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Comparison of loop mediated isothermal amplification with polymerase chain reaction for detection of *Staphylococcus aureus* in chevon

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Abstract
Staphylococcus aureus (*S. aureus*) is an important foodborne pathogen which can contaminate various food products and cause food poisoning due to the ingestion of preformed Staphylococcal Enterotoxins. Various molecular based approaches like Polymerase Chain Reaction (PCR) have been used to identify *S. aureus*, but as it needs expensive equipment, it is not suitable for routine testing. To overcome such limitation, several nucleic acid amplification methods like Loop Mediated Isothermal Amplification (LAMP) have been developed which is conducted under isothermal conditions, findings can be visually interpreted and there is no need of expensive equipment so, it is well suited for adoption as a field level diagnostic and poorly equipped laboratories. The present study demonstrated the comparison of PCR with LAMP for detection of *S. aureus* in chevon. On screening the 150 samples (raw chevon), 26 samples (17.33%) were positive for *S. aureus* by conventional methods. All the *S. aureus* isolates were then subjected to antibiotic susceptibility test using 12 different antibiotics, which revealed maximum resistance towards penicillin (88.46%) and 100% susceptibility to ceftriaxone, ciprofloxacin and vancomycin. By PCR and LAMP, out of 26, 23 and 24 isolates were confirmed as *S. aureus* respectively. The specificity of both the methods was similar which was 100%, but the sensitivity of LAMP was found to be 10 times more than that of PCR. Thus, LAMP assay is a convenient testing method for improving food sanitation, maintaining food safety as well as developing international trade.

Keywords: Chevon, loop mediated isothermal amplification, polymerase chain reaction, *Staphylococcus aureus*

Introduction
Food borne diseases are defined as diseases of an infectious/toxic nature caused by the ingestion of infected food or water. Bacteria (66%), chemicals (26%), viruses (4%) and parasites (4%) are the major causes of food borne illness (Newman *et al*., 2015) [1]. *Staphylococcus aureus* (*S. aureus*) is a Gram-positive coccus shaped facultative anaerobic bacteria which is a commensal on mucous membranes and skin and humans are the major reservoir of these organisms (Boucher *et al*., 2008) [2]. It is a common causative agent of certain infections like skin infections, respiratory infections and food poisoning. Staphylococcal food poisoning is a gastrointestinal illness which is caused by ingesting food which contain adequate amount of preformed staphylococcal enterotoxins (Fusco *et al*., 2018) [3]. It acts as one of the most important economic illness and is a major issue for the worldwide public health program (Alarcon *et al*., 2006) [4]. Following the consumption of contaminated food, staphylococcal food poisoning has a very quick onset (usually 3-5 hours). This is due to the different toxins which bacteria produces during growth at suitable temperatures (Le *et al*., 2003) [5]. The enterotoxins are highly stable, heat-resistant and ecologically resistant to conditions such as freezing and drying (Hennekinne *et al*., 2012) [6]. Characteristic of heat-stability of *S. aureus* poses a major threat to the food industry.
Standard detection and identification methods of *S. aureus* includes routine culture methods. Further to confirm the organism this is then followed by various biochemical tests for presumptive colonies (Xu *et al*., 2012) [7]. But the long recovery time & false negative tests posed questions about these conventional methodologies (Bsat *et al*., 1994) [8]. Polymerase Chain Reaction (PCR) is one of the most widely used methods in diagnostic applications because it allows sensitive and rapid diagnosis. However, this technique is not suitable for usual food safety testing as it requires expensive thermal cycler, complex DNA amplification operations and post amplification protocol such as electrophoresis.

So there is a need for a relatively rapid, cheap and user friendly technique to detect the *S. aureus* from the food samples.

Notomi *et al*. (2000) [9], developed Loop Mediated Isothermal Amplification (LAMP) assay which can amplify the target gene under isothermal conditions (60-65 °C) with high efficiency, specificity and sensitivity. This novel method can amplify a few copies of DNA to 10 copies in less than an hour. It serves as a useful tool to quickly detect and identify foodborne pathogens (Kokkinos *et al*., 2014) [10]. This method is based on the autocycling strand displacement nature of *Bst* DNA polymerase using a set of two specially designed inner and two outer primers. As it is conducted under isothermal conditions and findings can be visually interpreted, it is well suited for adoption as a field level diagnostic in developing countries and poorly equipped laboratories (Rekha *et al*., 2014) [11].

Hence, looking towards the scanty work in India regarding LAMP based diagnosis of *S. aureus* from chevon this study was planned with objectives to isolate and confirm *S. aureus* by PCR and LAMP technique and comparison of both techniques based on sensitivity and specificity.

