Food Microbiology 63 (2017) 147–152

Contents lists available at ScienceDirect

Food Microbiology

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

Occurrence and characterization of food-borne pathogens isolated from fruit, vegetables and sprouts retailed in the Czech Republic

Hana Vojkovská, Petra Myšková, Tereza Gelbíčová, Alena Skočková, Ivana Koláčková, Renáta Karpíšková*

Veterinary Research Institute, Department of Bacteriology, Hudcova 296/70, 621 00, Brno, Czechia

ARTICLE INFO

Article history:
Received 9 June 2016
Received in revised form
8 November 2016
Accepted 17 November 2016
Available online 19 November 2016

Keywords:
Salmonella
Listeria monocytogenes
STEC
MRSA
Foodborne

ABSTRACT

Food of non-animal origin is a major component of the human diet and has been considered to pose a low risk from the point of view of bacteriological safety. However, an increase in the number of outbreaks of illness caused by such pathogens and linked to the consumption of fresh fruit and vegetables have been reported from around the world recently. *Salmonella* spp., STEC (Shiga toxin producing *Escherichia coli*) and *Listeria monocytogenes* are among the most frequently identified agents. Additionally, the transmission of antibiotic resistant strains including also the methicillin resistant *S. aureus* (MRSA) to humans *via* the food chain is one of the greatest public health problems being confronted today. Therefore, we focused on the bacterial safety of fruit, vegetables and sprouts on sale in the Czech Republic. One strain (0.3%) of *Salmonella* Enteritidis phage type PT8, one strain (0.3%) of MRSA and 17 strains (5.0%) of *L. monocytogenes* were isolated from a total of 339 collected samples. The most problematic commodities were frozen fruit and vegetables (packed and unpacked) and fresh-cut vegetables. Our findings indicate deficiencies in hygiene practices during harvesting, processing and distribution of these commodities. Although sprouts and berries are the most likely to be contaminated by human pathogens, only two samples were positive for the presence of *L. monocytogenes*.

1. Introduction

Consumption of fresh fruit and vegetables is considered to be an efficient strategy in the prevention of a range of illnesses of modern society such as cancer, obesity and cardiovascular diseases (Berger et al., 2010; Heaton and Jones, 2008; Hoorfar, 2014). Additionally, fresh produce provides essential nutrients, such as vitamins, minerals and fibre (Maffei et al., 2013). From the food safety point of view, products of animal origin have attracted considerable attention of competent authorities and researchers so far (Berger et al., 2010), although food of non-animal origin is a major component of almost all meals (EFSA, 2013). However, an increase in number of outbreaks of human infections linked to the consumption of fresh fruits and vegetables was reported between 1995 and 2005 from around the world (Seow et al., 2012). Food of non-animal origin was associated with 10% of outbreaks, 26% of cases, 35% of hospitalizations and 46% of deaths between 2007 and 2011 in the EU (EFSA, 2013). It is generally accepted that this fact is a result of a gradual process with a combination of many contributing factors including an improvement in the detection methods (Beuchat, 1998; Heaton and Jones, 2008; Hoorfar, 2014).

Apart from 'traditional' contamination risk factors (e. g. unsatisfactory quality of sources of irrigation and rinsing water, application of improperly composted manures, secondary contamination during manipulation with products, changes in processing practices), the changes in society and in behaviour of consumers significantly contribute to the increased occurrence of infections associated with the consumption of fresh produce (Beuchat, 2002; Hoorfar, 2014). The reliance of today's consumers on restaurants, fast-foods or delis and public encouragement of healthy lifestyle has increased the demand for fresh produce and probably stimulated the growth of the fresh-cut industry and popularized the consumption of ready-to-eat (RTE) packed salads and fruit cups (Abadias et al., 2008; Harris et al., 2003; Hoorfar, 2014). The import and export of fruit and vegetables enables effortless spread of potential human pathogens across countries (Harris et al., 2003; Hoorfar, 2014).

It has been found that a number of outbreaks have been linked to imported produce, which can be caused by lower hygiene

* Corresponding author.
E-mail address: karpiskova@vri.cz (R. Karpíšková).

http://dx.doi.org/10.1016/j.fm.2016.11.012
0740-0020/

standards in these countries (Heaton and Jones, 2008; Hoorfar, 2014). Consumption of fresh vegetables, juices and other products thereof was associated with 5.0% of foodborne outbreaks in the EU in 2012. *Salmonella* spp. were together with viruses the most common causative agents (in total 48.7%) of reported cases for both pathogens (EFSA, 2014a). According to the reported outbreaks connected to fresh produce, *Salmonella* spp., STEC (Shiga toxin producing *Escherichia coli*) and *Listeria monocytogenes* are among the most frequently identified agents. Furthermore, it was found that specific types of produce, e. g. leafy greens, berries and sprouts, are more at risk of contamination and constituted an important source of pathogens in the documented outbreaks (Berger et al., 2010; Doyle and Erickson, 2008; EFSA, 2013; 2014b,c). Additionally, leafy greens often represent the main part of RTE packed salads, which were also identified as vehicles of pathogens in particular outbreaks (Little and Gillespie, 2008; Nguyenthe and Carlin, 1994). *Staphylococcus aureus* belongs to the less commonly identified pathogens in fresh produce associated outbreaks (3% of reported outbreaks; Doyle and Erickson, 2008).

