Prevalence and detection of *Stenotrophomonas maltophilia* carrying metallo-β-lactamase *bla*L1 in Beijing, China

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INTRODUCTION

*Stenotrophomonas maltophilia*, commonly associated with respiratory infections in children and adults, is an emerging Gram-negative MDRO (multi-drug-resistant organism) of global significance (Brooke, 2012). Currently, the incidence of *S. maltophilia* nosocomial infection is increasing, particularly for the immunocompromised (cancer, cystic fibrosis, drug addicts, and AIDS patients), dialysis patients, recipients of organ transplants, in addition to the reported cases of community-acquired *S. maltophilia*. The low outer membrane permeability of *S. maltophilia* renders it inherently resistant to most antibiotics, whilst the production of group 2e β-lactamase (L2) and group 3c β-lactamase (L1) confers resistance to β-lactam containing antibiotics (Alonso and Martínez, 1997). The *bla*L1 existed on a 200-kb plasmid, encoding a broad-spectrum metallo-β-lactamase which hydrolyzes carbapenems (Walsh et al., 1994; Avison et al., 2001), is usually produced at higher levels (induced) that hydrolyzes almost all known penicillins, cephalosporins, and carbapenems during β-lactam challenge. The ease of acquisition and spread of this antibiotic resistant gene of *bla*L1 in *S. maltophilia* emphasizes the need for antibiotic susceptibility testing of clinical isolates. At present, *bla*L1 has been identified in some clinical isolates of *S. maltophilia* with important drug resistance against carbapenems (Avison et al., 2001), so detection of *bla*L1 plays an important role to indicate the infection of *S. maltophilia* in the clinical work (Alonso and Martínez, 2006; Al-Jasser, 2006; Gould et al., 2006).

Recently, a number of molecular biology techniques have been used to detect different strains of *S. maltophilia* (Nakamura et al., 2010), PCR amplification of the 16S rRNA gene was used to detect *S. maltophilia* in blood samples of patients who are undergoing chemotherapy for acute leukemia or myelodysplastic syndrome (Nakamura et al., 2010). However, PCR requires specialized high-cost instruments and consumables. In addition, *Taq* DNA polymerase in PCR assays can be inactivated by inhibitors present in crude biological samples but the large fragment of *Bst* DNA polymerase (the large fragment of *Bst* DNA polymerase is part...
of Bacillus stearothermophilus DNA polymerase, it has 5′–3′ DNA polymerase activity) in loop-mediated isothermal amplification (LAMP) assays is more resistant to inhibitors present in crude biological samples (de Franchis et al., 1988; Kaneko et al., 2007). Thus, another rapid, simple and cost effective assay is needed to complement current PCR methods. The LAMP method which was developed in 2000 relies on auto-cycling strand displacement DNA synthesis which proceeds under isothermal conditions, typically within 60 min, and in the presence of Bst DNA polymerase (Notomi et al., 2000; Song et al., 2005). In this study, we develop this new method to detect the bla\textsubscript{L1} of S. maltophilia. The LAMP method has been shown to amplify target DNA with high-specificity, and it is used widely in the clinical detection of bacteria (Hara-Kudo et al., 2005; Ohtsuka et al., 2005), viruses (Okafuji et al., 2005), parasites (Chen et al., 2011; Kong et al., 2012), and for fetal sex identification (Hirayama et al., 2006).

Data on the prevalence of bla\textsubscript{L1} in S. maltophilia from ICU of Chinese hospitals are lacking. The objective of the current study is to develop a rapid, simple assay for S. maltophilia and to further investigate the infection status and the species distribution of bla\textsubscript{L1} in clinic. At first, we designed five primer sets which each set targets six or eight sequences on the bla\textsubscript{L1}. The specificity and sensitivity of the primers for bla\textsubscript{L1} was confirmed, and the LAMP method used for the detection of bla\textsubscript{L1} in clinical samples. Then, basing on this LAMP assays, dissemination and molecular characterization of L1-producing S. maltophilia isolates was investigated at ICU patients in three top hospitals (the hospitals that have large scale and many patients) in Beijing, China.

