Original Article

Genotypic and demographic characterization of invasive isolates of *Salmonella* Typhimurium in HIV co-infected patients in South Africa

Karen H. Keddy[1,2], Sarika Dwarika[1,2], Penny Crowther[3], Olga Perovic[4], Jeanette Wadula[4], Anwar Hoosen[5], Arvinda Sooka[1], Heather H. Crewe-Brown[4,6], Anthony M. Smith[1,2]

[1]*Enteric Diseases Reference Unit, National Institute for Communicable Diseases, Johannesburg, South Africa*
[2]*University of the Witwatersrand, Johannesburg, South Africa*
[3]*National Microbiology Surveillance Unit, National Institute for Communicable Diseases, Johannesburg, South Africa*
[4]*Department of Clinical Microbiology & Infectious Diseases, National Health Laboratory Service, University of Witwatersrand, Johannesburg, South Africa*
[5]*Department of Medical Microbiology, University of Pretoria and National Health Laboratory Service Tshwane Academic Division, Pretoria, South Africa*
[6]*National Health Service, Forth Valley, United Kingdom*

Abstract

Background: Non-typhoidal *Salmonella* are frequently food-borne zoonotic pathogens that may cause invasive disease in HIV-positive individuals.

Methodology: Invasive isolates (n = 652) of *Salmonella* Typhimurium from human patients in Gauteng Province of South Africa were investigated for the years 2006 and 2007. Bacteria were identified using standard microbiological techniques and serotyping was performed using commercially available antisera. Susceptibility testing to antimicrobial agents was determined by the E-test. Genotypic relatedness of isolates was investigated by pulsed-field gel electrophoresis analysis of digested genomic DNA.

Results: Forty-five clusters of isolates were identified, of which four (clusters 3, 5, 6 and 11) were major clusters. Most isolates originated from hospital H2 and most were isolated from patients in the age range of 15 to 64 years. Ninety-three percent (213/230) of patients with a known HIV status were HIV-positive. Most isolates showed resistance to multiple antibiotics. The most commonly expressed antibiotic resistance profiles were ACSSuNa (14%; 75/555) and ACSSuTNa (13%; 72/555). Some evidence was found for nosocomial acquisition of isolates. Of the isolates from the major clusters 3, 5, 6, and 11, 33% (8/24), 6% (7/117), 4% (1/26) and 6% (3/52) were of nosocomial origin, respectively.

Conclusions: In South Africa, *Salmonella* Typhimurium remains a major opportunistic infection of predominantly HIV-positive patients. Clonally diverse strains that are resistant to multiple antibiotics may circulate in patients aged between 15 and 64 years over prolonged periods within the hospital environment, adding to the health care burden.

Key words: *Salmonella* Typhimurium, HIV, invasive, nosocomial, South Africa

J Infect Dev Ctries 2009; 3(8):585-592.

Received 14 January 2009 - Accepted 21 August 2009

Introduction

Salmonellosis is primarily caused by the consumption of contaminated food and water, but *Salmonella* may also be transmitted via other routes including faecal-oral transmission (human-to-human) and direct contact with animals infected with *Salmonella* [1]. In immunocompromised patients [2], once *Salmonella* strains have entered the small bowel, the organisms break through the bowel mucosa and enter the Peyer's patches and associated lymph nodes, where they multiply and spread [1]. Immunocompromised persons are thus susceptible to life-threatening bacteraemia [3,4]. AIDS patients in sub-Saharan Africa infected with non-typhoidal *Salmonella* bacteraemia have a reported mortality rate of 35% to 60% [5]. Of the HIV-positive patients who survive, 25% to 45% suffer from recurrent non-typhoidal *Salmonella* bacteraemia about one to six months after the first non-typhoidal infection [5].

