Acta Tropica 130 (2014) 1–6

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Confirmed low prevalence of *Listeria* mastitis in she-camel milk delivers a safe, alternative milk for human consumption

Kamelia M. Osman[a,*], Ahmed Samir[a], Ahmed Orabi[a], Tara Rava Zolnikov[b]

[a] Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, P.O. Box 12211, Cairo, Egypt
[b] North Dakota State University, Developmental Science, Fargo, ND 58102, United States

ARTICLE INFO

Article history:
Received 29 July 2013
Received in revised form 4 August 2013
Accepted 1 October 2013
Available online xxx

Keywords:
Listeria species
She-camel
Mastitis
Virulence genes
Biofilm formation
Antibiotic resistance

ABSTRACT

She-camel milk is an alternative solution for people allergic to milk; unfortunately, potential harmful bacteria have not been tested in she-camel milk. *Listeria monocytogenes* is one harmful bacterium that causes adverse health effects if chronically or acutely ingested by humans. The purpose of this study was to estimate the prevalence, characterize the phenotypic, genetic characterization, virulence factors, and antibiopotential harmful bacteria resistance profile of *Listeria* isolated from the milk of she-camel. Udder milk samples were collected from 100 she-camels and screened for mastitis using the California mastitis test (46 healthy female camels, 24 subclinical mastitic animals and 30 clinical mastitic animals). Samples were then examined for the presence of pathogenic *Listeria* spp; if located, the isolation of *Listeria* was completed using the International Organization for Standards technique to test for pathogenicity. The isolates were subjected to PCR assay for virulence-associated genes. *Listeria* spp. were isolated from 4% of samples and only 1.0% was confirmed as *L. monocytogenes*. The results of this study provide evidence for the low prevalence of intramammary *Listeria* infection; additionally, this study concludes she-camel milk in healthy camels milked and harvested in proper hygienic conditions may be used as alternative milk for human consumption.

1. Introduction

The camel (*C. dromedarius*) plays an important subsistence role in arid and semi- arid regions of Africa and the Middle East (FO et al., 2012). Camels provide milk containing essential nutrients to human populations in these regions (FO et al., 2012). Camel milk is primarily consumed for nutrient value in a raw and unpasteurized form, but may also be used for therapeutic purposes. In the Middle East and India, camel milk has been used to counter the effects of type 1 diabetes, milk allergies, Crohn's disease, autism, dropsy, jaundice, chronic hepatitis, hepatitis C virus (HCV), tuberculosis, asthma, anemia, lung and spleen-related ailments, and piles; additionally, camel milk-based creams decrease inflammation on dermatological autoimmune diseases (Yagil, 2013). Traditionally, camel milk may be directly incorporated into diets at an early age due to the belief that milk promotes bone formation in infants and children. Another common belief and practice among the Bedouin of the Sinai Peninsula focuses on the healing properties of camel milk consumption toward any internal disease (Yagil, 2011). The indispensable roles fulfilled by camel milk emphasize the importance of its undeniable existence; camel milk continues to be used for dietary purposes and traditional or medicinal usage in native populations throughout the world.

Ingestion of contaminated milk is one of the major routes of infection by the human pathogen *Listeria monocytogenes* (Melo et al., 2013). *Listeria monocytogenes* causes cerebral listeriosis, a life-threatening disease in both animals and humans (Al-Swailem et al., 2010; Kasalica et al., 2011). Studies confirming *Listeria* infection in camel milk neither currently exist nor have been previously conducted. This study sought to estimate the prevalence, characterize the in vitro and in vivo pathogenicity, virulence-associated genes (*hly*A, *plc*B, *act*A and *iap*) and antibiotic resistance profile of *L. monocytogenes* and other *Listeria* spp. isolated from the milk of she-camel (Figs. 1 and 2).

The study took place in private dairy farms surrounding Cairo, Egypt. 100 she-camel milk samples were collected from visibly normal mammary glands. The animals had not been treated with an antibiotic for at least 30 days prior to collection. Milk samples were screened for mastitis using the California mastitis test (CMT) prior to isolation of *Listeria* spp. Udders and teats of the randomly selected, lactating animals were cleaned, dried, and disinfected with 70% ethanol before sample collection. Three streams of foremilk was expressed from each quarter and then discarded. During sample collection, 10–15 ml of milk from each quarter was manually expressed into separate sterile 25 ml universal tubes. After gently suspending each sample, the milk was poured into

* Corresponding author. Tel.: +20 233854762; fax: +20 233854762.
E-mail address: kamelia-osman@hotmail.com (K.M. Osman).

