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Full Length Research Paper

Development and evaluation of loop-mediated isothermal amplification (LAMP) for the rapid diagnosis of *Candida parapsilosis*

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The *Candida parapsilosis* family has emerged as a major opportunistic and nosocomial pathogen. It causes multifaceted pathology in immuno-compromised and normal hosts, notably low birth weight neonates. In the present study, a novel method, known as loop-mediated isothermal amplification (LAMP), was described for the rapid and specific detection of the species, using primer sets derived from the 5.8 S ribosomal RNA gene of *C. parapsilosis* (internal transcribed spacer 2, ITS2). Amplification products can be detected macroscopically by visual inspection in vials using SYBRGreen I as well as by electrophoresis on agarose gel. The LAMP assay resulted in specific amplification of the ITS2 of *C. parapsilosis* using pure cultures after a 45-min reaction at 65°C; no cross-reactivity with other fungi including other *Candida* species was observed. The detectable DNA limit was 0.01 pg fungal DNA per reaction, equivalent to 3.74 × 10^{-3}cfu/ml. In addition, specific amplification was achieved using 30 proven *C. parapsilosis* strains from patients samples. The method provides a powerful tool for rapid diagnostics in the clinical laboratory, and has potential for use in ecological studies.

Key words: Loop-mediated isothermal amplification, diagnosis, *Candida parapsilosis.*

INTRODUCTION

Over the past decade, the incidence of *Candida parapsilosis* has dramatically increased. In fact, reports indicate that *C. parapsilosis* is often the second most commonly isolated *Candida* species from blood cultures (Almirante et al., 2006; Brito et al., 2006), and *C. parapsilosis* even outranks *Candida albicans* in some European (Nakamura and Takahashi, 2006), Asian (Nakamura and Takahashi, 2006; Ng et al., 2001) and South American (Medrano et al., 2006) hospitals. This species has emerged as an important nosocomial pathogen, with clinical manifestations including fungemia, endocarditis, endophthalmitis, septic arthritis and peritonitis, all of which usually occur in association with invasive procedures or prosthetic devices. Outbreaks of *C. parapsilosis* infections have been caused by contamination of hyperalimentation solutions, intravascular pressure monitoring devices, and ophthalmic irrigating solution. Experimental studies have generally shown that *C. parapsilosis* is less virulent than

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C. albicans or *Candida tropicalis*. However, characteristics of *C. parapsilosis* that may relate to its increasing occurrence in nosocomial settings include frequent colonization of the skin (Bonassoli et al., 2005), particularly the subungual space, and an ability to proliferate in glucose-containing solutions, with a resultant increase in adherence to synthetic materials (Alonso-Valle et al., 2003).

Traditionally, *C. parapsilosis* strains have been identified based on morphological, physiological and biochemical characteristics (Van Asbeck et al., 2009). These methods are laborious and time consuming. Currently, matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) MS is reported as a reliable, rapid and simple technique for the identification of the *C. parapsilosis* group (Quiles-Melero et al., 2012). However, MALDI-TOF MS requires expensive equipment, which impedes it as an attractive tool for the routine of a clinical microbiology laboratory. Molecular methods based on the analysis of polymorphism in the DNA region that encodes the ribosomal RNA genes (5S, 5.8S, 18S and 26S) (Kurtzman and Robnett, 1998; Nosek et al., 2002; Sofair et al., 2006) and the non-coding internal transcribed spacers (ITS)(Cadez et al., 2002; Sabate et al., 2002) and IGS (Intergenic Spacer) regions (Diaz et al., 2000; Naumov et al., 2003) are being successfully used for the identification of many yeast species. Recently, developed molecular techniques may facilitate the continued exploration of the epidemiology and pathogenesis of *C. parapsilosis* infections. However, all have been developed based on cultured material, and require a fully equipped molecular laboratory. Thus, there is still a need for a rapid and simple technique that is able to deliver an unambiguous identification within a single day.

Loop-mediated isothermal amplification (LAMP) is a novel nucleic acid amplification method, which relies on autocycling strand displacement DNA synthesis performed by the *Bst* DNA polymerase large fragment (Mori et al., 2001; Nagamine et al., 2002; Notomi et al., 2000). The amplification products are stem-loop DNA structures with several inverted repeats of the target and cauliflower-like structures with multiple loops. LAMP has the following characteristics: (i) all reactions can be conducted under isothermal conditions ranging from 60 to 65°C by using only one type of enzyme; (ii) the specificity of the reaction is extremely high because it uses four primers recognizing six distinct regions on the target DNA; (iii) amplification can be performed in a shorter time than amplification by PCR because there is no time loss due to thermal cycling; and (iv) it produces extremely large amounts of amplified products and enables simple detection methods such as visual judgment by the turbidity or fluorescence of the reaction mixture, which is kept in the reaction tube (Mori and Notomi, 2009). With all these characteristics, LAMP of target DNA has emerged as a powerful tool to facilitate point-of-care genetic testing at the bedside. Recently, Nagamine et al. (2002) reported that when two more primers, termed loop primers, were added, the LAMP reaction time could be even less than half of that for the original LAMP method. In their procedure, six primers recognized eight distinct regions on the targeted DNA. In the present study, we introduced LAMP diagnostics for *C. parapsilosis*. The sensitivity, specificity and applicability of this method for *C. parapsilosis* from patient samples were evaluated. It is believed that the rapid detection and confirmation of *C. parapsilosis* in clinical specimens is essential for efficient management.

MATERIALS AND METHODS

Strains

Thirty proven strains of *C. parapsilosis* isolated from patients, 5 isolates of other reference strains including *C. albicans*, *C. tropicals*, *Candida glabrata*, *Candida krusei* and *Cryptococcus neoformans* and one *C. parapsilosis* type strain ATCC 22019 were used in this study. The 30 strains of *C. parapsilosis* and the 5 other reference strains were all collected in Department of Laboratory Medicine, The First Affiliated Hospital of Sun Yat-sen University during the period of January 2010 to December 2012. Cases from patients were confirmed by routine and molecular identification methods. All the isolates were cultured on Sabourand dextrose agar (SDA) at 37°C. Inoculated plates were examined after 48 h of incubation. Identification of *Candida* species were based on VITEK 2 system (bioMérieux, Marcy l'Etoile, France) and further identified by 18S rRNA gene sequencing as described by Zheng et al. (2013).

DNA extraction

Candida species were grown on SDA plates for 24 to 48 h at 30°C. Single colonies were inoculated into 200 ml of YPD broth (1% yeast extract, 2% peptone, 2% glucose) and incubated in a shaking water bath at 200 rpm and 30°C for 36 h. DNA was extracted from this culture by adaptation of the Lyticase-based method (10KU, Sigama, USA). DNA concentrations and A260/A280 ratios were determined using a spectrophotometer Lambda 1A (Perkin-Elmer, USA). An A260/A280 ratio of 1.8-2.1 was considered acceptable.

Design of LAMP primers

The target gene of the LAMP was the 5.8 S ribosomal RNA gene of *C. parapsilosis* (internal transcribed spacer 2, ITS2). The binding sites of all primer sets are located within the target gene and were designed by using PrimerExplorer software V4 (Eiken Chemical Co. Ltd.) in the database under the Accession No. KF313207. A set of sixLAMP primers was selected as follows: outer primers (F3 and B3), a forward inner primer (FIP), a backward inner primer (BIP) and loop primers (loop F and loop B) (Table 1).

LAMP reaction

The LAMP reaction was performed with a Loop amp DNA amplification kit (Eiken Chemical Co., Ltd., Tochigi, Japan). A reaction mixture (25 µl) containing 1.2 µM each inner primer (FIP and BIP), 0.2 µM each outer primer (F3 and B3), 0.8 µM each loop primer (F and B), 0.8 mM dNTPs, 1M betaine (Sigma), 1×ThermoPol Buffer, 4 mM $MgSO_4$, 8 U of Bst DNA large fragment polymerase (New England Biolabs), with 2 µl of crude DNA extract

Table 1. Sequences of primers used in the LAMP assay.

Primer name	Sequence(5'→ 3')
Forward outer (F3)	AACGAGAGTATCACTCACTAC
Backward outer (B3)	TCAACAATGGATCTCTTGGT
Forward inner primer (FIP)	ATTGCGCCCTCTGGTATTCCCAAACACAACGTGTTTGAGA
Backward inner primer (BIP)	GTGCGTTCAAAGATTCGATGATTCTCTCGCATCGATGAAGAAC
LF	CCTGTTTGAGCGTCATTTCCT
LB	ACGGAATTCTGCAATTCACATTACG

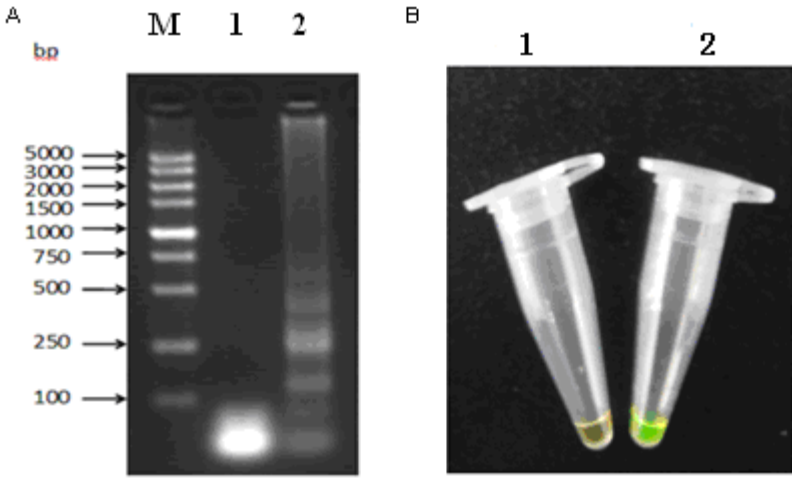

Figure 1. Identification specificities of the LAMP assay for *C. parapsilosis*. (A) Electrophoretic analysis of LAMP amplified products. Lane M, 100-bp ladder used as a size marker; Lane 1, negative control; Lane 2, *C. parapsilosis* ATCC22019. (B) Visual inspection of LAMP amplified products. Tube 1, negative control; Tube 2, *C. parapsilosis* ATCC22019.

as the template and the specified amounts of DNA lysates was incubated at 65°C for 45 min and was heated at more than 80°C for 2 min to terminate the reaction. Positive and negative controls were included in each run, and all precautions to prevent cross contamination were observed.

