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Rapid Detection of New Delhi Metallo-β-Lactamase Gene Using Recombinase-Aided Amplification Directly on Clinical Samples From Children

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New Delhi metallo-β-lactamase, a metallo-β-lactamase carbapenemase type, mediates resistance to most β-lactam antibiotics including penicillins, cephalosporins, and carbapenems. Therefore, it is important to detect *bla*$_{\text{NDM}}$ genes in children's clinical samples as quickly as possible and analyze their characteristics. Here, a recombinase-aided amplification (RAA) assay, which operates in a single one-step reaction tube at 39°C in 5–15 min, was established to target *bla*$_{\text{NDM}}$ genes in children's clinical samples. The analytical sensitivity of the RAA assay was 20 copies, and the various bacterial types without *bla*$_{\text{NDM}}$ genes did not amplify. This method was used to detect *bla*$_{\text{NDM}}$ genes in 112 children's stool samples, 10 of which were tested positive by both RAA and standard PCR. To further investigate the characteristics of carbapenem-resistant bacteria carrying *bla*$_{\text{NDM}}$ in children, 15 carbapenem-resistant bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Citrobacter freundii*, *Klebsiella oxytoca*, *Acinetobacter junii*, and *Proteus mirabilis*) were isolated from the 10 samples. Notably, more than one bacterial type was isolated from three samples. Most of these isolates were resistant to cephalosporins, cefoperazone-sulbactam, piperacillin-tazobactam, ticarcillin-clavulanic acid, aztreonam, co-trimoxazole, and carbapenems. *bla*$_{\text{NDM}-1}$ and *bla*$_{\text{NDM}-5}$ were the two main types in these samples. These data show that the RAA assay has potential to be a sensitive and rapid *bla*$_{\text{NDM}}$ gene screening test for clinical samples. The common existence of *bla*$_{\text{NDM}}$ and multi-drug resistance genes presents major challenges for pediatric treatment.

Keywords: carbapenemase, *bla*$_{\text{NDM}}$, recombinase-aided amplification, pediatrics, character

INTRODUCTION

Since their first use in 1985, broad-spectrum carbapenems have been commonly used in clinical practice (Lyon, 1985), but carbapenemase-producing *Enterobacterales* bacteria now pose an increasingly important threat to global health (Trecarichi and Tumbarello, 2017). Carbapenemases are β-lactamases belonging to three different Ambler classes (classes A, B, and D). Class A enzymes include the most common *Klebsiella pneumoniae* carbapenemase (KPC) family and the much less commonly encountered non-metallo-carbapenemase A (NmcA) enzyme, *Serratia marcescens* enzymes (SMEs), the imipenem-hydrolyzing β-lactamase (IMI), and the Guiana extended spectrum (GES) enzyme family. Class B enzymes are metallo-β-lactamases (MBL) and include New Delhi metallo-β-lactamases (NDM), imipenemase (IMP), and Verona integron-encoded metallo-β-lactamase (VIM). Class D enzymes are OXA-type carbapenemases (Tooke et al., 2019).

NDMs can hydrolyze most β-lactams including penicillins, cephalosporins, and carbapenems (Kumarasamy et al., 2010; Khan et al., 2017). The first clinical bacterial strain carrying this gene was isolated in 2009 from a Swedish patient who had traveled to India in 2007, where he acquired a *K. pneumoniae*-related urinary tract infection. Carbapenem resistance in this isolate was mediated through the production of a novel carbapenemase designated NDM-1 (Yong et al., 2009). Since then, NDM-1 has been found in various *Enterobacteriaceae*, and in *Acinetobacter* and *Pseudomonas* species, and 31 NDM variants have thus far been identified (Hong et al., 2015; Farhat and Khan, 2020).

NDM-positive bacteria, which include *Enterobacteriaceae*, *Pseudomonas*, and *Acinetobacter*, cause various infections with high mortality rates (Rahman et al., 2018; Yang et al., 2020). Therefore, the rapid and sensitive detection of these NDM-positive pathogens is required if appropriate therapy is to be administered. Recombinase-aided amplification (RAA) is a new assay based on isothermal amplification technology. The reaction system includes a recombinase (UvsX), a single-stranded DNA-binding protein, and a DNA polymerase. The amplification process is completed within 15–30 min at 39°C, making it suitable for clinical applications (Zhang et al., 2017; Fan et al., 2019; Qi et al., 2019; Shen et al., 2019; Wang et al., 2019; Xue et al., 2020a,b).

