Microbiological Analysis of Nontyphoidal Salmonella Strains Causing Distinct Syndromes of Bacteremia or Enteritis in HIV/AIDS Patients in San Diego, California

Michael J. Preziosi, a Sean M. Kandel, b Donald G. Guiney, a and Sara H. Browne a

Division of Infectious Diseases, Department of Medicine, University of California, San Diego, La Jolla, California, USA, a and Nova Southeastern University College of Osteopathic Medicine, Fort Lauderdale, Florida, USA b

Recurrent invasive nontyphoidal Salmonella (NTS) infection is an AIDS-defining illness that has become less common in the developed world in the era of highly active antiretroviral therapy (HAART), while it has emerged as a major public health problem in developing countries, particularly sub-Saharan Africa. We retrospectively analyzed Salmonella (NTS) infection in HIV/AIDS patients from June 2003 until December 2009 at the University of California, San Diego (UCSD), Medical Center. Bacterial isolates from all patients were tested for selected microbiological properties, including major Salmonella (NTS) virulence loci rpoS, sodC, spvB, and sseL. Fourteen percent of all Salmonella (NTS) cases recorded at the UCSD Medical Center during this period occurred in known HIV/AIDS patients. The clinical presentations in HIV patients fell into two distinct groups, bacteremia and enteritis. There was little clinical overlap between these two syndromes. All strains were positive for the presence of the rpoS and sodC virulence loci, and 75% of strains were positive for the presence of the spvB and sseL loci. Antibiotic susceptibility assay showed that all strains were susceptible totrimethoprim-sulfamethoxazole and ciprofloxacin. The clinical presentation did not have a clear relationship to the CD4+ cell count. Of the bacteremic isolates, all but one isolate, drawn from a patient with substantial enteric morbidities, had all of the virulence genes tested, but 66% of nonbacteremic, enteritis strains also contained all the tested virulence loci. In conclusion, neither patients’ CD4+ cell count nor bacterial strain properties necessarily predicted the clinical presentation of HIV/AIDS patients with Salmonella (NTS) infection, and AIDS patients can have episodes of Salmonella enteritis without dissemination.

Invasive nontyphoidal Salmonella (NTS) infection was recognized as a complication of AIDS in the early days of the HIV pandemic (4, 38, 40) but has become increasingly rare in developed countries in the era of highly active antiretroviral therapy (HAART) (24). In underdeveloped countries, especially those in sub-Saharan Africa, invasive NTS infection has emerged as a substantial problem with a high mortality in the AIDS population (6, 15, 27, 30, 36, 43). In the epidemic of invasive NTS in sub-Saharan Africa, the two most common serovars are Salmonella enterica serovar Typhimurium and S. Enteritidis, which often cause recurrent invasive disease in HIV-infected adults (15, 16). Several studies have shown a tendency for variants of these serotypes to emerge in different hosts and that these variants have differing pathogenicity (22, 41, 45). Sequence analysis of a common S. Typhimurium strain causing bacteremia in HIV patients in Malawi and Kenya showed the presence of a number of pseudogenes and deletions relative to standard reference strains, suggesting the possibility of host and/or specialized niche adaptation (30). Little is known of reasons for the decline of invasive NTS as a complication of AIDS in the developed world or whether variants of S. Typhimurium and S. Enteritidis with particular genetic characteristics occur in this population. There is an absence of recent studies investigating the clinical and microbiological features of NTS infection in HIV patients in an industrialized-country, urban setting.

Infection due to NTS has several clinical presentations ranging in severity from self-limited enteritis to fatal septicemia (6). One model for NTS infection in HIV/AIDS patients is that gastrointestinal infection, if left untreated, will disseminate (42). In this model, Salmonella infection begins in the intestinal tract, and local immune responses in healthy hosts are generally able to contain the disease (14). However, in the very young or in patients with immunosuppression, the infection typically spreads beyond the gastrointestinal tract and results in bacteremia, sometimes complicated by metastatic foci of infection in organs, including the bones, joints, liver, spleen, and meninges (6, 14). Several reports from Africa have commented on the lack of diarrhea in both children and HIV patients presenting with NTS bacteremia (2, 33, 36). A study of immunocompromised patients in England concluded that NTS bacteremia without enteritis is a sign of abnormal immune function (3). Remarkably, there has been little emphasis on the occurrence of Salmonella enteritis without systemic infection in HIV/AIDS patients.

