Comparison of *Salmonella enterica* serotype Infantis isolates from a veterinary teaching hospital

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**Introduction**

*Salmonella* spp. have often been associated with outbreaks of nosocomial infections among patients in veterinary hospitals (Castor *et al.* 1989; Walker *et al.* 1991; van Duijkeren *et al.* 1994; Tillotson *et al.* 1997; Anon. 2001; Schott *et al.* 2001). Nosocomial infections with *Salmonella* species have also been reported in human health care settings (Moraes *et al.* 2000; Spearing *et al.* 2000; Olsen *et al.* 2001; Bornemann *et al.* 2002). The Large Animal Hospital at the James L. Voss Veterinary Teaching Hospital (JLV-VTH) at Colorado State University (CSU) was closed in 1996 (June through August) and 2001 (August) in order to mitigate the nosocomial spread of *Salmonella* spp. among hospitalized large animals (Tillotson *et al.* 1997; Morley 2002). The 1996 outbreak resulted in partial and subsequent total closure of the large animal facility over a 3-month period and cost more than an estimated $500 000. Total losses were greater than this because this estimate only included expenses related to mitigation,
and did not include opportunity losses such as lost revenues (Morley 2002). The results of a survey conducted in 1997 among veterinary teaching hospitals showed that 12 of 18 responders reported 18 outbreaks of nosocomial disease in the period between 1985 and 1996. Seventy-eight per cent of outbreaks were attributed to Salmonella spp. infections and six of these outbreaks resulted in hospital closure (Morley 2002).

Both of the outbreaks at the JLV-VTH were associated with Salmonella enterica serotype Infantis. Isolates with the same serotype and antibiogram were also isolated from multiple patients and the hospital environment at other times through 2004. Salmonella Infantis was isolated from 59 animals during the 1996 outbreak at the JLV-VTH. In contrast, only 72 other equine S. Infantis isolates were submitted to the United States Department of Agriculture (USDA) National Veterinary Services Laboratories (NVSL) in 1996 and S. Infantis represented only 2.7% of all nonhuman isolates during that same year, being the twentieth most common serotype (CDC 1996; Tillotson et al. 1997). Between 1997 and 2004, out of 1632 Salmonella isolates from horses selected for testing as part of the U.S. National Antimicrobial Resistance Monitoring System (NARMS), only 19 (1.2%) were identified as serotype Infantis (USDA 2006). Similarly, between the years 1997 and 2003, S. Infantis was listed among the 15 most common serotypes only twice, in 1999 and 2001 when isolates comprised 2.3% and 2.1% of all veterinary Salmonella isolates, respectively (USDA 2006). Among human isolates from the United States, Salmonella Infantis comprised only 1–2.3% of all Salmonella isolates tested by the NARMS between the years 1996 and 2003 (CDC 2006).

In contrast, S. Infantis has been one of the predominant isolates associated with outbreaks of human salmonellosis in Japan (Murakami et al. 1999). It has also been reported as a common isolate in several European and South American countries (Wegener and Baggesen 1996; Lindqvist et al. 1999; Merino et al. 2003; Nogrady et al. 2005). In particular, S. Infantis has been reportedly isolated in association with clinical disease in human infants (Moraes et al. 2000; Liebana et al. 2004).

In 1996, S. Infantis was isolated from a number of faecal and environmental samples collected throughout the JLV-VTH (Tillotson et al. 1997). Following the outbreak, a number of improved biosecurity measures were introduced in order to reduce the risk of nosocomial infections (Tillotson et al. 1997; Morley 2002, 2004; Traub-Dargatz et al. 2004). These measures included active bacteriological surveillance of hospitalized large animal patients and the hospital environment. As a result of this surveillance program, we have isolated S. Infantis from both faecal samples and the environment through 2004, albeit with decreasing frequency. The purpose of the present study was to compare selected 1996 isolates of S. Infantis and isolates from subsequent years to determine whether these isolates were epidemiologically related or likely represented separate introductions of S. Infantis to the JLV-VTH environment.

