Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.3, No.17, 2013

Epidemiologic Attributes and Virulence Profile of *Salmonella* Tennessee isolates from Infections associated with Peanut Butter National Outbreak

Chau H. Nguyen[1,] , Seongbeom Cho[2, 3], and Mahdi A. Saeed*[1, 2]
1 Department of Epidemiology, Michigan State University, ,USA
2 Departments of Large Animal Clinical Sciences and Epidemiolog. Michigan State University,
3 College of Veterinary Medicine and Research Institute for Veterinary Science , Seoul National University, Seoul 151-742, Korea
* E-mail of the corresponding author: saeeda@msu.edu

Abstract
The multi-state outbreak of *Salmonella* serotype Tennessee infections associated with peanut butter during 2006-2007 was the first outbreak in the United States associated with this food vehicle. We investigated whether the outbreak-related strains had any distinct virulence attributes. We have analyzed 96 representative isolates from human and non-human sources from multiple states for attachment and invasion of caco-2 cell. In logistic regression analysis, we found that *Salmonella* Tennessee strains associated with the peanut butter outbreak were more likely to be highly invasive than strains from non-outbreak sources, OR 4.03 (95% CI 1.42, 11.41). Results from this study suggest that peanut butter could have provided an impetus for the expression of certain sets of virulence genes leading to the observed high level of invasiveness of the *Salmonella* Tennessee contaminants. The occurrence of this outbreak underscores the importance of hygienic practices in peanut butter manufacturing plants for the prevention of such mass contamination.
Keywords: *Salmonella* Tennessee; peanut butter; newly emerging food vehicles for *Salmonella*; risk factors for *Salmonella* Tennessee

1. Introduction

Salmonella serotypes are among the most common causes of outbreaks of foodborne illness (Bell and Kyriakides,2002) . There are over 2,500 identified *Salmonella* serotypes of which 20 are commonly associated with human salmonellosis (WHO, 1993; CDC, 2007). *Salmonella* Tennessee (*S*. Tennessee) is not common among all the *Salmonella* serotypes that are reported each year. In the U.S., the average reported cases for *S*. Tennessee infections represent 0.01% of all reported *Salmonella* serotypes(CDC, 2007). During 1994-2004, there were only 52 cases of foodborne infections that were attributable to *S*. Tennessee.. Historically, S*almonella* outbreaks are frequently associated with certain groups of food vehicles. However, there was one earlier outbreak of *S*. Tennessee infections that was associated with powdered milk products and infant formula in the United States and Canada, reported to the Centers for Disease Control (CDC) in 1993(CDC, 1993)..

In November 2006, a substantial increase in the incidence of *S*. Tennessee infections was reported from most of the states in the US. The outbreak caused more than 800 cases in 47 states, accounting for the observed increase in reported cases within the year(CDC, 1993;CDC, 2007;JAMA, 2007; CDC, 2009). Subsequent investigation by the states health authorities, CDC, and the Food and Drug Administration (FDA) demonstrated that peanut butter consumption of two specific brands was associated with the illness (JAMA, 2007;CDC, 2009). Both of the brands were produced at the same manufacturing plant and *S.* Tennessee strains were isolated from several unopened and opened jars of the two brands, underscoring the emergence of peanut butter as a new food vehicle for human salmonellosis in the US(CDC, 2007;CDC, 2009). On February 14, 2007, a recall was implemented for both brands, resulting in a decline of reported cases(CDC, 2009.

Peanut butter- derived isolates have unique PFGE profiles. *Salmonella*. Tennessee isolates from the 2006-2007 outbreak displays three closely related PFGE patterns: JNXX01.0010, .0011, and .0026(CDC, 2007;CDC, 2009;CDC, 2010). *S*. Typhimurium on the other hand, displayed 2 clusters of unusual PFGE patterns. The first cluster consisted of 13 *S*. Typhimurium isolates with PFGE pattern JPXX01.1818. The second cluster consisted of 41 *S*. Typhimurium isolate with PFGE patterns JPXX01.0459/JPXX01.1825(CDC, 2007;CDC, 2009;CDC, 2010). Isolates from the two clusters were noted to be similar in patterns and testing of the isolates confirmed that the two clusters displayed the same pattern: JPXA26.0462. A different sub-typing method, multilocus variable-number tandem-repeat analysis (MLVA) showed that the two isolates were indistinguishable and were epidemiologically similar. As a result, the two clusters were grouped together as a single outbreak strain(CDC, 2010).

we have conducted a descriptive epidemiologic and microbiologic study of *S*. Tennessee isolates associated with the national peanut butter outbreak to describe the demographic, molecular epidemiologic, and virulence

attributes of a group of *S*. Tennessee isolates collected from clinical cases of the peanut butter-associated disease outbreak and compared them with *S*. Tennessee isolates that were not related to the outbreak.

