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Evaluation of the Direct MacConkey Method for Identification of Carbapenem-Resistant Gram-Negative Organisms from Rectal Swabs: Reevaluating Zone Diameter Cutoffs

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ABSTRACT The optimal method to screen for gastrointestinal colonization with carbapenem-resistant organisms (CRO) has yet to be established. The direct MacConkey (direct MAC) plate method demonstrates high sensitivity for CRO detection, but established zone diameter (ZD) criteria for ertapenem (≤27 mm) and meropenem (≤32 mm) result in high rates of false positives upon confirmatory testing. To increase specificity, we screened for CRO in two high-risk wards using the direct MAC plate method, recorded ZDs for each sample, and generated receiver operating characteristic (ROC) curves to evaluate the optimal ZD cutoff criteria. Of 6,868 swabs obtained over an 18-month period, 4,766 (69%) had growth on MAC plates, and 2,500 (36%) met criteria for further evaluation based on previously established ZDs around the carbapenem disks. A total of 812 (12%) swabs were confirmed positive for at least one CRO and included 213 (3%) carbapenemase-producing organisms (CPO), resulting in a specificity of 78% for the direct MAC plate method. Reducing the ertapenem and meropenem ZDs to ≤25 mm improved specificity to 83%, decreasing the confirmatory testing workload by 32%. The sensitivities with the lower ZD criteria were 89% for CRO and 94% for CPO, respectively. The direct MAC plate method criteria for CRO testing can be modified to balance the sensitivity and specificity of CRO while reducing the burden on clinical microbiology laboratories. These modifications can be particularly helpful in regions with a low CRO prevalence.

KEYWORDS CRO, carbapenem-resistant organism, direct MacConkey Plate method, colonization, method

Carbapenem-resistant organisms (CRO) are well recognized as significant public health concerns. Several global health entities, including the Centers for Diseases Control and Prevention (CDC) and the World Health Organization, have designated these organisms as high threat and critical priority pathogens, respectively (1, 2). Rectal surveillance cultures for carbapenem-resistant *Enterobacterales* (CRE) is recommended by the CDC in limited scenarios (3). Although universal admission screening would be the optimal approach, allowing for rapid isolation of patients to limit the spread in the health care setting, both logistical and cost constraints prevent this approach from being widely adopted (4). Some institutions have opted to screen high-risk patients, such as intensive care unit (ICU) or oncology and transplant patients, where colonization and subsequent infection can cause the highest morbidity and mortality (5, 6).

The optimal method to screen for gastrointestinal colonization with CRO has yet to

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be established. Most methods focus on CRE detection or identification of the "big five" carbapenemase genes (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, and $bla_{OXA\text{-}48\text{-}like}$) (7). These methods include broth enrichment, direct selective culture, chromogenic media, and detection of carbapenemase genes using molecular methods from fecal samples (8–14). Our group previously demonstrated that the direct MacConkey (direct MAC) plate method using ertapenem disks had the highest sensitivity for CRO detection compared to broth enrichment methods, the ChromID Carba chromogenic medium (research use only [RUO] version; bioMérieux, France), and the Check-Direct CPE molecular assay (RUO version; Check-Points, Wageningen, The Netherlands) (14). The direct MAC plate method is an affordable and user-friendly approach that utilizes standard laboratory materials. However, this method—as currently implemented—lacks specificity, leading to frequent isolation of carbapenem-susceptible organisms (8, 11). Previously, we processed 6,868 CRO surveillance rectal swabs among two high-risk wards applying the direct MAC plate method, using an ertapenem disk in the first quadrant and a meropenem disk in the second quadrant. Confirmatory testing was performed on isolated organisms. The objective of the current study was to reevaluate the zone diameter criteria for ertapenem and meropenem to determine whether the direct MAC plate method's specificity can be increased while maintaining acceptable sensitivity. We also present the descriptive epidemiology of CRO among the study cohort.

MATERIALS AND METHODS

Specimens. From January 2016 to June 2017, consecutive rectal ESwabs (Copan Diagnostics, Murrieta, CA) from patients in the medical intensive care unit (MICU) and solid organ transplant unit at The Johns Hopkins Hospital were collected at unit admission and weekly thereafter. The swabs were collected as part of a long-standing vancomycin-resistant *Enterococcus* (VRE) surveillance program. At the completion of standard-of-care testing, and prior to disposal, the swabs were deidentified and held at 4°C for up to 4 days prior to further testing. This study was approved by The Johns Hopkins University School of Medicine Institutional Review Board, with a waiver of informed consent.

