JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2005, p. 1470–1472 Vol. 43, No. 3
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.3.1470–1472.2005

Typhoid Fever Associated with Acute Appendicitis Caused by an H1-j Strain of *Salmonella enterica* Serotype Typhi

Susanna K. P. Lau, Patrick C. Y. Woo, Clair Y. F. Chan, Wai-Lan Woo, Gibson K. S. Woo, and Kwok-Yung Yuen*

Department of Microbiology, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Received 2 September 2004/Returned for modification 24 October 2004/Accepted 3 November 2004

While most strains of *Salmonella enterica* serotype Typhi, the etiologic agent of typhoid fever, have only a phase 1 flagellar antigen, H1-d, variations of the flagellar antigen have been observed. Although H1-j strains (one of the flagellar antigen variants) account for 10 to 50% of *S. enterica* serotype Typhi strains found in Indonesia, there have been no published data to suggest its existence in other parts of the world. We describe a case of typhoid fever associated with acute appendicitis caused by an *S. enterica* serotype Typhi H1-j strain in a Chinese woman in Hong Kong. A gram-negative, motile rod was recovered from her blood and stool cultures. Conventional biochemical tests and the Vitek system (GNI+) showed that the bacterium was *S. enterica* serotype Typhi. The isolate agglutinated with poly(O), 9O, Vi and H1-j *Salmonella* antisera but not with poly(H) antisera. The patient developed antibodies against only *S. enterica* serotype Typhi O antigens but not against H1-d antigen by the Widal test. Flagellin C gene (*fliC*) sequencing showed a 261-bp deletion in the *fliC* gene of the isolate, confirming that the isolate possessed the H1-j antigen. The patient had no past history of travel to Indonesia or personal contact with any Indonesian. She recovered with appendectomy and antibiotic treatment. Further studies should be performed to determine the prevalence of this unusual *S. enterica* serotype Typhi strain in our locality.

CASE REPORT

A 52-year-old Chinese woman was admitted to the hospital because of right-lower-quadrant pain and fever for 1 day associated with vomiting, chills, and rigor. She also complained of having decreased appetite, diarrhea, and abdominal discomfort for 1 month. She had history of pelvic actinomycosis related to an intrauterine contraceptive device treated by surgical drainage and prolonged antibiotic treatment 5 years ago. She had no recent travel history or past history of travel to Indonesia. On admission, her oral temperature was 39.9°C. Physical examination revealed tenderness, guarding, and rebound tenderness over the right lower quadrant of the abdomen. Blood and stool cultures were performed. Her total leukocyte count was 6.9×10^9/liter (neutrophils, 5.7×10^9/liter; lymphocytes, 0.7×10^9/liter), her hemoglobin level was 13.6 g/dl, and her platelet count was 212×10^9/liter. She had hyponatremia (sodium, 127 mmol/liter), hypokalemia (potassium, 2.9 mmol/liter), and elevated liver enzymes (alkaline phosphatase, 181 U/liter; alanine aminotransferase, 182 U/liter; aspartate aminotransferase, 177 U/liter; and γ-glutaryl transferase, 136 U/liter). Her renal function tests were within normal limits. An abdominal radiograph revealed an appendicolith inferior to the right sacroiliac joint. Ultrasound scan of the abdomen showed a tubular lesion with hyperemia and target appearance on transverse section anterior to the cecum, suggestive of an inflamed appendix. Empirical intravenous cefuroxime and metronidazole were administered, and an emergency appendectomy was performed. At operation, an inflamed appendix with ulcerated mucosa was found. Histological examination of the appendix showed the presence of erosion and transmural inflammation extending into the subserosa, neutrophilic cryptitis, and crypt abscesses. Fever subsided and she recovered uneventfully after the operation. In view of her blood and stool culture results, blood was sent for a Widal test and she was given oral ciprofloxacin. She was discharged after 10 days of hospitalization.