Materials and methods

Study design

The isolates included in this retrospective study were collected either as part of diagnostic procedures for large animal patients or as part of an active hospital surveillance programme and were stored at the Veterinary Diagnostic Laboratory at CSU. During the study period, S. Infantis was isolated from 59 JLV-VTH patients in 1996, 17 in 1997, 2 in 1999, and 7 in 2001. In addition, S. Infantis was recovered from environmental samples collected from the JLV-VTH in 1996, 1997, 1999, 2000, 2001, and 2004. Isolates were routinely archived at the time of initial isolation, and a subset of these isolates was included in studies described herein.

Salmonella Infantis isolates

Fifty-six S. Infantis isolates collected from 1996 through 2004 were available for comparison. All isolates were stored at −70°C in a glycerin freezing medium. Salmonella Infantis ATCC® 51741 (American Tissue Culture Collection) was included in comparisons as an epidemiologically unrelated isolate, as were five isolates collected as part of surveillance efforts in Washington state [two human isolates, and one isolate each from bovine, porcine, and canine sources, all identified by ‘WSU’ (Washington State University) in the isolate number on Fig. 1]. The source, sample type, and date of isolation of all isolates are listed in Fig. 1. The 34 faecal isolates included in the study were recovered from 29 different patients; three isolates (isolate number 39, 40, and 41) were recovered from one patient, while two isolates were recovered from each of three different patients (isolate numbers 1 and 2, 8 and 9, 30 and 31, respectively).

Preparation of pulsed-field gel electrophoresis plugs

Salmonella Infantis isolates were thawed, streaked, streaked for isolation on blood agar (BA) plates (BBL™ trypticase soy agar with 5% sheep red cells; Becton Dickinson, Franklin Lakes, NJ, USA) and incubated overnight at 35°C. A single colony was picked from each plate, suspended in 0.5 ml of Luria Bertani (LB) broth (Difco™; Becton Dickinson) and spread evenly on a new BA plate. The plate was incubated overnight at 35°C. Bacterial cells were
were then washed once in distilled water at 50°C followed by four washes in 0.5% sodium dodecyl sulfate (SDS), cooled to 54°C, and dispensed into a plug mould (reusable plug mould; BioRad Laboratories). When set, plugs were incubated overnight in lysis buffer (50 mmol l⁻¹ Tris, 50 mmol l⁻¹ EDTA, 1% N-lauryl-sarcosine, 0.1 mg ml⁻¹ of proteinase K, pH 8.0) at 54°C in a shaking water bath. The plugs were then washed once in distilled water at 50°C, followed by four washes in 0.5 × TBE (4.45 mmol l⁻¹ Tris, 4.45 mmol borax, 0.1 mmol l⁻¹ EDTA, pH 8.3) and stored in 0.5 × TBE buffer at 4°C for further processing.

### Restriction enzyme digestion of bacterial DNA

Restriction enzyme digestions were performed using two restriction enzymes, XbaI (New England Biolabs, Ipswich, MA, USA) and SpeI (New England Biolabs) in two separate reactions. An approximately 3-mm strip of a plug was incubated in 200 µl of a respective restriction buffer (XbaI or SpeI) at 37°C for an hour. The buffer was exchanged for a 160 µl of restriction reaction mix containing 40 units of either XbaI or SpeI and incubated overnight at 37°C. The agarose strip was then washed twice in 0.5 × TBE buffer at room temperature for 30 min. After the second wash, the agarose strip was cut in half and loaded into a gel.

### Pulsed-field gel electrophoresis

PFGE was performed using a CHEF mapper XA system (BioRad Laboratories). Digested bacterial DNA embedded in agarose plugs was subjected to electrophoresis through 1% agarose (Certified Megabase Agarose; BioRad Laboratories) in 0.5 × TBE gels at 6 V cm⁻¹, 120 angle, with 2s–50s switch time over 22 h. Following electrophoresis, the gels were stained with Gel Star stain (Cambrex Bioproducts, East Rutherford, NJ, USA), washed in 0.5 × TBE and photographed. Two lanes with the lambda ladder molecular weight standard (BioRad Laboratories) were included on each gel to allow normalization of the gels.