In the present work, we developed an RAA assay to detect the bla_{NDM} gene in clinical samples. The analytical specificity and sensitivity of the assay were evaluated, and the detection results were PCR verified. To further analyze the characteristics of carbapenem-resistant strains with bla_{NDM} from children, the minimum inhibitory concentrations (MICs), bla_{NDM} genotypes and clinical diagnosis pertaining to these isolates were investigated.

MATERIALS AND METHODS

Bacteria

Altogether, 16 common *Enterobacterales* bacterial types (*Klebsiella pneumoniae* 2146, *Acinetobacter baumannii* 1 and 2, *Pseudomonas aeruginosa*, *K. pneumoniae* 700603, *Escherichia coli* 25922, *Klebsiella oxytoca*, *P. aeruginosa* ATCC27853, *Shigella sonnei*, *Salmonella enteritidis*, *Enterobacter aerogenes*, *Proteus mirabilis*, *Campylobacter jejuni*, *Enterobacter cloacae*, *S. marcescens*, *Citrobacter freundii*) were investigated in this study, the sources of which are listed in **Table 1**. These bacteria were cultured at 37°C in brain heart infusion medium (BHI, Oxoid Ltd., United Kingdom) broth. From them, *K. pneumoniae* 2146, *A. baumannii* 1, and *P. aeruginosa* contain bla_{NDM-1} genes, *A. baumannii* 2 contains bla_{KPC-2} gene, whereas the other bacterial types were all sensitive strains. All bacterial types included in this study were previously evaluated for the presence of bla_{NDM} by PCR (data not shown).

TABLE 1 | Bacterial types used in this study.

Species	Source
Klebsiella pneumoniae 2146	Our microorganism center
Acinetobacter baumannii 1	Clinical isolate
Pseudomonas aeruginosa	Clinical isolate
Escherichia coli ATCC 25922	Our microorganism center
K. pneumoniae ATCC 700603	Our microorganism center
Acinetobacter baumannii 2	Our microorganism center
Pseudomonas aeruginosa ATCC 27853	Our microorganism center
Shigella sonnei	Our microorganism center
Salmonella enteritidis	Our microorganism center
Klebsiella oxytoca	Clinical isolate
Enterobacter aerogenes	Clinical isolate
Proteus mirabilis	Clinical isolate
Enterobacter cloacae	Clinical isolate
Serratia marcescens	Clinical isolate
Campylobacter jejuni	Clinical isolate
Citrobacter freundii	Clinical isolate

Clinical Samples and Bacterial Cultures

One hundred and twelve stool samples were randomly collected from each inpatients in the Capital Institute of Pediatrics, Beijing, China. All specimens were first cultured on blood plates and the isolated bacteria were verified using the VITEK 2 compact system (BioMerieux, France). The rest were used for DNA isolation. A 200-mg sample was taken from each remaining fecal sample for DNA extraction, and the instructions from the kit (Tiangen Biotech Co., Ltd., Beijing, China) were followed.

Primer Design

The sequences of all 31 bla_{NDM} genes were downloaded from the National Center for Biotechnology Information (NCBI) GenBank database.[1] The conserved regions in these 31 genes were used to manually design the primers and the probe according to the principles relating to RAA primer and probe design (primer size between 30 and 35 bp, probe size between 46 and 52 bp, product size between 100 and 200 bp). The conserved region in the 16S rRNA gene was designed to act as an internal positive control. An NCBI primer-specific BLAST analysis was used to confirm the specificity of the primers and probe. Online OligoEvaluator software[2] was used to analyze the potential for primer dimers to occur and to identify hairpins. All primers and probes were synthesized and purified by Sangon Biotech (Shanghai, China) using high-performance liquid chromatography.

[1] http://www.ncbi.nlm.nih.gov/
[2] http://www.oligoevaluator.com

DNA Extraction

Total DNA was extracted from each bacterial type (**Table 1**) with the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. The DNA samples were eluted in 150 μL of nuclease-free water and stored at −80°C until use. *Klebsiella pneumoniae* 2146 DNA was 10-fold diluted from 10^{-7} copies/μL to 10^{0} copies/μL, as calculated using the following formula: DNA copy number (copy number/μL) = [6.02 × 10^{23} × plasmid concentration (ng/μL) × 10^{-9}]/[DNA length (in nucleotides) × 660], and then stored at −80°C until use.

