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Mexican unpasteurised fresh cheeses are contaminated with *Salmonella* spp., non-O157 Shiga toxin producing *Escherichia coli* and potential uropathogenic *E. coli* strains: A public health risk

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ABSTRACT

Fresh cheeses are a main garnish of Mexican food. Consumption of artisanal fresh cheeses is very common and most of them are made from unpasteurised cow milk. A total of 52 fresh unpasteurised cheeses of five different types were purchased from a variety of suppliers from Tabasco, Mexico. Using the most probable number method, 67% and 63% of samples were positive for faecal coliforms and *E. coli*, respectively; revealing their low microbiological quality. General hygienic conditions and practices of traditional cheese manufacturers were poor; most establishments had unclean cement floors, all lacked windows and doors screens, and none of the food-handlers wore aprons, surgical masks or bouffant caps. After analysing all *E. coli* isolates (121 strains) for the presence of 26 virulence genes, results showed that 9 (17%) samples were contaminated with diarrheagenic *E. coli* strains, 8 harboured non-O157 Shiga toxin producing *E. coli* (STEC), and one sample contained both STEC and diffusely adherent *E. coli* strains. All STEC strains carried the *stx*1 gene. Potential uropathogenic *E. coli* (UPEC) strains were isolated from 15 (29%) samples; the most frequent gene combination was *fim*A-*agn*43. Two samples were contaminated with *Salmonella*. The results demonstrated that unpasteurised fresh cheeses produced in Tabasco are of poor microbiological quality and may frequently harbour foodborne pathogens. Food safety authorities in Mexico need to conduct more rigorous surveillance of fresh cheeses. Furthermore, simple and inexpensive measures as establishing programs emphasizing good hand milking practices and hygienic manufacturing procedures may have a major effect on improving the microbiological quality of these food items.

1. Introduction

Foodborne diseases are a major public health problem worldwide and carry a large burden of disease. In 2013, a total of 818 foodborne outbreaks occurred in the United States (US) resulting in 13,360 illnesses, 1062 hospitalizations, and 16 deaths (CDC, 2013). Dairy products were implicated in 10% of these outbreaks. Moreover, from 1998 to 2011, a total of 90 outbreaks were associated to cheese consumption. Thirty-eight of these were linked to cheese made from unpasteurised milk, and 40% of them, to soft cheeses imported from Mexico (Gould et al., 2014). Few cheeseborne outbreaks have been reported in Mexico, likely due to the lack of an active surveillance system for foodborne outbreaks. Parrilla-Cerrillo et al. (1993), conducted a 9-year retrospective study of foodborne outbreaks in Mexico. Seventeen (29%) of the 58 reported outbreaks were associated with cheese consumption.

Microbial contamination can occur at any stage from cheese production to consumption, rendering cheese an important vehicle of foodborne illness, including diarrhoeal diseases, brucellosis and listeriosis (Cody et al., 1999; Farina et al., 2008; Koch et al., 2010; MacDonald et al., 2005; Méndez Martínez et al., 2003). *Salmonella* spp., *Campylobacter*, *Brucella* and diarrheagenic *Escherichia coli* (DEC), including *E. coli* O157:H7, are the most frequent pathogens associated with cheese outbreaks worldwide (Cody et al., 1999; Dominguez et al., 2009; Espie et al., 2006; Gould et al., 2014; Honish et al., 2005).

DEC groups are generally associated with mild to severe diarrhoea in humans. In addition to diarrhoeal illness, Shiga toxin producing *E. coli* (STEC) such as *E. coli* O157:H7, non-O157 serotypes and *E. coli* O104:H4, a hybrid DEC strain, can cause severe manifestations such as haemorrhagic colitis and haemolytic uremic syndrome (HUS) (Frank et al., 2011). DEC have been classified into several groups based on clinical, epidemiological and virulence traits: enterotoxigenic *E. coli* (ETEC), typical and atypical enteropathogenic *E. coli* (tEPEC, aEPEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli*

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(DAEC) and STEC; the latter group also encompasses the enterohaemorrhagic *E. coli* (EHEC) subgroup (Table 1) (Kaper et al., 2004; Nataro and Kaper, 1998; Patzi-Vargas et al., 2015).

