International Journal of Food Microbiology 199 (2015) 72–77

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Salmonella surveillance on fresh produce in retail in Turkey

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ARTICLE INFO

Article history:
Received 10 September 2014
Received in revised form 3 January 2015
Accepted 9 January 2015
Available online 17 January 2015

Keywords:
Salmonella
fresh produce
pulsed-field gel electrophoresis
multi-locus sequence typing

ABSTRACT

Although Turkey is one of the major producers of fruits and vegetables in the world, there has been no information available on the prevalence of pathogens in fresh produce. To fill this gap, we collected 503 fresh produce samples including tomato, parsley, iceberg lettuce, green-leaf lettuce and five different fresh pepper varieties (i.e., green, kapya, bell, mazamort and Charleston) from 3 major districts within 9 supermarkets and 3 bazaars in Ankara, Turkey to investigate the presence of *Salmonella*. *Salmonella* was detected in 0.8% (4/503) of samples by conventional culturing method with molecular confirmation conducted through polymerase chain reaction (PCR). For further characterization of isolates, serotyping, antimicrobial susceptibility testing, multi-locus sequence typing (MLST; *aroC, thrA, purE, sucA, hisD, hemD* and *dnaN*) and pulsed-field gel electrophoresis (PFGE) were performed. *Salmonella enterica* subsp. *enterica* serotypes Anatum, Charity, Enteritidis and Mikawasima were isolated from two parsley, one pepper and one lettuce samples, respectively. MLST resulted in 4 sequence types (STs) for each serotype, including one novel ST for serotype Mikawasima. Similarly, PFGE revealed four different *Xba*I PFGE patterns. The results of this survey, obtained by the most common subtyping methods (i.e. serotyping, MLST and PFGE) worldwide, contributes to the development of a national database in Turkey, which is essential for investigating the evolutionary pathways, geographical distribution and genetic diversity of *Salmonella* strains.

1. Introduction

Salmonella is an important human and animal pathogen worldwide, mainly transmitted to humans through contaminated food (Jay et al., 2005). Each year, nontyphoidal *Salmonella* spp. have been estimated to be responsible for 1.0 million (Scallan et al., 2011) and 80.3 million human salmonellosis cases (Majowicz et al., 2010) in the United States and globally, respectively.

Since fresh produce is one of the major components, such as essential vitamins, minerals, and fiber, the consumption of fresh produce has increased worldwide in recent years (EU, 2007). The number of outbreaks associated with the consumption of contaminated fresh produce, especially those caused by *Salmonella,* has also increased. In EU countries excluding Spain, a total of 37 Salmonellosis outbreaks have been linked to the consumption of food of non-animal origin including fresh produce have been reported between 2007 and 2011 (EFSA, 2013). Irrigation water contaminated with manure or animal waste is a common environmental source for the transmission of organisms into fresh produce (Olaimat and Holley, 2012). The first large multistate outbreak was caused by *Salmonella* Javiana in fresh produce (i.e., tomato); including 176 illnesses in four states in the USA in 1990 (Hedberg et al., 1999). In 2008, peppers (i.e., serrano and jalapeño) were associated with a multistate salmonellosis outbreak that occurred in the USA, which affected more than 1000 people, including 286 persons who were hospitalized including 2 deaths (CDC, 2008).

