Determining the Optimal Screening Protocol to Detect Hospitalized Patients Colonized with Carbapenem Resistant *Enterobacteriaceae*: A Comparison of Laboratory Methods

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ABSTRACT

Severe infections caused by multi-drug resistant gram negative rods (MDR-GNR) including carbapenem resistant *Enterobacteriaceae* (CRE) are a source of significant healthcare costs and mortality. Implementing laboratory surveillance programs to identify hospitalized patients colonized by CRE represents an important component of an overall strategy to minimize the spread of resistant bacteria. Several CRE screening methods have been described, but there is no consensus as to which method is most appropriate for identifying colonized patients. The purpose of this study was to directly compare the performance characteristics of available methods of detecting gastrointestinal colonization by CRE. Rectal swabs originally obtained from hospitalized patients for VRE surveillance were de-identified and subjected to CRE screening procedures. Of the methods tested, we found that chromogenic agar plates formulated to detect bacteria expressing extended spectrum β-lactamases were the least time consuming and most sensitive procedure for identifying colonization by CRE in this hospitalized patient population (80% sensitivity). The recommended Centers for Disease Control screening method demonstrated comparatively poor sensitivity (60%). Understanding the performance of available screening methods, as well as the limitations associated with each of these methods, is essential prior to initiating a hospital wide CRE colonization surveillance program.

BACKGROUND

In hospitals where extended spectrum β-lactamase (ESBL) expressing MDR-GNR infections are common, ESBL-stable carbapenem antibiotics are frequently prescribed for empiric antimicrobial therapy. Most drugs with some efficacy against CRE have suboptimal antimicrobial properties and side effects (1, 2). Unfortunately, the prevalence of CRE is increasing.

The CDC has recommended a limited assessment of CRE rates in US hospitals, but there is no consensus among infection control and public health officials on the role of active surveillance in different clinical scenarios (3). Additionally, there is no agreement within the microbiology laboratory community about how best to implement a screening program for CRE (4). Several CRE screening methods have been described, but few studies directly compare multiple available methods for CRE screening using patient specimens.

OBJECTIVE

The goal of this study was to compare multiple described methods to determine the optimal laboratory screening protocol to identify patients with gastrointestinal colonization by CREs in a non-outbreak setting. Based on available data, we hypothesized that HardyCHROM ESBL plates will be the most sensitive and efficient culture technique for CRE detection.

METHODS

Vancomycin-resistant enterococci (VRE) surveillance swabs were de-identified and retained for the purpose of this study. Swabs were submerged in 2 mL of tryptic soy broth (TSB) and vortexed for 30 seconds. This liquid media was reserved for use in the carbapenem resistance screening methods as described below. Additionally, 100 µl of inoculated TSB was plated directly to Columbia blood agar plates, incubated at 37°C overnight and examined for bacterial growth. Specimens that did not yield growth on Columbia blood agar or any of the three screening methods were considered inadequate for analysis and not evaluated further.

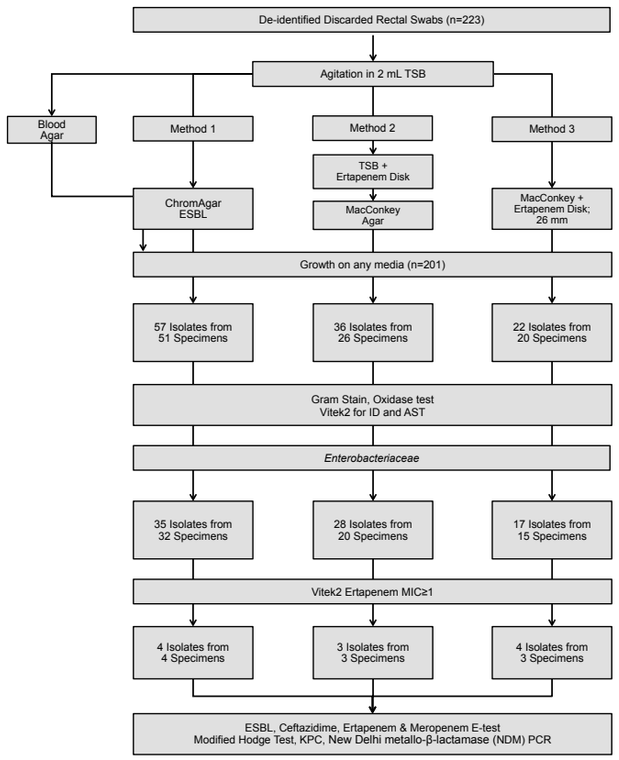

Figure 1. Overview of study workflow and procedures. Endpoint PCR for the KPC gene was performed directly on primary specimens.

