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Virulence and resistance on various pathogens mediated by mobile genetic integrons via high flux

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ABSTRACT

Background: Recognized as a resistance mechanism responsible for the emergence and prevalence of antimicrobial resistance, integron is widely distributed and spread among clinical microorganisms and play a key role in the dissemination of such antimicrobial resistance, which may eventually contribute to the unleashing of "Super Bugs" In this study, detection assays based on loop-mediated isothermal amplification (LAMP) methodologies targeting on class 1 to class 3 integrase genes was developed and evaluated.

Methods: LAMP methodology was employed to develop novel detection assays on class 1, 2 and 3 integrons. Firstly, this protocol was specifically designed to detect such integrons by targeting integrase genes *intI1*, *intI2* and *intI3*. Development, evaluation and optimization of such LAMP assays was studied, including the reaction temperature, volumn, time, sensitivity and specificity of both primers and targets. A total of 1082 strains, including 397 integron positive and 685 integron negative microorganisms, were included for the application verification of the established LAMP assays.

Results: The indispensability of each primer was confirmed, and the optimal amplification was obtained under 63 °C for 45 min, with 25 μl reaction found to be the most cost-efficient volume. As application was concerned, all of the 397 integron-positive isolates yielded positive amplicons and other 685 integron-negative bacteria were negative for the integron-LAMP assays, revealing totaling 100% detection rate and specificity.

Conclusions: The established integron-LAMP assays was demonstrated to be a valid and rapid detection method for integrons screening, which may aid in both the laboratory and clinical integron screening for microorganisms.

1. Backgrounds

In the past decades, indiscriminate abuse of existing antibiotics leads to proliferation of antibiotic resistance in microbes [50] and will consequently result in an increasing number of clinical failures in bacterial mediated diseases [1,2]. Several resistance mechanisms are responsible for the emergence and prevalence of antimicrobial resistance, such as plasmids and transposons.

As a novel resistance determinant, integron was firstly reported in 1989 [3], and its mechanism and mobility, such as the excision and integration for gene cassettes, were further investigated [3–12]. A complete integron platform may comprise three basic genetic elements, the integrase gene (*intI*), recombination site (*attI* and *attC*) and a promoter gene (*Pc*). Through specific excision and integration, gene cassettes become part of integron and mediated various functions for the hosts, with drug resistance mostly identified [13–15]. Integrons have been classified based on the differences and divergence in the sequences of *intI*. Up to date, four general classes of integrons have been identified and distinguished, and classes 1 to 3 integrons are known as multi-resistant integrons, which was reported to be capable of acquiring same gene cassettes via similar recombination platform [16–20]. Recently, the occurrence and prevalence of integrons in clinical microorganisms and the role played in antimicrobial resistance have been well studied, and currently integrons are considered to be widely distributed and spread among clinical microorganisms and play a key role in the dissemination of such antimicrobial resistance, which may eventually contribute to the unleashing of "Super Bugs" [21–23,54–60].

As screening and detection of integrons were concerned, PCR has been widely used as a common assay. However, the requirement for PCR thermal cycler and electrophoresis of PCR amplicons have

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Table 1
Standard strains used in the development of LAMP assay.

Strains	No.	*intI1*	*intI2*	*intI3*
Class 1 integron positive microorganisms				
E. coli	109	+	–	–
Acinetobacter	21	+	–	–
P. aeruginosa	51	+	–	–
K. pneumoniae	28	+	–	–
E. cloacae	13	+	–	–
S. aureus	92	+	–	–
S. epidermidis	16	+	–	–
S. haemolyticus	5	+	–	–
S. hominis	9	+	–	–
S. warneri	1	+	–	–
E. faecalis	9	+	–	–
E. faecium	2	+	–	–
Stretococcus	5	+	–	–
Class 2 integron positive microorganisms				
P. aeruginosa	20	–	+	–
E. coli	6	–	+	–
Proteus	2	–	+	–
Class 1 and 2 integron positive microorganisms				
P. aeruginosa	3	+	+	–
E. faecalis	2	+	+	–
E. coli	3	+	+	–
Integron negative microorganisms				
S. aureus	138	–	–	–
S. epidermidis	16	–	–	–
S. haemolyticus	7	–	–	–
S. hominis	8	–	–	–
S. capitis	1	–	–	–
S. saprophiticus	1	–	–	–
S. sciuri	1	–	–	–
S. schleiferi	1	–	–	–
S. intermedius	1	–	–	–
L. monocytogenes	58	–	–	–
L. invanovii	2	–	–	–
L. welshimeri	1	–	–	–
L. seeligeri	1	–	–	–
B. cereus	6	–	–	–
E. coli	121	–	–	–
V. parahaemolyticus	108	–	–	–
V. vulnificus	1	–	–	–
V. mimicus	1	–	–	–
P. aeruginosa	153	–	–	–
Salmonella enterica	44	–	–	–
Salmonella typhimurium	2	–	–	–
Salmonella choleraesuis	1	–	–	–
Salmonella enteritidis	2	–	–	–
Salmonella typhi	3	–	–	–
Salmonella paratyphi	1	–	–	–
Salmonella aberdeen	1	–	–	–
Salmonella gallinarum	1	–	–	–
K. pneumoniae	1	–	–	–
E. cloacae	1	–	–	–
Y. enterocolitica	2	–	–	–
Total	1082			

