Food Microbiology 46 (2015) 627–634

Contents lists available at ScienceDirect

Food Microbiology

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

Comparison of *Salmonella enterica* serovar Bovismorbificans 2011 hummus outbreak strains with non-outbreak strains

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ARTICLE INFO

Article history:
Received 19 June 2013
Received in revised form
4 February 2014
Accepted 18 February 2014
Available online 25 March 2014

Keywords:
Salmonella
Bovismorbificans
Hummus

ABSTRACT

Eleven *Salmonella enterica* serovar *Bovismorbificans* isolates obtained from the U.S. District of Columbia during a 2011 hummus-associated foodborne outbreak were compared to 12 non-outbreak isolates. All isolates from the outbreak demonstrated a single PFGE pattern that was distinctly different from other isolates of *S.* Bovismorbificans as recorded in the PulseNet Database. Results from molecular analyses of the hummus-associated *S.* Bovismorbificans isolates indicate that the isolates from the outbreak were unique and have acquired an 80–90 kb plasmid. The impact of this study is that the information gained will add and expand our knowledge of diversity of the *S.* Bovismorbificans serovar.

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1. Introduction

Outbreaks associated with a variety of *Salmonella enterica* serovars and food matrices continue to increase each year, making identification, differentiation and characterization of the various serotypes in foodborne outbreak a high priority for the field of food safety. Every year approximately 40,000 cases of salmonellosis are reported in the United States. There are over 2500 different serovars of *S. enterica*, and some of the most recent reported cases of *S. enterica* associated with foodborne outbreaks include: *S.* Typhimurium in ground beef, *S.* Heidelberg in Kosher broiled chicken livers and ground turkey, *S.* Enteritidis in Turkish pine nuts, eggs, alfalfa sprouts and spicy sprouts, *S.* Agona in fresh imported papayas, *S.* Hadar in turkey burgers, *S.* Panama in Cantaloupe, *S.* Bareilly in spicy tuna, *S.* Braenderup in mangoes, and *S.* Bredeney in Peanut Butter (CDC Outbreaks).

Human infections with *S. enterica* serovar Bovismorbificans are relatively infrequent in the United States. Prior to 2011, the last major outbreak associated with *S.* Bovismorbificans occurred in 2004 with 35 confirmed cases associated with the consumption of alfalfa sprouts (CDC Outbreaks). From 1999 to 2009, only 758 illnesses associated with *S.* Bovismorbificans were reported, compared to 1000 confirmed cases associated with *S.* Enteritidis in 1999 alone (Foodborne Outbreak Online Database, CDC, www.cdc.gov/salmonella/outbreaks.html). The majority of foodborne outbreaks associated with *S.* Bovismorbificans has occurred in Europe and have been traced to pork products, lettuce and sprouts (Rimhanen-Finne et al., 2011). In Finland in 1994, 201 cases were reported from a large sprout-associated *S.* Bovismorbificans outbreak (Puohiniemi et al., 1997). In Sweden in 1994, there was a large sprout-associated, *S.* Bovismorbificans outbreak (Pönkä et al., 1995) and a second nationwide outbreak occurred in 2009 with 42 clinical isolates identified and was also associated with ready-to-eat alfalfa sprouts. The sprouts samples were traced back to a domestic producer, but the seeds originated in Italy (Rimhanen-Finne et al., 2011). Over a 13 week period between November 2004 and March 2005, 525 cases of laboratory confirmed *S.* Bovismorbificans associated with raw pork were reported to The Robert Koch Institute (Rimhanen-Finne et al., 2011).

From August through November of 2011, sesame seed paste (tahini) and humus containing a rare serotype of *Salmonella* caused illness in 23 people in 7 states, including the District of Columbia

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http://dx.doi.org/10.1016/j.fm.2014.02.016
0740-0020/Published by Elsevier Ltd.

(Food Safety News). Reported cases were largely concentrated in the Mid-Atlantic region, with eight in Washington, D.C., seven in Maryland, three in Virginia and one each in Delaware and New Jersey. Three cases were also reported outside this region – one in California, one in Michigan and one in New Hampshire (Food Safety News). In this study, eleven *S.* Bovismorbificans isolates from clinical and hummus food samples were obtained from the DC Public Health Laboratory (DC_PHL) (Table 1). PFGE and conventional serotyping were used to identify the causal agent as a unique strain of *S.* Bovismorbificans. Hummus has not previously been associated as a source of *Salmonella* contamination. The DC_PHL and the FDA Office of Applied Research and Safety Assessment (OARSA) collaborated to characterize the outbreak strains. In addition to the original PFGE and the Kauffman White serotyping analyses, molecular serotyping using PCR, Optical Mapping, Multiple-locus variable-number tandem-repeats analysis (MLVA), antibiotic susceptibility testing, molecular fingerprinting using the Diversilab system, and plasmid analyses were used to characterize these outbreak strains.

