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Fecal Carriage of Genes Associated with Gram-Negative Multi-Drug Resistant Organisms

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Amended Abstract

Background: Screening for colonization with Gram-negative multidrug-resistant organisms (MDRO) is usually limited to the investigation of outbreaks. We screened stool samples from hospitalized patients for carbapenemase and extended-spectrum β-lactamase (ESBL) genes to determine the prevalence of MDRO colonization and association with infection.

Methods: Two-hundred stool samples received for *Clostridium difficile* PCR testing at Cleveland Clinic were also analyzed at OpGen using the Acuitas® MDRO Gene Test, a microfluidic PCR array targeting seven antimicrobial resistance gene families (CTX-M, KPC, NDM, VIM, IMP, OXA, VanA). Stool was incubated overnight in tryptic soy broth containing 3 ug/mL ceftriaxone prior to gene testing. The medical records of patients carrying genes associated with Gram-negative MDRO were reviewed.

Results: The positivity rate for *C. difficile* PCR was 16%. VanA was detected in 89 patients. Samples from 27 patients were positive for Gram-negative resistance genes. CTX-M genes were detected in the stool of 12 patients and infections due to ESBL-producing *Klebsiella pneumoniae* or *Escherichia coli* were documented in six of the patients. Gram-negative infections caused by carbapenem-resistant or AmpC phenotype organisms occurred in two patients with CTX-M carriage. KPC resistance genes were detected in nine stool samples and four of those patients had Gram-negative MDRO infections (two with carbapenem-resistant *K. pneumoniae*, one with ESBL *K. pneumoniae* and one with an AmpC phenotype *Enterobacter aerogenes*). Three of the five patients with VIM detected in the stool had infections caused by meropenem-resistant *Pseudomonas aeruginosa*. OXA genes were detected in the stool of two patients and the only documented infection was not caused by an MDRO.

Conclusions: Colonization with OXA, VIM, KPC, and CTX-M genes was observed in 1%, 3%, 5%, and 6% of inpatients, respectively. The majority of patients (52%) with carriage of these genes had documentation of multidrug-resistant Gram-negative infections in their medical record at variable time intervals relative to the date of sample collection.

Introduction

The emergence of multidrug-resistant organisms (MDRO) is an ongoing concern. Infections caused by carbapenemase-producing organisms are associated with high mortality and outbreaks. The carbapenemase detected most often in the United States is encoded by the *Klebsiella pneumoniae* carbapenemase (KPC) gene and usually found in *Enterobacteriaceae*, but has also been reported in *Pseudomonas aeruginosa* and *Acinetobacter* spp. Other enzymes responsible for carbapenem-resistant *Enterobacteriaceae* (CRE), *P. aeruginosa*, and *Acinetobacter* spp. include the New Delhi metallo-β-lactamase (NDM), the Verona integron-encoded metallo-β-lactamase (VIM), active on imipenem (IMP), and oxacillin-hydrolyzing (OXA) β-lactamase families. The activity of carbapenems is retained for *Enterobactericeae* harboring extended-spectrum β-lactamases (ESBL) such as CTX-M. Because screening for carriage of Gram-negative MDRO is usually performed for outbreak investigations, information regarding colonization rates is limited. The primary purpose of this study was to determine the fecal carriage rate of antimicrobial resistance genes by inpatients with stools being assessed for *Clostridium difficile* infection. A secondary objective was to determine the association of resistance gene carriage with MDRO infection.

Methods

Prior to initiation of the study, IRB approval was obtained at the Cleveland Clinic. Remnant liquid stool specimens (n=200) submitted to the Cleveland Clinic Microbiology laboratory for *Clostridium difficile* PCR testing were de-identified and sent to OpGen (Gaithersburg, MD). Overnight incubation of each stool sample in selective tryptic soy broth containing 3 µg/mL ceftriaxone was followed by genomic DNA extraction. Screening for CTX-M, KPC, NDM, VIM, IMP, OXA, and VanA antimicrobial resistance determinants was performed using the Acuitas® MDRO Gene Test, a PCR-based microfluidic array. A chart review of patients with Gram-negative resistance genes detected was performed to assess the relationship between fecal carriage of antimicrobial resistance genes and the susceptibility profile of organisms causing infections.