* Corresponding author. Mailing address: Department of Microbiology, The University of Hong Kong, University Pathology Building, Queen Mary Hospital, Hong Kong. Phone: (852) 28554892. Fax: (852) 28551241. E-mail: hkumicro@hkucc.hku.hk.

Salmonella enterica serotype Typhi, the causative agent of typhoid fever, is classified under the species *S. enterica*. Since there has been no good genotypic standard for classification within *S. enterica*, serotypes are defined traditionally by the possession of various somatic, flagellar, and capsular antigens (1, 2). We have previously described the identification of an *S. enterica* serotype Typhi variant by a combination of conventional serotyping and flagellin (*fliC*) and CDP-tyvelose epimerase (*rfbE*) gene sequencing (12). A recent study showed that flagellin genes may be useful targets for the molecular determination of flagellar antigen type (8). Identification of serotypes is important in understanding the epidemiology of *Salmonella* and implementing public health measures. While *S. enterica* serotype Typhi typically has the H1-d flagellar antigen, the H1-j serotype has only been described in Indonesia. In the present report, we describe the characterization of an *S. enterica* serotype Typhi H1-j strain isolated from a patient with typhoid fever and acute appendicitis in Hong Kong by both conventional microbiological tests and *fliC* gene sequencing.

Clinical and microbiological data. All clinical data were collected prospectively as described in our previous publication (7). Clinical specimens were collected and handled according to standard protocols, and all suspect colonies were identified

by standard conventional biochemical methods (9) and the Vitek system (GNI+) (bioMerieux Vitek, Durham, N.C.). On day 2 postincubation, the aerobic blood culture bottle turned positive with a gram-negative, motile rod. It grew on blood agar, chocolate agar, and MacConkey agar as colonies 4 mm in diameter after 24 h of incubation at 37°C in ambient air. It fermented glucose, reduced nitrate, and did not produce cytochrome oxidase, typical for a member of the family *Enterobacteriaceae*. Standard conventional biochemical tests and the Vitek system (GNI+) (bioMerieux Vitek) showed that the biochemical profile of the strain was compatible with *S. enterica* serotype Typhi. The isolate agglutinated with poly(O), 9O (by tube agglutination with antiserum diluted to 1:160), and Vi antisera but not with poly(H) *Salmonella* antisera (Murex Biotech Ltd., Temple Hill, Dartford, United Kingdom). When tested with individual H antisera, the isolate only agglutinated with Hj antisera (Statens Serum Institut, Artillerivej, Copenhagen, Denmark). The strain was sensitive to ampicillin, cephalothin, cefuroxime, ceftazidime, cefotaxime, ceftriaxone, ciprofloxacin, gentamicin, amikacin, cotrimoxazole, amoxicillin-clavulanic acid, piperacillin-tazobactam, and imipenem. Cultures of her stool collected on day 3 and 4 after admission also recovered a gram-negative rod of the same biochemical and antibiotic susceptibility profiles. The Widal test performed on sera obtained on 7 and 11 days after admission showed antibody titers of 1:400 for TO and < 1:50 for TH, AH, BH, and CH. Ciprofloxacin was continued for a total of 14 days. The patient has remained asymptomatic up to the time of writing, 10 months from discharge.

Flagellin C gene (*fliC*) sequencing. Bacterial DNA extraction was modified from our previous published protocol (12, 13). PCR amplification and DNA sequencing of the *fliC* gene were performed according to previous published protocols (4, 12), using primers LPW1856 (5′-ATGGCACAAGTCATTAA TACAAAC-3′) and LPW1857 (5′-TTAACGCAGTAAAGA GAGGACGTT-3′) (Gibco BRL, Rockville, Md.). The PCR product was gel purified with the QIAquick PCR purification kit (QIAgen, Hilden, Germany). Both strands of the PCR product were sequenced with an ABI 377 automated sequencer according to the manufacturers' instructions (Perkin-Elmer, Foster City, Calif.), using the PCR primers LPW1856 and LPW1857. The sequence of the PCR product was compared with known flagellin gene sequences in the GenBank database by multiple sequence alignment with the CLUSTAL W program (11). PCR of the flagellin gene of the bacteria showed a band at about 1,260 bp. There was a 261-bp deletion in the *fliC* gene of the isolate, showing that the isolate possessed the H1-j antigen.