PCR reaction

To compare the detection sensitivities of LAMP and PCR, PCR using F3 and B3 primers which amplify a 446-bp product was carried out in a total reaction volume of 25 µl containing 1 µl of the fungal DNA, 2 µl of a pair of appropriate primers (0.1 mM), 2 µl dNTPs mixture (0.8 mM), 2.5 U ExTaq^{TM} DNA polymerase ((TaKaRa, Shiga, Japan) with the corresponding polymerase buffer were mixed. PCR conditions consisted of an initial denaturation of 94°C for 4 min and 30 cycles of 94°C for 60 s, 58°C for 60 s, 72°C for 90 s and a final extension of 72°Cfor 4 min in a DNA thermal cycler 9700 (Applied Biosystems, Foster City, CA). The amplified products (4µl) were then analyzed by 1% agarose gel.

Analysis of LAMP products

Amplified products were analyzed by electrophoresis on 1% agarose gels, stained with ethidium bromide and photographed. A 100-bp DNA ladder was used as the molecular weight standard. LAMP amplicons in the reaction tube were directly detected with the naked eye by adding 1.0 µl of 1/10-diluted original SYBR Green I (Molecular Probes Inc.) to the tube and observing the color of the solution. The solution turned green in the presence of a LAMP amplicon, while it remained orange with no amplification. The sensitivities of electrophoresis and SYBR Green I inspection with the naked eye were compared by using seriallydiluted LAMP products.

RESULTS

Specificity of LAMP assay

The specificity of LAMP was tested using fungal DNA extracted from *C. parapsilosis* ATCC22019, 5 proven isolates of *C. parapsilosis* and 5 isolates of non-*C. parapsilosis*, including *C. albicans*, *C. tropicals*, *C. glabrata*, *C. krusei* and *Cryptococcus neoformans*. After incubation at 65°C for 45 min, all the *C. parapsilosis* isolates were positively detected, whereas no cross-reactivity with other fungi including other *Candida* species such as *C. albicans* was observed (Figure 1). The products

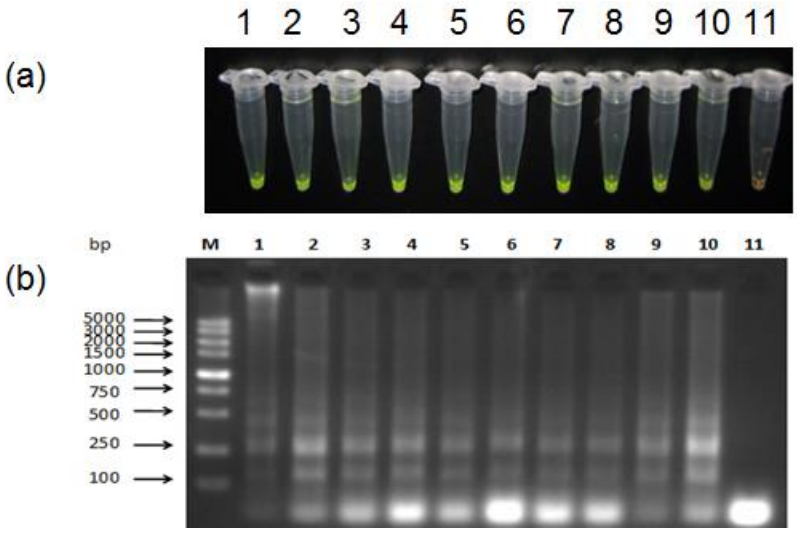

Figure 2. Sensitivities of visual inspection (a) and electrophoretic analysis (b) of LAMP amplified products. The number above each tube or lanes 1-11 represents the dilution of the LAMP product: 1, no dilution; 2-11: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10} dilutions of DNA templates from *C. parapsilosis*.

of the LAMP reaction could be detected by electrophoresis on 1% agarose gels and showed ladder-like patterns (1). The products could also be made visible to the naked eye directly in Eppendorf vials or under UV transillumination after adding SYBR Green I dye. Positive reactions showed bright green fluorescence, whereas negative reactions remained light orange. These results indicate that the LAMP method is highly specific for *C. parapsilosis* in the study.

Sensitivity of the LAMP assay

To assess the detection sensitivity of the LAMP assay for the detection of *C. parapsilosis*, the reaction was tested using 1-µl tenfold serial dilutions of fungal DNA (1 µg/ml) and compared with the PCR assay. The LAMP reaction was able to detect *C. parapsilosis* up to 0.01 pg fungal DNA per reaction, equivalent to 3.74×10^{-3} cfu/ml. However, PCR could only detect *C. parapsilosis* up to 0.1 pg fungal DNA per reaction. LAMP amplification products were analyzed visually by addition 1 µl SYBR Green I and by 2% agarose gel electrophoresis (Figure 2). The results indicate a tenfold higher sensitivity of LAMP than the standard PCR method.

Identification of *Candida* strains isolated from clinical samples

Clinical samples were first discriminated by VITEK 2 system and further identified by 18SrRNA gene sequencing, and then assessed by LAMP established in this study. The results showed that all the 30 proven *C. parapsilosis* strains were detected, suggesting that the established LAMP assay for *C. parapsilosis* represented a great consistency with conventional PCR and VITEK 2 system (Figure 3).

DISCUSSION

LAMP is a powerful innovative gene amplification technique providing a simple and rapid tool for early detection and identification of microbial diseases. In the present study, we developed and evaluated the LAMP assay, exemplified by the detection and identification of *C. parapsilosis* in DNA from pure cultures. The LAMP assay is a simple detection tool in which the reaction is performed in a single tube by mixing the thermopol buffer, primers, and *Bst* DNA polymerase, and incubation of the mixture at 65°C for 45 min. The LAMP reaction is done under isothermal conditions and it does not require expensive equipment. The only equipment needed for the LAMP reaction is a regular laboratory water bath or a heating block that can provide a constant temperature of65°C. Moreover, the amplification efficiency is extremely high because there is no time loss because of thermal cycling and inhibition reactions at later stages are less likely to occur unlike in standard PCR. In addition, LAMP amplifies DNA to higher concentrations than PCR making it convenient for visualizing the products after addition of SYBR Green I without gel electrophoresis. Hence, the LAMP assay could be developed into a field

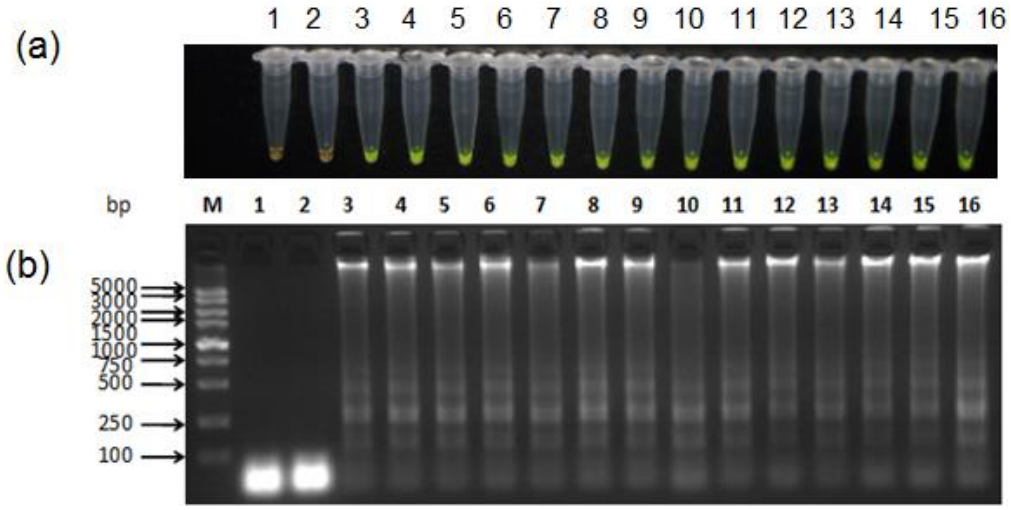

Figure 3. Identification of *Candida* strains isolated from clinical samples. (a) Electrophoretic analysis of LAMP amplified products. (b) Visual inspection of LAMP amplified products. The number above each Lane 1 represents *Candida albicans* strain; Lane 2 represents *Candida tropical* strain; Tube or Lane 3-16 represent DNA templates from proven *C. parapsilosis* strains.

test and made available to empower active efforts to identify *C. parapsilosis*.

During the past decade, various nucleic acid amplification based methods have been developed to address the need for rapid and sensitive diagnosis of *C. parapsilosis* (Burton et al., 2011). These methods require either precision instruments for the amplification or elaborate methods for detection of the amplified products, which are the major obstacles to wide use of these methods in relatively small scale clinical laboratories (Carolis et al., 2014; Del et al., 2011; Hays et al., 2011). In this regard, the LAMP-based assay developed in this study has the advantages of rapid reaction, simple operation and easy detection.

In this study, the LAMP method detecting *C. parapsilosis* was found to be highly sensitive, as it could detect *C. parapsilosis* up to 0.01 pg fungal DNA per reaction, equivalent to 3.74×10^{-3} cfu/ml, whereas by PCR, the detection of *C. parapsilosis* was possible up to 0.1 pg fungal DNA per reaction. This indicates that the sensitivity of LAMP is ten times more than that of the standard PCR. This increased sensitivity makes LAMP a better choice than PCR for the detection of *C. parapsilosis* in cases where lower fungal concentrations are expected.

Identification of the species of *C. parapsilosis* isolates is another critical requirement for clinical laboratories. In the present study, the results showed that all the 30 proven *C. parapsilosis* isolates from clinical samples were detected by the LAMP assay, suggesting that the established LAMP assay for *C. parapsilosis* represented a great consistency with conventional PCR and VITEK 2 system. The conventional biochemical tests for identification of *C. parapsilosis* are relatively time-consuming. The LAMP-based assay can identify *C. parapsilosis* in 80 min: 30 min for DNA extraction, 45 min for the LAMP reaction and 1 min for detection.