Materials and Methods

Sample collection

A total of 150 raw chevon samples were collected aseptically from different retail meat shops in Anand district of Gujarat. They were then immediately transferred on ice at 4°C to the laboratory of Department of Veterinary Public Health & Epidemiology, College of Veterinary Science and Animal Husbandry, Anand for further processing.

Isolation and identification

Enrichment of all the samples was carried out in Peptone Water enrichment broth at 37 ºC for 24 hrs. The selective media used for isolation of *S. aureus* was Mannitol Salt Agar (MSA) and Baird Parker Agar (BPA). A loopful of inoculum was taken from enrichment broth and was streaked on MSA and also on BPA medium supplemented with Egg Yolk Emulsion and 3.5% Potassium Tellurite solution. The inoculated plates were then incubated for 24-48 hrs at 37 ºC. The isolates suspected to be *S. aureus* were subjected to morphological and biochemical tests. Presumed *S. aureus* isolates were further confirmed by Gram's staining (HiMedia Gram Staining Kit) and biochemical tests.

Antibiotic susceptibility testing

The antibiotic sensitivity test was carried out against 12 antibiotics procured from HiMedia Laboratories Pvt. Ltd., Mumbai, India as per method described by Bauer (1966) [12]. The antibiotics were ampicillin (10 mcg), ceftriaxone (30 mcg), ciprofloxacin (30 mcg), chloramphenicol (5 mcg), enrofloxacin (10 mcg), gentamicin (10 mcg), methicillin (5 mcg), oxacillin (1 mcg), penicillin G (10 units), streptomycin (10 mcg), tetracycline (30 mcg) and vancomycin (30 mcg). A loopful of isolated colonies of *S. aureus* were taken and were inoculated in Brain Heart Infusion (BHI) broth and incubated at 37 ºC till the turbidity of broth matches the turbidity of the 0.5 McFarland standard. After this the sterilized plates of Mueller Hinton Agar (MHA) were inoculated by streaking over the entire agar surface for two or more times. Then by using sterile forceps different commercially available antibiotic discs were placed on the inoculated agar surface about 2 cm apart. The plates were incubated at 37 °C for 18-24 hrs. After the incubation period, the diameter of inhibition zones was measured and the antibiotics were graded as sensitive, intermediate and resistant according to guidelines provided by Clinical Laboratory Standard Institute (CLSI).

Polymerase chain reaction

Culturally and biochemically positive isolates of *S. aureus* were subjected for molecular characterization using PCR for confirmation by targeting species specific *sau* gene. The DNA from *S. aureus* isolates was extracted by boiling method. A loopful of pure culture was suspended in 100 µl nuclease free water in a sterilized micro centrifuge tube. The suspension was vortexed and then heated at 95 ˚C for 10 mins in thermal cycler. This was then centrifuged at 10000 rpm for 6 mins so that the cell debris settle down. The upper aqueous phase was used as a DNA template for PCR and LAMP. The reaction mixture for PCR was prepared in 200 µl PCR tubes on ice to a final volume of 25 µL and the amplification to screen the *sau* gene was done by using Thermocycler PCR machine (Eppendorf Mastercycler gradient, Germany). The reaction mixture contained 12.5 µL PCR master mix (2X), 1 µL each of forward and reverse primer (10pmol), 5.5 µL nuclease free water and 5 µL template. The details of oligonucleotide primers for *sau* gene and of thermal profiling of PCR are mentioned in Table 1 and Table 2 respectively. The final amplified product was analyzed by agarose gel electrophoresis on 1% agarose gel and visualized under gel documentation system.

Table 1: Description of primer used for detection of *Staphylococcus aureus*

Sr. No.	Target gene	Primer sequence (5'-3')	Product Size (Base pairs)	Reference
1.	*sau*	F: GGA CGA CAT TAG ACG AAT CA R: CGG GCA CCT ATT TTC TAT CT	1318 bp	Riffon *et al.* (2001) [13]

Table 2: PCR conditions for detection of *sau* gene

Cycling Conditions		Temperature	Time
Initial Denaturation		94 ºC	5 min
34 cycles	Denaturation	94 ºC	30 sec
	Annealing	51.1 ºC	30 sec
	Extension	72 ºC	30 sec
Final Extension		72 ºC	5 min