Recombinant Plasmid Construction

The full-length *bla*$_{NDM-1}$ gene (*K. pneumoniae* 2146, GenBank Accession No. CP006659) was PCR-amplified and cloned into vector pUC57 (Tiangen Biotech Co., Ltd., Beijing, China). The standard recombinant plasmids with 10-fold concentrations ranging from 10^{7} copies/μL to 10^{0} copies/μL were prepared and stored at −80°C until use.

RAA Assay

RAA assays were performed in 50-μL reaction volumes using a commercial RAA kit (Jiangsu Qitian Bio-Tech Co., Ltd., China). The reaction mixtures contained 2 μL of extracted DNA template, 25 μL of reaction buffer, 15.7 μL of DNase-free water, 2.1 μL of primer F (10 μM), 2.1 μL of primer R (10 μM), 0.6 μL of the probe (10 μM), and 2.5 μL of 280 mM magnesium acetate. The reaction mixture was added to a tube containing the RAA enzyme mix in a lyophilized form. Tubes were placed into a B6100 Oscillation mixer (QT-RAA-B6100, Jiangsu Qitian Bio-Tech Co., Ltd., China) and incubated for 4 min, mixed briefly, centrifuged, and finally transferred to a fluorescence detector (QT-RAA-1620, Jiangsu Qitian Bio-Tech Co., Ltd.) to be measured for 20 min at 39°C.

Analytical Sensitivity and Specificity of the RAA Assay

The analytical sensitivity of the RAA assay was determined using 10-fold serial dilutions of the recombinant plasmid ranging from 10^{7} copies/μL to 10^{0} copies/μL. Assay specificity was evaluated by testing the bacterial panel described in **Table 1**. *K. pneumoniae* 2146 and two other *bla*$_{NDM-1}$-containing clinical isolates (*A. baumannii* 1 and *P. aeruginosa*) were used as the positive controls for testing the specificity of the RAA assay toward *bla*$_{NDM}$. Distilled water was used as the negative control.

PCR Detection and Sequencing

A 25-μL reaction volume containing the following components was used for all the PCRs: 12.5 μL of PCR Master Mix reagent (Tiangen Biotech Co., Ltd., Beijing, China), 9.5 μL of double-distilled water, 0.5 μL of 10 μM NDM-F primer (5′-ATGGAATTGCCCAATATTAT-3′) and NDM-R primer (5′-TCAGCGCAGCTTGTCGGCCA-3′), and 2 μL of DNA template. The PCR cycling conditions were 94°C for 2 min, followed by 35 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 45 s. The final extension step was 72°C for 10 min. PCR products were electrophoretically separated on 1.5% agarose gels and stained with ethidium bromide. Images were documented on the Gel Doc EQ imaging system (Bio-Rad). PCR products were sequenced at Sangon Biotech. The resultant sequences were entered into DNAStar software (DNASTAR Inc., Madison, WI, United States), and sequence alignments were performed by the ClustalW method.

Evaluation of the RAA Assay Using Clinical Samples

To evaluate the performance of the RAA assay directly on clinical samples, 112 clinical stool samples were tested using the established RAA methods in this study for identifying *bla*$_{NDM}$ genes. All the RAA test results were compared with those from the standard PCR assay.

Phenotypes of the NDM-Positive Clinical Isolates

Antimicrobial susceptibility testing of the *bla*$_{NDM}$-positive isolates was initially performed using the VITEK 2 compact system. The MIC values for amikacin, tobramycin, ciprofloxacin, levofloxacin, tigecycline, doxycycline, minocycline, co-trimoxazole, aztreonam, cefepime, ceftazidime, ceftriaxone, cefuroxime, cefoperazone/sulbactam, ticarcillin/clavulanic acid, piperacillin/tazobactam, imipenem, and meropenem were determined by VITEK 2 AST-N335 cards. *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853 were used for quality control. The concentration gradient-based E-test strip method was used to double check the MIC values for imipenem and meropenem in the *in vitro* susceptibility tests. The 2020 Clinical Laboratory Standards Institute's threshold was used to as reference.