In conclusion, the LAMP method described in this study represents a new sensitive, specific and rapid protocol for the detection of *C. parapsilosis*. Due to its easy operation without sophisticated equipment, it will be simple enough to use in small-scale hospitals, primary care facilities and clinical laboratories in developing countries if the remaining issues such as nucleic acid extraction and cross-contamination controls are addressed. Our next direction in developing this promising method for wider clinical use would be to detect *C. parapsilosis* in clinical specimens such as blood, urine and sputum.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

The prevalence of *Brucella abortus* DNA in seropositive bovine sera in Bangladesh

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Prevalence of brucellosis has been widely investigated on the basis of serological test in livestock but the information on the prevalence of *Brucella* species is scarce in Bangladesh. The objective of this work was to determine the prevalence of *Brucella* species in cattle and buffaloes in Bangladesh. For these purpose, a total of 799 serum samples of cattle and buffaloes were collected from different districts of Bangladesh. Out of 799 serum samples, 45 serum samples reacted positively to the Rose Bengal test (RBT); among the RBT positive serum, 14 sera were found to contain *Brucella* DNA by genus specific IS711 screening using quantitative real time PCR (qRT-PCR); and all the 14 qRT-PCR positive samples were found to contain specifically *Brucella abortus* DNA. This report confirms that *B. abortus* is endemic in cattle and buffaloes in Bangladesh. A combination of SAT-iLEISA and PCR could be effective for future eradication programmes.

Key words: Brucellosis, cattle, buffalo, Bangladesh, serology, polymerase chain reaction (PCR).

INTRODUCTION

Brucellosis is considered to be the most widespread zoonosis throughout the world and is caused by different species of the genus *Brucella* (OIE, 2008). In animals, brucellosis mainly affects reproduction and fertility, with abortion, birth of weak offspring and reduced milk yield (Sewel and Blocklesby, 1990). In man, the clinical picture resembles many other febrile diseases, but sacroiliitis and hepato-splenomegaly are the most prominent symptoms. Severe complications are endocarditis and neurological disorders (Colmenero et al., 1996). Numerous serological tests, that is, Rose Bengal Test (RBT), serum agglutination test (SAT), complement fixation test (CFT) and ELISA are used for detecting *Brucella* antibodies in cattle and small ruminants at herd level. Presently, quantitative real time (qRT) PCR methods are used to corroborate serological diagnostics. *Brucella* DNA can

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Table 1. Oligonucleotide primers and probes in the real-time multiplex PCR assay for the detection of *Brucella* spp., *B. abortus*, *B. melitensis*.

Species	Forward primer[a]	Reverse primer[a]
Brucella spp.	GCTCGGTTGCCAATATCAATGC	GGGTAAAGCGTCGCCAGAAG
Brucella spp. TagManprobe[ab]	6FAMAAATCTTCCACCTTGCCCTTGCCATCABHQ1	
B. abortus	GCGGCTTTTCTATCACGGTATTC	CATGCGCTATGATCTGGTTACG
B. abortus Tag Manprobe[ab]	HEXCGCTCATGCTCGCCAGACTTCAATGBHQ1	
B. melitensis	AACAAGCGGCACCCCTAAAA	CATGCGCTATGATCTGGTTACG
*B. melitensis*TagMan probe[ab]	Texas RedCAGGAGTGTTTCGGCTCAGAATAATCCACABHQ2	

[a]Oligonucleotide sequence provided in 5´to 3´orientation. 5´Fluorophonre/3´quencher[b]: 6-FAM: 6-carboxyfluorescein; HEX: 6-hexachlorofluorescein; BHQ1: Black Hole Quencher 1; BHQ2: Black Hole Quencher 2.

readily be detected in serum of infected animals when blood culture fails, and species differentiation is done using serum and the IS711 species specific qRT-PCR is possible (Gwida et al., 2011).

In the agro-based economy of Bangladesh, livestock contribute 2.73% of the total gross domestic product (GDP) and 75% of rural people are directly or indirectly involved in livestock rearing including 23.4 million cattle and 1.86 million buffaloes. Brucellosis was first identified serologically in cattle in 1967 (Mia and Aslam, 1967), and in buffalo in 1997 (Rahman et al., 1997). Besides, the serological prevalence of brucellosis has been reported in man and animals in Bangladesh (Nahar and Ahmed, 2009; Muhammad et al., 2010; Rahman et al., 2006; 2011; 2012). Pharo et al. (1981) for the first time in Bangladesh described the isolation of *Brucella abortus* from two cows both of which were MRT and RBT positive. In the same year, Rahman and Rahman (1981) claimed to isolate *Brucella* spp. from MRT positive milk in sub-clinical mastitic udder. Unfortunately, the detail procedure to validate the isolates as *Brucella* spp. is missing in these papers. Moreover, these isolates were not preserved in any laboratory in Bangladesh for further analysis. The culture of *Brucella* spp. requires BSL 3 facilities, highly skilled personnel and it has also high health risk to laboratory workers. However, real time PCR techniques are available to identify *Brucella* at species level which is more sensitive, specific, faster, safe and relatively cheaper than culture technique (Alton et al., 1988; Al Dahouk et al., 2007). Therefore, the aim of this study was to determine the species of *Brucella* in Bangladesh using sophisticated and sensitive technique, quantitative real time PCR.

MATERIALS AND METHODS

Blood samples from 99 adult buffaloes and 700 cattle were randomly collected between May and October 2011 for a preliminary study. RBT, SAT, CFT (all Pourquier, IDEXX, Montpellier, France) and the IDEXX Brucellosis Serum X2 Ab Test (IDEXX, Liebefeld-Bern, Switzerland) were performed according to the procedures described by the manufacturers. The RBT positive sera were re-tested with SAT, CFT, ELISA and qRT-PCR. For the qRT-PCR, DNA was isolated from 200 µL of seropositive serum using the High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. *Brucella* IS711 targeting genus specific qRT-PCR was done according to the established and routine protocol (Tomaso et al., 2010) on a light cycler 2.0 instrument (Roche, Mannheim, Germany). Cycle threshold values (CT) ≤ 40 were interpreted as positive. Positive samples were then typed with the *Brucella* IS711species specific qRT-PCRs for *B. abortus* and *Brucella melitensis* according to Probert et al. (2004). CT values were calculated by the instrument's software MxPro3000P v 4.01. CT values ≤42 were interpreted as positive. The details primers list could be found in Table 1.

Statistical analysis

Descriptive statistics, 95% confidence interval of prevalence and Fisher Exact test to determine the level of significance between *B. abortus* detection level among RBT positive cattle and buffalo serum were performed in R 3.1.0 (The R foundation for Statistical Computing).

RESULTS

Out of total 700 cattle and 99 buffalo sera, 38 cattle and seven buffalo sera showed positive reaction to RBT with the overall prevalence of brucellosis 5.42% (95% Confidence Interval (CI): 3.87-7.38) in cattle and 7.07% (95% CI: 2.89-14.03) in buffalo (Table 2). Out of 38 RBT positive sera of cattle, 23.68% were *B. abortus* positive whereas out of 7 RBT positive buffalo sera, 71.43% were *B. abortus* positives. The difference in detection level of *B. abortus* from cattle and buffalo sera was statistically significant (p=0.02). The odds of getting *B. abortus* DNA from RBT positive buffalo sera was 7.61 times higher than the same from cattle sera (Table 2). Figure 1 shows the amplification plots of *B. abortus* specific real time PCR based on seropositive cattle and buffalo sera.

Out of 45 sera tested, six samples were three tests positive and can be considered as acute and active infection. Among 799 sera samples, 36 were positive only to RBT but negative to the other two tests (Table 3).

The relationship between serological tests and PCR is shown in Table 4. Out of nine *B. abortus* specific rt PCR positive cattle samples, 7 were positive only to RBT but negative to other two tests. On the other hand, out of five buffalo *B. abortus* specific rt PCR positive buffalo

Table 2. Prevalence of brucellosis and *B. abortus* infection in cattle and buffalo based on RBT and rt PCR.

Sera	Tested	Positive in RBT	Prevalence (95% CI)	*B. abortus* detected	Detection percentage (95% CI)	Fisher Exact Test Odd ratio (95% CI)
Cattle	700	38	5.42 (3.87-7.38)	9	23.68 (11.4-40.24)	1
Buffalo	99	7	7.07 (2.89-14.03)	5	71.43 (29.04-96.33)	7.61 (1.03-92.99)

*p-value=0.02.

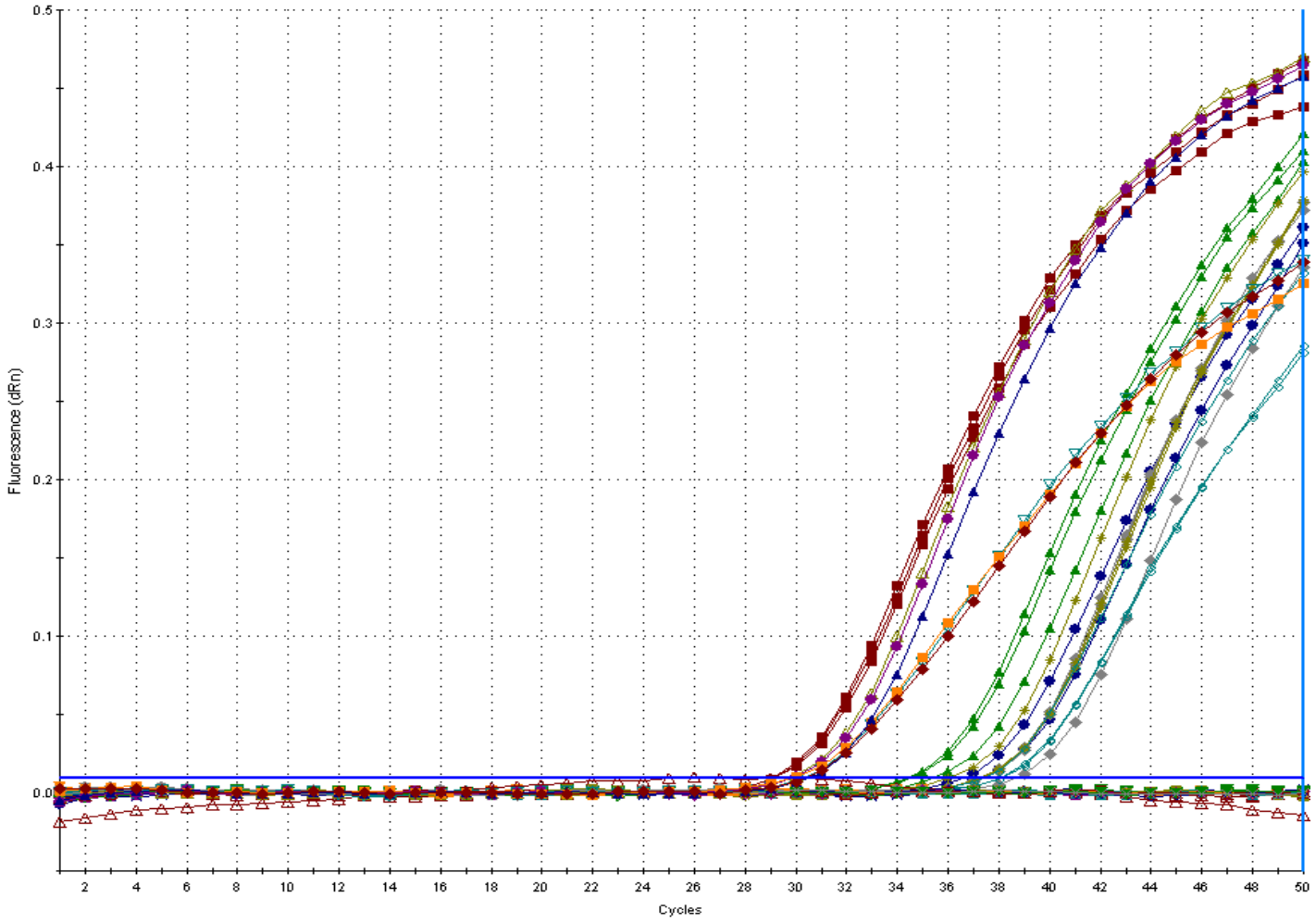

Figure 1. Amplification plots of *B. abortus* specific real time PCR with the DNA extracted from sera of cattle and buffalo in Bangladesh.

samples, only one was positive to RBT but negative to the other two tests. The genus specific screening by PCR detected *Brucella* DNA in 14 sera, the species specific IS711 PCR also detected *B. abortus* DNA from all the14 sera samples tested.

