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Research Article

Rapid Detection of Methecillin Resistant *Staphylococcus Aureus* Using Loop Mediated Isothermal Amplification (LAMP)

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Abstract

Background: *Staphylococcus aureus*, including methicillin-resistant *S. aureus* (MRSA), is a major bacterial pathogen associated with nosocomial and community-acquired *S. aureus* infections all over the world.

Aim: The aim of this study was to establish the loop mediated isothermal amplification technique as a rapid detection method for methicillin resistant S. aureus.

Methods: The study was carried out in Omdurman Military hospital, Alshorta hospital, and Alneelain university dental clinic. A total of 60 samples were collected and cultured, All confirmed *S. aureus* isolates were tested against oxacillin antibiotic by the disk diffusion method to detect MRSA isolates LAMP and PCR assays were then used to detect mec A gene directly from the samples and from culture isolates.

Results: Among 60 (wound, dental plaque and urine) seven samples (11.6%) MRSA were detected by culture, 5% were found positive for mec A using LAMP and 3.3% using PCR. LAMP detected mec A in 100% of the seven MRSA isolates, PCR detected mec A in 14.3%. The LAMP results were confirmed using melting analysis.

Conclusion: The LAMP described herein is a reliable and rapid method for detection of the mec A gene. Generally, these findings are useful for future studies since there is little available information about MRSA infection in Sudan. LAMP can be used in a hospital setting for rapid diagnosis of MSRA, this should help better management and treatment of the affected patients in addition to rapid initiation of infection control procedures.

Keywords: *S. aureus*; mec A; LAMP; PCR

Abbreviations

PCR: Polymerase Chain Reaction; LAMP: Loop Mediated Isothermal Amplification; MRSA: Methicillin Resistant Staphylococcus aureus; BST: Bacillus stearothermophilus; SCCMEC: Staphylococcal Chromosomal Cassette mec mobile genetic element; UV: Ultra Violet; DNA: Deoxyribonucleic Acid

Introduction

Staphylococcus aureus is an infectious pathogen that can survive on inanimate environmental surfaces. It can colonize skin, mucous membranes, and the anterior nares in about 30% of healthy individuals [1]. They grow well under conditions of high osmotic pressure and low moisture, making them able to grow and survive in

nasal secretions and on the skin [2]. *Staphylococcus aureus* is found in a wide range of habitats including environmental surfaces, in nasal nares of domesticated animals like dogs, cats and horses and on human body surfaces as part of normal microflora [3]. The human body is a natural reservoir for this bacterium, and studies have shown that the anterior nares are where it is often found than in the other parts of the body [4]. Carriers may be divided into three groups: persistent, intermittent, and non-carriers Persistent carriers usually carry only one strain and make up 20% of the population. Sixty percent of intermittent carriers harbor multiple strains of S. aureus for weeks at a time. Twenty percent are non-carriers and may yield negative results in the laboratory cultures on repeat swabs over time [5]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is associated with substantial morbidity and mortality in many regions of the world [1]. Methicillin-susceptible *S. aureus* becomes methicillin-resistant *S. aureus* (MRSA) upon acquisition of the *Staphylococcal* Chromosomal Cassette mec (SCC mec) mobile genetic element. SCC mec harbors mec A or mec C, both of which encode alternate penicillin- binding proteins, which mediate resistance to almost all β-lactam antibiotics [6]. Rapid identification of MRSA in hospitalized patients is essential for administration of appropriate antibiotic therapy and infection control regimens. In addition, the increasing numbers of community-acquired infections necessitate the screening of carriers, rather than simply detecting the infected patient [7]. *Staphylococcus* strains have commonly been identified via routine standard procedures including colony morphology, Gram staining, testing of catalase, and coagulase [8], MRSA is identified via susceptibility tests [9], The disadvantage of the phenotypic methods are that they are time consuming and have a turnaround time of 2-3 days [10].

Identification of MRSA by molecular methods is widely used and based; in general; on the detection of *S. aureus* specific gene target the mec A gene [11]. For the rapid examination of methicillin resistance in Staphylococci, polymerase chain reaction (PCR)-based molecular techniques have been developed by targeting the mecA, the gene for penicillin-binding protein 2a [12], and real-time PCR is now widely used for the diagnosis [13]. In general, compared to the conventional culture method, PCR can be performed in relatively rapid and simple fashion, but special reagents and apparatus such as thermal cycler are needed for the method. In the last decade, loop-mediated isothermal amplification (LAMP) had been reported as a novel nucleic acid amplification method and applied to the detection of various pathogenic organisms [8]. LAMP which is based on auto cycling strand displacement DNA synthesis using the Bst DNA polymerase enzyme was developed by Notomi., *et al* [14]. The Bst DNA polymerase was isolated from *Bacillus stearothermophilus*, which possesses a 5′ →3′ exonuclease along with 5′→3′ polymerase activity and needs high concentration of magnesium for optimum activity [15]. The LAMP reaction can be conducted under isothermal condition ranging from 60 to 65 C [16,17], and specificity is attributable to four primers that recognize six distinct sequences [14-18]. Continuous amplification under isothermal condition produces an extremely large amount of target DNA within less than an hour [7,19], and the method enables simple visual (naked-eye) judgment of the DNA amplification through a white precipitate [20] or color change of the reaction mixture with SYBR green I [21]. A number of LAMP tests to detect MRSA have been designed and used successfully [8,22]. The rapidity, specificity, and simplicity of the technique make it appealing for use in MRSA screening. The purpose of the present study was establishment of a LAMP test for detection of MRSA based on the mecA gene and to compare it with a PCR method.

