elements on the same plasmid suggests that the CP-Hv pathotype could spread rapidly through horizontal transfer. This discovery demonstrates the critical role of genomic characterization of emerging resistance and virulence phenotypes by the AR Lab Network as part of US containment efforts.

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604. Gram-Negative Bacilli Carrying Multiple Carbapenemases: the United States, 2012–2018

David Ham, MD, MPH[1]; Garrett Mahon, MPH[2]; Sandeep Bhaurla, MPH, CIC[3]; Sam Horwich-Scholefield, MPH[4]; Liore Klein, MSPH[5]; Nychie Dotson, MPH[6]; James Rasheed, PhD[2]; Jennifer Huang, MPH[2]; Allison C. Brown, PhD MPH[2]; Alexander Kallen, MD[2] and Maroya S. Walters, PhD[7]; [1]Centers for Disease Control and Prevention, Atlanta, Georgia; [2]CDC, Atlanta, Georgia; [3]Los Angeles County Department of Public Health, Los Angeles, California; [4]California Department of Public Health, Richmond, California; [5]Maryland Department of Health, Baltimore, Maryland; [6]Florida Department of Health, Tallahassee, Florida; [7]Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, Atlanta, Georgia

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Background. Gram-negative bacilli carrying multiple carbapenemase genes (multi-CP-GNB) present an emerging public health threat; to date, most isolates reported in the literature have been from outside the United States. We reviewed multi-CP-GNB reported to CDC.

Methods. Reports of multi-CP-GNB isolates carrying genes encoding >1 targeted carbapenemases (i.e., KPC, NDM, OXA-48-type, VIM, or IMP) were received from healthcare facilities, health departments, and public health laboratories, and included isolates tested through the AR Laboratory Network (ARLN) beginning in 2017 as well as isolates sent to CDC for reference testing. Epidemiologic data were gathered by health departments during public health investigations.

Results. From October 2012 to November 2018, 111 multi-CP-GNB isolates from 71 patients in 20 states were identified. Two patients had three different multi-CP-GNB and one patient had two different multi-CP-GNB. The majority of cases (76%) were reported in 2017 or later, after ARLN testing began. Among patients with multi-CP-GNB, the most common organism-mechanisms combination was *Klebsiella pneumoniae* carrying NDM and OXA-48-type enzymes (table). Urine (44%) and rectal (20%) were the most frequent specimen sources for isolates. The median age of patients was 63 years (range 2–89 years); most had specimens collected at acute care hospitals (87%) or post-acute care facilities (9%). Of 50 patients with information available, 37 traveled internationally in the 12 months prior to culture collection. Among these, 88% were hospitalized for ≥1 night while outside the United States with 10 countries reported, of which India was most common (n = 18). All 5 patients with *Pseudomonas aeruginosa* co-carrying carbapenemases reported recent hospitalization outside the United States.

Conclusion. The multi-CP-GNB reported to CDC include diverse organisms and carbapenemase combinations and often harbored carbapenemases from different β-lactamase classes, which may severely limit treatment options. Healthcare exposures outside the United States were common; providers should ask about this exposure at healthcare admission and, when present, institute interventions to stop transmission in order to slow further US emergence.

Table: Unique Patients With Gram Negative Bacilli Producing Multiple Carbapenemases, by Organism and Mechanism, N=76**

Organism	IMP+NDM+ (n=2)	KPC+NDM+ (n=17)	KPC+OXA+ (n=1)	KPC+VIM+ (n=7)	NDM+OXA+ (n=47)	NDM+VIM+ (n=2)
Enterobacteriaceae (n=71)	0	17	1	6	47	0
E. coli (n=10)		2			8	
Enterobacter (n=10)		8		2		
*K. Pneumoniae** (n=48)		7	1	2	38	
K. Oxytoca (n=1)				1		
Citrobacter freundii (n=1)				1		
Providencia rettgeri (n=1)					1	
P. aeruginosa (n=5)	2			1		2

*Includes organisms now classified as *Klebsiella aerogenes*

**71 patients had 76 isolates with unique organism-mechanism combinations

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605. Identification of a Novel CMY-Variant Enzyme in a Clinical *Escherichia coli* Strain with Treatment-Emergent Ceftazidime–Avibactam Resistance

