Risk Factors for and Mechanisms of Colistin Resistance Among Enterobacterales: Getting at the CORE of the Issue

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Abstract

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, MI. Patients with CORE isolates from January 1st 2016 to March 31st 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 WGS, 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; 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.

Key words: colistin resistance, polymyxin resistance, Enterobacterales
INTRODUCTION

Polymyxins possess broad-spectrum activity against many aerobic Gram-negative pathogens and remain agents of “last resort” for some multi-drug 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 highlights 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) to identify risk factors for infection or colonization with colistin-resistant Enterobacterales (CORE) in patients > 18 years of age between January 1\(^{st}\) 2016 and March 31\(^{st}\) 2017.
Study definitions and data collection: Enterobacterales with a colistin minimum inhibitory concentration (MIC) $\geq 4$ 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 (i.e. *Escherichia coli*, *K. pneumoniae*, or *Enterobacter* species), anatomical site of culture collection, geographic location of culture collection (inpatient versus outpatient), and year of culture collection. Additionally, case group #2 was matched to case group #1 based on bacterial species. 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 day 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) at the Michigan Medicine Clinical Microbiology Laboratory according to Clinical Laboratory Standards Institute (CLSI) Performance Standards for Antimicrobial Susceptibility Testing (M100). For isolates with MIC of $\geq 4$ 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 (WGS):** 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 three 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) 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 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 to controls and COSE to controls using Fischer’s exact test and Wilcoxon rank sum test to calculate 95% confidence intervals (CIs) and $P$ values. Variables with $P < 0.10$ were considered for inclusion in multivariable logistic regression model comparing CORE to controls and COSE to controls. Backward stepwise selection was performed to create a final explanatory model. All models were adjusted for confounding and assessed for collinearity. Values with $P < 0.05$ were considered significant. Statistical analysis was performed using STATA v16.0 (Statacorp, College Station, TX).

**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 COSE group. There were 103 COSE and 102 control isolates (one control isolate 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 to a mean of 4.5 in controls.
CORE vs control patients: On bivariate analysis, CORE patients were more likely to be > 55 years, to suffer from diabetes, 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 > 55 years (OR 4.06, 95% CIs 2.24-7.36) and have received antibiotics within the prior 90 days (OR 2.22, 95% CIs 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 > 55 years (OR 3.11, 95% CIs 1.63-5.93), have received antibiotics in the prior 90 days (OR 4.43, 95% CIs 2.34-8.38) and were more likely to have a history of cerebrovascular disease (OR 2.52, 95% CIs 1.17-5.42) (Table 2).

Comparing and contrasting the two 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 CORE and COSE groups were relatively low (Table 3). Ceftriaxone susceptibility was detected in 48/58 (83%) of CORE isolates versus 52/56 (93%) of COSE isolates ($p = 0.094$). Ciprofloxacin resistance was more common among CORE patients, with 78/102 (76%) CORE isolates ciprofloxacin susceptible versus 91/99 (92%) of COSE isolates ($p=0.003$).

Molecular analysis of isolates: Thirty-two CORE isolates were available for WGS. WGS revealed that resistant isolates belonged to several different species including: nine *E. coli*...
(30.3%), eight *K. pneumoniae* (24.2%), five *Enterobacter cloacae sp. cloacae* (15.2%), five *E. roggenkampii* (15.2%), two *E. absburiae* (6.1%), one *E. kobei* (3%), one *E. cloacae sp. dissolvens* (3%), and one *Morganella morganii* (3%) (Table 4).

Sequenced *E. coli* isolates (n=9) belonged to eight different 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 four additional isolates. Amino acid substitutions were found at 6 positions in PmrA, 10 positions in PmrB, one 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 one beta-lactamase gene (e.g., *blaEC, blaTEM, blaCTX-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 mechanism 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 one had a single substitution T21P. Amino acid variations were found at 3 positions in PmrA, 12 positions in PmrB, one position in PhoP and one position in PhoQ. However, only two mutations in *K. pneumoniae* (PmrA A41T and PmrB E57G) have been previously reported in colistin-resistant isolates. All isolates carried one *blaSHV* ESBL gene, and 4/8 carried additional beta-lactamase genes (including *blaOXA-1, blaCMY, blaCTX-M*); other resistance genes included *oqxA, oqxB, sul, fosA, aph(3′)-Ib, aph(6)-Id, aac(3)-Ila, 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 cloaceae 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. 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 also 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. 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 7,000 tested E. coli and K. pneumoniae North American isolates between 2006-2009 as part of the SENTRY surveillance program. More population-based colistin resistance surveillance data will be
needed to identify any meaningful trends, particularly in light of increasing reports of worldwide \textit{mcr}-1 identification.

