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Risk Factors for and Mechanisms of *CO*listin *R*esistance Among *E*nterobacterales: Getting at the CORE of the Issue

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Background. Despite the recent emergence of plasmid-mediated colistin resistance, the epidemiology and mechanisms of colistin-resistant Enterobacterales (CORE) infections remain poorly understood.

Methods. A case–case–control study was conducted utilizing routine clinical isolates obtained at a single tertiary health system in Ann Arbor, Michigan. Patients with CORE isolates from January 1, 2016, to March 31, 2017, were matched 1:1 with patients with colistin-susceptible Enterobacterales (COSE) and uninfected controls. Multivariable logistic regression was used to compare clinical and microbiologic features of patients with CORE and COSE to controls. A subset of available CORE isolates underwent whole-genome sequencing to identify putative colistin resistance genes.

Results. Of 16 373 tested clinical isolates, 166 (0.99%) were colistin-resistant, representing 103 unique patients. Among 103 CORE isolates, 103 COSE isolates, and 102 uninfected controls, antibiotic exposure in the antecedent 90 days and age >55 years were predictors of both CORE and COSE. Of 33 isolates that underwent whole-genome sequencing, a large variety of mutations associated with colistin resistance were identified, including 4 *mcr*-1/*mcr*-1.1 genes and 4 *pmrA/B* mutations among 9 *Escherichia coli* isolates and 5 *mgrB* and 3 *PmrA* mutations among 8 *Klebsiella pneumoniae* isolates. Genetic mutations found in *Enterobacter* species were not associated with known phenotypic colistin resistance.

Conclusions. Increased age and prior antibiotic receipt were associated with increased risk for patients with CORE and for patients with COSE. *Mcr*-1, *pmrA/B*, and *mgrB* were the predominant colistin resistance–associated mutations identified among *E. coli* and *K. pneumoniae*, respectively. Mechanisms of colistin resistance among *Enterobacter* species could not be determined.

Keywords. colistin resistance; enterobacterales; polymyxin resistance.

Polymyxins possess broad-spectrum activity against many aerobic gram-negative pathogens and remain agents of "last resort" for some multidrug- and extensively drug-resistant gram-negative bacteria (MDR and XDR-GNB). Despite recent approval of several novel antibiotic agents such as cefiderocol, eravacycline, and plazomicin, there remain important treatment niches for the polymyxins. For example, few of the newer agents provide reliable coverage for pathogens such as New Delhi Metallo β-lactamase (NDM)–producing *Klebsiella pneumoniae* and *Acinetobacter baumannii* [1].

Although clinical experience with polymyxins began in 1959 and therapeutic use for MDR-GNB has dramatically increased in recent years, sparse data exist on baseline prevalence of colistin resistance among Enterobacterales, particularly in the United States [2, 3]. Furthermore, colistin susceptibility testing is challenging, with unreliable results produced by automated methods utilized in many clinical microbiology laboratories [4, 5]. The recent discovery and rapid global dissemination of the mobile colistin resistance (*mcr*) gene highlight the importance of improved population-level data regarding prevalence and epidemiology of polymyxin resistance [6, 7]. This study aimed to determine the overall prevalence of colistin resistance among Enterobacterales, along with predictors and primary mechanisms of colistin resistance in a population of patients in Southeast Michigan.

METHODS

Study Setting

A retrospective case–case–control study was performed at Michigan Medicine (Ann Arbor, MI, USA) to identify risk

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factors for infection or colonization with colistin-resistant Enterobacterales (CORE) in patients >18 years of age between January 1, 2016, and March 31, 2017.

Study Definitions and Data Collection

Enterobacterales with a colistin minimum inhibitory concentration (MIC) ≥4 mg/L on repeat broth microdilution (BMD) testing were considered colistin-resistant. Isolates with colistin MIC <4 mg/L were considered colistin-susceptible. Case group #1 (CORE) consisted of patients who possessed colistin-resistant isolates recovered from clinical cultures. Case group #2 (COSE) consisted of patients with colistin-susceptible isolates recovered from clinical cultures. The control group consisted of patients with clinical cultures that were negative for bacterial growth. Case group #2 and the control group were matched in a 1:1 ratio by random selection to case group #1 by the following variables: bacterial genus and species (ie, *Escherichia coli*, *K. pneumoniae*, or *Enterobacter* species), anatomical site of culture collection, geographic location of culture collection (inpatient vs outpatient), and year of culture collection. Enterobacterales species possessing intrinsic colistin resistance were excluded.

The following data were extracted from the electronic medical record: demographics, comorbidities, admission source, antibiotic exposure over the prior 90 days, and invasive device use within 72 hours of culture collection, with the goal of identifying clinical characteristics associated with CORE infection or colonization.

Laboratory Testing

During the study period, all clinical Enterobacterales isolates were identified by matrix-assisted laser desorption ionization-time of flight (Bruker Daltonik, Bremen, Germany) and were tested for colistin and other antibiotic susceptibilities by automated BMD (TREK Sensititre, Thermo Fisher, Oakwood Village, OH, USA) at the Michigan Medicine Clinical Microbiology Laboratory according to Clinical Laboratory Standards Institute (CLSI) Performance Standards for Antimicrobial Susceptibility Testing (M100). For isolates with an MIC of ≥4 mg/L, resistance was confirmed by repeat BMD testing through the Michigan Department of Health and Human Services (MDHHS) Bureau of Laboratories. Beginning in July 2016, CORE isolates were tested for *mcr*-1 by polymerase chain reaction (PCR) at MDHHS BOL according to Centers for Disease Control and Prevention (CDC) protocols [8].

