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Understanding the routes of contamination of ready-to-eat vegetables in the Middle East

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ABSTRACT

In the developing countries, inaccessibility to safe water, lack of agricultural infrastructures and limitations to implementing good agricultural practices (GAP) are persistent challenges. To understand the spread of hazards and identify critical areas of transmission in the food chain, a total of 90 samples of raw salad vegetables (parsley, lettuce, radish) were collected from farms and post-harvest washing facilities (n = 12) in an extensively cultivated area in Lebanon, the Bekaa Valley and from wholesale market stalls traced back to surveyed fields. Our results showed high geometric mean indicator levels ranging from <0.7 to 7 log CFU/g (*Escherichia coli*), 1.69–8.16 log CFU/g (total coliforms), <0.7–8.39 log CFU/g (*Staphylococcus aureus*). The mean counts of total coliforms and *E. coli* on fresh produce followed an increasing trend from fields to the markets indicating potential sources of faecal contamination throughout the food chain. Of more concern was the presence of pathogens *Listeria monocytogenes* (14%) and *S. aureus* (45.5%) in fresh produce from harvest to retail, and *Salmonella* spp. was detected in 6.7% of the raw vegetables from the post-harvest washing areas. These results along with our observations highlight shortfalls in hygienic farming and postharvest practices, including the use of inappropriately treated manure and chicken litter to fertilize the crops on the fields which contributed to the high levels of *S. aureus* in the product at retail. Unregulated use of wash water, inadequate transportation and storage conditions with risks of cross contamination was also identified. Suggested control measures should mitigate the risks at the source and put emphasis on developing strict policies on monitoring the safety of water sources and on the application of the good agricultural and hygienic practices (GAP, GHP) on primary production stages, washing, transportation and storage at retail.

1. Introduction

In the Middle East, many types of vegetables are eaten raw in salads or used as garnishes in appetizers and traditional dishes, and also increasingly because of their perceived healthy attributes. Yet, they have been in recent years a major contributor to foodborne illnesses in other parts of the world (Callejón et al., 2015; Lynch, Tauxe, & Hedberg, 2009; Painter et al., 2013). In the United States (U.S.), leafy greens were identified at the top of the 10 riskiest foods regulated by the Food and Drug Administration (FDA) accounting for almost 40% of foodborne outbreaks based on data derived from the Centers for Disease Control and Prevention (CDC) (CSPI, 2009). Pathogens identified as hazards on fresh vegetables include *Shigella* spp., *Listeria monocytogenes*, *Staphylococcus aureus, Aeromonas hydrophila* and the spore-formers *Bacillus cereus*, *Clostridium botulinum* and *C. perfringens.* However, the ones implicated in most outbreaks involving fresh fruits and vegetables are *Salmonella*, *Escherichia coli* O157:H7 (Buck, Walcott, & Beuchat, 2003; European Commission, 2002) with reported doses as low as 10 cells and 2–2000 cells, respectively (Harris et al., 2003; Kisluk, Hoover, Kneil, & Yaron, 2012). Norovirus is also among the pathogens of greatest concern that are associated with fresh produce outbreaks (Todd & Greig, 2015) and the high likelihood of inflicting illnesses is attributed to its low infectious doses10-100 viral particles as reported by D'Souza and Su (2010) and Barrabeig et al. (2010). The reportedly held rationale that increased consumption of fresh vegetables is actually the reason for the in-

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creased foodborne illnesses has been challenged in a American Society for Microbiology (2008) report stating that the proportion of outbreaks due to leafy greens has increased beyond what can be explained by increased consumption. This leads us to focus on the primary production stages on farms and subsequent processing as the main contamination sources, although no doubt coupled with enhanced epidemiological and surveillance programs (CSPI, 2009) and the expanded interaction of the local and international markets of fresh produce.

Perishable fruits and vegetables are now transported long distances from growing to retail markets with a wide product distribution range to meet consumer demand. Thus, any associated illnesses could be widely dispersed within or beyond national borders, requiring sophisticated surveillance tools like PulseNet to identify these, while traceability to origin remains a challenge in such an extended supply chain (Sivapalasingam, Friedman, Cohen, & Tauxe, 2004). This may be beyond the resources of many developing countries including those in MENA (Middle East North Africa), where illnesses related to leafy greens may be underestimated or rarely reported. In this Region prompt concerted research efforts to understand, prevent and control risks of illnesses arising from consumption of contaminated salad vegetables and fruits are lagging behind those in other regions. Throughout the farm to fork continuum, fresh produce is subjected to numerous opportunities for microbial contamination due to a range of handling, processing, storage and transportation activities which in the event of unfavorable conditions may lead to the presence of microbial hazards (Gil et al., 2015).

