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Original article

Evaluation of Loop-Mediated Isothermal Amplification (LAMP) Assay in Detection of Methicillin-Resistant Staphylococcus Aureus (MRSA) among Health Care Providers in Benha University Hospitals

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Abstract

Background: Methicillin-Resistant Staphylococcus aureus (MRSA) has emerged as an important pathogen of public health importance causing significant morbidity. This study aimed at evaluation of the sensitivity, specificity and efficacy of loop-mediated isothermal amplification (LAMP) assay in detection of methicillin-resistance staphylococcus aureus (MRSA) in healthcare providers at Benha University Hospitals in comparison to conventional microbiological methods. **Methods:** A cross-sectional study was conducted on 50 healthcare workers in the ICU in Benha University Hospital. The collected nasal swabs were cultured on mannitol salt and blood agars, the obtained colonies were gram stained. Staphylococcal colonies were conventionally identified by catalase and coagulase tests and anti-microbial sensitivity was performed to identify the *S. aureus* methicillin sensitivity. Simultaneously, the obtained colonies undergone DNA extraction and amplification using LAMP assay. **Results:** LAMP assay showed 100% sensitivity, 83.3% specificity, 86.7% positive predictive value (PPV), and 100% negative predictive value (NPV) in detecting MRSA colonies in studied subjects compared to conventional microbiological method taking the former shorter time and simpler procedure in consider. **Conclusion:** the use of LAMP assay along with conventional microbiological methods is considered as acceptable diagnostic strategy especially in resource-limited areas.

Keywords: LAMP; Staphylococcus Aureus; MRSA; Health Care Providers.

Introduction

The increasing prevalence of methicillin-resistant staphylococcal aureus (MRSA) and its ability to spread in both hospitals and community have posed a major challenge for infection control [(1)]. Hand contamination is the most important mode of MRSA transmission. The airborne dispersal of staphylococci in association with an upper respiratory tract infection is considered the alternative mechanism of MRSA transmission [(2)]. Healthcare workers (HCWs) have an important role in MRSA transmission, by acting more frequently as vectors, rather than being the main sources of MRSA transmission. HCWs are most often transiently colonized by MRSA, but they may become persistent carriers if they have chronic dermatitis or sinusitis, and this may lead to prolonged MRSA transmission [(3)].

Conventional MRSA detection techniques, including growth-based assays, colony morphology, and microdilution resistance tests, are time-consuming. It may take about 48 h in case of a positive nasal swab culture [(4).] Thus, clinical laboratories have focused on rapidly identifying and determining the antimicrobial susceptibility patterns of bacterial isolates through developing a rapid reliable methods for accurate detection and differentiation of methicillin susceptible and resistant S. aureus (MSSA and MRSA) isolates [(5)].

Loop-mediated isothermal amplification (LAMP), a novel nucleic acid amplification technique, was applied to detect MRSA directly from positive blood culture bottles. In which DNA is amplified under isothermal conditions (63°C) with high specificity by using a set of four specially designed inner and outer primers on the target DNA [(6).] MRSA-LAMP targets the *mecA* gene which encodes the PBP2a protein, can detect MRSA within 2 h after the nasal swab culture signal become positive [(7)]. LAMP advantages over PCR assays are being simple, rapid, specific and sensitive (10-1000 times higher than PCR) [(4)], what makes LAMP assay a promising alternative method for the rapid identification of S. aureus especially in resource-limited laboratories [(8)].

In the current work, we aimed at evaluating the sensitivity, specificity and efficacy of LAMP assay in detecting methicillin-resistance Staphylococcus aureus (MRSA) in healthcare providers at Benha University

Hospitals compared to the conventional microbiological methods.

Subjects and Methods

Study design

A cross-sectional study was conducted on 50 healthcare workers (HCWs) in intensive care units (ICU) at Benha University Hospitals between January and August 2020. The protocol of this study is in accordance with the ethical guidelines of the 2004 Declaration of Helsinki and was approved by the local ethical committee of Faculty of Medicine, Benha University. Informed consent was obtained from each participant before enrollment in the study.