The bacterium isolated from our patient was confirmed to be an H1-j strain of *S. enterica* serotype Typhi by a combination of phenotypic and genotypic tests. Conventional biochemical tests and commercially available kits showed that the isolate was compatible with *S. enterica* serotype Typhi. However, the isolate did not agglutinate with poly(H) (which did not include H1-j antigen), and the patient did not show an antibody response to H1-d antigen. Further testing with individual H antisera and flagellin gene sequencing showed that the isolate possessed an unusual phase 1 flagellar antigen, the H1-j antigen, encoded by the *fliC-j* gene, which was believed to have arisen from a 261-bp deletion of *fliC-d* gene (4). In fact, the isolate was less motile than the usual *S. enterica* serotype Typhi isolates, which is in line with H1-j strains previously described (4, 5).

The present report represents the first documented case of *S. enterica* serotype Typhi infection due to an H1-j strain outside Indonesia. *S. enterica* serotype Typhi typically only has a phase 1 flagellar antigen, H1-d. Variants of *S. enterica* serotype Typhi possessing the H1-j antigen instead of the H1-d antigen were first identified as laboratory mutants from serum selection in 1936. In 1981, clinical isolates of H1-j strains were found in Indonesia, with some possessing a second flagellar antigen, z66, which is still not well characterized (4, 6). Subsequently, Frankel et al. identified a 261-bp deletion in the central antigenic determinant part of the *fliC-d* gene of H1-j strains responsible for the flagellar antigen variation and proposed that such a deletion was the result of an intragenic homologous recombination involving two 11-bp direct repeats (4). Although H1-j strains account for 10 to 50% of all *S. enterica* serotype Typhi isolates in Indonesia (4, 5), there have been no published data to suggest the existence of this particular serotype in other parts of the world. In a study from Korea, only 1 of the 375 *S. enterica* serotype Typhi isolates tested was shown to possess the H1-j antigen. However, the isolate was cultured from a Korea-Indonesian man who has already been symptomatic in Indonesia and therefore was an Indonesian strain (10). Our patient has never traveled to Indonesia nor has personal contact with any Indonesian. Therefore, the origin of the present isolate remains to be determined. Further studies are required to determine whether it has arisen from mutations of the *fliC* gene in a local strain to H1-j or been imported from Indonesia. Since there are more than 60,000 Indonesian domestic helpers working in Hong Kong, an H1-j strain of *S. enterica* serotype Typhi may have been imported and become endemic by person-to-person transmission. To better understand the epidemiology and potential for emergence of this atypical serotype, studies should be performed to determine the prevalence of H1-j variants in our locality.

Although *S. enterica* serotype Typhi is known to cause an appendicitis-like syndrome due to mesenteric adenitis, genuine appendicitis due to *S. enterica* serotype Typhi has not been reported in the literature. The present report documents a case of typhoid fever associated with acute appendicitis confirmed by histology. However, it is not known if the H1-j antigen in the present isolate contributes to its pathogenesis. In a previous study comparing the invasiveness and clinical illness of H1-d and H1-j flagellar serotypes of *S. enterica* serotype Typhi isolated from patients with typhoid fever, it was found that patients with H1-j infection were older and had milder clinical illness. Moreover, H1-j isolates were less motile and less invasive than H1-d isolates in the Hep-2 cell culture system (5). However, earlier studies were unable to make similar correlation (3). Further work is required to clarify the potential relationship between antigenic properties and pathogenicity. Although the present isolate is also less motile, it caused a severe and prolonged course of disease associated with appendicitis in our patient. Testing the present isolate along with other H1-j and H1-d strains of *S. enterica* serotype Typhi in cell culture system may provide useful data on its relative invasiveness.