### Analysis of PFGE results

Cluster analysis of PFGE gel images was performed in Bionumerics (Applied Maths, Sint-Martens-Latem) using unweighted pair group mean analysis (UPGMA) of dice similarity coefficients. Position tolerances for XbaI and SpeI profiles were 2-0% and 1-5%, respectively. Percent optimization was 1-0 for both analyses. A composite cluster analysis was then calculated based on arithmetic averages of dice similarity coefficients from the two separate cluster analyses.

### Antimicrobial drug susceptibility testing

Susceptibility profiles were determined by a disc diffusion method in accordance with guidelines published by the Clinical and Laboratory Standards Institute (formerly National Committee on Clinical Laboratory Standards, NCCLS 2002). Isolates were plated onto Mueller-Hinton agar (BBL™, Becton Dickinson) and the following discs (BBL™ Sensi-Disc™ Susceptibility Test Discs; Becton Dickinson) were used (with the amount of drug contained in discs): tetracycline (Ts) – 30 µg, amikacin (Ak) – 30 µg, amoxicillin-clavulanate (Ao) – 10 µg, amoxicillin-clavulanate (Ao) – 30 µg, streptomycin (St) – 10 µg, cephalothin (Cp) – 30 µg, chloramphenicol (Ch) – 30 µg, enrofloxacin (En) – 5 µg, cefotaxin (Cf) – 30 µg, gentamicin (G) – 10 µg, sulfonamides (Su) – 0-25 µg, and trimethoprim-sulfamethoxazole (Ts) – 1-25/23-75 µg.

### Integron polymerase chain reaction

DNA was extracted from 200 µl of each S. Infantis overnight culture using a High Pure Polymerase Chain Reaction (PCR) template preparation kit (Roche Diagnostics) according to the manufacturer’s instructions. DNA was amplified in a reaction mixture (25 µl) containing approximately 50 ng of template DNA, 2.5 mmol MgCl₂, and 0.4 mmol of each deoxynucleoside triphosphate with 50 pM of each primer, and 2-5 unit of Taq DNA polymerase in a 1× reaction buffer (Expand High Fidelity PCR system; Roche Diagnostics) using a thermocycler (MJ Research PTC-200 DNA Engine thermal cycler; BioRad Laboratories). The following primers were used: int1 F: 5’-ACATGTGATGGGCACGCACGA-3’ and int1 R: 5’-AT- TTCTGTCTGGCTTGCGA-3’ (GenBank accession number AY551331). The primers were derived from a conserved region of the int1 gene coding for type 1 integrase and were predicted to amplify a 568-bp product. Cycling conditions consisted of 30 cycles of denaturation (94°C, 60 s), annealing (54°C, 60 s), and elongation (72°C, 60 s) with a final elongation step (72°C, 5 min). Amplified DNA products were subjected to electrophoresis through a 1% agarose gel, visualized by staining with ethidium bromide and photographed. As the presence of type 1 integrons has been previously associated with Su resistance (Antunes et al. 2005), this association was investigated using the chi-square test and by calcula-
ting the odds ratio (OR) and 95% confidence interval (95% CI).

