Misidentification of a mucoid strain of *Salmonella enterica* serotype Choleraesuis as *Hafnia alvei* by the Vitek GNI+ card

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ABSTRACT

Nontyphoidal salmonellae are some of the commonest causes of bacterial gastroenteritis worldwide. They are also notable causes of extraintestinal infections including bacteremia and vascular infections. *Salmonella enterica* serotype *Choleraesuis* is typically associated with invasive infections. We report a patient who had an infected intra-abdominal aortic aneurysm due to an unusually mucoid strain of *Salmonella enterica* serotype *Choleraesuis*. The isolate was erroneously identified as *Hafnia alvei* by the Vitek GNI+ card. A blood culture isolate from the same patient 9 months earlier was also identified as *H. alvei* by the Vitek GNI+ card. Despite an apparent cure with intravenous amoxicillin-clavulanic acid at that time, the *Salmonella* infection had not been cleared, and manifested as ruptured infected abdominal aortic aneurysm. Repeated passage of the strain yielded non-mucoid colonies, which were correctly identified by the API and PHOENIX systems. The isolates from the aneurysm and the former bacteremic episode were found to be identical using pulsed field gel electrophoresis. The fallibility of automated bacterial identification systems are highlighted. Such errors are especially important for isolates in which *in vitro* antibiotic susceptibility testing does not correlate with clinical success of treatment, as illustrated by *Salmonella* infections.
CASE REPORT

A 75-year-old man was admitted to our hospital in March 2006 because of persistent back pain and abdominal distention for 2 days. He had history of hypertension, transient ischemic accident, and pulmonary tuberculosis complicated with bronchiectasis. On this admission, his oral temperature was 37.5°C, with blood pressure of 163/87 mmHg and heart rate of 85 beats/minutes. Physical examination revealed distension and tenderness over the lower abdomen. His total leukocyte count was $13.6 \times 10^9$ /liter (neutrophils, $11.8 \times 10^9$ /liter; lymphocytes, $0.8 \times 10^9$ /liter), and his platelet count was $306 \times 10^9$ /liter. The hemoglobin level dropped from 13.7 to 10.7 g/dl within 10 hours after admission, but the clotting profile, liver and renal function tests were all within normal limits. An abdominal radiograph revealed widening of the left psoas shadow. An urgent computed tomography scan revealed an aneurysmal dilatation of the infra-renal aorta extending to aortic bifurcation, with intra-mural thrombus and retroperitoneal hematoma, compatible with ruptured infra-renal abdominal aortic aneurysm. Suturing of the aneurismal sac with interposition tube graft by-pass was performed. The intra-mural thrombus was removed and sent for culture. A Gram-negative bacillus was recovered from the thrombus culture, which was initially identified as *Hafnia alvei*. Review of the patient’s hospital record showed that he had an episode of fever and *H. alvei* bacteremia 9 months ago, which resolved with 2 weeks of intravenous amoxicillin-clavulanic acid. He remained afebrile post-operatively. In view of his intramural thrombus culture result, blood was sent for bacterial culture and Widal test as a serological test for typhoidal salmonellosis while the definitive identification of the bacterial isolates were being performed. He was given intravenous ceftriaxone, and discharged after 22 days of hospitalization.
All clinical specimens were collected and handled according to standard protocols (1). Gram smear of the intramural thrombus revealed the presence of Gram-negative rods, which grew on blood agar, chocolate agar, and MacConkey agar as mucoid colonies of 4 mm in diameter after 24 h of incubation at 37°C in ambient air (Fig. 1A). It fermented glucose, reduced nitrate, and did not produce cytochrome oxidase, typical for a member of the family *Enterobacteriaceae*. Both standard conventional biochemical tests and the Vitek system (GNI+) with software version R09.01 (bioMerieux Vitek, Durham, N.C.) and PHOENIX system with software version 3.34A/3.52F (BD, Sparks, Md.) failed to identify the mucoid strain which did not fit the typical profiles of known bacterial species (1). The former identified the strain as an inactive *Escherichia coli* (31%), and the latter identified the strain as CDC group EF-4a (90%). The isolate also did not agglutinate with poly(O) and poly(H) *Salmonella* antisera (Murex Biotech Ltd., Temple Hill, Dartford, United Kingdom). API 20E (bioMerieux Vitek, Hazelwood, Mo.) was not performed on the mucoid strain. The bacterium was then repeatedly passaged on blood agar until it reverted to a non-mucoid form (Fig. 1B). On conventional biochemical testing, the non-mucoid strain was indole-negative, lactose-non-fermenting, decarboxylated lysine and ornithine, and did not utilize citrate or produce hydrogen sulfide. Vitek GNI+ card identified the non-mucoid strain as *Hafnia alvei* (97%), identical to the strain isolated from blood 9 months ago, which was not mucoid. No additional tests were recommended by the system. Nonetheless, in view of the atypical clinical presentation, testing of the non-mucoid strain with API 20E (bioMerieux Vitek, Hazelwood, Mo.) and PHOENIX (BD, Sparks, Md.) identified the bacterium as...
Salmonella enterica serotype Choleraesuis. Serotyping with Salmonella antisera was performed, which gave an antigenic formula of 6,7:c:1,5 and negative for the Vi antigen. Widal test performed on serum obtained on 20 days after admission showed antibody titers of 200 for CH and < 50 for TO, TH, AH, and BH. As the antigenic formula of Salmonella enterica serotype Choleraesuis (6,7:c:1,5) is similar to that of Salmonella enterica serotype Paratyhi C (6,7,[Vi]:c:1,5), the elevated CH titre was likely to be associated with the cross-reactivity between the two serotypes. The patient’s strain is not Salmonella enterica serotype Paratyphi C because it is negative for the Vi antigen and does not ferment arabinose and trehalose. Ceftriaxone was continued for a total of 21 days. The patient has remained asymptomatic up to the time of writing, 3 months after discharge.