This work was partly supported by the University Development Fund and Committee of Research and Conference Grants, The University of Hong Kong, and Tung Wah Fund for Research in Infectious Disease.

REFERENCES

1. **Brenner, F. W., R. G. Villar, F. J. Angulo, R. Tauxe, and B. Swaminathan.** 2000. *Salmonella* nomenclature. J. Clin. Microbiol. **38:**2465–2467.
2. **Christensen, H., S. Nordentoft, and J. E. Olsen.** 1998. Phylogenetic relationships of *Salmonella* based on rRNA sequences. J. Syst. Bacteriol. **48:**605–610.
3. **Franco, A., C. Gonzalez, O. S. Levine, R. Lagos, R. H. Hall, S. L. Hoffman, M. A. Moechtar, E. Gotuzzo, M. M. Levine, and D. M. Hone.** 1992. Further consideration of the clonal nature of *Salmonella typhi*: evaluation of molecular and clinical characteristics of strains from Indonesia and Peru. J. Clin. Microbiol. **30:**2187–2190.
4. **Frankel, G., S. M. Newton, G. K. Schoolnik, and B. A. Stocker.** 1989. Intragenic recombination in a flagellin gene: characterization of the *H1-j* gene of *Salmonella typhi*. EMBO J. **8:**3149–3152.
5. **Grossman, D. A., N. D. Witham, D. H. Burr, M. Lesmana, F. A. Rubin, G. K. Schoolnik, and J. Parsonnet.** 1995. Flagellar serotypes *of Salmonella typhi* in Indonesia: relationships among motility, invasiveness, and clinical illness. J. Infect. Dis. **171:**212–216.
6. **Guinee, P. A., W. H. Jansen, H. M. Maas, L. Le Minor, and R. Beaud.** 1981. An unusual H antigen (Z66) in strains of *Salmonella typhi*. Ann. Microbiol. (Paris) **132:**331–334.
7. **Luk, W. K., S. S. Y. Wong, K. Y. Yuen, P. L. Ho, P. C. Y. Woo, R. A. Lee, and P. Y. Chau.** 1998. Inpatient emergencies encountered by an infectious disease concultative service. Clin. Infect. Dis. **26:**695–701.
8. **McQuiston, J. R., R. Parrenas, M. Ortiz-Rivera, L. Gheesling, F. Brenner, and P. I. Fields.** 2004. Sequencing and comparative analysis of flagellin genes *fliC*, fljB, and *flpA* from *Salmonella*. J. Clin. Microbiol. **42:**1923–1932.
9. **Murray, P. R., E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Yolken.** 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
10. **Song, J. H., H. Cho, M. Y. Park, Y. S. Kim, H. B. Moon, Y. K. Kim, and C. H. Pai.** 1994. Detection of the H1-j strain of *Salmonella typhi* among Korean isolates by the polymerase chain reaction. Am. J. Trop. Med. Hyg. **50:**608–611.
11. **Thompson, J. D., D. G. Higgins, and T. J. Gibson.** 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:**4673–4680.
12. **Woo, P. C. Y., A. M. Y. Fung, S. S. Y. Wong, H.-W. Tsoi, and K.-Y. Yuen.** 2001. Isolation and characterization of a *Salmonella enterica* serotype Typhi variant and its clinical and public health implications. J. Clin. Microbiol. **39:**1190–1194.
13. **Yuen, K.-Y., P. C. Y. Woo, J. L. L. Teng, K.-W. Leung, M. K. M. Wong, and S. K. P. Lau.** 2001. *Laribacter hongkongensis* gen. nov., sp. nov., a novel gram-negative bacterium isolated from a cirrhotic patient with bacteremia and empyema. J. Clin. Microbiol. **39:**4227–4232.