Direct MacConkey plate method. The ESwabs were vortexed for 10 s, and a 100 μl aliquot of the liquid Amies broth was inoculated to a MacConkey (MAC) plate and streaked for isolation. An ertapenem disk was placed in the first quadrant and a meropenem disk in the second quadrant, followed by incubation overnight (18 to 24 h) at 35°C. The zone diameters (ZDs) around the carbapenem disks were recorded. If growth did not reach the meropenem disk, only the ertapenem zone diameter was assessed. Any Gram-negative bacilli (GNB) growing within a ZD of $\leq$27 mm of the ertapenem disk or $\leq$32 mm of the meropenem disk were further subcultured (8, 11) and identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Inc., Billerica, MA). Carbapenem antimicrobial susceptibility testing (AST) of GNB growing within the predefined carbapenem ZD was performed by disk diffusion, including ertapenem, meropenem, and imipenem for the *Enterobacterales* and meropenem and imipenem for glucose-nonfermenting organisms. Clinical and Laboratory Standards Institute (CLSI) interpretive criteria were applied (15). Carbapenem disk diffusion testing was not performed for *Stenotrophomonas maltophilia*, as this organism is intrinsically resistant to carbapenems due to a chromosomally encoded metallo-β-lactamase.

Carbapenemase detection. Carbapenemase production was determined using the modified carbapenem inactivation method (mCIM) for all GNB not susceptible (intermediate or resistant) to the carbapenems, as previously described (16–18). For isolates that were mCIM indeterminate or positive, genomic DNA was extracted using the DNeasy blood and tissue kit (Qiagen, Inc., Valencia, California). Identification of β-lactamase genes was performed using the Check-MDR CT103XL microarray-based assay (Check-Points, Wageningen, The Netherlands).

A convenience sample of 60 randomly selected swabs, that were found to be negative by the direct MAC method and for which remnant ESwab broth (300 μl) was available, were tested by the CARBA-R assay (Cepheid, Sunnyvale, CA) using an in-house-validated method. The convenience sample was chosen to confirm that swabs near the ZD cutoff (ertapenem ZD = 28 mm) for further workup were indeed negative for the most common carbapenemase genes (bla_{KPC}, bla_{NDM}, $bla_{OXA\text{-}48\text{-}like}$, bla_{VIM}, and bla_{IMP}).

Statistical analysis. The sensitivity and specificity of alternative ZD cutoffs, individually and in combination, for detecting CRO, carbapenemase-producing organisms (CPO), CRE, and carbapenemase-producing *Enterobacterales* (CPE) using the cultured isolate phenotypic and/or genotypic results as the reference standard were determined. Any direct MAC plate with no growth or with carbapenem ZDs that did not qualify for further workup was considered negative. Receiver operating characteristic (ROC) curves were generated for ertapenem and meropenem ZDs to evaluate the optimal ZDs for detection of CRO, CPO, CRE, and CPE.

TABLE 1 Distribution of carbapenemase-producing organisms isolated from 6,868 rectal swabs using the direct MacConkey plate method

Organism group or species	No. of organisms isolated	Carbapenemase (CP) gene(s) detected[b]
CPE isolates (n = 108)		
Citrobacter amalonaticus	7	bla_{KPC}
	2	bla_{KPC} and bla_{NDM}
	1	bla_{NDM}
	2	No CP detected
Citrobacter freundii	1	bla_{KPC}
	2	No CP detected (bla_{CMY-2})
Enterobacter cloacae	8	bla_{KPC}
	1	bla_{KPC} and $bla_{OXA-48-like}$
	26[a]	No CP detected (18 bla_{ACT} or bla_{MIR}, 8 negative)
Escherichia coli	6	bla_{KPC}
	2	No CP detected
Hafnia alvei	1	bla_{NDM}
Klebsiella (*Enterobacter*) *aerogenes*	1	bla_{KPC}
Klebsiella pneumoniae	26	bla_{KPC}
	13	$bla_{OXA-48-like}$ and bla_{NDM}
	5	bla_{NDM}
	1	No CP detected
Morganella morganii	3	No CP detected (1 bla_{ACT} or bla_{MIR}, 1 bla_{DHA}, and 1 negative)
CP-NF isolates (n = 105)		
Acinetobacter baumannii complex	12	bla_{OXA-24}
	1	bla_{OXA-23}
	6	No CP detected
Acinetobacter radioresistans	1	bla_{OXA-23}
Pseudomonas aeruginosa	3	bla_{VIM}
	7	No CP detected
Pseudomonas otitidis	1	No CP detected
Stenotrophomonas maltophilia	74	Not tested due to chromosomally encoded L1 metallo-β-lactamase

[a]A total of 14 isolates were indeterminate by the mCIM.
[b]Where no CP genes were identified, other β-lactamase genes detected are listed in parentheses.