Statistical Analysis

The trials were performed in triplicate. The kappa and *p*-values of the RAA and standard PCR assays (with sequencing) were calculated. The statistical analysis was conducted with SPSS 21.0 (IBM, Armonk, NY, United States).

RESULTS

Primer Design for the RAA Assay

Since the first NDM sequence (*bla*$_{NDM-1}$) was released in 2009 (GenBank accession number KU341526.1), 31 variants have been identified. The genome sequences of the *bla*$_{NDM}$ genes are almost identical. The primers and probe were manually designed to bind within the conserved regions according to the principles of RAA primer and probe design (**Figure 1** and **Table 2**).

Analytical Specificity of the RAA Assay

As shown in **Figures 2A–C**, in contrast with the other bacterial samples and water control, only *K. pneumoniae* 2146-, *A. baumannii*-, and *P. aeruginosa* DNA-containing samples produced amplification signals. However, all the bacterial types produced amplification signals from the 16S rRNA gene

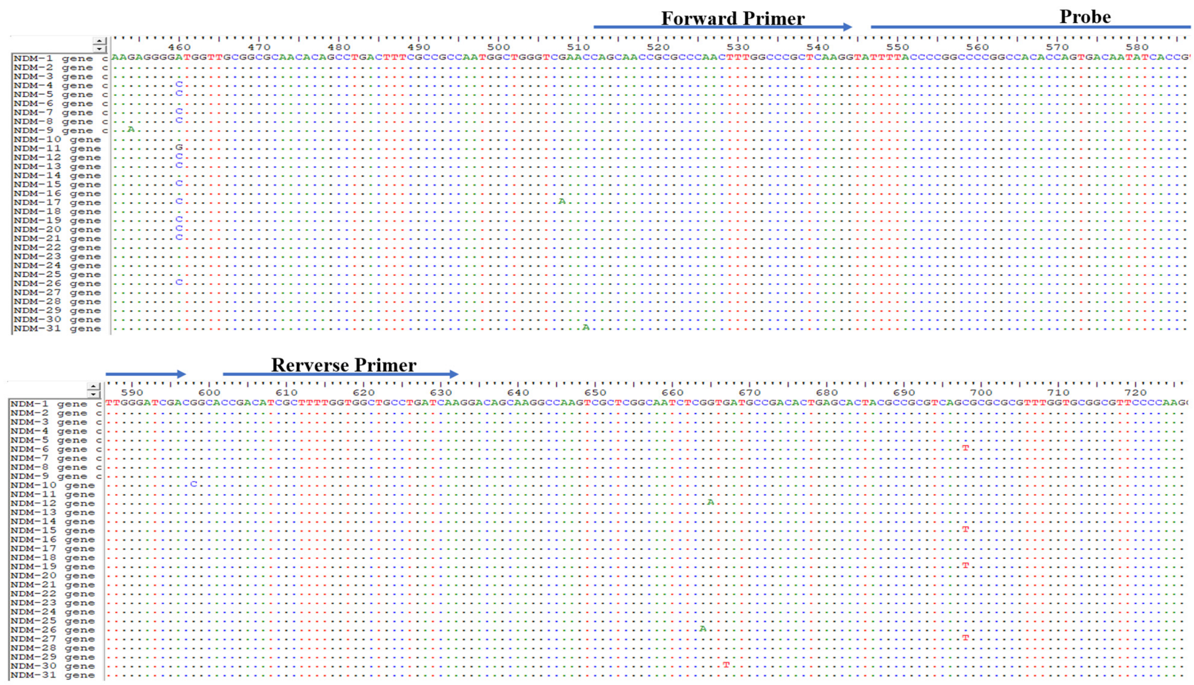

FIGURE 1 | Primer and probe regions in the 31 *bla*NDM variants.

(**Figures 2D–F**). Hence, the RAA assay for the detection of *bla*NDM was specific (100%).

Analytical Sensitivity of the RAA Assay

The sensitivity of the RAA assay for *bla*NDM detection was determined using a panel of serially diluted recombinant plasmids and bacterial genomic DNA containing the *bla*NDM gene. As shown in **Figure 3**, an increase in the fluorescence signal was observed from 1×10^7 to 1×10^1 copies/reaction. The detection limit of the RAA assay was 20 copies per reaction.