The burden of HIV disease in South Africa is extremely high [G. Pembrey, http://www.avert.org/aidssouthafrica.htm]. Very little epidemiological data exists for human *Salmonella* isolates recovered in South Africa and the association with HIV. In South Africa in 1998 through to 1999,

multidrug resistant *Salmonella* Typhimurium phage type DT104 strains were isolated from HIV-positive patients at the Chris Hani Baragwanath Hospital in Gauteng Province [Crewe-Brown *et al.*, ICAAC conference proceedings, September 2000, Toronto, Canada]. In 2004, a study by Kruger *et al.* on non-typhoidal *Salmonella* isolates collected from December 2002 to March 2003 showed increased resistance to extended-spectrum cephalosporins [6]. In 2006, multidrug resistant *Salmonella* Isangi were isolated from patients who were admitted to a tertiary hospital in Durban, South Africa [7].

A contributing factor of human-to-human transmission of salmonellosis is nosocomial infection: secondary infection acquired while under medical care, 48 hours and more after the patient has been admitted to a long-term care facility or hospital [8]. There has been an alarming increase in nosocomial outbreaks reported in the last 10 years [8]. Nosocomial infections have been reported in Russia and Belarus in the 1990s through to 2003 [8], in the United States from 1996 to 1998 [9], in Italy from 1998 to 2000 [10], in Spain from 1999 to 2000 [11] and in Romania in 2002 [12].

The aims of this study were to clarify the molecular epidemiology of invasive *Salmonella* Typhimurium isolates in Gauteng Province, South Africa, for the years 2006 and 2007 in association with HIV, to enhance our understanding of the nosocomial nature of this organism, and to identify epidemiological clusters that may assist in the interventions to stop further spread of disease.

Materials and Methods

Case definition and selection of isolates

Invasive nosocomial salmonellosis was defined as a positive culture from a patient for *Salmonella* two or more days after admission from a normally sterile body site. *Salmonella* Typhimurium isolates from normally sterile body sites in human patients were collected from clinical laboratories in Gauteng. Isolates were stored at -75°C in tryptic soy broth with 10% (vol/vol) glycerol (Diagnostic Media Products, Sandringham, South Africa). Surveillance officers appointed to four sentinel surveillance sites in Gauteng, South Africa, completed basic patient information by interviewing patients or reviewing patient records. Information was recorded in the surveillance database using the EpiInfo (version 6.04d) software (CDC, Atlanta, USA).

Bacterial identification and serotyping

Phenotypic and genotypic characterization of *Salmonella* Typhimurium isolates referred by laboratories in Gauteng was performed by the Enteric Diseases Reference Unit (EDRU) of the National Institute for Communicable Diseases (NICD) in Johannesburg, South Africa. Bacterial isolates were identified using standard microbiological techniques. Specific antisera (Statens Serum Institut, Copenhagen, Denmark; Remel Europe Ltd, Dartford, Kent, UK; and BioMérieux, Marcy-I'Étoile, France) were used to serotype *Salmonella* Typhimurium isolates, according to the Kauffman-White scheme.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined using E-tests (AB Biodisk, Solna, Sweden) according to the manufacturer's instructions. The following antibiotics were tested: chloramphenicol (C), streptomycin (S), tetracycline (T), nalidixic acid (Na), ciprofloxacin (CI), trimethoprim (TR), sulfamethoxazole (Su), kanamycin (KM), ampicillin (A), imipenem (IP), ceftriaxone (TX), cefepime (PM) and ceftazidime (TZ). For the present study, we particularly focused on the following six antibiotics: ampicillin (A), chloramphenicol (C), streptomycin (S), sulfamethoxazole (Su), tetracycline (T) and nalidixic acid (Na) (ACSSuTNa) and production of extended spectrum beta-lactamase tests using the double disk method per the manufacturer's instructions (Mast Diagnostics, Merseyside, United Kingdom).

Pulsed-field gel electrophoresis (PFGE) analysis of isolates

PFGE analysis was performed on isolates as previously described [13]. Genomic DNA were digested with restriction enzyme *Xba*I (Roche Diagnostics GmbH, Mannheim, Germany) and thereafter separated on a 1% agarose gel (SeaKem Gold agarose, Lonza, Rockland, ME, USA). Electrophoresis was performed using the CHEF-DR electrophoresis systems (Bio-Rad Laboratories Inc., USA). The following run parameters were used: A voltage of 6 volts, at a run temperature of 14°C, a run time of 21 hours, an initial switch time of 2.2 seconds and a final switch time of 63.8 seconds. These patterns were then visualized by UV illumination after staining the agarose gels with ethidium bromide. Fingerprint patterns were analysed using BioNumerics (version 5.1) software (Applied Maths, Sint-Martens-Latem, Belgium). Patterns were normalized against the reference pattern for *S.*