RESULTS

Direct MAC culture results. A total of 6,868 rectal swabs from 2,968 patients (median of 2 swabs per patient; range, 1 to 35) were cultured for CRO by the direct MAC plate method. Of those, 4,766 (69.4%) had growth on MAC plates, with 2,500 (36%) meeting criteria for additional CRO evaluation based on the ZDs around the carbapenem disks. Of the 1,869 (27.2%) plates considered negative based on ertapenem ZD of >27 mm and meropenem ZD of >32 mm, a convenience sample of 60 swabs with direct MAC results near the ZD cutoff were selected and found to be negative for carbapenemase genes by the CARBA-R assay. Of the 2,500 requiring further workup, 1,542 (24.5%) qualified based on both carbapenem ZDs, whereas 557 (8.1%) qualified based solely on ertapenem ZD criteria and 401 (5.8%) qualified based solely on meropenem ZD criteria. Of note, as the meropenem disk was placed in the second quadrant of growth, there were some instances (n = 115) where meropenem ZDs could not be assessed due to no bacterial growth in the relevant quadrant. A total of 812 (11.8%) swabs were confirmed positive for at least one CRO based on the carbapenem AST (853 CRO isolates total) results, including 482 (7.1%) CRE, 362 (5.3%) carbapenem-resistant nonfermenters (CR-NF), and 9 (0.1%) carbapenem-resistant *Aeromonas* species (here grouped with the CR-NF). There were 41 (0.6%) swabs growing both CRE and CR-NF. Based on confirmed CRO by AST of recovered isolates, the specificity of the direct MAC plate method for detection of CRO using existing ZD criteria was 78.2% [6,056 (true negative)/6,056 + 1,688 (false positive) × 100] in this study.

Detection of carbapenemase producers. Of the 853 CRO isolated, 213 (3.1%) CPO were identified by the phenotypic mCIM method, including 108 (1.6%) CPE and 105 (1.5%) CP-CR-NF. Three swabs had both a CPE and carbapenemase-producing CR-NF (CP-NF) isolated. The CPO isolated in this study are described in Table 1. The most common CPEs were *Klebsiella pneumoniae* (n = 45; 41.7%), followed by *Enterobacter cloacae* complex (n = 35; 32.7%), *Citrobacter amalonaticus* (n = 12; 11.2%), and *Esche-*