2. Materials and methods

2.1. Strains

A total of 23 isolates were examined in this study (Table 1). DC_PHL identified 11 *S.* Bovismorbificans isolates from clinical and food samples (hummus) associated with the 2011 Washington DC outbreak. Two additional isolates associated with the outbreak were obtained from the Michigan and Delaware Departments of Health, Division of Public Health laboratories. Ten additional *S.* Bovismorbificans isolates, not associated with the outbreak, were also examined; and these isolates were obtained from the FDA Center for Food Safety and Applied Nutrition (CFSAN) and the Center for Veterinary Medicine (CVM) Salmonella culture collections. Two outlier strains of *Salmonella*: *S.* Typhimurium and *S.* Newport were also included in the studies (Table 1).

2.2. Molecular serotyping (PCR analysis)

To evaluate the molecular serotyping PCR method's usefulness during an outbreak, all isolates used in this study were serotyped using the conventional serotyping method and a PCR serotyping method described by Jean-Gilles Beaubrun et al. (2012) and Kim et al. (2006). Genomic DNA was isolated using the NucliSENS EasyMag instrument (BioMerieux, Inc. Hazelwood, MO) according to the manufacturer's instructions. PCR products were visualized using the Agilent 2100 Bio-analyzer (Agilent Technologies, Waldbronn, Germany), and the DNA 1000 Reagents kit (Agilent Technologies) following the manufacturer's protocol.

2.3. Pulsed field gel electrophoresis

All isolates of *S.* Bovismorbificans were analyzed for genetic relatedness using pulsed-field gel electrophoresis (PFGE) with XbaI and BlnI according to the U.S. CDC PulseNet protocol (CDC Protocols). Electrophoresis was performed with a CHEF-DR system (Bio-Rad Laboratories, Hercules, California) using 1% SeaKem agarose in 0.5× Tris–borate–EDTA at 180 V. Running conditions consisted of one phase from 2.2 s to 63.8 s at a run time of 18 h. PFGE profiles were analyzed using Bionumerics software v3.5 (Applied Maths, Sint-Martens-Latem, Belgium) (BioNumerics).

2.4. Antimicrobial susceptibility testing

All of the *S.* Bovismorbificans isolates were tested for susceptibility to standardized panels of antimicrobial drugs by the broth microdilution method (Sensititre® panel type: CMV1AGNF, Trek Diagnostic Systems, Westlake, OH) and the analysis was conducted according to the rigorously standardized Clinical and Laboratory Standards Institute (CLSI) protocols (Clinical and Laboratory Standards Institute, 2002, 2004, 2007). CLSI approved interpretive criteria were used when available; otherwise provisional National Antimicrobial Resistance Monitoring System (NARMS) breakpoints were used (Clinical and Laboratory Standards Institute, 2002, 2004, 2007).

2.5. Optical mapping

Genome mapping was conducted using Optical mapping. High molecular weight DNA was prepared from bacterial colonies following the manufacturer's procedures (OpGen, Gaithersburg,

Table 1
Summary of all the strains used in this study and the appropriate identification from various laboratories.

ID	DMB	CVM	DCPHL	Source	Serotypes	Location	Outbreak
SAL194	SARA2 LT2				Typhimurium		
SAL609	SL1489	41673	11-399	Stool-RF	Bovismorbificans	DC	Hummus
SAL610	SL1490	41674	11-405	Stool-RF	Bovismorbificans	DC	Hummus
SAL611	SL1491	41675	11-440	Stool-RF	Bovismorbificans	DC	Hummus
SAL612	SL1492	41676	11-441	Stool-RF	Bovismorbificans	DC	Hummus
SAL613	SL1493	41677	11-466	Stool-RF	Bovismorbificans	DC	Hummus
SAL614	SL1494	41678	11-495	Stool-RF	Bovismorbificans	DC	Hummus
SAL615	SL1495	41679	11-510	Stool-RF	Bovismorbificans	DC	Hummus
SAL616	SL1496	41680	11-528	Food	Bovismorbificans	DC	Hummus
SAL617	SL1497	41681	11-538	Food	Bovismorbificans	DC	Hummus
SAL618	SL1498	41682	11-539	Food	Bovismorbificans	DC	Hummus
SAL619	SL1499	41683	11-565	Stool-RF	Bovismorbificans	DC	Hummus
SAL676	SL1542	41838	12-180	Clinical	Bovismorbificans	DE	Hummus
SAL677	SL1543	41839	12-198	Clinical	Bovismorbificans	MI	N/A
SAL678	SL1544	41840	12-199	Clinical	Bovismorbificans	MI	N/A
SAL679	SL1545	41841	12-200	Clinical	Bovismorbificans	MI	N/A
SAL680	SL1546	41842	12-201	Clinical	Bovismorbificans	MI	N/A
SAL681	SL1547	41843	12-202	Clinical	Bovismorbificans	MI	N/A
SAL682	SL1548	41844	12-203	Clinical	Bovismorbificans	MI	Hummus
SAL683	SL1549	41845	12-204	Clinical	Bovismorbificans	MI	N/A
SAL185	SL0062	41672		Clinical	Bovismorbificans	MI	N/A
SAL644	SL1541	17906		Human	Bovismorbificans	CVM	N/A
SAL155	SL1541			Tomato Farm	Newport	VA	N/A