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Short Communication

Characterization of *Salmonella* isolates from retail foods based on serotyping, pulse field gel electrophoresis, antibiotic resistance and other phenotypic properties

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ABSTRACT

Sixteen *Salmonella* strains isolated from a variety of foods during 2000 and 2003 by the Florida State Department of Agriculture were characterized by various genotypic and phenotypic tests. Among 16 isolates, 15 different serotypes were identified. Pulse-field gel electrophoresis (PFGE) fingerprinting profiles obtained using restriction endonucleases XbaI and BlnI revealed that 16 *Salmonella* isolates were genetically diverse with 16 unique PFGE patterns. The PFGE pattern of eight isolates matched with the CDC/FDA data base of previous outbreaks and clinical isolates indicating their potential to cause disease. With the exception of isolates obtained from alligator meat (tetracycline resistant) and orange juice (chloramphenicol and sulfisoxazole resistant), the remainder of the isolates were susceptible to the panel of 15 antimicrobials tested. Molecular subtyping was further complimented by a variety of phenotypic tests such as acid-tolerance, Caco-2 cell invasion and biofilm formation which have often been used as a gauge of virulence and infection potential of *Salmonella* isolates. The induced acid tolerance level of the isolate obtained from orange juice was not significantly different from the laboratory reference strain *S. enterica* serovar Typhimurium SL1344. Six isolates exhibited very low levels of constitutive acid-tolerance, of which four isolates failed to infect differentiated Caco-2 cells. Although all isolates formed biofilms, there was no clear relation between the ability to form biofilms, infect differentiated Caco-2 cells and induce acid-tolerance. This study indicated that different serotypes of *Salmonella* were present in a variety of retail foods and exhibited diverse phenotypic characteristics.

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1. Introduction

Infections by *Salmonella* serovars is a major health concern world wide and it is estimated that 95% of these infections are food-borne. Notyphoidal salmonellosis is usually acquired by ingestion of contaminated water or food, and poultry products are a major source in many developed countries (Hald et al., 2004). The number of cases based on recent FoodNet data (CDC, 2005) is 147 per million persons, with 40% of those cases occurring in children under 15 years old.

Pulse field gel electrophoresis (PFGE) profiling is a DNA fingerprinting method which is based on the restriction digestion of purified genomic DNA. It is currently considered the gold-standard method for subtyping food-borne pathogens (Whittam and Bergholz, 2007). PFGE forms the basis for PulseNet, a national molecular subtyping network that was established in 1996 by the Center for Disease Control (CDC) and is now utilized by all state public health laboratories and food safety laboratories at the Food and Drugs Administration (FDA) and the United States Department of Agriculture (USDA). Currently PFGE data are considered reliable and a sensitive way to detect differences between closely related strains, so that isolates with indistinguishable PFGE profiles can be classified as epidemiologically linked with a high degree of confidence (Whittam and Bergholz, 2007). The broad applicability and informativeness of PFGE, however, is limited since it does not offer phenotypic characterization of pathogens. Our knowledge on routes of foodborne transmission of *Salmonella* has been acquired mostly through the study of epidemiological data from various prevalence studies and outbreak investigations. It is important to carryout phenotypic characterization of strains isolated in outbreak as well as sporadic cases since bacteria can change with or without altering the PFGE pattern (Shen et al., 2006) and thereby contributing to loss of information about the history of divergence.

In order to expand the advantages of PFGE beyond outbreak investigations we analyzed *Salmonella* isolates obtained by the Florida State Department of Agriculture through their routine survey over the period of 2000 and 2003. We reasoned that molecular subtyping in combination with phenotypic characterization will contribute

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towards quantifying the various sources of hazards and risks from sporadic occurrence of *Salmonella* strains. The four main objectives of this study were (i) to determine the pulse field gel electrophoresis patterns as well as antimicrobial susceptibility profiles of the isolates; (ii) to examine and characterize the isolates' ability to withstand synthetic gastric juice acid challenge; (iii) to determine the isolates' ability to infect differentiated Caco-2 cells; and (iv) to examine the biofilm formation ability of the isolates.

2. Materials and methods

2.1. Bacterial strains and growth media

During the years 2000–2003, approximately 3000 food samples collected in Florida using a standard randomized protocol were screened for *Salmonella* (USDA–FSIS, October 01, 2004). Sixteen *Salmonella* isolates from 11 different samples were identified using conventional microbiological criteria (Table 1). All strains were maintained at −70 °C and streaked on LB agar medium prior to use. Single colony obtained after overnight growth at 37 °C was used to inoculate various growth media such as LB-MOPS (morpholinepropanesulfonic acid, 100 mM, pH 8.0) or LB-MES (morpholineethanesulfonic acid, 100 mM, pH 5.5), or minimal E medium with 0.4% glucose. Liquid cultures were grown in shaker–incubator with 220 rpm, at 37 °C for 20 h.

2.2. Serotyping and Pulsed-field gel electrophoresis (PFGE)

All isolates were further confirmed as *Salmonella* using VITEK Gram-negative identification cards (BioMerieux Inc., Hazelwood, MO) following the manufacturer's instructions. *Salmonella* isolates were further serotyped by Kauffman–White classification scheme (Brenner, 1998) for O and H antigens using either commercially available Difco (Becton, Dickinson and Company, Sparks, MD) or CDC (Atlanta, GA) antisera.