MATERIALS AND METHODS

BACTERIAL ISOLATES, IDENTIFICATION, MLST TYPING, AND ANTIMICROBIAL SUSCEPTIBILITY TESTING

A total of 37 bacterial strains were used in this study to develop the LAMP assays, and their sources are listed in Table 1. S. maltophilia K279a carrying bla\textsubscript{L1} and bla\textsubscript{L2} with the typical antimicrobial resistance properties was used as the positive control. The other species including common clinical infectious species and homologous species with S. maltophilia stored at our laboratory were used for estimating the sensitivity and specificity of the LAMP assay. 105 clinical nasopharyngeal swabs and sputum samples were collected from ICU hospitalized patients with clinically suspected multi-resistant infections in the 307 hospital, 302 hospital, and 301 hospital in China, and species identification was carried out using an automated system (Phoenix and BD systems) and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). 16S rDNA and bla\textsubscript{L1} were validated by PCR-based sequencing, and their sequence showed 100% (for 16S rDNA) and 98–100% (for bla\textsubscript{L1}) identity with the sequences of previously reported genes, respectively. The allele number for each gene was assigned on the basis of the information in the MLST database\textsuperscript{1}. A combination of the allelic sequences of the seven genes yielded the allelic profile. Antimicrobial susceptibility testing was performed by microbroth dilution according to the Clinical and Laboratory Standards Institute (CLSI, Clinical and Laboratory Standards Institute Performance standards for antimicrobial susceptibility testing; Twentieth informational supplement CLSI Document M100-S20, Wayne, PA, USA 2010.), and Etest strips (bioMérieux) for carbapenem. The carbapenemase activity of isolates was assessed by Etest MBls.

The strains were screened for the presence of known MBL genes (\textit{bla\textsubscript{TEM}}, \textit{bla\textsubscript{IMP}}, \textit{bla\textsubscript{SPM}}, \textit{bla\textsubscript{MDM}}, \textit{bla\textsubscript{NDM}}, \textit{bla\textsubscript{JMB}}, \textit{bla\textsubscript{ZMB}}, \textit{bla\textsubscript{NDM}) by PCR with primers as reported previously (Patzer et al., 2009). The strains were also screened for the presence of other \beta-lactamase genes (\textit{bla\textsubscript{CTX}}, \textit{bla\textsubscript{CMY}, etc.; Poiré et al., 2007).}

ISOLATION OF GENOMIC DNA

The 37 bacterial strains and the 105 clinical samples were cultured in brain heart infusion (BHI) broth at 37°C according to a standard protocol. Chelex\textsuperscript{®} 100 was used to extract total genomic DNA (including plasmid DNA) from 5 ml overnight bacterial cultures. Briefly, 500 μl bacterial suspension was centrifuged at 10,000 × g for 2 min and the supernatant discarded. The pellet was resuspended in 500 μl distilled water and 500 μl Chelex DNA extraction buffer (25 mM NaOH, 10 mM Tris-HCl, 1% Triton X-100, 1% NP-40, 0.1 mM EDTA, 2% Chelex-100) added. The cell suspension was heated in boiling water for 10 min, held on ice for 5 min, and centrifuged at 14,000 × g for 2 min. The extracted DNA was used as template in the LAMP and PCR reactions.

As for isolation of DNA from clinical sputum samples, DNA was extracted directly from 200 μl clinical sputum samples with the TIANamp Genomic DNA Kit (TIANGEN Biotech Co., Ltd., Beijing, China). The DNA was purified with the SV Gel and PCR Clean-Up System (Promega Co., USA). The DNA concentration was detected using the Spectrophotometer ND-1000 (Thermo Fisher Scientific Inc., USA).

PRIMER DESIGN

A total of 20 \textit{bla\textsubscript{L1}} in the NCBI GenBank database (Gen-Bank: HQ822273.1; EF126060.1; EF126061.1; AM743169.1; AB294542.1; AJ251814.1; JF705927.1; JF705926.1; EF126051.1; EF126054.1; EF126053.1; EF601224.1; AB294547.1; AB294545.1; AJ291672.1; AF010282.1; AB194306.1; AJ289085.1; AJ289086.1; AB194305.1) were compared, then the sequences of conserved regions were chosen to design the primer sets. Primer Explorer V4 software\textsuperscript{2} was used to design the outer forward primer (F3), outer backward primer (B3), forward inner primer (FIP), backward inner primer (BIP) and backward loop primer (loop B), used to accelerate the amplification reaction. Mergers of bases are used to circumvent the mutational site (Table 2). The FIP and BIP primers were linked by a four thymidine spacer (TTTT). Conventional PCR was performed using primers labeled L1-23-F3 and L1-23-B3. The primers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China).