During outbreak investigation, phenotypic and genotypic subtyping of *Salmonella* is essential for source identification, diagnosis and treatment. Serotyping is a basic method commonly used in epidemiological surveillance and outbreak investigations of *Salmonella*. Antimicrobial susceptibility test, another phenotypic characterization method, yields antimicrobial resistance patterns of *Salmonella* strains against a panel of antimicrobials, and therefore, provides sub-characterization. In addition to phenotypic methods, many DNA-based genotyping methods can be used to discriminate *Salmonella* isolates beyond species and subspecies level due to their high discriminative powers (Foley et al., 2006). These molecular methods can be divided into three basic groups; DNA banding pattern, DNA sequencing and DNA hybridization–based methods (Li et al., 2012). Pulsed field gel electrophoresis (PFGE), a DNA banding pattern–based method, is generally referred as the "gold standard" due to its ability to discriminate genetic differences and lineage among bacterial strains of the same species in many epidemiological studies (Levin, 2010; Swaminathan et al., 2006). PFGE offers many advantages such as interpretation of the entire bacterial genome in a single gel, high discrimination, reproducibility, and high degree of standardization (Gorman and Adley, 2004). However, PFGE results cannot be used in phylogenetic analyses for evolutionary purposes. On the other hand, multi-locus sequence typing (MLST), a DNA sequencing–based method, even with less discriminative power, can be used for phylogenetic analysis (Li et al., 2009). The main advantage of MLST is

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http://dx.doi.org/10.1016/j.ijfoodmicro.2015.01.010
0168-1605/

that allelic profiles of *Salmonella* can readily be compared to those in a MLST database via the Internet (Foley et al., 2006).

Turkey is one of the major producers of fruits and vegetables in the world. According to the latest forecasts of the Turkish Statistical Institute (TurkStat) Statistics (2013), tomatoes and peppers have an important place in the Turkish agricultural sector, with the highest amounts of production and exportation among all fresh fruit and vegetables.

There is limited information on foodborne diseases, since there is no active national foodborne pathogen surveillance system that collects information on foodborne pathogens in Turkey. Being one of the world's largest producers and exporters of fresh produce, Turkey needs a strong surveillance system for the early detection of potential foodborne outbreaks. In order to fill this gap, we aimed to determine the prevalence of a common foodborne pathogen, *Salmonella*, in fresh produce (tomato, leafy greens and 5 different pepper varieties) at the last chain of the farm to fork chain in Ankara. The diversity of *Salmonella* isolates was determined by phenotypic and genotypic methods (serotyping, MLST and PFGE), which are the most commonly used subtyping methods for *Salmonella* in the world.

2. Materials and Methods

2.1. Study design and sample collection

During the study period (from July to October 2012), a total of 503 samples including tomato ($n = 62$), parsley ($n = 62$), iceberg lettuce ($n = 62$) and green leaf lettuce ($n = 62$), as well as 255 fresh pepper samples representing 5 different varieties (green pepper, kapya pepper, bell pepper, mazamort pepper and charleston) were collected from 3 districts within 9 supermarkets and 3 bazaars in Ankara, Turkey (Table 1). The most populated districts (i.e., Yenimahalle, Çankaya and Keçiören) were chosen in Ankara city taking into consideration the results of the Ankara 2012 population census. The selection of supermarkets and bazaars was based on the availability of fresh produce samples during the period and their geographical location, situated in western, northern and central Ankara. According to availability of the peppers, 3 bazaars and 9 supermarkets in 3 districts were visited to collect fresh produce per week.

Table 1
Distribution of *Salmonella*-positive fresh produce samples among 3 major districts in Ankara.

Districts	Type of retail	Fresh produce	No. of samples		Serotype
			Total	Containing *Salmonella*	
Cankaya	Supermarkets ($n = 3$)	Pepper	70	0	Anatum
		Tomato	18	0	
		Parsley	18	1	
		Iceberg lettuce	18	0	
		Green leaf lettuce	18	0	
	Bazaar ($n = 1$)	Pepper	13	0	Mikawasima
		Tomato	5	0	
		Parsley	5	0	
		Iceberg lettuce	5	1	
		Green leaf lettuce	5	0	
Kecioren	Supermarkets ($n = 3$)	Pepper	60	1	Enteritidis
		Tomato	13	0	
		Parsley	13	1	Charity
		Iceberg lettuce	13	0	
		Green leaf lettuce	13	0	
	Bazaar ($n = 1$)	Pepper	26	0	
		Tomato	5	0	
		Parsley	5	0	
		Iceberg lettuce	5	0	
		Green leaf lettuce	5	0	
Yenimahalle	Supermarkets ($n = 3$)	Pepper	65	0	
		Tomato	15	0	
		Parsley	15	0	
		Iceberg lettuce	15	0	
		Green leaf lettuce	15	0	
	Bazaar ($n = 1$)	Pepper	21	0	
		Tomato	6	0	
		Parsley	6	0	
		Iceberg lettuce	6	0	
		Green leaf lettuce	6	0	