Whole-Genome Sequencing

Before performing WGS, resistance to polymyxin was confirmed by broth macrodilution following CLSI guidelines using glass sterile tubes, as previously described [9]. A subset of 33 available CORE isolates then underwent WGS to identify mechanisms associated with polymyxin resistance. A method for saving CORE isolates in our clinical microbiology laboratory began in September 2016, and therefore isolates before that time were not available for WGS. The results of 3 *mcr*-1-harboring *E. coli* isolates have previously been described [10]. For the remaining isolates, total DNA from resistant isolates was extracted using the MasterPure Gram Positive DNA purification kit following the manufacturer's instructions (Epicentre, Madison, WI, USA). Libraries were prepared for sequencing using the Illumina NexteraXT kit (Illumina Inc., San Diego, CA, USA) and sequenced using an Illumina NextSeq550 at the Genomics Core at Case Western Reserve University. De novo assembly and annotation were performed using PATRIC (Pathosystems Resource Integration Center) [11, 12]. Species type was confirmed through StrainSeeker; resistome type and multilocus sequence type (MLST) were determined using ResFinder 2.0 and MLST 2.0, respectively (available at the Center for Genomic Epidemiology: http://www.genomicepidemiology.org) [13]. Isolates were deposited under BioProject PRJNA699920.

The following genes associated with polymyxin resistance were queried for mutations or insertions: *mgrB, phoP, phoQ, crrA, crrB, pmrA,* and *pmrB*. *E. coli* K12 substr. MC4100 (Genbank accession number HG738867.1) and *K. pneumoniae* subsp. pneumoniae HS11286 (Genbank accession number HG738867.1) were used as reference genomes. Due to the variability in species/subspecies, a single reference could not be used for *Enterobacter* spp.; instead, genes of interest were compared between the Enterobacter isolates by means of multiple alignments in order to determine significant polymorphisms.

Statistical Analysis

Descriptive statistics were performed to characterize the study population. Bivariable analysis of clinical characteristics was performed comparing CORE with controls and COSE with controls using the Fisther exact test and Wilcoxon rank-sum test to calculate 95% CIs and *P* values. Variables with $P < .10$ were considered for inclusion in the multivariable logistic regression model comparing CORE with controls and COSE with controls. Backward stepwise selection was performed to create a final explanatory model. All models were adjusted for confounding and assessed for collinearity. Values with $P < .05$ were considered significant. Statistical analysis was performed using STATA, version 16.0 (Statacorp, College Station, TX, USA).

Patient Consent Statement

This study was approved by the University of Michigan Institutional Review Board (HUM00133470) with a waiver of written informed consent.

RESULTS

Of 16 373 tested clinical isolates, 166 (0.99%) were colistin-resistant, representing 149 unique patients. Forty-six patients

were excluded because the isolates were from a referral lab without any available medical records. The 103 included CORE specimens were comprised of 45 (44%) *Enterobacter* species, 31 (30%) *Escherichia coli*, and 27 (26%) *Klebsiella* species. Sources of isolates were predominantly urinary (77%), followed by wound (14%) (Table 1). These proportions were similar in the

Table 1. Bivariable Analysis of Risk Factors for CORE or COSE Infection or Colonization