Material and methodology

Study design

This is a Cross sectional study carried out in Khartoum State's hospitals.

Clinical samples

60 miscellaneous samples were kindly provided by the following:

Omdurman military hospital (20 wound samples), Alshorta hospital (20 urine samples)., Alneelain university dental clinic (20 dental plaque samples). The patients did not have a medical history of MRSA-infection.

Isolation, identification, and antimicrobial susceptibility testing

The samples were cultured using standard methods on mannitol salt agar at 37°C for 24 hours, Isolation of *S. aureus* was based on the morphology of colonies, mannitol fermentation ability, catalase and coagulase tests. All confirmed *S. aureus* isolates were tested against oxacillin antibiotic by the disk diffusion method to detect MRSA isolates.

DNA Extraction

DNA was extracted from positive-MRSA isolates, directly from the samples using boiling method.

PCR

For PCR, backward and forward primers mentioned in (Table 1) were used to amplify mecA region,The reaction was performed in 20 µl volume using Solis Bio dyne master mix.(Solis Bio dyne, Estonia) The volume included: 4 µl master mix, 1 µl forward primer, 1 µl reverse primer, 5 µl extracted DNA and 9 µl distilled water. The DNA was amplified in thermocycling conditions using PCR machine Techno (Japan) as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 62°C for 1 min and extension at 72°C for 1 min, with a final extension at 72°C for 7 min. Each amplicon was run in 2% agarose gel. MRSA clinical samples and culture isolates that were successfully amplified gave bands of 210 bp when the gel was visualized under UV light, this was considered as positive result for mecA gene presence.

Primers	Sequence
Forward	5′-TGCTATCCACCCTCAAACA GG-3′
Reverse	5′-AACGTTGTAACCACCCCAAGA-3′

Table 1: PCRuniversal primers sequence used for the amplification of mecA gene [7].

LAMP primers Mix

Primers mix that contains four primers (FIP, BIP, F3, B3) which were derived from the sequence of the mecA gene [7] were used. Final concentration of FIP and BIP was 40 pmol each, F3 and B3 5 pmol each. The sequences of these primers are shown in (Table 2).

Primer	Sequence
F3	5′-aagatggcaaagatattcaact-3′
B3	5′-aggttctttttttatcttcggtta-3′
FIB	5′-acctgtttgagggtggatagcatgatgctaaagttcaaaagagt-3′
BIP	5′-gcacttgtaagcacaccttcacttcgttactcatgccatac-3′

Table 2: LAMP Primers used in this study [7].

LAMP condition

The reaction was performed in a final volume of 25 µl using LAMP regents (Mast, Reinfeld, Germany) which contained 12.5 µl 2x LAMP reaction buffer, 1 µl of Bst DNA polymerase, 2 µl primer mix (PM) included (40 pmol each FIP and BIP primers, 5 pmol each F3 and B3 primers), 1 µl fluorescence dye (FD), 6.5 µl H2O and 2 µl of target DNA. The mixture was incubated in a real-time PCR at 64o C for 60 minutes and the results were visualized using the FAM channel.

Result

Antimicrobial Susceptibilities Test

Among a total of 60 samples 7(11.6%) MRSA were isolated.

PCR detection of mecA gene among MRSA isolates and clinical samples

PCR amplification showed that two out of sixty (3.3%) samples were positive for mecA gene for the directly extracted samples, and one out of seven(14.3%) samples was positive for mecA gene for the isolated MRSA.

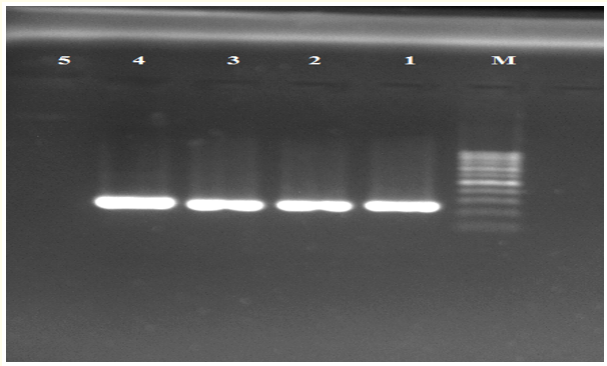

Figure 1: MecA PCR result (210bp) on 2% agrose gel. Lane1and 2shows positive control , lanes , 3 and 4 show results in two patients, lane 5 show negative control, M: 100bp DNA marker.

