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A Pooling Strategy for Detecting Carbapenem Resistance Genes by the Xpert Carba-R Test in Rectal Swab Specimens

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ABSTRACT Rapid and accurate detection of carriers of carbapenemase-producing organisms (CPO) in hospitalized patients is critical for infection control and prevention. This study aimed to evaluate a pooling strategy for the detection of carbapenem resistance genes (CRG) in multiple specimens using the Xpert Carba-R test. Two rectal swabs each were collected from 415 unique patients. One swab was tested by Carba-R on the five specimen-pooled strategy. The other swab was tested individually by culture followed by DNA sequence analysis for CRG as the reference. At the first 5:1 pooling testing, 22 of 83 pools were positive, which yielded 34 positives from individual specimens when positive pools were subsequently retested. All individual specimens in the 61 negative pools were retested as negative by Carba-R. Among the 34 Carba-R-positive samples, 30 and four were positive and negative, respectively, by culture and sequencing. The remaining 381 Carba-R-negative specimens were also negative by culture and sequencing. Overall sensitivity, specificity, positive predictive value, and negative predictive value of the 5:1 pooled screening were 100.0% (95% confidence interval [CI] = 85.9% to 100%), 99.0% (95% CI = 97.2% to 99.7%), 88.2% (95% CI = 71.6% to 96.2%), and 100.0% (95% CI = 98.8% to 100%), respectively. Using the 5:1 pooling strategy, our study completed CRG screening in 414 patients with 193 reagents with significant cost savings. The 5:1 pooling strategy using the Carba-R test showed a potential method for screening CRG from rectal swabs with good sensitivity and decreased cost.

KEYWORDS carbapenemase-producing organisms, carbapenem resistance genes, pooling, Xpert Carba-R test, screening

Infections caused by carbapenemase-producing organisms (CPO) have received widespread attention internationally for their high mortality and challenging treatment regimens (1, 2). Molecular epidemiological studies have shown that acquired carbapenem resistance genes were the primary mechanism for carbapenem resistance (3, 4). Patients in the intensive care unit (ICU) or hematopoietic stem cell transplantation (HSCT) wards are at higher risk of infection if they are colonized with CPO in their gastrointestinal tracts (5–7). Therefore, rapid and accurate screening to identify CPO carriers among hospitalized patients is critical for infection control and prevention activities. At present, the detection of CPO depends primarily on phenotypic methods based on culture and antimicrobial susceptibility testing (AST). The modified carbapenem inactivation method (mCIM) has performed exceptionally well in detecting CPO in Enterobacterales (8) and with *Pseudomonas aeruginosa*, though the performance with *Acinetobacter baumannii* has been shown to be less reliable (9). Commercially available chromogenic media, in conjunction with the mCIM, EDTA-mCIM (eCIM), and Carba NP, can all be used to identify CPO phenotypically (10, 11). However, these methods rely

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The authors declare a conflict of interest. X.G., F.C.T., and Y.-W.T. are employees of Cepheid, the commercial manufacturer of the Xpert Carba-R test. The other authors declare no competing interests.

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on the isolation of CPO strains from clinical specimens prior to testing with phenotypic methods, which can delay the time to results from 48 h to 72 h.

The Xpert Carba-R test (Cepheid, Sunnyvale, CA) is a molecular test based on real-time PCR that provides fast and accurate results from clinical specimens, including rectal and peri-rectal swabs, as well as pure bacteria cultures. Jin et al. conducted a study with the Xpert Carba-R test screening 2,404 rectal swab specimens from unique patients and reported overall positive and negative agreements ranging from 94.5% to 100% and from 94.8% to 99.9% for bla_{NDM}, bla_{KPC}, bla_{IMP}, bla_{VIM}, and bla_{OXA-48}, respectively (12). Subsequent studies have also been conducted to evaluate the performance of the Xpert Carba-R test, and it proved to perform well for both clinical specimens and cultured isolates (12–15).