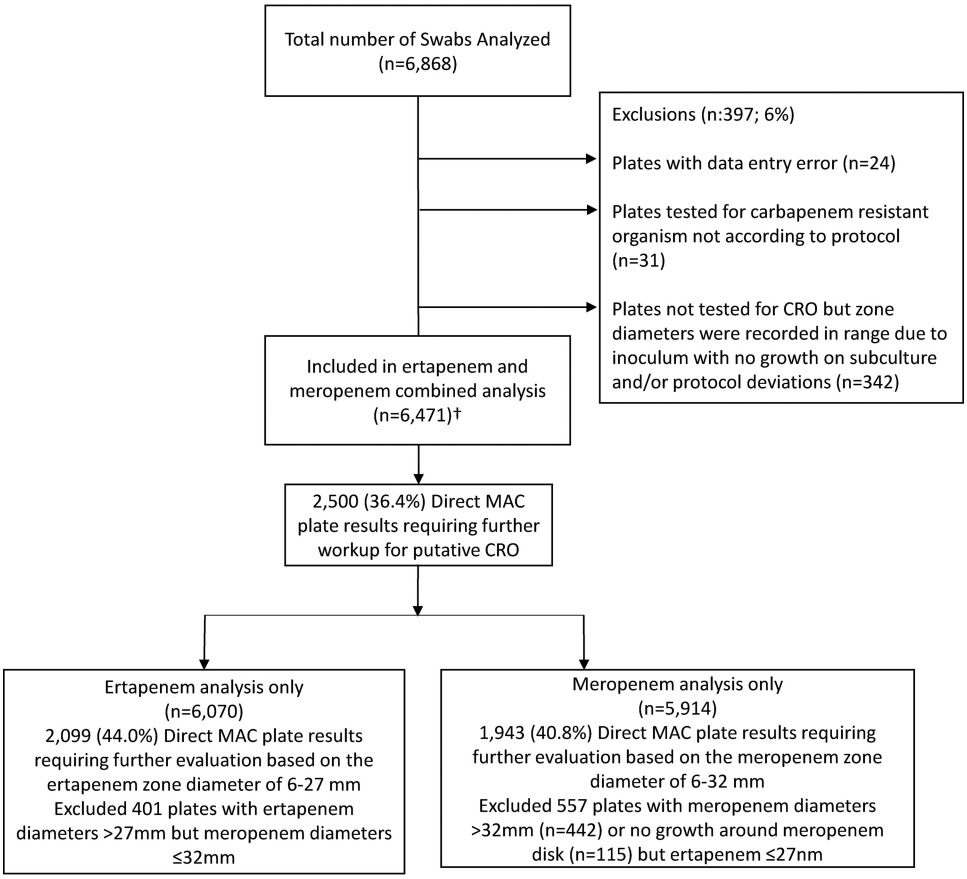

FIG 1 Receiver operator curve study design. The analysis marked with † included 2,500 (36.4%) plates with ertapenem diameters of 6 to 27 mm or meropenem diameters of 6 to 32 mm, 2,102 (30.6%) plates without MAC growth, and 1,869 (27.2%) plates with ertapenem diameters of >27 mm and meropenem diameters of >32 mm.

richia coli ($n = 8$; 7.5%). The most common carbapenemase genes identified among CPE were 53 (49.5%) bla_{KPC}, 12 (11.2%) $bla_{OXA\text{-}48\text{-}like}$ and bla_{NDM}, 9 (8.4%) bla_{NDM}, 2 (1.9%) bla_{KPC} and $bla_{NDM,}$ and 1 (0.9%) bla_{KPC} and $bla_{OXA\text{-}48\text{-}like}$. *S. maltophilia* ($n = 74$; 70.5%) was the most common CP-NF encountered and was presumably mediated by the chromosomally encoded L1 metallo-β-lactamase; it was followed by *Acinetobacter baumannii* ($n = 19$; 18.1%), with the great majority being OXA-24 producers. Of note, of 238 carbapenem-resistant *Pseudomonas aeruginosa* isolates, only 10 (4.2%) were identified as carbapenemase producers, with 3 VIM producers identified (1.3%).

Direct MAC plate ZDs for detecting CRO. Analysis of the median ZD was limited to results for which both ertapenem and meropenem disk criteria were available, which included 2,500 swabs requiring further workup and 2,102 swabs that were negative by the direct MAC plate method based on the carbapenem ZDs (Fig. 1). Median ertapenem and meropenem ZDs were 16 mm (interquartile range [IQR], 6 to 23) and 26 mm (IQR, 20 to 30) for direct MAC plates positive for CRO, versus 25 mm (IQR, 21 to 26) and 30 mm (IQR, 28 to 32) for direct MAC plates negative for CRO, respectively (P values <0.001). For CPO, the median ertapenem and meropenem ZDs were 11 mm (IQR, 6 to 20) and 20 mm (IQR, 6 to 28) for positives versus 24 mm (IQR, 18 to 25) and 30 mm (IQR, 27 to 31) for negatives, respectively (P values <0.001). Further breakdown of the median ZDs based on carbapenem-resistant, carbapenemase-producing, and carbapenemase genotype status are summarized in Table 2. Histograms of the direct MAC plate ertapenem and meropenem ZDs that were positive or negative for CRO indicated a distribution of both CRO-positive and CRO-negative cultures across all ZDs, but with a tendency for CRO-positive cultures to cluster at lower ZDs and CRO-negative cultures to cluster at higher ZDs (Fig. 2).

TABLE 2 Median ertapenem and meropenem zone diameter by carbapenem-resistant organism and carbapenemase-producing organism status

	Ertapenem (ZD = 6–27 mm, *n* = 2,099)			Meropenem (ZD = 6–32 mm, *n* = 1,943)		
		Median (IQR) diams (mm)			Median (IQR) diams (mm)	
Organism status	No. positive (%)	Positive	Negative	No. positive (%)	Positive	Negative
CRO	777 (37)	16 (6–23)	25 (21–26)	636 (32.7)	26 (20–30)	30 (28–32)
Organisms						
CRE	454 (21.6)	20 (11–24)	24 (18–26)	385 (19.8)	28 (22–30)	30 (27–32)
CR-NF	363 (17.3)	10 (6–20)	24 (20–26)	289 (14.9)	24 (17–30)	30 (28–32)
Mechanisms						
CPO	202 (9.6)	11 (6–20)	24 (18–25)	174 (9)	20 (6–28)	30 (27–31)
CPE	105 (5)	12 (6–20)	23 (17–25)	94 (4.8)	20 (6–28)	30 (26–31)
CP-NF	103 (4.9)	10 (6–20)	23 (17–25)	86 (4.4)	19 (6–28)	30 (26–31)
KPC CP-CRE	51 (2.4)	6 (6–17)	23 (16–25)	46 (2.4)	16 (6–25)	30 (26–31)
NDM CP-CRE	21 (1)	6 (6–12)	23 (16–25)	19 (1)	6 (6–18)	30 (26–31)
OXA-48-like CP-CRE	13 (0.6)	6 (6–6)	23 (16–25)	12 (0.6)	6 (6–12)	30 (26–31)