In view of the discrepancy in Vitek system with other commercial systems and the agglutination test result, 16S rRNA sequencing using MicroSeq® 500 16S rRNA Bacterial Identification Kit (Perkin-Elmer Applied Biosystems Division, Foster City, Calif.) was performed according to the manufacturer’s instructions. The DNA sequence was compared with known 16S rRNA gene sequences in the GenBank by multiple sequence alignment using the CLUSTAL W program (2). The strain was identified as S. enterica, with 1% discrepancy from the isolate sequences of S. enterica serotype Choleraesuis.

The so-called H. alvei isolated in July 2005 was re-examined by conventional biochemical tests, Vitek GNI+ system, and the PHOENIX. A discrepancy between the Vitek and PHOENIX systems in identification of the strain was noted again. Agglutination with Salmonella was performed, which gave an identical antigenic formula as the strain isolated from the thrombus. The susceptibility testing by disk diffusion test on this strain of S. enterica serotype Choleraesuis and the non-mucoid...
strain isolated during the current admission was performed according to the Clinical
and Laboratory Standards Institute standard (3), which was identically susceptible to
ampicillin, cefotaxime, ceftriaxone, chloramphenicol, ciprofloxacin, cotrimoxazole and
nalidixic acid. Pulsed-field gel electrophoresis (PFGE) of this patient’s *S. enterica*
serotype Choleraesuis isolates and a strain from another patient was performed.
Protocol for PFGE analysis has been described previously (4). *XbaI* was used for
digestion of DNA in the PFGE analysis. The result, as demonstrated in Fig. 1C,
confirmed that the strains isolated from the blood and the intramural thrombus (both
mucoid and non-mucoid phenotypes) in this patient was identical, but different from
an unrelated strain.
Non-typhoidal salmonellae are associated with self-limiting gastrointestinal
tract infection and other extraintestinal infections, such as intravascular infections,
arthritis, osteomyelitis and bacteremia. Severe or recurrent infections are often seen in
the elderly or immunocompromised hosts. The Choleraesuis serotype is notable in
that it is typically associated with invasive extra-intestinal infections rather than
gastroenteritis (5). The infection is frequently characterized by bacteremia and severe
sepsis with localized pyogenic infections such as osteomyelitis, arthritis, and vascular
infections such as aortitis and mycotic aneurysm. These focal infections require
prolonged and high doses of antibiotics for treatments. Correct identification of this
serotype is therefore crucial to alert the clinician to search for an infective focus and
for proper management of the patients. On the other hand, the role of *Hafnia alvei* as a
human pathogen is less well-established. Sites most likely to yield *Hafnia* spp. as sole
pathogen include urine and blood. *H. alvei* has been reported to be associated with
bacteremia, gastroenteritis, nosocomial pneumonia and urinary tract infections (6), but
has never been reported to cause intravascular infections.
The present report represents the first documented case of misidentification of 
*S. enterica* serotype Choleraesuis to *H. alvei* by the Vitek GNI+ system. The accuracy 
of the system in identifying species of the *Enterobacteriaceae* family has been 
reported to range from 75% to 94.4% (7-10). Table 1 presents a comparison of current 
case report and four other studies on the Vitek GNI+ card having unidentified or 
incorrect identification of non-typhoidal salmonellae. Even though Knight *et al.* (11) 
reported that the accuracy of Vitek system in identifying *Salmonella* spp. from food to 
be as high as 96.7%, the accuracy from human isolates from the studies is only 72%. 
The most commonly documented misidentification is incorrectly identified 
*Salmonella* spp. as *Escherichia coli*. There was also one isolate of *S. enterica* subsp. 
*arizonae* being mistaken as *H. alvei*. We noticed that the reason for misidentification 
was associated with aberrant results of 4 tests: acid formation from sorbitol and 
arabinose, fermentation of O-nitrophenyl-beta-D-galacto-pyranoside and hydrogen 
sulfide production (Table 2). We suggest that these tests be repeated by conventional 
methods if identification of strains is doubtful or atypical.