In countries with insufficient resources, the cost of using the Xpert Carba-R test is high, which limits its clinical application. Our goal was to seek a test strategy to reduce the overall cost of CRG screening while preserving the test performance, including sensitivity, specificity, and accuracy. The concept of pooling specimens for testing was introduced by Dorfman in 1943 to efficiently detect a small number of defective members in a large number of populations (16). A formula to calculate the optimal size of the pool was presented in that study. For SARS-CoV-2 detection among 26,576 samples, Ben-Ami et al. achieved a 7.3-fold increase in test efficiency while preserving clinical sensitivity by implementing the Dorfman pooling strategy with a pool size of eight specimens (17). The pooling strategy also performed well in other studies of SARS-CoV-2 detection (18–21). In countries or regions with a low infection rate, pooling of specimens is more economical and time-saving for achieving a diagnosis (18–21). However, to our knowledge, there has been no study of a pooling strategy to detect CPO in clinical specimens. Therefore, we adopted this strategy for the detection of CPO carriers with the goal of improving the cost-effectiveness of testing the Xpert Carba-R test, decreasing the cost of testing while maintaining the test performance.

MATERIALS AND METHODS

Sample collection. Between November 2021 and April 2022, 448 pairs of flocked swabs in transport media (COPAN Diagnostics Inc., Murrieta, CA) were collected from the patients of the Peking University People's Hospital (PKUPH). All specimens were kept in transport media (Fig. S1), stored at 4°C, and processed within 24 h after collection. This study was approved by the Medical Ethics Committee of PKUPH (approval number: 2021PHB266).

Study design. For each swab pair, one swab was tested by Carba-R test in the pool of five samples (5:1 strategy). The other swab was tested individually by culture followed by DNA sequence analysis for CRG as the reference method (Fig. 1). In addition to the 5:1 pooling strategy, we also used Carba-R test on the second swab (the one used for culture) to verify the negative results of pooling sample testing. Finally, the results reported by Carba-R test were compared with the culture followed by sequencing results to calculate sensitivity, specificity, accuracy, positive predictive value, and negative predictive value of the 5:1 pooling strategy.

Pooling and individual test by Xpert Carba-R test. For the pooling test, five swabs from different patients were taken out from transport media and pooled in a 5-mL Sample Reagent vial supplied with Carba-R test kit and vortexed for 30 s. Then, 1.7 mL of the liquid was transferred into the Xpert Carba-R cartridge. Finally, the cartridge was placed into the GeneXpert instrument (Catalog #: GXXVI-16-L, Cepheid, CA, USA) as previously described (12). All samples included in this pool were reported as negative when the pool result was reported as negative. If the pool result was positive, all five samples in the pool were retested individually using the second swab to identify the positive sample(s). For the individual test, a single swab was placed in a single 5-mL Sample Reagent vial and tested in the same manner as the pooling tests.

Quality control for the Xpert Carba-R test was conducted every workday with one negative-control isolate and two positive-control isolates selected from 10 positive-control isolates each containing target genes (two containing bla_{NDM}, two with bla_{KPC}, two with bla_{IMP}, two with bla_{VIM}, and two with bla_{OXA-48}).

Culture, antimicrobial susceptibility testing, and sequencing. Swabs were cultured on a China Blue Agar (Thermo Fisher Scientific, MA, USA) containing 0.5 μg/mL of imipenem. After 24 h to 48 h of incubation at 35°C, colonies were identified with a Matrix-Assisted Laser Desorption-Ionization Time of Flight Mass Spectrometry analyzer (MALDI-TOF MS) (Bruker Daltonics, MA, USA). *Enterobacterales* were subcultured on Columbia blood agar (Thermo Fisher Scientific, MA, USA) for antimicrobial susceptibility testing, and mCIM and eCIM tests were conducted for the detection of carbapenemases and metallo-beta-lactamases (MBLs). Ten isolates of carbapenemase-producing *Enterobacterales* were selected from frozen isolates as positive controls. The isolates were analyzed by DNA sequencing to confirm the presence of bla_{NDM}, bla_{KPC}, bla_{IMP}, bla_{VIM}, and bla_{OXA-48} genes as previously described (22, 23).

RESULTS

Prevalence and determination of optimal pool size. From November 1, 2020 to October 31, 2021, a total of 1,304 patients underwent rectal swab cultures in PKUPH; 69

