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Bacteremia Caused by a Lactose-Fermenting, Multiply Resistant *Salmonella typhi* Strain in a Patient Recovering from Typhoid Fever

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A female patient suffered a typical attack of typhoid fever due to a lactose-negative, fully susceptible *Salmonella typhi* strain. During convalescence she became febrile, and a lactose-fermenting *S. typhi* strain resistant to ampicillin, chloramphenicol, tetracycline, and trimethoprim was isolated from blood culture. This isolate was shown to harbor a plasmid which cotransferred lactose fermentation and antibiotic resistance.

Salmonella typhi is an endemic pathogen in many regions of South Africa. The majority of isolates in South Africa, as elsewhere, are lactose negative and susceptible to the antibiotics used in the treatment of typhoid fever. However, numerous reports have appeared in the literature that describe clinical isolates of *S. typhi* which are resistant to one or more antibiotics (1, 4, 7, 12, 15, 18, 20). Although lactose-fermenting salmonellae are not unusual (5, 8), the natural occurrence of lactose fermentation in *S. typhi* strains is rare. This phenomenon was first reported by Falkow and Baron in 1962 (6). A second case of typhoid fever due to a lactose-fermenting *S. typhi* strain was described by Kunz and Ewing in 1965 (13), but this was thought to be due to a manipulated laboratory strain of *S. typhi*. Recently, Kohbata et al. (12) encountered a case of typhoid fever caused by an *S. typhi* strain which was both lactose fermenting and resistant to many antibiotics. This was the first time such an isolate had been recorded. In this paper we describe a case of typhoid fever in which a similar organism was isolated.

CASE REPORT

A 15-year-old girl, who had been in a typhoid-endemic region the previous month, was admitted to hospital on 31 January 1985 with symptoms typical of typhoid fever. She had been ill for 3 weeks prior to admission. A fully susceptible, lactose-negative *S. typhi* strain was isolated from the patient's blood. The Widal test showed antibodies to *S. typhi* O and H antigens at titers of 1:3,200 and 1:800, respectively. *S. typhi* was not isolated from stool specimens, which contained blood and mucus, but microscopy of these specimens revealed trophozoites of *Entamoeba histolytica* which exhibited erythrophagocytosis.

Initially the patient was treated with ampicillin (2 g intravenously every 6 h [q6h]), cloxacillin (2 g intravenously q6h), and co-trimoxazole (10 ml, containing 160 mg of trimethoprim and 800 mg of sulfamethoxazole, intravenously twice a day) to ensure broad-spectrum antibiotic cover. At 3 days after admission her therapy was changed to metronidazole (500 mg perorally q8h) to treat her amebic dysentery and chloramphenicol (1 g intravenously q6h) to treat her typhoid fever. Ampicillin therapy was continued. She failed to respond to therapy, and her condition deteriorated. She developed a generalized ileus, suggesting a perforation of the bowel, for which she was treated supportively and without surgical intervention. On day 10 after admission she became afebrile, and thereafter her condition steadily improved. Treatment with chloramphenicol and metronidazole was terminated on day 20, but the ampicillin therapy (1 g perorally q6h) was continued. During her illness she developed a large decubitus ulcer, and she therefore remained in hospital for further treatment. At 5 weeks after initial presentation, she became markedly febrile again with a temperature of 40.7°C. This suggested a relapse of typhoid fever. Two gram-negative organisms, one lactose fermenting and the other lactose negative, were isolated from blood culture. Identification tests showed that the lactose-negative strain, designated *S. typhi* LN, was a typical *S. typhi* strain. The lactose-fermenting strain had the same profile as *S. typhi* LN, except that it was lactose positive and Kligler iron agar negative. The lactose-fermenting strain was designated *S. typhi* LF and was shown to be multiply resistant. Treatment with cefotaxime was initiated, and ampicillin therapy was discontinued. The patient rapidly became afebrile and made an uneventful recovery. Fecal cultures performed after the initiation of cefotaxime therapy failed to yield *S. typhi*. The patient was investigated at this stage for localized foci of infection, but both the bone scan and the abdominal sonar scan performed were negative.

The isolation of a lactose-fermenting, multiply resistant *S. typhi* strain prompted further investigation of this organism and the genetic basis of its unusual properties.

MATERIALS AND METHODS

On subculturing the lactose-fermenting, multiply resistant *S. typhi* strain, it was noted that it spontaneously segregated into lactose-positive and lactose-negative elements within a single clone. Both elements were individually propagated, and the lactose-fermenting segregant was maintained on medium containing ampicillin.

The strain designated *S. typhi* LN was the original lactose-negative organism, *S. typhi* LF was the lactose-fermenting, multiply resistant isolate, and *S. typhi* LF rev was the spontaneous, lactose-negative revertant from *S. typhi* LF. *Escherichia coli* CSH 56 (F^- *lac*$^-$) was obtained from the Cold Spring Harbor collection and used as a recipient in conjugation studies. *S. typhi* LN* was a naladixic acid-resistant mutant generated from *S. typhi* LN.

Serotyping. Serotyping was performed with commercially prepared antisera (obtained from Hoechst-Roussel Pharmaceuticals Inc. and Wellcome Laboratories) by the methods described by Kauffman (10).