DISCUSSION

We found seroprevalences of 5.42 and 7.07% in cattle and buffalo by RBT, respectively (Table 2). The seroprevalence of brucellosis in cattle in Bangladesh is reported to lie between 2.4 - 18.4% at animal level and at 62.5% at herd level. Serological prevalence in buffaloes was reported to be 2.87% (Rahmanet al., 1997; Amin et al., 2005).

About 13.3% (6/45) RBT positive bovines were found to be acutely infected with brucellosis. These animals were positive to both IgG (iELISA) and IgM (SAT) detecting tests. The IgM and IgG are produced respectively in early and later stage of the disease. So, if a sample is positive in SAT and ELISA, it is considered as an active and acute infection. Whereas, if a sample is positive only to

Table 3. Summary of three serological test results.

RBT	SAT	iELISA	Number	Remarks
1+	+	+	0	
1+	+	-	3	Probably false positive (if RBT detected IgG)/Acute infection (if RBT detected IgM)
1+	-	-	33	Probably false positive
2+	+	+	4	Acute infection*
2+	+	-	0	
2+	-	-	3	Probably false positive
3+	+	+	2	Acute infection*
3+	+	-	0	
3+	-	-	0	
Sub-total			45	Tested by genus and species specific rt PCR
Suspicious	ND	Negative	93	Probably false positive
Suspicious	ND	ND	15	Probably false positive
Negative	ND	Negative	50	
Negative	ND	ND	596	
Total			799	

ND: Not done, only two sera were tested by CFT and found positive. They were positive in at least 2+ in RBT and also in iLEISa and SAT.

Table 4. Relationship of serological tests and PCR.

Sample	Area	RBT	SAT	iELISA	BCSP genus specific rt PCR	IS711 genus specific rt PCR	*B. abortus* specific IS711 rtpcr	Number
Cattle serum	Kurigram	1+	Negative	Negative	Positive	Not done	Positive	7
Cattle serum	Kurigram	2+	Positive	Positive	Positive	Positive	Positive	1
Cattle serum	Kurigram	3+	Positive	Positive	Negative	Positive	Positive	1
Buffalo serum	Mymensingh	1+	Negative	Negative	Positive	Not done	Positive	1
Buffalo serum	Mymensingh	2+	Positive	Positive	Positive	Positive	Positive	1
Buffalo serum	Bagerhat	2+	Positive	Positive	Positive	Positive	Positive	2
Buffalo serum	Bagerhat	3+	Positive	Positive	Positive	Positive	Positive	1
Total								14

IgG ELISA, it is considered as chronic infection. A sample positive to only agglutination test like SAT cannot be considered as brucellosis unless confirmed by an IgG detecting test like IgG ELISA within one week (Godfroid et al., 2010; Seleem et al., 2010). However, it requires repeated sampling from the same animal which was not possible and also not the purpose of this study. From all cattle and buffalo sera investigated, only two cattle sera from Kurigram could be analysed by CFT due to the low quality of the sera. These two sera were also positive in the ELISA.

Out of 9 cattle sera from where *B. abortus* DNA were detected 7 had negative test results both in SAT and iELSA. The biological explanation of this phenomenon is not clear. However, these animals were positive to RBT (1+). The infection in these animals may be in the very early stage which was detected by the qualitative test (RBT) but not by the quantitative tests like SAT and iLEISA for the presence of antibody below cut-off level. Similarly, for buffalo sera only one sample was positive to RBT but negative to SAT and iELISA. In humans, presence of *Brucella* DNA after a long time after clinical cure was also reported by Navarro et al. (2006). This indicates that the presence of only *Brucella* DNA does not indicate acute infection. Similar phenomenon may also occur in animals as we have notice in this study. Contrarily, serological cross reactivity with other abortion causing agents could explain the high number of RBT 'positives'which is regularly reported for females older than four years (Chantal and Thomas, 1976). However, the low number of animals investigated in this study does not allow statistical proof of these assumptions.

The major shortcoming of PCR based techniques is that the biovar cannot be determined. Cultivation from sera often fails and was thus not attempted in our preliminary study but has to be part of future investigations. It can be concluded that a combination of real-time PCR with SAT and iELISA should be applied to

detect brucellosis in cattle and buffalo from Bangladesh in a future eradication program. This paper for the first time detected the presence of *B. abortus* using real time PCR technique in the cattle and buffalo populations in Bangladesh.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Verification of molecular characterization of coagulase positive *Staphylococcus* from bovine mastitis with matrix-assisted laser desorption ionization, time-offlight mass spectrometry (MALDI-TOF MS) mass spectrometry

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Besides *Staphylococcus aureus*, other coagulase-positive *Staphylococcus* (CPS) species such as *Staphylococcus hyicus* and *Staphylococcus intermedius* are implicated in bovine mastitis etiology. These species are often misdiagnosed as *S. aureus*. Also, some atypical *S. aureus* isolates can test negative for coagulase production and consequently be misdiagnosed as coagulase-negative *Staphylococcus* (CNS). Several currently available methods for the identification of *Staphylococcus* spp., including molecular techniques, are widely used worldwide. Recently, matrix-assisted laser desorption ionization, time-offlight mass spectrometry (MALDI-TOF MS) has been attracting attention for its fast and precise identification of several microorganisms at the species level. The present work evaluated the efficiency of a protocol for *S. aureus* characterization using PCR and M-PCR procedures. MALDI-TOF was considered the gold standard test to evaluate the sensitivity and specificity of the proposed identification protocol. Seventy-two *Staphylococcus* spp., isolates were evaluated. All samples were submitted to PCR for *coa*, *nuc* and 23S rDNA. Out of 33 isolates, genotypically characterized as *S. aureus* and confirmed by MALDI-TOF MS, 2 (6.1%) tested negative for coagulase production. Three isolates were identified as *S. hyicus* (2) and *S. intermedius* (1) by MALDI-TOF MS. The proposed molecular identification schedule achieved 100% sensitivity and specificity as compared to MALDI-TOF MS.

Key words: Bovine mastitis, coagulase-positive *Staphylococcus*, matrix-assisted laser desorption ionization, time-offlight mass spectrometry (MALDI-TOF MS), molecular identification.

INTRODUCTION

Bovine mastitis is an inflammatory disease usually caused by bacterial and mycotic pathogens (Capurro, 2009). It is recognized as a major disease affecting milk production and consequently dairy enterprises. Among the infectious

agents implicated in the etiology of mastitis, *Staphylococcus* spp. are usually the most frequent bacteria (Taponen and Pyörälä, 2009).

According to the List of Prokaryotic Names with Standing in Nomenclature (http://www.bacterio.net/staphylococcus.html), the genus *Staphylococcus* comprises 49 species and 26 subspecies, separated into two distinct groups based on their ability to produce coagulase. The coagulase-negative *Staphylococcus* (CNS) was long regarded as non pathogenic species assembled in an undistinguishable group. Today, their importance in animal infections is becoming clear and there are several reports implicating CNS in bovine mastitis.

Eight coagulase-positive *Staphylococcus* species have been reported: *Staphylococcus aureus*, *Staphylococcus intermedius*, *Staphylococcus delphini*, *Staphylococcus pseudintermedius*, *Staphylococcus schleiferi* subsp. *coagulans*, *Staphylococcus hyicus*, *Staphylococcus lutrae* and *Staphylococcus agnetis* (Freney et al., 1999; Devriese et al., 2005; Sasaki et al., 2010; Taponen et al., 2012). *S. aureus* is the most frequent species isolated from bovine mastitis samples. *S. intermedius* and *S. hyicus* are rarely identified and the other CPS seems to be misidentified as *S. aureus* (Capurro, 2009).

The failures in the identification protocol are mostly related to phenotypic procedures, since distinguishing between species is a difficult task. The use of molecular markers has greatly improved species differentiation and allows the elucidation of the taxonomy of *Staphylococcus* spp., (Lange et al., 2011). Description of new species (Foster et al., 1997; Devriese et al., 2005) and reclassification of known ones have happened as a consequence of new methods and techniques (Sasaki et al., 2007; Blaiotta et al., 2010).

Molecular identification methods are keys to achieving phenotypic identification spaces as gene specific markers are being recognized. Nucleic acid-based detection approaches offer rapid and sensitive methods that are easily reproducible. Several identification schedules considering the amplification of *nuc*, *coa* and 23S rDNA genes have been previously reported for *S. aureus* (Hookey et al., 1998; Straub et al., 1999; Ciftci et al., 2009). Sasaki et al. (2010) developed a *multiplex* PCR (M-PCR) of *nuc* gene which encodes for thermonuclease in different *Staphylococcus* species.

Recently, matrix-assisted laser desorptionionization, time off light mass spectrometry (MALDI-TOF MS) has been attracting attention for its fast and precise identification of several microorganisms at the species level, even in mixed cultures (Bizzini and Greub, 2010; Bannoehr and Guardabassi, 2012). Mass spectrometry (MS) is a technique based on the analysis of ionized molecules in a gaseous phase. Decristophoris et al. (2011) reported high specificity (95%) and sensitivity (100%) in the identification of species of the SIG group, the *S. intermedius* reclassification proposed by Devriese et al. (2005), that comprises *S. intermedius,* the new species *S. pseudintermedius* and *S. delphini*. Böhme et al. (2012) also reported its use for *S. aureus* identification.

