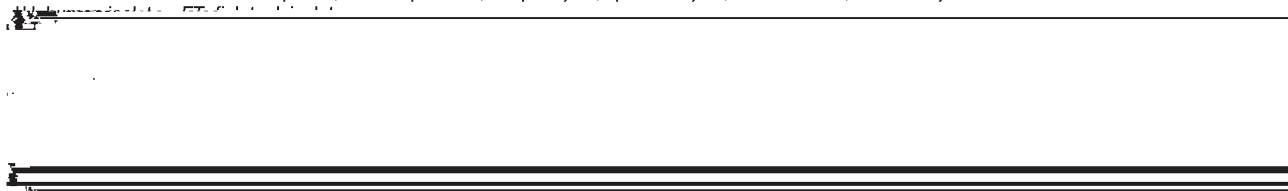
# Aquariums as Reservoirs for Multidrug-resistant *Salmonella* Paratyphi B

Table. Genetic characteristics of *Salmonella enterica* serovar Paratyphi B dT+ isolates used in this study

| Isolate no.* | Source† | Phage type‡ | State§ | Date of isolation | Age, y/Sex | SGI1¶ |
|---|---|---|---|---|---|---|
| Set 1 | | | | | | |
| SRC229 | H | Aus2 | ACT | 2000 | <1/F | + |
| SRC230 | H | Aus2 | ACT | 2000 | 1/M | + |
| SRC231 | FT | Aus2 | ACT | 2000 | – | + |
| Set 2 | | | | | | |
| SRC232# | H | Aus2 | Vic | 2000 | 11/F | + |
| SRC233# | H | Aus2 | Vic | 2000 | 11/F | + |
| SRC233A | FT | Aus2 | Vic | 2000 | – | ND |
| Set 3 | | | | | | |
| SRC145 | H | Aus3 | Vic | 2003 | 74/F | + |
| SRC142 | FT | Aus3 | Vic | 2003 | – | + |
| SRC143 | FT | Aus3 | Vic | 2003 | – | + |
| Set 4 | | | | | | |
| SRC149 | H | Aus3 | Vic | 2003 | 12/M | + |
| SRC147 | FT | Aus3 | Vic | 2003 | – | + |
| SRC148 | FT | Aus3 | Vic | 2003 | – | + |
| Control | | | | | | |
| SRC50 | H | RDNC | Vic | 2001 | 14/M | +** |

*All isolates were resistant to ampicillin, chloramphenicol, streptomycin, spectinomycin, sulfonamides, and tetracycline.



aquariums of 5 patients with RDNC Aus3-type infections, and identical isolates were recovered from each fish tank. Four matched sets of isolates, 2 from 2000 and 2 from 2003, were further examined (Table). One isolate (SRC50) characterized previously (*7*) was used as a control (Table).

To determine if the resistance phenotype of these strains was due to SGI1 (*4,5,7,8,11*), polymerase chain reaction (PCR) with primer pairs shown in Figure 1 was used as previously described (*7*). The left and right junctions of SGI1 with the chromosome and of In104 with SGI1 were present in all cases. Regions containing the gene cassettes were amplified by using standard primers (L1 and R1) in the 5′- and 3′-conserved segments of class 1 integrons. Fragments of 1.0 and 1.2 kb were amplified from all isolates, and digestion of these amplicons with *Rsa*I generated a profile (data not shown) that was indistinguishable from the pattern for the 2 amplicons containing the *aadA2* and *blaP1* cassettes found in In104 and *S*. Paratyphi B dT+ isolates SRC49 and SRC50 from 2001 (*7*). The *aadA2* gene cassette was linked to SO26 in the SGI1 backbone, which indicates that it is on the left, as in In104, and the expected 1.8-kb PCR fragment was generated by using primers in *groEL* and *blaP1* (Figure 1), which places the *blaP1* cassette on the right. Southern hybridization of *Xba*I–digested whole-cell DNA with a probe for the *floR* gene as described previously (*7*) identified a band of ≈12 kb, which is consistent with an SGI1 structure identical to that reported previously (*7,8,11*) and the *groEL-blaP1* amplicon linked this 12-kb *Xba*I fragment with the adjacent 4.3-kb *Xba*I fragment (Figure 1).