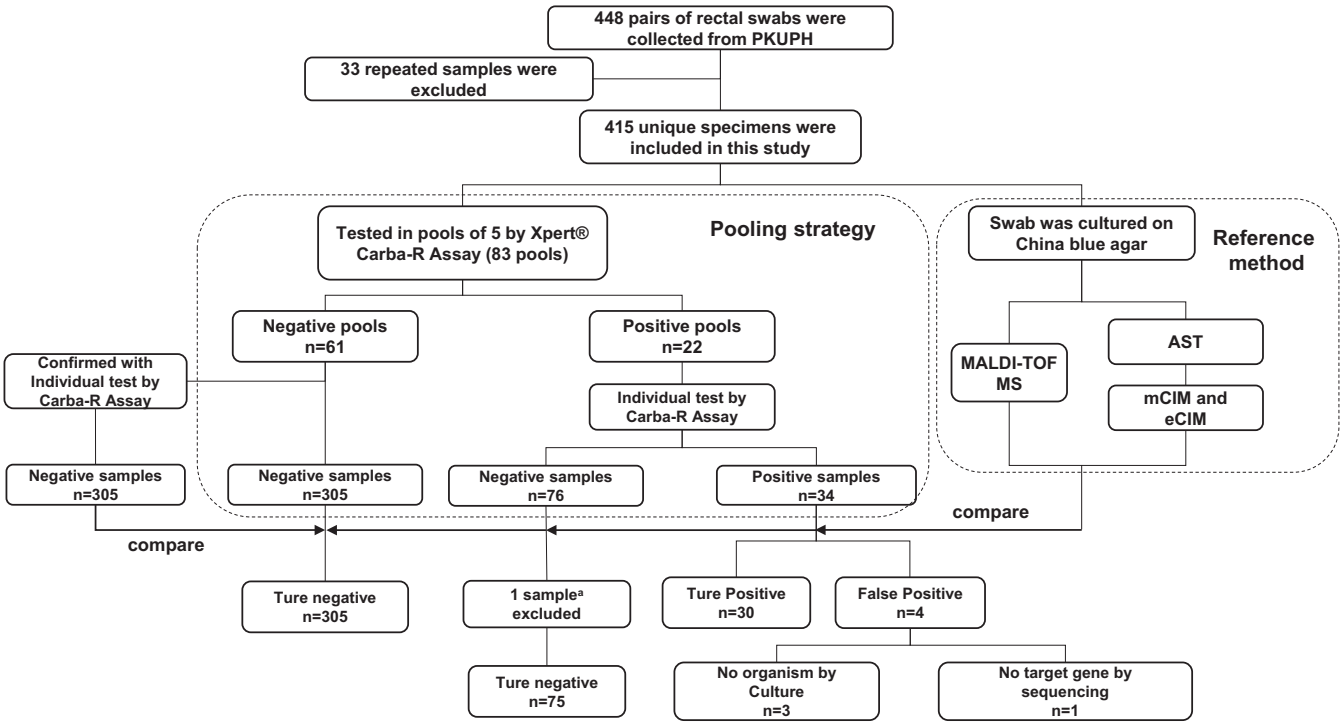

FIG 1 Detection flowchart of rectal swabs. [a]One sample was excluded because a carbapenem-resistant organism isolate was cultured from the specimen but no target gene was detected by either Xpert testing or sequencing, suggesting that carbapenem resistance was mediated by another mechanism.

patients were positive with CRG by culture. The prevalence of intestinal colonization with CRG carrier was 5.3% (69/1,304). We built a model according to Dorfman's pooling theory (16) to determine which size of the pool (from two to nine) would be the most efficient at prevalence ranging from 0% to 15%. This model revealed that pooling five samples would likely be the most efficient given the prevalence of 5.3% in the hospital (Fig. 2).

Results of pooling using Xpert Carba-R test for detecting CRG in rectal swabs. In this study, 415 swabs with complete data were collected between November 2021 and April 2022 (Fig. 1). The samples were divided into 83 pools; 22 pools were positive with the Xpert Carba-R test. Thirty-four swabs from the pools were positive when tested individually, and 380 out of 414 samples were CRG negative. Among the 34 positive samples, 30 were positive by culture and sequencing (including 26 samples with one targeted gene and four with two targeted genes). These included two with bla_{NDM} plus bla_{IMP}, one with bla_{VIM} plus bla_{IMP}, and one with bla_{KPC} and bla_{NDM}. Four samples were negative by culture. All samples that were contained in negative pools were negative by culture and sequencing.

Overall, to screen 414 samples for CRG, 193 tests were conducted in pooling strategy and 216 were avoided compared with individual tests (414 tests), resulting in the efficiency of the pooling strategy of 215%, which means 53.5% cost could be saved. The overall sensitivity, specificity, accuracy, positive predictive value, and negative predictive value compared with reference method are presented in Table 1. Table 2 shows the performance of the pooling test strategy by Xpert Carba-R test when analyzed by target genes, respectively.

