Jpn. J. Infect. Dis., 56, 2003

Laboratory and Epidemiology Communications

A New Clonal Line of *Salmonella* Saintpaul Having Emerged and Prevailed since 1999 in Aichi, Japan

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Communicated by Yutaka Miyazaki

(Accepted May 9, 2003)

Though having long been a rare *Salmonella* serotype in Japan, *Salmonella enterica* serotype Saintpaul (antigenic formula, 4:eh:1,2) has become one of the most frequent serotypes throughout Japan including Aichi Prefecture since 1999. In the list of 15 most common *Salmonella* serovars from human sources in Japan (National Institute of Infectious Diseases website http://idsc.nih.go.jp in 2002), *Salmonella* Saintpaul first appeared in 1997 as ranking 9th, and since 1999, its rank has risen every year, i.e.; 1999: 9th, 2000: 7th, 2001: 5th, 2002: 4th. In 2001, Shiga Prefecture experienced a food poisoning outbreak caused by this serotype (1). A nationwide food poisoning outbreak in Germany in 1993 involved multiple *Salmonella* serotypes including Saintpaul (2).

The epidemiology of this serotype in humans is not well known and the reason this serotype has recently become predominant is unknown.

The present study examines the clonal relationship among apparently epidemiologically unrelated Saintpaul serotypes isolated in Aichi Prefecture in 1981 - 2002. We performed pulsed-field gel electrophoresis (PFGE) analysis of *Bln*I- and *Xba*I-DNA digests, antibiotic sensitivity tests, and plasmid profiling on 80 such isolates. Five were obtained from

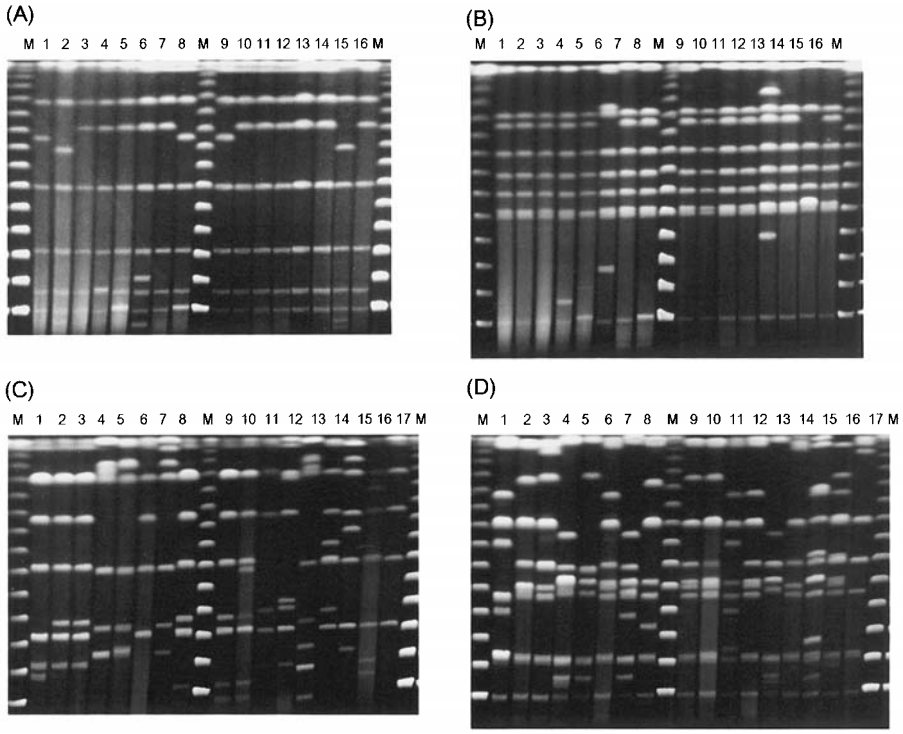

Fig. 1. Examples of PFGE gels of *Bln*I (A, C) and *Xba*I (B, D) macrorestriction fragments of isolates. Figs. A and B: isolates after 1999 (1999 inclusive). 1: 01-35, 2: 01-37, 3: 01-44, 4: 01-62, 5: 01-103, 6: 01-162, 7: 02-5, 8: 02-70, 9: 02-118, 10: 02-152, 11: 02-154, 12: 02-155, 13: 02-212, 14: 02-222, 15: 02-229, 16: 02-235. Figs. C and D: isolates before 1999. 1: 81-23, 2: 81-175, 3: 84-147, 4: 86-133, 5: 87-29, 6: 87-43, 7: 87-227, 8: 88-46, 9: 88-47, 10: 88-48, 11: 93-331, 12: 94-1, 13: 94-158, 14: 95-98, 15: 95-176, 16: 95-178, 17: 96-179. The first two letters of ID numbers denote the year isolated (e.g., 99:1999, 02:2002). Lane M, λ DNA standard (48.5-kb concatemer).