**Results**

**PFGE profiles**

The *XbaI* restriction and electrophoresis produced 14−17 visible bands that ranged in size from c. 20 kbp to c. 600 kbp. *SpeI* restriction produced 17−21 bands in the same size range as the *XbaI* digest. Only bands ≥48 kbp were included in analyses. Relationships between the 56 *S. Infantis* isolates based on combined results from *XbaI* and *SpeI* digests are shown in Fig. 1. *Salmonella Infantis* ATCC 51741, as well as five *S. Infantis* isolates from WSU were included as the outside isolates for comparison. The *XbaI* digest produced 20 patterns that differed from each other by 1–12 bands (Fig. 1). The *SpeI* digest produced at least 14 patterns with 1–12 band differences and was useful in separating some isolates that were indistinguishable by *XbaI* digest (e.g., isolate numbers 9 and 43 or numbers 39 and 53) (Fig. 1). Overall, all but two isolates from the JLV-VTH (numbers 34 and 54) appeared to be more closely related to each other than to non-JLV-VTH isolates. Three main clusters were identified with more than 90% similarity each (Cluster A, B, and C, Fig. 1). Cluster A comprised 23 isolates, 18 of which showed more than 97% similarity to each other. Eleven isolates from 2001 had 100% similarity by both enzymes based on the bionumerics analysis. The remaining isolates in cluster A contained both 2001 and 1996 isolates with three 1996 isolates (numbers 20, 22, 25) showing 100% similarity to two 2001 isolates (numbers 1 and 2). Cluster B comprised 31 isolates that could be separated into three subclusters. While isolates within a cluster were more often recovered during the same year, one cluster with extremely high similarity contained two faecal isolates from 2001 and two environmental isolates from 2004. Isolates from various species and environmental sources were within the same cluster. For example, a cluster of 2001 isolates contained isolates from equine, bovine, and camelid faecal samples as well as from the environment. Cluster C comprised external reference isolates, but none of the isolates recovered as part of this study (Fig. 1).

**Antimicrobial drug susceptibility profiles**

There were 11 different resistance phenotypes observed among 56 *S. Infantis* isolates obtained from patients or the environment of the JLV-VTH (Fig. 1, Table 1). The most common resistance phenotype was identified in 19 isolates (33.9%) that were resistant to ten AMD (TTApAoStCpChCfGSuTs) (Table 1). These isolates were recovered in 1996 and 2001 and were represented in clusters A and B (Fig. 1). The next most common pattern of resistance included 11 (19.6%) isolates that were susceptible to all AMD evaluated and were recovered in 1997, 2001, and 2004. There were two patterns of resistance among isolates resistant to eight AMDs; 11 isolates (19.6%) were resistant to TTApAoStCpChCfSu and were isolated in 1996, 2000, and 2001, and one isolate (1.8%) recovered in 2004 was resistant to TTApAoStCpChCfTs. Five isolates (8.9%) had the same pattern of resistance to nine AMD (TTApAoStCpChCfSuTs). There were three resistance patterns among the six isolates (10.7%) that were resistant to four AMD, two patterns for the two isolates (3.6%) that were resistant to six AMD, and one isolate (1.8%) was resistant to five AMD. Overall, 45/56 (80.3%) were resistant to at least one AMD and 38/56 (67.8%) were resistant to ≥5 AMD. More than 50% of all *S. Infantis* isolates were resistant to Tt, Ap, Ao, St, Cp, Ch, Cf, and Su, with the highest percentage of isolates being resistant to St and Su. None of the isolates was resistant to Ak or En (Table 2).

**Presence of class 1 integrons**

Forty out of 54 (77.8%) isolates were positive by PCR for the presence of class 1 integrons (Fig. 1). Among the eight groups of isolates that were indistinguishable using PFGE in these analyses (Fig. 1), isolates from five groups had the same integron status (i.e. isolates from four clusters were all positive and isolates from one cluster were all negative), and the remaining three clusters consisted of isolates with mixed integron status.
Table 1 Frequency of antimicrobial drug (AMD) resistance patterns among James L. Voss Veterinary Teaching Hospital (JLV-VTH) (n = 56)