As in most countries worldwide, DECs are not routinely included in the microbiological analyses of human clinical and food samples in Mexico, despite the fact that these pathogens are the main causal agents of diarrhoea in children less than five years of age (Patzi-Vargas et al., 2015). Furthermore, non-O157 STEC and atypical EPEC (aEPEC) are the most prevalent DEC groups isolated from food and beverages in our country (Canizalez-Roman et al., 2013; Cerna-Cortes et al., 2012a, 2012b; Lopez-Saucedo et al., 2010).

Extraintestinal *E. coli* (ExPEC) is another *E. coli* group that commonly causes serious illness and death worldwide. Urinary tract infection (UTI) is the most common illness caused by ExPEC (Foxman, 2014). Most UTIs can be attributed to a highly heterogeneous group of ExPEC known as uropathogenic *E. coli* (UPEC). UPEC harbour specific virulence factors that permit their successful transition from the intestine to the urinary tract (Flores-Mireles et al., 2015). These include fimbrial adhesins and iron acquisition systems that allow the establishment of infection in the bladder or kidney, toxins that cause damage to host cells, and biofilm formation and bacterial aggregates that contribute to UPEC persistence (Anderson et al., 2003, 2010; Flores-Mireles et al., 2015; Travis et al., 2008). In recent years, investigators have hypothesized that food plays a major role in the transmission of ExPEC strains (Aslam et al., 2014; Nordstrom et al., 2013; Vincent et al., 2010). However, their detection in food items remains difficult as there are no specific markers for UPEC, and isolation of the strain from the urine of a symptomatic patient remains the gold standard for identification.

Due to the paucity of data on the microbial contamination of cheese in Mexico, we conducted the current study with the following aims: 1) to evaluate the overall microbiological quality and prevalence of *E. coli* and *Salmonella* in fresh cheeses produced in the state of Tabasco and 2) to identify the prevalence of diarrheagenic *E. coli* groups and potential uropathogenic *E. coli* strains.

2. Materials and methods

2.1. Location

Cheese samples were collected from 17 municipalities in Tabasco, a state located in southeast Mexico. Tabasco has a hot and humid tropical weather, with temperatures that reach up to 36 °C and 90% humidity (http://www.cuentame.inegi.org.mx/monografias/informacion/tab/territorio/clima.aspx?tema=me&e=27).

2.2. Sample collection

Between February and June 2011 a total of 52 local fresh cheeses were purchased from municipal markets, convenience stores, traditional cheese manufacturers and supermarkets. We recorded if cheeses were stored in refrigeration or at room temperature. All samples were transported in iceboxes to the laboratory and processed the same day; pH was measured on arrival with a pH meter (HANNA, Rhode Island, US). Four traditional cheese manufacturers (local cheese production establishments) were visited to evaluate general hygienic conditions and practices.

2.3. Cheese characteristics

Five different types of artisanal local fresh cheeses that are manufactured under similar conditions were analysed: 1) "Queso Fresco", a high-moisture creamy cheese with a salty taste, 2) "Queso Crema", a soft cheese with a relatively high salt content, 3) "Queso Doble Crema", a soft creamy cheese, 4) "Queso Panela", a cheese with high moisture content and 5) "Queso Poro", a cheese variety with a sour salty taste that is limited to the state of Tabasco.

