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 (aspartate aminotransferase, 181 U/liter; alanine aminotransferase, 182 U/liter; aspartate aminotransferase, 177 U/liter; and γ-glutamyl transferase, 136 U/liter). Her renal function tests were within normal limits. An appendicolith inferior to the right sacroiliac joint. Ultrasound scan of the abdomen revealed an appendicolith inferior to the right sacroiliac joint and showed a tubular lesion with hyperemia and target appearance 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.

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 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 cyto-
chrome oxidase, typical for a member of the family Enterobac-
teraceae. Standard conventional biochemical tests and the
Vitek system (GNI+) (bioMerieux Vitek) showed that the bio-
chemical profile of the strain was compatible with S. en-
terica serotype Typhi. The isolate agglutinated with poly(O),
9O (by tube agglutination with antisera 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, Copen-
hagen, Denmark). The strain was sensitive to ampicillin, ceph-
alothin, cefuroxime, cefazidime, cefotaxime, ceftriaxone,
ciprofloxacin, gentamicin, amikacin, cotrimoxazole, amoxicil-
lin-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 (flfC) sequencing. Bacterial DNA extrac-
tion was modified from our previous published protocol (12,
13). PCR amplification and DNA sequencing of the flfC gene
were performed according to previous published protocols (4,
12), using primers LPW1856 (5′-ATGGCAACCGTCTATTG
TACAAAC-3′) and LPW1857 (5′-TTAACCGAGTAAAGA
GAGGACGT-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 se-
quencer according to the manufacturers’ instructions (Perkin-
Elmer, Foster City, Calif.), using the PCR primers LPW1856
and LPW1857. The sequence of the PCR product was com-
pared 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 flfC gene of the isolate, showing that the isolate pos-
sessed 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 re-
response to H1-d antigen. Further testing with individual H an-
tisera and flagellin gene sequencing showed that the isolate
possessed an unusual phase 1 flagellar antigen, the H1-j anti-
gen, encoded by the flfC-j gene, which was believed to have
arisen from a 261-bp deletion of flfC-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 out-
side 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 selec-
tion in 1936. In 1981, clinical isolates of H1-j strains were
found in Indonesia, with some possessing a second flagellar
antigen, 266, which is still not well characterized (4, 6). Subse-
sequently, Frankel et al. identified a 261-bp deletion in the cen-
tral antigenic determinant part of the flfC-d gene of H1-j
strains responsible for the flagellar antigen variation and pro-
posed 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. en-
terica serotype Typhi isolates in Indonesia (4, 5), there have
been no published data to suggest the existence of this partic-
ular serumotype 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 In-
donesia nor has personal contact with any Indonesian. The-
fore, the origin of the present isolate remains to be deter-
mined. Further studies are required to determine whether it
has arisen from mutations of the flfC 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 po-
tential 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 iso-
lated from patients with typhoid fever, it was found that pa-
tients with H1-j infection were older and had milder clinical
illness. Moreover, H1-j isolates were less motile and less inva-
sive than H1-d isolates in the Hep-2 cell culture system (5).
However, earlier studies were unable to make similar correla-
tion (3). Further work is required to clarify the potential rela-
tionship between antigenic properties and pathogenicity. Al-
though 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