Samuel L. Aitken, PharmD[1]; Samuel L. Aitken, PharmD[1]; Bradley T. Endres, PhD[2]; Ayesha Khan, BSc[3]; William C. Shropshire, MPH[4]; Jovan Borjan, PharmD[1]; Micah M. Bhatti, MD, PhD[1]; Pranoti V. Sahasrabhojane, MS[1]; Yohei Doi, MD, PhD[5]; Ryan K. Shields, PharmD, MS[6]; Samuel A. Shelburne, MD, PhD[1] and Samuel A. Shelburne, MD, PhD[1]; [1]The University of Texas MD Anderson Cancer Center, Houston, Texas; [2]University of Houston College of Pharmacy, Houston, Texas; [3]UTHealth McGovern Medical School, Houston, Texas; [4]UTHealth School of Public Health, Houston, Texas; [5]University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; [6]University of Pittsburgh, Pittsburgh, Pennsylvania

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Background. Ceftazidime–avibactam (CZA) is a novel β-lactam / β-lactamase inhibitor with *in vitro* activity against multidrug-resistant Gram-negatives, including those harboring CMY-2 enzymes. Treatment-emergent resistance to CZA has been described in KPC-producing *Klebsiella pneumoniae* but has not been described in non-carbapenemase-producing, carbapenem-resistant *Enterobacteriaceae* (CRE).

Methods. A patient with an intra-abdominal infection due to a carbapenem-resistant *E. coli* (ertapenem MIC 16 μg/mL; meropenem MIC 2 μg/mL; CZA MIC 2 μg/mL; carbapenemase negative) was treated with CZA. On day 48 of therapy, a CZA resistant, carbapenem-sensitive *E. coli* was identified from abdominal drainage (CZA MIC ≥256 μg/mL; meropenem MIC 0.19 μg/mL). Illumina MiSeq whole-genome sequencing (WGS) was performed on both isolates to identify potential resistance mechanisms. The ResFinder database was used to identify known β-lactamase enzymes, and *in silico* modeling of β-lactamase structure was assessed.

Results. WGS revealed that both isolates were ST410 *E. coli*, with the sole difference in β-lactam resistance determinants between the two being a novel CMY β-lactamase harbored on an Inc1-type conjugative plasmid in the second isolate. The novel CMY has 4 amino acid substitutions relative to CMY-2: A134E, Q140K, V231S, and N366Y. The V231S substitution is found in CMY-42 and has previously been associated with increased ceftazidime hydrolysis. The remaining three substitutions have not previously been identified. Previous studies have identified that substitutions at position 366 influence the rate of ceftazidime hydrolysis rate. Preliminary protein structure analysis suggests that positions 140 and 366 are in the active site. No other differences in β-lactam resistance determinants were identified between the first and second isolates.

Conclusion. To our knowledge, we have identified the first case of CMY-associated CZA resistance. Given the widespread and transferrable nature of CMY enzymes, this finding raises concern for additional cases of resistance with increasing usage of CZA. Further analysis is needed to identify the mechanism by which this enzyme confers CZA resistance.

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606. Identification and Characterization of HMB-2, a Novel Metallo-β-Lactamase in a *Pseudomonas aeruginosa* Isolate

Wenming Zhu[1]; Gillian A. McAllister, BS[2]; Maria Jose Machado, MS[3]; Davina Campbell, MS[4]; Maria Karlsson, PhD[2]; James Rasheed, PhD[3]; Marion A. Kainer, MBBS, MPH, FRACP, FSHEA[5]; Daniel Muleta, MD, MPH[5]; Maroya S. Walters, PhD[3]; Julian E. Grass, MPH[4]; Alison L. Halpin, PhD[2] and Richard A. Stanton, PhD[4]; [1]Centers for Disease Control, Atlanta, Georgia; [2]Centers for Disease Control and Prevention, Atlanta, Georgia; [3]CDC, Atlanta, Georgia; [4]Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, Atlanta, Georgia; [5]Tennessee Department of Health, Nashville, Tennessee

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Background. Carbapenemases, a global health threat, are a diverse group of β-lactamases active against cephalosporins and carbapenems, which are often last resort treatments for multidrug-resistant gram-negative infections. The most common carbapenemases reported among *Pseudomonas aeruginosa* are metallo-β-lactamase (MBLs). We describe a novel MBL (designated HMB-2) identified in a *P. aeruginosa* isolate from a urine specimen collected in 2015 as part of CDC's Emerging Infections Program.