Inclusion and exclusion criteria

Any health care provider who had no absence for the last 6 months and did not take antibiotics during the last 3 days and accepted to participate were included in the study. If the HCW was a pregnant female, diseased and hospitalized within the previous 60 days, immunocompromised (diabetes mellitus, malignancy, current chemotherapy, chronic oral steroid use) and/ or having nasal mucosal injuries were excluded.

Sampling

Nasal swabs were taken from all participants using a standardized small, soft-tipped nylon swab with plastic shaft that was inserted into one or both nostrils and twirled few times until it was covered with secretions. The swab was placed in a sterile tube containing 0.5 mm of broth. The cap was placed on the tube. Standard precautions of hand wash, hand rub and wearing personal protective equipment (PPE) as gloves and face mask was followed strictly.

Conventional microbiological isolation and identification of MRSA:

The collected nasal swabs were cultured on Mannitol Salt agar (MSA); a selective and differential medium for the isolation and identification of *S. aureus* from clinical specimens; and blood agar; a differential media to detect hemolysis by cytolytic toxins (hemolysins) secreted by bacteria. Staphylococcus is usually either beta hemolytic (cause partial hemolysis) or gamma hemolytic (not causing hemolysis at all). The growing colonies subsequently underwent the following: a) Morphological identification by gram staining to detect the gram-positive staphylococci arranged in clusters. b) Biochemical identification of staphylococcal colonies was done by positive catalase test. The coagulase test was used to identify the coagulase positive S. aureus and coagulase negative staphylococci

(CoNS). c) Anti-microbial susceptibility for all isolated staphylococcal colonies was done by minimal inhibitory concentration (MIC) cefoxitin disk diffusion test under CLSI 2019 guidelines in order to assess the methicillin resistance.

LAMP assay to detect MRSA

Bacterial DNA was extracted from all isolated colonies using GeneJET Genomic DNA Purification Kit (Thermo Scientific, Cat. # K0721, USA) after pretreatment by lysozyme solution (50 mg/mL) (Thermo Scientific, Cat. # 90082, USA). The isothermal LAMP assay was carried out by (WarmStart® LAMP Kit (DNA & RNA), NEB #E1700S/L, New England Biolabs, USA) to amplify the *MecA* gene. The target sequence of *S. aureus mecA X52593* was obtained from GenBank and four primers were assembled by (Invitrogen, USA) as previously published [9]. At the end of the procedure, the amplification in the reaction tube was detected by visualizing the turbidity. Turbid tube corresponds to positive *S. aureus mecA* gene amplification.

Statistical analysis

Data were analyzed using SPSS software, version 22.0 for Windows (IBM, Armonk, NY, USA). Categorical data were presented as number and percentages. Comparison between groups were test by Chi-square (X^2) or exact Fisher test (EFT) as appropriate. Quantitative data were tested for normality using Shapiro-Wilks's test assuming normality at P >0.05. Normally distributed variables were expressed as mean ±standard deviation (SD) and comparison between means was done by student T test. Kappa test of significance was used to assess agreement between categories. ROC curve was constructed to assess the performance of LAMP assay in detection of MRSA. P ≤0.05 was considered significant in this work.

Results

Nasal swabs were obtained from 50 healthcare providers in ICU at Benha University hospital. They were 37 (74%) females and 13 (26%) males with mean age as 33.4±7.6 years. They were 31 (62%) nurses, 13 (26%) physicians and 6 (12%) workers. **(Table 1)**

Culturing of nasal swabs on mannitol salt agar resulted in 20 cases (40%) were pink colonies and 30 cases (60%) were yellow colonies and on blood agar; 30 cases (60%) showed hemolysis and 20 cases (40%) did not show any sign of hemolysis. Morphological identification of obtained colonies after gram staining, revealed that 35 cases (70%) were gram positive cocci.