Loop mediated isothermal amplification

LAMP assay using primers for *arcC* for *S. aureus* was performed. The details of the primers are mentioned in Table 3. Total 25 µL of LAMP reaction mixture was prepared which consisted of 1.50 µL of isothermal buffer (10X), 1.50 µL Mg_2SO_4 (100 mM), 3.50 µL dNTP (10 mM), 4 µL each of inner primer (FIP, BIP), 0.50 µL each of outer primer (F3, B3), 1 µL *Bst* DNA polymerase, 2 µL DNA template and 6.50 µL nuclease free water. The reaction mixture was prepared in 200 µL PCR tubes and then it was incubated in water bath for isothermal amplification at 65 ºC for 60 mins and further heated to 80 °C for 2 min to terminate the reaction. The LAMP products were visualized either by visual detection after addition of dyes like SYBR green or by agarose gel electrophoresis in which a ladder like pattern is seen. After the amplification of DNA, 1 µl of SYBR Green (1:100) was

added to each LAMP reaction tube for the visual detection of amplified product. Amplified DNA were also analyzed on 2% agarose gel by electrophoresis at 100 V for 45 mins and then observed under U.V. transilluminator of gel documentation system (Biovis, India).

Table 3: Description of primers used for detection of *Staphylococcus aureus* by LAMP

Sr. No.	Target gene	Primer sequence (5'-3')	Reference
1.	*arcC*	**F3:** CACTATTCATTTCAGTTAAAATGCG. **B3:** CGATGCAAAACCTTAAACCT. **FIP:** ATCGAACAGTGACACAACGCCACCAATAGCCTATCATACCCT. **BIP:** TAAACTTCCAATTTGTGGGCCATTATTTGATTCACCAGCGC.	Chavan (2015) [14]

Detection of specificity of LAMP assay and PCR

For checking the specificity of LAMP and PCR, DNA was extracted from *S. aureus* isolates and some other bacterial strains like *E. coli*, *Salmonella* spp. *Bacillus cereus* and *Klebsiella* spp. *S. aureus* specific LAMP and PCR reaction was performed for all these bacteria according to the above-mentioned procedures and then the results were compared.

Detection of sensitivity of LAMP assay and PCR

Sensitivity was assessed by diluting the template DNA followed by LAMP and PCR. The DNA was extracted and then serially diluted to get concentrations 100 ng, 10 ng, 1 ng, 100 pg, 10 pg and 1 pg. Then 3 µL of DNA was taken from each dilution and *S. aureus* specific LAMP and PCR was performed making the resultant concentrations of 300 ng/tube, 30 ng/tube, 3 ng/tube, 300 pg/tube, 30 pg/tube and 3 pg/tube DNA. Finally, the results of both the techniques were compared.

Results

Isolation & identification

Out of the total 150 samples, 26 samples (17.33%) were isolated as *S. aureus* based on colony morphology, bacteria morphology and biochemical characterization. They produced jet black and golden yellow colonies on BPA and MSA respectively, were gram positive cocci in bunches and were positive for catalase, methyl red and VP.

Antibiotic susceptibility testing

The antimicrobial resistance profile of the tested *S. aureus* isolates revealed that all the isolates were susceptible to ceftriaxone, ciprofloxacin, and vancomycin. The highest resistance was shown to penicillin (88.46%) followed by streptomycin (46.15%), tetracycline (42.30%), methicillin, oxacillin (19.23% each), gentamicin (15.38%), ampicillin and enrofloxcin (7.69% each) and chloramphenicol (3.84%). Further description is given in Table 4.

Table 4: Antibiotic drug resistance pattern of *Staphylococcus aureus* isolates

Sr. No	Name of the antibiotic	Sensitive	Intermediate	Resistant
1.	Ampicillin	24 (92.30%)	-	2 (7.69%)
2.	Ceftriaxone	26 (100%)	-	-
3.	Chloramphenicol	23 (88.46%)	2 (7.69%)	1 (3.84%)
4.	Ciprofloxacin	26 (100%)	-	-
6.	Enrofloxacin	20 (76.92%)	4 (15.38%)	2 (7.69%)
7.	Gentamicin	22 (84.61%)	-	4 (15.38%)
8.	Methicillin	21 (80.76%)	-	5 (19.23%)
9.	Oxacillin	21 (80.76%)	-	5 (19.23%)
10.	Penicillin	3 (11.53%)	-	23 (88.46%)
11.	Streptomycin	12 (46.15%)	2 (7.69%)	12 (46.15%)
12.	Tetracycline	10 (38.46%)	5 (19.23%)	11 (42.30%)
13.	Vancomycin	26 (100%)	-	-

Ploymerase chain reaction

Out of the 26 positive isolates of *S. aureus* which were obtained by conventional culture method, 23 (15.33%) samples were confirmed by PCR shown in Fig 1.

