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SUSCEPTIBILITY

Imipenem-Relebactam Susceptibility and Genotypic Characteristics of Carbapenem-Resistant *Enterobacterales* Identified during Population-Based Surveillance

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ABSTRACT Laboratories submit all carbapenem-resistant *Enterobacter*, *Escherichia coli*, and *Klebsiella* species to the Alameda County Public Health Department (ACPHD). ACPHD evaluated 75 isolates submitted during 9 months for susceptibility to imipenem-relebactam (I-R) and, using whole-genome sequencing, identified β-lactamase genes. Of 60 (80%) isolates susceptible to I-R, 8 (13%) had detectable carbapenemase genes, including 4 KPC, two NDM, and two OXA-48-like; we described the relationship between the presence of β-lactamase resistance genes and susceptibility to I-R.

KEYWORDS β-lactamase inhibitor, NDM, carbapenem resistance, imipenem-relebactam, whole-genome sequencing

The U.S. Centers for Disease Control and Prevention (CDC) has designated carbapenem-resistant *Enterobacterales* (CRE), a group of organisms that cause infections with limited treatment options, as a top-tier public health threat that requires urgent and aggressive action, including development of new antibiotics (1). Although β-lactam combination agents such as ceftazidime-avibactam or meropenem-vaborbactam have become available, resistance to these agents has emerged; thus, evaluating resistance to other combination agents is a growing need (2–4).

While imipenem is an effective treatment for infections caused by class C cephalosporinases such as AmpC and extended-spectrum β-lactamase-(ESBL) enzymes, studies have shown that the combination of imipenem with relebactam (I-R) may be an effective treatment for patients with infections caused by bacteria with class A carbapenemases, such as the *Klebsiella pneumoniae* carbapenemase (KPC) (5–8). I-R has not been shown to be effective against bacteria with class B metallo-β-lactamase (MBL) genes, such as the New Delhi metallo-β-lactamase (NDM), and some studies have concluded that pathogens positive for class D oxacillinase carbapenemase genes (OXA) are also nonsusceptible (9–11).

Most studies examining I-R susceptibility have focused on isolates from single health care centers or large-scale sentinel surveillance programs (12, 13). Several publications utilized whole-genome sequencing (WGS) to identify genetic markers from isolates collected in multiple countries and academic centers (9, 12, 14).

This study is the first reported population-based assessment of CRE susceptibility to I-R and corresponding genetic characteristics in a U.S. health jurisdiction. Alameda County, population 1.67 million, is in the San Francisco Bay Area of California. Since June 2017, the Alameda County Public Health Department (ACPHD) mandates submittal of all isolates from *E. coli*, *Klebsiella* spp., and *Enterobacter* spp. identified by the clinical laboratory as resistant to one or more carbapenems to the ACPHD public health laboratory, where they undergo further genetic testing (15). Resistance genes are

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ascertained by WGS on the Illumina MiSeq platform. β-Lactamase genes are identified by a customized gene-calling workflow, built into Geneious software version 10.2, and confirmed in Resfinder version 3.2 (CGE webserver).

ACPHD assessed 75 CRE isolates submitted from July 2019 to April 2020 for antimicrobial resistance genetic markers using WGS and subsequently tested for susceptibility to I-R by broth microdilution per CLSI M100 guidelines using established MIC interpretative breakpoints for imipenem (13). We used the BD PhoenixSpec nephelometer, which is designed to measure the turbidity of microbial suspensions equivalent to standardized bacterial suspensions, and colonies were kept in a range between 0.49 and 0.51 for all samples. Isolates with detectable NDM or OXA carbapenemase genes were tested in triplicate to confirm susceptibility, and the median result was included in the analysis. Genomic data were deposited with links to accession number PRJNA788096 in the NCBI BioProject database.

To assess genetic relatedness, a phylogenetic analysis was performed using the k-mer distance method with the Complete algorithm for clustering, and the tree was drawn using Geneious version 10.2, created by Biomatters. Fisher's exact test was used to assess statistically significant differences between proportions; all analyses were conducted in R version 4.0 (R Core Team).

Out of 75 specimens, 27% had detectable carbapenemase genes, including 9 (60%) of 15 *Klebsiella pneumoniae*, 10 (42%) of 24 *E. coli*, and 1 (3%) of 29 *Enterobacter cloacae* complex isolates, representing 7 different carbapenemase alleles (Table 1). Out of 20 isolates with detectable carbapenemase genes, 14 (70%) also carried an ESBL gene, which was significantly greater than the proportion of isolates without carbapenemase genes that carried an ESBL gene (20%; $P < 0.01$) (see Table S1 in the supplemental material).

A total of 60 (80%) isolates were susceptible to I-R, with an MIC of less than 2 μg/ml (MIC_{50}, <0.5; MIC_{90}, 8.0; range, <0.5 to >32). Among 75 isolates, 1 (1%) showed intermediate resistance (MIC, 2 μg/ml), and 14 (19%) were resistant to I-R (MIC, >2 μg/ml).

A significantly smaller proportion of isolates with detectable carbapenemase genes were susceptible to I-R (40%) compared to isolates without detectable genes (95%) ($P < 0.01$); of the 8 I-R-susceptible isolates with detectable carbapenemase genes, we identified 2 NDM, 2 OXA-48-like, and 4 KPC genes (Fig. 1). The single resistant isolate with OXA-48-like genes also contained an NDM-5 gene.