2. Materials and Methods

Clinical isolates of S. Tennessee

Salmonella Tennessee isolates from clinical cases of the disease and other sources were collected from several participating states departments of health along with epidemiological data, when available. A total of 194 isolates were procured from all sources at the time of the investigation. Sources are listed below:

A total of 96 isolates were profiled. Table 1 displays the frequency counts and percentages of the profiled *S*. Tennessee isolates sources.

Attachment and invasion assay of *S*. Tennessee isolates with Caco-2 cells grown in tissue culture:

Attachment and invasion assays for 96 isolates of *S*. Tennessee on Caco-2 cells were performed. The Caco-2 cells grown in tissue culture were obtained from American Type Culture (ATCC HTB-37), and expresses characteristics of enterocytic differentiation upon reaching 80% confluency(Engle et al.,1998; Lee et al.,2009). Caco-2 cells were grown in complete growth medium consisted of Dulbecco's Modified Eagles Medium (DMEM) supplemented with 20% (vol/vol) Fetal Bovine Serum (FBS), 1% (vol/vol) nonessential amino acids. To prevent bacterial contamination, penicillin, at final concentrations of 100 ug/ml and streptomycin, at a final concentration of 100 ug/ml, were added. Cells were maintained at 37^{o}C in 5% CO_2 and 95% air in T-75 flasks (Sarstedt, Numbrecht, Germany) containing 10 ml of complete growth media for Caco-2 cell lines. Cells were grown, feed every 2-4 days, and sub-cultured when reached 80% confluency using Trypsin to detach cells from flask walls(Kirsop and Doyle,1991; Unchern ,2009).

Availability of epidemiologic information with each isolate and whether only few isolates were available from a given source were the bases for the selection of 96 isolates from a variety of sources including humans, food, animals, animal feed, and environmental sources. Samples were labeled before preparation for attachment and invasion assay. Two days prior to attachment or invasion assay, selected *S*. Tennessee isolates were grown on Tryptic Soy Agar (TSA) plates. Next day, an isolated colony was picked and inoculated into 10 ml Brain Heart Infusion (BHI) broth tubes for bacterial growth overnight. Prior to attachment or invasion assay, each BHI tube was vortexted and each bacterial isolate was plated to determine bacterial counts by plate count assay.

Attachment assays were performed using isolates grown with and without 1% D-Mannose. For attachment assays, each individual wells in 24well- plates were seeded with 3.31 x 10^5 Caco-2 cells and grown to form a monolayer on sterile cover slips to 80% confluency. Prior to attachment, wells were washed twice with incomplete DMEM (no FBS or antibiotics) and approximately 5 x 10^8 bacterial cells grown with and without 1% D-mannose were added to cover slips in each well with 2 ml of complete DMEM containing no antibiotics. Twenty four- well plates were then incubated for 1 hr in a 5% CO_2 atmosphere at 37 °C. Cells were then washed 3 times with sterile Phosphate Buffer Saline (PBS), fixed with methanol for 15 minutes, and stained with a 1:7 dilution Giemsa stain (Sigma) for 1 hour. Each cover slip was then removed from the wells, rinsed with PBS twice and a final rinse with distilled water and hung in coupling jars overnight to dry. Cover slips were mounted using "Permount" (Fishers) on microscope slides and left to dry overnight before examination by light microscopy the following day(Kirsop and Doyle,1991; Unchern ,2009) .

Invasion assays with Caco-2 cells were performed as described above except incubation period was 3 hours. Twenty four- well plates were then washed once with complete DMEM and intracellular growth medium (IGM) consisting of DMEM, 20% FBS, and 1 ml of Gentamicin per 100 ml was added and plates incubated. IGM was replaced in each well with fresh IGM every hour for 3 hours. Plates were then processed as described above for attachment assay. The addition of Gentamicin in invasion assays was meant to kill any extra-cellular bacteria without affecting growth of intracellular bacteria(Gahring et al.,1990).