Variable	CORE (n = 103)	COSE (n = 103)	Controls (n = 102)	CORE vs Control	*P* Value	COSE vs Control	*P* Value	CORE vs COSE	*P* Value
Escherichia coli	31 (30%)	31 (30%)	—	—	—	—	—	1.00 (0.52–1.89)	1.00
Klebsiella pneumoniae	27 (26%)	27 (26%)	—	—	—	—	—	1.00 (0.51–1.95)	1.00
Enterobacter spp.	45 (44%)	45 (44%)	—	—	—	—	—	1.00 (0.55–1.80)	1.00
Urinary culture	79 (77%)	73 (71%)	79 (77%)	0.96 (0.47–1.94)	.90	0.71 (0.36–1.39)	0.28	1.35 (0.70–2.66)	.34
Wound culture	14 (14%)	19 (18%)	13 (13%)	1.08 (0.44–2.64)	.86	1.55 (0.68–3.63)	0.26	0.70 (0.30–1.57)	.34
Respiratory culture	5 (5%)	5 (5%)	5 (5%)	0.99 (0.22–4.45)	.99	0.99 (0.22–4.45)	0.99	1.00 (0.22–4.49)	1.00
Blood culture	4 (4%)	4 (4%)	3 (3%)	1.33 (0.22–9.32)	.71	1.33 (0.22–9.32)	0.71	1.00 (0.18–5.53)	1.00
Other culture[a]	1 (1%)	2 (2%)	2 (2%)	0.49 (0.01–9.59)	.56	0.99 (0.07–13.90)	0.99	0.50 (0.01–9.68)	.56
Inpatient culture	28 (27%)	28 (27%)	24 (24%)	1.21 (0.62–2.40)	.55	1.21 (0.62–2.40)	0.55	1.00 (0.52–1.94)	1.00
Outpatient culture	50 (49%)	50 (49%)	58 (57%)	0.72 (0.40–1.29)	.23	0.72 (0.40–1.29)	0.23	1.00 (0.56–1.79)	1.00
Emergency dept culture	25 (24%)	25 (24%)	20 (20%)	1.31 (0.64–2.71)	.42	1.31 (0.64–2.71)	0.42	1.00 (0.50–1.99)	1.00
Age, mean, y	60.5	60.9	48.5	—	<.01	—	<0.01		.75
Female	71 (69%)	67 (65%)	63 (62%)	1.37 (0.74 – 2.55)	.28	1.15 (0.63 – 2.12)	0.63	1.19 (0.64–2.22)	.33
Non-White race	25 (24%)	24 (23%)	21 (21%)	1.22 (0.60 – 2.50)	.55	1.16 (0.57 – 2.38)	0.67	1.06 (0.53–2.11)	.87
Charlson Index, median (IQR)	6 (3–11)	8 (4–12)	3 (0–7)	—	<.01	—	<0.01	2.43 (0.53–14.92)	.19
Cerebrovasc-ular dis-ease	27 (26%)	37 (36%)	14 (14%)	2.23 (1.04–4.94)	.04	3.52 (1.69–7.62)	<0.01	0.63 (0.33–1.20)	.09
Congestive heart failure	23 (22%)	29 (28%)	18 (18%)	1.34 (0.64–2.85)	.49	1.82 (0.90–3.79)	0.10	0.73 (0.37–1.44)	.21
Dementia	8 (8%)	2 (2%)	2 (2%)	4.21 (0.81–41.43)	.10	0.99 (0.71–13.90)	1.00	4.25 (0.81–41.83)	.05
Diabetes with compli-cation	21 (20%)	24 (23%)	11 (11%)	2.12 (0.91–5.16)	.06	2.51 (1.09–6.04)	0.03	0.84 (0.41–1.72)	.37
Diabetes without com-plication	34 (33%)	41 40%)	22 (22%)	1.79 (0.92–3.53)	.08	2.40 (1.25–4.68)	<0.01	0.75 (0.40–1.37)	.19
Malignancy	30 (29%)	38 (37%)	22 (22%)	1.49 (0.76–2.97)	.26	2.13 (1.10–4.16)	0.02	0.70 (0.38–1.31)	.15
Metastatic solid tumor	25 (24%)	26 (25%)	14 (14%)	2.01 (0.93–4.49)	.07	2.12 (0.98–4.71)	0.05	0.95 (0.48–1.88)	.50
Moderate/severe liver disease	7 (7%)	6 (6%)	1 (1%)	7.36 (0.91–335.07)	.07	6.25 (0.73–290.10)	0.12	1.18 (0.33–4.41)	.50
Chronic renal disease	35 (34%)	36 (35%)	21 (21%)	1.99 (1.01–3.93)	.04	2.07 (1.06–4.10)	0.03	0.96 (0.52–1.77)	.50
Chronic pulmonary disease	39 (38%)	41 (40%)	33 (32%)	1.27 (0.69–2.36)	.47	1.38 (0.75–2.55)	0.31	0.92 (0.51–1.68)	.44