2.2. Salmonella detection and isolation

The procedure for the detection and isolation of *Salmonella* was carried out according to the techniques recommended by the International Organization for Standardization (ISO 6579). Fresh produce samples (25 g) were weighed and homogenized with 225 ml of buffered peptone water in a stomacher and incubated at 37 °C for 16–20 h. A total of 0.1 ml of the pre-enrichment sample was transferred to 10 ml of Rappaport Vassiliadis soy peptone (RVS) broth in duplicate and this was incubated at 41.5 ± 1 °C for 24 ± 3 h. A total of 10 μl of inoculum in RVS broth was added onto the XLD and BGA agar and incubated at 37 ± 1 °C for 24 ± 3 h. All suspicious *Salmonella* colonies (colonies with slightly transparent zones of reddish color and a black center on XLD agar and grey-reddish/pink colonies on BGA agar) were inoculated on BHI agar and incubated at 37 ± 1 °C for 24 ± 3 h to molecular confirmation. The *invA* (F: 5′-GAA TCC TCA GTT TTC AGT TTC-3′; R: 5′-TAG CCG TAA CAA CCA ATA CAA ATG-3′) gene of *Salmonella* was used to confirm the identity of the presumptive *Salmonella* (Kim et al., 2007).

2.3. Serotyping

Salmonella isolates were serotyped according to the White–Kauffmann–Le minor scheme in the laboratory of Public Health Agency of Turkey, in Ankara.

2.4. Antimicrobial susceptibility testing

The resistance of all isolates to antimicrobial drugs was evaluated by disk diffusion method (CLSI, 2013). Isolates were transferred to 4 ml Mueller–Hinton broth by sterile loop and the broths were incubated at 37 °C for 18 h. After incubation, bacterial cell suspensions were transferred into Mueller–Hinton agar. Paper discs (6 mm) that contained antimicrobials were put into the surface of agar and the petri dishes were incubated at 37 °C for 16–18 h. For the disk diffusion method, 18 different antimicrobial elements (Oxoid Ltd., Basingstoke, UK) were analyzed: amikacin 30 μg (AK), gentamicin 10 μg (GN), kanamycin 30 μg (K), streptomycin 10 μg (S), ciprofloxacin 5 μg (CIP), nalidixic acid 30 μg (N), ampicillin 10 μg (AMP), amoxicillin-clavulanic acid 20/10 μg (AMC), tetracycline 30 μg (TE), cefoxitin 30 μg (FOX), cephalothin 30 μg (KF), ertapenem 10 μg (ETP), ceftriaxone 30 μg (CRO), ceftiofur 30 μg (EFT), sulfisoxazole (SF), sulfamethoxazole-trimethoprim (SXT), chloramphenicol (C) and imipenem 10 μg (IPM). The quality control strain was *E. coli* ATCC 25922 for the tests. The susceptibility limits of antimicrobial agents except ceftiofur were determined by the Clinical Laboratory Standards Institute (CLSI, 2013) and the limits for ceftiofur were set according to a 2002 CLSI report.

2.5. Multi-locus sequence typing (MLST) and nucleotide analysis

Spin column–based DNA isolation of *Salmonella* isolates was carried out with NanoBiz Bacterial Genomic DNA Isolation Kit (NanoBiz, Ankara). PCR amplification of 7 housekeeping genes (*aroC, thrA, purE, sucA, hisD, hemD* and *dnaN*) of *Salmonella* were carried out according to the protocol of *Salmonella enterica* MLST Database of the University of Warwick Medical School (UoW) (available on http://mlst.warwick.ac.uk/mlst/dbs/Senterica).