

Isolation of *Salmonella* serotypes in wastewater and effluent: Effect of treatment and potential risk

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Abstract

The characteristics of strains of *Salmonella* isolated in wastewater were compared before and after treatment with activated sludge (conventional sewage treatment). Of the 21 strains studied, 11 came from raw wastewater and 10 from treated water. Our objective was to determine if the treatment affected all serotypes to the same extent, and whether it had an effect on the plasmidic composition and selection of resistances to antibiotics and disinfectants. The serotypes of all strains were identified, and we also determined the sensitivity to antibiotics, the minimal bactericidal concentration of three disinfectants (peracetic acid, glutaraldehyde, and sodium hypochlorite), and plasmidic content. Results showed no significant differences in the isolated strains before versus after treatment, thus indicating that the conventional treatment of sewage does not constitute a risk factor in the selection of *Salmonella* strains with a greater pathogenic potential.

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Introduction

Foods and water still play the main roles in the transmission of *Salmonella*, though wastewater is particularly important.

Surface water is often contaminated by urban wastewater, by effluents of meat industries and by wastewater from livestock ranches, involving different serotypes of *Salmonella* (Rusin et al., 2000). The serotypes isolated from human samples do not always coincide with the serotypes isolated from wastewater, yet this is not always the case in Spain, where *Salmonella enteritidis* (50.7%), *S. typhimurium* (23.2%), and *S. hadar* (4.7%) were the most frequent serotypes isolated from clinical

human samples in the year 2000, and together with *S. anatum* were also the ones most often isolated from water samples (Usera et al., 2001).

Although wastewater and drinking water are treated to eliminate pathogenic microorganisms and prevent waterborne transmission, numerous studies indicate that conventional wastewater treatment does not guarantee their complete elimination. Our research group recently found treated water to contain a *Salmonella* MPN of 45/100 ml (Howard et al., 2004).

The survival of *Salmonella* despite treatment implies the possibility of selection of the most resistant strains, or the acquisition of resistance through the transference of genetic material. Thus a double public health risk may exist: the presence of *Salmonella* itself in the effluent, and the predominance of more pathogenic strains.

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We studied strains of *Salmonella* isolated in wastewater to observe the effect of sanitary treatment on the selection of serotypes and resistances to a number of antibiotics and disinfectants.

Material and methods

Bacterial strains

This study involved 21 strains of *Salmonella* isolated from 42 samples obtained at the wastewater treatment plant of Granada (Southern Spain) during the period November 1999–March 2000. The samples were obtained within a 24 h period, with two samples taken simultaneously: one of raw wastewater and the other of the effluent after aerobic biological treatment. From the 21 samples of raw wastewater, 11 strains were isolated (52.4% of positive samples), and 10 strains proceeded from the 21 samples (47.6% positive) of the effluent that had undergone activated sludge treatment (Howard et al., 2004).

Salmonella strains were isolated using the selective Rappaport–Vassiliadis medium (Oxoid) and *Salmonella–Shigella* agar (Oxoid). Biochemical identification was performed with analysis of serotypes based on slide seroagglutination using somatic and flagellar *Salmonella* antisera (Centro Nacional de Microbiología del Instituto de Salud Carlos III, Majadahonda, Madrid, Spain).

The following reference strains were used: *Pseudomonas aeruginosa* CIP A22 and *Escherichia coli* CIP 54127 for disinfectant activity testing; and *E. coli* V517 as the marker of molecular weight of plasmids.

Antibiotic susceptibility testing

Antibiotic susceptibility was determined by the agar diffusion method, initially described by Kirby and Bauer, and standardized by the US Federal Drug Administration (FDA) and by the World Health Organization (Acar and Goldstein, 1996). The following antimicrobial susceptibility test disks were used: amikacin (An), amoxicillin/clavulanic acid (AmC), ampicillin (Ap), azthreonam (Atm), cefepime (Fep), cefotaxime (Ctx), cefoxitin (Fox), cefuroxime (Cxm), cephalothin (Cf), ciprofloxacin (Cip), chloramphenicol (C), fosfomicin (Fos), gentamicin (Gm), imipenem (Ipm), nalidixic acid (Na), neomycin (N), nitrofurantoin (Fm), piperacillin (Pip), sulfisoxazole (Su), tetracycline (Te), and trimethoprim/sulfamethoxazole (Sxt).

Disinfectant activity testing

Three high level commercial disinfectants were used: 2% glutaraldehyde (Instrunet[®]), 0.26% peracetic acid

(Perasafe[®]), and sodium hypochlorite (liquid bleach). The available chlorine in sodium hypochlorite solutions was determined using the iodometric method (APHA, 1989).

Disinfectant activity was tested in accordance with the norms UNE-EN 1040 and AFNOR NF T 72-150 for the evaluation of bactericidal activity by means of the dilution-neutralization method (AFNOR, 1995; European Standardization Committee, 1997; Álvarez Alcántara et al., 2001). The temperature of the assay was 20 °C and the time of contact was 5 min. From the concentration of use, a series of 1/2 dilutions of the disinfectants was tested to determine the minimal bactericidal concentration (MBC). The composition of 1000 ml of the neutralizer at a double concentration ($\times 2$) was as follows: Tween 80, 129 g (120 ml); 40% sodium bisulphite, 25 ml; sodium thiosulphate pentahydrate, 15.69 g; adjusted to pH 7.0 and sterilized by filtration (Espigares et al., 2003).

Plasmid analysis

Plasmids were isolated from the bacterial strains according the Quantum Prep kit instructions (Bio-Rad). The isolated plasmids were characterized by 0.7% agarose gel electrophoresis (Maniatis et al., 1989). The molecular weights of the plasmids were obtained by comparing their relative mobilities to standard DNA markers.

Statistical analysis

The statistical treatment of the data was done with the SPSS for Windows software package (SPSS Inc., Chicago, USA). After basic statistical analysis of data, the Student's *t*-test was applied for two independent samples.

Results and discussion

Because this study involves urban wastewater, it was to be expected that the strains of *Salmonella* isolated would coincide with those most frequently isolated from humans over the same geographical area. Data published for Spain show that the four most frequent serotypes in clinical samples of human origin within the year 1999 were: 46.1% of *S. enteritidis*, 20.2% of *S. typhimurium*, 8% of *S. hadar*, 4.6% of *S. 4,5,12:i:-(I)*, and 3.0% of *S. virchow* (Usera et al., 2000). Similarly, for the period 1999–2000 the serotypes most frequently isolated in Europe were *S. enteritidis*, *S. typhimurium*, *S. hadar*, and *S. virchow* (Fisher, 2000), while in the United States *S. enteritidis* and *S. typhimurium* comprised 42%

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