LAMP detection of mecA gene among MRSA isolates and clinical samples

LAMP amplification showed that three out of sixty (5%) samples were positive for mecA gene for the directly extracted samples, and seven out of seven (100%) samples was positive for mecA gene for the isolated MRSA.

	Culture Samples			Clinical Samples	
	Culture	**LAMP**	**PCR**	**LAMP**	**PCR**
Positive	7 (11.6%)	7 (100%)	1 (14.3%)	3 (5%)	2 (3.3%)
Negative	53 (88.4%)	0 (0%)	6 (85.7%)	57 (95%)	58 (96.7%)
Total	60 (100%)	7 (100%)	7 (100%)	60 (100%)	60 (100%)

Table3: Comparison of the results for mecA LAMP and mecA PCR using clinical samples and MRSA isolates.

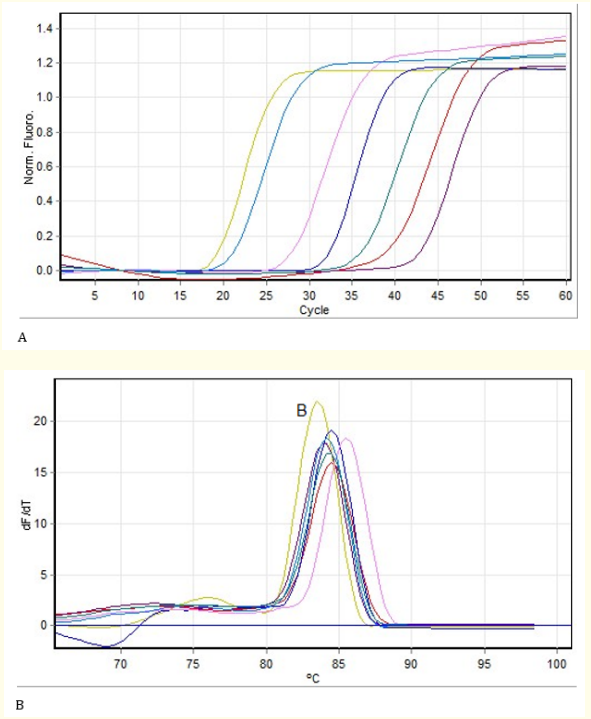

Figure 2: Examples for Amplification plot and melt curve of LAMP. A. amplification analysis, B. melting-curve analysis. Beak B in yellow color indicate the positive control.

Discussion

In the current study, LAMP assay was used for the rapid identification of MRSA from pure cultures and directly from wound, dental plaque, and urine samples using simple boiling DNA extraction method. A rapid and accurate LAMP assay for detection of MRSA is described. Compared with conventional PCR, which consumes four hours until reading the result under UV, LAMP reactions are characteristically rapid and are performed to higher amplification efficiency within sixty minutes. This is due to the isothermal nature of LAMP, as it proceeds at the optimum temperature of the polymerase, with no loss of time resulting from changes in the temperature of the reaction system [23].

LAMP detected mecA gene in all seven MRSA culture isolates while PCR detected mecA gene in only one sample which is similar to the results of [24,25] who reported that LAMP is 10 times more sensitive than standard PCR. The lesser sensitivity of PCR than the LAMP method may be due to the presence of PCR inhibitors, such as lipid and protein components [26,27].

Regarding direct detection in analyzing wound samples LAMP detected mecA gene in one sample (no.5), this sample was excluded as non mannitol fermenting staphylococcus and it may be *S. epidermidis* that is carrier for mecA gene[3,7,24, 28] This sample was not detected by PCR. The direct detection results also support LAMP sensitivity over the PCR. However, both LAMP and PCR use in directly extracted samples failed to detect mecA gene in six (85.7%) out of the total seven samples that were MRSA according to culture method (six wound samples and one dental plaque sample). The reason might be that directly extracted samples using boiling method produced insufficient amounts of DNA. LAMP and PCR perform better when using DNA extraction kit in direct detection of MRSA from dental plaque samples [7]. In direct analysis of the urine samples, the LAMP and PCR demonstrated identical results, suggesting the clinical applicability of the method for urine samples, However, this sample (one positive sample detected by LAMP and PCR) was excluded as coagulase negative *Staphylococcus*.

Conclusion

Identification of MRSA and resistance of mec A gene is necessary for the surveillance of their transmission in hospitals and to overcome the problems associated with Gram+ve MRSA resistance. The LAMP described here is a reliable and rapid method for detection of the mec A gene in cultured organisms obviating the need for using antibiotics sensitivity testing.

Generally, these findings. should help faster and better management and treatment of the affected patients, in addition to rapid initiation of infection control procedures in health facilities. The results should also encourage future country wide epidemiological studies since there is little available information about MRSA infection in Sudan.

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