The biochemical identification of the gram-positive cocci isolates showed that all isolates were catalase positive, and 30 isolates (85.7%) were coagulase positive. Cefoxitin disk diffusion test was performed to identify the methicillin susceptibility in gram positive cocci isolates. Four isolates (11.4%) were methicillin resistant coagulase negative (MRCoN), 1 isolate (2.9%) was methicillin sensitive coagulase negative (MSCoN), 26 isolates (74.3%) were methicillin resistant staphylococcus aureus (MRSA), and 4 isolates (11.4%) were methicillin sensitive staphylococcus aureus (MSSA). LAMP assay was performed on colonies from the 50 isolates. Result interpretation was done by visual assessment of turbidity in LAMP reaction tube. Sixty percent (30 isolates) were turbid and 40% (20 isolates) were clear. **(Table 2)**

It was found that that all non-staphylococcus bacteria, methicillin sensitive coagulase negative gram-positive cocci and methicillin sensitive staphylococcus aureus detected by conventional microbiological methods for bacterial identification and phenotyping were clear by LAMP assay, and all the methicillin resistant coagulase negative gram-positive cocci and methicillin resistant staphylococcus aureus were turbid by LAMP assay. A significant 92% agreement between LAMP assay and conventional microbiological methods in detecting MRSA in studied isolates was detected (P <0.001). **(Table 3)**

ROC curve analysis was conducted to assess the performance of LAMP assay in detecting MRSA in studied isolates. It showed that LAMP assay can significantly detect MRSA with an excellent area under ROC curve (AUC) 0.917 (95% CI 0.83-1.0) with a 100% sensitivity, 83.3% specificity, 86.7% positive predictive value and 100% negative predictive value. **(Fig. 1)**

By studying the relation between MRSA detected by LAMP assay and socio-demographic characters of studied subjects, it was found that neither gender nor age of studied subjects showed any significant difference regarding MRSA detected with LAMP (P 0.148, 0.710 respectively). While MRSA detection by LAMP was significantly higher in nurses rather than physicians and workers (P <0.001). **(Table 4)**

Table 1: Socio-demographic characters of the studied subjects.

Variable		N (n= 50)	% (100%)
Gender	**Female**	37	74.0%
	Male	13	26.0%
Age (years)	**Mean ±SD**		33.4±7.6
	Range		21−48
Occupation	**Physician**	13	26.0%
	Nurse	31	62.0%
	Worker	6	12.0%

Table 2: Microbiological and molecular testing of nasal swabs.

Variable		N (n=50)	% (100%)
Culture			
Mannitol Salt Agar	**Pink colonies**	20	40.0
	Yellow colonies	30	60.0
Blood Agar	**No hemolysis**	20	40.0
	Hemolysis	30	60.0
Gram stain			
Morphology (cocci)	**Gm negative**	15	30.0
	Gm positive	35	70.0
Biochemical identification of Gm positive Cocci			
		(n= 35)	**(100%)**
Catalase test	**Negative**	0	0.0
	Positive	35	100.0
Coagulase test	**Negative**	5	14.3
	Positive	30	85.7
Anti-microbial susceptibility by MIC			
Cefoxitin disk diffusion	**MRCoN**	4	11.4
	MSCoN	1	2.9
	MRSA	26	74.3
	MSSA	4	11.4
LAMP assay for MRSA			
		(n= 50)	**(100%)**
Visual examination of LAMP reaction tube	**Turbid**	30	60
	Clear	20	40

MRCoN; methicillin resistance coagulase negative Gm positive cocci, MSCoN; methicillin sensitive coagulase negative Gm positive cocci, MRSA; methicillin resistance S. aureus, MSSA; methicillin sensitive S. aureus.

