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Short communication

Escherichia coli hyperepidemic clone ST410-A harboring $bla_{CTX\text{-}M\text{-}15}$ isolated from fresh vegetables in a municipal market in Quito-Ecuador

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ABSTRACT

Dissemination of Extended spectrum β-lactamases (ESBL) Enterobacteriaceae is a major medical threat. Vegetables and fruits, which are usually consumed raw, are a very suitable pathway for the spread of these bacteria from farm-to-fork. However, limited information exists regarding resistant bacteria and epidemic clones that are disseminated in vegetables and tap water in South America. We processed a total of 90 samples in triplicate of nine typically consumed raw vegetables from a central municipal market, and tap water samples were processed from twenty-one locations in Quito, Ecuador. The samples were analyzed for total coliforms and ESBL Enterobacteriaceae contamination using the dilution filtration method. ESBL *Escherichia coli* isolates were phenotypically and genotypically characterized. The water was free of Enterobacteriaceae, but all the vegetables and fruits (except for blackberries) presented total coliform counts. Watercress had the highest load of total coliforms (3.3 × 10E4). ESBL *E. coli* was detected in alfalfa, leaf lettuce and parsley/cilantro samples. Alfalfa had the highest load of ESBL *E. coli*/total coliforms (1/3.3 × 10E2). We identified *E. coli* ST44-A and ST410-A harboring $bla_{CTX\text{-}M\text{-}15}$ downstream of *ISEcp1*. Alfalfa and parsley/cilantro were contaminated with hyperepidemic *E. coli* ST410-A, which was resistant to quinolones and harbored $bla_{CTX\text{-}M\text{-}15}$. For the first time, we report ESBL *E. coli* ST410-A from vegetables and express an alert regarding the health risk this could represent.

1. Introduction

Rapid dissemination of Enterobacteriaceae producing Extended-Spectrum β-lactamases (ESBL) is a major medical threat. This is mainly because these strains increase the therapeutic use of last-resort antimicrobials such as carbapenems, tigecycline and colistin. Therefore, antimicrobial resistance increases, and new mechanisms emerge (Iovleva and Doi, 2017; Srinivas and Rivard, 2017; Talaga-Ćwiertnia et al., 2017). The most frequent plasmid mediated ESBL include CTX-M, TEM, SHV and OXA types, and all of them are reported in clinical and environmental isolates of gram-negative bacilli. However, the CTX-M family is the most important enzymatic mechanism of resistance to third generation cephalosporins (3GC) in Enterobacteriaceae (Cantón et al., 2012).

To date, > 170 variants of $bla_{CTX\text{-}M}$ have been reported (http://www.lahey.org; last accessed October 2017) belonging to five phylogenetic groups (CTX-M-1, 2, 9, 8 and 25) based on their amino acid sequences (Poirel et al., 2008). Worldwide, $bla_{CTX\text{-}M\text{-}15}$, which is classified into the CTX-M-1 group, is most frequently reported in *Escherichia coli* isolated from clinical and environmental settings. The successful dissemination and establishment of this variant is related to its association with the pandemic clone known as ST131, transposition element IS*Ecp1* and its location in conjugative plasmids (Mathers et al., 2015).

Additionally, regarding the clinical location of *E. coli* producing CTX-M-15, an increase in reports of *E. coli* harboring the $bla_{CTX\text{-}M\text{-}15}$ gene isolated from the environment and food have exposed a complex model of dissemination and variety of reservoirs of this resistance mechanism (Randall et al., 2016; Röderova et al., 2017). The principal risk for public health is the evolution and dissemination of multidrug resistant hyperepidemic clones with capacity for spread from environment and animals to humans where cause difficult treatment infections (Müller et al., 2016; Schaufler et al., 2017). In this context, fresh vegetables are especially important because their production process, i.e., the contact with manure or sewage irrigation contaminated water, may lead to their contamination with resistant *E. coli* (Araújo et al., 2017).

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Moreover, these products are commonly consumed raw, which results in a very suitable vehicle for human gut colonization [9].

To establish the contribution of vegetables and fruits in the dissemination of ESBL Enterobacteriaceae, identification of the pathways of bacterial spread to humans from farm-to-fork is needed. However, few reports of ESBL coliforms in raw vegetables exist, and to the best of our knowledge, there are no reports from South America in the indexed literature. Therefore, the aim of this pilot study was to detect ESBL coliforms on fresh vegetables commercialized at a central municipal market (CMM) in Quito, Ecuador.

2. Materials and methods

2.1. Study design

Quito is in the Andean Region of Ecuador (0°13′07″S and 78°30′35″W) and located at an altitude of 2810 m. Agricultural products that supply the city come mostly from the surrounding valleys and highlands and from the coastal region of the country. Because municipal markets are the main distribution system of vegetables and fruits for retail stores, we decided to sample from a CMM in Quito. To detect coliform contamination, we selected nine fresh vegetables and fruits that are mostly consumed raw in salads and juices: iceberg lettuce, leaf lettuce, watercress, alfalfa, parsley/cilantro, green pepper, tomato, strawberry, and blackberry. Additionally, tap water is the main source of water used to wash and prepare food. Therefore, we considered the coliform evaluation of tap water for possible source of contamination.

