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ORIGINAL ARTICLE

# Comparison of *Salmonella enterica* serovar distribution and antibiotic resistance patterns in wastewater at municipal water treatment plants in two California cities

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## Abstract

**Aim:** To determine *Salmonella enterica* serovars and antibiotic resistance (ABR) in the human waste stream.

**Methods and Results:** Sampling of influent wastewater at municipal treatment plants in two California cities was performed by collecting composite samples, over a 24-h period, from the treatment plants on five to six occasions. Serial water quantities were filtered and cultured with a *Salmonella* selective method and an oxytetracycline-supplemented *Salmonella* selective method. Antibiotic susceptibilities to 12 antibiotics were determined and the isolates were grouped based on ABR patterns. From 983 *S. enterica* isolated, 102 represented unique sampling-serovar-ABR patterns. Thirty-five different serovars were identified to be distributed over 17 different ABR patterns. The serovar distribution differed between the sampling sites, whereas there was no significant trend in levels of multiple ABR.

**Conclusions:** *Salmonella enterica* was recovered with ease from small sample volumes of wastewater received by municipal water treatment plants. A large variety of serovars and ABR profiles were represented in the recovered *Salmonella*.

**Significance and Impact of the Study:** The ease of sampling and recovery of *Salmonella* from municipal wastewater from treatment plants makes it a valuable sampling approach for monitoring the presence of *Salmonella* in the human population.

## Introduction

Hippocrates, in 400 B.C., wrote '*Whoever wishes to investigate medicine properly, should proceed thus: …We must also consider the qualities of the waters, for as they differ from one another in taste and weight, so also do they differ much in their qualities.*' (http://classics.mit.edu//hippocrates/airwatpl.html). *Salmonella enterica* serovar *enterica* (*S. enterica*), while known to be a common cause of food-borne, water-borne and zoonotic illness, is mainly reported as part of multiperson outbreaks and rarely as individual disease (Bean *et al.* 1990). The exceptions to this are reports from reptile-associated salmonellosis, and even these are likely under-reported (Austin and Wilkins 1998). The current passive surveillance system for *Salmonella* rarely observes diffuse outbreaks and collects little detail on sporadic cases (Tauxe 1997). In animal populations, 'community' prevalence of *Salmonella* has been described and it is known that carrier and nonclinical states exist (Dargatz *et al.* 2003; Edrington *et al.* 2004; Fossler *et al.* 2004). In contrast, neither the community prevalence nor the carriage rate of *Salmonella* in the human population has been described in the published literature (Voetsch *et al.* 2004).

*Salmonella* has been isolated from water released into the environment from human wastewater treatment

 

plants. A study of effluent water from 12 wastewater treatment sites in California reported that *Salmonella* was recovered downstream of 11 plants (Kinde *et al.* 1997). Several studies investigating salmonellosis outbreaks have linked the use of water contaminated with human faeces or animal manure to the outbreaks (Oosterom 1991; Beuchat and Ryu 1997; Threlfall 2002). In addition, an increasing number of these *Salmonella*-associated epidemics involves multiple-antibiotic-resistant serovars (Threlfall 2002). A Finnish study reported high recovery of *Salmonella* from influent water into four conventional wastewater treatment plants (100% of 100 ml samples were *Salmonella* positive) and *Salmonella* reduction through the procedures indicated that wastewater treatment without efficient tertiary treatment, such as filtration or disinfection, may constitute a risk for public health, especially with regard to antibiotic-resistant strains (Koivunen *et al.* 2003). From these studies, it is clear that water can serve to transport *Salmonella* within the environment and that there are likely multiple sources for these *Salmonella*, including the human community.