<table>
<thead>
<tr>
<th>Resistance number</th>
<th>Resistance pattern</th>
<th>Number of isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>TtApAoStCpCfGsuTs</td>
<td>19 (33.9)</td>
</tr>
<tr>
<td>9</td>
<td>TtApAoStCpCfGsTs</td>
<td>5 (8.9)</td>
</tr>
<tr>
<td>8</td>
<td>TtApAoStCpGsTs</td>
<td>11 (19.6)</td>
</tr>
<tr>
<td>8</td>
<td>TtApStCpGsTs</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>6</td>
<td>TtApStCpGsTs</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>6</td>
<td>TtApStCfGsSu</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>5</td>
<td>TtApStGsu</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>4</td>
<td>TtStChSu</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>4</td>
<td>ApStGsSu</td>
<td>4 (7.1)</td>
</tr>
<tr>
<td>4</td>
<td>TtApStSu</td>
<td>1 (1.8)</td>
</tr>
<tr>
<td>0</td>
<td>None</td>
<td>11 (19.6)</td>
</tr>
</tbody>
</table>

In the column ‘Resistance pattern’, AMD to which isolates were resistant to are listed: tetracycline (Tt), ampicillin (Ap), amoxicillin-clavulanate (Ao), streptomycin (St), cephalotin (Cp), chloramphenicol (Ch), ceftiofur (Cf), gentamicin (G), sulfonamides (Su) and trimethoprim-sulfamethoxazole (Ts). Resistance number indicates the number of AMDs to which the isolates were resistant.

Table 2 Frequency of resistance to 12 antimicrobial drugs among Salmonella enterica serotype Infantis isolates from James L. Voss Veterinary Teaching Hospital (JLV-VTH) (n = 56)

<table>
<thead>
<tr>
<th>Antimicrobial drug</th>
<th>Drug class</th>
<th>Number resistant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline</td>
<td>Tetracyclines</td>
<td>41 (73.2)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>Aminoglycosides</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>Penicillins</td>
<td>44 (78.6)</td>
</tr>
<tr>
<td>Amoxicillin-clavulanate</td>
<td>Penicillins</td>
<td>36 (64.3)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>Aminoglycosides</td>
<td>45 (80.3)</td>
</tr>
<tr>
<td>Cephalotin</td>
<td>Cephalosporins</td>
<td>37 (66.1)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Phenicols</td>
<td>37 (66.1)</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>Fluoroquinolones</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ceftiofur</td>
<td>Cephalosporins</td>
<td>36 (64.3)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>Aminoglycosides</td>
<td>26 (46.4)</td>
</tr>
<tr>
<td>Sulfasoxazole</td>
<td>Sulfonamides</td>
<td>45 (80.3)</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>Sulfonamides</td>
<td>25 (44.6)</td>
</tr>
</tbody>
</table>

(Fig. 1). Isolates that had class 1 integrons were nearly seven times more likely to be resistant to Su than were isolates without integrons (OR = 6.8, 95% CI = 1.68–27.72).

Discussion

The principle aim of this study was to determine whether there had been separate introductions of S. Infantis from 1996 through 2004, or whether there was evidence supporting the possibility of environmental persistence and nosocomial transmission. On several occasions during this study, isolates classified as indistinguishable by genetic analysis were obtained from different sources including different species of animals and environment. This is particularly evident in the subcluster of isolates with 100% similarity from 2001 within cluster A, which were obtained from faecal samples of bovids, equids, camelids, and the hospital environment within a period of 1 month. This is highly suggestive of nosocomial spread of this particular clone among patients and the environment in August 2001. The PFGE patterns of another two equine isolates (numbers 1 and 2, both from the same horse) recovered during the same month were only slightly different (98% similarity). These isolates also had a less resistant phenotype, so minor differences detected by PFGE may be related to differences associated with loss or acquisition of resistance genes. In addition, several clusters contained indistinguishable isolates from different years, e.g. isolates 20, 22, and 25 from 1996 showed indistinguishable PFGE profiles from isolates 1 and 2 from 2001. Similarly, isolates 17 and 18 from 2001 showed 100% similarity to isolates 63 and 66 from 2004. Thus, detection of indistinguishable isolates over a period of time and from a variety of sources suggests that S. Infantis appear to have persisted despite ongoing rigorous cleaning and disinfection aimed at minimizing persistence of bacterial burden in the hospital environment.