Misidentification of *Salmonella* spp. is of great concern, especially when the 
bacterium is isolated from extraintestinal sites. When incorrectly identified as *E. coli* 
and *H. alvei*, the choice of antibiotic treatment will be misled. Treatment with 
ampicillin and third-generation cephalosporins (such as ceftriaxone) should clear up 
the bacteremia. However, it is known that using antibiotics such as the first- or 
second-generation cephalosporins – despite apparent in vitro susceptibility – is prone 
to failure and relapse owing to a low intracellular concentrations within the 
phagocytes (12). The third-generation cephalosporins and fluoroquinolones are 
therefore generally considered to be the drug of choice owing to their better 
intracellular penetration. Whether the aorta had already been infected or not during
the earlier episode of bacteremia is unknown. The atherosclerotic aortic might be
seeded by *Salmonella* during that earlier episode of bacteremia, or the aorta might
have been infected prior to that. To address the problem of misidentification, we
suggest that all *Enterobacteriaceae* isolates with unusual biochemical profiles being
identified as *H. alvei* or *E. coli* should be correlated with patients’ clinical picture.
The biochemical profile should be confirmed with conventional testing methods and
alternative identification tests be performed. Serotyping of these isolates should be
performed in situations where *Salmonella* infections are suspected.

Isolation of mucoid variants of *Salmonella* in clinical specimens is unusual,
although they have been found among survivors of selective agents or detrimental
conditions (13). The presence of large amounts of capsular material in mucoid isolates
may interfere with the slide agglutination test. A correct bacterial inoculum is
essential for antibiotic susceptibility testing and biochemical tests. It is notoriously
difficult to prepare an accurate inoculum for highly mucoid strains and this may result
in aberrant biochemical profiles, especially when tested by automated systems (14).
Theses strains must be handled with care. The increased utilization of 16S rRNA
sequencing in clinical laboratories could allow more accurate identification of these
unusual isolates.
ACKNOWLEDGEMENT

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REFERENCES


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<th>Authors (reference)</th>
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<td>R05.01</td>
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<td>53</td>
<td>38 (71.7%)</td>
<td>5 (9.4%)</td>
<td>10 (18.9%)</td>
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TABLE 2. Results of 4 biochemical tests of the patient’s isolate (non-mucoid strain), *Salmonella enterica* serotype Choleraesuis and *Hafnia alvei*.

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<td>Expected results for <em>Hafnia alvei</em></td>
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LEGENDS

FIG. 1. Characteristics of the isolate from thrombus. (A) Mucoid colonies on blood agar after 24 h of incubation at 37°C in ambient air. (B) Change of colony morphology to non-mucoid colonies on blood agar after repeated passaging. (C) PFGE patterns of *Salmonella enterica* serotype Choleraesuis from patients, using *XbaI* for chromosomal DNA digestion. Lane 1 was the mucoid strain isolated from this patient’s intramural thrombus; lane 2 was the non-mucoid strain transformed from the mucoid one; lane 3 was the blood isolate from the same patient 9 months ago; lane 4 was another strain of *Salmonella enterica* serotype Choleraesuis isolated from blood of another unrelated patient, which served as a control for comparison, and lane 5 was 48.5 kb-lambda ladder.