We hypothesize that there is a detectable prevalence of *Salmonella* in human communities and that municipal wastewater reflects this prevalence. Furthermore, the community *Salmonella* prevalence may be influenced by the environment that surrounds communities. The aim of this study was to describe and compare the *S. enterica* serovar diversity and antibiotic resistance (ABR) patterns from wastewater at municipal sewage treatment plants in two communities, one with a high density of animal agriculture (Visalia, CA) to a community with a low density of animal agriculture (Davis, CA).

## Materials and methods

### Study site location

Wastewater influents from two municipalities were compared. The first (Visalia, CA) represented a community with an intense and varied agricultural economy with a dairy emphasis. The city of Visalia (population: 95 800 in 2002) lies within Tulare County (population: 379 000) and is home to over 279 000 dairy cows in more than 300 dairies. In 2002, Tulare County ranked one in the state and nation in total milk production (6·4 billion pounds). The second site (Davis, CA) represented a community with a significant crop-based agricultural economy but little livestock agriculture. Davis (population: 63 300 in 2002) is located within Yolo County (population: 176 000) where the agricultural focus is on crops such as corn or grain (ranked two in CA). Although Yolo County does not contain a large population, Davis is less than 20 miles from the Sacramento municipal region, which has a population in excess of 1 300 000 persons. The per capita county inventory for cattle is 16 times higher in Tulare than in Yolo County; per capita hog inventory is 38 times higher in Tulare relation to Yolo County. Both wastewater treatment sites received wastewater from households, schools, hospitals, retail and light businesses. For Visalia, there was only one wastewater treatment plant. Davis had a separate treatment plant for the University of California campus. All households within the municipalities were connected to the municipal wastewater stream, whereas no agricultural premises were connected to this stream.

### Sample collection

Twenty-four-hour composite samples from the influent wet well were collected as part of the standard operating procedures at each municipal waste treatment plant. These samples were flow proportional (calibrated to automatically collect 200 ml every 20 000 gal over a 24-h period) and were collected into a refrigerated 3-gal (11·4 l) container. The 3-gal composite collection container was hand shaken prior to transferring a 1-l sample to a sterile 1-l bottle. Samples were transported chill to the laboratory within 24 h of collection. Six samples were obtained from the Visalia water treatment plant and five samples from the Davis plant, with 1-week intervals during the autumn of 2002.

### Bacteriological techniques

A pilot trial indicated a very high recovery rate of *Salmonella* as well as a diverse set of serovars. Based on these studies, four different volumes (1, 3, 20 and 40 ml) of the influent samples were filtered through a 47 mm × 0·45 $\mu$m pore size membrane filter (Millipore, Molsheim, France). Three replicates of each filter volume were performed. For some of the larger volumes of water, more than one filter was required and they were processed as one sample. The filters were subjected to three culture methods. In the first method, a filter was placed in 40 ml buffered peptone broth (BPB) (Difco Laboratories, Detroit, MI, USA) and incubated at 37°C for 20 h. From the incubated BPB, 100 $\mu$l was transferred into 10 ml of Rappaport–Vasilliadis (RV) broth (Difco Laboratories) and incubated at 42°C for 20 h. Approximately, 0·1 ml of the RV broth was streaked onto xylose-lysine-deoxycholate (XLD; Hardy Diagnostics, Santa Maria, CA, USA) and brilliant green (BG; Hardy Diagnostics) agar plates using a sterile cotton-tip swab and streaked for isolation using a 10-$\mu$l disposable loop. The plates were incubated at 37°C for 20 h and examined for *Salmonella* suspect colonies. For the second method, a filter was initially

incubated in 40 ml BPB supplemented with 4 μg oxytetracycline per ml. The sample was subsequently processed as described earlier. For the third method, filters were processed as described for the first method but were plated onto XLD and BG supplemented with 4 μg oxytetracycline per ml agar.