Table 1. Continued

Variable	CORE (n = 103)	COSE (n = 103)	Controls (n = 102)	CORE vs Control	*P* Value	COSE vs Control	*P* Value	CORE vs COSE	*P* Value
Transplant	7 (7%)	10 (10%)	4 (4%)	1.79 (0.44–8.57)	.54	1.79 (0.44–8.57)	0.16	0.68 (0.21–2.07)	.31
Leukemia prior 12 mo	5 (5%)	5 (5%)	3 (3%)	1.69 (0.32–11.10)	.72	1.69 (0.31–11.10)	0.72	1.0 (0.22–4.49)	.63
Urinary catheter	22 (21%)	15 (15%)	19 (19%)	1.19 (0.56–2.51)	.38	0.74 (0.33–1.66)	0.28	1.59 (0.73–3.54)	.14
Feeding tube	3 (3%)	3 (3%)	3 (3%)	0.99 (0.13–7.57)	.65	0.99 (0.13–7.57)	0.65	1.00 (0.13–7.65)	.66
Hospital days before culture, median (IQR)	14 (3–28)	9 (3–18)	2 (1–8)		.001		0.005		.486
Hospital-onset culture (>48 h)[b]	22 (21%)	23 (22%)	10 (10%)	2.50 (1.05–6.25)	.018	2.65 (1.12–6.59)	0.012	0.94 (0.46–1.93)	.500
Survival to discharge	45/47 (96%)	50/51 (98%)	34/38 (90%)		.23		0.01		.47
Readmission 30 d	13/45 (29%)	22/50 (44%)	6/34 (18%)	1.84 (0.56–6.66)	.202	3.38 (1.09–11.64)	0.016	0.54 (0.21–1.38)	.116
Antibiotic DOT prior 90 d, median (IQR)	1 (0 – 8)	3 (0 - 18)	0 (0 – 2)		<.01		<0.01		<.01
Any antibiotic prior 90 d (dichotomous)	60 (58%)	75 (73%)	41 (40%)	2.08 (1.15–3.77)	.007	3.99 (2.13–7.50)	0.001	0.52 (0.28–0.97)	.02
Ciprofloxacin prior 90 d	12 (12%)	9 (9%)	5 (5%)	2.56 (0.80–9.60)	.07	1.86 (0.53–7.30)	0.21	1.38 (0.50–3.89)	.32
TMP-SMX prior 90 d	10 (10%)	14 (14%)	7 (7%)	1.46 (0.48–4.71)	.31	2.13 (0.76–6.53)	0.09	0.68 (0.26–1.76)	.26
Amoxicillin-clavulanate prior 90 d	4 (4%)	10 (10%)	6 (6%)	0.64 (0.13–2.83)	.37	1.72 (0.54–5.99)	0.22	0.38 (0.08–1.36)	.08
Piperacillin-tazobactam prior 90 d	6 (6%)	3 (3%)	2 (2%)	3.09 (0.53–31.89)	.14	1.50 (0.17–18.28)	0.51	2.06 (0.42–13.05)	.25
Ceftriaxone prior 90 d	2 (2%)	9 (9%)	3 (3%)	0.65 (0.05–5.84)	.50	3.16 (0.75–18.59)	0.07	0.21 (0.21–1.04)	.03
Cefepime prior 90 d	5 (5%)	6 (6%)	5 (5%)	0.99 (0.22–4.45)	.62	1.20 (0.29–5.14)	0.51	0.82 (0.19 – 3.37)	.50
Cephalexin prior 90 d	9 (9%)	12 (12%)	3 (3%)	3.16 (0.75–18.59)	.07	4.35 (1.12–24.63)	0.02	0.73 (0.26–1.98)	.32
Meropenem prior 90 d	1 (1%)	2 (2%)	0 (0%)	—	.50	—	0.25	0.50 (0.01–9.68)	.50
Nitrofurantoin 90 d	10 (10%)	9 (9%)	2 (2%)	5.38 (1.10–51.37)	.02	4.79 (0.95–46.34)	0.03	1.12 (0.39–3.28)	.50
Clindamycin prior 90 d	2 (2%)	13 (13%)	2 (2%)	0.99 (0.07–13.90)	.69	7.22 (1.56–67.11)	0.01	0.14 (0.01–0.64)	.01
Metronidazole prior 90 d	10 (10%)	3 (35)	8 (8%)	1.26 (0.43–3.86)	.41	0.35 (0.06–1.53)	0.10	3.58 (0.88–20.77)	.04
Colistin prior 90 d	0 (0%)	0 (0%)	0 (0%)	—	—	—	—	—	—

Abbreviations: CORE, colistin-resistant Enterobacterales; COSE, colistin-resistant Enterobacterales; DOT, days of therapy; IQR, interquartile range; TMP-SMX, trimethoprim-sulfamethoxazole.

[a]Includes rectal swabs, synovial fluid, and corneal scrapings.

[b]Denominators: CORE: 47 COSE: 51 control: 38.

COSE group. There were 103 COSE isolates and 102 control subjects (1 control was excluded due to ineligibility).

Overall, the mean age of study patients was 56.7 years, and 65.3% were female. Both the CORE and COSE groups had a relatively high severity of underlying illness, with mean Charlson scores of 7.6 and 8.1, respectively, as compared with a mean of 4.5 in controls.

CORE vs Control Patients

On bivariate analysis, CORE patients were more likely to be age >55 years, to suffer from diabetes, to have cerebrovascular, renal, and liver disease, to have the isolate be acquired in the hospital, and to have antibiotic exposure in the prior 90 days (Table 1). On multivariable analysis, CORE patients were more likely to be age >55 years (odds ratio [OR], 4.06; 95% CI, 2.24–7.36) and

to have received antibiotics within the prior 90 days (OR, 2.22; 95% CI, 1.23–4.03) (Table 2).

COSE vs Control Patients

Bivariate predictors for COSE patients included age >55 years and antibiotic exposure in the prior 90 days. (Table 1) On multivariable analysis, COSE patients were more likely to be age >55 years (OR, 3.11; 95% CI, 1.63–5.93), to have received antibiotics in the prior 90 days (OR, 4.43; 95% CI, 2.34–8.38), and were more likely to have a history of cerebrovascular disease (OR, 2.52; 95% CI, 1.17–5.42) (Table 2).

Comparing and Contrasting the 2 Models

Multivariable models for CORE and COSE were both adjusted for moderate to severe liver disease, which was identified as a potential confounder during backward stepwise variable selection. Independent risk factors for CORE and COSE were similar, with antecedent antibiotic exposure and age >55 years being the predominant risk factors. Additionally, cerebrovascular disease was identified as a risk factor for COSE but not CORE.

Antimicrobial Resistance Among CORE and COSE Isolates

Rates of beta-lactam resistance among both the CORE and COSE groups were relatively low (Table 3). Ceftriaxone susceptibility was detected in 48/58 (83%) CORE isolates vs 52/56 (93%) COSE isolates ($P = .094$). Ciprofloxacin resistance was more common among CORE patients, with 78/102 (76%) CORE isolates being ciprofloxacin susceptible vs 91/99 (92%) of COSE isolates ($P = .003$).