Although all 1996 isolates showed more than 88% similarity, there were some distinct differences among isolates, and some appeared to have closer relationships to isolates from subsequent years than to each other. This may indicate that more than one strain was present in the hospital in 1996, and persistence in the environment led to subsequent patient exposures. Both clusters A and B contained isolates from 1996, as well as isolates from other years. Thus, it appeared that 1996 isolates from different clusters were less similar to each other than to isolates from subsequent years belonging to the same cluster. In addition, all but two isolates (numbers 37 and 42) from July 1996 showed clearly distinguishable restriction patterns with both XbaI and SpeI despite the fact that these isolates were recovered from hospitalized horses within 1 month and some were isolated from the same animal at different time points (isolates 39, 40, and 41). Similarly, 2001 small ruminant isolates (numbers 16, 17, and 18) from cluster B were more similar to each other than to other 2001 isolates from cluster A. Also, one 1996 isolate (number 34) clustered on its own, separately from clusters A and B. This suggests that either the 1996 outbreak was associated with multiple introductions of different strains of S. Infantis, or alternatively, it represents normal variability because of mutations and exchange of genetic material between bacteria throughout the time S. Infantis persisted in the hospital environment, perhaps related to selection pressures imposed by the use of AMD and disinfectants in the hospital (Alyaseen et al. 2005; Graffunder et al. 2005).
Although PFGE has been shown to have good discriminatory power for the purpose of differentiating between individual isolates as compared with other methods, it is not without pitfalls (Levesque et al. 1995). One of the problems associated with differentiation between *Salmonella* isolates is the high level of clonality observed in some *Salmonella* serotypes, with similar genotypes obtained from a variety of sources. For example, most of the *S. Infantis* isolates from Danish cattle farms and feed collected over a period of three years (1992–1995) belonged to one predominant genotype (Lindqvist et al. 1999). In another study, indistinguishable PFGE patterns were found in *S. Infantis* obtained from dog treat samples from different geographic locations including domestic and imported products (White et al. 2003). In contrast, other investigators showed that a *S. Infantis* clone incriminated in the outbreak of human salmonellosis could be differentiated by PFGE of bacterial DNA digested with *Xba*1 from human isolates obtained before and after the outbreak. The same clone was also recovered from meat incriminated in the outbreak, although 20 additional patterns were recorded for unrelated isolates (Wegener and Baggesen 1996). Also, examination of *S. Infantis* PFGE patterns submitted to a VetNet project over the years 1997–2004 revealed 46 unique patterns among 146 patterns (Jonathan Frye, unpublished data). As such, reaching conclusions regarding the source and relatedness of clinically relevant isolates based on molecular analysis still remains a challenge (Levesque et al. 1995).

The results of this study cannot differentiate with certainty whether the 1996 outbreak was a result of clonal spread following a single introduction of *S. Infantis* to the hospital environment or a result of multiple introductions of several different clones. However, considering the high rate of isolation of *S. Infantis* from patients and the environment in 1996 in comparison with national data, as well as the presence of several indistinguishable PFGE patterns among isolates from the current study, our results strongly suggest that at least some of the infections were because of a clonal spread in those situations where indistinguishable PFGE patterns were obtained with both restriction enzymes from samples collected from various animals and environmental sources over a short period of time. Further, results of this investigation give the strong impression that contamination that arose from the 1996 outbreak persisted for years despite very rigorous hygiene and biosecurity precautions. This is analogous to the persistence of a single stable clone of *Salmonella enterica* serotype Heidelberg in an equine hospital in Australia over a period of 6 years that has been described (Amavisit et al. 2001). This has important implications for other veterinary facilities that have experienced nosocomial outbreaks of *Salmonella* and also underscores the importance of environmental contamination in the spread of nosocomial *Salmonella* infections.