2.2. Sampling

During samples acquisition, we also asked sellers about the farming source of the products. Samplings of vegetables and fruits were conducted at three-time periods: June, July and August 2015. We collected approximately 500 g of vegetables and fruits three times each product (in the first week of each sampling months) to a total of 27 samples and triplicate water samples from twenty-one locations around the city were obtained during August 2015. A detailed map showing the selected sampling sites was constructed in ArcGIS software using waypoints registered with a GPS eTrex 10 (Garmin) over a set of Pleiades high-resolution satellite images (ortho, RGB, 2012–2013, and pixel 0.5 × 0.5 m); another complementary map shows the general location and was generated using Google Maps with open data (https://www.google.com.ec/maps) (Fig. 1).

2.3. Quantification and phenotypic characterization

Vegetable and fruit samples were acquired and immediately packaged aseptically in sterile polythene zip bags, and water samples were collected in sterile plastic flasks. The samples were transported in a cold box for analysis within 2–3 h. Forty grams of the edible parts of each sample were selected to simulate salad or juice preparation; the samples were not washed, and the inedible or damaged parts were removed using a sterile knife. The selected parts were suspended in 460 mL of peptone water and shaken for 30 min at room temperature before coliform analysis.

Quantitative evaluation of total and 3GC resistant coliforms was conducted by a filtration method on McConkey plates. Briefly, 10 µL, 100 µL, 1 mL and 10 mL of each sample was mixed with 100 mL of saline water and filtered through a 50 µm filter (Millipore). The membranes were deposited on McConkey plates without antimicrobial selection for counts of total coliforms and deposited on plates supplemented with cefotaxime (5 µg/mL) for isolation of ESBL coliforms. The plates were incubated at 37 °C for 24 h. Enumeration was manually conducted. All coliform-like colonies were enumerated to establish total and 3GC resistant coliform loads. Fisher's exact test was used to identify

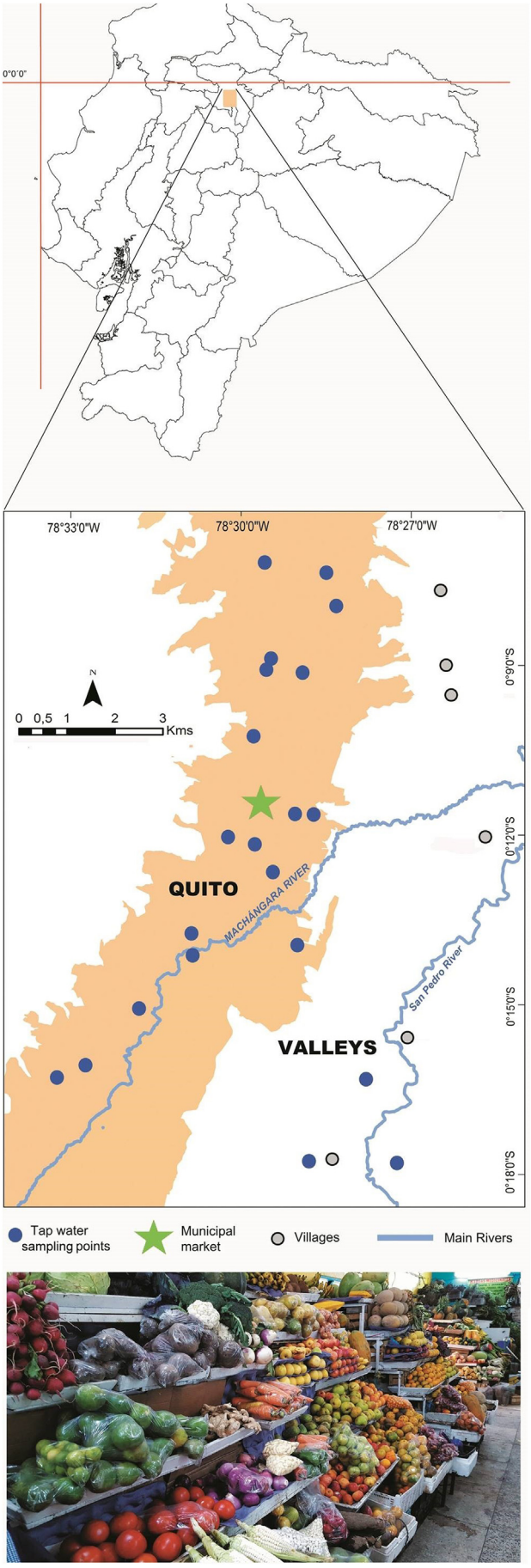

Fig. 1. Study area and location of sampling points.