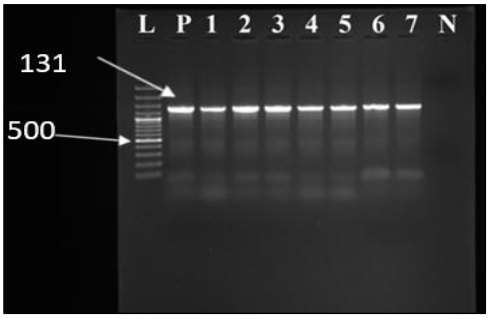

Fig 1: Agarose gel showing amplification product of *sau* gene (Approxi.1318 bp) L:100bp DNA ladder, P: Positive control, Lane 1-7: Positive samples, N: Negative control

Loop Mediated Isothermal Amplification

After subjecting all the 26 positive isolates of *S. aureus* to LAMP, it was observed that 24 isolates were found positive (92.30%) using LAMP technique for *S. aureus* (Fig 2a & 2b).

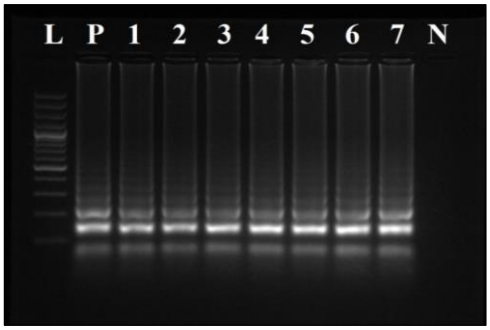

Fig 2a: Ladder like pattern of LAMP products on 2% agarose gel for *S. aureus* L: 100 bp DNA ladder, P: Positive control, Lane 1-7: Ladder like pattern of LAMP products of *S. aureus*, N: Negative control

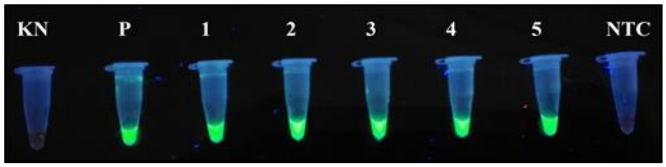

Fig 2b: Visualization of LAMP products under UV light for fluorescence for *S. aureus* KN: Known negative, P: Positive control, 1-5: Positive samples, NTC: Negative Template Control

Comparison

In the present research work, the PCR technique could detect 88.46% (23/26) while LAMP technique could detect 92.30% (24/26) of *S. aureus* isolates out of the culturally positive isolates. For specificity it was observed that both LAMP assay and PCR successfully gave positive result only for DNA isolates of standard *S. aureus*. The specificity of both PCR and LAMP assay was found to be 100% (Fig 3a & 3b). The current study showed that LAMP could detect up to 3 ng/tube concentration of DNA for *S. aureus* whereas PCR could detect the DNA up to 30 ng/tube of DNA. Thus, the sensitivity of the LAMP assay was found 10 folds greater than that of PCR (Fig 4a & 4b).

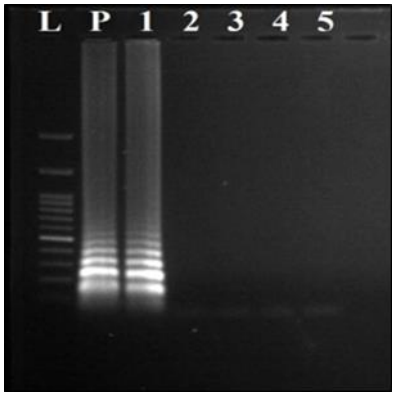

Fig 3a: LAMP assay specificity confirmation for *S. aureus* by electrophoresis LAMP reaction with different bacterial DNA template L: 100bp DNA Ladder, P: Positive control, 1: *Staphylococcus aureus*, 2: *Salmonella spp.*, 3: *Bacillus cereus*, 4: *Klebsiella* spp., 5: *Escherichia coli*

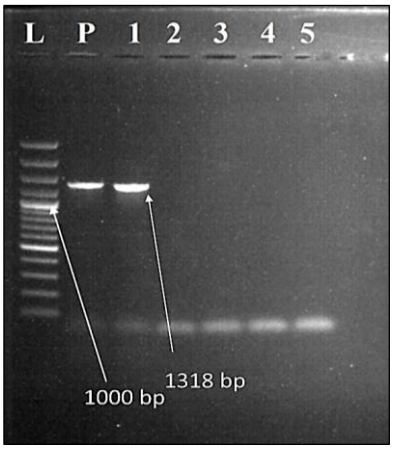

Fig 3b: PCR assay specificity confirmation for *S. aureus* by electrophoresis PCR reaction with different bacterial DNA template L: 100bp DNA Ladder, P: Positive control, 1: *Staphylococcus aureus*, 2: *Salmonella spp.*, 3: *Bacillus cereus*, 4: *Klebsiella* spp., 5: *Escherichia coli*