enterica serovar Braenderup (strain H9812). Dendrograms were produced by using the unweighted pair group method with arithmetic means. Analysis of the band patterns was performed with the dice-coefficient at an optimization setting and position tolerance setting of 0.5% and 1.5%, respectively. For the purpose of this study, three or more isolates with a similarity value of ≥ 90% was defined as a PFGE cluster. The clusters were numbered 1, 2, 3, etc. for referral purposes.

Statistical analysis

In addition to descriptive analysis, univariate logistic regression was performed to determine which individual explanatory variables were significantly associated with the outcome variables – HIV status and nosocomial infections – by calculation of unadjusted odds ratios and 95% confidence intervals.

Results

Analysis of isolates was restricted to Gauteng for the years 2006 and 2007. A total of 1,410 *Salmonella* isolates that were isolated from human patients were received by EDRU, of which 61% (857/1,410) were serotyped *Salmonella* Typhimurium. Of the 857 *Salmonella* Typhimurium isolates, 652 were invasive, accounting for 46% (652/1,410) of all *Salmonella* isolates. In the current study, all invasive isolates were analysed.

PFGE analysis could separate 85% (555/652) of the invasive human isolates into distinctive clusters. These data are summarized graphically in Figures 1a-c. A total of 45 clusters were identified amongst the 555 isolates. Of the 45 clusters identified, four clusters (3, 5, 6 and 11) were primary clusters represented by 83 (15%), 119 (21%), 59 (11%) and 82 (15%) of 555 isolates, respectively. Four secondary clusters (7, 10, 18, and 20) were categorized ranging from 16/555 (3%) to 22/555 (4%) isolates. Small numbers of isolates (n ≤ 9 isolates) made up the remaining 37 clusters.

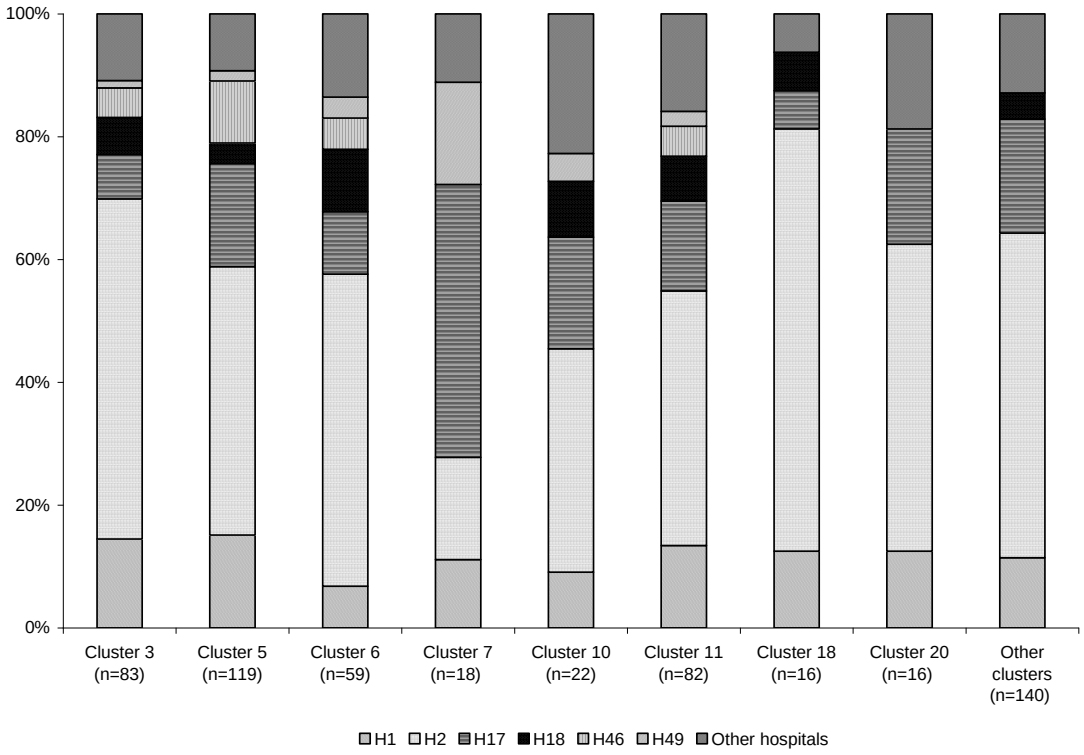
Isolates were sourced from 21 hospitals in Gauteng Province. Six hospitals (29%) (H1, H2, H17, H18, H46 and H49) accounted for 87% (485/555) of all isolates (Figure 1a). Most isolates (48%; 266/555) originated from hospital H2, a major public and teaching hospital. For three hospitals (H1, H2 and H49), evidence was found for nosocomial acquisition of isolates: hospital H2 accounted for most nosocomial isolates. Cluster 5 was a predominant cluster (21%; 119/555) in all 21 hospitals in Gauteng for the years 2006 and 2007 and was responsible for most of the infections caused by *Salmonella* Typhimurium during these years.