The biosecurity practices at JLV-VTH are strict and stalls are routinely disinfected using a three-step process in which stalls are scrubbed with a bleach/detergent solution, cleaned with high pressure steam and very hot water, and disinfected with a quaternary ammonium product. All stalls in which *Salmonella*-positive animals were housed are cultured before being made available for use with other patients. The entire large animal facility is also routinely disinfected by applying peroxygen disinfectant to all surfaces using small particle mists (Patterson et al. 2005). Additionally, numerous other biosecurity practices are used as redundant protective ‘barriers’ in order to minimize nosocomial spread of pathogens (Dunowska et al. 2005; Morley et al. 2005; Patterson et al. 2005; CSU 2006; Dunowska et al. 2006; Traub-Dargatz et al. 2006). However, it is impossible to eliminate all opportunities for nosocomial transmission of pathogens. Interestingly, one of the 1996 isolates (number 44) was recovered from a mouse suggesting a possible role for rodents in transmission of *Salmonella* between different hospital areas or in re-introduction of *Salmonella* into clean areas over a period of time. Rodents have been associated with persistence of *Salmonella* infections on chicken farms (Rose et al. 2000; Liebana et al. 2003) and mice can be persistently infected with *Salmonella* without showing any clinical signs for prolonged periods of time (Monack et al. 2004).

The majority (74–1%) of the tested isolates were positive for type 1 integrons. Type 1 integrons are mobile elements that facilitate transfer and acquisition of resistance genes (Levesque et al. 1995). Type 1 integrons previously have been associated with Su resistance (Antunes et al. 2005). This is consistent with the observation that 80% of the isolates from JLV-VTH were resistant to sulfamethoxazole and isolates that tested positive for the presence of integrons were 6–8 times more likely to be sulfamethoxazole-resistant.

Phenotypic markers alone are not reliable for differentiation of isolates because of the ease with which bacteria can exchange resistance traits (Arbeit 1995; Merino et al. 2003). In this study, some isolates determined to have high similarity by PFGE analysis showed very different susceptibility patterns (Fig. 1). Such variability in phenotypic characteristics is not unusual and may be associated with different expression of resistance traits, chromosomal mutations not affecting the PFGE pattern, or exchange of resistance plasmids that would produce fragments of a size below PFGE detection capability (c. 50 kb) (Arbeit 1995).

A large percentage of the isolates were resistant to multiple AMD with 67–8% being resistant to >5 AMD.
This contrasts sharply with results reported for veterinary Salmonella isolates in 2003 by NARMS, where nearly 50% of Salmonella isolates were sensitive to all AMD tested and only 2.2% were resistant to >5 AMD (USDA 2006). The prevalence of multidrug resistance in this small set of isolates is likely related to their clonality, although a higher prevalence of multidrug resistance among S. Infantis isolates has been also reported by others (Moraes et al. 2000; Hamada et al. 2002, 2003a,b; Liebana et al. 2004; Nogradly et al. 2005). The resistance to AMD tested among equine NARMS isolates ranged from 0-4% (ceftriaxone) to 30-0% (Tt) and thus was much lower than that observed among the JLV-VTH S. Infantis isolates (USDA 2006, Table 2). Similarly, the highest percentage of AMD-resistant veterinary S. Infantis isolates from the NARMS database during the years when S. Infantis was listed as one of most common 15 isolates was only 6.5% for St in 1999 and 18.5% to Su in 2001 (USDA 2006). Also, in contrast to our results, a majority (90.0%) of NARMS S. Infantis human isolates recovered from 1996 to 2002 were susceptible to all 16 antimicrobials tested (CDC 2006). Although several of the AMD to which S. Infantis isolates were resistant have been used for treatment of large animals in the JLV-VTH, others such as Ch or St have not been used in the population or this facility for many years. This indicates that many resistance genes in S. Infantis isolates may be genetically linked and transferred together, so that they may persist in the bacterial genome in the absence of selection pressure.

In summary, results of genetic analysis in combination with clear epidemiological links give the strong impression that there was nosocomial transmission of S. Infantis during the 1996 outbreak, and that contamination arising from 1996 outbreak persisted across years despite rigorous hygiene and biosecurity precautions and may have led to subsequent nosocomial infections.

Acknowledgements

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