Statistical analysis:

SAS systems ver 9.1.3 was used to perform descriptive epidemiologic study of the association between certain demographic epidemiologic attributes and invasive S. Tennessee strains. Frequency and percentages were computed for variables of interest. Cross tabulation tables were used to test for association of distribution of isolation sites of isolates by age groups and gender of cases.

Variables were categorized as binary, ordinal, or multinomial. Proc logistic procedure was used to perform unadjusted analyses of variables of interest with the outcome. S. Tennessee strains with PFGE pattern ending in JNXX01.0010, .0011, or .0026 were classified as strains associated with cases having a positive exposure to peanut butter and related to the outbreak. The outcome variable attachment was coded as 0 for negative attachment results and 1 for positive attachment results of bacterial cells with Caco-2 cell models after interaction was allowed following reported procedures. The interaction was assessed by microscopic examination. Invasion results were assigned to 2 categories based on the percentage of Caco-2 cells containing bacterial micro-colonies as observed under the microscope. Outcome for invasion was coded as either highly

invasive (equal to or greater than 75% of Caco-2 cells containing bacterial micro-colonies) or invasive (between 25 and 75% of Caco-2 cells containing bacterial micro-colonies).

3. Results

Descriptive analysis:

Of the 96 isolates profiled, 29 (43.28%) were related to the peanut butter outbreak compared to 38 (56.72%) non-related isolates and 29 isolates were unknown. Fifty- five isolates profiled were from human sources. The PFGE patterns of profiled human isolates in comparison to all profiled isolates are displayed in table 3. Approximately half of the profiled human isolates had missing information for the PFGE pattern. Of the 27 isolates with known PFGE patterns, 16 of the isolates displayed one of the 3 closely related PFGE pattern reported from the outbreak strain by CDC. Of all profiled isolates, 24 of the isolates displayed the 3 closely related PFGE pattern associated with the outbreak.

A total of 23 USDA samples were received of which 22 were from animal sources (including cattle, poultry, and swine) and 1 was from animal feed. All 22 *S.* Tennessee animal isolates displayed Mannose resistant local attachment under microscopic observation. An equal number of profiled isolates (50%, 11/22) were observed to be invasive and highly invasive.

Descriptive epidemiologic analysis of S. Tennessee Human isolates

A total of 55 human isolates were randomly chosen for attachment and invasion of Caco-2 cells in tissue culture. Age information was available for 46 patients. The mean age of patients was 43 years of age, ranging from 1- 94 years. Approximately 36.36% of human isolates were isolated from patients between 16- 64 years of age and 23.64% were from patients 65 years and over. The most common site of isolation was from stool (49.09%). Cross tabulation table of gender by site of isolation revealed that 49.09% (27/55) percent of human samples tested were from females of which 100% (27/27) were recovered from stool and urine versus 82.35% (14/17) recovered from stool and urine for males (p-value= 0.0152). Table 5 compares the gender and site distribution of profiled isolates and non-profiled S. Tennessee isolates. Additionally, Table 6 displays the frequency counts and percentages of the outcome invasion with selected variables of interest.

Univariate analysis

The univariate analysis of outcome invasion with selected predictor variables of interest for profiled S. Tennessee human isolates and all profiled S. Tennessee isolates are displayed in table 7. For profiled human isolates, outbreak, age, and gender were found to be insignificant at the 5% alpha value. However, for all profiled S. Tennessee isolates, it was observed that S. Tennessee strains associated with the peanut butter outbreak were more likely to be highly invasive than strains from non-outbreak sources OR= 4.03 (p-value=0.0088, 95% confidence interval (CI): 1.42, 11.41). Comparison of invasion patterns of all profiled S. Tennessee strains associated with the outbreak revealed that 75.86% (22/29) were observed to be highly invasive under microscopic examination.

Interaction of *S.* Tennessee with Caco-2 cells

Ninety-three of the isolates tested displayed a positive attachment to the Caco-2 cells. There were no differences observed in attachment patterns of isolates from different sources under a light microscope as most isolate demonstrated a pattern of Mannose resistant- local attachment. Of the 96 isolates tested, 3 were observed to be Mannose sensitive and 93 isolates expressed local attachment in the presence of 1% Mannose (Mannose-resistant). Table 8 displays the No. and percentages for attachment and invasion outcome of profiled isolates. Approximately 57% (55/96) of the isolates tested were invasive, compared to 43% (41/96) for highly invasive.