In the present study, we proposed a molecular schedule based on PCR amplification of the *nuc*, 23S rDNA and *coa* genes in coagulase-positive *Staphylococcus* isolated from dairy farms. The results obtained were compared with those yielded by MALDI-TOF MS, considered the gold standard technique due to its reliability and speed.

MATERIALS AND METHODS

Sampling

The 72 *Staphylococcus* spp. isolates evaluated in this study were obtained from samples of mastitic cow's milk and dairy workers' hands, obtained from dairy farms in the state of Rio de Janeiro, Brazil.

The samples were first inoculated on blood agar (blood agar base enriched with 5% sheep blood) and incubated at 35°C (± 2°C) for 24 h. Then, the isolates were submitted to routine microbiological diagnostics, including inoculation in selective medium for analysis of cultural properties and catalase and coagulase production. The coagulase-positive samples were evaluated for maltose and $_D$-mannitol fermentation, acetoin production and nitrate reduction (Winn et al., 2006). Coagulase-negative isolates were stored in 45% glycerol added to Brain Heart Infusion (BHI) broth for complementary analysis. To its identification, a modified scheme based on Cunha et al., (2004) was used, comprising the following tests: fermentation of the sugars xylose, arabinose, sucrose, trehalose, maltose, mannitol, lactose, xylitol, ribose, fructose and mannose; production of hemolysin; presence of urease; and resistance to novobiocin 5 mcg.

Molecular and proteomic analysis

After phenotypic identification, all strains including CNSs, were submitted to polymerase chain reaction for 16S rRNA to confirm the presence of *Staphylococcus* spp. (Zhang et al., 2004). PCR for *coa* (Hookey et al., 1998), *nuc* (Ciftci et al., 2009) and 23S rDNA (Straub et al., 1999) genes were performed to characterize *S. aureus* (Table 1). *S. aureus* standard strain ATCC29213 was used as control.

Multiplex PCR (M-PCR) for *nuc* gene was performed according to Sasaki et al. (2010) to characterize coagulase-positive *Staphylococcus* species (Table 1). Strains ATCC 29213 *S. aureus* and ATCC 29663 *S. intermedius* and two strains from UFRJ culture collection, the *S. hyicus* 5368 and *S. schleiferi* 3975 were used as quality controls.

Furthermore, all 72 isolates were evaluated by the MALDI-TOF MS. To perform this procedure, the samples were inoculated in BHI agar at 37°C for 24 h. Each culture was transferred to a microplate

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Table 1. Primers and cycles employed to identify *S. aureus* and other CPS species.

Gene (fragment)	Species	Primer Sequence (5'-3')	Cycling*
16S rRNA(756 pb)	*Staphylococcus* spp.	AAC TCT GTT ATT AGG GAA GAA CA CCA CCT TCC TCC GGT TTG TCA CC	1
23S *rDNA*(1250 pb)	*S. aureus*	ACG GAG TTA CAA AGG ACG AC AGC TCA GCC TTA ACG AGT AC	1
coa (Variável)	*S. aureus*	ATA GAG ATG CTG GTA CAG G GCT TCC GAT TGT TCG ATG C	2
nuc (279 pb)	*S. aureus*	GCG ATT GAT GGT GAT ACG GTT AGC CAA GCC TTG ACG AAC TAA AGC	3
nuc (359 pb)	*S. aureus*	TCG CTT GCT ATG ATT GTG G GCC AAT GTT CTA CCA TAG C	4
nuc (430 pb)	*S. intermedius*	CAT GTC ATA TTA TTG CGA ATG A AGG ACC ATC ACC ATT GAC ATA TTG AAA CC	4
nuc (526 pb)	*S. schleiferi* sub sp. *coagulans*	AAT GGC TAC AAT GAT AAT CAC TAA CAT ATC TGT CTT TCG GCG CG	4
nuc (661 pb)	*S. delphini* group A	TGA AGG CAT ATT GTA GAA CAA CGR TAC TTT TCG TTA GGT CG	4
nuc (1135 pb)	*S. delphini* group B	GGA AGR TTC GTT TTT CCT AGA C TAT GCG ATT CAA GAA CTG A	4
nuc (793 pb)	*S. hyicus*	CAT TAT ATG ATT TGA ACG TG GAA TCA ATA TCG TAA AGT TGC	4
nuc (926 pb)	*S. pseudintermedius*	TRG GCA GTA GGA TTC GTT AA CTT TTG TGC TYC MTT TTG G	4

*1. 94°C 5 min (94ºC 1 min, 55°C 1 min, 72°C 1 min) x 30 and 72ºC 10 min; 2. 94°C 4 min (94ºC 1 min, 60°C 1 min, 72°C 1 min) x 30 and 72ºC 5 min; 3. 94°C 5 min (94°C 45 s, 68°C 45 sec and 72°C 90 s) x 30 and 72°C 10 min; 4. 95°C 2 min (95°C 30 sec, 56°C 35 sec and 72°C 1 min) x 30 and 72°C 2 min.

(96 MSP, Bruker® - Billerica, USA). Each bacterial sediment was covered by a lysis solution (70% formic acid; Sigma-Aldrich®). Additionally, a 1-µL aliquot of matrix solution (alpha-ciano-4-hidroxi-cinamic acid diluted in 50% acetonitrile and 2.5% trifluoracetic acid, Sigma-Aldrich®) was added to each sediment. The spectra of each sample were generated in a mass spectrometer (MALDI-TOF LT Microflex, Bruker®) equipped with a 337 nm nitrogen laser in a linear path, controlled by the FlexControl 3.3 (Bruker®) program. The spectra were collected in a mass range between 2,000-20,000 m/s, and then were analyzed by the MALDI Biotyper 2.0 (Bruker®) program, using the standard configuration for bacteria identification, by which the spectrum of the sample is compared with the references in the database. The results vary on a 0-3 scale, where the highest value means a more precise match and reliable identification (Table 2). In this study, we accepted values for matching greater than or equal to 2.

The percentage of sensitivity, specificity and positive and negative predictive values for the employed molecular methods were measured considering MALDI-TOF MS proteomic analysis as the gold standard technique in this study.

RESULTS

Out of a total of 72 *Staphylococcus* spp. isolates evaluated in this study, 52.8% (38/72) tested negative for the phenotypic coagulase production test, so they were initially considered to be coagulase-negative *Staphylococcus*. Phenotypic identification of the 47.2% (34/72) of isolates that tested positive for coagulase production demonstrated that 79.4% (27/34) were *S. aureus*. Seven coagulase-positive isolates (20.6%) from the 34 could not be phenotypically identified.

PCR amplification of the 16S rRNA gene (756 pb) tested positive in all 72 isolates, corroborating the *Staphylococcus* spp., phenotypic identification. Additionally, PCRs for *coa*, nuc and 23S rDNA genes were carried out for all 72 isolates to characterize *S. aureus*. The decision to evaluate even the phenotypic coagulase-negative strains was due to the report of the detection of atypical coagulase-negative *S. aureus* strains misdiagnosed as CNSs (Akineden et al., 2011). The *coa* gene was detected in 41.7% (30/72) isolates, yielding variable size amplicons. Each *nuc* (279 pb) and 23S rDNA (1250 pb) gene was detected in 37.5% (27/72) of the isolates. Strains were characterized as *S. aureus* when positive for the amplification of at least one of these specific genes, consisting of 45.8% (33/72) of the samples. Interestingly, 6.1% (2/33) tested negative for phenotypic coagulase production. Also, none of the studied genes were detected in 4.2% (3/72) of the coagulase-positive isolates. These isolates were submitted to M-PCR for *nuc* genes of *S. intermedius*,

Table 2. Comparison of the phenotype, genotype and proteomic identification of 36 coagulase-positive *Staphylococcus* isolates analyzed by the MALDI-TOF MS technique and their respective scores.

Isolate	Phenotype identification	PCR genes *coa*, *nuc*, 23S rRNA	M-PCR genes *nuc*	MALDI-TOF MS (score)
1	*S. aureus*	*S. aureus*	*S. intermedius*	*S. aureus*(2.354)
2	CPS	*S. aureus*	*S. aureus*	*S. aureus*(2.258)
3	CPS	*S. aureus*	*S. aureus*	*S. aureus*(2.380)
4	CNS	*S. aureus*	ND	*S. aureus*(2.329)
5	*S. aureus*	*S. aureus*	ND	*S. aureus*(2.403)
6	CNS	*S. aureus*	ND	*S. aureus*(2.317)
7	*S. aureus*	*S. aureus*	ND	*S. aureus*(2.459)
8	*S. aureus*	*S. aureus*	ND	*S. aureus*(2.367)
9	*S. aureus*	*S. aureus*	ND	*S. aureus*(2.408)
10	*S. aureus*	*S. aureus*	ND	*S. aureus*(2.381)
11	*S. aureus*	*S. aureus*	ND	*S. aureus*(2.441)
12	*S. aureus*	*S. aureus*	ND	*S. aureus*(2.424)
13	S. aureus	*S. aureus*	ND	*S. aureus*(2.443)
14	*S. aureus*	*S. aureus*	ND	*S. aureus*(2.351)
15	*S. aureus*	*S. aureus*	ND	*S. aureus*(2.405)
16	CPS	*S. aureus*	ND	*S. aureus*(2.419)
17	*S. aureus*	*S. aureus*	ND	*S. aureus*(2.371)
18	*S. aureus*	*S. aureus*	ND	*S. aureus*(2.418)
19	*S. aureus*	*S. aureus*	ND	*S. aureus*(2.426)
20	*S. aureus*	*S. aureus*	ND	*S. aureus*(2.450)
21	*S. aureus*	*S. aureus*	ND	*S. aureus*(2.455)
22	*S. aureus*	*S. aureus*	ND	*S. aureus*(2.410)
23	*S. aureus*	*S. aureus*	ND	*S. aureus*(2.461)
24	*S. aureus*	*S. aureus*	ND	*S. aureus*(2.379)
25	*S. aureus*	*S. aureus*	ND	*S. aureus*(2.428)
26	*S. aureus*	*S. aureus*	ND	*S. aureus*(2.410)
27	CPS	*S. aureus*	ND	*S. aureus*(2.477)
28	*S. aureus*	*S. aureus*	ND	*S. aureus*(2.442)
29	*S. aureus*	*S. aureus*	ND	*S. aureus*(2.451)
30	*S. aureus*	*S. aureus*	ND	*S. aureus*(2.397)
31	CPS	*S. aureus*	ND	*S. aureus*(2.425)
32	*S. aureus*	*S. aureus*	ND	*S. aureus*(2.422)
33	*S. aureus*	*S. aureus*	ND	*S. aureus*(2.424)
34	CPS	Negative (CPS)	Negative	*S. hyicus*(2.157)
35	CPS	Negative (CPS)	Negative	*S. hyicus*(2.116)
36	*S. aureus*	Negative (CPS)	Nonspecific fragment	*S. intermedius*(2.178)

*CPS: Coagulase-positive *Staphylococcus*; CNS: coagulase-negative *Staphylococcus*; ND: not determined.