Most of the isolates (54%; 299/555) came from patients between 15 and 64 years of age (Figure 1b). The majority (64%; 192/299) of the isolates from patients in this age range were represented in clusters 3 (19%), 5 (20%), 6 (10%) and 11 (14%). Thirty-one percent (172/555) of the isolates came from patients aged four years and younger (Figure 1b). The majority (59%; 102/172) of the isolates from this young age group were represented in clusters 3 (6%), 5 (23%), 6 (9%) and 11 (21%), the same four clusters that represent the majority of isolates in the older age group of 15 to 64 years. Of the 555 isolates, only two isolates were recovered from patients aged 65 years and older.

HIV status was available for 41% (230/555) of patients: 93% (213/230) were HIV-positive (Figure 1c). Patients in the 15 to 64 years age group were 13 times more likely to be HIV-positive than those in the 0-4 years age group ($p < 0.001$, 95% CI 2.8-59.3). The majority (63%; 134/213) of the isolates from HIV-positive patients fell in clusters 3 (16%), 5 (23%), 6 (11%) and 11 (12%).

The most commonly expressed antibiotic resistance profiles of isolates from all the PFGE clusters were: ACSSuNa (14%; 75/555) and ACSSuTNa (13%; 72/555) (Table 1). Antibiotic resistance trends in the predominant clusters (3, 5, 6 and 11) were as follows: in cluster 3, 92% (76/83) of the isolates were resistant to three or more antibiotics; in cluster 5, 90% (107/119) of the isolates were resistant to three or more antibiotics; in cluster 6, 76% (45/59) of the isolates were resistant to three or more antibiotics. Ciprofloxacin resistance was rare, however, and only noted in 3/555 (0.5% isolates). Extended-spectrum beta-lactamase (ESBL) production was present in 12% of isolates evaluated (data not shown). Patients with ESBL-producing *Salmonella* Typhimurium were 4.1 times more likely to have invasive nosocomial infections than patients with non-ESBL producing *Salmonella* Typhimurium ($P < 0.005$, 95% CI 1.7-10.0). Cluster 11 (n = 82) included the largest group of antibiotic susceptible isolates.

Figure 1a. Number of patients in Gauteng hospitals, represented as a percentage, falling within each PFGE cluster.



H1, Hospital 1; H2, Hospital 2; H17, Hospital 17; H18, Hospital 18; H46, Hospital 46; H49, Hospital 49.

Figure 1b. Age ranges of patients in Gauteng hospitals, represented as a percentage, falling within each PFGE cluster.