Up to ten suspect *Salmonella* isolates per plate were biochemically tested using triple sugar iron and urea agar slants (Hardy Diagnostics). Isolates biochemically confirmed, as *Salmonella* were restreaked onto brain heart infusion agar plates (Hardy Diagnostics) and serogrouped using polyvalent antisera O, A, B, C1, C2, D and E (Difco Laboratories). All isolates were serotyped at the California Food Animal Health Laboratory System in San Bernardino or at the Veterinary Medicine Teaching and Research Center (VMTRC) *Salmonella* serotyping laboratory using the modified Kauffmann–White scheme (Popoff and Le Minor 1997; Popoff *et al.* 2000).

Antibiograms were performed on all isolates using the disc diffusion assay in accordance with the CLSI (NCCLS) guidelines (Bauer *et al.* 1966; NCCLS 2002). Twelve antibiotic discs (Difco, Becten Dickenson, Sparks, MD, USA) were used: amikacin 30 μg, amoxicillin/clavulanic acid 20/10 μg, ampicillin 10 μg, cephalotin 30 μg, ceftiofur 30 μg, chloramphenicol 30 μg, gentamicin 10 μg, nalidixic acid 30 μg, streptomycin 10 μg, sulfisoxazole 250 μg, tetracycline 30 μg and sulfamethoxazole/trimethoprim 23·75/1·25 μg. For each batch of isolates tested, two quality control strains were included in the assay set, *Escherichia coli* ATCC 25922 (ATCC, Manassas, VA, USA) and a VMTRC strain of *S. enterica* serovar Typhimurium. The inhibition zone diameters were read using a digital calibrated measuring device and recorded directly into a spreadsheet.

### Data analysis

From the antibiograms, all isolates had a profile consisting of the measured inhibition zone size for each of the 12 evaluated antibiotics. Using cluster analysis methods (SAS, version 8; SAS Institute Inc., Cary, NC, USA), *Salmonella* isolates with similar inhibition zone patterns were formed into ABR clusters (Berge *et al.* 2003). The dissimilarity measure used was the squared Euclidean distance. The clusters were determined using the average linkage algorithm also referred to as the unweighted pair-group average method. The clusters were ordered based on a decreasing sum of the mean zone sizes to the 12 antibiotics. For descriptive and illustrative purposes, the isolates are described as susceptible or resistant to individual antibiotics in accordance with CLSI–published breakpoints for human isolates of *E. coli* (NCCLS 2002).

Because of the selective and enrichment methods used in this study, we were unable to estimate the prevalence and only observed the occurrence of *Salmonella*. As multiple isolates of the same serovar were obtained from each of the sampling occasions and different cultivation procedures, only a single isolate representing each serovar and ABR cluster per sampling occasion and site was retained for these analyses. Distribution of serovars between the two sampling sites was compared using Fisher's exact test (StatXact-4; Cytel Software Corporation, Cambridge, MA, USA). The null hypothesis tested was that there was no difference in serovar distribution between the two sites. To assess differences in levels of multiple resistance in isolates between the two sites, the distribution of isolates from the most susceptible cluster to the most resistant cluster was tested using a Monte Carlo simulation of the nonparametric Kruskal–Wallis test based on sampling 10 000 tables and providing 99% confidence intervals (CI) for *P*-value estimates (StatXact-4; Cytel Software Corporation).

Information regarding *Salmonella* serovars obtained from human clinical submissions during 2002 obtained from Tulare and Yolo County authorities were included for comparison.

## Results

From the 11 sampling occasions, 983 isolates of *S. enterica* were confirmed biochemically, serotyped and antibiotic susceptibility tested to 12 antibiotics. A total of 102 isolates, representing unique *Salmonella* serovar and ABR cluster combinations for each sampling occasion and site, were retained for this descriptive study (Table 1).