Molecular Analysis of Isolates

Thirty-two CORE isolates were available for WGS. WGS revealed that resistant isolates belonged to several different species including: 9 *E. coli* (30.3%), 8 *K. pneumoniae* (24.2%), 5 *Enterobacter cloacae* sp. *cloacae* (15.2%), 5 *E. roggenkampii* (15.2%), 2 *E. absburiae* (6.1%), 1 *E. kobei* (3%), 1 *E.cloacae* sp. *dissolvens* (3%), and 1 *Morganella morganii* (3%) (Table 4).

Sequenced *E. coli* isolates (n = 9) belonged to 8 different sequence types (STs) (Table 4). Colistin resistance mechanisms were identified in 8/9 isolates. *Mcr* genes were found on 4 *E. coli* isolates: 3 carried *mcr-1* and 1 carried *mcr*-1.1. Mutations in *pmrA/B* associated with colistin resistance were also identified in 4 additional isolates. Amino acid substitutions were found at 6 positions in PmrA, 10 positions in PmrB, 1 position in PhoP, and 4 positions in PhoQ. Several substitutions had been previously reported; however, most of them were reported on both colistin-susceptible and colistin-resistant isolates, whereas only a few were exclusively reported on colistin-resistant isolates including PmrA L105P and PmrB G22E, E126D, D315N. Most isolates carried at least 1 beta-lactamase gene (eg, bla_{EC}, bla_{TEM}, bla_{CTX-M}), and other resistance genes included *qacEΔ1, catB3, mph(A), sul1, aadA5, dfrA17, tet(B), floR, dfrA1, fosA3, ant(3”), aph(3’)-IIa*.

Sequenced *K. pneumoniae* isolates (n = 8) belonged to diverse STs including ST13, ST17, ST37, ST230, ST307, and ST1401. One or more putative colistin resistance mechanisms were identified in 7/8 isolates. Regarding *mgrB* mutations, 2/5 contained early stop codons, 2/5 had the gene interrupted by insertion sequences, and 1 had a single substitution, T21P. Amino acid variations were found at 3 positions in PmrA, 12 positions in PmrB, 1 position in PhoP, and 1 position in PhoQ. However, only 2 mutations in *K. pneumoniae* (PmrA A41T and PmrB E57G) have been previously reported in colistin-resistant isolates. All isolates carried 1 bla_{SHV} ESBL gene, and 4/8 carried additional beta-lactamase genes (including bla_{OXA-1}, bla_{CMY}, bla_{CTX-M}); other resistance genes included *oqxA, oqxB, sul, fosA, aph(3’)-Ib, aph(6)-Id, aac(3)-IIa, tet(A), qnrS1, ere(A)*.

Enterobacter spp. isolates (n = 15) presented amino acid variations in 15 positions in PmrA, 49 positions in PmrB, 8 positions in PhoP, and 50 positions in PhoQ; none of these isolates had prior colistin exposure. However, due to the great diversity within the *Enterobacter cloacae* complex (ECC), the low number of isolates per species, and the lack of a well-characterized reference strain for each species, any association between mutations in those genes with particular *Enterobacter* species could not be inferred. Also, in spite of the observed variations in these genes known for their role in colistin resistance, it was not possible to establish whether specific residue changes were directly responsible for colistin resistance. Furthermore, in addition to the chromosomally encoded *ampC*, all isolates carried *oqxA* and *oqxB*; other resistance genes included *mdf(A)* and *fosA*.

DISCUSSION

This is one of the first studies to provide large-scale colistin resistance data on clinical Enterobacterales isolates that were routinely tested for colistin susceptibility in the United States.

Table 2. Multivariable Analysis of Risk Factors for CORE or COSE Infection or Colonization[a]

Variable	CORE vs Control Odds Ratio (95% CIs)	*P* Value	COSE vs Control Odds Ratio (95% CIs)	*P* Value
Age >55 y	4.06 (2.24–7.36)	<.001	3.11 (1.63–5.93)	.001
Cerebrovascular disease	—	—	2.52 (1.17–5.42)	.018
Antibiotic exposure prior 90 d	2.22 (1.23–4.03)	.008	4.43 (2.34–8.38)	<.001

Abbreviations: CORE, colistin-resistant Enterobacterales; COSE, colistin-resistant Enterobacterales.

[a]Adjusted for moderate/severe liver disease.