4. Discussion

The emergence of *S.* Tennessee in peanut butter has caused over 800 cases, perhaps many more. The emergence of *S.* Typhimurium again in 2008-2009 lead to a higher number of cases compared to *S.* Tennessee(CDC, 2009). Newly recognized emerging foodborne bacterial pathogens as well as well recognized pathogens can be associated with new food vehicles (. Most foodborne pathogens are foodborne zoonoses, having an animal reservoir from which they spread to humans(Tauxe, 1997) . Additionally, the spread of these foodborne bacterial pathogens can reach to a global level. Pathogens such as *Salmonella* has spread around the world since the 1980s, whereas *S.* Typhimurium DT 104 is appearing in North America and Europe(Tauxe, 1997) . New foodborne bacterial pathogens are being identified at an increasing rate, suggesting that many more remain to be discovered(Tauxe, 1997) .

In this study, descriptive epidemiologic analysis of a subset of the cases from the national peanut butter outbreak and virulence profiling of S. Tennessee isolates for their attachment and invasion of Caco-2 cells were conducted as an assessment of the distinct virulence of the *S.* Tennessee strains associated with the peanut butter outbreak, 2006-2007.

Descriptive epidemiologic study of clinical cases revealed that a higher number of S. Tennessee strains were

isolated from females than from males. Similarly, CDC reported a higher percentage of female patients (73%) compared to males(JAMA,2007). S. Tennessee infections are rare, however, they are more likely than other serotypes to infect the urinary tract(CDC, 2007). Urinary tract infections are common among females(Hohmann ,2001). Therefore, it is conceivable that the high proportion of isolates from the urine, may explain the high number of cases among females in comparison to males. *Salmonella* infections are more prevalent among the young, immunocompromised, and elderly. We observed a high number of isolate samples obtained from patients 65 years of age and above. The number of isolate samples obtained for young individuals were lower than other age groups. However, information for age was unknown for 9 clinical samples out of the 55 profiled isolate samples.

The use of Caco-2 cells models to perform attachment assays with S. Tennessee bacterial strains revealed a high percentage of attachment. All profiled S. Tennessee isolates procured from participating health departments displayed attachment to the Caco-2 cells model grown in tissue culture, an essential initial step leading to invasiveness of the strains. Furthermore, S. Tennessee strains were Mannose- resistant and displayed attachment to Caco-2 cells in the presence of 1% Mannose in the medium. The observed high percentage of Mannose-resistant-attachment suggests that strains of *Salmonella* enterica serovar Tennessee expresses specific surface structures other than Type 1 pili which may mediate their adherence to Caco-2 cells and explain their invasiveness to the clinically infected subjects during the outbreak(Fadle et al., 2002;Lee et al., 2009; Mellor et al.,2009). This observation has lead to the conclusion that S. Tennessee strains associated with the outbreak are likely to be more invasive than strains from non-outbreak sources. Whether the high level of attachment and invasiveness observed within the S. Tennessee strains associated with the peanut butter outbreak could have been attributed to the growth in peanut butter, remained to be investigated.

All S. Tennessee strains tested for their attachment and invasion of caco-2 cells were found to be invasive. However, S. Tennessee strains associated with the peanut butter outbreak were found to be highly invasive when compared with strains not associated with the outbreak. Whether peanut butter may have provided an impetus for optimal growth and expression of certain surface structures that enhances the strain's attachment and invasiveness as observed in the S. Tennessee outbreak strains, needs further investigation.

The strengths of the study were that the laboratory personnel performing attachment and invasion assays were blinded to the source of the isolates. Laboratory personnel were specially trained for the performance of attachment and invasion assays. This study has limitations in that we were limited in the number of isolates procured from participating states department of health and there were missing information for several isolates that led to their exclusion from the study. The emergence of known or newly recognized serotypes appear to be a continuous challenging process for health communities as seen in the S. Tennessee outbreak and again in S. Typhimurium, in which both occurred in a high fat low water content food vehicle. The occurrence of these two outbreaks suggests that our perceptions need to be reviewed and examined with an eye to correction. Food sources such as peanut butter previously thought safe are now considered hazardous. Consumption of peanut butter can be a risk factor in the etiology of sporadic non-typhoidal *Salmonella* infections among adults and children. It is not sufficient enough to only educate food producers, handlers, and consumers in basic food safety. More in house prevention programs and testing should be sought to omit public exposure to emerging contaminated food items and protecting consumers from severe illnesses resulting from foodborne bacterial pathogens.