Using the conventional media, *Salmonella* was recovered from each site and at each sampling time and from nearly all sampling volumes. The single exception was one sampling volume at a single sampling occasion when we were unable to recover *Salmonella*. Using the antibiotic-supplemented media, *Salmonella* was recovered at

**Table 1** The total number of *Salmonella enterica* isolated per sampling occasion and the number of unique serovar-antibiotic resistance types recovered from influent wastewater collected at two municipal treatment plants in California

| | Davis | | Visalia | |
|---|---|---|---|---|
| Sampling occasion | Total | Unique | Total | Unique |
| 1 | 42 | 10 | 108 | 14 |
| 2 | 55 | 12 | 123 | 11 |
| 3 | 89 | 6 | 119 | 11 |
| 4 | 36 | 2 | 55 | 9 |
| 5 | 89 | 10 | 70 | 8 |
| 6 | – | – | 95 | 9 |
| | 311 | 40 | 570 | 62 |

 

each site, sampling time and volume. We did not detect any difference in serovar distribution between the two tetracycline-supplemented methods.

In total, 35 different *Salmonella* serovars were identified (Table 2). Fifteen serovars were only identified on a single sampling occasion. Six serovars (*S.* Derby, Hadar, Havana, Montevideo, Saint Paul and Typhimurium) were found on five or more sampling occasions. The *Salmonella* serovar distribution between the two water treatment plants was different (Fisher's Exact $P$ value = 0·0003).

**Table 2** *Salmonella enterica* isolates from two municipal wastewater treatment plants in California sampled in the autumn of 2002 and serovars recovered from human Salmonellosis cases in Tulare and Yolo County during 2002. Wastewater isolates represent unique serovar-antibiotic resistance pattern at each sampling time

| | Davis | | Visalia | |
|---|---|---|---|---|
| Serovar | Wastewater | Clinical | Wastewater | Clinical |
| Agona | 2 | 2 | | 3 |
| Albany | | | 1 | |
| Anatum | | | 1 | |
| Banana | 1 | | | |
| Barranquilla | 1 | | | |
| Bovismorbificans | | | 1 | |
| Braenderup | | | 1 | |
| Cerro | | | 2 | |
| Derby | 1 | | 8 | 1 |
| Edinburg | 5 | | | |
| Fluntern | 1 | | | |
| Hadar | 5 | 2 | 2 | 2 |
| Havana | 5 | | 2 | |
| Heidelberg | 1 | 3 | 1 | 3 |
| Infantis | | 1 | 2 | 2 |
| Kiambu | 2 | | | |
| Manhattan | | | 1 | |
| Mbandaka | 1 | | | |
| Meleagridis | | | 1 | |
| Montevideo | | 2 | 5 | 1 |
| Muenchen | | | 1 | |
| Muenster | | | 1 | |
| Newport | | | 3 | 19 |
| Oranienburg | 2 | 1 | | |
| Panama | | | 2 | |
| Pomona | 1 | 1 | | |
| Reading | 1 | | 1 | |
| Saint Paul | 7 | | 9 | |
| Senftenberg | | | 2 | |
| Stanley | | | 1 | 1 |
| Thompson | | 3 | 3 | 1 |
| Typhimurium | 3 | 7 | 6 | 16 |
| Typhimurium 4,5,12:i:- | 1 | | 1 | |
| Uganda | | | 1 | |
| Virchow | | | 3 | |
| Other | | 10 | | 6 |
| Total | 40 | 32 | 62 | 55 |

Nine serovars were unique to the Davis study site, 19 serovars were unique to Visalia and eight serovars were found at both water treatment plants. Of the eight serovars found in common between the study sites, five of them (*S.* Derby, Hadar, Havana, Saint Paul and Typhimurium) were all recovered across multiple sampling times.

Some of the *Salmonella* serovars recovered from human clinical cases in the two counties were also found in wastewater (Table 3). The most frequent clinical source serovars, *S.* Typhimurium (Visalia and Davis) and *S.* Newport (Visalia), were found in the wastewater for the respective sites. However, the most common wastewater serovar, *S.* Saint Paul, was not seen in the clinical isolates.

