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Detection and Characterization of β-Lactamase Encoding Genes in Carbapenem Non-Susceptible Gram-Negative Bacteria and Susceptibility of Isolates to Ceftazidime-Avibactam at a New York City Community Hospital

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Abstract

A surveillance study was undertaken to identify prominent β-lactamase encoding genes in 131 carbapenem non-susceptible gram-negative clinical isolates at a New York City community hospital. KPC carbapenemases were detected in 89% of Enterobacteriaceae as well as additional TEM, SHV, and CTX-M class A enzymes. OXA-23 and OXA-24 were the prevalent class D carbapenemases identified in *Acinetobacter* species. One OXA-23 in *M. morganii* and one OXA-48 in *K. pneumoniae* were also identified. Among class C β-lactamases CMY, ACT/MIR, DHA, and FOX were detected. The *in vitro* activity of ceftazidime-avibactam by E-test methodology was tested with minimal inhibitory concentrations (MIC) of ≤3 μg/ml for 97.8% of all Enterobacteriaceae, $MIC_{50/90}$ of 16/>256 μg/ml for carbapenem non-susceptible *Acinetobacter*, and 3/6 μg/ml for carbapenem non-susceptible *Pseudomonas aeruginosa*. Periodic surveillance of isolates to characterize current and emerging β-lactamase genotypes present in local isolates may help identify outbreak situations, provide assistance to infection control and antibiotic stewardship programs, and potentially improve patient outcomes.

Keywords

Carbapenem Non-Susceptible, Check-MDR CT103 XL Microarray, β-Lactamase

Detection, Resistance Mechanisms, Ceftazidime-Avibactam

1. Introduction

Carbapenem resistant Enterobacteriaceae (CRE), MDR *Pseudomonas aeruginosa* (MDRPSA) and MDR *Acinetobacter* (MDRAC) are urgent and serious threats as designated by the Centers for Disease Control and Prevention (CDC) [1]. Phenotypic expression of carbapenem resistance (reported by many clinical microbiology laboratories) can be due to numerous mechanisms with β-lactam hydrolyzing enzymes being the major contributor [2]. Therefore, it is important to classify which enzyme(s) is (are) present since detection is a vital component in defining their epidemiology, controlling spread, as well as aiding appropriate early therapeutic interventions. Since our hospital has a long history of β-lactam resistance present in multi-drug resistant gram-negative bacteria, an investigation was undertaken to characterize current enzymes associated with carbapenem resistant gram-negative bacteria. We also assessed the *in vitro* activity of ceftazidime-avibactam (CA), which has been shown to inhibit a wide spectrum of β-lactamase producing organisms, including those with KPC enzymes.

2. Materials and Methods

2.1. Microbiology and Susceptibility

One-hundred and thirty-one carbapenem non-susceptible gram-negative single-patient clinical isolates from blood, urine, sputum, and stool were identified in our Clinical Microbiology Laboratory using the Vitek 2® GN (gram-negative) ID card (bioMerieux, Durham, NC). Isolates identified from community and hospitalized patients as well as residents of an affiliated long-term care facility (LTCF) were selected from January 2012 through December 2015. Of these, 87 were Enterobacteriaceae consisting of 9 *Citrobacter* species, 29 *Escherichia coli*, 27 *Enterobacter* species, 13 *Klebsiella pneumoniae*, 8 *Serratia marcescens* and 1 *Morganella morganii*; 44 were non-fermenters which included 24 carbapenem non-susceptible *Pseudomonas aeruginosa* and 20 carbapenem non-susceptible *Acinetobacter*. Susceptibility to CA by E-test methodology was performed according to the manufacturer's specifications (bioMerieux, Durham, NC). *Klebsiella pneumoniae* ATCC 700603 was tested against CA and ceftazidime alone by E-test methodology to confirm the activity of avibactam.

2.2. DNA Isolation and Amplification

Bacteria were grown overnight on TSA II 5% blood agar plates and DNA was extracted from colonies using the DNeasy® blood and tissue kit (Qiagen Sciences, Germantown, MD). The Check-MDR CT103 XL microarray system was used for detection of β-lactamases according to manufacturer's instructions (Check points, Wegeningen, Netherlands). A description of the microarray technology and β-lactamases that can be detected

has been described in detail [3] [4] [5].