Resistance rates to other antimicrobials did not differ between the two 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.\textsuperscript{15}

Mechanisms of colistin resistance among the subset of tested isolates were diverse. Among \textit{E. coli}, \textit{mcr}-1/\textit{mcr}-1.1 was identified in 4/9 isolates and previously described polymyxin-associated \textit{pmrA}/\textit{B} mutations were identified in 4/9 isolates, respectively. In \textit{K. pneumoniae} at least one \textit{mgrB}, \textit{phoP}/\textit{Q}, or \textit{pmrA}/\textit{B} mutation was found in each isolate, however, only two \textit{pmrA} mutations were previously associated with colistin resistance. This high diversity of mutations in functional polymyxin resistance genes echoes prior studies in \textit{K. pneumoniae}, though our cohort was unique due to lack of prior colistin exposure.\textsuperscript{16,17} Mutations that have been identified will need to be functionally validated in order to assess their true contribution to colistin resistance.\textsuperscript{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 \textit{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.\textsuperscript{27,28} It is possible that non-polymyxin antimicrobials may promote similar selective pressure leading to polymyxin resistance. Interestingly, ciprofloxacin resistance occurred more frequently in the CORE group compared to the COSE group ($p=0.003$) 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.\textsuperscript{29,30} Development of antimicrobial resistance with exposure to structurally unrelated agents has been previously observed with other bacteria, most notably *Pseudomonas aeruginosa*.\textsuperscript{31-33}

Limitations of this study include the limited number of isolates available for WGS and inability to identify genetic etiology of colistin resistance among *Enterobacter* species. However, the available data provide important information regarding polymorphisms in functional colistin resistance 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.\textsuperscript{34-36} Increased age and antibiotic receipt in 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 non-polymyxin antimicrobials.
Author Contributions:
JPM, RAB, and KSK were involved in the conception and design of the work. JPM and LN performed the data collection. MAB was involved in performing microbiologic experiments. LJ, SH, SDR, AMH, RAB were involved in performance and interpretation of whole genome sequencing. JPM and LJ wrote the first draft of the manuscript. All authors were involved in drafting the work and revising it critically.

Potential conflicts of Interest:
K. S. K. has served as a consultant for Xellia, Merck, Spero, Shionogi, and Entasis.