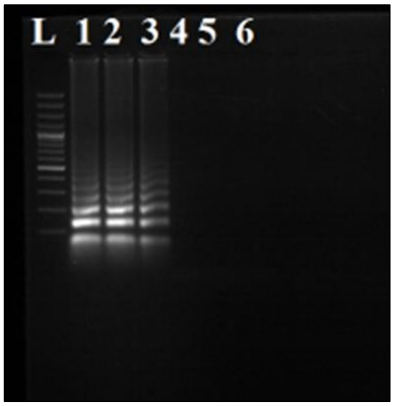

Fig 4a: LAMP assay sensitivity confirmation for *S. aureus* by electrophoresis LAMP carried out at different concentrations of DNA L: 100bp DNA Ladder, 1: 300 ng/tube, 2: 30 ng/tube, 3: 3 ng/tube, 4: 300 pg/tube, 5: 30 pg/tube, 6: 3 pg/tube

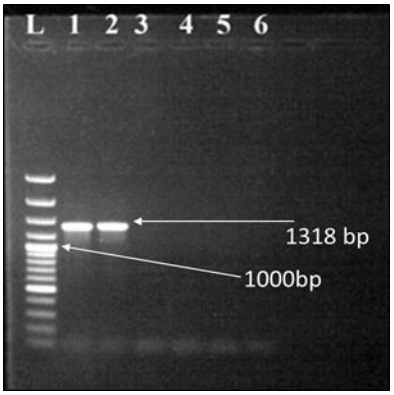

Fig 4b: PCR assay sensitivity confirmation for *S. aureus* by electrophoresis PCR carried out at different concentrations of DNA L: 100bp DNA Ladder, 1: 300 ng/tube, 2: 30 ng/tube, 3: 3 ng/tube, 4: 300 pg/tube, 5: 30 pg/tube, 6: 3 pg/tube

Discussion

Isolation & identification

The prevalence of *S. aureus* from chevon 17.33% (26/150) in the present study was similar to the findings of Zehra *et al*. (2019) [15] and Tefera *et al*. (2019) [16] who reported 17.70% and 16% prevalence rate respectively. In contrast to the present findings high prevalence rate of *S. aureus* was observed i.e. 40% by Latha *et al*. (2017) [17] and 55% by Sangeetha *et al*. (2020) [18]. But low prevalence rate was also reported, 2% by Iroha *et al*. (2011) [19] and 6% by Baghbaderani *et al*. (2020) [20].

Antibiotic susceptibility testing

In the present study the overall resistance of isolates was highest for penicillin (88.46%) which was almost similar to the findings of Zehra *et al*. (2019) [15], Tefera *et al*. (2019) [16] and Das & Mazumder (2016) [21] who reported 86.21%, 86.90% and 73.33% resistance respectively. Sergelidis *et al*. (2015) [22] detected 100% resistance in *S. aureus* isolates towards penicillin which is higher as compared to the results of present study. Streptomycin was 46.15% resistant to the *S. aureus* isolates in the present study, which is slightly less than the results given by Sangeetha *et al*. (2020) [18], who reported 33% resistance of *S. aureus* isolates towards streptomycin. The resistance pattern of *S. aureus* towards tetracycline (42.30%) is almost similar to the findings of Klimesova *et al*. (2017) [23] and Zehra *et al*. (2019) [15] who reported 34.60% and 37.93% resistance to tetracycline respectively. However