Food industries should use the occurrence of this outbreak to identify lessons to be learned and develop applicable procedures for use among their industries. An increase in more regular and sensitive testing policies for peanut butter and other food items for *Salmonella*, prior to the release, will improve the safety of these food products, prevent distribution of contaminated peanut butter jars and future damaging outbreaks caused by *Salmonella*.

5. Conclusion

We concluded that peanut butter could have provided an impetus for the expression of certain sets of virulence genes leading to the observed high level of invasiveness of the *Salmonella*. Tennessee contaminants. The occurrence of this outbreak underscores the importance of hygienic practices in peanut butter manufacturing plants for the prevention of such mass contamination.

Acknowledgments

This study was funded, in part, by the National Institute of Allergy
and Infectious Disease (NIAID), National Institute of Health (NIH), US Department of Health and
Human Services under contract NO1-A1-30058.
We acknowledge Kristin Evon of Microbiology Research Unit (MRU) at Michigan State
University's National Food Safety & Toxicology Building, and Dr. Hwan Chung of the

Department of Epidemiology at Michigan State University for their assistance and coordination. We would like also to acknowledge the support of the following State agencies for providing the S. Tennessee isolates used in this study:

1. Michigan Department of Community Health.
1. Cornell University and New York Department of Health
2. Indiana State Department of Health
3. Tennessee Department of Health
4. Minnesota Department of Health
5. University of Pennsylvania
6. USDA National Veterinary Service Laboratory (NVSL) Ames, Iowa

References

Bell, C.; Kyriakides, *A. Salmonella: a practical approach to the organism and its control in foods*. Blackwell Science: United Kingdom, 2002.

CDC, Centers for Disease Control and Prevention. Multistate outbreak of Salmonella serotype Tennessee infections associated with peanut butter- United States, 2006-2007. *Morb. Mortal Wkly. Rep.* **2007**, 56, 521-524.

WHO, World Health Organization. Drug-resistant Salmonella. Available at: http://www.who.int/mediacentre/factsheets/fs139/en/print.html [Accessed on June 23, 2009].

CDC, Centers for Disease Control and Prevention. Salmonella serotype Tennessee in powdered milk products and infant formula- Canada and United States, 1993. *Morb. Mortal Wkly. Rep.* **1993**, 42, 516-517.

CDC, Centers for Disease Control and Prevention. Multistate outbreak of Salmonella associated with peanut butter and peanut butter containing products- United States, 2008-2009. *Morb. Mortal Wkly. Rep.* **2009**, 58, 1-6.

Multistate outbreak of Salmonella serotype Tennessee infections associated with peanut butter United State, 2006-2007. *J. Am. Med. Assoc.* **2007**, 298, 33-35.

Salmonella outbreak taints peanut butter sales. Available at: http://blog.nielsen.com/nielsenwire/consumer/salmonella-outbreak-taints-peanut-butter-sales/ [Accessed on January 11, 2010].

Peanut butter and other peanut butter containing products recall list. Available at: http://www.accessdata.gov/scripts/peanutbutterrecall/index.cfm [Accessed on January 11, 2010].

CDC, Centers for Disease Control and Prevention. Salmonella strains tables for outbreak related to peanut butter and peanut butter containing products. Available at: http://www.cdc.gov/salmonella/typhimurium/strains_table.html [Accessed on January 19, 2010].

Lee, I.J.; Hom, K.; Bai, G.; Shapiro, M. NMR metabolic analysis of Caco-2 cell differentiation. *J. of Proteome Res.* **2009,** 30, A-E.

Engle, M.J.; Goetz, G.S.; Alpers, D.H. Caco-2 cells express a combination of colonocyte and enterocyte phenotypes. *J. of Cell. Physiol.* **1998**, 174, 362-369.

Kirsop, B.; Doyle, A. *Maintenance of microorganisms and cultured cells: a manual of laboratory methods*, 2nd ed.; Academic Press Inc: California, United States, 1991.

Unchern, S. Basic techniques in animal cell cultures. Available at: http://www.pharm.chula.ac.th/surachai/academic/stu/cell culture handbook.pdf [Accessed on June 23, 2009].

Gahring L.; Heffron, F.; Finlay, B.B. et al. Invasion and replication of Salmonella Typhimurium in animal cells. *Infect. and Immun.* **1990**, 58, 443-448.

Tauxe, R. Food safety and irradiation: protecting the public from foodborne infections. *Emerg. Infect. Diseases* **2001**, 7, 516-521.