Results

C. difficile PCR was positive for 16% of patients

Stool samples from 27 patients (14%) were positive for Gram-negative resistance genes (Fig 1). VanA was detected in 89 patients

Results of the chart review correlating Gram-negative resistance gene carriage with infection are outlined in Table 1 and summarized below:

CTX-M gene carriage (12 patients)
- ESBL-producing *K. pneumoniae* or *Escherichia coli* infections (n=6, 50%)
- Carbapenem-resistant or AmpC phenotype infections (n=2)

KPC gene carriage (9 patients)
- Carbapenem-resistant *K. pneumoniae* infections (n=2, 22%)
- ESBL-producing *K. pneumoniae* infection (n=1)
- AmpC phenotype *Enterobacter aerogenes* (n=1; same patient with CTX-M)

VIM gene carriage (5 patients)
- Infections caused by meropenem-resistant *P. aeruginosa* (n=3, 60%)

OXA gene carriage (2 patients)
- No MDRO infections

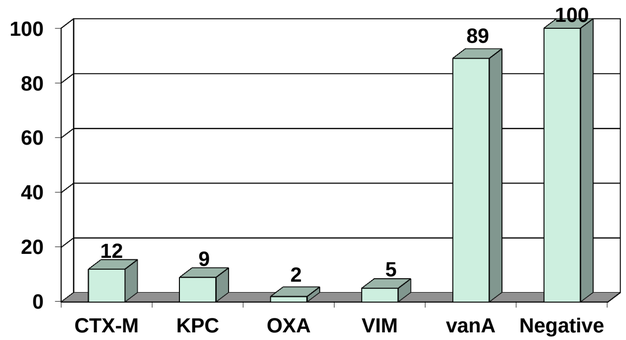

Fig 1. MDRO Gene Test results for 200 patients

Table 1. Correlation of resistance gene detection in 200 stool samples submitted for *C. difficile* testing with Gram-negative MDRO infections

Patient	Fecal MDRO gene test results					*C. difficile* PCR results	Clinical isolates from culture	
	KPC	CTX-M	OXA	VIM	VanA		MDRO phenotype	Organism (site)
42	X				X		CR	*K. pneumoniae* (abdom drain; 4 days earlier), *P. aeruginosa* (blood), VRE (urine)
82	X				X		CRE	*K. pneumoniae* (10-50 K cfu/ml urine; 2 years earlier)
50	X						No	No cultures ordered
73	X						No	Negative cultures
153	X					Pos	No	Negative cultures
155	X				X		No	MRSA bacteremia
157	X				X		ESBL	*K. pneumoniae* (blood; 12 days earlier)
167	X						No	Negative cultures
48	X	X			X		AmpC	*E. aerogenes* & VRE (peritoneal fluid; 1 week later)
2		X			X		ESBL	*K. pneumoniae* (blood; 9 days earlier)
27		X			X	Pos	ESBL	*K. pneumoniae* (respiratory; 3 months earlier)
35		X			X		CRE	*K. pneumoniae* (wound; 2 months earlier)
37		X			X	Pos	ESBL	*E. coli* (>100 K cfu/ml urine; 32 days later)
80		X					ESBL	*E. coli* (>100 K cfu/ml urine; recurrent starting 2 years earlier)
149		X			X		ESBL	*K. pneumoniae* (>100 K cfu/ml urine; 33 days later)
150		X			X		No	Susceptible *P. aeruginosa* (pacemaker lead, tissue pocket)
154		X			X		No	Negative wound culture
161		X					No	Only culture (stool) negative
168		X			X		ESBL	*K. pneumoniae* (ascites fluid; SBP + *C. freundii*, VRE; 21 days later)
202		X					No	Susceptible *P. mirabilis* (>100 K cfu/ml urine; 79 days later)
30			X				No	Amp resistant *E. coli* (>100 K cfu/ml urine; concurrent and 3 months later)
183			X		X		No	Negative cultures
94				X	X		CR	*P. aeruginosa* (sternal wound; recurrent starting 46 days earlier)
102				X	X		CR	*P. aeruginosa* (trach asp; 13 days later; CF; fatal resp failure)
105				X	X		No	Susceptible *E. cloacae* (10-50 K cfu/ml urine; 5 days earlier)
106				X	X		No	MSSA (blood)
165				X	X		CR	*P. aeruginosa* (blood; 2 days later; fatal septic shock)
Other patients					71	29		
Total (%)	9 (4.4)	12 (5.9)	2 (1.0)	6 (2.9)	91 (44.6)	32 (15.7)		

Conclusions

Colonization with CTX-M, KPC, VIM, and OXA genes was observed in 6%, 5%, 3%, and 1% of inpatients, respectively. Multidrug-resistant Gram-negative infections were documented in the medical record at variable time intervals before and/or after sample collection for 52% of patients colonized with Gram-negative resistance genes.

References

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