LAMP REACTION

A 25 μl reaction volume was used for all LAMP reactions and contained the following components (final concentration): 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 10 mM (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}, 0.1% Tween 20, 0.8 M betaine, 8 mM MgSO\textsubscript{4}, 1.4 mM each dNTP, and 8 U Bst DNA polymerase. The amount of primer per reaction was 40 pmol.

\textsuperscript{1}http://pubmlst.org/smaltophilia/

\textsuperscript{2}http://primerexplorer.jp/e/
Table 1 | Bacterial strains used in the current study.

| Species                          | Source                        |
|----------------------------------|-------------------------------|
| Stenotrophomonas maltophilia-2   | Clinical isolate              |
| S. maltophilia-17                | Clinical isolate              |
| S. maltophilia-24                | Clinical isolate              |
| S. maltophilia-25                | Clinical isolate              |
| S. maltophilia-36                | Clinical isolate              |
| S. maltophilia-41                | Clinical isolate              |
| S. maltophilia-51                | Clinical isolate              |
| S. maltophilia-58                | Clinical isolate              |
| S. maltophilia-63                | Clinical isolate              |
| S. maltophilia-66                | Clinical isolate              |
| S. maltophilia-67                | Clinical isolate              |
| S. maltophilia-3859              | Clinical isolate              |
| S. maltophilia-4621              | Clinical isolate              |
| S. maltophilia-WU2               | Clinical isolate              |
| S. maltophilia-K279a             | Our microorganism center      |
| Acinetobacter baumannii B260     | Our microorganism center      |
| A. baumannii H18                 | Our microorganism center      |
| Bacillus megatherium 4623        | Our microorganism center      |
| Beta hemolytic Streptococcus     | Our microorganism center      |
| group A CMCC32213                |                               |
| Bordetella pertussis ATCC 18530  | Our microorganism center      |
| Brucella suis 3572               | Clinical isolate              |
| Corynebacterium diphtheriae CMCC38001 | Clinical isolate |
| Enterotoxigenic Escherichia coli | Our microorganism center      |
| 44824                            |                               |
| Mycobacterium tuberculosis 8362  | Our microorganism center      |
| Neisseria meningitidis group B   | Our microorganism center      |
| CMCC29022                        |                               |
| Salmonella aberdeen 9264         | Our microorganism center      |
| Salmonella enteritidis 50326-1   | Our microorganism center      |
| Salmonella paratyphi B 86423     | Our microorganism center      |
| Shigella flexneri 4536           | Our microorganism center      |
| Shigella sonnei 2531             | Our microorganism center      |
| Staphylococcus aureus 2740       | Our microorganism center      |
| Vibrio carchariae 5732           | Our microorganism center      |
| V. cholera 3802                  | Our microorganism center      |
| V. parahaemolyticus 5474         | Our microorganism center      |
| Yersinia enterocolitica 1836     | Our microorganism center      |
| Y. pestis 2638                   | Our microorganism center      |

FIP and BIP, 20 pmol LB, 5 pmol F3 and B3. The appropriate amount of DNA template was included in the reaction volume. The LAMP assay proceeded in a reaction tube (Eiken Chemical Co., Ltd., Tochigi, Japan) for 60 min at 65°C.

DETECTION OF LAMP PRODUCTS

Two independent methods, based on either sample turbidity or fluorescence were used to detect LAMP products. Real-time changes in turbidity were monitored by measuring the optical density (\(\lambda_{650\ nm}\)) at 6 s intervals, for each LAMP reaction in a Loopamp real-time turbidimeter (LA-320c; Eiken Chemical Co., Ltd.). The changes in turbidity arose from the presence of the amplification by-product \(Mg_2P_2O_7\) (a white precipitate).

The second method used direct visual inspection to assess color changes in the presence of the fluorescent metal ion indicator calcein/Mn\(^{2+}\) complex. One microliter of calcein/Mn\(^{2+}\) complex (Eiken Chemical Co., Ltd.) was added to 25\(\mu\)l LAMP reaction volume prior to the commencement of the LAMP assay. On completion of the reaction a change in color from orange to green indicated a positive reaction, whilst no color change indicated a negative reaction. The color change was observed by the naked eye under natural light or under UV light at 365 nm.