2.4. Microbiological analyses

2.4.1. Presence of faecal coliforms and E. coli

Each sample was tested for the presence of faecal coliforms (FC) and *E. coli* using the Food and Drug Administration most-probable number (MPN) method (FDA, 2002). Briefly, 10 g of cheese were added to 90 mL of a sterile phosphate-buffered saline and homogenized at low speed in a sterile blender for 1 min. One millilitre of this homogenate was used for preparing serial dilutions (10^{-2}, 10^{-3}) in 9 mL of sterile phosphate-buffered saline. One millilitre of each dilution was inoculated into lactose broth and fermentation tubes in triplicate. After incubation at 37 °C for 48 h, a loopful of suspension from positive cultures (determined by turbidity and gas production) was transferred to tubes containing *Escherichia coli* broth (EC) and incubated at 44.5 °C ± 0.2 °C/48 h. Tubes with turbidity (growth) and gas production were considered positive for FC. Positive EC broths were streaked onto Eosin Methylene Blue Agar (EMB) and incubated at 37 °C/48 h. Metallic green colonies were selected and biochemically characterised by the IMViC test for *E. coli* confirmation. All confirmed *E. coli* colonies were further characterised for the presence of genes that define each DEC group, DEC toxins, and UPEC virulence genes (VG), as described below.

2.4.2. Salmonella

Salmonella was identified by procedures specified in both the Mexican Official Standard Guidelines (Secretaria de Salud, 2010) and the FDA Bacteriological Analytical Manual (FDA, 2014). Briefly, 25 g of the cheese samples were added to 225 mL of buffered peptone water and homogenized in a sterile blender for 1 min. The homogenized samples were incubated at 37 °C for 24 h. After incubation, 1 mL of each sample was transferred to 10 mL of both selenite broth and tetrathionate broth, respectively. After incubation for another 24 h, a loopful of each broth was streaked onto Hektoen enteric, *Salmonella-Shigella*, and xylose lysine desoxycholate agars and incubated at 37 °C for 24 h. Three to five typical *Salmonella* colonies were randomly chosen from each selective medium and identified using urea broth, lysine iron agar and triple sugar iron agar. *Salmonella* spp. were identified by serological analysis

Table 1
Phenotypic and genotypic characteristics used to identify diarrheagenic *Escherichia coli* groups (DEC).

DEC	Defining characteristic(s)	Target genes
tEPEC	Presence of both intimin (as a marker of the pathogenic island LEE) and the BFP contained in the EAF plasmid	*eae*A, *bfp*A
aEPEC	Presence of intimin (as a marker of LEE); absence of the EAF plasmid and Shiga toxins 1 and 2	*eae*A
ETEC	Presence of thermo labile (LT) or/and thermo stable (ST) toxins	*lt*, *st*
EIEC	Presence of the invasion-associated locus (IAL) of the invasion plasmid	*ial*
STEC	Presence of Shiga toxin 1 (STX1) and/or 2 (STX2)	*stx*1, *stx*2
STEC/EHEC	Presence of Shiga toxin 1 (STX1) and/or 2 (STX2); plus presence of intimin (as a marker of LEE)	*stx*1, *stx*2, *eae*A
EAEC	Presence of AggR master regulon most genes associated with the aggregative adherence (AA) and EAEC virulence are controlled by this regulon.	*agg*R
DAEC	Presence of surface afimbrial adhesins as AfaE-I and AfaE-III, that are encoded on the Afa/dr/daa operon	*afa*C

DEC: diarrheagenic *Escherichia coli* groups tEPEC: typical enteropathogenic *E. coli*, aEPEC: atypical enteropathogenic *E. coli*, ETEC: enterotoxigenic *E. coli*, EIEC: enteroinvasive *E. coli*, STEC: Shiga toxin producing *E. coli*, EHEC: enterohaemorrhagic *E. coli*, EAEC: enteroaggregative *E. coli*, DAEC: diffusely adherent *E. coli*, LEE: locus of enterocyte effacement, BFP: bundle-forming pilus, EAF: EPEC adherence factor plasmid, *eae*A and *afa*C: genes encoding for intimin and Afa adhesin usher, respectively.