S. pseudintermedius, *S. schleiferi* subsp. *coagulans*, *S. delphini* group A and B, *S. hyicus* and *S. aureus* (Sasaki et al., 2010). Out of these three CPSs isolates evaluated, just one presented an atypical amplicon bigger than 1000 pb. The other two isolates and the *S. hyicus* 5368 standard strain could not be identified by this technique. MALDI-TOF MS confirmed the 33 isolates previously identified as *S. aureus* (45.8%), even the strain misidentified as *S. intermedius* by the M-PCR assay. Three isolates, previously identified as CPSs, were identified by MALDI-TOF MS as *S. hyicus* (2) and *S. intermedius*. The M-PCR assay for the *nuc* gene was not able to distinguish these strains. All 36 isolates previously identified as CNSs (45.8%) were confirmed by the MALDI-TOF MS proteomic analysis. *S. chromogenes* and *S. sciuri* were the prevalent species. The genotypic identification schedule based simultaneously on the detection of coa, *nuc* and 23S rDNA genes and showed correspondence of 100% with the MALDI-TOF MS technique.

Table 3. Percentages of sensitivity, specificity, positive predictive value and negative predictive value found for the proposed identification of *S. aureus*.

Genes	Values (%)			
	Sensitivity	Specificity	PPV	NPV
coa, *nuc* e 23S rDNA	100	100	100	100
coa	90.9	100	100	92.8
nuc	81.8	100	100	86.7
23S rDNA	81.8	100	100	86.7

*PPV: Positive predictive value; NPV: negative predictive value.

DISCUSSION

The phenotypic differentiation of CPS species is a difficult task due to the absence of specific biochemical markers. To overcome this problem, the use of molecular tools has become routine in human and veterinary microbiology diagnosis. Nonetheless, genotypic assays are relatively expensive, time consuming and most important may provide results that are difficult to analyze.

To evaluate susceptibility patterns, it is necessary to establish a reliable identification procedure of CPS species involved in several infections of distinct hosts. Parameters such as oxacillin minimum inhibitory concentration, antimicrobial susceptibility, incubation time and inhibition zones are specific to different *Staphylococcus* species (Sasaki et al., 2010).

In the present study, MALDI-TOF MS proteomic analysis was carried out to evaluate the sensitivity, specificity and positive and negative predictive values of a molecular identification schedule for *S. aureus* based on the *coa*, *nuc* and 23S rDNA genes. It proved to be an efficient tool for distinguishing *Staphylococcus* species. Also, it has high potential for routine automated analysis, allowing the identification of isolates from clinical sources on a large scale (CLSI) (2013). Nevertheless, although it proved to be a fast and easy method with high specificity and sensitivity, the equipment is very expensive and requires skilled staff, so it is not suitable for small laboratories.

The proposed genotypic identification schedule based on the *coa*, *nuc* and 23S rDNA genes achieved 100% sensitivity and specificity as compared to MALDI-TOF MS, the gold standard tool in this study (Table 3). So, this proposed identification schedule is reliable to characterize *S. aureus*, even the atypical coagulase-negative strains, and can be used in small research laboratories.

Despite the fact that it was reported as a 99.8% sensitive and a 100% specific method, the M-PCR technique, established by Sasaki et al. (2010) was not able to distinguish among the other CPS strains. In fact, although several molecular approaches have been suggested for the proper identification of CPS, since phenotypic methods are time consuming and unreliable for animal samples, this is still a goal to be achieved.

Conflict of interest

The author(s) have not declared any conflict of interests.

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Full Length Research Paper

Microbiological quality and safety of street vended raw meat in Jijiga town of Somali Regional State, southeast Ethiopia

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A cross sectional study was conducted to determine the microbial quality and safety of street vended raw meats in Jijiga town, Ethiopia. Questionnaire was used to assess the profile of 33 street vendors. A total of 60 meat samples (30 beef and 30 goats) were collected. The pH and holding temperature were measured. Six microbial groups were counted using standard methods. The aerobic mesophilic flora was characterized. Vendors had very little degree of awareness on food safety and food borne diseases. The sanitary condition of the vending environment was poor. The mean pH values were 6.03 and 5.98 for beef and goat meat samples, respectively. The samples were held in a temperature range of 17.5-27.5°C. Total mesophilic bacteria, Enterobacteriaceae and coliforms, *Staphylococci*, lactic acid bacteria, yeasts and moulds had counts of >7, 4, 6, 4 and 4log cfu/g respectively for both species. The aerobic plate counts were dominated by *Staphylococcus* spp. followed by Enterobacteriaceae. *Salmonellae* were also isolated from 5 (8.3%) meat samples. There were significant differences (P<0.05) between goat and beef samples in total mesophilic bacteria and *Staphylococci* counts. The samples harbored high counts of microorganisms. Trainings, inspections, infrastructures and code of practice are recommended.

Key words: Jijiga, raw meat, street vendors, quality, safety.

INTRODUCTION

Food is essential for survival. However, occasionally, human beings consume undesirable chemical and biological agents and toxins resulting in food borne illness. Consequently, in many countries food safety and quality is becoming a matter of increasing concern. Food safety problems are particularly becoming an increasingly serious threat to public health in developing countries. Lack of adequate regulations related to food safety as

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reflected in many unrecognized cases of food borne illnesses puts especially children and infants at high risk (Unnevehr and Hirschhorn, 2000). Biological contaminants, largely bacteria, viruses and parasites constitute the major cause of food-borne diseases (Kaferstein, 2003).

Vending foods on the street is a common aspect of lifestyle both in industrialized as well as countries in which there are high unemployment, low salaries and limited work opportunities (Bryan et al., 1988). Street vendors provide an essential service to people of all walks of life by selling raw foods, complete meals, refreshing drinks and snacks (WHO, 1996).

In spite of numerous advantages offered by street vended foods, there are also several hazards associated with this sector of the economy. Multiple line evidence revealed that foods exposed for sale on the roadside may become contaminated by either spoilage or pathogenic microorganisms (Mogessie, 1995). This constitutes serious health hazards, particularly in economically disadvantaged countries where food surveillance are undeveloped or not there at all. Evidently, street vended foods have shown epidemiological link with illness (Van Kampen et al., 1998; Mogessie , 1995) and laboratory results have also shown high counts of microorganisms and presence of food borne pathogens (Umoh et al., 1984, 1985; Mogessie, 1995). Some foods like meats, rice, fish and fruits have been frequently identified as vehicles in outbreaks of food borne diseases in countries where food-borne surveillance data are available (Davey, 1985; Bryan et al., 1988). Among the most common street vended foods, meat and meat products were known to be the major in either processed or unprocessed form (WHO, 1996). Retailing unprocessed raw meat in the street or in an open air market for the public is common in Africa as well as in some parts of Asian countries (WHO, 1996). Studies made in Africa, Asia and Latin America (FAO, 1995) pointed out that the important aspect of street vended food is their safety and understanding the possible ways of contamination.

Microbial contamination of street vended foods could occur due to different possible reasons such as storing food in cheap utensils, holding food at a temperature that would permit bacterial growth, utilization of water of questionable hygienic quality, using packing materials that were not of food-grade quality, vending site that had no facilities for waste disposal and utilization of unclean utensils (Deriba and Mogessie, 2001).). In addition, street food vendors are unaware of the basic importance of personal cleanliness, thus their products are usually vulnerable to gross contamination by flies, insects, rodents, dust and other dirt (Deriba and Mogessie, 2001). It is also indicated that street-food vendors are often poor and uneducated and lack appreciation for safe food handling (Bryan et al., 1988).

Although vending raw meat is not common in most parts of Ethiopia, there are some areas in which vending raw meat in an open market is practiced. Jijiga town is one of these areas where raw meat street vendors are available in most parts of the town and highly populated at the center of the town. Raw meats of different animals (such as sheep, goat, camel and cattle) are commonly retailed and vending and purchasing activities are carried out every day in a week.

Studies concerning various street vended foods in Ethiopia showed the presence of pathogens or existence of good conditions in street foods to allow growth of pathogens in them (Mogessie, 1994, Deriba and Mogessie, 2002). However, information on the microbial quality and safety of street vended raw meats in Jijiga town is scant. The purpose of this study was therefore to determine the microbiological quality and safety of raw beef and raw goat meats as these types of meats were the most common and widely vended meats in the study area.

MATERIALS AND METHODS

Study area

The study was conducted at Jijiga town, the capital city of Somali Regional State, located about 80 km east of Harar and 620 km southeast of Addis Ababa. Its geographical coordinates are 9° 21' North, 42° 48' East. The majority of the region has an altitude of 900 m above sea level and in some areas the altitude reaches 1600 m. Of the total area size of the state, approximately 80% is flat and 7% mountainous. Regarding climate, 80% of the region is classified as "Kolla" (lowlands), 5% highland ("Dega") and 15% of the area fall under temperate ("Woyna Dega") category. The maximum temperature reaches 32-40°C. In the temperate ("Woyna Dega") areas, the temperature is within 20-28°C. The mean annual rainfall of the state is estimated to be 300-500 mm.

Study design and data collection

The current cross-sectional study was carried out at Jijiga town from December, 2010 to March, 2011 with the aim of evaluating the microbiological quality and safety of street vended beef and goat meat in the town. Questionnaire and direct observation were used as tools to collect data. Content of the questionnaire included issues addressing socio-demographic characteristics, health status and personnel hygiene, food handling practices and food safety knowledge of the vendors and access to hygienic water supply and other sanitary facilities. Standard microbiological methods were also used to assess the microbiological quality and safety of street vended raw meats.