PCR DETECTION

A 25 \(\mu\)l reaction volume was used for all PCR reactions and contained the following components: 12.5 \(\mu\)l PCR Taq MasterMix (Tiangen Biotech Co., Ltd.), 9.5 \(\mu\)l double distilled water, 1 \(\mu\)M L1-23F3 and L1-23B3 primers, and DNA template. The oligonucleotide primers used for cloning \(bla_{L1}\) are:

- F: \(5'-atgcgttctaccctgctcgccttcgcc-3'\)
- R: \(5'-tcagcgggccccggcgtttcttgccag-3'\)
- LAMP reaction volume was used for all PCR reactions and
- \(\lambda_{650\ nm}\) at 6 s intervals, for each LAMP reaction in a Loopamp real-time turbidimeter (LA-320c; Eiken Chemical Co., Ltd.). The changes in turbidity arose from the presence of the amplification by-product \(Mg_2P_2O_7\) (a white precipitate).

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RESULTS

THE OPTIMAL PRIMER SETS FOR LAMP ASSAY

Five primer sets were detected in the same reaction condition using real-time turbidimeter and their turbidity curves were draw at 650 nm according to the amplified results. The optimal primer sets amplified the target sequence with the shortest time among them was chosen for further investigation (see Table 2).

SENSITIVITY OF THE LAMP METHOD FOR \(bla_{L1}\) DETECTION

The sensitivity of the LAMP method for detecting \(bla_{L1}\) was evaluated using genomic DNA extracted from \(S.\ maltophilia\) K279a (Wizard Genomic DNA purification Kit), serially diluted 10-fold from 379 ng/\(\mu\)l to 0.00379 pg/\(\mu\)l. As shown in Figure 2A, the detection limit of the LAMP assay for \(bla_{L1}\) was 3.790 pg/\(\mu\)l. Visual inspection of the color change, post-LAMP assay, and in the presence of calcein/Mn\(^{2+}\) complex confirmed reactions positive (green) and negative (orange) for \(bla_{L1}\) (Figure 2B). The results from the two detection methods were in agreement with sensitivity for \(bla_{L1}\). PCR reactions on the serially diluted DNA using primers
Table 2 | Primers used for the amplification of bla_L1.

| Primer | Type               | Sequence (5’–3’)                        |
|--------|--------------------|----------------------------------------|
| L1-23F3 | Forward outer     | CGGCAAGCCACAGATGG                     |
| L1-23B3 | Backward outer    | GCAAGCAGCCTGTTTCT                      |
| L1-23FIP | Forward inner    | TCAATGCAAGTCCTGGGTTTTCGGTCACCTGCTGGACAAC |
| L1-23BIP | Backward inner   | TGY(C/T)AGCCATGCGCAY(T/C)GCS(C/G)GATTTTGCATTGGCCGCCACATG |
| L1-23LB  | Loop backward     | TCGCGAGCTCAAGCGT                      |

**FIGURE 1 | Specificity of the LAMP method for bla_L1 gene detection.** It has two parts, (A) is the graphic and (B) is the photography of microtubes. The reaction proceeded at 65°C for 65 min. Turbidity was monitored in the Loopamp real-time turbidimeter and the OD_{660nm} recorded at 6 s intervals. L1, *Brucella suis* 3572; L2, *Bacillus megatherium* 4623; L3, *Vibrio carchariae* 5732; L4, *Acinetobacter baumannii* B260; L5, *Corynebacterium diphtheriae* CMCC38001; L6, *Acinetobacter baumannii* H18; L7, *Mycobacterium tuberculosis* 8362; L8, *Shigella sonnei* 2531; L9, *Shigella flexneri* 4536; L10, *Salmonella enteritidis* 50326-1; L11, *Yersinia enterocolitica* 1836; L12, *Vibrio parahaemolyticus* 5474; L13, *Salmonella paratyphi* 86423; L14, *Neisseria meningitidis* group B CMCC29022; L15, *Enterotoxigenic E. coli* 44824; L16, *Beta hemolytic Streptococcus* group A CMCC2213; L17, *Yersinia pestis* 2638; L18, *Salmonella aberdeen* 9264; L19, *Vibrio cholerae* 3802; L20, *Staphylococcus aureus* 2740; L21, *Bordetella pertussis* ATCC 18530; L22, positive control (*S. maltophilia* K279a); L23, negative control (distilled water).

L1-23F3 and L1-23B3 were also conducted, and the detection limit for bla_L1 was established as 379 pg/μl (Figure 2C).