TABLE 2 | Primers used to amplify the *bla*NDM genes.

Primer	Sequence	Position
NDM-F	CAGCAACCGCGCCCAACTTTG GCCCGCTCAAGG	512-544[a]
NDM-R	TTGATCAGGCAGCCACCAAAA GCGATGTCGG	602-632[a]
NDM-P	TTTTACCCCGGCCCCGGCC ACACCAGTGACAA[FAM-dt][THF][BHQ-dt]CACCGTTGGGATCGAC[3′-block]	547-597[a]
16S-F	TGGAGCATGTGGTTTAATTC GATGCAACGC	1022285-1022314[b]
16S-R	GGATAAGGGTTGCGCTCGTT GCGGGACTTAA	1022432-1022462[b]
16S-P	TGACATCCACAGAACTTTCCAGA GATGGATTGG[FAM-dT]G[THF]C [BHQ-dT] TCGGGAACTGTGAGAC [3′-block]	1022334-1022387[b]

[a]GenBank accession number KU341526.1.
[b]GenBank accession number NZ_CP006659.2.
FAM, 6-carboxyfluorescein; THF, tetrahydrofuran; BHQ, black hole quencher; C3-spacer, 3′-phosphate blocker.

Evaluating the RAA Assay on Clinical Samples

The RAA assay was then evaluated with 112 stool samples, and the results were verified by standard PCR. From these 112 clinical samples, 10 were positive for *bla*NDM. All the results were 100% consistent with the results from the standard PCR assay. No significant differences between the detection results from RAA and PCR were observed (**Figure 4**).

Culture and Isolation of the *bla*NDM-Positive Clinical Samples

Fifteen carbapenem-resistant bacterial types were isolated from the 10 aforementioned samples including *E. coli*, *K. pneumoniae*, *A. baumannii*, *C. freundii*, *K. oxytoca*, *A. junii*, and *P. mirabilis*. Interestingly, more than one bacterial type was identified in three samples, and *bla*NDM-1 and *bla*NDM-5 existed in one patient's sample. After comparing the sequences of these blaNDM genes in GenBank, 11 *bla*NDM-1 and 4 *bla*NDM-5 types were confirmed (**Table 3**).

Clinical Information on *bla*NDM-Positive Isolates From Patients

To further understand the characteristics of *bla*NDM, the clinical characteristics of the three patients (ESY1, ESY2, and ESY3)