higher resistance of 60% towards tetracycline was reported by Bantawa *et al*. (2019) [24], whereas lower resistance of 26.66% was reported by Das & Mazumder (2016) [21]. The resistance to oxacillin (19.23%) in the present study was somewhat similar to the resistance pattern reported by Das & Mazumder (2016) [21] 23.33% but slightly higher than Zehra *et al*. (2019) [15] 10.34%. Tefera *et al*. (2019) [16] reported a higher resistance of 62.30% of *S. aureus* isolates as compared to the findings of present study. The resistance of *S. aureus* isolates to methicillin (19.23%) is much lower than the findings of Sangeetha *et al*. (2020) [18] who reported 100% resistance towards methicillin. The findings of the present study revealed 15.38% resistance to gentamicin which is somewhat similar to the findings of Zehra *et al*. (2019) [15] who reported 10.34% resistance, but it is higher than the findings of Tefera *et al*. (2019) [16] who reported 3.30% resistance towards gentamicin. Ampicillin was found to be 92.30% sensitive to *S. aureus* isolates which is similar to the results of Sangeetha *et al*. (2020) [18] who reported 100% sensitivity towards ampicillin. Tefera *et al*. (2019) [16] reported 80.30% resistance towards ampicillin which is much higher than the results of the present study. The sensitivity of chloramphenicol (88.46%) in the present study is similar to the findings of Bantawa *et al*. (2019) [24], Tefera *et al*. (2019) [16] and Das & Mazumder (2016) [21] who reported 95%, 90.20% and 90% sensitivity respectively towards chloramphenicol. The present findings recorded 100% sensitivity to ciprofloxacin. Other workers like Bantawa *et al*. (2019) [24], Sangeetha *et al*. (2020) [18] and Das & Mazumder (2016) [21] also reported 100%, 100% and 83.33% sensitivity respectively to ciprofloxacin. All the isolates showed sensitivity to ceftriaxone. Similar results were obtained by Sangeetha *et al*. (2020) [18] but Tefera *et al*. (2019) [16] reported a lower sensitivity rate of 32.80% towards ceftriaxone. Similar to ceftriaxone all the *S. aureus* isolates were sensitive to vancomycin also which is similar to the findings of Tefera *et al*. (2019) [16]. Das & Mazumder (2016) [21] reported 96.66% sensitivity towards vancomycin. The results of vancomycin resistance in the present study are totally opposite to those reported by Sangeetha *et al*. (2020) [18], who reported 100% resistance of *S. aureus* isolates towards vancomycin.

Ploymerase chain reaction

The finding of the present study is similar to Zehra *et al*. (2019) [15] (17.70%). In comparison to this study higher prevalence of *S. aureus* in chevon 40% was reported by Latha *et al*. (2017) [17].

Loop mediated isothermal amplification

The findings of the present study are similar to those reported by Xu *et al*. (2012) [7], Su *et al*. (2014) [25] and Lin *et al*. (2017) [26] who reported 98.50%, 98.40% and 97.20% detection rate of *S. aureus* by LAMP assay. In contrast Sudhaharan *et al*. (2015) [27] reported 82% detection rate of *S. aureus* by LAMP which is lower than the results of the present findings. Chavan (2015) [14] reported a detection rate of 100% for *S. aureus* by LAMP which is slightly higher than the findings of the present study.

Comparison

Su *et al*. (2014) [25] reported a detection rate of 98.40% and 91.70% by LAMP and PCR respectively. Chavan (2015) [14] reported cent percent detection rate by LAMP and 96.96% by PCR. Slightly lower detection rate of 82% was reported by Sudhaharan *et al*. (2015) [27] by LAMP and similar results were also obtained by PCR. The specificity results (100%) observed in present study are in accordance with Suwanampai *et al*. (2011) [28], Lim *et al*. (2013) [29] and Sheet *et al*. (2016) [30] who reported 100% specificity of LAMP as well as PCR. Yang *et al*. (2011) [31] reported 97.93% specificity of LAMP assay which is slightly lower than the specificity in the present study. The results of sensitivity in the present study are similar to those of Deng *et al*. (2019) [32], Xiong *et al*. (2020) [33] and Priya *et al*. (2021) [34] who also reported the sensitivity of LAMP assay to be 10 folds greater than that of PCR. Goto *et al*. (2007) [35], Nagarajappa *et al*. (2012) [36] and Zhao *et al*. (2012) [37] have reported the senstivity of LAMP assay to be 100 folds greater than that of PCR.

Conclusions

On screening 150 chevon samples collected from various meat shops in and around Anand 26 samples were isolated as *S. aureus* showing a prevalence of 17.33%. 23/26 (15.33%) were confirmed as *S. aureus* by PCR by targeting species specific *sau* gene. *S. aureus* isolates were completely sensitive to ceftriaxone, ciprofloxacin and vancomycin. High degree of resistance was observed towards penicillin (88.46%) followed by streptomycin (46.15%), tetracycline (42.30%), while moderate resistance activity was observed towards methicillin, oxacillin (19.23% for each) and gentamicin (15.38%). However low resistance was observed towards ampicillin and enrofloxcin (7.69% each) and chloramphenicol (3.84%). In case of LAMP 24/26 isolates (92.30%) were confirmed as *S. aureus*. The specificity of LAMP and PCR assay was found to be 100%. The sensitivity (detection limit) of the LAMP assay was noted to be 10 fold greater than that of PCR. Thus, LAMP assay is a convenient testing method for detection of S. aureus with reliable sensitivity and specificity.