**SPECIFICITY OF THE LAMP METHOD FOR bla_L1 DETECTION**

The specificity of the LAMP method for detecting bla_L1 was evaluated using *S. maltophilia* K279a with bla_L1 as the positive control, distilled water as the negative control, and 21 strains without carrying bla_L1 including common clinical infectious species and homologous species with *S. maltophilia* as test subjects. As shown in Figure 1A, turbidity increased only when *S. maltophilia* K279a with bla_L1 was used as template DNA in the LAMP assay. When distilled water and the 21 remaining bacterial species were used as template, no changes in turbidity were recorded. These results suggest that the primers had good specificity for bla_L1. In addition, these results are consistent with those obtained using the fluorescent indicator calcein/Mn^{2+} complex. Whereby, only the LAMP assay with *S. maltophilia* K279a with bla_L1 recorded a change from orange to green, indicative of a positive reaction (Figure 1B). Whilst, all test samples negative for bla_L1 and the negative control remained orange, indicative of a negative reaction.
FIGURE 2 | Comparison of the sensitivities for blaL1 gene detection by LAMP and conventional PCR methods. Pure genomic DNA extracted from S. maltophilia-K279a was diluted tenfold (379.0 ng/μl to 0.00379 pg/μl) and the DNA assayed by LAMP (A,B) and PCR (C). (A) Turbidity was monitored using the Loopamp real-time turbidimeter and the OD recorded at 650 nm, at 6 s intervals. (B) Visual inspection of the color change, post-LAMP assay, and in the presence of calcein/Mn2+ complex. (C) PCR products were analyzed by 2% agarose gel electrophoresis and stained with ethidium bromide. The DNA marker is D2000 DNA Marker (Tiangen Biotech Co., Ltd.) The size is about 179 bp.

DISSEMINATION OF L1-PRODUCING S. maltophilia IN CLINICAL
A total of 105 clinical sputum samples and nasopharyngeal swabs were collected for LAMP-based surveillance of blaL1 from 105 ICU patients with clinically suspected multi-resistant infections from the department of Respiratory Diseases in three top hospitals. Ten pairs of sputum samples and nasopharyngeal swabs from healthy people were collected as controls.

All clinical samples were analyzed by LAMP and PCR simultaneously. Of the 105 patients samples, 22 were confirmed to be infected with S. maltophilia with blaL1 and 83 negative samples by the LAMP assay (Figure 3), whilst the PCR assay detected 13 positive samples, and 92 negative samples. Then 22 strains of S. maltophilia were isolated and identified from all of clinical sputum samples and swabs samples, which the positive samples were in accordance with those in LAMP assay. S. maltophilia with blaL1 was positively identified 100% by LAMP and 86.7% by PCR, respectively. None of the samples from healthy people was tested as positive for blaL1. Thus, the results showed the LAMP assays is more sensitive and the specific than PCR for diagnosis of S. maltophilia in clinical practice.

The sequence analysis of the blaL1 genes from S. maltophilia isolates confirmed conservation with the nucleotide sequences of reported genes or with only a few sites mutated. In the MLST analysis of S. maltophilia, the results of seven housekeeper genes recovered that 22 strains belonged to different sequence type (ST) including ST4, ST8, ST 25, ST 28, ST29, or ST31, respectively. To further characterize the 22 strains carrying blaL1 genes, the susceptibility pattern was detected and clearly showed that all isolates had highly resistant to β-lactam antibiotics. The isolates also tested positive for MBLs in both the imipenem-EDTA double-disk synergy test (DDST) and modified Hodge test (MHT).

Furthermore, PCR screening of the isolates were performed for the known MBL genes including blaNDM−1, blaVIM, blaIMP, blaSPM−1, blaGIM−1, blaSIM−1, blaAIM−1, and blaL2 (Patzer et al., 2009). PCR yielded products of 22 isolates with expected sizes for blaL1, and sequencing of these genes showed 100% identities with previously reported genes. It’s interesting to note that the isolate named as S. maltophilia DCPS-01 contained L1 and L2 β-lactamase genes with a novel blaNDM−1 which has attracted wide attention because of its superior resistance to all β-lactam antibiotics, which presented increased carbapenemase activity to all β-lactams (MIC >128 μg/mL for imipenem and meropenem), aminoglycosides and quinolones, and was only susceptible to tigecycline and colistin.