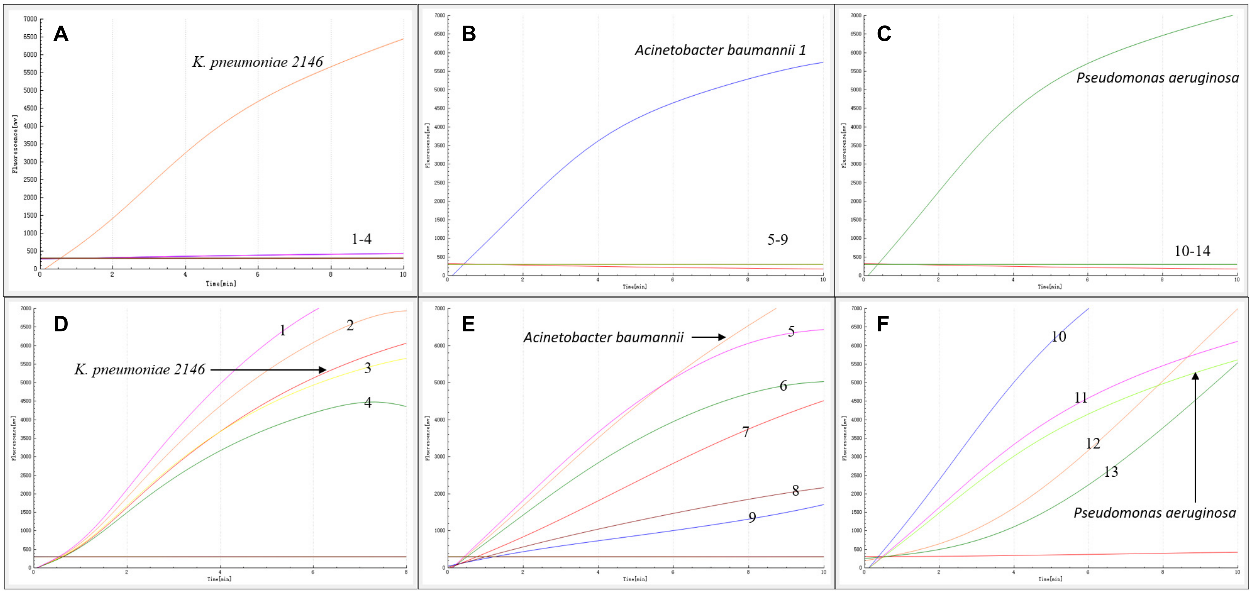

FIGURE 2 | Specificity of the RAA assay. *Klebsiella pneumoniae* 2146, *Acinetobacter baumannii* 1 and *Pseudomonas aeruginosa* produced amplification signals, while the other *bla*$_{NDM}$-lacking bacterial types were negative **(A–C)**. All the bacterial types produced 16S rRNA gene amplification signals **(D–F)**. 1: *K. oxytoca*, 2: *A. baumannii 2*, 3: *K. pneumoniae* 700603, 4: *P. aeruginosa* ATCC27853, 5: *S. sonnei*, 6: *E. coli* 25922, 7: *C. jejuni*, 8: *E. aerogenes*, 9: *P. mirabilis*, 10: *S. enteritidis*, 11: *E. cloacae*, 12: *C. freundii*, 13: *P. mirabilis*.

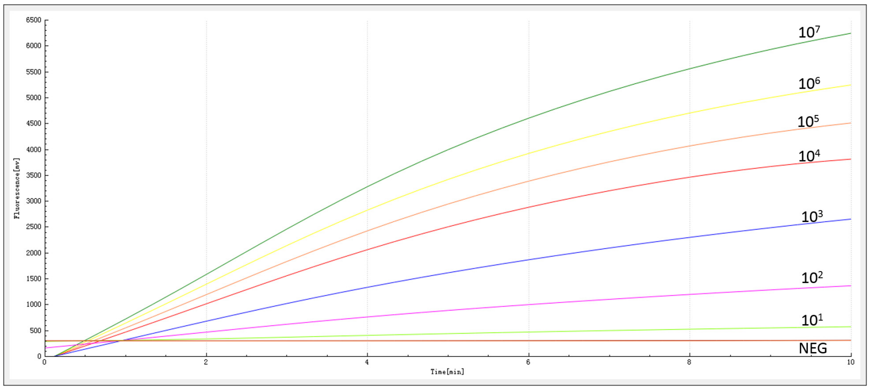

FIGURE 3 | Sensitivity of the RAA assay. An increase in the fluorescence signal was observed from 1 × 10^7 to 1 × 10^1 copies/reaction.

from whom more than one *bla*$_{NDM}$-positive isolates and *bla*$_{NDM}$ variants were identified were investigated (**Supplementary Table 1**). The diagnoses of these patients included chylous ascites and leukemia. All of these three patients had long histories of antibiotic therapy. Their clinical characteristics and antibiotic treatments were consistent with the culture and RAA detection results.

Susceptibility Test Results for the *bla*$_{NDM}$-Positive Bacteria

Following testing with the VITEK 2 compact system, most of the carbapenem-resistant *Enterobacterales* (CRE) types were resistant to cephalosporins, cefoperazone-sulbactam, piperacillin-tazobactam, ticarcillin-clavulanic acid, aztreonam, co-trimoxazole, and carbapenems. Overall, five, nine, and six bacteria types, respectively, were resistant to tobramycin, ciprofloxacin, and levofloxacin. Seven of the bacterial types were susceptible to doxycycline and minocycline. Amikacin and tigecycline showed excellent antibacterial activity against the CRE types (**Supplementary Table 2**).

DISCUSSION

In recent years, with the widespread use of carbapenems, CRE have become prevalent in the clinic (van Loon et al., 2017; Potter et al., 2016). The successful clinical treatment and control of CRE-related infections has faced great challenges.