To obtain further evidence for the identity of the matched human and fish tank isolates, macrorestriction analyses of *Xba*I-digested whole-cell DNA by pulsed-field gel electrophoresis (PFGE) were performed as previously described (*12*). Several studies (*3–6,13*) suggest that *S*. Paratyphi B dT+ isolates possess considerable genetic heterogeneity. However, the SGI1-containing isolates appear to be homogeneous. The band patterns for all SGI1-containing *S*. Paratyphi B dT+ were identical from humans and fish tanks with phage type RDNC Aus3 (Figure 2A) and Aus2 (data not shown). IS*200* profiles were also analyzed by hybridization of an IS*200* probe with *Pst* I-digested whole-cell DNA as described elsewhere (*6*). Again, all strains showed identical profiles (Figure 2B and data not shown) that differed by 1 band from profile IP1 recently described (*6*). Thus, matched isolates from humans and their fish tanks were indistinguishable from each other.

An unusual observation in this study was that isolates with different phage types showed identical PFGE and IS*200* profiles, indicating that they represented a clonal cluster. The control strain SRC50 (RDNC) also displayed the same patterns, demonstrating that it also is a member of the same clone. Thus, variation in phage type (Table) appears to have occurred within a single clone. Variation in phage type has also been reported in other studies of multidrug-resistant *S*. Paratyphi B dT+ strains (*4–6*), although

a number of related but slightly different *Xba*I PFGE patterns were observed in those studies. This finding suggests that all multidrug-resistant *S.* Paratyphi B dT+ found globally have a single origin, but that variations, possibly because of acquisition of other temperate phages or plasmids, have arisen over time. However, direct comparisons of strains from different countries will be needed to confirm this hypothesis.

## Conclusions

This is the first definitive report showing that ornamental fish tanks are a reservoir for multidrug-resistant *S*. Paratyphi B dT+ (ApCmSmSpSuTc phenotype) containing SGI1 that causes severe disease in humans, particularly young children. In addition to containing SGI1, the matched isolates from humans and their fish tanks had the same phage type and the same *Xba*I macrorestriction digest pattern and IS*200* profile. These findings identify home aquariums containing tropical fish as the most important, although not necessarily the only, source of multidrug-resistant *S*. Paratyphi B dT+. The fact that 12%–14% of Australian households have ornamental fish (*14*) and as many as 12 million American and 1 million Canadian families own domestic aquariums (*9*), together with the young age of most affected patients, indicate that multidrug-resistant *S*. Paratyphi B dT+ in home aquariums is a risk factor for *Salmonella* infection and thus becomes a public health issue.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## References


1. Chart H. The pathogenicity of strains of *Salmonella* Paratyphi B and *Salmonella* Java. J Appl Microbiol. 2003;94:340–8.
2. Van Pelt W, van der Zee H, Wannet WJ, van de Giessen AW, Mevius DJ, Bolder NM, et al. Explosive increase of *Salmonella* Java in poultry in the Netherlands: consequences for public health. Euro Surveill. 2003;8:31–5.
3. Miko A, Guerra B, Schroeter A, Dorn C, Helmuth R. Molecular characterization of multiresistant d-tartrate-positive *Salmonella enterica* serovar Paratyphi B isolates. J Clin Microbiol. 2002;40:3184–91.

8. Boyd D, Peters GA, Cloeckaert A, Boumedine KS, Chaslus-Dancla E, Imberechts H, et al. Complete nucleotide sequence of a 43-kilobase genomic island associated with the multidrug resistance region of *Salmonella enterica* serovar Typhimurium DT104 and its identification in phage type DT120 and serovar Agona. J Bacteriol. 2001;183:5725–32.
9. Gaulin C, Vincent C, Alain L, Ismail J. Outbreak of *Salmonella* Paratyphi B linked to aquariums in the province of Quebec, 2000. Can Commun Dis Rep. 2002;28:89–93,96.
10. Senanayake SN, Ferson MJ, Botham SJ, Belinfante RT. A child with *Salmonella enterica* serotype Paratyphi B infection acquired from a fish tank. Med J Aust. 2004;180:250.
11. Meunier D, Boyd D, Mulvey MR, Baucheron S, Mammina C, Nastasi A, et al. *Salmonella enterica* serotype Typhimurium DT104 antibiotic resistance genomic island I in serotype Paratyphi B. Emerg Infect Dis. 2002;8:430–3.
12. Thong KL, Ngeow YF, Altwegg M, Navaratnam P, Pang T. Molecular analysis of *Salmonella* Enteritidis by pulsed-field gel electrophoresis and ribotyping. J Clin Microbiol. 1995;33:1070–4.
13. Goh YL, Yasin R, Puthucheary SD, Koh YT, Lim VK, Taib Z, et al. DNA fingerprinting of human isolates of *Salmonella enterica* serotype Paratyphi B in Malaysia. J Appl Microbiol. 2003;95:1134–42.
14. Lehane L, Rawlin GT. Topically acquired bacterial zoonoses from fish: a review. Med J Aust. 2000;173:256–9.

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