The microbial quality of fresh produce has also been investigated in other studies (Abadias et al., 2008; Badosa et al., 2008; Santos et al., 2012; Seo et al., 2010a). However, contamination of products of non-animal origin retailed in many countries including the Czech Republic has not yet been well documented. The aim of this study was to investigate the prevalence of the main bacterial foodborne pathogens (*L. monocytogenes*, *Salmonella* spp., STEC and MRSA) in food of non-animal origin (fruit, vegetables and sprouts) accessible on the market of the Czech Republic. Additionally, the further detailed characterization of bacterial isolates was conducted.

2. Material and methods

2.1. Sample collection

A total of 339 samples of fruit and vegetables (fresh-cut, whole, frozen), and sprouts were randomly collected from different supermarkets, local stores and green markets during the whole year 2014 in nine cities of the Czech Republic. All samples were transported refrigerated to the laboratory where they were processed within 24 h. The samples examined in this study are listed in Tables 1 and 2.

2.2. Bacteriological analysis

Twenty-five grams of each sample were homogenized in 225 ml of buffered peptone water (BPW; Oxoid, UK) and incubated for 24 h at 37 °C. Further, the samples were processed according to corresponding regulations. In the case of *L. monocytogenes* enumeration 10 g of each sample was tested according to EN ISO 11290–2.

The detection of *Salmonella* spp. was performed according to EN ISO 6579 guideline using selective enrichment medium Rappaport-Vassiliadis (RVS, Oxoid), Mueller-Kauffmann Tetrathionate-Novobiocin Broth (Oxoid), XLD (Oxoid) and Rambach medium (Merck, Germany). Detection and enumeration of *L. monocytogenes* was performed according to EN ISO 11290-1 and 2:1996 using half and full Fraser broth (Oxoid), and ALOA medium (BioRad, France). Detection of STEC was performed according to ISO TS 13136. For detection of MRSA, the Brilliance MRSA 2 agar (Oxoid) was inoculated straight from the pre-enriched sample and incubated at 37 °C for 24 h.

2.3. Confirmation and characterisation of suspect colonies

2.3.1. Salmonella spp.

All suspect colonies of the genus *Salmonella* with typical growth characteristics on selective agar were confirmed by the genus *Salmonella* specific PCR (Olsen et al., 1995). *Salmonella* isolates were serotyped by slide agglutination method with commercial antisera (Denka Seiken, Japan and BioRad, France) and the final antigenic structure was obtained according to the Kauffmann-White-Le Minor scheme (Grimont and Weil, 2007). Phage typing was performed according to previously published protocols (Anderson et al., 1977; Ward et al., 1987) using HPA Colindale set of phages.

2.3.2. Listeria monocytogenes

Typical *L. monocytogenes* colonies (blue with PI-PLC activity) were serotyped by slide agglutination method with commercial antisera (Denka Seiken, Japan). The final serotype of isolates was obtained by the combination of this method and multiplex PCR (Borucki and Call, 2003; Doumith et al., 2004). Macrorestriction analysis using endonuclease *Asc*I (New England BioLabs, USA) was performed according to the EU RL protocol (ANSES, Paris, France).

2.3.3. MRSA

Suspect colonies of MRSA were confirmed by PCR for *S. aureus* specific fragment SA442 (Martineau et al., 1998) and screened for the presence of *mecA* gene according to Stegger et al. (2012). All MRSA isolates were subjected to the ST398-specific PCR to identify strains belonging to the livestock-associated clonal complex 398 (van Wamel et al., 2010). *spa* typing was performed as described previously according to the protocol on the website (http://www.SeqNet.org) using the Ridom StaphType software (Harmsen et al., 2003). Multilocus sequence genotyping (MLST) was performed on MRSA isolates according to published protocols (Enright et al., 2000). PCR products of the *spa* gene and 7 housekeeping genes for MLST were sequenced at the sequencing facility of Eurofins MWG Operon (Ebersberg, Germany).

Table 1
Analysed samples of fruit within this study.

Category	Fruit type	Number of samples	Country of origin	Selling place
Fresh-cut fruit	Pineapple	1	Unknown	Supermarket
Berries	Blueberry	10	Argentina, Chile, Czech Republic, Slovakia, Spain, South Africa	Supermarket, green market
	Cranberry	1	Poland	Supermarket
	Strawberry	59	Czech Republic, Netherlands	Supermarket, green market
	Raspberry	6	Czech Republic, Morocco, Portugal, Spain	Supermarket, green market
	Blackberry	4	Guatemala, Netherlands	Supermarket
Frozen fruit	Mixed	2	unknown	Supermarket
	Blueberry	1	unknown	Supermarket
	Strawberry	2	unknown	Supermarket
	Plum	1	unknown	Supermarket
	Black cherry	1	unknown	Supermarket
	Raspberry	2	unknown	Supermarket