Comparison of cycle threshold values tested by pooling and individual. Table S1 shows the 38 cycle threshold (Ct) values reported by pooling and individual testing, respectively. The 29 numbers in the first column represent 29 pooling samples. We calculated the ΔCt between the result for the pool and the result for the individual test. When there are two or more individual positive samples in a pooled sample, the ΔCt only calculates between the pooled sample and the individual sample with high CRG concentration (i.e., the lower Ct value). Therefore, the individual Cts for 11 samples were marked as not applicable because they were pooled with higher concentrations of positive samples. For a pooled sample, the average loss of Ct value was 1.08 (95% CI = 0.56 to 1.59) compared with the sample detected individually (Fig. 3).

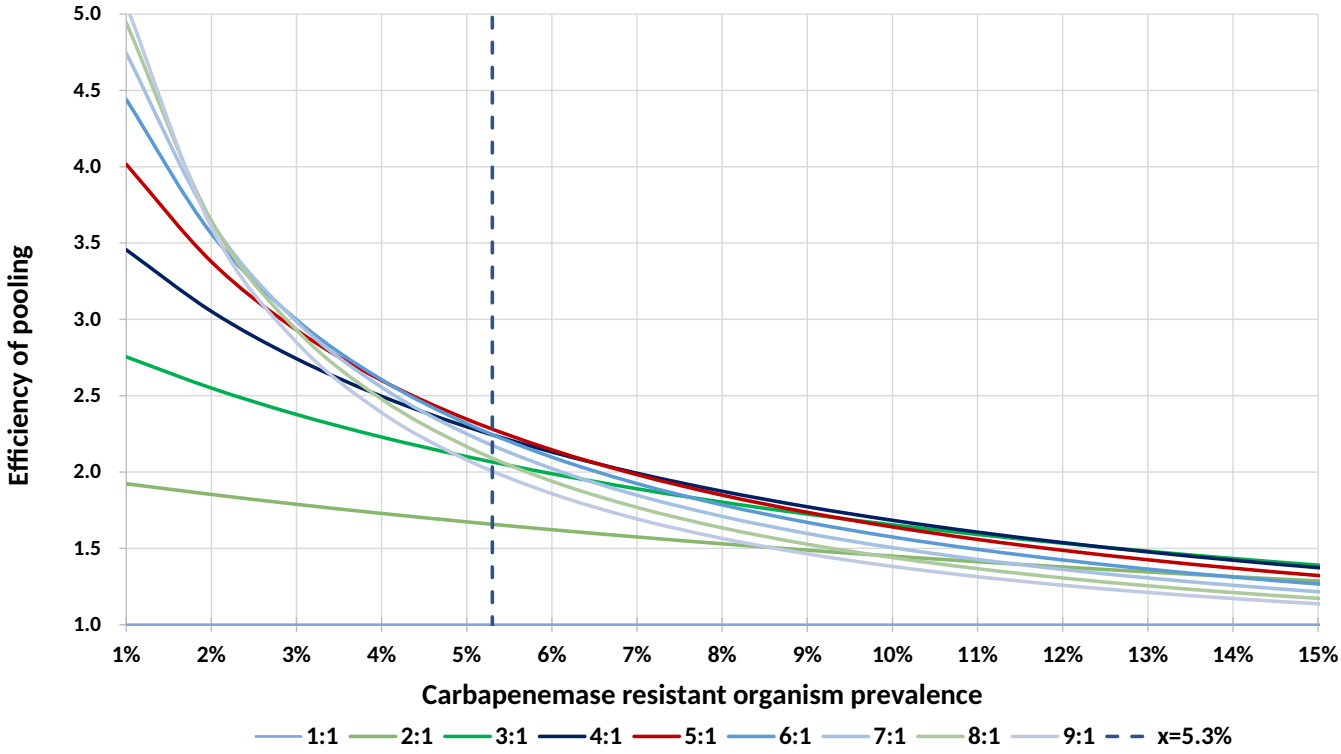

FIG 2 Efficiency model about pooling size and prevalence. Lines of various colors represent different ratios of pooling strategies, from 1:1 to 9:1. The dotted line represents the actual incidence of CRO colonization screening in our hospital, which was 5.3%.