Salmonella strains contain a large number of genes that affect the virulence phenotype. Genomic studies are leading to an increased appreciation of the considerable variation in the virulence genes carried by individual clinical isolates and the potential for a wide range of virulence phenotypes (25). There is a poor understanding of the current clinical spectrum of NTS disease and the microbiological features of the causative strains seen in clinical cases in a developed country. In this review of NTS infections in
TABLE 1 Primers used

<table>
<thead>
<tr>
<th>Gene</th>
<th>Direction or purpose</th>
<th>Primer</th>
<th>Amplicon size (bp)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>spvA</td>
<td>Forward</td>
<td>GACTATCTTTTCCACAAATGAAACC</td>
<td>~500</td>
<td>28, 39</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>GTACTTATGAGTTGAGTACCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sseI</td>
<td>Forward</td>
<td>TCCGGCGATAACCTTATGTTG</td>
<td>~1,000</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>CTGTCATCTGATAGTGTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sodCI</td>
<td>Forward</td>
<td>TATGGGATTGATTGCTAAGG</td>
<td>~300</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>ACAATTGTGCGCGGTGAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rpoS</td>
<td>Forward</td>
<td>TGCTGGCGAAGAACGAGGG</td>
<td>~1,000</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>TGAAGCTCTGAGTGCTGAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Internal sequencing</td>
<td>CACGTGTTACACAGCAGAAAGG</td>
<td></td>
<td>37</td>
</tr>
</tbody>
</table>

*M —, sseI primers were created on the basis of a published sequence (NCBI gene accession number 1252569) and the MacVector (version 11.0) primer design program.

the HIV population at the University of California, San Diego (UCSD), during a 6-year period, we were able to identify the key clinical features of the disease processes, as well as selected microbiological properties of all strains of Salmonella isolated from these patients.

We chose to examine the isolates for the presence of four distinct virulence loci that have been reported to have a variable distribution and/or polymorphisms in Salmonella strains: spvB, rpoS, sodCI, and sseI. The spv locus greatly enhances the ability of NTS to cause extraintestinal infection, including bacteremia (12, 18). spvB is required for the spv-mediated virulence phenotype and encodes a cytotoxin that depolymerizes cellular actin (32). rpoS is an important virulence locus in Salmonella that controls spv expression, catalase production, resistance to peroxide, and survival under various stress conditions, including stationary phase (9, 21). The sodCI gene encodes a superoxide dismutase important in resistance to oxidative stress and intracellular survival of Salmonella (8). We also chose to look at the sseI gene, which is associated with dissemination and chronic infection in animal models (35), because it is mutated in certain epidemic strains of S. Typhimurium isolated from bacteremic HIV patients in sub-Saharan Africa (30). Both the sodCI and sseI genes are located on the lysogenic Gifsy-2 prophage.

**CASE REPORT**

One patient’s case was of particular interest because her outcome was better than expected, given her severe immunosuppression and seemingly prolonged infection with a Salmonella isolate. The patient was a 34-year-old female with a history of HIV infection diagnosed in 1995 and nonadherence to antiretroviral therapy who presented to the UCSD emergency department in December of 2008 complaining of 3 days of frequent watery diarrhea and abdominal pain. Her CD4 count was 43 cells/µl, and her viral load had risen to 57,498 copies/ml. She was treated with intravenous fluids and declined admission to the hospital. No antibiotics were given, and a stool sample for culture sent from the emergency room grew a stool sample for culture sent from the emergency room grew Salmonella Typhimurium (isolate 8; see Tables 2 and 3). Blood cultures from both episodes were negative. On neither occasion had she been taking antiretrovirals or antibiotics other than dapsone for pneumocystis jirovecii pneumonia prophylaxis. For this second episode, she was admitted to the hospital for 2 days and received intravenous ciprofloxacin and metronidazole, with resolution of her symptoms. She was switched to oral antibiotics and discharged.

**MATERIALS AND METHODS**

**Patients.** The UCSD microbiology laboratory comprehensively documents all Salmonella infections seen at the UCSD Medical Center and Clinics and sends reports to the public health department. We performed a retrospective review of its database for all Salmonella infections at the UCSD Medical Center from June 2003 through December 2009. Salmonella isolates for all but two of the HIV-positive patients were available for analysis from San Diego County Public Health. The two patients for whom corresponding Salmonella isolates were not available were excluded from our analysis. Altogether, our study population included 16 HIV-positive patients and their corresponding Salmonella isolates.