3. Results

3.1. β-Lactamase Genotypes Identified by Check-MDR CT103 XL Microarray System

Table 1 lists the enzymes characterized in this study. KPC β-lactamases were present in 79/87 (91%) of CRE with 28/29 (97%) in *E. coli*, 11/13 (85%) in *K. pneumoniae*, 9/9 (100%) in *Citrobacter* species (8 *Citrobacter freundii,* and 1 *Citrobacter koserii*), 4/8 (50%) in *Serratia marcescens* and 19/27 (70%) in *Enterobacter* species (4 *Enterobacter aerogenes*, 21 *Enterobacter cloacae*, 1 *Enterobacter asburiae*, and 1 *Enterobacter gergoviae*). Among *E. coli* isolates, TEM (18/29, 62%), SHV (5/29, 17%), ESBLs (7/29, 24%), and plasmid-mediated AMP-C (1/29, 3% ACT/MIR and 1/29, 3% CMY) enzymes were documented. *K. pneumoniae* isolates harbored TEM (9/13, 69%), SHV (12/13, 92%), CTX-M (5/13, 38%), OXA-48 (1/13, 8%), and ESBLs (5/13, 38%) β-lactamases. In *Citrobacter* isolates, enzymes detected were TEM (7/9, 78%), SHV (2/9, 22%), ESBLs (2/9, 22%), and plasmid-mediated AMP-C (2/9, 22% ACT/MIR and 1/9, 11% CMY). In *Enterobacter* species, 10/27 (37%) harbored ESBLs and 20/27 (74%) possessed plasmid mediated β-lactamases including ACT/MIR (17/27, 63%), DHA (2/27, 7%) and FOX (1/27, 4%). In *S. marcescens*, TEM (8/10, 80%), CTX-M (1/10, 10%), KPC (4/8, 50%) and ESBLs (4/8, 50%) were characterized. The microarray also characterized OXA-23 in one *M. morganii* isolate and OXA-48 in one *K. pneumoniae* isolate.

Among non-fermenters, TEM or SHV enzymes (5/20, 25%), OXA-23 (14/20, 70%), and OXA-24 (3/20, 15%) were characterized in *Acinetobacter* species. TEM (3/24, 13%), and ESBL (1/24, 4%) were also found in *P. aeruginosa*.

No NDM, VIM, IMP, GIM or SPM metallo-β-lactamases were identified in any of the isolates.

Table 1. β-lactamase genotypes identified in 131 carbapenem non-susceptible gram-negative bacteria by Check-MDR CT103 XL microarray system.

Bacteria (no. of isolates)	β-lactamase Genotypes											
	TEM	SHV	CTX-M	ESBL	KPC	OXA-23	OXA-24	OXA-48	ACT/MIR	DHA	FOX	CMY
Escherichia coli (29)	18/29	5/29		7/29	28/29				1/29			1/29
Klebsiella pneumoniae (13)	9/13	12/13	5/13	5/13	11/13			1/13				
Enterobacter species (27)				10/27	19/27				17/27	2/27	1/27	
Morganella morganii (1)						1/1						
Citrobacter species (9)	7/9	2/9		2/9	9/9				2/9			1/9
Acinetobacter species (20)	2/20	3/20		3/20		14/20	3/20					
Serratia marcescens (8)	8/8		1/8	4/8	4/8							
Pseudomonas aeruginosa (24)	3/24			1/24								

3.2. Minimal Inhibitory Concentration of Ceftazidime and Ceftazidime-Avibactam by E-Test Methodology

Minimal inhibitory concentration (MIC) for CA by E-test (Table 2) was ≤2 μg/ml for 88/89 (98.9%) Enterobacteriaceae tested with one *Enterobacter cloacae* isolate having an MIC of 3 μg/ml. Among carbapenem non-susceptible *Pseudomonas aeruginosa,* $MIC_{50/90}$ of 12 μg/ml/24μg/ml for ceftazidime, 3 μg/ml/6μg/ml for CA and >256/>256, 16/>256 μg/ml for carbapenem non-susceptible *Acinetobacter* respectively, were recorded.

4. Discussion

In this surveillance study designed to identify current *β*-lactamases in a New York City hospital, KPC *β*-lactamases were the predominant enzymes characterized in carbapenem non-susceptible Enterobacteriaceae and reinforces our previously published studies recognizing such enzymes in hospitalized and community patients and in residents of our associated LTCF [6] [7] [8] [9]. The detection of OXA-48 in *K. pneumoniae* and OXA-23 in *Morganella morganii* are novel findings in our isolates. Although OXA-23 has previously been reported in *Proteus mirabilis* and in *E. coli*, OXA-23 has never been reported in *M. morganii* to our knowledge [10] [11]. Among class C enzymes, the detection of DHA and FOX in *Enterobacter* species and CMY in *E. coli* and *Citrobacter* species were also new findings in our isolates. CMY-II, ACT-1, FOX, and DHA plasmid mediated *amp C* enzymes in conjunction with loss of outer membrane porins, can lead to treatment failure with carbapenem monotherapy [12] [13] [14] [15]. Recognition of these enzymes with genotypic methods may also support more timely infection control intervention as we and others have previously demonstrated in the setting of carbapenem resistant *K. pneumoniae* outbreaks [14] [15]. The detection of OXA-23 in *M. morganii* was a serendipitous finding and would not have been recognized with earlier

Table 2. Minimal inhibitory concentration of ceftazidime and ceftazidime-avibactam by E-test methodology.