Survey

Survey using direct observation and questionnaire was undertaken throughout the study period in order to obtain data on socio-demography, food safety knowledge and food handling practices of street raw meat vendors. For this study, vendors selling mainly raw meat of goat and cattle were included. From the total of 44 raw meat vendors recognized by the city administration office and operating in the major open air market in a fixed place, only 33 food vendors were recruited using simple random sampling technique. Written consent was obtained by reading a statement to prospective respondents seeking permission for the data gathering. Data were collected only after getting willingness of the vendors

and confidentiality was ensured using data coding system.

Sample collection for microbiological analysis

About 60 (30 from each meat type) samples of raw meat were collected from 30 different street vendors as made available to the consumers. Collection and transportation of the meat samples was carried out following the procedures used by Mogessie (1994) and Deriba and Mogessie (2002).

Plating and enumeration of microorganisms from raw meat samples

Plating of samples and microbial enumeration was conducted based on well established procedures (Diane et al., 2003). Twenty five grams of raw meat and 225 ml of 0.1% sterile buffered peptone water (BPW) was homogenized in a stomacher bag after the meat was chopped using sterile scissors. A volume of 0.1 ml sample from appropriate dilutions was plated on the following culture media (all from Oxoid) for microbial count: Aerobic mesophilic bacteria were counted on plate count (PC) agar after incubation at 32°C for 24-48 h. Violet Red Bile agar was used to count coliforms. After 24 h incubation at 32°C, purplish red colonies surrounded by red zone of precipitated bile were counted as coliforms. Violet Red Bile Glucose agar plates were used to count enterobacteriacae. The seeded culture plates were incubated at 30-32°C for 20-24 h after which pink to red purple colonies with or without haloes of precipitation were enumerated as members of enterobacteriaceae. Staphylococci were counted on Mannitol Salt agar after incubation at 32°C for 36 h. Lactic acid bacteria were counted on de-Mann, Rogossa and Sharp (MRS) agar plates after incubation in an anaerobic jar at 32°C for 48 h. Yeasts and moulds were counted on potato dextrose agar plates. Colonies were counted after incubation at 28-30°C for five days (Diane et al., 2003).

Mezophilic flora analysis: After enumeration of aerobic mesophilic bacteria, about 10-20 colonies were picked randomly from countable plates and inoculated into tubes containing about 5 ml Nutrient Broth (Oxoid). The broth cultures were incubated at 37°C overnight. Cultures were further purified by repeated plating and differentiated to various bacterial groups. Cell morphology and clustering pattern, presence or absence of endospores and motility were examined under a microscope. Gram reaction was determined using the KOH test as indicated by Gregerson (1978). Furthermore, the presence of cytochrome oxidase (Kovacs, 1956) and catalase (Deriba and Mogessie, 2001) and oxidation-fermentation test (Hugh and Leifson, 1953) for glucose metabolism were also employed to characterize the microbial flora to their respective genus and/or species level.

Isolation of *Salmonella* spp. from meat samples: Isolation and identification of *Salmonella* was done according ISO 6579 (Muinde and Kuri, 2005). Briefly, 25 g sample was mixed with 225 ml buffered peptone water (BPW) and homogenized in a stomacher bag after the meat was chopped using sterile scissors. The homogenized solution was incubated at 37°C for 18-24 h for primary enrichment. For secondary enrichment, 0.1 ml of the solution was added in a tube containing 10 ml Rappaport-Vassiliadis broth (Oxoid) and incubated at 42°C for 24 h. A loopful of culture from the enrichment broth was inoculated into xylose lysine deoxycholate (XLD) medium (Oxoid) and incubated at 37°C for 18-24 h. Characteristic colonies from XLD medium were picked and further purified and tested biochemically using the following media: Triple Sugar Iron (TSI) agar, Lysine Iron (LI) agar, Urea agar, Simmon's Citrate agar and Sulphur-Indole-Motility (SIM) medium. For all media, incubation was done at 37°C for 18-24 h (Diane et al., 2003).

Data management and statistical analysis

All data collected form survey and laboratory investigations were double entered into Microsoft Spread Sheet data storage program. For the analysis, data generated from the questionnaire was analyzed using SPSS version 15.0. All microbial counts were converted to $\log_{10}$ colony forming unit (cfu) per gram values. Difference in microbial counts among meat samples of the two meat types was analyzed by analysis of variance (ANOVA). Significance was determined at the 5% of confidence level.

RESULTS

Survey

Survey results indicated that the majority of the food vendors were females (78.8%). Fifty-eight percent of the respondents were in the age range of 31-45 years. Only 30.3% of the vendors were literate (elementary school). Most of them (58%) were involved in vending meat for 5-10 years.

The sanitary condition of the vending environment was poor as it was dusty and full of remains of slaughtered animals such as bones, horn, head and other body parts. House flies were also very prevalent throughout the vending area and even on the raw meats displayed for sale by street vendors. All street vendors included in our study had no access to clean potable water. Forced by the situation, they simply reuse the water that they brought from their home.

It was also observed that the raw meats were displayed uncovered for more than 6 h for sale at ambient temperature on a table or a carton which would be used again and again.

All food handlers have a basic task to maintain a high degree of personal cleanliness and observe hygienic and safe food handling practices. Only 67% of the vendors had relatively good personal hygiene with respect to cleanness of their cloths and visible body parts. None of raw meat street vendors evaluated in our study wore appropriate working garment (overcoat). The majority (70%) of street vendors wore jewelers on their hands, ear and different body parts.

Microbiological analysis

Mean pH values for the meat samples investigated in our study ranged between 5.98 and 6.03. The raw meat samples analyzed in our study were held within a temperature range of 17.5-27.5°C during the time of vending and they were also possibly displayed for more than 6 h.

The mean values of aerobic mesophilic counts of street

Table 1. Microbial counts (log cfu/g) of street vended raw beef and goat meat samples in Jijiga town, 2011.

Microbial group	RBM			RGM		
	Mean	S.D.	Range	Mean	S.D.	Range
AMB	8.07	0.75	6.20-9.40	7.59	0.76	6.00-9.00
TC	4.71	1.32	2.30-7.70	4.31	1.12	2.00-6.10
Enterobacteriaceae	4.45	1.31	2.00-6.90	4.10	1.14	2.30-6.60
Staphylococci	6.74	0.37	5.80-7.50	6.23	0.40	5.30-7.00
LAB	5.16	0.88	3.30-7.40	4.82	0.81	2.90-6.40
Yeasts & Moulds	4.62	1.06	2.70-7.00	4.66	0.87	2.30-6.10

AMB, Aerobic mesophilic bacteria; TC, total coliforms; LAB, lactic acid bacteria; S.D, standard deviation; RBM, raw beef meat; RGM, raw goat meat.

Table 2. Frequency distribution of mezophilic bacteria in meats collected from street vendors in Jijiga town, 2011

Meat type	No. of isolates	*Staphylococcus* spp.	*Micrococcus* spp.	*Other G+ ve rods*	*EB*	*Pseudomonas* spp.	*Alcaligenes* spp.	*Acinetobacter* spp.	*Aeromonas* spp.
Beef	149	77(52)	10(6.7)	11(7.4)	33(22.2)	4(2.7)	7(4.7)	4(2.7)	3(2.0)
Goat	153	73(47.7)	11(7.2)	16(10.5)	36(23.5)	3(2.0)	10(6.5)	3(2.0)	1(0.7)
Sum	302	150(49)	21(7)	27(8.9)	69(22.8)	7(2.3)	17(5.6)	7(2.3)	4(1.3)

Where: EB, Entrobacteriaceae; Numbers in the parenthesis are percentage of the total isolates of respective species.

vended raw meat obtained in this study were 8.07 log cfu/g (ranged from 6.20 to 9.40 log cfu/g) and 7.59 log cfu/g (ranged 6.00-9.00 log cfu/g) for raw beef and raw goat meat, respectively (Table 1).

Enterobacteriaceae and coliforms were also encountered in our samples frequently (Table 1). The mean count of enterobacteriaceae and coliforms in our raw beef and raw goat meat samples was as high as log 4 cfu/g. Both raw meat samples analyzed in the present study had staphylococci counts ≥ 6log cfu/g (Table 1).

Counts of lactic acid bacteria in our study were also high with mean counts as high as log 5.16 cfu/g for raw beef meat and 4.82 log cfu/g for raw goat meat. The presence of such high counts of lactic acid bacteria (LAB) in the meat samples might indicate improper handling of the meats and inadequate storage conditions. Since lactic acid bacteria (LAB) are meat spoilers (Jay, 2005), the presence of such high counts in the samples may limit the keeping quality of the raw meats. The mean count of yeasts and moulds for raw beef and goat meat samples analyzed in our study were log 4.62 cfu/g and log 4.66 cfu/g, respectively (Table 1).

In our study, a total of 302 bacterial groups (149 isolates from raw beef and 153 isolates from raw goat meat) were isolated and characterized to various genera and bacterial groups (Table 2). In both types of meats, the aerobic mesophilic flora was dominated by staphylococci followed by enterobacteriacae and other Gram positive rods. *Pseudomonas* spp., *Alcaligenes* spp., *Acinetobacter* spp, and *Aeromonas* spp. were also among the aerobic mesophilic bacterial groups isolated in beef and goat meat samples although they were not significant in their number. *Salmonella* was isolated from 5 meat samples (8.3%) (3 from goat meat and 2 from beef samples) (Table 2).

Statistical analysis with one-way ANOVA revealed that there were significant differences ($P< 0.05$) between goat and beef raw meat samples with regard to aerobic mesospheric count and staphylococci count (Table 3). However, significant

Table 3. ANOVA for microbial counts (log cfu/g) of raw beef and raw goat meat samples collected from street vendors in Jijiga Town, 2011.

Bacterial groups	Log cfu/g (Mean+S.D.)	
	RBM	RGM
AM B	8.07±0.75[a]	7.59±0.76[b]
TC	4.45±1.31[a]	4.10±1.14[a]
Enterobacteriaceae	4.71±1.32[a]	4.31±1.12[a]
Staphylococci	6.74±0.37[a]	6.23±0.40[b]
LAB	5.16±0.88[a]	4.82±0.81[a]
Yeasts and moulds	4.62±1.06[a]	4.66±0.87[a]

AMB, Aerobic mesophilic bacteria; TC, total coliforms; LAB, lactic acid bacteria; RBM, raw beef meat; RGM, raw goat meat; NB: Rows followed by the same letters are not significantly different (P > 0.05).

differences were not observed in the counts of other microbial groups (P >0.05).