We reviewed charts for vital signs, complete blood count as well as CD4+ cell counts, HIV load, fecal leukocytes and fecal occult blood, use of antiretroviral drugs, and prophylaxis for opportunistic infections. The presence of the systemic inflammatory response syndrome (SIRS) at the time of infection was determined if at least two of the following were documented: temperature of <36°C or >38°C, heart rate of >90/min, respiratory rate of >20/min, white blood cell count of <4 × 10^9/liter or >12 × 10^9/liter, or 10% bands (1). Charts were also reviewed for comorbidities and any mention of diarrhea, abdominal pain, headache, and fever at the time of positive Salmonella cultures, as well as the type and duration of administration of any antibiotics. Institutional review board approval was obtained from the Human Subjects Protection Program at UCSD.

**Identification and antimicrobial susceptibilities.** The Salmonella isolates were initially identified by standard laboratory methods and were then identified at the serovar level according to the Kauffman and White scheme using somatic and flagellar antigens in the San Diego County Public Health laboratories. Antimicrobial susceptibilities were determined using the Kirby-Bauer disc diffusion method and confirmed with the Vitek 2 system, version 5.01 (bioMérieux), using card type AST-GN47 and the CLSI 2011 MIC interpretation guideline (23). In isolates resistant to ceftriaxone, the double-disc diffusion method was used to test for the extended-spectrum-B-lactamase (ESBL) phenotype (26). Isolates were screened for reduced susceptibility to ciprofloxacin by testing for nalidixic acid resistance with the Kirby-Bauer method; in resistant isolates, a ciprofloxacin Etest was done to determine the MIC (20).

**Identification of virulence factors.** DNA was extracted from single colonies incubated overnight in Luria-Bertani (LB) broth using a Qiagen DNeasy kit. Each isolate was tested for PCR for the spvB, sseI, and rpoS genes with 1× PCR buffer, 0.25 mM deoxynucleoside triphosphates, 1.5 mM MgCl_2, and 1.5 U Qiagen Taq DNA polymerase for 30 cycles (Perkin-Elmer 480 thermocycler) (28, 39). The parameters for PCR were 1 min at 94°C for denaturing, 3 min at 55°C for annealing, and 1 min at 72°C for extension. PCR products were immediately run on 1.5% agarose gels, stained with ethidium bromide for 1 h at 50 to 70 V, and visualized under UV light. All primers, listed in Table 1, were made by Integrated DNA
Technologies (Coralville, IA). DNA sequencing of the rpoS gene was performed by the DNA Sequencing Shared Resource, UCSD Cancer Center, which is funded in part by NCI Cancer Center support grant 2 P30 CA023100-23, using an ABI Prism 3100 genetic analyzer, a fully automated multicolor fluorescence-based DNA analysis system using capillary electrophoresis with 16 capillaries operating in parallel. RpoS expression and size were confirmed by immunoblotting using a monoclonal antibody as described previously (7).

RESULTS

There were 3,247 total cases of salmonellosis in San Diego County from 2003 to 2009, during which the county population was roughly 1.3 million people and the HIV prevalence was 1% (www.sdcounty.ca.gov/hhsa/). One hundred twenty-nine patients with stool and/or blood cultures positive for NTS were identified. Forty-one percent of HIV-positive patients with NTS infections (7/17) and 15% of HIV-negative patients (17/112) had documented bacteremia (χ² = 6.59; P < 0.05).

Clinical findings. Most patients presented with signs of either systemic illness or enteritis, but not both (Table 2). Clinical evidence of SIRS was present in 100% of patients with bacteremia but only three of the eight patients (37%) with diarrhea and negative blood cultures. Only one of the eight patients (12%) with bacteremia reported diarrhea, and stool culture in that patient was negative, while 100% of patients with diarrhea and no documented bacteremia had positive stool cultures. However, we acknowledge that the presence of diarrhea was likely a major indication to obtain a stool sample for culture. One patient grew Salmonella (NTS) from both stool and blood sources; there was no clinical report of diarrhea in this case. Patients with low and high CD4⁺ cell counts were distributed into each distinct clinical presentation (Table 2). The majority of patients were not on antiretroviral therapy at the time of Salmonella infection. A variety of clinical, personal, and socioeconomic factors contributed to this relatively low incidence of antiretroviral therapy. None of the patients were on trimethoprim-sulfamethoxazole (TMP-SMX) or other antibiotic prophylaxis that would have been active against Salmonella.