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| 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) |
| **Klebsiella pneumoniae**      | 27 (26%)   | 27 (26%)   | --             | --             | --      | --             | --      | 1.00         | (0.51-1.95) |
| **Enterobacter spp.**          | 45 (44%)   | 45 (44%)   | --             | --             | --      | --             | --      | 1.00         | (0.55-1.80) |
| **Urinary Culture**            | 79 (77%)   | 73 (71%)   | 79 (77%)       | 0.96           | (0.47-1.94) | 0.90           | 0.71     | (0.36-1.39) | 0.28     | 1.35         | (0.70-2.66) |
| **Wound Culture**              | 14 (14%)   | 19 (18%)   | 13 (13%)       | 1.08           | (0.44-2.64) | 0.86           | 1.55     | (0.68-3.63) | 0.26     | 0.70         | (0.30-1.57) |
| **Respiratory Culture**        | 5 (5%)     | 5 (5%)     | 5 (5%)         | 0.99           | (0.22-4.45) | 0.99           | 0.99     | (0.22-4.45) | 0.99     | 1.00         | (0.22-4.49) |
| **Blood Culture**              | 4 (4%)     | 4 (4%)     | 3 (3%)         | 1.33           | (0.22-9.32) | 0.71           | 1.33     | (0.22-9.32) | 0.71     | 1.00         | (0.18-5.53) |
| **Other Culture*               | 1 (1%)     | 2 (2%)     | 2 (2%)         | 0.49           | (0.01-9.59) | 0.56           | 0.99     | (0.07-13.90) | 0.99     | 0.50         | (0.01-9.68) |
| **Inpatient Culture**          | 28 (27%)   | 28 (27%)   | 24 (24%)       | 1.21           | (0.62-2.40) | 0.55           | 1.21     | (0.62-2.40) | 0.55     | 1.00         | (0.52-1.94) |
| **Outpatient Culture**         | 50 (49%)   | 50 (49%)   | 58 (57%)       | 0.72           | (0.40-1.29)  | 0.23           | 0.72     | (0.40-1.29) | 0.23     | 1.00         | (0.56-1.79) |
| **Emergency Dept Culture**     | 25 (24%)   | 25 (24%)   | 20 (20%)       | 1.31           | (0.64-2.71)  | 0.42           | 1.31     | (0.64-2.71) | 0.42     | 1.00         | (0.50-1.99) |
| **Age, mean**                  | 60.5       | 60.9       | 48.5           | --             | <0.01    | --             | <0.01   | 0.75         |
| **Female**                     | 71 (69%)   | 67 (65%)   | 63 (62%)       | 1.37           | (0.74-2.55) | 0.28           | 1.15     | (0.63-2.12) | 0.63     | 1.19         | (0.64-2.22) |
| **Non-white race**             | 25 (24%)   | 24 (23%)   | 21 (21%)       | 1.22           | (0.60-2.50) | 0.55           | 1.16     | (0.57-2.38) | 0.67     | 1.06         | (0.53-2.11) |
| **Charlson Index, median (IQR)**| 6 (3-11)  | 8 (4-12)   | 3 (0-7)        | --             | <0.01    | --             | <0.01   | 2.43         | (0.53-14.92) |
| **Cerebrovascular disease**    | 27 (26%)   | 37 (36%)   | 14 (14%)       | 2.23           | (1.04-4.94) | 0.04           | 3.52     | (1.69-7.62) | <0.01   | 0.63         | (0.33-1.20) |
| **Congestive Heart Failure**   | 23 (22%)   | 29 (28%)   | 18 (18%)       | 1.34           | (0.64-2.85) | 0.49           | 1.82     | (0.90-3.79) | 0.10     | 0.73         | (0.37-1.44) |
| **Dementia**                   | 8 (8%)     | 2 (2%)     | 2 (2%)         | 4.21           | (0.81-41.43)| 0.10           | 0.99     | (0.71-13.90) | 1.00     | 4.25         | (0.81-41.83) |
| **Diabetes with**              | 21 (20%)   | 24 (22%)   | 11 (11%)       | 2.12           | 0.06      | 2.51           | 0.03     | 0.84         | 0.37     | 0.84         | (0.37-2.11) |
| complication                        | (23%) | (0.91-5.16) | (1.09-6.04) | (0.41-1.72) | 0.19 |
|------------------------------------|-------|-------------|-------------|-------------|------|
| Diabetes without complication      | 34 (33%) | 0.08 | 2.40 (1.25-4.68) | <0.01 | 0.75 (0.40-1.37) |
| Malignancy                         | 30 (29%) | 0.26 | 2.13 (1.10-4.16) | 0.02 | 0.70 (0.38-1.31) |
| Metastatic Solid Tumor             | 25 (24%) | 0.07 | 2.12 (0.98-4.71) | 0.05 | 0.95 (0.48-1.88) |
| Moderate/severe Liver Disease      | 7 (7%) | 0.07 | 6.25 (0.73-290.10) | 0.12 | 1.18 (0.33-4.81) |
| Chronic renal disease              | 35 (34%) | 0.04 | 2.07 (1.06-4.10) | 0.03 | 0.96 (0.52-1.77) |
| Chronic pulmonary disease          | 39 (38%) | 0.47 | 1.38 (0.75-2.55) | 0.31 | 0.92 (0.51-1.68) |
| Transplant                         | 7 (7%) | 0.54 | 1.79 (0.44-8.57) | 0.16 | 0.68 (0.21-2.07) |
| Leukemia prior 12 months           | 5 (5%) | 0.72 | 1.69 (0.32-11.10) | 0.72 | 1.0 (0.22-4.49) |