Bacteria (no. of isolates)	Ceftazidime $MIC_{50/90}$ (μg/ml)	Range	Ceftazidime avibactam $MIC_{50/90}$ (μg/ml)	Range	Ceftazidime avibactam breakpoints susceptible/resistant (μg/ml)
Escherichia coli (29)	96.0/>256	8.0->256	0.5/1	0.016-2	≤8/4/≥16/4
Klebsiella pneumoniae (13)	64.0/>256	12.0->256	1.0/2	0.25-2	≤8/4/≥16/4
Enterobacter species (27)	32.0/256	0.38->256	0.5/1.5	0.19-3	≤8/4/≥16/4
Morganella morganii (1)	N/A	3	N/A	3	≤8/4/≥16/4
Citrobacter species (9)	N/A	1->256	N/A	0.94-1.5	≤8/4/≥16/4
Acinetobacter species (20)	>256/>256	0.75.0->256	24.0->256	0.75->256	N/A
Serratia marcescens (8)	N/A	1.0->256	N/A	0.064-192	≤8/4/≥16/4
Pseudomonas aeruginosa (24)	12/24	1.5->256	3.0/6	0.75-8	≤8/4/≥16/4
Klebsiella pneumoniae ATCC 700603 control	24	N/A	1	N/A	≤2/>16

N/A = not applicable.

Check MDR microarray systems or other commercially available systems. These enzymes are not inhibited by any current β-lactamase inhibitors, including avibactam. This is delineated in **Table 2** with no difference in MICs (3 μg/ml) between ceftazidime alone and CA. This is in contrast to the OXA-48 enzyme found in *K. pneumoniae* which is inhibited by avibactam, (MIC for ceftazidime = 128 μg/ml, MIC for CA = 0.75 μg/ml). Among *Acinetobacter* isolates, OXA-23 and OXA-24 were the predominant carbapenemases, a finding consistent with our earlier studies [16] [17]. While all *P. aeruginosa* in this study were reported as carbapenem resistant by the Vitek 2® system, the Check-MDR CT103 XL Microarray reported no carbapenem resistant genotypes likely due to lack of specific *ampC* primers for *Pseudomonas aeruginosa*. To our knowledge, no commercial system is available to detect such enzymes.

Carbapenem resistance in *Pseudomonas aeruginosa* can be due to the interplay of up regulated efflux, chromosomal *ampC* β-lactamase production, and diminished *oprD* [18]. Thus, both phenotypic and genotypic testing may be necessary for better prediction of antibacterial resistance and opportunity for better directed therapy in these and additional isolates expressing these determinants.

An earlier study evaluating the *in vitro* activity of CA against all *Enterobacteriaceae* isolates from US hospitals with characterization of associated β-lactamases, documented $MIC_{50/90}$ of 0.12/0.25 μg/ml for CA [19]. In contrast, we found $MIC_{50/90}$ of 0.5/1.5μg/ml for carbapenem non-susceptible strains. Another survey, which also characterized such isolates and their susceptibility to CA, reported $MIC_{50/90}$, of 1/1μg/ml [20]. Although not assessed in our study, elevated MICs have been associated with KPC-3 strains of *K. pneumoniae* as well as strains with ESBLs and porin mutations and this is an area for future investigation [21].

We have previously used other molecular techniques to characterize β-lactamase encoding genes in 24% of the isolates analyzed in this study [7] [8] [22] [23] LaBombardi VJ, unpublished results]. However, recent studies have demonstrated sensitivities and specificities of 100% and accuracies of 94% for the detection of known resistance genes using the Check-Points MDR CT103 XL microarray kit (4, 5).

In conclusion, we have identified class A, C and D β-lactamase encoding genes in carbapenem non-susceptible gram-negative clinical isolates at our community hospital in New York City. Molecular characterization in conjunction with phenotypic reporting by clinical microbiology laboratories can provide a "snapshot" of enzymes present within a geographical area, can support antibiogram data and reveal β-lactamases that are "silently disseminating" [24] [25]. Genotypic results from systems such as the one used in this study may answer the question: "are susceptibility tests enough, or should laboratories still seek ESBLs and carbapenemases directly?" [26]. Periodic sampling such as this may be beneficial to describe epidemiologic evolution of carbapenem resistant organisms. Combining phenotypic and genotypic data has significant potential for identifying unrecognized reservoirs in both hospital and LTCFs, can be instrumental in detecting outbreaks, and provide assistance to infection control and antibiotic stewardship programs which can potentially improve patient outcomes.

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