Microbiology findings. S. Enteritidis was the most common serovar isolated from HIV patients (6 strains), while S. Typhimurium was the next most common (4 strains counting both isolates at different times from the same patient). These two serovars were also the most commonly identified from the 111 non-HIV patients, although complete serotype data were not available for all isolates. S. Dublin comprised 2 isolates in HIV patients, while the S. Agona isolate was sensitive to TMP-SMX. On August 29, 2017 by guest

### TABLE 2 Clinical characteristics of UCSD HIV patients and corresponding Salmonella serovars

<table>
<thead>
<tr>
<th>Patient group and isolate</th>
<th>Salmonella serovar</th>
<th>Culture result</th>
<th>Presence of:</th>
<th>CD4 count (no. of cells/ml)</th>
<th>Receipt of:</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive blood culture and negative or absent stool culture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Dublin</td>
<td>ND</td>
<td>+</td>
<td>Yes</td>
<td>No</td>
<td>538</td>
</tr>
<tr>
<td>6a</td>
<td>Newport</td>
<td>–</td>
<td>+</td>
<td>Yes</td>
<td>No</td>
<td>207</td>
</tr>
<tr>
<td>11</td>
<td>Enteritidis</td>
<td>–</td>
<td>+</td>
<td>Yes</td>
<td>Yes</td>
<td>44</td>
</tr>
<tr>
<td>12</td>
<td>Enteritidis</td>
<td>ND</td>
<td>+</td>
<td>Yes</td>
<td>No</td>
<td>43</td>
</tr>
<tr>
<td>13</td>
<td>Enteritidis</td>
<td>ND</td>
<td>+</td>
<td>Yes</td>
<td>No</td>
<td>14</td>
</tr>
<tr>
<td>16</td>
<td>Dublin</td>
<td>–</td>
<td>+</td>
<td>Yes</td>
<td>No</td>
<td>113</td>
</tr>
<tr>
<td>17</td>
<td>Typhimurium</td>
<td>ND</td>
<td>+</td>
<td>Yes</td>
<td>No</td>
<td>51</td>
</tr>
<tr>
<td>Positive blood and stool cultures, 10</td>
<td>Enteritidis</td>
<td>+</td>
<td>+</td>
<td>Yes</td>
<td>No</td>
<td>64</td>
</tr>
</tbody>
</table>

### Notes:
- SIRS, systemic inflammatory response syndrome; ARVs, antiretrovirals; TMP-SMX, trimethoprim-sulfamethoxazole; ND, not done.
- a This patient had multiple comorbidities, including Kaposi’s sarcoma, Burkitt’s lymphoma, C. difficile colitis, severe anemia, and tuberculosis enteritis.
- b This patient had a sample for culture drawn after ceftriaxone was started.
- c This patient had a sample for culture drawn after ceftriaxone was started.
- d This patient was started on prophylactic TMP-SMX on the day that his stool sample was sent for culture. His blood sample for culture was not drawn until 2 weeks later. He appeared septic at the time that his blood sample for culture was drawn, and his Salmonella isolate was sensitive to TMP-SMX.
Correspondence between the...same host is a puzzle.

Interestingly, there was an absolute correlation of the


carriage of the


rpoS


locus or RpoS genes were produced, the mechanisms of pathogenesis, and the genomic composition. While typhoid fever strains do not show a predilection to infect the HIV population, NTS strains cause invasive disease much more frequently in HIV patients (43). The majority of literature on NTS is concerned either with the clinical and epidemiologic aspects of infection or with the microbiologic characteristics of the organisms. There are few, if any, studies which pair microbiologic analysis of isolates with clinical information from individual patients. The spv locus is a marker for the spv genes to disseminated infection often lack clinical data from their hosts. Studies that do link Salmonella virulence factors such as spv genes to disseminated infection often lack clinical data from individual patients (10, 11, 17, 19, 31, 44). In this case series, we included information on NTS isolates and the HIV patients from whom they were isolated in an attempt to characterize the current spectrum of Salmonella infection in the HIV population in an industrialized-country setting.