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patients in sporadic cases and the remaining 75 from healthy carriers. Among these 80 strains, 21 strains were isolated in 1981-1998, and the remaining 59 in 1999-2002.

PFGE typing of the Saintpaul serotype was very difficult due to degradation of DNA during the procedure as in the case of many *Clostridium difficile* or *Pseudomonas aeruginosa* strains. Recently, Corkill and others reported that degradation of the DNA of such bacteria was prevented by the addition of 50 μM thiourea in the gel buffer (3,4). The addition of thiourea in the running buffer was effective in our analysis as well. PFGE of DNA digests was performed using Pulsaphor (Pharmacia Biotech AB, Uppsala, Sweden) through 1% agarose in TBE buffer. The pulse times were increased from 5 to 40 s for 20 h.

Strains isolated in 1999-2002 exhibited similar PFGE patterns (Figs. 1A and 1B; representative patterns of 2001-2002 isolates are shown). In contrast, strains isolated before 1999 exhibited diverse PFGE patterns (Figs. 1C and 1D).

Inspection of PFGE patterns of *Bln*I-digests and those of *Xba*I-digests identified 20 patterns among 21 strains isolated before 1999, i.e., only one pattern was shared by two isolates, and other patterns were unique to the isolates. Meanwhile, the PFGE patterns of isolates after 1999 were largely similar; we identified 27 non-identical but similar patterns for 59 isolates. The 21 isolates before 1999 and 27 isolates after 1999 (1999 inclusive) with representative PFGE patterns were subjected to cluster analysis by Fingerprinting II software (Bio-Rad Laborotories, Hercules, Calif., USA).

All of the strains except one, i.e., 58/59, isolated after 1999 (1999 inclusive) were grouped into one, cluster I (Fig. 2). Sub-cluster A with over 77% similarity was the majority (91%) of cluster I. Several isolates were identical in the cluster analysis (sub-sub-clusters a, b, c in Fig. 2). All isolates before 1999, except one (98-298), belonged to the clusters other than the cluster I, and shared less than 48% similarity with those in the cluster I. Isolates later than 1999 were probably clonal in origin.

In Japan, the most prevalent serotype in human salmonellosis is Enteritidis (5) and is often associated with contaminated eggs. As in the case of nationwide food poisoning in 1999 attributed to cuttlefish contaminated by *S.* Oranienburg and *S.* Chester (6), the surge in the prevalence of Saintpaul serotype could be brought about consumption of a particular contaminated food by a large population.

We tested several other typing methods for their applicability to epidemiological investigation of Saintpaul serotypes. As shown below, none of them worked as well as PFGE.

(1) Ribotyping with the 16S rRNA gene probe using several enzymes such as *Eco*RI, *Sma*I, *Pvu*II, and *Pst*I revealed only one or two band differences among the strain examined, suggesting poor discrimination.

(2) Most serotypes contain a 708-bp long, *Salmonella*-specific insertion element IS*200*, which can be applicable for molecular epidemiology (7). *S.* Saintpaul lacked this IS element.

(3) Plasmids were detected in seven out of 80 strains (Fig. 3). Five were isolated before 1999; no particular plasmid pattern predominated. The plasmid profiles of two isolates were similar (lanes 6: 99-318 and 7: 00-162). They were isolated after 1999 and shared an 87% similarity in PFGE (Fig. 2). As plasmids were detected only in a minority, 5/21 before 1999 and 2/59 after 1999, plasmid profiling was not of much value for the Saintpaul