Seventeen ABR clusters described the antibiotic susceptibility patterns of the 102 *Salmonella* isolates (Table 3). Fourteen of the clusters were multiple resistant to two or more of the tested antibiotics. No isolates were resistant to amikacin or sulfisoxazole/trimethoprim. The distribution of serovars between the ABR clusters A–Q indicated that there were susceptibility patterns, both shared and unique to serovars across the two study sites (Table 4).

There was a marked difference in the ABR clusters of recovered *Salmonella* depending on the culture method used. Using the nonselective method 86% of 647 *Salmonella* isolates were pan sensitive, 4% were monoresistant and 10% were multiple resistant. The same samples cultured on tetracycline-supplemented media recovered only multiple resistant serovars, belonging to ABR clusters F–Q.

*Salmonella enterica* serovars resistant to four or more antibiotics included *S.* Braenderup, Derby, Edinburg, Hadar, Saint Paul, Typhimurium, Typhimurium 4,5,12:i:-, Uganda and Virchow. Four of the multiple resistant serovars were furthermore resistant to ceftiofur, a third generation cephalosporin. These included Typhimurium 4,5,12:i:-, Newport, Saint Paul and Uganda.

There was no difference in levels of multiple resistance in *Salmonella* isolates obtained from Davis and Visalia (Kruskal–Wallis: Monte Carlo estimate of $P$ value = 0·46, 99% CI: 0·45–0·47). This set of 102 isolates distributed over 35 serovars did not allow for a within-serovar comparison of ABR cluster distribution for the two study sites.

## Discussion

This study has shown that a wide variety of *S. enterica* serovars and ABR patterns were observed in municipal wastewater. *Salmonella enterica* isolates were easily recovered from municipal wastewater even in filtering volumes as small as 1 ml. While the study indicated that there were significant differences in serovar distribution

**Table 3** *Salmonella enterica* isolates from two municipal wastewater treatment plants grouped into 17 antibiotic resistance (ABR) clusters based on the inhibition zone sizes to the 12 antibiotics

| | | Antibiotics – mean inhibition zone sizes in mm | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ABR cluster* | No. of isolates | AMP | AMC | CEF | XNL | STR | GEN | AMI | SULF | SXT | TET | CHL | NAL |
| A | 52 | 23 | 25 | 23 | 24 | 14 | 21 | 22 | 21 | 25 | 19 | 24 | 21 |
| B | 1 | 18 | 19 | 18 | 22 | **7** | 21 | 20 | 23 | 26 | 21 | 25 | 20 |
| C | 1 | 26 | 29 | 26 | 27 | 17 | 23 | 24 | 23 | 28 | **6** | 25 | 23 |
| D | 2 | 24 | 26 | 24 | 24 | 14 | 21 | 22 | 19 | 26 | 19 | 22 | **9** |
| E | 2 | **6** | 14 | 25 | 25 | 15 | 23 | 23 | 26 | 29 | 21 | 25 | 22 |
| F | 7 | 24 | 26 | 24 | 24 | **7** | 22 | 23 | 23 | 26 | **6** | 25 | 21 |
| G | 2 | **6** | 20 | 20 | 25 | **8** | **11** | 25 | 26 | 27 | 23 | 24 | 21 |
| H | 12 | 23 | 24 | 22 | 23 | **7** | 20 | 20 | **7** | 20 | **6** | 23 | 20 |
| I | 1 | **6** | **7** | **6** | 23 | 14 | 20 | 22 | 26 | 30 | 18 | 19 | 20 |
| J | 1 | 27 | 30 | 25 | 28 | **6** | 24 | 26 | **6** | 23 | **6** | **6** | 23 |
| K | 2 | **6** | 19 | 20 | 25 | **9** | **11** | 24 | **6** | 26 | 21 | 24 | 20 |
| L | 1 | **6** | 18 | 16 | 24 | **6** | **6** | 21 | 25 | 27 | **8** | 26 | 21 |
| M | 2 | **6** | **9** | **6** | **14** | 15 | 22 | 22 | 20 | 27 | 19 | 23 | 19 |
| N | 2 | **6** | 19 | 19 | 23 | 15 | **13** | 21 | **6** | 19 | 20 | 20 | **6** |
| O | 5 | **6** | 17 | 17 | 24 | **6** | 21 | 22 | **6** | 21 | **6** | 23 | 21 |
| P | 2 | **6** | 15 | 23 | 25 | **6** | 23 | 21 | **6** | 23 | **8** | **6** | 22 |
| Q | 7 | **6** | **7** | **6** | **14** | **6** | 22 | 23 | **6** | 22 | **6** | **6** | 22 |
| Sum | 102 | | | | | | | | | | | | |