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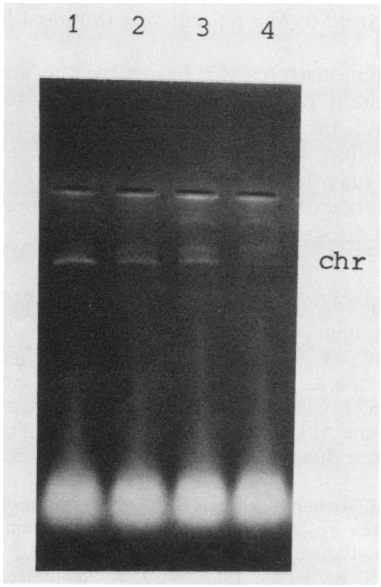

FIG. 1. Gel electrophoresis of DNA extracted from *S. typhi* LN (lane 1), *S. typhi* LF rev (lane 2), *S. typhi* LF (lane 3), and *S. typhi* LF-*E. coli* transconjugant (lane 4). chr, Chromosomal DNA.

MICs. After screening for antimicrobial susceptibility by disk diffusion by the method of Bauer et al. (2), we determined MICs of antibiotics to which the organism was resistant. MICs of the following antimicrobial agents were determined by the microdilution broth method as approved by the National Committee for Clinical Laboratory Standards (19): ampicillin (Beecham Laboratories), tetracycline hydrochloride (Lederle Laboratories), chloramphenicol (Parke, Davis & Co.), and trimethoprim (Wellcome).

Bacteriophage typing. Phage typing was performed by the method of Craigie and Felix (3) with bacteriophage preparations supplied by the Public Health Laboratory Services, Colindale, England.

Conjugation studies. Conjugation experiments were performed by the method described by Miller (17). Briefly, donor and recipient organisms were grown in Mueller-Hinton broth (Oxoid) overnight at 37°C. (All growth media for *S. typhi* LF contained 20 mg of ampicillin per liter to eliminate antibiotic-susceptible segregants.) Fresh broth media were inoculated 1:50 with the overnight cultures, and the cultures were grown at 37°C until they reached 2×10^8 to 3×10^8 CFU/ml. Donor organisms were spun down, washed, and suspended in fresh medium without ampicillin. The conjugation mixture was prepared by adding 1 ml of the donor cell culture to 9 ml of the recipient cell culture and was incubated at 37°C for 2 h. Portions (1 ml) were removed, vortexed, and added to 19 ml of Mueller-Hinton agar (Oxoid) containing the appropriate antibiotics. Conjugants were selected by adding nalidixic acid (50 mg/liter) and tetracycline (50 mg/liter) to the media. The plates were incubated at 37°C and read after 24 h.

DNA extraction and electrophoresis. Screening for plasmids was performed by the method of Kado and Liu (9), and the DNA was separated by gel electrophoresis with 0.7% agarose gels as described by Maniatis et al. (16).

Screening for potential plasmid-donating bacteria in ward contacts. Stool specimens from the patient and her ward contacts were obtained and screened for lactose-fermenting bacteria with antibiograms similar to the resistance pattern of the *S. typhi* LF isolate. Candidate strains were subsequently examined for plasmids by the techniques described above.

RESULTS

Serotyping of *S. typhi* LN, *S. typhi* LF, and *S. typhi* LF rev demonstrated the presence of the O, H, and Vi antigens on all three isolates, confirming the identification.

MIC determinations showed that *S. typhi* LN and *S. typhi* LF rev were fully susceptible to all antibiotics tested, while *S. typhi* LF was resistant to ampicillin (MIC, 256 mg/liter), tetracycline (256 mg/liter), chloramphenicol (256 mg/liter), and trimethoprim (>512 mg/liter).

Phage typing of each organism showed that *S. typhi* LN and *S. typhi* LF rev were phage type A and that *S. typhi* LF was a degraded Vi strain.

The fact that the *S. typhi* LF spontaneously segregated into lactose-fermenting and lactose-nonfermenting components with different antibiograms suggested that both determinants are located on a plasmid which was unstable. Plasmid screens of these organisms showed the presence of a single plasmid (>200 kilobases) in the *S. typhi* LF strains but not in the other two organisms (Fig. 1).

Conjugation studies with *S. typhi* LF as donor and *E. coli* CSH 56 and *S. typhi* LN* as recipients revealed that the plasmid was self-transmissible and that the lactose fermentation and antibiotic resistance determinants were cotransferred. The transfer frequency was 3×10^{-6} for both recipients. Subsequent phage typing and screening for plasmids of *S. typhi* LN* transconjugants showed them to be Vi degraded and to contain a plasmid (Fig. 1). The results are summarized in Table 1.

In our attempts to find the origin of the *S. typhi* LF plasmid, five possible candidates were obtained. There were three isolates of *E. coli* from ward contacts and two *Klebsiella pneumoniae* strains from the patient. When these isolates were screened for plasmids, it was found that none of them contained plasmids identical in size to that in *S. typhi* LF (Fig. 2).

