chemother 59:786–790. https://doi.org/10.1093/jac/dkl562.
7. Band VI, Crispell EK, Napier BA, Herrera CM, Tharp GK, Vavikolanu K, Pohl J, Read TD, Bosinger SE, Trent MS, Burd EM, Weiss DS. 2016. Antibiotic failure mediated by a resistant subpopulation in Enterobacter cloacae. Nat Microbiol 1:16053. https://doi.org/10.1038/nmicrobiol.2016.53.
8. Band VI, Satola SW, Burd EM, Farley MM, Jacob JT, Weiss DS. 2018. Carbapenem-resistant *Klebsiella pneumoniae* exhibiting clinically undetected colistin heteroresistance leads to treatment failure in a murine model of infection. mBio 9:e02448-17. https://doi.org/10.1128/mBio.02448-17.
9. Guh AY, Bulens SN, Mu Y, Jacob JT, Reno J, Scott J, Wilson LE, Vaeth E, Lynfield R, Shaw KM, Vagnone PMS, Bamberg WM, Janelle SJ, Dumyati G, Concannon C, Beldavs Z, Cunningham M, Cassidy PM, Phipps EC, Kenslow N, Travis T, Lonsway D, Rasheed JK, Limbago BM, Kallen AJ. 2015. Epidemiology of carbapenem-resistant Enterobacteriaceae in 7 US communities, 2012–2013. JAMA 314:1479–1487. https://doi.org/10.1001/jama.2015.12480.
10. Centers for Disease Control and Prevention. 2016. Multi-site Gram-negative surveillance initiative. Centers for Disease Control and Prevention, Atlanta, GA. https://www.cdc.gov/hai/eip/mugsi.html. Accessed 20 March 2020.
11. Guérin F, Isnard C, Sinel C, Morand P, Dhalluin A, Cattoir V, Giard J-C. 2016. Cluster-dependent colistin hetero-resistance in Enterobacter cloacae complex. J Antimicrob Chemother 71:3058–3061. https://doi.org/10.1093/jac/dkw260.
12. Gunn JS, Lim KB, Krueger J, Kim K, Guo L, Hackett M, Miller SI. 1998. PmrA-PmrB-regulated genes necessary for 4-aminoarabinose lipid A modification and polymyxin resistance. Mol Microbiol 27:1171–1182. https://doi.org/10.1046/j.1365-2958.1998.00757.x.
13. Helander IM, Kato Y, Kilpeläinen I, Kostiainen R, Lindner B, Nummila K, Sugiyama T, Yokochi T. 1996. Characterization of lipopolysaccharides of polymyxin-resistant and polymyxin-sensitive Klebsiella pneumoniae O3. Eur J Biochem 237:272–278. https://doi.org/10.1111/j.1432-1033.1996.0272n.x.
14. Ernst RK, Yi EC, Guo L, Lim KB, Burns JL, Hackett M, Miller SI. 1999. Specific lipopolysaccharide found in cystic fibrosis airway Pseudomonas aeruginosa. Science 286:1561–1565. https://doi.org/10.1126/science.286.5444.1561.
15. Shi Y, Cromie MJ, Hsu F-F, Turk J, Groisman EA. 2004. PhoP-regulated

Salmonella resistance to the antimicrobial peptides magainin 2 and polymyxin B. Mol Microbiol 53:229–241. https://doi.org/10.1111/j.1365-2958.2004.04107.x.
16. Sorensen M, Chandler CE, Gardner FM, Ramadan S, Khot PD, Leung LM, Farrance CE, Goodlett DR, Ernst RK, Nilsson E. 2020. Rapid microbial identification and colistin resistance detection via MALDI-TOF MS using a novel on-target extraction of membrane lipids. Sci Rep 10(1):21536. https://doi.org/10.1038/s41598-020-78401-3.
17. El Hamidi A, Tirsoaga A, Novikov A, Hussein A, Caroff M. 2005. Microextraction of bacterial lipid A: easy and rapid method for mass spectrometric characterization. J Lipid Res 46:1773–1778. https://doi.org/10.1194/jlr.D500014-JLR200.
18. Kang KN, Klein DR, Kazi MI, Guérin F, Cattoir V, Brodbelt JS, Boll JM. 2019. Colistin heteroresistance in Enterobacter cloacae is regulated by PhoPQ-dependent 4-amino-4-deoxy-l-arabinose addition to lipid A. Mol Microbiol 111:1604–1616. https://doi.org/10.1111/mmi.14240.