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References

1. Newman KL, Leon JS, Rebolledo PA, Scallan E. The impact of socioeconomic status on foodborne illness in high-income countries: A systematic review. Epidemiology & Infection. 2015;143(12):2473-2485.
2. Boucher HW, Corey GR. Epidemiology of methicillin-resistant *Staphylococcus aureus*. Clinical Infectious Diseases. 2008;46(5):S344-S349.
3. Fusco V, Blaiotta G, Becker K. Staphylococcal food poisoning, in Food safety and preservation. Academic Press, 2018, 353-390.
4. Alarcon B, Vicedo B, Aznar R. PCR-based procedures for detection and quantification of *Staphylococcus aureus* and their application in food. Journal of Applied Microbiology. 2006;100(2):352-364.
5. Le Loir Y, Baron F, Gautier M. *Staphylococcus aureus* and food poisoning. Genetics and Molecular Research. 2003;2(1):63-76.
6. Hennekinne JA, De Buyser ML, Dragacci S. *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. FEMS Microbiology Reviews. 2012;36(4):815-836.
7. Xu Z, Li L, Chu J, Peters BM, Harris ML, Li B, *et al*.

Development and application of loop-mediated isothermal amplification assays on rapid detection of various types of staphylococci strains. Food Research International. 2012;47(2):166-173.
8. Bsat N, Wiedmann M, Czajka J, Barany F, Piani M. Food safety applications of nuclei acid-based assays: Applications of immunobiosensors and bioelectronics in food sciences and quality control. Food technology (Chicago). 1994;48(6):142-145.
9. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, *et al*. Loop-mediated isothermal amplification of DNA. Nucleic Acids Research. 2000;28(12):e63-e63.
10. Kokkinos PA, Ziros PG, Bellou M, Vantarakis A. Loop-mediated isothermal amplification (LAMP) for the detection of *Salmonella* in food. Food Analytical Methods. 2014;7(2):512-526.
11. Rekha V, Rana R, Arun TR, Aswathi PB, Kalluvila J, John DG, *et al*. Loop mediated isothermal amplification (LAMP) test-a novel nucleic acid-based assay for disease diagnosis. Advances in Animal and Veterinary Sciences. 2014;2:344-350.
12. Bauer AW. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology. 1966;45:149-158.
13. Riffon R, Sayasith K, Khalil H, Dubreuil P, Drolet M, Lagace J. Development of a rapid and sensitive test for identification of major pathogens in bovine mastitis by PCR. Journal of Clinical Microbiology. 2001;39(7):2584-2589.
14. Chavan PB. Comparative Studies on Loop-Mediated Isothermal Amplification (LAMP) Method and Conventional PCR for Detection of *Staphylococcus aureus* from Animal Origin Foods (M.V.Sc. thesis, MAFSU, Nagpur), 2015.
15. Zehra A, Gulzar M, Singh R, Kaur S, Gill JPS. Prevalence, multidrug resistance and molecular typing of methicillin-resistant *Staphylococcus aureus* (MRSA) in retail meat from Punjab, India. Journal of Global Antimicrobial Resistance. 2019;16:152-158.
16. Tefera M, Aleme H, Girma S, Ali A, Gugsa G, Abera F, *et al*. Antimicrobial Susceptibility Pattern of Isolated from Sheep and Goat Carcasses. The Open Microbiology Journal, 2019, 13(1).
17. Latha C, Anu CJ, Ajaykumar VJ, Sunil B. Prevalence of *Listeria monocytogenes*, *Yersinia enterocolitica*, *Staphylococcus aureus* and *Salmonella enterica* Typhimurium in meat and meat products using multiplex polymerase chain reaction. Veterinary World. 2017;10(8):927.
18. Sangeetha A, Balakrishnan S, Kowsalya P, Manimaran K, Dhanalakshmi M, Sivakumar T. Microbial safety of meat sold in Orathanadu region, Thanjavur. Journal of Entomology and Zoology Studies. 2020;8(1):811-814.
19. Iroha IR, Ugbo EC, Ilang DC, Oji AE, Ayogu TE. Bacteria contamination of raw meat sold in Abakaliki, Ebonyi State Nigeria. Journal of Public Health and Epidemiology. 2011;3(2):49-53.
20. Baghbaderani ZT, Shakerian A, Rahimi E. Phenotypic and Genotypic Assessment of Antibiotic Resistance of *Staphylococcus aureus* Bacteria Isolated from Retail Meat. Infection and Drug Resistance. 2020;13:13-39.