AMP, ampicillin; AMC, amoxicillin–clavulanic acid; CEF, cephalothin; XNL, ceftiofur; STR, streptomycin; GEN, gentamicin; AMI, amikacin; SULF, sulfisoxazole; SXT, sulfamethoxazole-trimethoprim; TET, tetracycline; CHL, chloramphenicol; NAL, nalidixic acid.
*The zone sizes in boldface indicate resistance according to the CLSI guidelines for human *E. coli*.

between the two sites, with a greater variety isolated from Visalia compared with Davis, it also demonstrated that there were a set of serovars (*S.* Derby, Hadar, Havana, Saint Paul and Typhimurium) that were common at both sites and consistently isolated during the course of the study. A set of *Salmonella* serovars were multiple antibiotic resistant with many serovars sharing the resistant phenotypes. There was no significant difference in the levels of multiple ABR between the two study sites.

The principle differences between the study sites were the intensity and type of agriculture and the population dynamics of the region. Visalia is in the centre of an intense agricultural region, with significant animal agriculture. It is the largest city in the county and the economy is based on agriculture. Davis is also located in an agricultural county, but the agriculture is based primarily on crops with little animal agriculture. Davis is a relatively small city but is part of the greater Sacramento area, which is a large metropolitan centre for the state of California. The influence of agriculture may explain some of the differences observed in the variability of *Salmonella* in municipal wastewater at the two sites. Three of the unique serovars recovered from Visalia, *S.* Montevideo, Meleagridis and Newport, were also noted in diagnostic and surveillance samples from dairy cattle and their environments. (William Sischo, pers. comm.). Newport has also been associated with clinical disease in bovine animals and humans in 2002 (Berge *et al.* 2004).

The source of the *Salmonella* isolates recovered from the wastewater treatment plants cannot be determined. From the counties' passive surveillance systems for clinical disease, the most frequent human clinical isolates were *S.* Newport (Visalia) and *S.* Typhimurium (Davis and Visalia). While *S.* Newport was found in the Visalia wastewater samples and *S.* Typhimurium was found at both sites, there was far more variability in serovars isolated from the wastewater than that was observed in the reported human clinical isolates. This suggests that the isolates originated from other sources, which may include unreported clinical cases and human subclinical infections. The under-reporting of human clinical salmonellosis is well known, and it is believed that for every reported case observed in Food-Net there are 38 additional cases in the community (Voetsch *et al.* 2004). This does not account for nonclinical cases. Our data support this belief, as the most common serovar *S.* Saint Paul, which was identified in samples from both Davis and Visalia and at multiple sampling times, was not observed as a clinical isolate. As clinical submissions may be linked to the pathogenicity of the serovar or strain, it is possible that the less pathogenic *Salmonella* are not observed by public health officials. We also