Table 3. Antimicrobial Susceptibility of Enterobacterales Isolates

	CORE				COSE			
	All Isolates[a] (n = 103)	*E. coli* (n = 31)	*K. pneumoniae* (n = 27)	*E. cloacae* (n = 45)	All Isolates[a] (n = 103)	*E. coli* (n = 31)	*K. pneumoniae* (n = 27)	*E. cloacae* (n = 45)
Ertapenem	80/83 (96%)	25/25 (100%)	20/21 (95%)	35/37 (95%)	60/64 (94%)	13/13 (100%)	11/11 (100%)	36/40 (90%)
Meropenem	102/103 (99%)	31/31 (100%)	26/27 (96%)	45/45 (100%)	99/99 (100%)	30/30 (100%)	27/27 (100%)	42/42 (100%)
Ceftriaxone	48/58 (83%)	27/31 (87%)	21/27 (78%)	—	52/56 (93%)	28/30 (93%)	24/26 (92%)	—
Cefepime	97/102 (95%)	29/31 (94%)	25/27 (93%)	43/44 (98%)	94/100 (94%)	25/27 (93%)	26/28 (93%)	40/42 (95%)
Piperacillin/ tazobactam	90/101 (89%)	29/31 (94%)	23/27 (85%)	38/43 (88%)	87/101 (86%)	29/30 (97%)	24/27 (89%)	34/43 (79%)
Ciprofloxacin	78/102 (76%)	18/30 (60%)	19/27 (70%)	41/45 (91%)	91/99 (92%)	26/30 (87%)	25/27 (93%)	40/42 (95%)
TMP/SMX	86/102 (84%)	22/30 (73%)	21/27 (78%)	43/45 (96%)	80/99 (81%)	25/30 (83%)	21/27 (78%)	34/42 (81%)

Abbreviations: CORE, colistin-resistant Enterobacterales; COSE, colistin-resistant Enterobacterales; TMP/SMX, trimethoprim-sulfamethoxazole.

[a]Total number of tested isolates does not always add to 103 due to instances of suppressed or missing data. For example, ceftriaxone susceptibility is not routinely reported for *Enterobacter* species due to the presence of AmpC beta-lactamases.

Approximately 1% colistin resistance was identified among 16 000 Enterobacterales isolates tested by BMD. Antibiotic exposure in the antecedent 90 days and age >55 years were predictors of CORE and of COSE. Notably, none of the 103 patients with CORE were exposed to colistin before culture collection.

Independent risk factors for isolation of CORE and COSE in this study were similar. This echoes prior data from Europe, where the characteristics of patients with colistin-resistant and colistin-susceptible *E. coli* or *K. pneumonia* did not differ; prior meropenem exposure was the only variable uniquely associated with colistin-resistant isolates [14]. Of note, meropenem use was uncommon in our current cohort. The prevalence of 1% colistin resistance was comparable to the 0.1% and 1.8% resistance found among 7000 tested *E. coli* and *K. pneumoniae* North American isolates between 2006 and 2009 as part of the SENTRY surveillance program [3]. More population-based colistin resistance surveillance data will be needed to identify any meaningful trends, particularly in light of increasing reports of worldwide *mcr*-1 identification.

Resistance rates to other antimicrobials did not differ between the 2 groups, with the exception of higher rates of ciprofloxacin resistance found among CORE isolates. Our overall rates of drug resistance were low, unlike many prior studies, which selectively assessed for colistin resistance among multidrug-resistant gram-negative bacteria. The reason that unique risk factors identified for colistin resistance were not identified remains unclear, but unstudied factors, such as variations in dietary practices, including consumption of colistin-exposed meat sources, could potentially play a role [15].

Mechanisms of colistin resistance among the subset of tested isolates were diverse. Among *E. coli*, *mcr*-1/*mcr*-1.1 was identified in 4/9 isolates, and previously described polymyxin-associated *pmrA/B* mutations were identified in 4/9 isolates, respectively. In *K. pneumoniae* at least 1 *mgrB*, *phoP/Q*, or *pmrA/B* mutation was found in each isolate; however, only 2 *pmrA* mutations were previously associated with colistin resistance. This high diversity of mutations in functional polymyxin resistance genes echoes prior studies in *K. pneumoniae,* though our cohort was unique due to lack of prior colistin exposure [16, 17]. Mutations that have been identified will need to be functionally validated in order to assess their true contribution to colistin resistance [18–26]. Mechanisms of polymyxin resistance among Enterobacter isolates could not be identified due to the tremendous genetic variability within the genus, making it difficult to identify a single reference strain.

Of particular interest was the fact that though the majority of patients in the CORE group had received antibiotics in the 30 days before the collection of the isolates, none of them were exposed to polymyxin therapy. This raises the possibility that either collateral antimicrobial selective pressure or stochastic development of mutations in colistin resistance–associated genes resulting from exposure to other antibiotics occurred, leading to de novo polymyxin resistance. Various environmental stressors, such as cationic antimicrobial peptides, reduced pH, and Mg^{2+}, have been identified to be activators of the PhoPQ and PmrAB systems [27, 28]. It is possible that nonpolymyxin antimicrobials may promote similar selective pressure, leading to polymyxin resistance. Interestingly, ciprofloxacin resistance occurred more frequently in the CORE group compared with the COSE group ($P = .003$), and ciprofloxacin exposure was more common in the CORE group. Perhaps the bacterial stress response associated with quinolone exposure leads to accelerated mutations rates in these strains through activation of SOS response or potentially through other mechanisms [29, 30]. Development of antimicrobial resistance with exposure to structurally unrelated agents has been previously observed with other bacteria, most notably *Pseudomonas aeruginosa* [31–33].