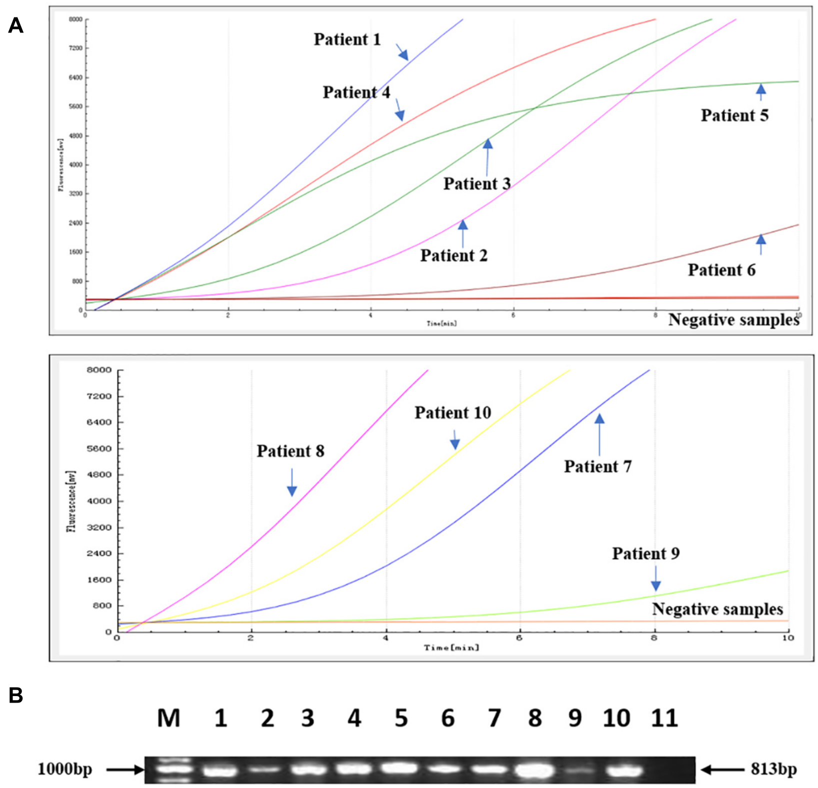

FIGURE 4 | Comparison of the RAA detection results with standard PCR on 112 clinical samples. Ten positive samples were identified. **(A)** The RAA detection results from the 10 positive samples. **(B)** The PCR results from the same 10 positive samples. M: Marker, 1–10: The 10 positive samples for patients 1–10; 11: negative control.

bla$_{NDM}$ is an MBL carbapenemase type usually seen in Asia (Safavi et al., 2020). The prevalence of NDM-producing *E. coli* was reported to be 82.6, 12.9, 1.5, 1.0, and 2.0% in Asia, Europe, America, Africa, and Oceania, respectively (Dadashi et al., 2019). The Chinese CRE Network showed that the *bla*$_{DM}$ production rate for carbapenemase-producing clinical isolates was 33.5% and these isolates were widely distributed in *K. pneumoniae*, *E. coli*, *E. cloacae*, and other species (Zhang et al., 2018). Dissemination of *bla*$_{NDM}$ through horizontal gene transfer is a potential threat to society. The detection rate for *bla*$_{NDM}$ in children was found to be comparably higher than in adults, with rates of 49% for CRE strains in children and 20.6% in adults (Fan et al., 2019). Development of a sensitive and reliable test to identify *bla*$_{NDM}$ directly in pediatric samples is therefore a priority for early diagnosis and infection control.

TABLE 3 | RAA detection results for clinical samples.

Sample ID	RAA	PCR	Culture	Sequencing
EYS1-1	+	+	*E. coli*	NDM-1
ESY1-2	+	+	*E. coli*	NDM-5,
ESY1-3	+	+	*K. pneumoniae*	NDM-1
ESY2-1	+	+	*K. pneumoniae*	NDM-1
ESY2-2	+	+	*K. oxytoca*	NDM-1
ESY2-3	+	+	*C. freundii*	NDM-1
ESY3-1	+	+	*C. freund*	NDM-1
ESY3-2	+	+	*C. freund*	NDM-5
ESY4	+	+	*A. junii*	NDM-1
ESY5	+	+	*K. pneumoniae*	NDM-1
ESY6	+	+	*K. pneumoniae*	NDM-5
ESY7	+	+	*E. coli*	NDM-5
ESY8	+	+	*A. baumannii*	NDM-1
ESY9	+	+	*A. baumannii*	NDM-1
ESY10	+	+	*P. mirabilis*	NDM-1