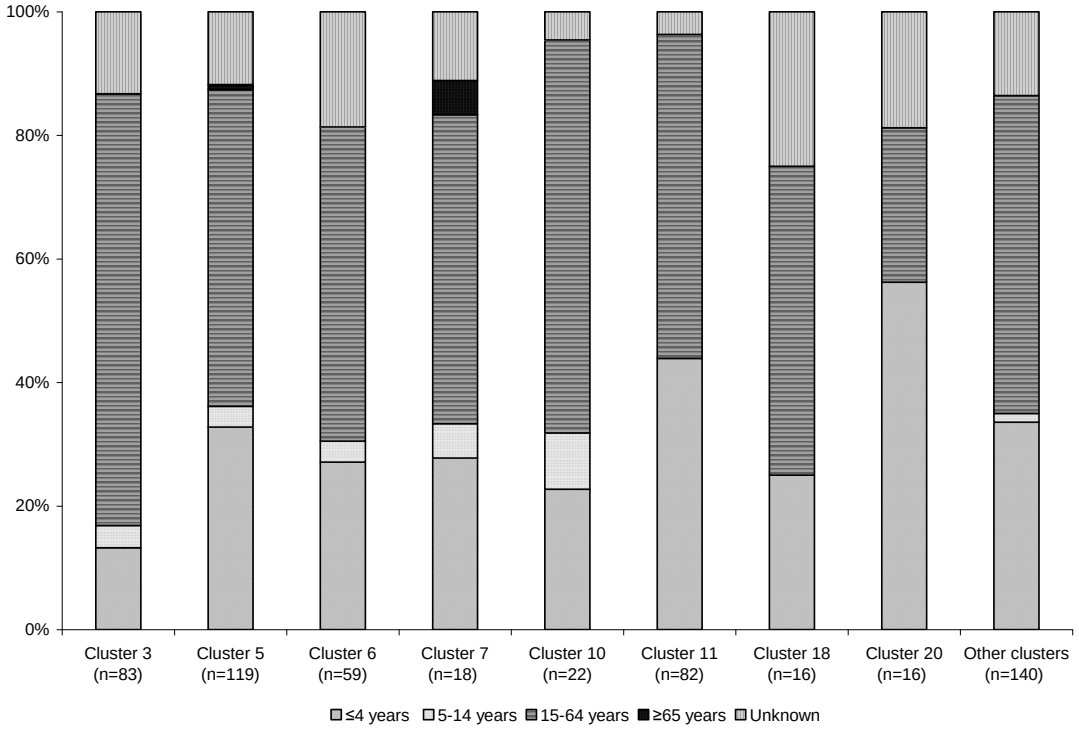
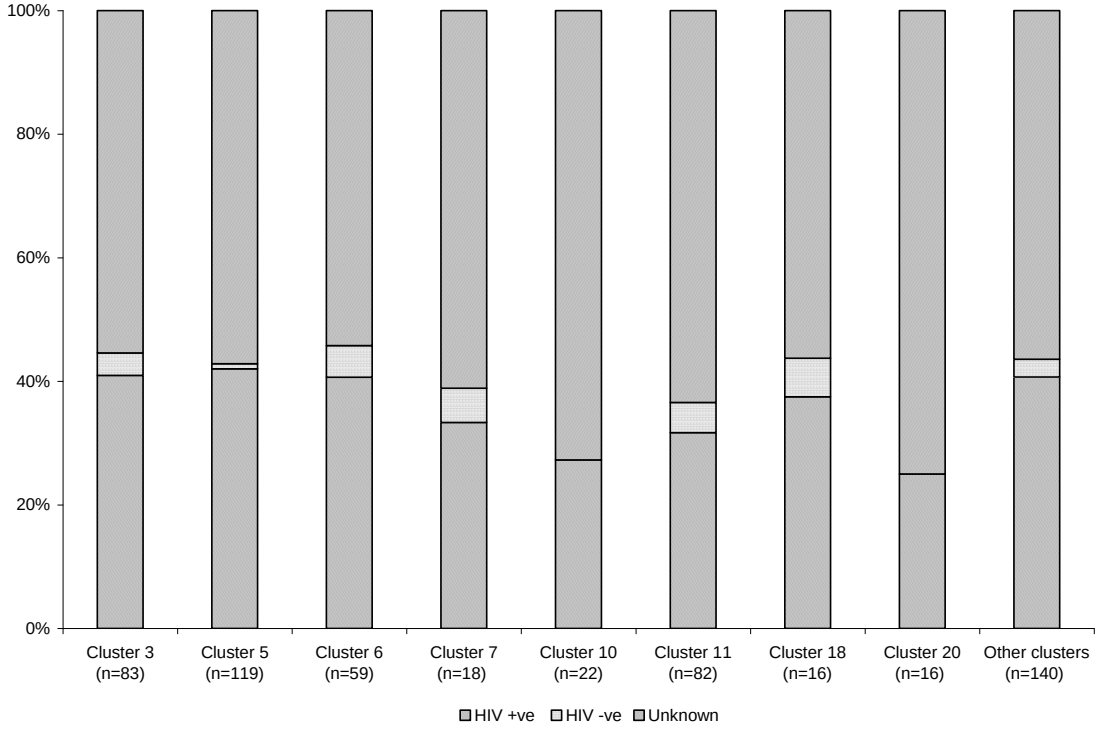