Therefore, our data showed the diversity genotypic features of S. maltophilia carrying blaL1 indicated wide spread in the
respiratory infections. Importantly, the emergence of these powerful co-occurring resistance mechanisms described here provides warning that future therapeutic options may be seriously limited.

**DISCUSSION**

*Stenotrophomonas maltophilia* is a widespread environmental bacterium that has become a nosocomial pathogen of increasing importance. It is currently the third most common nosocomial non-fermenting bacteria, behind *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, and is associated with crude mortality rates ranging from 14 to 69% in patients with bacteraemia (Jang et al., 1992; Victor et al., 1994). Of the 1661 antibiotic resistant strains of *S. maltophilia* recovered from 14 hospitals across several regions of China during 2010, the majority (97.5%) were isolated from patients. Approximately 68.3% of strains were isolated from patients ≥ 60 years of age, whilst only 4.8% were from patients < 18 years of age. Most (83.0%) isolates were recovered from sputum and respiratory tract secretions (The data and information from CHINET: CHINET 2010 surveillance of antibiotic resistance in *S. maltophilia* in China). Within hospitals, the bacterium is most often found in water sources, and can be a contaminant of hospital equipment such as nebulizers and intravenous catheters. From these sources, the organism can infect patients, resulting in a wide spectrum of symptoms dependent upon the site of infection, though, most commonly, *S. maltophilia* causes bacteraemia or respiratory tract infections (Denton and Kerr, 1998). β-Lactam resistance is due to the expression of the β-lactamases L1, which together hydrolyze the full range of β-lactam drugs, with the exception of monobactams (Walsh et al., 1994). It is therefore necessary to detect and monitor antibiotic resistance, persistence and spread of *S. maltophilia* within the community and in health care settings.

Loop-mediated isothermal amplification assays are generally less time and labor intensive compared with traditional methods of pathogen detection, in part because the amplification of the target gene is performed at a constant temperature, and the reaction times are usually less than an hour. To date, a method for detecting *S. maltophilia* based on LAMP assays has not been reported. In the current study, we designed primers specific for the metallo-β-lactamase bla₃_1 for use in a LAMP assay to detect *S. maltophilia* in clinical samples. Results from the specificity and sensitivity analyses demonstrated that the LAMP method detected genomic DNA at 3.79 pg/μl, and was specific for the β-lactamase bla₁. In the specificity and sensitivity detection, we only use the bla₃_1 of *S. maltophilia* K279a as the target gene, although many other *S. maltophilia* strains do not have the same sequence of bla₃_1, the most conserve regions of bla₁ were choses to design the decisive primers. In LAMP reaction, it is not true that all the primers should combine with the target sequence. The decisive primers are the FIP and BIP, if the FIP and BIP can combine with the target sequence, the reaction is certain to occur, well, of course, more novel experiments should be made to ensure this conclusion. Although the LAMP method has complex amplification principle, the assay is rapid, easy to operate, highly sensitive and specific, and proceeds under isothermal conditions. We believe this assay would be suitable for use in inspection and quarantine departments and in health care units to test for *S. maltophilia*, and we anticipate its routine use in hospital testing regimes, particularly for rapid clinical testing.

A drawback of the LAMP method is the relatively high false-positive rates; a consequence of the assay's high sensitivity (Notomi et al., 2000). Strict spatial separation of reagent preparation from the testing area is necessary to avoid contamination. In the current study, a sealing agent was applied to the reaction tube once the
reaction mixture had been prepared, and its presence is useful in preventing contamination.

In conclusion, we designed a detection method based on LAMP for the specific, sensitive, rapid, and effective detection of the metallo-β-lactamase βlma1 of S. maltophilia. We believe this technique would greatly benefit hospitals and health units, and we anticipate that LAMP will become the gold standard for the rapid detection of pathogens in clinical samples. At the same time, this report provides new insights into the mechanisms of drug resistance and warning that future therapeutic options may be seriously limited.

AUTHOR CONTRIBUTIONS
Jing Yuan helped conceive project and designed experiments. Zhan Yang, Wei Liu, and Qian Cui performed and wrote the manuscript. Huan Li, Xiangna Zhao, Xiao Wei, Wenkai Niu, Changqing Bai, Yan Li, and Liuyuan Huang designed and executed experiments. Simo Huang, Derong Dong, and Sijing Lu helped to edit the manuscript.

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