DISCUSSION

The acquisition by *S. typhi* of R plasmids conferring resistance to many antibiotics is well documented. The patterns of resistance vary considerably: some isolates are resistant to one antibiotic only (14), while others have been reported to be resistant to four or five antibiotics (7, 11; E. J. Threlfall, E. R. Ward, B. Rowe, and R. Robins-Brown, Letter, Lancet i:740, 1982). The plasmids conferring resistance have also been classed in a variety of incompatibility groups including C, F_{II}, H_I, and W. Until recently, there were no reports of *S. typhi* isolates which were both multiply

TABLE 1. Characteristics of original and experimental strains of *S. typhi*

Strain	Antibiogram	Lactose fermentation	Phage type	Plasmid
S. typhi LN	Susceptible	−	A	None
S. typhi LF	Ap^r Cm^r Tc^r Tp^r	+	Vi-deg[a]	>200 kb
S. typhi LF rev	Susceptible	−	A	None
S. typhi LF-*E. coli* CSH 56	Ap^r Cm^r Tc^r Tp^r	+		>200 kb
S. typhi LF-*S. typhi* LN[a]	Ap^r Cm^r Tc^r Tp^r	+	Vi-deg	>200 kb

[a] Vi-deg, Vi-degraded strain.

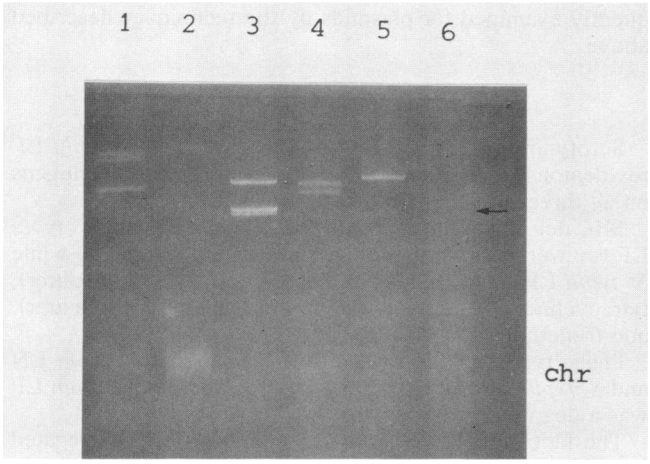

FIG. 2. Gel electrophoresis of DNA extracted from *K. pneumoniae* (lane 1), *S. typhi* LF (lane 2), *E. coli* 1 (lane 3), *E. coli* 2 (lane 4), *E. coli* 3 (lane 5), and *Agrobacterium tumefasciens* C58 (lane 6). Arrow denotes 200-kb plasmid marker. chr, Chromosomal DNA.

resistant and lactose fermenting. However, in 1983 Kohbata et al. (12) reported the isolation of an *S. typhi* strain which was resistant to ampicillin, chloramphenicol, sulfonamides, trimethoprim, gentamicin, kanamycin, tobramycin, and cephalosporins and which was capable of fermenting lactose. The lactose fermentation and some drug resistance determinants were transferable, which suggested that these characteristics are plasmid borne. However, details of this plasmid have not yet been published. To the best of our knowledge, the *S. typhi* strain described here is only the second such isolate to be reported. The resistance profile of this isolate (Ap^r Cm^r Tc^r Tp^r) differs somewhat from that of the Japanese isolate, but they are similar in a number of other respects. Both plasmids contain the lactose fermentation determinant, both are transmissible, and both are unstable in the organism. The incompatibility groups of the plasmids have not been established.

With regard to clinical aspects of the two cases of thyphoid fever caused by multiply resistant, lactose-fermenting strains of *S. typhi*, the patient reported by Kohbata et al. (12) underwent cholecystectomy prior to his episode of typhoid fever, which uncharacteristically presented with diarrhea. The authors unfortunately did not state whether the patient received antimicrobial therapy during or after his gall bladder operation. Clinically, our patient's second illness, which was relatively mild and of short duration, was not a typical relapse of typhoid fever. The most likely source of the *S. typhi* LF strain during the second bacteremic illness was the gastrointestinal tract, and this episode was caused by an organism that was similar to, but not identical with, the original invasive strain.

It appears from the investigations performed that the original antibiotic-susceptible *S. typhi* LN isolate acquired a plasmid in vivo which altered the antibiotic resistance profile, phage type, and lactose-fermenting ability of the organism. Such in vivo acquisition of R plasmids has been described previously (4; Threlfall et al., letter). Although an attempt was made to determine the source of the plasmid, it was unsuccessful. There were no other cases of typhoid fever in the ward at the time, and no *S. typhi* LF strains were detected in samples taken from ward contacts. It can therefore be speculated that a member of the family *Enterobacteriaceae* harbored by the patient was the most likely donor of the plasmid.

This case demonstrates the hazard of the emergence of antibiotic-resistant pathogenic bacteria as a result of the concurrent and prolonged use of several antimicrobial agents. In addition, the occurrence of a lactose-fermenting *S. typhi* strain may delay the diagnosis of typhoid fever, and when the presence of the organism is not suspected, it may be overlooked altogether.

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