Figure 1c. HIV status of patients in Gauteng hospitals, represented as a percentage, falling within each PFGE cluster.



Only 317 of 555 isolates had data which allowed us to draw conclusions as to possible nosocomial acquisition of the isolate. Of these 317 isolates, 31 (10%) were identified as nosocomial; these fell into 16 clusters (Figure 1a-c and Table 1). The majority of these nosocomial isolates were from HIV-positive patients (97%; 28/29) and from patients in the age range of 15 to 64 years (68%; 21/31). The nosocomial isolates were identified from three hospitals (H1, H2 and H49). Patients from hospital H49 were 17.5 times more likely to have nosocomial infections than patients from hospital H1 ($p < 0.01$, 95% CI 2.1-149.2), and 7.4 times more likely than patients from hospital H2 ($p < 0.05$, 95% CI 1.2-46.8). Hospital H2 accounted for the majority (81%; 25/31) of the nosocomial isolates, belonging to multiple clusters. Most of our 31 nosocomial isolates were encompassed in clusters 3 (26%), 5 (23%) and 11 (10%). Patients with isolates in cluster 3 were 7.9 times more likely than patients in cluster 5 to have nosocomial infections ($P < 0.001$), 12.5 times more likely than patients in cluster 6 to have nosocomial infections ($P < 0.03$) and 8.2 times more likely than patients in cluster 11 ($P < 0.005$) to have nosocomial infections.

Discussion

Salmonella infections are common in a hospital environment due to overcrowding of patients and insufficient medical staff [14]. In addition, *Salmonella* infections in hospitals may be associated with the use of contaminated medical equipment, as well as with the consumption of contaminated meals served at hospitals resulting from poor hand hygiene practices and inadequate cooking of meals by kitchen staff [14]. In the absence of timely investigation, these factors may have accounted for possible routes by which the majority of clusters of *Salmonella* Typhimurium could have circulated in Gauteng hospitals.

The predominance of invasive salmonellosis in young patients (31%) is unsurprising, as young patients have an immature immune system and are more prone to *Salmonella* infections [2,15]. Similarly, the number of infections and nosocomial infections in the 15 to 64 years age range correlates well with published HIV-positive incidence rates in South Africa [16]. That the same four PFGE clusters (clusters 3, 5, 6 and 11) were represented in both of these age groups and at three hospitals allows us to speculate that nosocomial exposure, either through

transfer of staff or of patients between hospitals and possibly in association with increased virulence of patients caused by numerous undefined reasons [1] or to the overwhelming salmonellosis identified in

Table 1. Attributes of nosocomial isolates of *Salmonella* Typhimurium. Important findings are emphasised in bold face.