Twelve (86%) resistant isolates ($n = 14$) had detectable NDM genes. Interestingly, two (14%) of 14 NDM-positive isolates tested susceptible to I-R, indicating that the presence of an NDM gene does not always confer resistance against I-R (Table S1). A potential explanation for this finding is that genotype may not always lead to phenotypic expression of the carbapenemase; while it was beyond the scope of the current study, additional research should assess the expression of MBL genes using phenotypic and genotypic methods.

Conversely, genotypic factors such as porin mutations or undetected MBLs could play a role in explaining why 3 (20%) of 15 I-R-nonsusceptible isolates had no carbapenemase genes (16). We screened any carbapenemase-negative isolate with resistance to either imipenem and/or meropenem ($n = 16$) for the porin genes *ompA*, *ompC*, *ompF*, *ompK35*, and *ompK36*. The three nonsusceptible isolates without carbapenemase genes had at least one porin gene that was either absent or the open reading frame was interrupted by a stop codon, indicating the possible role of porins in I-R resistance (Table S1).

Although isolates were obtained through population-based surveillance of a single geographic area, we did not observe substantial relatedness among CRE isolates. The multiplicity of alleles and sequence types indicate a high level of genetic diversity, which is reflected by the overall lack of clustering in the phylogenetic tree (Fig. S1).

There are limitations to our study. Although our public health laboratory conducts routine genotypic surveillance of all CRE, we did not perform confirmatory antimicrobial susceptibility testing (AST) on isolates submitted by clinical laboratories; clinical lab AST results

TABLE 1 β-Lactamase genes and imipenem-relebactam susceptibility by genus and species

Genus/species	No. of isolates (%)	I-R susceptible (%)[a]	No. of carbapenemase genes, $n = 20$ (27%)[b]							No. of ESBL genes, $n = 25$, (33%)[c]		No. of other genes, $n = 60$ (80%)[d]		
			KPC-2	KPC-3	NDM-1	NDM-5	NDM-7	OXA-48	OXA-181	SHV	CTX-M	TEM-1	AmpC	OXA
E. cloacae complex	29 (39)	28 (97)	1	0	0	0	0	0	0	1	0	5	28	0
E. coli	24 (32)	18 (75)	0	0	1	6	1	1	1	0	10	9	8	2
K. pneumoniae	15 (20)	7 (47)	1	2	4	1	1	0	1	14	2	5	3	5
K. aerogenes	7 (9)	7 (100)	0	0	0	0	0	0	0	0	0	0	4	0
Total	75	60 (80%)	2	2	5	7	2	1	2	15	12	19	43	7

[a]Susceptible isolates were defined as having MIC less than 2 μg/ml; intermediate resistance was 2 μg/ml, and resistance was greater than 2 μg/ml.

[b]Note that one *K. pneumoniae* isolate had detectable NDM-5 and OXA-181 genes. Columns are not mutually exclusive; a single isolate may have multiple ESBL or other genes present.

[c]ESBL genes are defined as bla_{SHV} or $bla_{CTX\text{-}M}$.

[d]Other classifications includes ACT group, $bla_{TEM\text{-}1}$, AmpC, and noncarbapenemase, non-ESBL OXA genes (e.g., OXA-1, OXA-9).

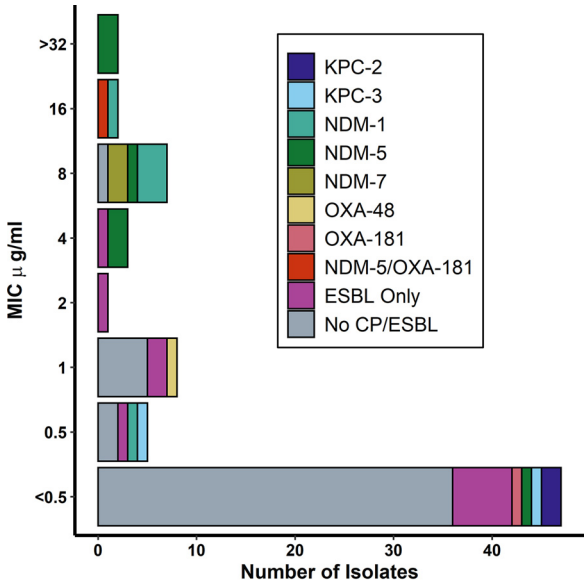

FIG 1 Carbapenemase and ESBL genes by imipenem-relebactam MIC (n = 75). Of 14 (19%) isolates that were resistant to I-R, 5 had detectable genes for NDM-5, 4 had NDM-1, 2 had NDM-7, and 1 was positive for both NDM-5 and OXA-18 genes. Two resistant isolates did not have detectable carbapenemase genes.

are listed for 71 (95%) isolates in Table S1. In addition, the number of isolates tested was not sufficient to make causal inferences about the association between antimicrobial resistance genes and I-R susceptibility, and our routine procedures for detection of antimicrobial resistance genes encoding β-lactamases did not exclude the possibility of efflux pumps, which also play a role in carbapenem resistance (17).

Despite these limitations, the genotypic and phenotypic assessment of a population-based sample of carbapenem-resistant *Enterobacter* spp., *E. coli*, and *Klebsiella* spp. demonstrated that certain genetic characteristics, such as the presence of NDM or OXA-48-like carbapenemases, may not be a sufficient cause of I-R resistance.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.6 MB.

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