DISCUSSION

Idowu and Rowland (2006) reported that in countries like Nigeria, Ghana, Uganda and Botswana, the majority of vendors are women who balance the income-generating opportunities of street vending. On the other hand, Muinde and Kuri (2005) have reported that 60% of the vendors surveyed in Nairobi were male. Although the quality and safety of raw meats sold by males and females was not assessed in our study, however, Ohiokpehai (2003) reported that female vendors sold food of better quality than their male counterparts. Klontz et al. (1995) also reported that in the United States, safer food preparations were consistently reported by persons who were female, at least 40 years old, with at least high school education and experience in the sector. In this survey, the experience and the age is consistent with that indicated by Klontz et al. (1995). However, there were significant percentage of youngsters under the age of 16-25 and inexperienced (0-4 years) vendors had also participated at vending activities in addition, their higher percentage of illiteracy would influence the good handling practice so does the safety of raw meat.

The presence of animals, insects, liquid waste and solid waste in all of food vending areas is similar to a study conducted elsewhere (FAO, 1988). The linkage between houseflies and diarrheal diseases has been also documented (Smith and Rose, 1998).

Reused water would have dissolved organic material in it to serve as a 'culture medium' favoring the growth of array of microorganisms including pathogens (Bryan et al., 1992c). For instance, in Ibadan, Nigeria, water was considered to be the major source of food contamination (Yah et al., 2009).

It has been mentioned that holding foods for more than 4-6 h is one of the main contributing factors of high possible microbial counts (El-Sherbeeny et al., 1985; Bryan et al., 1992a, b, c). Deriba and Mogessie (2001) also indicated that foods that are held at ambient temperatures of 15-45°C for more than about 4 h present a considerable public health risk.

All food handlers have a basic task to maintain a high degree of personal cleanliness and observed hygienic and safe food handling practices. Keeping hands clean, shortening fingernails, wearing clean working garment and hair cover (hair net and cap) are some of the precautions that a food handler must maintain (Kinfe and Abera, 2005). However, none of raw meat street vendors evaluated in our study wore appropriate working garment (over coat).

Jewelries observed especially on vendor's hand were very high (70%) as compared to street food vendors assessed in other areas of Ethiopia such as Mekele (35.7%) and Awassa (28.7%) (Kinfe and Abera, 2005). Thus, the culture might have also its own effect on food safety in relation to jewelries and clothing.

Several studies have shown that skin under rings is more heavily colonized by microorganisms as compared to fingers without rings (Jacobson et al., 1985). Hands are the most important vehicle for the transfer of organisms from faeces, nose, skin or other sites to food (WHO, 1984). Epidemiological studies of *Salmonella typhi, non-typhi salmonellae, Campylobacter* and *Escherichia coli* have demonstrated that these organisms can survive on finger tips and other surfaces for varying periods of time and in some cases after hand washing (Pether and Gilbert, 1971; WHO, 1984). Hands are important agents when it comes to transmitting micro-organisms and intestinal parasites to food. Therefore, they should always be washed before starting work, immediately after using the bathroom, after handling contaminated material or any other material that could possibly transmit diseases, and whenever necessary (Goh et al.,1993). WHO (1984) also indicated that food vendors should wash their hands in hot soapy water before preparing or touching foods and after using bathroom. However, washing hands was not a common practice by raw meat street vendors in Jijiga town. Absence of clean water and washing facilities in the vending environment and lack of awareness of the vendors about food handling and safety might be possible reasons for the poor handling practice of vendors observed in this study. Van-Kampen et al. (1998) reported that the lack of available hand washing facilities and poor knowledge concerning hygiene were correlated with improper food handling practices of street food vendors in Jakarta, Indonesia. On the other hand, a study conducted by Azanza et al. (2005) in Philippines showed that street vendors had good practice of washing hands during handling foods due to the relatively high level of knowledge in hand washing and the availability of a number of hand washing facilities within the area.

Microbiological analysis

These mean pH values (6.03 and 5.98) for beef and goat meat samples respectively might make these products susceptible to bacteria as well as mold and yeast spoilage (Jay, 1996) and could allow the multiplication of several bacterial pathogens (Ferrari and Torres, 2002). Freese et al. (1998) also indicated that pH above 4.4 and 5.0 would promote growth of pathogens.

Food that is not maintained within the safety temperature zone acts as an incubator for pathogenic bacteria whether the food is raw, partially cooked or fully done (Roller, 1999). According to Van Kampen et al. (1998) and Joseph and Doser (1999), time-temperature abuse was considered particularly potentially hazardous and initiate microbial proliferation. Freese et al. (1998) also indicated that storing foods at a temperature range of 15–47°C could promote growth of pathogens.

The mean values of total areobic mesophilic counts were relatively higher than that reported by Okonko et al. (2009) for fresh meats sold in Calabar metropolis, Nigeria which had a mean aerobic mesophilic count of 4 log cfu/g. Comparable results with our study were reported by Kumar et al. (2010) for raw beef meat marketed in some parts of Tigray region as samples had areobic mesophilic counts >7log cfu/g. According to Jay (2005), foods kept at ambient temperature, will stimulates the growth of aerobic mesophilic organisms, including most of the pathogens. Thus, high aerobic mesophilic count recorded in this study might reflect the time temperature abuse during displaying the meats for sale. ICMSF (1980) also indicated that high total bacterial count might be attributed to the contamination of the product from different sources or unsatisfactory processing and it may be due to unsuitable temperature during storage.

Although, there are no standards or guidelines regarding the microbial contamination of street vended raw meat in Ethiopia, HPA (2009) indicated that aerobic mesophilic count must be < 7 log cfu/g for raw meats. However, in this study, the mean counts of raw beef and raw goat meat samples were 8.07 and 7.59 log cfu/g, respectively. These mean values, thus exceeded the typical guideline for aerobic mesophilic count. Total bacterial count is considered an index of quality, which gives an idea about the hygienic measures during processing and helps in the determination of the keeping quality of the product Aberle et al. (2001). Comparable results were also reported by Mukhopadhyay et al. (2009) as most of goat meat and beef meat samples showed aerobic plate counts above 7.00 log cfu/g. Thus, it can be also said that most of the meat samples analyzed in this study were in a condition at which spoilage of meat can occur since they had aerobic mesophilic counts greater than 7log cfu/g (Warriss, 2001).

Comparable Enterobacteriaceae counts were also reported by Khalafalla et al. (1993) for ground beef meat samples. However, the mean values of our samples were higher than that reported by Mehmet and Hilmi (2005) for ground beef samples in Turkey which had mean count of Enterobacteriaceae and coliforms as low as 3log cfu/g. According to Cathy (1997) and HPA (2009) a raw meat is categorized as unacceptable if the count of Enterobacteriaceae and coliforms is > 4log cfu/g. Based on this, it can be said that both species of meat samples were found to be unacceptable as they had counts of these microbial groups >4log cfu/g. The presence of such high counts in the investigated samples could indicate time/temperature abuse during handling or inadequate storage and displaying conditions during sale. As these microbial groups are safety indicators, the presence of high counts may indicate possible presence of pathogens (Jay, 1996).

Staphylococci counts obtained were comparable with results obtained for ground beef by Tekinsen et al. (1980). However, the mean values of our samples were by far greater than that reported for ground meat obtained at retail (2log cfu/g) (Mehmet and Hilmi, 2005). Khalafalla et al. (1993) also reported lower counts of staphylococci (3log cfu/g) for ground beef meat samples. Staphylococci are common in unprocessed animal products and in products handled by bare hands. The high count of staphylococci in our meat samples indicates the presence of cross contamination, which is usually related to human skin, hand touch, discharge from human and clothing because of faulty handling activities, as they are typical contaminants from hands, clothes and utensils (Postgate, 2000).

The presence of such high counts of lactic acid bacteria (LAB) in this study might indicate improper handling of the meats and inadequate storage conditions. Since lactic acid bacteria (LAB) are spoilers (Jay, 2005), the presence of such high counts in the samples may limit their keeping quality.

In contrast with our finding, Selvan et al. (2007) reported that the mean total viable count was significantly greater in goat meat than other products (chicken and beef) studied in Chennai City, India. Another study in India by Mukhopadhyay et al. (2009) also indicated that coliform count was slightly lower in beef than goat meat samples (mean 5.84 and 6.40 log cfu/g). The presence of low microbial counts in raw goat meat samples as compared to raw beef samples in this study can be explained by the relatively short display time of goat meat at retail due to consumer preference for goat meat. In addition to this, trimming and cutting which usually enhance microbial contamination was minimized during sale of goat meat as compared to beef meat. These differences may be explained by personal hygiene, individual difference in awareness and safe food handling practice, displaying period and intrinsic characteristics of the two meat species.

The aerobic mesophilic flora was dominated by staphylococci followed by enterobacteriacae. Deriba and Mogessie (2001) reported that the microflora of ‘kitfo’ a

traditional Ethiopian spiced, minced meat samples collected from street vendors in Addis Ababa were also dominated by various bacterial genera, *Staphylococcus* spp.

Isolation of *Staphylococcus* spp. and Enterobacteriaceae from the street vended meat can be worrying because certain strain of these bacteria cause food-borne infections (Mogessie, 1994). Thus, the raw meat samples investigated were under question from food safety point of view.

Salmonella was isolated from 5 meat samples (8.3%) quite far as compared to the study in Jimma town by Tasew et al. (2010) for minced meat in which rate of *Salmonella* isolation was 2 (1.2%). However, our samples had lower prevalence of salmonella as compared to other findings where rate of isolation from raw meat at retail was 20% in Gaborone, Botswana (Mrema et al., 2006), 9% in raw meat obtained from butchers shop in Awassa, Ethiopia (Mogessie, 1994) and 42% from raw "kitfo" (minced meat) in Addis Ababa (Mezgebu and Mogessie, 1998). The variation in the prevalence of *Salmonella* contamination could be partly due to differences in sample type, sampling techniques, distribution of *Salmonellae* in a lot examined and the detection methods employed.

In general, the majority of raw meats considered in this study had high microbial load and in some cases, even pathogens were isolated. Time/temperature abuse during vending on the street or cross contamination due to improper handling of meat or inappropriate vending practices or a combination of these factors might contribute to the presence of high microbial counts. Furthermore, the absence of clean potable water and receptacles, and also the poor sanitary condition of the vending area revealed inadequacies concerning quality and safety of the meats analyzed in this study. Training and inspections are important. Moreover, provision of basic infrastructures and establishment of code of practice for the sector are also recommended.

Conflict of interest

The author(s) have not declared any conflict of interests.

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