Isolate number	PFGE cluster	Hospital of origin[a]	Month/year of isolation	Antibiotic resistance profile[b]	Age-range of patients (years)	HIV Status of patients
N600	**3**	**H2**	**February 2006**	**ASSuTNa**	5-14	-ve
N637	**3**	**H2**	February 2006	ASSuT	≤4	+ve
N503	**3**	**H2**	April 2006	**ACSuTNa**	15-64	+ve
N282	**3**	**H2**	May 2006	**ACSSuTNa**	15-64	+ve
N093	**3**	**H2**	June 2006	**ACSSuTNa**	15-64	unknown
N196	**3**	**H2**	June 2006	**ACSSuTNa**	15-64	+ve
N591	**3**	**H2**	August 2006	**ACSuTNa**	15-64	+ve
N636	**3**	**H2**	**August 2007**	ASuNa	15-64	+ve
N200	**5**	**H2**	**March 2006**	**ACSSuNa**	15-64	+ve
N038	**5**	**H2**	February 2007	**ACSSuNa**	15-64	+ve
N859	**5**	**H2**	February 2007	**ACSSuTNa**	≤4	+ve
N810	**5**	**H49**	February 2007	ASu	≤4	+ve
N767	**5**	**H2**	May 2007	**ACSSuTNa**	≤4	+ve
N520	**5**	**H2**	July 2007	ASSuT	15-64	+ve
N537	**5**	**H2**	**July 2007**	ASSuT	15-64	+ve
N599	**11**	**H2**	**May 2006**	susceptible	15-64	+ve
N511	**11**	**H2**	July 2007	Su	≤4	+ve
N213	**11**	**H49**	**October 2007**	Su	15-64	+ve
N282	4	**H2**	May 2006	**ACSSuTNa**	15-64	+ve
N039	6	**H2**	January 2007	ASu	≤4	+ve
N801	7	**H49**	June 2006	Su	≤4	+ve
N402	10	**H1**	November 2007	Su	15-64	unknown
N787	21	**H2**	June 2006	susceptible	15-64	+ve
N771	22	**H2**	June 2006	ASSu	≤4	+ve
N511	25	**H2**	July 2007	Su	≤4	+ve
N520	27	**H2**	July 2007	ASSuT	15-64	+ve
N312	33	**H2**	May 2006	ASSuT	15-64	+ve
N800	35	**H1**	August 2005	**ACSuTNa**	15-64	+ve
N708	36	**H2**	September 2007	ASuNa	15-64	+ve
N076	38	**H2**	December 2007	**ACSSuTNa**	15-64	+ve
N721	45	**H1**	November 2007	susceptible	15-64	+ve

[a] H1, Hospital 1; H2, Hospital 2; H49, Hospital 49.
[b] A, Ampicillin; C, Chloramphenicol; S, Streptomycin; Su, Sulfamethoxazole, T, Tetracycline; Na, Nalidixic acid; susceptible, susceptible to all six of the former mentioned antibiotics.

these *Salmonella* strains, permitted these strains to dominate. We have no data to confirm whether such transfers occurred, and cannot account for the ubiquitous presence of these clusters. Older patients are usually more predisposed to acquiring salmonellosis as a secondary infection as a result of their weakened immunity [1]. Conversely, the results in this study suggest salmonellosis in elderly patients in Gauteng for 2006 and 2007 occurred less frequently. These results could possibly be accounted for by the underreporting of *Salmonella* cases in older patients of other age groups (less than 65 years) due to the high burden of HIV infection in these age groups [2,5].

HIV is a life-threatening epidemic in South Africa and accounts for up to 1,000 deaths of AIDS patients daily. Statistical reports have shown that in South Africa at the end of 2007, approximately 5.7 million people were living with HIV [G. Pembrey, website publication, http://www.avert.org/aidssouthafrica.htm]. HIV-

positive patients are 20 times more likely to acquire non-typhoidal *Salmonella* infection compared with immunocompetent patients [1,17]. Patient demographic information, such as HIV status and age, was not available for all patients; the lack of this information may have skewed results and may have been a limitation of this study. Despite the unknown HIV status for 59% (325/555) of the patients in the current study, the results still suggest that *Salmonella* Typhimurium may be responsible for extensive co-morbidity suffered by HIV-positive patients living in Gauteng Province.

Multidrug resistance was common in the nosocomial clusters. We showed that ESBL production was in fact a risk factor for invasive nosocomial salmonellosis. In clusters 3, 5, and 6, the majority of the isolates were multidrug resistant isolates (> 75%); antimicrobial management of such patients with invasive *Salmonella* Typhimurium may be compromised, resulting in longer morbidity periods and possibly higher death rates among patients. Previous studies have shown that the pentaresistant ACSSuT pattern that we observed is frequently reported in *Salmonella* Typhimurium strains isolated in the United Kingdom (UK), France and North America [18,19]. An increase in resistance to the quinolone class of antimicrobials (nalidixic acid), such as we noted, in addition to the pentaresistant pattern, has also been documented in an international survey performed on representative isolates of *Salmonella* Typhimurium for the years 1992 to 2001 [19]. Cluster 11 included 36 isolates showing susceptibility to all six antibiotics and included 23 isolates showing resistance to sulfamethoxazole only. For this cluster of isolates, the treatment given to patients may be uncomplicated and morbidity may be less severe.