Receiver operating characteristic curves. ROC curves were constructed to evaluate the optimal cutoff criteria to balance sensitivity and specificity for implementation of the direct MAC plate method. The ROC curves for CRO using the direct MAC plate method showed an area under the curve (AUC) of 0.93 (95% confidence interval [CI], 0.92 to 0.94) for ertapenem and an AUC of 0.87 (95% CI, 0.85 to 0.89) for meropenem (Fig. 3). The ROC curves for CPO using the direct MAC plate method indicated an AUC of 0.92 (95% CI, 0.90 to 0.94) for ertapenem and an AUC of 0.91 (95% CI, 0.88 to 0.93) for meropenem (Fig. 3). Table 3 summarizes the sensitivity and specificity of the combined ertapenem and meropenem ZD cutoffs for the various CRO groups when a ZD could be measured.

Establishing new zone diameter criteria—balancing sensitivity and specificity. Due to the reduced specificity of the direct MAC plate method (36% of swabs required further workup, while only 12% were positive for CRO), we assessed whether we could establish new ZD criteria for when further testing would be necessary. We generated individual and combined ertapenem and meropenem ZD charts to assess the impact of narrowing the ZD on both sensitivity and specificity (Table 3; see also Tables S1 and S2). Using these charts, we found that an ertapenem and meropenem ZD of ≤25 mm provided sensitivities for CRO and CPO detection of 89% and 94%, respectively. The

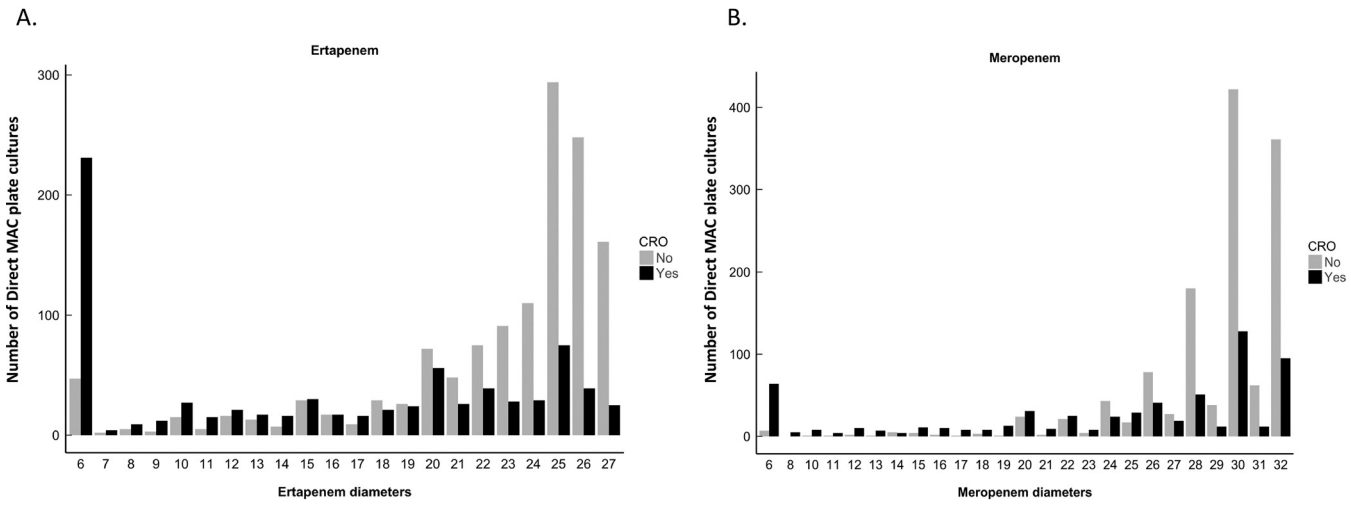

FIG 2 Histograms demonstrating the distribution of the direct MacConkey plate method ertapenem and meropenem zone diameters that were determined to be positive or negative for CRO.