Water is recognized as one of the most important vectors of enteric human pathogens on vegetable crops (Park et al., 2012), This is exacerbated by the fact that water scarcity impacts the quality of the water used for irrigation coming from uncertain sources which may harbor pathogens (Leifert, Ball, Volakakis, & Cooper, 2008). Facing multiple challenges, i.e., political, economic, climate change, unfortunately many developing countries are increasingly reverting to the use of untreated waste water for irrigation and processing of vegetables (Aiat Melloul & Hassani, 1999; AL-Jaboobi, Tijane, EL-Ariqi, & Bouksaim, 2013; Castro-Rosas et al., 2012; De Bon, Parrot, & Moustier, 2010; Ensink, Mahmood, & Dalsgaard, 2007; Thurston-Enriquez et al., 2002). One example for this is the produce industry in Lebanon, where agricultural production is concentrated in the Bekaa Valley, both the most cultivated area and the most affected by water pollution (Halablab, Sheet, & Holail, 2011; Jurdi, 1992). Almost 146 farms use the surface water of the Litani River to irrigate various vegetables as reported in 2011 in local news (retrieved from http://english.al-akhbar.com/node/2617). This river is frequently polluted by untreated sewage, domestic solid waste, and industrial effluents (Houri & El Jeblawi, 2007) and as result, leafy greens in that area have been found to pose health risks to consumers (Halablab et al., 2011). In addition, export potential for produce may be increasingly at risk because importing countries are demanding higher standards. Despite the fact that risks of foodborne illness are likely to be higher in the developing countries of the MENA regions where the waste water treatment is still underdeveloped and use of untreated water for irrigation is illegal, most research on the microbiological safety of fresh vegetables and fruits has been carried out in developed nations (Allen et al., 2013; Johnston et al., 2005; Lehto, Kuisma, Määttä, Kymäläinen, & Mäki, 2011; Seow, Ágoston, Phua, & Yuk, 2012; Wood, Chen, Friesen, Delaquis, & Allen, 2015). Certainly, very little has been done in Lebanon (Halablab et al., 2011; Khatib, Olama, & Khawaja, 2015), may be because the surveillance data for foodborne illness is lacking, and partly because of lack priority for research funding. There can be no doubt that foodborne infections originating from contaminated fruits and leafy green vegetables do occur in the MENA region including Lebanon, based on surveillance data from other regions since they are frequently eaten at most meals (EFSA, 2014; European Commission, 2002; Painter et al., 2013).

To address this lack of understanding of what and how microorganisms of concern are transmitted across the food chain, we conducted a study of risk factors contributing to microbial contamination of vegetables eaten raw, represented by flat leaf parsley (*Petroselinum crispum*. var. *neapolitanum*), romaine lettuce (*Lactuca sativa* L. var. *longifolia*), and small red radish (*Raphanus sativus*) from farms in the Bekaa Valley, Lebanon, to the central market of fresh vegetables in Beirut, and recommended mitigation strategies.

2. Materials and methods

2.1. Study design and sample collection

Sampling sites comprised 10 major farms in the Bekaa Valley, 2 crop washing facilities and the wholesale market in Beirut which receives most of farmers' crops and a major supply point of fresh raw vegetables for supermarkets, distributors, groceries and restaurants in Beirut. Target commodities included leafy greens and radish.

The study was planned to obtain samples from different points of the chain to reflect the farm-to-retail contamination and microbial growth potential.

Table 1 shows samples distribution across different sampling locations.

Samples of lettuce, parsley and radish ($n = 90$) were collected in July–August 2013 and July 2014, a relatively hot and dry season in the Bekaa. A whole head of lettuce, and a bundle of parsley or radishes was considered as one sample; sampling of each type was done from different points of the same field. Water samples ($n = 5$ of 1 L-samples each collected in 250 ml portions from different points of the crop washing ponds or in 1 L bulk from the wells and $n = 6$ of 100 ml samples from water streams) were collected in polystyrene sterile bottles/cup. We noted in our on-farm assessment survey that non-potable river water was used for irrigation and post-harvest washing. However, when water sources declined in the summer, farmers were forced to use private wells for irrigation and filling the washing ponds. In two of the farms, sewage water was used both as irrigation and nutrient fertilizer for economic reasons.

Samples were placed in insulated coolers with ice-packs and transported 135 km to the laboratory the same day. Logistically it was not feasible to process all the food samples on the same day, and these were stored in freezers at −18 °C to be analysed on subsequent days, whereas the water samples were analysed that day.

2.2. Bacteriological analysis

For irrigation and wash water microbiological assessment, *E. coli* designated as Hygiene Criterion indicating faecal contamination (EFSA, 2014) and total coliforms (TC) were tested. The group TC comprises the genera *Escherichia*, *Citrobacter*, *Enterobacter* and *Klebsiella*, indicator organisms that indicate the general sanitary level of water and possible contamination by different pathogens (Pachepsky, Shelton, McLain, Patel, & Mandrell, 2011; WHO, 2006). The enumeration of bacteria was performed according to the filtration method following EN ISO 9308-1:2000 using selective enrichment and RAPID'*E. coli* chromogenic media (Bio-Rad Laboratories Ltd., Hemel Hempstead, UK).

Fresh produce samples were analysed for the presence of pathogens and hygienic indicator organisms, i.e., *S. aureus*, *Salmonella* spp., *L. monocytogenes*, and for total plate counts (APC)