**Table 4** *Salmonella enterica* serovars obtained from two municipal wastewater treatment plants in two California cities grouped by ABR cluster*

| ABR cluster | Davis | Visalia | *Salmonella enterica* serovars |
|---|---|---|---|
| A | 20 | 32 | Albany, Anatum, Banana, Barranquila, Bovismorficans, Cerro, Derby, Edinburg, Fluntern, Havana, Heidelberg, Infantis, Kiambu, Manhattan, Meleagridis, Montevideo, Muenchen, Muenster, Oranienburg, Panama, Pomona, Saint Paul, Senftenberg, Stanley, Thompson, Typhimurium |
| B | 1 | 0 | Hadar |
| C | 1 | 0 | Reading |
| D | 1 | 1 | Mbandaka, Thompson |
| E | 2 | 0 | Havana |
| F | 3 | 4 | Derby, Hadar, Heidelberg, Saint Paul |
| G | 2 | 0 | Edinburg |
| H | 3 | 9 | Agona, Derby, Hadar, Reading, Saint Paul, Virchow |
| I | 1 | 0 | Havana |
| J | 0 | 1 | Typhimurium |
| K | 2 | 0 | Edinburg, Saint Paul |
| L | 1 | 0 | Hadar |
| M | 0 | 2 | Typhimurium 4,5,12:i:-, Braenderup |
| N | 0 | 2 | Virchow |
| O | 1 | 4 | Derby, Typhimurium |
| P | 1 | 1 | Typhimurium |
| Q | 1 | 6 | Typhimurium 4,5,12:i:-, Newport, Saint Paul, Uganda |
| Sum | 40 | 62 | |

*ABR clusters as defined in Table 3.

cannot rule out that these isolates came from nonhuman sources including pet and feral animal clinical cases, nonclinical pet and feral animal cases and environmental sources.

The distribution of serovars recovered from four Finnish water treatment plants did not correspond to the serovars most commonly associated with human clinical salmonellosis cases. In this study, it was hypothesized that there may be differential survival of serovars in wastewater (Koivunen *et al.* 2003). As *Salmonella* shedding prevalence in the California human population has not been determined, it is difficult to know the relationship between our finding, *Salmonella* in the human effluent water, and shedding patterns in the human population. Although faecal shedding in clinical salmonellosis can contaminate large quantities of water (Kinde *et al.* 1997), the large variability in serovars recovered indicates multiple sources of the isolates.

In an analogous study conducted on dairy farms, *Salmonella* was recovered from 74% of 561 flush water drain samples, whereas only 10% of 8091 individual cow faecal swabs were *Salmonella* positive. The odds of finding *Salmonella* in flush water was 3·5–181 times more likely for various serovars than to find the bacteria in individual cow faecal swabs (William Sischo, pers. comm.). The ease of recovery of a wide range of *Salmonella* serovars in flush water compared with individual animal faecal swabs indicates that *Salmonella* is able to survive outside the host animal and that wastewater is a valuable sampling commodity to signal the prevalence and diversity of *Salmonella*. Warnick *et al.* (2003) also demonstrated that environmental farm samples were more efficient for identifying infected premises than individual bovine faecal samples.

Assuming that humans were the major source of *Salmonella* in these samples, the human demographics of the sampling sites may be compared with assessed potential differences in exposure to *Salmonella*. Employment in the two cities may result in a differential exposure to environmental *Salmonella*, with more Visalia residents being directly or indirectly exposed to agricultural operations than Davis residents. In addition to differential environmental exposure, local differences in food-borne bacterial exposure may also be a factor. Because of the highly centralized distribution of food products in the United States, bacterial contaminants of grocery-purchased food are likely similar in Davis and Visalia. However, produce from farmer's market and specialty food represent differential food exposures between the two communities. It is also possible that differing socioeconomic levels and cultural influences in the two cities may contribute to differences in environmental and food exposure to *Salmonella*.