0001-706X/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.actatropica.2013.10.001

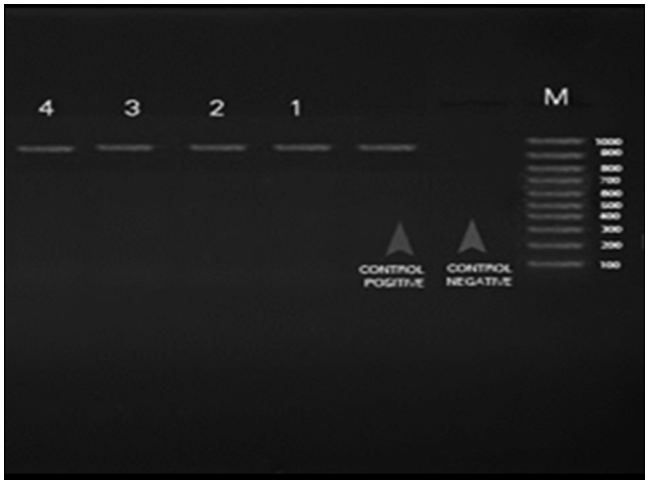

Fig. 1. 165 rDNA-RFLP profiles obtained by 3% agarose gel electrophoresis of digests of the amplified 165 rDNA after restriction with *Alul*. M: DNA Marker 100 bp (100–1500 bp); Control negative: *E. coli* strain ATCC 25922; Control positive: *L. monocytogenes* ATCC 7494 (bp 938); Lane 1: *L. monocytogenes* (bp 938); Lanes 2 and 3: *L .welshimeri* (2 isolates) (bp 938); Lane 4: *L. seelegeri* (bp 938).

a separate, unused container. As indicated by the Forsbäck et al. (2010) a composite milk sample representing one udder was created; this sample was comprised of all four quarters in one collection vial. A subsample of 15 ml of milk, taken from the composite milk sample, was transferred to sterile universal bottles. The milk samples were quickly transported to the laboratory under chilled conditions and stored at 4 °C until bacteriologically analyzed.

For the isolation and identification of *Listeria* species in the milk samples, the techniques recommended by the International Organization for Standards (ISO) (ISO 11290-1, 1996) were implemented. ISO steps included primary and secondary selective enrichment. Primary selective enrichment involved 500 ml of Half Fraser Enrichment Broth Antibiotic supplement CCFA (Oxoid; CM0895B and SR0166E) mixed with 25 ml of each milk sample and incubated at 30 °C for 48 h. The pre-enrichment culture (half Fraser broth), 0.1 ml was then transferred into 10 ml of Fraser broth (Oxoid; CM0895B and SR0156E) and incubated at 35 °C for 48 h; this step concluded secondary selective enrichment of the *Listeria* species. Culture and strain characterization sequentially followed the enrichment phases. A loopful of the culture obtained from the Fraser brother was streaked onto PALCAM agar plates (Oxoid; CM0877B and SR0156E) and incubated at 37 °C for 24 to 48 h.

Identification of *Listeria* species on PALCAM agar plates was based on aesculin hydrolysis and mannitol fermentation. All *Listeria* species hydrolyse aesculin were visually confirmed by a blackening of the medium. Mannitol fermentation was demonstrated by a color change in the colony and/or surrounding medium from red or gray to yellow due to the production of acidic end products. The selectivity of the PALCAM medium was achieved through the presence of lithium chloride, polymixin B sulphate and acriflavine hydrochloride present in the medium base and ceftazidime provided by PALCAM antimicrobial supplement. These agents effectively suppress growth of most commonly occurring non-*Listeria* bacteria species present in typical food samples.