Altekruse, S.F.; Cohen, M.L.; Swerdlow, D.L. Emerging foodborne disease. *Emerg. Infect. Diseases* **1997**, 3, 285-293.

Tauxe, R. Emerging foodborne diseases: an evolving public health challenge. *Emerg. Infect. Dieases* **1997**, 3, 425-434.

Fadl, A.A.; Venkitanarayanan, K.S.; Khan, M.I. Identification of Salmonella Enteritidis outer membrane proteins expressed during attachment to human intestinal epithelial cells. *J. of Appl. Microbiol.* **2002**, 92, 180-186.

Mellor, E.G.; Goulter, M.R.; Chia, T.W.R.; Dykes, A.G. Comparative analysis of attachment of shiga- toxigenic Escherichia coli and Salmonella strains to cultured HT-29 and Caco-2 cell lines. *Appl. and Environ. Microbiol.* **2009**, 75, 1796-1799.

Hohmann, E. Nontyphoidal salmonellosis. *Clin. Infect. Diseases* **2001**, 32, 263-269.

Table 1. Frequency counts and percents of profiled isolates with corresponding sources. One *S*. Tennessee isolate source was unknown.

Source	No.	%
Human	55	57.30
Animal	23	23.96
Food	14	14.58
Feed	1	1.04
Environmental	2	2.08
Missing	1	1.04
Total	96	100

Table 2. PFGE patterns of profiled human *S*. Tennessee isolates compared to all profiled *S*.Tennessee isolates.

PFGE patterns associated with S. Tennessee outbreak	Profiled human isolates		All profiled isolates	
	No.	(%)	No.	(%)
JNXX01.0010	3	5.45	4	4.17
JNXX01.0011	9	16.36	16	16.67
JNXX01.0026	4	7.27	4	4.17
Other PFGE patterns				
JNXX01.0001	3	5.45	4	4.17
JNXX01.0002	1	1.82	1	1.04
JNXX01.0012	2	3.64	2	2.08
JNXX01.0014	1	1.82	3	3.13
JNXX01.0030	1	1.82	1	1.04
JNXX01.0039	1	1.82	1	1.04
JNXX01.0049	2	3.64	2	2.08
Missing PFGE	28	50.91	58	60.42
Total	55	100	96	100

Table 3. Gender and site distribution of profiled S. Tennessee from human source.

Gender	Profiled human isolates (N, %)	Site of isolation for profiled human isolates			
		Stool (N, %)	Urine (N, %)	Blood (N, %)	Wound (N, %)
Female	27 (40.1)	15 (53.9)	1 (46.2)	0	0
Male	17 (30.9)	12 (70.6)	2 (11.8)	1 (5.9)	2 (11.8)
Missing	11 (20)				
Total	55 (100)				

Table 4. Univariable analysis of outcome highly invasive with variable outbreak for all profiled *S*. Tennessee isolates.

Variable	Invasion Outcome			
	Invasive		Highly invasive	
	No.	%	No.	%
Gender:				
Female	12	70.54	15	55.56
Male	5	29.41	12	44.44
Outbreak:				
PB associated	6	54.55	16	84.21
Non-PB associated	5	45.45	3	15.79
Site:				
Stool	9	50.00	20	64.52
Urine	7	38.89	10	32.26
Blood	0	0.00	1	3.23
Wound	2	11.11	0	0.00
Age:				
< 5 years	2	11.11	4	14.29
≥ 5 and < 16 years	2	11.11	5	17.86
≥ 16 and < 65 years	8	44.44	12	42.86
≥ 65 years	6	33.33	7	25.00

Table 5. Univariable analysis of outcome invasion with selected predictor variables for profiled human S. Tennessee isolates and all profiled S. Tennessee isolates.

Variable	Profiled human isolates			All profiled isolates		
	OR (95% CI)	P-value	Missing	OR (95% CI)	P-value	Missing
Outbreak	4.44 (0.80, 24.61)	0.089	25	4.03 (1.42, 11.41)	0.009	29
Gender	0.52 (0.14, 1.89)	0.32	11			
Age	1.01 (0.99, 1.03)	0.39	9			

Table 6. Attachment and invasion patterns of the 96 *S.* Tennessee isolates selected from different sources. Three isolates were classified to be Mannose sensitive.

Profile	Outcome
	N (%)
Attachment positive	93 (98)
Attachment negative	3 (2)
ToTotal	96 (10)
Highly invasive	41 (43)
Invasive	55 (57)
ToTotal	96 (100)