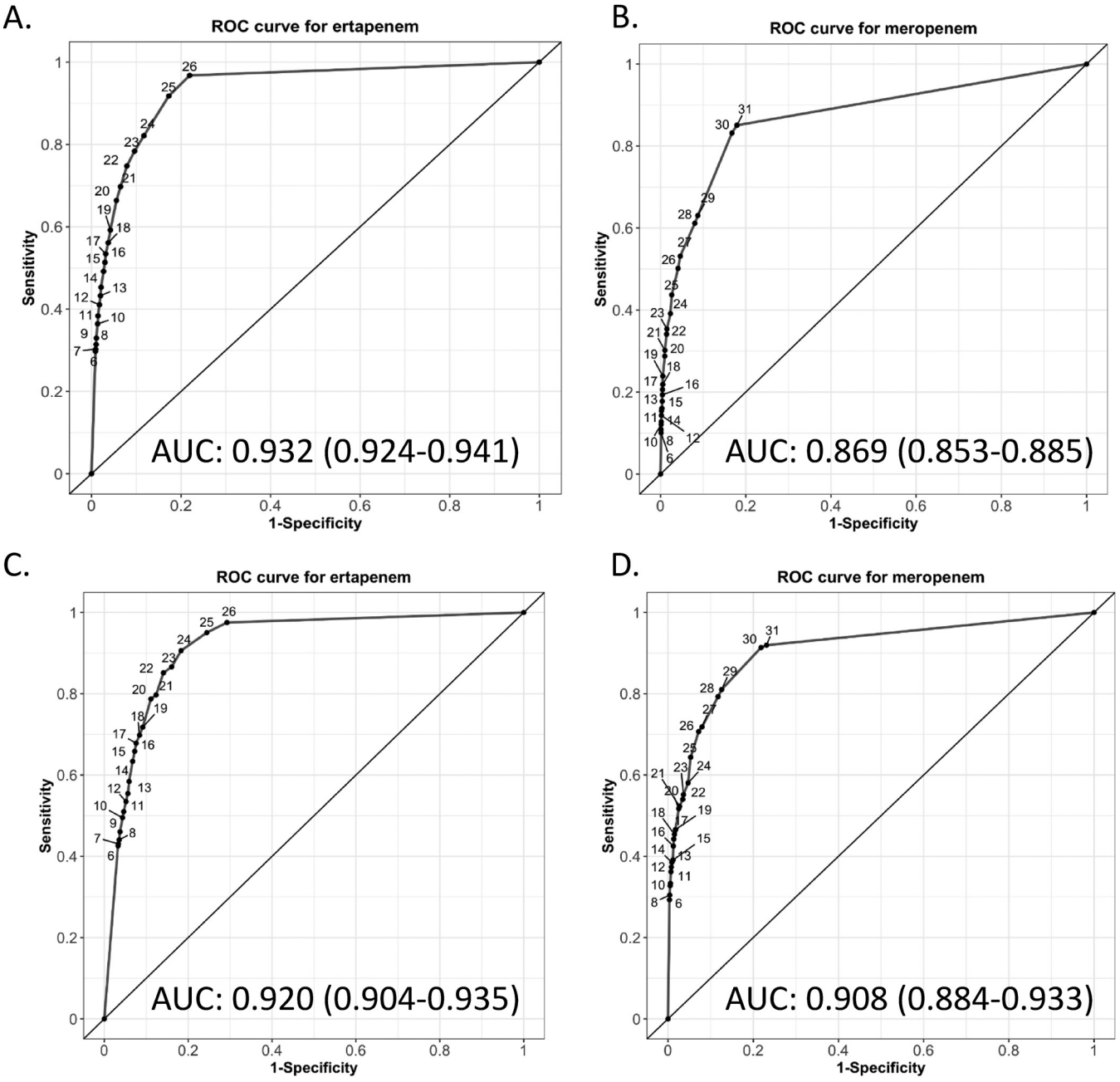

FIG 3 Receiver operator curves for ertapenem and meropenem zone diameters to detect carbapenem-resistant organisms and carbapenemase-producing organisms by the direct MacConkey plate method. ROC curves for (A) ertapenem and (B) meropenem for detection of carbapenem-resistant organisms (CRO). ROC curves for (C) ertapenem and (D) meropenem for detection of carbapenemase-producing organisms (CPO). The numerical points on the ROC represent different zone diameter readings for the direct MAC cultures. AUC, area under the curve.

specificities for CRO and CPO detection were 83% and 76%, respectively. Of the 2,500 cultures requiring additional evaluation, the smaller ZDs would have reclassified 814 as negative, resulting in an increased specificity from 76% to 83% for CRO and a 32% reduction in workload. When narrowing the ZD size to 25 mm, 89 swabs positive for 92 CRO would have been missed, including 79 that were positive for one or multiple non-CP-CRO and 10 that were positive for CPO. The 10 positive CPO swabs included 6 *S. maltophilia*, 2 carbapenemase-producing *K. pneumoniae* (1 KPC and 1 NDM), 1 NDM-producing *Hafnia alvei*, and 1 *A. baumannii* isolate. The non-CP-CRO isolates were mostly CRE ($n = 34$) that were intermediate or resistant to ertapenem only, or the

TABLE 3 Performance characteristics of the combined ertapenem and meropenem zone diameter results for the direct MAC plate method