Discrepant results. Three samples were positive in the pooled sample but no organisms grew on the culture medium. One sample was positive and a carbapenem-resistant colony was obtained on a China Blue Agar containing 0.5 μg/mL of imipenem, but there was no carbapenemase gene detected in this isolate by DNA sequence analysis. All four samples were treated as false positives as shown in Table S2. Furthermore, there was one disagreement between the result from pooling and that from the individual test: the pool was positive for both bla_{IMP} (Ct 37.8) and bla_{NDM} (Ct 36.6) but when five samples were tested individually, only one sample was reported as bla_{IMP} positive (Ct 35.8) but no

TABLE 1 Statistics and performance of pooling test strategy[a]

Item	Efficiency of pooling
Total sample size	414
No. of pools (5 in 1)	83
No. of positive pools	22
Reagent consumption (including individual test)	193 (22*5 + 83)
Practical efficiency	215% (414/193)

	Culture and sequencing (reference method)	
Item	No. of positive	No. of negative
Pooling		
No. of positive	30	4
No. of negative	0	380

Statistic	Performance	95% CI
Sensitivity (95% CI)[a]	100%	85.9% to 100%
Specificity (95% CI)	99.0%	97.2% to 99.7%
Accuracy (95% CI)	99.1%	97.4% to 99.7%
PPV (95% CI)	88.2%	71.6% to 96.2%
NPV (95% CI)	100%	98.8% to 100%

[a]CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

TABLE 2 Sensitivity, specificity, accuracy, PPV, and NPV of Xpert Carba-R pooling strategy for individual CRG in comparison with culture and sequencing[a]

	Culture and sequencing (reference method)									
Xpert Carba-R	bla_{NDM}+	bla_{NDM}−	bla_{KPC}+	bla_{KPC}−	bla_{VIM}+	bla_{VIM}−	bla_{IMP}+	bla_{IMP}−	bla_{OXA}+	bla_{OXA}−
No. of positive	23	2	4	1	1	0	6	1	0	0
No. of negative	0	389	0	409	0	413	0	407	0	414
Sensitivity (95% CI)	100% (82.2% to 100%)		100% (39.6% to 100%)		100% (5.5% to 100%)		100% (51.7% to 100%)		NA	
Specificity (95% CI)	99.5% (98.0% to 99.9%)		99.8% (98.4% to 99.9%)		100% (98.9% to 100%)		99.8% (98.4% to 99.8%)		100% (98.9% to 100%)	
Accuracy (95% CI)	99.5% (98.0% to 99.9%)		99.8% (98.5% to 99.9%)		100% (98.9% to 100%)		99.8% (98.5% to 99.9%)		100% (98.9% to 100%)	
PPV (95% CI)	92.0% (72.5% to 98.6%)		80.0% (29.9% to 99.0%)		100% (5.5% to 100%)		85.7% (42.0% to 99.2%)		NA	
NPV (95% CI)	100% (98.8% to 100%)		100% (988% to 100%)		100% (98.9% to 100%)		100% (98.8% to 100%)		100% (98.9% to 100%)	

[a]NA, not applicable; PPV, positive predictive value; NPV, negative predictive value.

bla_{NDM} positive was reported. Moreover, no bla_{NDM} positive sample was found by reference method.

DISCUSSION

When using a specimen pooling strategy, fewer pools of larger pool sizes at a low prevalence achieve higher efficiency because most pools will result as negative. As prevalence increases, a smaller pool is preferable to retest fewer samples when a positive pool is detected (23). It is at the discretion of the laboratory to determine the best pooling ration according to its resistance gene prevalence.

In terms of potential loss of performance, sensitivity loss is the greatest concern for sample pooling (18). In our study, the pooling strategy showed both a high sensitivity (100%, 85.9% to 100%) and negative predictive value (100%, 98.8% to 100%) compared with the reference method. This means that few positive samples would be missed when screening for CRG by pooling. We conclude, based on our data, that the pooling strategy using Xpert Carba-R test is suitable for rapid and accurate screening of CRG in regions with a low prevalence of carbapenem resistance. Pooling also reduced turnaround time (TAT) for generating results from screening from 48 h to no more than 2 h for samples with a negative result. Given the high mortality of CRO infection (24) and challenges of treatment for multidrug resistant infections, the reduction of TAT could aid in selecting therapy and in the prevention of CRO spread in the hospital (4), especially benefiting transplant patients (25) and patients in ICUs. Meanwhile,