In this study, we developed an RAA assay to detect *bla*$_{NDM}$ directly in clinical specimens. This assay has proved to be highly specific and sensitive, as shown by its successful use in identifying SARS-CoV-2, hepatitis B virus, adenovirus, respiratory syncytial virus, salmonella, and other pathogens (Zhang et al., 2017; Fan et al., 2019; Qi et al., 2019; Shen et al., 2019; Wang et al., 2019; Xue et al., 2020a,b). In our tests, its sensitivity was 20 copies per reaction (0.1 pg/μL), which is comparable with the detection levels of other rapid detection methods, such as loop-mediated isothermal amplification (2.6–25.8 copies/reaction, 10.7 pg/μL) (Naas et al., 2011; Liu et al., 2012; Vasoo et al., 2013; Rathinasabapathi et al., 2015; Lund et al., 2018; Moreira et al., 2018; Bordin et al., 2019), and multiplex real-time PCR (7.5–1,000 cfu/ml). The primers we designed targeted the conserved region of the *bla*$_{NDM}$ gene among 28 variants, thereby avoiding any potential cross amplification with other genes. The results confirm that our RAA assay is highly specific by only providing a positive result for the bla_{NDM} gene, and no cross-reactions in other bacterial types without bla_{NDM} genes.

Even though multiplex real-time PCR and LAMP are also highly sensitive and specific, their whole reaction times are relatively long at approximately 1–2 h. The RAA detection method takes the shortest time, producing its results within 20 min, which makes it superior to these other methods. In addition, RAA is cheaper than other isothermal amplification techniques, such as LAMP and nucleic acid sequence-based amplification (NASBA).

To investigate the use of this method in clinical samples, we tested 112 clinical pediatric samples. Ten of them were bla_{NDM}-positive, and the RAA assay showed 100% percent agreement with the PCR method. These results confirm the validity of the RAA method for the rapid detection of bla_{NDM} in clinical samples.

We further investigated the characteristics of bla_{NDM}-positive carbapenem-resistant bacteria in children using the 15 bacteria types we identified (which included 4 *K. pneumoniae*, 3 *E. coli*, 3 *C. freundii*, 2 *A. baumannii*, 1 *A. junii*, 1 *K. oxytoca*, and 1 *P. mirabilis*) in the 10 samples. Our findings are consistent with those from other studies showing that *K. pneumoniae*, *E. coli*, and *C. freundii* were the most prevalent types in CRE strains (Fan et al., 2019). Here, the sequencing results showed that four of the bacteria types were positive for $bla_{NDM\text{-}1}$ and two for $bla_{NDM\text{-}5}$, which was consistence with the other studies showing that $bla_{NDM\text{-}1}$ and $bla_{NDM\text{-}5}$ are disseminated among *Enterobacterales* in children in China (Fan et al., 2019; Li et al., 2020; Tian et al., 2020).

Interestingly, we isolated more than one carbapenem-resistant type from three patients and found that bla_{NDM-1} and bla_{NDM-5} coexisted in one patient. The clinical characteristics of the three patients showed that two were patients from the hematology department and one was a patient in the intensive care unit where multiple antibiotics are often used. We then analyzed the antibiotic resistance characteristics of the 15 isolates. The MIC results showed these isolates were highly resistant to cephalosporins, cefoperazone-sulbactam, piperacillin-tazobactam, ticarcillin-clavulanic acid, aztreonam, co-trimoxazole, and carbapenems but susceptible to tigecycline and amikacin. The histories of antibiotic use in these patients were consistent with the detection results.

In summary, the RAA assay has high specificity and sensitivity in detecting bla_{NDM} genes in clinical samples. By providing a simple, rapid, and reliable method for bla_{NDM} detection, this assay may prove a great help for clinical treatment and antibiotic use. The common existence of bla_{NDM} and multi-drug resistance remains a big challenge for the clinical treatment of infections.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

JY, YW, and DQ designed the study. YF, GX, CY, JC, HZ, RZ, LG, and WX performed the experiments. NL, SL, SD, WZ, HY, JT, LM, and TZ analyzed the results. YF and GX wrote the manuscript. JY, YW, and DQ revised the manuscript. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.691289/full#supplementary-material

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