The limitations of this study include the limited number of isolates available for WGS and the inability to identify the genetic etiology of colistin resistance among *Enterobacter* species. However, the available data provide important information regarding polymorphisms in functional colistin resistance

Table 4. Summary of Molecular Features of CORE Isolates Found by WGS

ID	Species	MLST[a]	PolB MIC	*mcr*	*mgrB*	*phoP*	*phoQ*	*pmrA*	*pmrB*	*ccrA*	*ccrB*	Resistome	Antibiotics Received, No. of d
	E. coli	410	4	*mcr-1*	WT	N/A							TZP (2), MERO (4), VAN (2)
	E.coli	10	4	*mcr-1*	WT								NIT (1)
	E. coli	1196	4	*mcr-1*	WT								None
CORE_ Eco1	*E. coli*	1196	>8	*mcr-1.11*	WT	**I44L***	WT	**S29G***	D256G, Y361N	N/A		*bla*$_{CTX-M-55}$, *qacL, floR, dfrA1, sul, fosA3, ant(3″), aph(3′)-IIa*	FEP (1), SXT (6), VAN (1)
CORE_ Eco2	*E. coli*	131	8	Neg	**V8A***	**I44L***	WT	**S29G***, **T31S***	**H5R***, *G22E*, *E126D*, D256G, **V354I***	N/A		*bla*$_{TEM-1}$	None
CORE_ Eco3	*E. coli*	1193	8	Neg	**V8A***	**I44L***	WT	**S29G***, **T31S***, R81A, **I128N***, **G144S***	**H5R***, *E126D*, D256G	N/A		*bla*$_{EC—5}$, *aph(3)-Id*	None
CORE_ Eco4	*E. coli*	73	8	Neg	**V8A***	**I44L***	R6H	**S29G*,T31S***, **I128N*, G144S***	**H5R***, *E126D*, D256G, *D315N*, **V354I***	N/A		*bla*$_{CTX-M-27}$, *bla*$_{EC—5}$	AMOX (11), NIT (11)
CORE_ Eco5	*E. coli*	8582	>8	Neg	WT	**I44L***	L239I, **A482T***	**S29G*,T31S***, *L105P*, **G144S***	**H5R***, S205L, D256G	N/A		*bla*$_{EC—13}$	None
CORE_ Eco6	*E. coli*	648	8	Neg	WT	**I44L***	L467M	**S29G***	**H5R***, del_A70-M70, D256G, **A363V***	N/A		*qacEΔ1, catB3, mph(A), sul1, aadA5, dfrA17, tet(B)*	AMP (2), LEX (39)
CORE_ Kpn1	*K. pneumoniae*	230	>8	Neg	L3 stop	WT	WT	WT	A147E, G233R	insMHVVISTVEEN	G306V	*bla*$_{SHV—27}$, *oqxB19*	AZM (4), FOS (1)
CORE_ Kpn2	*K. pneumoniae*	13	>8	Neg	T21P	WT	WT	WT	M152V, A223T, G233R	NF	NF	*bla*$_{SHV-5}$, *oqxA, oqxB25, qnrS1, fosA*	None
CORE_ Kpn3	*K. pneumoniae*	17	>8	Neg	IS ins	WT	WT	WT	*E57G*, G233R	insMHVVISTVEEN	WT	*bla*$_{SHV-28}$, *bla*$_{OXA-1}$, *oqxA, qnrB1, tet(A)*	AMX (14), TZP (7), VAN (14)
CORE_ Kpn4	*K. pneumoniae*	37	4	Neg	Q30 stop	S173F	WT	WT	WT	insMHVVISTVEEN	WT	*bla*$_{SHV-11}$, *bla*$_{CMY-2}$, *oqxA*	TZP (1), METRO (3)
CORE_ Kpn5	*K. pneumoniae*	1401	>8	Neg	IS ins	WT	P182A	R113H	R113H, G233R, G336D	NF	NF	*bla*$_{SHV-36}$, *fosA*	None
CORE_ Kpn6	*K. pneumoniae*	307	>8	Neg	WT	WT	WT	*A41T*	A41T, L190M, G233R	insMHVVISTVEEN	WT	*bla*$_{CTX-M-15}$, *bla*$_{SHV—28}$, *bla*$_{OXA-1}$, *oqxA, oqxB19, sul2, fosA, aph(3′)-Ib, aph(6)-Id, aac(3)-IIa*	LEX (10), VAN (35)
CORE_ Kpn7	*K. pneumoniae*	307	>8	Neg	WT	WT	WT	*A41T*	T134P, L190M, G233R	insMHVVISTVEEN	WT	*bla*$_{SHV-28}$, *bla*$_{OXA-1}$, *oqxA, oqxB19, qnrB1, fosA, tet(A)*	None
CORE_ Kpn8	*K. pneumoniae*	New ST	>8	Neg	WT	WT	WT	**M66I***	M66I, G233R, V325G	insMHVVISTVEEN, P88V	WT	*bla*$_{SHV-11}$, ere(A), *quacL, qnrS1, aadA2*	CIP (45), METRO (45)
CORE_ Ent1	*E. roggenkampii*	N/A	>8	Neg	—	—	—	—	—	—	—	*bla*$_{CMG}$, mdf(A), *oqxA, oqxB, fosA*	FEP (1), CLIN (1), VAN (7), METRO (1)
CORE_ Ent2	*E. roggenkampii*	N/A	>8	Neg	—	—	—	—	—	—	—	*bla*$_{MIR-1/5/6}$, mdf(A), *oqxA, oqxB, fosA*	None
CORE_ Ent3	*E. roggenkampii*	N/A	>8	Neg	—	—	—	—	—	—	—	*bla*$_{MIR-5}$, mdf(A), *oqxA, oqxB, fosA*	N/D