Table 3: Degree of agreement between MRSA detected by microbiological and LAMP molecular testing

		MRSA identification by microbiological tests		
		Negative	**Positive**	**Total**
MRSA detected by LAMP assay	**Negative**	20 (83.3%)	0 (0.0%)	20 (40.0%)
	Positive	4 (16.7%)	26 (100.0%)	30 (60.0%)
	Total	24 (100.0%)	26 (100.0%)	50 (100.0%)
Kappa test			0.838	
Degree of agreement			92%	
P			**<0.001**	

Table 4: Relation between MRSA detected by LAMP assay and socio-demographic characters of studied subjects.

		MRSA by LAMP		Test	*P*
		Negative	**Positive**		
Gender N (%)	**Female**	20 (54.1%)	17 (45.9%)	X^2=2.09	0.148
	Male	4 (30.8%)	9 (69.2%)		
Age (years) mean± SD		32.9±8.050	33.7±6.93	T= 0.37	0.710
Occupation N (%)	**Physician**	9 (69.2%)	4 (30.8%)		
	Nurse	9 (29.0%)	22 (71.0%)	FET=13.3	**<0.001**
	Worker	6 (100.0%)	0 (0.0%)		

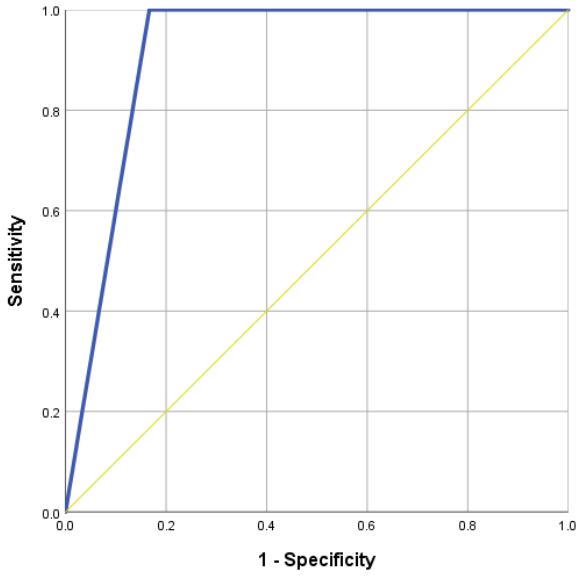

Figure 1: ROC curve for LAMP to detect MRSA in studied is

Discussion

In this work methicillin susceptibility for gram positive cocci isolates was done by cefoxitin disk diffusion (MIC) test and revealed that 4 isolates were MRCoN (11.4%), 1 isolate was MSCoN (2.9%), 4 isolates were MSSA (11.4%).and 26 isolates were MRSA with a prevalence of 74.3% MRSA among studied healthcare workers. The prevalence of MRSA carriers among healthcare workers was variable. In Egypt, other studies reported the frequency of nasal carriage of MRSA among healthcare workers by 27.6% (45/163), and the frequency of methicillin resistance among isolated S. aureus was 93.8% (45/48) [(10)].

These results were comparable to the study done by other researchers in the surgical intensive care unit of El-Demerdash Hospital, Ain Shams University, where the colonization frequency of nasal MRSA in healthcare workers was 28.6% [(11)]. On the contrary, a study by other studiers was conducted on all employees (N= 575) of the heart center (Herzzentrum Dresden GmbH), the specialized cardiology care and cardiac surgery center of the Technische Universität Dresden's teaching hospital in the period between July 2014 to May 2015 revealed a low prevalence of MRSA in healthcare workers of less than 1% (1 in 149 HCW) [(12)]. Moreover, in the USA a study reported that the carriage rate of MRSA in healthcare workers approximates 5% with concerns of transmission of this pathogen to patients [(13).] These variable prevalence rates might owe to the strength of infection control programs applied in each hospital. The gender associated predominance of MRSA colonization is also variable. Hand-hygiene behavior varies according to gender. Males are less compliant, which in turn may predispose them to higher colonization and infection rates. Female hormones such as estrogen affect the expression of virulence factors of the bacteria [(14)].