21. Das P, Mazumder PB. Prevalence of Staphylococcus in raw meat samples in Southern Assam, India. IOSR Journal of Agriculture and Veterinary Science. 2016;9(1):23-29.
22. Sergelidis D, Papadopoulos T, Komodromos D, Sergelidou E, Lazou T, Papagianni M, *et al*. Isolation of methicillin-resistant *Staphylococcus aureus* from small ruminants and their meat at slaughter and retail level in Greece. Letters in Applied Microbiology. 2015;61(5):498-503.
23. Klimesova M, Manga I, Nejeschlebova L, Horacek J, Ponizil A, Vondruskova E. Occurrence of *Staphylococcus aureus* in cattle, sheep, goat and pig rearing in the Czech Republic. Acta Veterinaria Brno. 2017;86(1):3-10.
24. Bantawa K, Sah SN, Limbu DS, Subba P, Ghimire A. Antibiotic resistance patterns of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella*, *Shigella* and *Vibrio* isolated from chicken, pork, buffalo and goat meat in eastern Nepal. BMC Research Notes. 2019;12(1):1-6.
25. Su J, Liu X, Cui H, Li Y, Chen D, Li Y, *et al*. Rapid and simple detection of methicillin-resistant *Staphylococcus aureus* by *orfX* loop-mediated isothermal amplification assay. Bmc Biotechnology. 2014;14(1):8.
26. Lin Q, Xu P, Li J, Chen Y, Feng J. Direct bacterial loop-mediated isothermal amplification detection on the pathogenic features of the nosocomial pathogen-Methicillin resistant *Staphylococcus aureus* strains with respiratory origins. Microbial Pathogenesis. 2017;109:183-188.
27. Sudhaharan S, Vanjari L, Mamidi N, Ede N, Vemu L. Evaluation of LAMP assay using phenotypic tests and conventional PCR for detection of *nuc* and *mecA* genes among clinical isolates of *Staphylococcus* spp. Journal of Clinical and Diagnostic Research. 2015;9(8):DC06.
28. Suwanampai T, Pattaragulvanit K, Pattanamahakul P, Sutheinkul O, Okada K, Honda T, *et al*. Evaluation of loop-mediated isothermal amplification method for detecting enterotoxin A gene of *Staphylococcus aureus* in pork. Southeast Asian Journal of Tropical Medicine and Public Health. 2011;42(6):14-89.
29. Lim KT, Teh CSJ, Thong KL. Loop-mediated isothermal amplification assay for the rapid detection of *Staphylococcus aureus*. Bio Med Research International, 2013, 1-5.
30. Sheet OH, Grabowski NT, Klein G, Abdulmawjood A. Development and validation of a loop mediated isothermal amplification (LAMP) assay for the detection of *Staphylococcus aureus* in bovine mastitis milk samples. Molecular and Cellular Probes. 2016;30(5):320-325.
31. Yang H, Ma X, Zhang X, Wang Y, Zhang W. Development and evaluation of a loop-mediated isothermal amplification assay for the rapid detection of *Staphylococcus aureus* in food. European Food Research and Technology. 2011;232(5):769-776.
32. Deng Y, Liu Y, Jiang Z, Wang J, Zhang Q, Qian Y, *et al*. A multiplex loop-mediated isothermal amplification assay for rapid detection of *Bacillus cereus* and *Staphylococcus aureus*. Bioscience trends, 2019.
33. Xiong J, Huang B, Xu JS, Huang WS. A Closed-Tube Loop-Mediated Isothermal Amplification Assay for the Visual Detection of *Staphylococcus aureus*. Applied Biochemistry and Biotechnology, 2020, 1-11.
34. Priya GB, Agrawal RK, Milton AA, Mendiratta SK, Singh BR, Kumar D, *et al*. Isothermal amplification assay

for visual on-site detection of *Staphylococcus aureus* in Chevon. Food Biotechnology. 2021;35(3):221-36.
35. Goto M, Hayashidani H, Takatori K, Hara-Kudo Y. Rapid detection of enterotoxigenic *Staphylococcus aureus* harbouring genes for four classical enterotoxins, SEA, SEB, SEC and SED, by loop-mediated isothermal amplification assay. Letters in Applied Microbiology. 2007;45(1):100-107.
36. Nagarajappa S, Thakur MS, Manonmani HK. Detection of enterotoxigenic staphylococci by loop-mediated isothermal amplification method. Journal of Food Safety. 2012;32(1):59-65.
37. Zhao X, Li Y, Park M, Wang J, Zhang Y, He X, *et al*. Loop-mediated isothermal amplification assay targeting the *femA* gene for rapid detection of *Staphylococcus aureus* from clinical and food samples. Journal of Microbiology and Biotechnology. 2013;23(2):246-250.