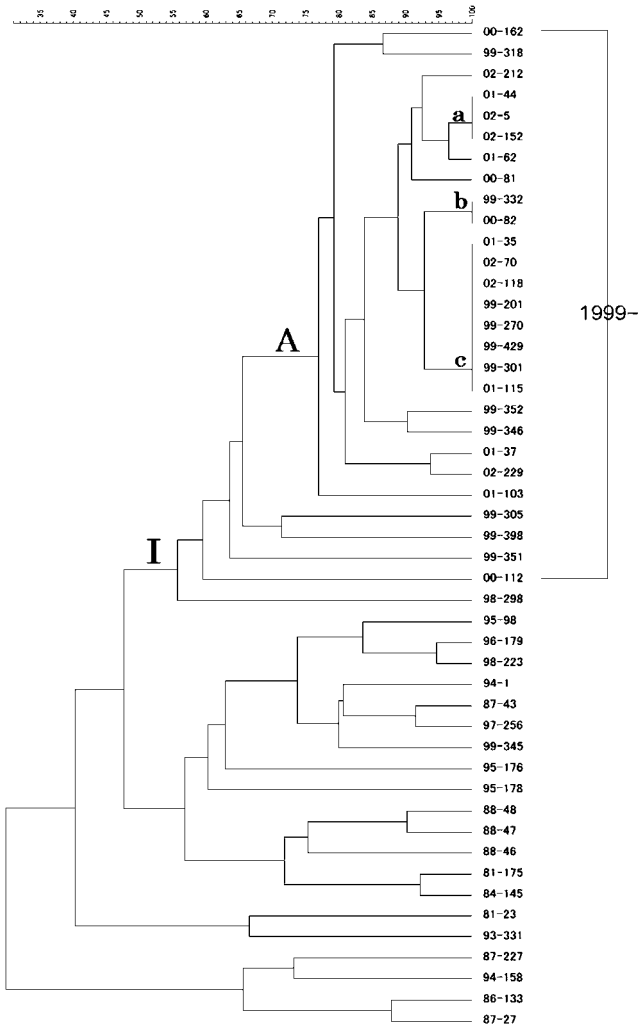

Fig. 2. Dendrogram generated by Fingerprinting II software showing the relationship of 48 representative fingerprints (*Bln*I and *Xba*I) for 80 isolates of *S.* Saintpaul. The first two letters of the ID numbers represent the year isolated. The analysis of the generated bands was performed using the Dice coefficient and the unweighted pair group method with arithmetic averages (UPGMA). Scale indicates percentage of similarity. The strains isolated from 1999 to 2002 belong to cluster I, while the strains isolated before 1999 belong to other clusters.

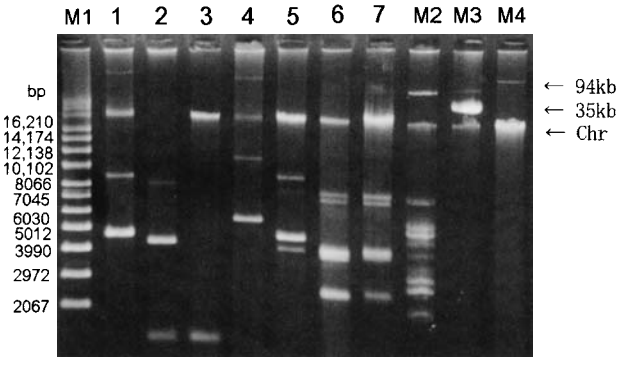

Fig. 3. Plasmid profile. Plasmids were detected in 7 isolates. Five of them were isolated before 1999. Two isolates were resistant to drugs. Lane 1: 81-23, 2: 86-133, 3: 87-27, 4: 94-158 (resistant to streptomycin, tetracycline, minocycline), 5: 95-176, 6: 99-318, 7: 00-162 (resistant to tetracycline, minocycline), M1: Supercoiled DNA ladder, M2~4: Plasmids of known size; M2: V517 (54, 7.3, 5.6, 5.2, 4.0, 3.0, 2.7, and 2.1 kb), M3: 291/s-a (35 kb), M4: CSH-2/NR2 (94 kb). Chr: chromosomal DNA.

serotype.

(4) We examined patterns of antimicrobiotic sensitivity for ampicillin, cefaloridine, kanamycin, streptomycin, tetracycline, minocycline, fosfomycin, chloramphenicol, colistin, nalidixic acid, pipemidic acid, and norfloxacin by using Antibiotic Disks (KB disc, Eiken Chemical Co., Tokyo). Among 80 isolates, only two showed resistance to drugs. One isolate (00-162) was resistant to tetracycline and minocycline, and the other (94-158) to streptomycin, tetracycline, and minocycline. Both strains harbored plasmids (Fig. 3, lanes 4 and 7).

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