In the current study, the colonies obtained were tested by the LAMP assay for the presence of the *MecA* gene encoding oxacillin resistance. All (100%) non-cocci bacteria, MSCoN and MSSA tested negative (clear reaction tube) by LAMP assay and all (100%) of MRCoN and MRSA tested positive (turbid reaction tube) by LAMP assay. Accordingly, the LAMP assay can significantly detect MRSA with 100% sensitivity, 83.3% specificity, 86.7% positive predictive value and 100% negative predictive value with significant 92%

agreement with the conventional microbiological method for detecting MRSA in studied isolates.

This was in agreement with another study which found the sensitivity, specificity, positive predictive value, and negative predictive value of LAMP for the assessment of the *mecA* gene were 100%, 75%, 90.2%, and 100%, respectively [(15)]. Also, our results were in line with another one (16) which observed the LAMP sensitivity 100%, specificity 99.72%, PPV 100%, NPV 100% for MRSA detection and for *mecA* detection sensitivity 96%, specificity 100%, PPV 100% and NPV 90.89%, and with others who reported a 100% sensitivity, 99.72% specificity for LAMP in detecting MRSA(17). However, they found lower concordance between *mecA* LAMP signals and phenotypic oxacillin susceptibility testing with 18 false-positive and 19 false-negative LAMP signals lowering the sensitivity and the specificity to 94.71% and 95.89% respectively. Most of their false-positive signals (n= 16) derived from blood cultures growing coagulase- negative Staphylococci, while two false-positive *mecA* LAMP signals occurred in blood cultures growing S. aureus.

That was similar to values reported in a previous study as *orfX*-LAMP assay was applied for detection of 667 clinical Staphylococcus strains, including 566 MRSA, 25 MSSA, 53 MRCNS and 23 MSCNS strains, with comparative validation by standard PCR assay, giving the detection rate, positive predictive value (PPV) and negative predictive value (NPV) of *orfX*-LAMP were 98.4%, 100% and 92.7% respectively(4).

In the current work, we found that neither gender nor age of the studied subjects showed any significant difference regarding MRSA detected with LAMP. While LAMP detected MRSA was significantly higher in nurses (71%) rather than physicians (30.8%). It was reported in more current study that *S. aureus* carriage rate was highest among doctors (20.8%) whereas MRSA carriage rate was highest among nurses (7.8%)(18). The high risk of colonization with MRSA strains among nurses could be due to their frequent patient contact. Recently, higher prevalence of MRSA isolates was detected among females (82.2%) and among nurses (55.6%) and a higher MRSA isolates among HCWs in the surgery department (20%) [(10)].

In other study(12), women predominately participated (68.9%). Most participants were between 40 and 49 (31.1%) and 30–39 (24.4%) years old, worked as a nurse (55.6%). Thus, nurses are more frequently colonized with MRSA than other HCWs (e.g., physicians). This might be attributed to their closer physical contact with patients.

Our results were in accordance with reports of a 1.9% carriage rate of MRSA in doctors, 7.5% in nurses and no MRSA strains were isolated from cleaners [(19)]. On contrary, other reports revealed that MRSA colonization were the highest among doctors (50%) followed by nurses (25%), with higher MRSA colonization frequency in females (51.28%)(20). This high rate of methicillin resistance among nurses could be due to lack of knowledge, practice and follow up of infection control measures, particularly hand hygiene and contact precautions.

Conclusion

From the present study we could conclude that the novel time- and labor-saving LAMP based MRSA detection assay can detect MRSA from culture isolates with 100% sensitivity and 83.3% specificity providing the benefit of obtaining reliable results within a time frame of 2 to 3 hours after culture compared to days by conventional microbiological methods. Thus, the use of the LAMP assay along with conventional microbiological methods is considered as acceptable diagnostic strategy especially in resource-limited areas.

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