Ertapenem zone diameter (mm)	Meropenem zone diameter (mm)	CRO (%)		CPO (%)	
		Sensitivity	Specificity	Sensitivity	Specificity
24	24	81	88	91	82
24	25	81	88	91	82
24	26	81	87	91	81
24	27	82	87	91	81
24	28	84	85	92	78
24	29	84	84	92	78
24	30	89	79	94	73
24	31	90	79	95	72
25	24	89	83	94	76
25	25	89	83	94	76
25	26	89	83	94	76
25	27	90	82	94	76
25	28	91	81	94	74
25	29	91	81	94	74
25	30	94	77	97	70
25	31	94	76	97	69
26	24	94	79	97	72
26	25	94	79	97	72
26	26	94	79	97	72
26	27	94	79	97	72
26	28	94	78	97	71
26	29	94	77	97	71
26	30	97	75	99	68
26	31	97	74	99	67

Morganella-Proteus-Providencia group ($n = 19$) that demonstrate intrinsically elevated MICs to imipenem.

Investigating CPE missed using a 25-mm zone diameter cutoff for carbapenems. The most concerning misses using the smaller ZD cutoff of 25 mm for both carbapenems were the 3 CPE-positive swabs. The one KPC-producing *K. pneumoniae* isolate (ertapenem ZD, 40 mm; meropenem ZD, 32 mm) was from a patient with multiple admissions that throughout the course of the study had 7 swabs with only a single positive. The NDM-producing *K. pneumoniae* (ertapenem ZD, 26 mm; meropenem ZD, 32 mm) was from a patient that had 32 swabs throughout multiple admissions. Both patients had swabs that were negative before and after the one that was found to be positive. The NDM-producing *H. alvei* isolate (ertapenem ZD, 26 mm; meropenem ZD, 32 mm) was from a patient who was admitted for a month for which 5 swabs were collected over the admission, and the last swab was the one that was found to be positive.

DISCUSSION

Gastrointestinal colonization with CRO is a mounting public health concern, regardless of the underlying mechanism of carbapenem resistance or the particular Gram-negative organism. Detection of carbapenemase producers has received considerable attention, as carbapenemase genes are often located on readily transmissible plasmids and colonization with these organisms has fueled notable outbreaks (19, 20). However, colonization with other CRO, such as CR *P. aeruginosa* or CR *A. baumannii*, can serve as a reservoir for transmission and lead to devastating infections in high-risk populations (5, 6). Currently, there are no clear recommendations on optimal approaches to screen for CRO gastrointestinal colonization (7). Furthermore, screening decisions (e.g., carbapenemase genes versus CRE versus all CRO) are prevalence- and setting-dependent and require a balance between costs and risk that are subject to differing interpretations (4). Reevaluating the carbapenem ZD cutoffs to improve the specificity of the direct MAC plate method by testing 6,968 rectal swabs, we found that 11.8% of swabs were positive for at least one CRO, including 7.1% positive for CRE and 5.4% positive for CR-NF. Among CRO isolates, 3.1% were positive for CPO, including 1.6% positive for CPE

and 1.5% positive for CP-NF. Furthermore, by balancing sensitivity and specificity, we found that reducing the ertapenem and meropenem ZDs to ≤25 mm improved specificity to 83%, decreasing the confirmatory testing workload by 32%.

The CRO prevalence estimates from the current work are similar to those in our point-prevalence study; however, the distribution of organisms and mechanisms of carbapenem resistance was much more heterogeneous in the current study (14). Not surprisingly, KPC-producing *K. pneumoniae* was the most common CPE isolate identified; however, we were surprised to identify multiple other carbapenemase variants, including those less commonly encountered in the United States, such as NDM and OXA-48-like carbapenemases. Interestingly, *C. amalonaticus* was the third most common CPE encountered, with isolates harboring KPC, NDM, or KPC and NDM. A review of clinical culture results over the study period did not yield any positive cultures with CP *C. amalonaticus*, for which only a single positive bacteremia case reported from our hospital over the last 5 years (21). Thus, *C. amalonaticus* may play a role as a "silent" reservoir in the GI tract for carbapenemase genes, with the potential to share mobile genetic elements with other, more virulent Gram-negative colonizers. Moreover, we report a high rate of colonization with glucose-nonfermenting organisms, which is not surprising among the MICU and solid organ transplant populations being screened. Among the CR-NF isolates OXA-24-producing *A. baumannii* and non-CP-CRO *P. aeruginosa* isolates were the most commonly encountered. Rates of CP *P. aeruginosa* were low, and all were VIM producers (when a carbapenemase gene was identified). *S. maltophilia* accounted for the large majority of CP-NF isolates.