restricted its further application, especially in clinical laboratory [24,25]. In the latest decade, loop-mediated isothermal amplification (LAMP), as a novel nucleic acid amplification method, was reported [26–28] and applied to the detection of various pathogenic organisms [29–45]. In this study, LAMP assays on resistance integrons (class 1, 2 and 3 integrons) screening was developed, evaluated, optimized and further applied to the detection of a large scale of clinical isolates, with approximately 60 min required for the entire process.

2. Methods

2.1. Bacterial strains

For development and evaluation of the integrons screening LAMP assays, *Vibro cholera* (*V. cholera*) *O1* strain SK-10, *Escherichia coli* (*E. coli*) strain harboring an R483:Tn7 plasmid and *Serratia marcescen* (*S. marcescens*) AK9373 were used as a positive control for classes 1, 2 and 3 integrons, respectively. For application of the established LAMP assays, a total of 1082 strains were studied, including various species of Gram-negative and Gram-positive isolates (Table 1) with 397 integron positive microorganism and 685 integron negative microorganisms. All tested bacteria were previously identified using standard procedures, including colony morphology, Gram staining, Vitek 2 automated system and the API commercial kit.

Table 2
List of oligonucleotide primer sequences.

Target	Sequence (5′ to 3′)	Size	Position	GenBank
intI1				AF550415
F3	CGAGGCATTTCTGTCCTGG	19	185–203	
B3	ACCACTTCATCCGGGGTC	18	353–370	
FIP	AGAAGAACAGCAAGGCCGCC-ACGAGCGCAAGGTTTCG	37	211-227, 251-270	
BIP	AAGGTGCTGTGCACGGATCTG-AGCACCACCGGCAAGC	37	276-296, 337-352	
LF	AATGCCTGACGATGCGTGGA	20	231–250	
LB	CCCTGGCTTCAGGAGATCG	20	297–316	
intI2				AP002527
F3	TATGGTGCAGGTTTGCGC	18	390–407	
B3	CCCTACGCCTTGTAAGTTGT	20	574–593	
FIP	ACCGTCATGCACAGTGATGCAG-GAATGCTTGCGTTTGCGG	40	414-431, 458-479	
BIP	AGAAACAGCCTACTGCCCACG-TCTTGCTGAATAAGCCGCG	40	495-515, 553-571	
LF	CCATTATCAAAATCAAAATC	20	438–507	
LB	CGCCTAATCCCAGCAATAAA	20	516–535	
intI3				AY219651
F3	CCACAAACCGGCGTTGAG	18	753–770	
B3	GTCCGAATGCCCCAACAA	18	927–944	
FIP	GCCAGCCTGAACTACCGCTTTT-CGCCGCCACCACTTGTT	39	771-787, 815-836	
BIP	CACGTATCTGTCCACACCCTGC-GGATGTCTGTGCCTGCTTG	41	846-867, 894-912	
LF	TTTAGTTGCCGGTTCAGTCT	20	795–814	
LB	GCCACTCATTCGCCACCCAC	20	868–887	

2.2. Primer design

The protocol of the LAMP assays for integrons screening were specifically designed targeting *intI1*, *intI2* and *intI3* region on integron (Table 2). LAMP primers were designed via PrimerExplorer (V4), including forward inner primer (FIP) with the complementary sequence of F1 (F1c), a T–T–T–T linker and F2, backward inner primer (BIP) with the complementary sequence of B1 (B1c), a T–T–T–T linker and B2, the outer primers F3 and B3 located outside of the F2 and B2 regions, loop primers LF and LB located between F2 and F1 or B1 and B2, respectively.

2.3. DNA extraction

Template DNA from isolates used for development, evaluation and optimization of LAMP assays were prepared as described previously [46–49]. In brief, strains were inoculated and incubated in overnight Luria-Bertani (LB) broth cultures at 37 °C with shaking, followed by dilution with 10-fold in 10 mM Tris-HCl (pH 8.0) containing 1 mM EDTA and boiling of the suspension for 10 min then kept on ice. After centrifugation at 12,000 rpm for 3 min [51–53], the resulting supernatant was used as template for LAMP amplification. Template DNA for every single strain were further confirmed by OD_{260}/OD_{280} under spectrophometer and electrophoresis, and those DNAs with OD_{260}/OD_{280} ranging from 1.6 to 1.8 and typical genomic DNA bands on agarose gel were qualified and subjected to LAMP assays.