Colonies suspected to be *Listeria* were transferred onto pre-dried plates of trytic soya yeast extract agar (TSYEA) (Difco, Bacton, USA) and incubated at 30 °C for 18–24 h. Those putative *Listeria* colonies were characterized using Gram's staining, tumbling motility at 20–25 °C, catalase test, methyl red–Voges Proskauer (MR–VP) reactions, characteristics of haemolysis on 5% sheep blood agar (SBA), carbohydrate utilization and CAMP test. The CAMP test was undertaken using *L. monocytogenes* (ATCC 7494), *S. aureus* (ATCC 7494) and *Rhodococcus equi* (ATCC 6939) and *E. coli* (ATCC 25922). For the carbohydrate utilization test, isolated colonies from TSYEA were transferred into test tubes containing xylose, rhamnose and mannitol and incubated at 37 °C for up to 5 days. At the same time, strains were identified using the API® *Listeria* system (bioMe′rieux, Marcy l'Etoile, France) and the Oxoid Microbact™ *Listeria* 12L (MB1128A) The Microbact™ *Listeria* 12L system was intended to be used for the identification of *Listeria* spp. isolated from the milk samples.

All the biochemically characterized *Listeria* isolates were assayed for PI-PLC activity as described by Notermans et al. (1991a). The plates were covered with the substrate 1% l-α-phosphatidylinositol (Sigma) in 0.7% agarose and observed for 6 days; at this time, development of turbid haloes (the insoluble diacylglycerol) around the colonies were identified.

The DL-alanine-β-naphthylamide HCl (DLABN) and Dalanine-*p*-nitroanilide (DAPN) tests were performed as described by Clark and McLauchlin (1997) in order to differentiate *L. monocytogenes* from other species of *Listeria*. Free β-naphthylamine was detected by observing the deep yellow-orange complex, which developed within 5 min. Hydrolysis of the DAPN was visible by the liberation of the intense yellow *p*-nitrophenol.

Serotyping was carried out on *L. monocytogenes* strains using commercial specific antisera (Behringwerke AG) against the serovars 1 and 4, following the manufacturer's instructions.

Four identified *Listeria* species (*L. monocytogenes L. seelegeriat* and *L. welshimeri*) were tested by disk diffusion (Oxoid Ltd., Basingstoke, UK) for antimicrobial susceptibility tests. Antimicrobials were selected for testing based on the licensing for mastitis treatment in cattle, use in human medicine and potential resistant-determinant phenotypes located on genetic mobile elements (TET and ERY) (FAO/WHO/OIE, 2008; WHO, 2009). Resistance was

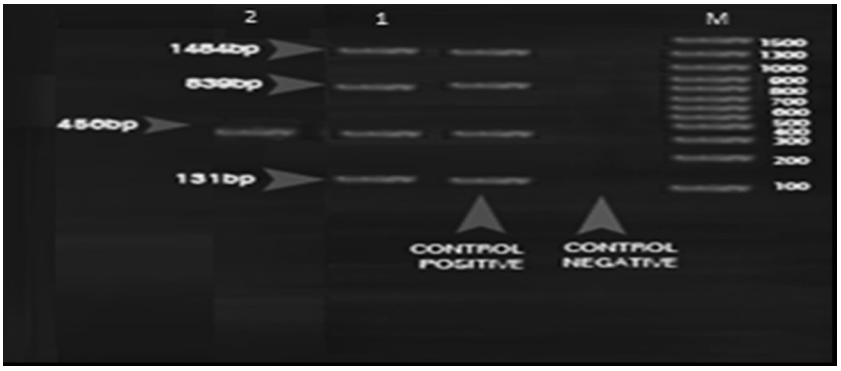

Fig. 2. Multiplex PCR of four virulence associated genes for standard *L. monocytogenes* (ATCC 7494) and isolated *Listeria* species. M: DNA Marker (100 to 1500 bp); Control negative: *E. coli* strain ATCC 25922; Control positive: amplified products of four virulence associated genes of standard *L. monocytogenes* (ATCC 7494): *plc*B gene (bp 1484); *act*A gene (bp 839); *hly*A gene (bp 456); *iap* gene (bp 131); Lane 1: amplified products of four virulence associated genes of the isolated *L. monocytogenes*; Lane 2: amplified products of one virulence associated gene *hly*A gene (bp 456) of the isolated *L. seelegeri*.