While there has been increasing concern over the emergence of antibiotic-resistant *Salmonella* (Threlfall 2002), the necessity to add oxytetracycline to the culture media in order to increase the recovery of resistant isolates indicates the dominance of pan-susceptible strains in wastewater. More than 85% of the isolates from the nonselective *Salmonella* culture method were pan susceptible. Accounting for the recovery from both nonselective and selective culture methods, 43% (44/102) of the isolates in the data set were multiple antibiotic resistant. These multiple resistant patterns were often found in several serovars and from both treatment plants. Antibiotic selection in *Salmonella* culture may be of value in situations similar to ours, where there are high levels of *Salmonella* in the sample matrix and recovery of antibiotic-resistant serovars is of interest.

Resistance to the third generation cephalosporins in *S. enterica* has been emerging and causing increasing concerns, as these antibiotics are one of the first drugs of choice to treat human salmonellosis, particularly paediatric cases (Gupta *et al.* 2003). Several of the ABR clusters exhibited resistance to the third generation cephalosporin, ceftiofur, and included *S.* Typhimurium 4,5,12:i:-, New-

port, Saint Paul and Uganda, which were isolated multiple times from the Visalia study site. In addition, a ceftiofur-resistant *S.* Typhimurium 4,5,12:i:- was recovered from the Davis site. It has been speculated that the use of ceftiofur in bovine animal production may create selection pressure for cephalosporin resistance in *S. enterica* (Gupta *et al.* 2003). This study is equivocal in its support of this hypothesis. More important is the observation that the phenotype that included the third generation cephalosporin resistance was observed in four serovars across multiple time points in two distinct regional sites. The mechanism for the third generation cephalosporin resistance has been described as $bla_{cmy2}$ and is observed on a plasmid (Winokur *et al.* 2001). The resistance has been shown to be transferable via *in vitro* electrophoresis and conjugation (Giles *et al.* 2004). Our data support the notion that the resistance is observed in a number of different serovars and perhaps easily transferred between bacterial species and serovars *in vivo*.

Studies have found *Salmonella* in post-treatment samples. Kinde *et al.* (1997) found *Salmonella* in the effluent water from 11 of the 12 wastewater treatment sites in California. There are beliefs that ABR patterns are transient characteristics that may fluctuate, as the water moves through the treatment plan (Andersen 1993). A study comparing enterococcal and coliform composition pre- and post-treatment in six water treatment plants did not discover any differential survival in resistant strains compared with the susceptible strains (Villanova *et al.* 2004). It would be of interest to determine whether there is a differential survival between *Salmonella* serovars and ABR patterns during wastewater treatment. Several studies have indicated that the transfer of ABR plasmids occurs in wastewater (Mach and Grimes 1982; Alcaide and Garay 1984). The high diversity and recovery of *Salmonella* with different ABR patterns in this study indicates that there may be a potential for new highly resistant serovars to emerge in municipal wastewater plants. We performed monthly sampling at water samples downstream of the municipal plant in Visalia, the same location during 2002, using similar nonsupplemented methodology as described, without recovery of *Salmonella* (William Sischo, pers. comm.). The ability of the treatment plant to effectively reduce the numbers of *S. enterica* from the influent grey water, therefore, seems to be a crucial component in minimizing the environmental contamination with this pathogen.

This study indicates that municipal wastewater would be of interest to further monitor the community prevalence of subclinical or nonreported *S. enterica* infection. Wastewater or human septic tank samples would facilitate monitoring of bacteria in human populations compared with the difficulties associated with obtaining individual faecal samples (Sayah *et al.* 2005). Wastewater samples may be good indicators of emerging antimicrobial resistance in the community as was shown by the detection of glycopeptide resistance in *Enterococcus faecium* in Germany (Klare *et al.* 1993). The use of wastewater sampling could be incorporated into national surveillance programs to monitor *S. enterica* as well as other pathogens in order to obtain further knowledge of serovar shifts and their ABR patterns that are not detected in our current passive surveillance system. Influent samples at wastewater treatment plants are routinely and systematically collected on a daily basis and could easily be obtained from the wastewater treatment facilities. The procedures for sampling and culture could be standardized for a national scheme.

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