| Urinary Catheter                   | 22 (21%) | 0.38 | 0.74 (0.33-1.66) | 0.28 | 1.59 (0.73-3.54) |
| Feeding Tube                       | 3 (3%) | 0.65 | 0.99 (0.13-7.57) | 0.65 | 1.00 (0.13-7.65) |
| Hospital days prior to culture, median (IQR) | 14 (3-28) | 0.001 | 0.005 | 0.486 |
| Hospital Onset Culture (>48 hrs) + | 22 (21%) | 0.018 | 2.65 (1.12-6.59) | 0.012 | 0.94 (0.46-1.93) |
| Survival to Discharge              | 45/47 (96%) | 0.23 | 0.01 | 0.47 |
| Readmission 30 days                | 13/45 (29%) | 0.202 | 3.38 (1.09-11.64) | 0.016 | 0.54 (0.21-1.38) |
| Antibiotic DOT prior 90 days, median (IQR) | 1 (0 – 8) | <0.01 | <0.01 | <0.01 |
| Any Antibiotic Prior 90 days (dichotomous) | 60 (58%) | 0.007 | 3.99 (2.13-7.50) | 0.001 | 0.52 (0.28-0.97) |
| Ciprofloxacin Prior 90d            | 12 (12%) | 0.07 | 1.86 (0.53-7.30) | 0.21 | 1.38 (0.50-3.89) |
| TMP-SMX‡ Prior 90d                  | 10 (10%) | 0.31 | 2.13 (0.76-0.09 | 0.09 | 0.68 (0.26-0.26) |
| Drug                          | Prior 90d |     | 4.71) | 6.53) | 1.76) | 0.08 |
|-------------------------------|-----------|-----|--------|--------|--------|------|
| Amoxicillin-clavulanate       | 4 (4%)    | 10  | 6 (6%) | 0.64   | 0.37   | 0.22 |
|                              |           | (10%)|        | (0.13-2.83) |        |      |
|                              |           | 6   |        | 1.72   |        | 0.38 |
|                              |           |     |        | (0.54-5.99) |        | (0.08-1.36) |
| Piperacillin-tazobactam       | 6 (6%)    | 3   | 2 (2%) | 3.09   | 0.14   | 0.51 |
| Prior 90d                     |           | (3%) |        | (0.53-31.89) |        |      |
|                              |           | 2   |        | 1.50   |        | 2.06 |
|                              |           |     |        | (0.17-18.28) |        | (0.42-13.05) |
| Ceftriaxone Prior 90d         | 2 (2%)    | 9   | 3 (3%) | 0.65   | 0.50   | 0.07 |
|                              |           | (9%) |        | (0.05-5.84) |        |      |
|                              |           | 3   |        | 3.16   |        | 0.21 |
|                              |           |     |        | (0.75-18.59) |        | (0.21-1.04) |
| Cefepime Prior 90d            | 5 (5%)    | 6   | 5 (5%) | 0.99   | 0.62   | 0.51 |
|                              |           | (6%) |        | (0.22-4.45) |        |      |
|                              |           | 5   |        | 1.20   |        | 0.82 |
|                              |           |     |        | (0.29-5.14) |        | (0.19-3.37) |
| Cephalexin Prior 90d          | 9 (9%)    | 12  | 3 (3%) | 3.16   | 0.07   | 0.02 |
|                              |           | (12%)|        | (0.75-18.59) |        |      |
|                              |           | 3   |        | 4.35   |        | 0.73 |
|                              |           |     |        | (1.12-24.63) |        | (0.26-1.98) |
| Meropenem Prior 90d           | 1 (1%)    | 2   | 0 (0%) | 0.50   | 0.25   | 0.50 |
|                              |           | (2%) |        |        |        | (0.01-9.68) |
|                              |           | 0   |        |        |        |      |
| Nitrofurantoin 90d            | 10 (10%)  | 9   | 2 (2%) | 5.38   | 0.02   | 1.12 |
|                              |           | (9%) |        | (1.10-51.37) |        |      |
|                              |           | 2   |        | 4.79   |        | 0.39 |
|                              |           |     |        | (0.95-46.34) |        | (0.39-3.28) |
| Clindamycin Prior 90d         | 2 (2%)    | 13  | 2 (2%) | 0.99   | 0.69   | 0.01 |
|                              |           | (13%)|        | (0.07-13.90) |        |      |
|                              |           | 2   |        | 7.22   |        | 0.14 |
|                              |           |     |        | (1.56-67.11) |        | (0.01-0.64) |
| Metronidazole Prior 90d       | 10 (10%)  | 3   | 8 (8%) | 1.26   | 0.41   | 0.10 |
|                              |           | (35) |        | (0.43-3.86) |        |      |
|                              |           | 8   |        | 0.35   |        | 3.58 |
|                              |           |     |        | (0.06-1.53) |        | (0.88-20.77) |
| Colistin Prior 90d            | 0 (0%)    | 0   | 0 (0%) |        |        |      |
|                              |           | (0%) |        |        |        |      |