The most striking finding from our study was the separation of clinical syndromes into distinct presentations of bacteremia and enteritis. Only one patient with bacteremia had diarrhea, and none of the three enteritis patients with SIRS had positive blood cultures. Surprisingly, there was no clear relationship between CD4+ cell count and the type of clinical presentation. Patients with high or very low CD4+ cell counts were present in each clinical group.

We examined the Salmonella isolates for the presence of virulence genes that might contribute to the particular clinical presentations of individual patients. The spv locus was confirmed in every isolate associated with bacteremia (save one from a patient with disseminated S. Newport infection with probable compromised intestinal mucosa due to tuberculous enteritis, C. difficile colitis, and Kaposi’s sarcoma). This finding supports the hypothesis that the spv locus promotes disseminated infection in HIV patients, but we found significant exceptions to this model. Most patients with enteritis (6 out of 9) without documented bacteremia were infected with spv-containing strains. The most dramatic example was the case associated with S. Typhimurium isolates 8 and 9, described in the case report. This patient had poorly controlled HIV infection due to noncompliance and had repeatedly low CD4+ cell numbers. Despite this clinical setting, she presented twice with enteritis without bacteremia. Remarkably, she was not even treated for her initial episode. Analysis of the two S. Typhimurium isolates failed to reveal any defects in spvB, rpoS, sodCI, or sseL.

All isolates contained the sodCI gene. This was somewhat surprising, since many Salmonella serovars lack this virulence gene (8). Experimentally, the sodCI gene enhances the virulence of Salmonella in mouse models of systemic infection. The significance of...
the correspondence between spvB gene carriage and ssel is unknown. Since ssel is encoded on the same Gifsy-2 prophage as sodCI, our results suggest that the ssel-deficient strains still contain the portion of Gifsy-2 encoding sodCI. Such a regional deletion was found in the African epidemic strain described previously, and other deletions and polymorphisms in the ssel region have been described (30). The functional significance of these variations is not known, but experimental evidence indicates that ssel affects the mobility of host phagocytic cells and the dissemination of Salmonella from the intestinal tract, as well as long-term survival in a mouse model of chronic Salmonella infection (35).

Also of interest in this series are the antibiotic susceptibilities of the NTS isolates. Specifically, all of the isolates were susceptible to TMP-SMX, consistent with the susceptibilities of Salmonella isolates from immunocompetent patients in the UCSD database over the same time period but in stark contrast to those of the epidemic strains found in Africa (2, 6, 33, 34, 36, 46). It has been observed that genes for antibiotic resistance in NTS reside on virulence plasmids and that use of antibiotics over time may select not only for antibiotic resistance but for increased virulence as well (30, 48). Notably, none of the patients in this analysis had been taking prophylactic TMP-SMX, which may provide a protective effect against Salmonella infection in developed countries. In contrast, patients who take TMP-SMX prophylaxis in Africa have been observed to be at greater risk for infection with resistant organisms (5). Also of note is that 2 of the 17 isolates, an Edinburgh isolate and an S. Agona isolate, were resistant to extended-spectrum cephalosporins.

Our analysis of virulence factors is by no means exhaustive, and mutations in genes that we did not examine may account for some of the clinical findings presented here. Still, despite the fact that the limited number of patients and isolates studied here prohibits broad conclusions, our review shows that it is possible for HIV patients, even those with low CD4 counts, our review shows that it is possible for HIV-infected patients to harbor resistant organisms.

ACKNOWLEDGMENTS

We thank Joshua Fierer for antimicrobial susceptibility testing, Patricia Hasegawa for excellent technical assistance, and Lizanne Keays for her invaluable assistance. We are very grateful to Patricia McCay and the San Diego County Public Health laboratories for providing strains used in this study.

This study was supported in part by NIH grants AI032178 and AI077661 to D. G. Guiney and K08 AI062273 to S. H. Browne.

REFERENCES


27. Kankwatira AM, Mwafulirwa GA, Gordon MA. November 2012 Volume 50 Number 11 jcm.asm.org


30. Krause M, Fang FC, Guiney DG.

31. Krause M, Fang FC, Guiney DG.

32. Lesnick ML, Reiner NE, Fierer J, Guiney DG.


35. Mandomando I, et al.