Methods. We performed antimicrobial susceptibility testing (AST) by broth microdilution, real-time PCR to screen for common carbapenemases (IMP, KPC, NDM, VIM, and OXA-48), and modified carbapenem inactivation method (mCIM) to test for carbapenemase production. The isolate underwent whole-genome sequencing (WGS) using Illumina MiSeq and PacBio RS II (Pacific Biosciences) platforms. Long read sequences were polished using Quiver and corrected by Pilon utilizing Illumina reads. We further characterized a putative novel MBL identified in WGS data by amplifying and cloning the gene into the pCR2.1-TOPO II vector (Invitrogen), which was then sub-cloned into a pET21 expression vector (Sigma–Aldrich). The resulting *hmb2*+ pET21 plasmid was transformed into a susceptible *Escherichia coli* for AST, including the imipenem-EDTA method to confirm MBL activity.

Results. The isolate displayed resistance to carbapenems and demonstrated phenotypic carbapenemase activity (mCIM positive), but was negative for carbapenemase genes by PCR. WGS analyses identified a putative MBL gene located on the chromosome. The gene shared 98% DNA and protein sequence identity with an MBL reported in 2016 in a *P. aeruginosa* isolate from Germany (HMB-1) and thus was named *hmb*-2. The cloned *hmb*-2 gene conferred resistance to carbapenems (meropenem and ertapenem) and third-generation cephalosporins (cefotaxime and ceftazidime) in transformed *E. coli*. The Minimum Inhibitory Concentrationratio for the imipenem-EDTA method was ≥4.

Conclusion. A putative, novel β-lactamase gene, $bla_{\text{HMB-2}}$, was identified and cloned. The imipenem-EDTA results indicated that HMB-2 is an MBL. This discovery

underscores the important role WGS plays in identifying new mechanisms of antimicrobial resistance.

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607. Scope and Predictive Genetic/Phenotypic Signatures of "Bicarbonate [$NaHCO_3$]-Responsivity" and β-Lactam Sensitization among Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Selvi Ceren. Ersoy, BS, MA, PhD[1]; Brianne Zapata-Davila, BS, MS[1]; Mariam Otmishi, BS[2]; Vanessa Milan, BS[1]; Liang Li, PhD[3]; Henry Chambers, BA, MD[4]; Henry Chambers, BA, MD[4]; Yan Xiong, MD, PhD[1] and Arnold Bayer, MD[3]; [1]Los Angeles Biomedical Research Institute, Los Angeles, California; [2]CSU Dominguez Hills, Torrance, California; [3]LABioMed at Harbor-UCLA Medical Center, Torrance, California; [4]UC San Francisco School of Medicine, San Francisco, California

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Background. Selected MRSA strains become susceptible to β-lactams (e.g., oxacillin [OX]; cefazolin [CFZ]) in vitro when tested in a standard medium (cation-adjusted Mueller–Hinton Broth; CA-MHB) supplemented with $NaHCO_3$ ("$NaHCO_3$-responsivity"). In vivo activity of β-lactams was demonstrated for MRSA strains with this phenotype in a rabbit endocarditis model (Ersoy et al Antimicrob Agents Chemother 2019). The current study was designed to: (i) determine the prevalence of the NaHCO3-responsive phenotype in a large collection of clinical MRSA isolates; and (ii) identify genetic and phenotypic predictors of this phenotype. ***Methods.*** 58 recent MRSA bloodstream isolates representing contemporary clonal complex (CC) genotypes were screened for the $NaHCO_3$-responsive phenotype by broth microdilution MICs in CA-MHB, with or without $NaHCO_3$ supplementation (25–44 mM).

Methods. 58 recent MRSA bloodstream isolates representing contemporary clonal complex (CC) genotypes were screened for the NaHCO3-responsive phenotype by broth microdilution MICs in CA-MHB, with or without NaHCO3 supplementation (25–44 mM).