In the present work, we attempt to establish optimal ZD cutoffs for the direct MAC plate method as a screening method for identifying CRO from rectal swabs. Initially, Lolans and colleagues described the direct MAC plate method using an ertapenem disk with a ≤27 mm zone diameter cutoff for detection of KPC-producing *K. pneumoniae* and *E. coli*. They tested the method with 149 rectal swabs, for which 38 KPC producers were identified with a sensitivity of 97% and specificity of 90% (11). Other studies have evaluated the direct MAC plate method in comparison to alternative screening methods for identifying KPC-producing *Enterobacterales* from fecal specimens by various carbapenem disks (ertapenem and/or meropenem and/or imipenem) and zone diameter cutoffs (range, 21 to 27 mm) with sensitivities ranging from 75 to 97% and specificities from 78 to 96% (11, 22–24). These differences in performance characteristics are mostly explained by variations in the direct MAC plate method applied and the comparator used in the studies. Blackburn et al. then used spiked simulated specimens to assess the optimal zone diameters for screening CRO by ROC analysis and defined optimal zone diameters as ≤24 mm for ertapenem, ≤34 mm for meropenem, and ≤32 mm for imipenem (8). We evaluated sensitivity, specificity, and ROC curves for CRO and performed subanalyses for CRE, CR-NF, CPO, CPE, and CP-NF. The zone diameters for CRO-positive and CRO-negative cultures overlapped, but cultures positive for CRO tended to cluster with smaller zone diameters, whereas cultures negative for CRO clustered with larger zone diameters; differences in median ZDs between CRO-positive and CRO-negative organisms were statistically significant. The variability in zone diameters is not surprising, as specimen sampling, inoculum, and diversity of the microbiota all contribute to ZD variation. Overall, when evaluating the carbapenems separately for identification of CRO, ertapenem was found to provide increased sensitivity, whereas meropenem provided increased specificity. Intrinsic resistance among NF to ertapenem did contribute to the lower specificity of ertapenem in distinguishing CRO-positive from CRO-negative cultures (Fig. 2A).

For the detection of CRO using the direct MAC plate method, we recommend implementing a cutoff ZD of 25 mm for both ertapenem and meropenem to maximize sensitivity and specificity. Furthermore, applying the same ZD cutoff for both carbapenems allows for ease of use and remembrance of the cutoff. At these ZDs, the sensitivity and specificity of the method for CRO are 89% and 83% and for CPO are 94% and 76%, respectively, for cultures with growth on MAC plates. Under these newly proposed ZDs, the number of cultures requiring further workup would decrease by 32%

(814 cultures would be reclassified as negative), significantly reducing the laboratory workload. Although 3 CPE isolates among the 814 cultures were missed under the narrowed ZD criteria, there was no evidence of transmission from these patients to others, and in 2 cases subsequent follow-up swabs tested CRO negative. The larger zone diameters in these cases likely reflect a low burden of colonization with the CPE identified and perhaps reflect transient colonization. Although studies evaluating the burden of colonization and further transmission have yet to be completed for CRO, it is thought that a low burden of colonization presents a lower risk for spread, similarly to VRE (25). Thus, the additional work to find low-level colonization in these patients is likely outweighed by the presumably low potential for onward transmission. That said, each institution could weigh the risk with their individual procedures and populations based on the data that was generated in this study to determine the ZD criteria to apply (Table 3; see also Tables S1 and S2 in the supplemental material).

Limitations of this study include (i) some ESwabs were processed after the recommended 48-h period (up to 96 h after collection), (ii) the predefined ZD cutoffs may have underestimated the number of patients colonized with CRO due to organisms growing outside these ZDs not undergoing additional testing, (iii) the mCIM was used to detect CP among all nonfermenters and may not be the ideal method for organisms other than *P. aeruginosa*, and (iv) mCIM was indeterminate/positive among a large number of *E. cloacae* isolates in which no carbapenemase was detected by the Check-Direct multidrug resistance (MDR) assay. Whole-genome sequencing of these isolates is necessary to confirm the presence or absence of carbapenemase genes. Lastly, this study was limited to a single hospital setting and should be repeated in a broader population with different CRO prevalence to be generalizable to other settings.

In conclusion, we present the largest study to date evaluating the direct MAC plate method for detection of CRO colonization among high-risk inpatient wards over an 18-month period. We found similar rates of CRO (11.8%) and CPO (3.1%) colonization to our point-prevalence study, but with greater heterogeneity in carbapenemase production among various organisms. Our data indicate that the direct MAC plate method can be modified to balance the sensitivity and specificity of CRO detection, and we recommend reducing the zone diameter size for further workup to 25 mm for both ertapenem and meropenem. As CRO colonization screening assumes increasing importance, the data from this study can help institutions balance resource allocation and costs/benefits of using the direct MAC plate method.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.01127-19.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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