Table 1: Summary of Methods

	Primary Incubation	Secondary Incubation	Preliminary Screen Positive	Reference
Method 1	HardyCHROM ESBL	None	Any growth at 24 or 48 h	Product Insert
Method 2	5 mL TSB with 10 µg ertapenem disk	MacConkey Agar	Any growth on MacConkey agar	(5)
Method 3	MacConkey Agar with 10 µg ertapenem disk	None	Any growth within 26 mm zone of ertapenem	(6)

Table 1. General method descriptions and definitions of screen positive specimens.

RESULTS

Table 2: Subcultured Microorganisms from Screen-Positive Specimens

	Enterobacteriaceae	Non-Fermenting GNR	Other
Method 1 (n=57)	35	14	8
Method 2 (n=36)	28	8	0
Method 3 (n=22)	17	5	0
Other Includes: GPC (5), Yeast (2), GPR (1)			

Table 2. Comparison of numbers and types of microorganisms isolated from screen-positive specimens by each method.

Table 3: Colony Characteristics of CA ESBL Isolates

Color	*Enterobacteriaceae* (n=35)	NF GNR (n=14)	GPC / GPR (n=6)	Yeast (n=2)
Pink / Magenta	22	0	1	1
Blue	9	1	5	0
Clear / White	3	11	0	1
Green	1	2	0	0

Table 3. Colony characteristics of isolates from HardyCHROM ESBL plates. Per manufacturer instructions, *E. coli* produces pink/magenta colonies. *Citrobacter*, *Klebsiella* and *Enterobacter* spp. produce blue colonies. *Proteus* and *Morganella* spp. produce clear colonies, and occasional *Proteus vulgaris* isolates produce green colonies.

Table 4: Performance Characteristics of Methods

		Ertapenem Resistant					
		Positive	Negative				
Method 1	Positive	4	46	Sens	80	PPV	8
	Negative	1	150	Spec	76.5	NPV	99.3
Method 2	Positive	3	22	Sens	60	PPV	12
	Negative	2	174	Spec	88.8	NPV	98.9
Method 3	Positive	3	16	Sens	60	PPV	15.8
	Negative	2	180	Spec	91.8	NPV	98.9
Method 1**	Positive	4	38	Sens	80	PPV	9.5
	Negative	1	158	Spec	80.6	NPV	99.4
** Exclude non-GNR by Gram stain							

Table 4. Method performance characteristics for identifying CRE. While Method 1 (HardyCHROM ESBL plates) was most sensitive for detecting CRE, it also yielded many false positives. The other methods had many false positives as well, and thus the PPV is low.

Table 5: Ceftazidime MIC by Vitek2 for False-Positive *Enterobacteriaceae*

	≤ 4 µg/mL	= 8 µg/mL	≥16 µg/mL
Method 1 (n=31)	10	2	19
Method 2 (n=25)	21	1	3
Method 3 (n=13)	12	0	1
p < 0.001 by Fisher Exact Probability Test			

Table 5. Ceftazidime MIC for false-positive *Enterobacteriaceae*. While Method 1 identified the most false positive CRE isolates, these isolates generally had a high ceftazidime MIC, and were often characterized as ESBL-positive.

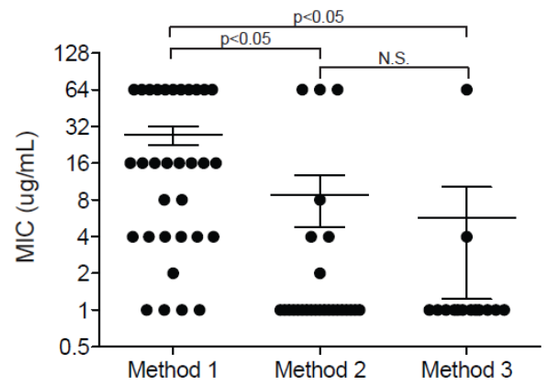

Figure 2. Comparison of ceftazidime MICs for false-positive CRE isolates for each method. Both the rates of ceftazidime resistance and the median MIC for ceftazidime were significantly higher for false-positive isolates identified by Method 1 than they were by either Methods 2 or 3.

CONCLUSIONS

- HardyCHROM ESBL plates with a confirmatory antibiotic susceptibility test perform better than other published CRE detection methods.
- HardyCHROM ESBL plates were the most sensitive technique for the detection of CRE in rectal surveillance swabs.
- HardyCHROM ESBL plates yielded a number of "false positives," likely as a consequence of using these plates to screen for CRE instead of ESBL-expressing *Enterobacteriaceae*.
- Isolates recovered from the HardyCHROM ESBL plates had the highest rates of ceftazidime and ceftriaxone resistance, and many of these isolates were ESBL-positive by Vitek2.
- The other methods tested were more specific, but still generated substantial false positives. In contrast to the isolates recovered from the HardyCHROM ESBL plates, the false positives from Methods 2 and 3 tended to be pansusceptible GNR.

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