* Include rectal swabs, synovial fluid, and corneal scrapings.
**Denominators: CORE: 47 COSE: 51 control: 38
†DOT = days of therapy
‡TMP-SMX = trimethoprim-sulfamethoxazole
Table 2: Multivariable Analysis of Risk Factors for CORE or COSE Infection or Colonization*

| Variable                     | CORE vs Control Odds Ratio (95% CIs) | P value | COSE vs Control Odds Ratio (95% CIs) | P value |
|------------------------------|--------------------------------------|---------|--------------------------------------|---------|
| Age > 55                     | 4.06 (2.24-7.36)                     | <0.001  | 3.11 (1.63-5.93)                     | 0.001   |
| Cerebrovascular Disease      | --                                   | --      | 2.52 (1.17-5.42)                     | 0.018   |
| Antibiotic Exposure Prior 90 days | 2.22 (1.23-4.03)                    | 0.008   | 4.43 (2.34-8.38)                     | <0.001  |

*Adjusted for moderate/severe liver disease
Table 3: Antimicrobial Susceptibility of Enterobacterales isolates

|                | CORE |               |               | COSE |               |               |
|----------------|------|---------------|---------------|------|---------------|---------------|
|                | All  | E. coli       | K. pneumoniae | E.   | All           | E. coli       | K. pneumoniae | E. cloacae    |
|                | isolates* | (n=31)      | (n=27)        | cloacae (n=45) | Isolates* | (n=31)      | (n=27)        | (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%) |

*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 presence of AmpC beta-lactamases.
### Table 4. Summary of molecular features of CORE isolates found by WGS.

| ID   | Species | MLST | Pred MIC | mic | regB | PreP | PhenX | PhenS | Pred | CodB | CodB | Resistance | Antibiotic resisted (Dashes) |
|------|---------|------|----------|-----|------|------|--------|--------|------|------|------|------------|-------------------------------|
| E.coli | E. coli | 1190 | -6 | 3,4,7 | WT | 1190 | -6 | 3,4,7 | WT | NA | | | | | |
| CORE001 | E. coli | 1190 | -6 | 3,4,7 | WT | 1190 | -6 | 3,4,7 | WT | NA | | | | | |
| CORE002 | E. coli | 1190 | -6 | 3,4,7 | WT | 1190 | -6 | 3,4,7 | WT | NA | | | | | |
| CORE003 | E. coli | 1190 | -6 | 3,4,7 | WT | 1190 | -6 | 3,4,7 | WT | NA | | | | | |
| CORE004 | E. coli | 1190 | -6 | 3,4,7 | WT | 1190 | -6 | 3,4,7 | WT | NA | | | | | |
| CORE005 | E. coli | 1190 | -6 | 3,4,7 | WT | 1190 | -6 | 3,4,7 | WT | NA | | | | | |
| CORE006 | E. coli | 1190 | -6 | 3,4,7 | WT | 1190 | -6 | 3,4,7 | WT | NA | | | | | |
| CORE007 | E. coli | 1190 | -6 | 3,4,7 | WT | 1190 | -6 | 3,4,7 | WT | NA | | | | | |

**Table 4.** Summary of molecular features of CORE isolates found by WGS.

| ID   | Species | MLST | Pred MIC | mic | regB | PreP | PhenX | PhenS | Pred | CodB | CodB | Resistance | Antibiotic resisted (Dashes) |
|------|---------|------|----------|-----|------|------|--------|--------|------|------|------|------------|-------------------------------|
| E.coli | E. coli | 1190 | -6 | 3,4,7 | WT | 1190 | -6 | 3,4,7 | WT | NA | | | | | |
| CORE001 | E. coli | 1190 | -6 | 3,4,7 | WT | 1190 | -6 | 3,4,7 | WT | NA | | | | | |
| CORE002 | E. coli | 1190 | -6 | 3,4,7 | WT | 1190 | -6 | 3,4,7 | WT | NA | | | | | |
| CORE003 | E. coli | 1190 | -6 | 3,4,7 | WT | 1190 | -6 | 3,4,7 | WT | NA | | | | | |
| CORE004 | E. coli | 1190 | -6 | 3,4,7 | WT | 1190 | -6 | 3,4,7 | WT | NA | | | | | |
| CORE005 | E. coli | 1190 | -6 | 3,4,7 | WT | 1190 | -6 | 3,4,7 | WT | NA | | | | | |
| CORE006 | E. coli | 1190 | -6 | 3,4,7 | WT | 1190 | -6 | 3,4,7 | WT | NA | | | | | |
| CORE007 | E. coli | 1190 | -6 | 3,4,7 | WT | 1190 | -6 | 3,4,7 | WT | NA | | | | | |

**Table 4.** Summary of molecular features of CORE isolates found by WGS.

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. TZP, piperacillin-tazobactam; MERO, meropenem; VAN, vancomycin; NIT, nitrofurantoin; FEP, cefepime; SXT, trimethoprim-sulfamethoxazole; AMOX, amoxicillin; AMP, ampicillin; LEX, cephalaxin; AZM, aztreonam; FOS, fosfomycin; TOB, tobramycin; RIF, rifampicin; CLIN, clindamycin; CIP, ciprofloxacin; CFZ, ceftazolin; AMX, amoxicillin; METRO, metronidazole. N/A: not analyzed; NF: not found. MLST: Achtman scheme used for E. coli.
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