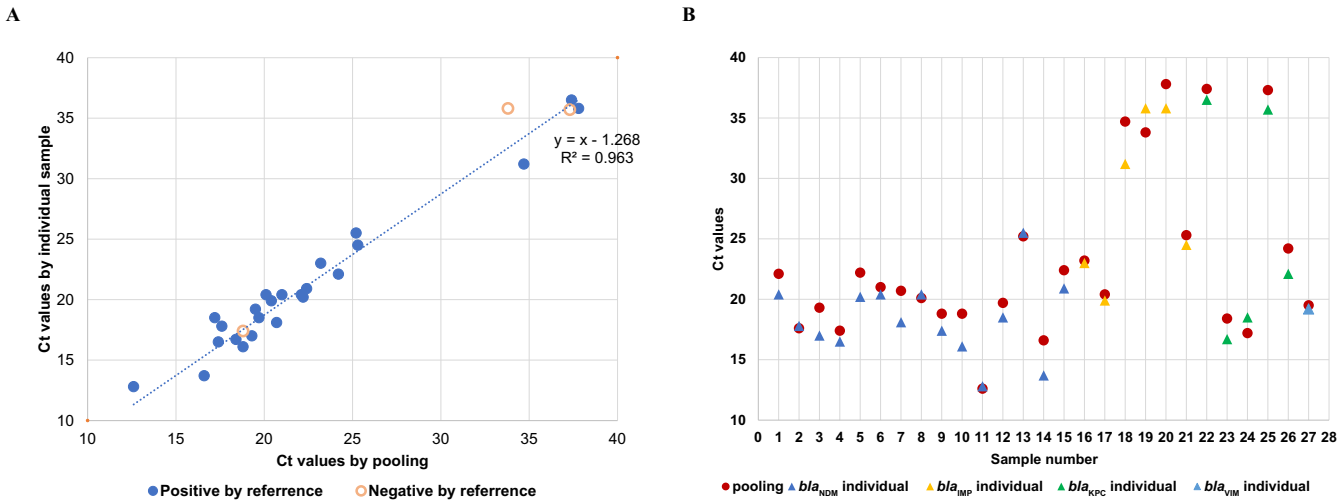

FIG 3 Comparison of Ct values tested by pooling and individual from positive samples. (A) The Ct value of the pooling strategy is on the *x* axis, the Ct value of the individual sample detection is on the *y* axis, the distribution position relative to y = x, and its linear analysis; the solid points represent the samples detected as positive by the reference method, and the hollow points represent the negative by the reference method. (B) Comparison between pooling strategy Ct and individual detection Ct values.

an increase in efficiency decreases the cost of testing and reduces the financial burden on patients in countries where patients pay for the costs of their healthcare.

In our study, we used culture and sequencing as the reference method. Although we conducted AST, mCIM, and eCIM tests together with culture, the sensitivity of the reference method is not 100% (10). Three of the discrepant results demonstrated CRG detected by Xpert Carba-R with late Ct values, but the samples were negative by culture. The late Ct values may indicate a low organism burden in the samples, which may explain why the cultures were negative. It is also possible that the target genes were present but not expressed in the host organisms and, thus, would have been carbapenem susceptible and would not grow on the culture media. The reference method could be modified by culturing samples in broth instead of on a plate to achieve a higher sensitivity (12, 26) and plated on a nonselective media with a meropenem disk in the inoculum area to detect organism with low expression levels. Sequencing nucleic acid extracted from the sample reagent containing the pooled samples may also have confirmed the presence of the resistance gene in the original sample pool.

There are some limitations to our study. First, although there was no sample judged as a false negative in our study, the loss of Ct values (1.08) compared with the individual tests is not negligible, indicating a risk of false-negative results by pooling in samples with low concentration of organisms. Second, there were four samples where the ΔCt between the pooled sample and the individual samples ranged from 7 to 18, suggesting that the target resistance gene concentration was much higher in the pool than in the individual organism that was isolated. This suggests that additional organisms may have been missed by culture. Third, our study was conducted in one center, which may have biased the results, manifested in the numbers of samples with the various targeted genes that were detected. There were more bla_{NDM} positive samples than other targeted genes. This resulted in insufficient data for analysis of some genes, such as bla_{OXA-48}, which was not found during our study. Fourth, we did not calculate the specific labor costs because the Carba-R tests were implemented as a research method by a researcher. Given the limitations, we still feel that the pooling strategy would be cost-effective, with little loss of sensitivity compared with testing individual rectal swab samples.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

ACKNOWLEDGMENTS

X.G., F.C.T., and Y.-W.T. are employees of Cepheid, the commercial manufacturer of the Xpert Carba-R test. The other authors declare no competing interests.

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