Results. 43/58 (74.1%) and 21/58 (36.2%) were rendered susceptible to CFZ and OX, respectively, in the presence of $NaHCO_3$; 20 of the 21 OX-susceptible strains were also susceptible to CFZ in the presence of $NaHCO_3$. High baseline β-lactam MICs (i.e., MICs in CA-MHB alone ≥64 μg/mL) was not predictive of $NaHCO_3$ responsivity. The CC8 genotype was correlated with $NaHCO_3$ responsivity for OX, but not CFZ ($P < 0.05$).

Conclusion. The $NaHCO_3$-responsive phenotype is relatively common for both OX and especially CFZ among clinical MRSA isolates. Identification of specific genetic factors linked to this phenotype remains ongoing. Confirmation in relevant animal models that this phenotype is predictive of β-lactam efficacy *in vivo* could provide a solid foundation for a paradigm shift in antimicrobial susceptibility testing of MRSA.

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608. Emerging Methicillin Resistance Mechanism in *mec* Gene-Negative Staphylococci not Detected by Reference Methods

Geraldine Durand, PharmD, PhD[1]; Celine Dupieux-Chabert, PharmD[2]; Claude-Alexandre Gustave, MD[2]; Michèle Bes, PhD[2]; Berangere Fruiquière, MSc[1]; Corine Fulchiron, MSc[1]; Lorette Munoz, Tech[1]; Sarah Rivat, Tech[1]; Anne-gaelle Ranc, Pharm D[2]; Francois Vandenesch, MD, Pr[2]; Frederic Laurent, Pharm D, Pr[2]; Anne Tristan, Pharm D, PhD[2] and Patricia Martins-Simoes, PhD[2]; [1]bioMérieux, La Balme Les Grottes, Rhone-Alpes, France; [2]French National Reference Center for Staphylococci, Lyon, Rhone-Alpes, France

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Background. B-lactam resistance in Staphylococci is mediated by *mec* genes usually diagnosed by disc diffusion Cefoxitin test (DDFOX) and PCR testing. Here, we report methicillin-resistant *Staphylococcus lugdunensis* and *Staphylococcus aureus* strains lacking *mec* gene misdiagnosed by reference methods. Since the strains are not B-lactamase hyperproducers we investigated the molecular basis of the methicillin resistance.

Methods. We tested 2 *S. lugdunensis* isolates (SL1, SL2) collected from distinct blood cultures of the same patient and 2 *S. aureus* isolates (SA1, SA2): (i) by DDFOX, (ii) for Oxacillin MIC by agar dilution (AD), (iii) by VITEK®2 (bioMérieux) for Oxacillin MIC (V2 OXA) and Cefoxitin Screen Test (V2 OXSF), (iv) for *mec*A, B, C genes by PCR and (v) by whole-genome sequencing (WGS).

Results. The 4 isolates were methicillin susceptible by DD FOX and *mec* negative. However, all the isolates displayed variable results for V2 OXA MIC (0.5 to ≥4 mg/L) and for V2 OXSF (POSITIVE, NEGATIVE). For SL1 and SL2 isolates, the V2 OXSF growth curve atypical pattern has led to investigating the OXSF wells. The plates inoculated with the broth extracted from the OXSF well showed 2 colony morphotypes (small "P" and regular "G") for both isolates. The small colonies (SL1P, SL2P) were Oxacillin resistant (V2 OXA MIC≥4; AD MIC = 4) and V2 OXSF POSITIVE whereas the regular colonies (SL1G, SL2G) were Oxacillin susceptible (V2 OXA MIC = 2; AD MIC = 0.5) and V2 OXSF NEGATIVE. The 4 morphotypes were cefoxitin susceptible by DDFOX and *mec* negative. Interestingly, WGS revealed a GdpP truncation in the N-terminal domain only found in *S. lugdunensis* small colonies (SL1P, SL2P) phenotypically resistant to Oxacillin. GdpP is a cyclic diadenosine monophosphate phosphodiesterase enzyme which function is the hydrolysis of a signaling nucleotide.