Pulsed-field gel electrophoresis was performed according to the protocol developed by the Centers for Disease Control and Prevention (CDC) (Centers for Disease Control and Prevention, 1998). PFGE results were analyzed using the BioNumerics Software (Applied-Maths, Kortrijk, Belgium); banding patterns were compared using Dice coefficients with a 1.5% band position tolerance.

2.3. Antimicrobial susceptibility testing

Antimicrobial minimum inhibitory concentrations (MIC) were determined using the Sensititre automated antimicrobial susceptibility system in accordance with the manufacturer's instructions (Trek Diagnostic Systems, Cleveland, OH) and the Clinical and Laboratory Standards (CLSI) standards (Clinical and Laboratory Standards Institute, 2002). Results were interpreted in accordance with interpretive criteria provided by CLSI for those antimicrobial agents for whom CLSI had interpretive criteria. (Clinical and Laboratory Standards Institute, 2006). For those antimicrobial agents for whom there were no CLSI interpretive criteria (nalidixic acid and streptomycin) the interpretive criteria used by the National Antimicrobial Resistance Monitoring System (NARMS) were used. The following antimicrobials were tested: amikacin, amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim/sulfamethoxazole. *Escherichia coli* ATCC 25922 and ATCC 35218, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control organisms in antimicrobial MIC determinations.

2.4. Caco-2 invasion assay

The human Caco-2 intestinal cell line was obtained from the ATCC (Manassas, VA). The tissue culture cells were cultivated at 37 °C in a 94% air/ 5%CO_2 atmosphere in MEM supplemented with Earle's salts, 20% Fetal bovine serum, 4 mM L-glutamine, 0.1 mM nonessential amino acids, 100 units/ml penicillin, 100 µg/ml streptomycin, and amphotericin B 2.5 µg/ml. All cell culture media and supplements were obtained from GIBCO (Invitrogen, or Quality Biologicals, Inc., Gaithersburg, MD). Caco-2 cells were maintained as confluent monolayers for four weeks prior to use to allow the cells to differentiate prior to use.

The invasion assays were performed essentially as described earlier by Huang et al. (1998) and as modified by Knodler et al. (2002). All assays were conducted in quadruplicate and independently repeated at least twice. Results are expressed as an average of the replicate experiments (+/− represent standard deviation of mean values). Recovery data percentages were calculated and analyzed as described

Table 1
Acid-tolerance characteristic of *Salmonella* spp. isolates

Strain designation	Date of isolation	Source	Acid-tolerance characteristics (% survival after synthetic gastric juice challenge at pH 3.0, 2 h at 37 °C)[a,b]	
			Inducible acid-tolerance	Constitutive acid-tolerance
SL1344, Typhimurium	NA	NA	28.8±0.4 (B)	3.1±1.5 (A)
LT2, Typhimurium	NA	NA	4.7±1.0 (C)	0.01±0.01 (D)
92	6/26/2000	Paprika	32.0±4.0 (B)	0.28±0.05 (C)
93	6/27/2000	Irrigation water	31.0±3.7 (B)	0.22±0.12 (C)
94	7/14/2000	Paprika (raw imported material)	28.5±1.8 (B)	0.88±0.16 (B)
95	9/25/2000	Alligator meat	39.7±6.6 (B)	3.3±0.56 (A)
96	9/26/2000	Processed catfish	40.7±0.67 (B)	0.32±0.09 (C)
97	11/29/2000	Organic legume mix	65.8±6.8 (A)	1.1±0.57 (B)
98	1/12/2001	Paprika	27.2±2.32 (B)	0.28±0.01 (C)
99	1/24/2001	Processed catfish	39.2±9.2 (B)	0.44±0.06 (B,C)
100	1/25/2001	Alligator meat	30.9±9.6 (B)	0.07±03 (D)
101	1/8/2002	Spices	24.5±1.6 (B)	0.02±0.02 (D)
102	3/19/2002	Sesame seed alonjoli	0.26±0.09 (D)	0.02±0.01 (D)
103	4/2/2002	Dry white cheese	37.2±6.4 (B)	0.04±0.01 (D)
104	4/12/2002	Sage Spice	25.7±3.2 (B)	0.06±0.04 (D)
105	6/27/2003	Ground cumin	29.2±0.32 (B)	0.34±0.08 (C)
106	7/14/2003	Ground cumin	27.9±9.1 (B)	0.02±0.01 (D)
107	12/3/2002	Fresh Squeezed orange juice	26.9±3.9 (B)	1.4±0.21 (B)

[a]Mean values ($n=3$) in each column that are not followed by the same letter in the parenthesis indicate significant ($P<0.05$) differences. ± denotes standard deviation of mean.
[b]Inducible acid-tolerance was measured by subjecting cells grown in mild acidic conditions for 24 h (LB-MES, pH 5.5) to synthetic gastric juice challenge and constitutive acid-tolerance was measure by subjecting cells grown in LB-MOPS (pH 8.0) to synthetic gastric juice challenge.