Table 4. Continued

ID	Species	MLST[a]	PolB MIC	*mcr*	*mgrB*	*phoP*	*phoQ*	*pmrA*	*pmrB*	*ccrA*	*ccrB*	Resistome	Antibiotics Received, No. of d
CORE_Ent4	*E. roggenkampii*	N/A	>8	Neg	—	—	—	—	—	—	—	$bla_{MIR-1/5}$, mdf(A), *oqxA, oqxB*	SXT (1)
CORE_Ent5	*E. roggenkampii*	N/A	4	Neg	—	—	—	—	—	—	—	$bla_{MIR-1/5}$, mdf(A), *oqxA, oqxB, fosA*	METRO (59)
CORE_Ent6	*E. cloacae* sp. *Cloacae*	N/A	>8	Neg	—	—	—	—	—	—	—	bla_{CMH-3}, mdf(A), *oqxA, oqxB, fosA*	CIP (76), FOS (1)
CORE_Ent7	*E. cloacae* sp. *cloacae*	N/A	>8	Neg	—	—	—	—	—	—	—	bla_{CMH-3}, mdf(A), *oqxA, oqxB, fosA*	AMX (6), FOX (1)
CORE_Ent8	*E. cloacae* sp. *cloacae*	N/A	>8	Neg	—	—	—	—	—	—	—	bla_{CMH-3}, mdf(A), *oqxA, oqxB, fosA*	LEX (8), CFZ (2), CIP (1), NIT (12), METRO (2)
CORE_Ent9	*E. cloacae* sp. *Cloacae*	N/A	>8	Neg	—	—	—	—	—	—	—	bla_{CMH-3}, mdf(A), *oqxA, oqxB, fosA*	None
CORE_Ent10	*E. cloacae* sp. *cloacae*	N/A	>8	Neg	—	—	—	—	—	—	—	bla_{CMH-3}, mdf(A), *oqxA, oqxB, fosA, aph(3")-Ib, aph(6)-Id*	CLIN (2), NIT (1)
CORE_Ent11	*E. cloacae* sp. *dissolvens*	N/A	>8	Neg	—	—	—	—	—	—	—	bla_{CMH-3}, *oqxA, oqxB, qnrE1, fosA*	TZP (1), VAN (6), DAP (2)
CORE_Ent12	*E. absburiae*	N/A	>8	Neg	—	—	—	—	—	—	—	bla_{ACT-4}, mdf(A), *oqxA, oqxB, fosA*	LEX (1)
CORE_Ent13	*E. absburiae*	N/A	>8	Neg	—	—	—	—	—	—	—	bla_{ACT-6}, *oqxA, oqxB, fosA*	TZP (26), TOB (2), VAN (2) RIF (26)
CORE_Ent14	*E. kobei*	N/A	>8	Neg	—	—	—	—	—	—	—	bla_{ACT-9}, mdf(A), *oqxA, oqxB, fosA*	AMP (1)
CORE_Ent15	*E. hormaechei*	N/A	>8	Neg	—	—	—	—	—	—	—	bla_{ACT-7}, mdf(A), *oqxA, oqxB, fosA*	None
CORE_Mmo1	*Morganella morganii*	—	>8	Neg	Intrinsically resistant							bla_{DHA-14}, sul1, *sul2, qnrS1,* tet(A), aph(3')-Ia, *aph(3')-Ib, aph(6)-Id, aac(3)-Iid, aadA1*	—

Bold indicates that the substitution has been previously described in the literature. Asterisks indicate that it has also been found on colistin-susceptible isolates. Underlined and bold substitutions have been reported exclusively on resistant isolates and/or have been functionally validated.

Abbreviations: AMOX, amoxicillin; AMP, ampicillin; AMX, amoxicillin; AZM, aztreonam; CIP, ciprofloxacin; CLIN, clindamycin; CFZ, cefazolin; FEP, cefepime; FOS, fosfomycin; LEX, cephalexin; MERO, meropenem; METRO, metronidazole; N/A, not analyzed; NF, not found; NIT, nitrofurantoin; RIF, rifampicin; SXT, trimethoprim-sulfamethoxazole; TOB, tobramycin; TZP, piperacillin-tazobactam; VAN, vancomycin.

[a]MLST: Achtman scheme was used for *E. coli*.

genes found among *Enterobacter* isolates that can aid future investigations.

In conclusion, we identified a low prevalence of colistin resistance among a large collection of Enterobacterales isolates in Southeast Michigan, a region with a historically high incidence of emerging multidrug-resistant pathogens [34–36]. Increased age and antibiotic receipt in the antecedent 90 days were independently associated with increased risk for patients with CORE, as well as for patients with COSE. *Mcr*-1 and *mgrB* mutations were the predominant causes among *E. coli* and *K. pneumoniae,* respectively, but the mechanisms of resistance in *Enterobacter* isolates were unclear. Further studies are needed to determine the drivers of and determinants of polymyxin resistance among Enterobacterales, including exposure to nonpolymyxin antimicrobials.

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