Conclusion. We described *mec* negative *S. lugdunensis* and *S. aureus* strains expressing heterogeneous methicillin resistance detected by the VITEK2 OXSF test. *S. lugdunensis* subpopulation of small colonies resistant to oxacillin is associated with a truncation of GdpP protein previously described in *S. aureus*. Interestingly GdpP loss of function in Staphylococci is associated with a reduced growth and may arise as a result of the selective pressure of exposure to B Lactams.

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609. Differing Genotypic Contexts Between *E. coli* and *A. baumannii* Modulate the Role of blaADC-7 in the Development of Antibiotic Collateral Sensitivity

Erin McClure, BS[1]; Julia Newman[2]; Nikhil Krishnan, BS[3]; Joseph Rutter[4]; Andrea M. Hujer, BS[5]; Mark D. Adams, PhD[6]; Jacob Scott, MD, DPhil[7] and Robert A. Bonomo, MD[4]; [1]Cleveland Clinic Foundation, Cleveland Heights, Ohio; [2]Hawken School, Gates Mills, Ohio; [3]Case Western Reserve University School of Medicine, Cleveland, Ohio; [4]Louis Stokes Cleveland VA Medical Center, Cleveland, Ohio; [5]Louis Stokes VA Medical Center, Cleveland, Ohio; [6]The Jackson Laboratory for Genomic Medicine, Bar Harbor, Maine; [7]Cleveland Clinic Lerner Research Institute, Cleveland, Ohio

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Background. Antibiotic resistance is a global health crisis. While persistent drug discovery of novel antibiotics has previously been relied upon to thwart resistance, evolution inevitably perseveres. While genes conferring antibiotic resistance have previously been characterized, it is unclear how varying genetic contexts can change the antibiotic resistance phenotype a given gene confers.

Methods. The DH10B strain of *E. coli* was transformed with a bla_{ADC-7} plasmid. In 12 evolutionary replicates, the modified *E. coli* strain and a clinical strain of *A. baumannii* containing the same resistance gene were passaged daily for 10 days on cefepime gradient agar plates with gradually increasing concentrations of cefepime. MICs of cefepime and a diverse set of 15 other drugs were determined for the parental strains and after the final passage passage. MIC of cefepime after intermediary passages were determined for select replicates. Lastly the bla_{ADC-7} gene after the final passage was sequenced.

Results. At the end of 10 passages, collateral sensitivity in *A. baumannii* was observed to tigecycline and fosfomycin in 5 and 6 replicates respectively, out of 12 total. 4 out of 12 *E. coli* replicates displayed collateral sensitivity to minocycline (Figure 1). In the third *E. coli* replicate, Sanger sequencing revealed a novel S286R mutation in bla_{ADC-7} appearing in passage seven which preceded a several log fold increase in the MIC of cefepime (Figures 2 and 3). No additional mutations were found in the other evolutionary replicates.

Conclusion. Patterns of resistance varied among antibiotics of the same class, (e.g., tetracyclines, fourth-generation cephalosporins) in both *E. coli* and *A. baumannii*; however, *A. baumannii* expressed less widespread collateral resistance than *E. coli*. A previously undiscovered S286R mutation in bla_{ADC-7} coincided with a pronounced increased in resistance to cefepime. Further studies are required to determine whether this mutation gives rise to a structural change in the protein product. Given that no other mutations were found, resistance to cefepime and subsequent collateral resistance to other antibiotics may have developed due to epigenetic changes or mutations outside the bla_{ADC-7} genes. Indeed, future experiments with whole-genome sequencing may reveal such changes.

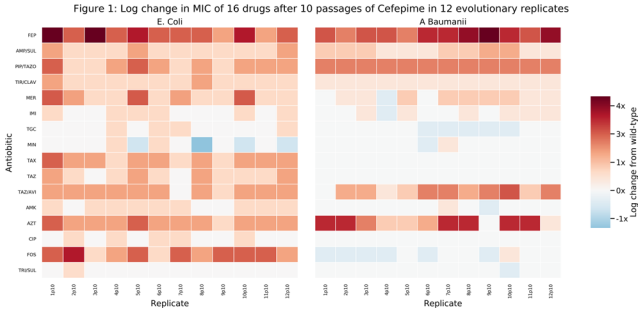

Figure 2: MIC of cefepime for *E. coli* replicate 3