Compared with isolates in the other clusters, isolates in cluster 3 were the most likely to be nosocomial. Past studies have shown that nosocomial infections occurred most commonly when there was an over-population of patients and fewer health care workers. There tends to be a reduction in infection control practiced by health care professionals to comply with the demand of seeing and treating an increased number of patients [14], which could possibly account for the high number of nosocomial isolates in hospital H2, a large academic hospital which accommodates not only patients living in the surrounding areas in Gauteng Province, but also serves as a referral hospital for a large part of South Africa and neighbouring African countries. Hospital infections could also have been acquired through direct contact (person-to-person) or through contact with common contaminated surfaces in the hospital environment [1].

The predominance of HIV-positive patients in whom nosocomial infection was identified supports the observation of previous studies that immunocompromised patients, such as HIV-positive patients, are more susceptible to nosocomial infections [1]. The extensive time period over which these isolates were sourced suggests that these nosocomial clusters were circulating in these three hospitals (H1, H2 and H49) for many months.

In conclusion, invasive *Salmonella* Typhimurium isolates in Gauteng demonstrated an extensive genetic diversity as shown by PFGE analysis, which segregated 555 isolates into 45 clusters. Most isolates showed resistance to multiple antibiotics, making these patients challenging to treat. Most isolates were from patients aged between 15 and 64 years, and patients were mostly HIV-positive. The occurrence of certain clusters over a prolonged period of time is cause for serious concern: it suggests that appropriate infection control measures have been inadequate in those hospitals for extended periods or are only intermittently followed, with resultant stresses both on health care systems as well patient morbidity. This hypothesis is supported by the extensive number of PFGE clusters, which suggests that these organisms are being repeatedly introduced into the hospital environment. In South Africa, invasive *Salmonella* Typhimurium remains an important opportunistic infection particularly associated with HIV-positive patients and is associated with nosocomial transmission.

Acknowledgements

This study was funded by grants from the Medical Research Council of South Africa and in part by cooperative agreements from the HHS Centers for Disease Control and Prevention (CDC), the National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention (NCHHSTP), and the Global AIDS Program (GAP) Cooperative Agreement U62/PSO022901. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of CDC.

Members of GERMS-SA

Sandeep Vasaikar (University of Transkei, Mthatha, Eastern Cape); Nolan Janse van Rensberg, André Möller, Anne-Marie Pretorius, Peter Smith (University of the Free State,

Bloemfontein, Free State); Khatija Ahmed, Anwar Hoosen, Ruth Lekalakala, Donald Ngwira, Pyu Pyu Sein (University of Limpopo – Medunsa Campus, Garankuwa, Gauteng); Heather Crewe-Brown, Charles Feldman, Alan Karstaedt, Olga Perovic, Jeannette Wadula (University of the Witwatersrand, Johannesburg, Gauteng); Mike Dove, Kathy Lindeque, Linda Meyer, Gerhard Weldhagen (University of Pretoria, Pretoria, Gauteng); Prathna Bhola, Prashini Moodley, Sharona Seetharam, Sindiswe Sithole, Wim Sturm, Trusha Vanmali (University of KwaZulu Natal, Durban, KwaZulu Natal); Ken Hamese (Polokwane/Mankweng Hospital Complex, Polokwane, Limpopo); Keith Bauer, Greta Hoyland, Jacob Lebudi, Charles Mutanda (National Health Laboratory Service, Mpumalanga); Rena Hoffmann, Lynne Liebowitz, Elizabeth Wasserman (University of Stellenbosch, Stellenbosch, Western Cape), Denise Roditi, John Simpson, Andrew Whitelaw (University of Cape Town, Cape Town, Western Cape); Adrian Brink (AMPATH laboratories, Johannesburg, Gauteng); Claire Heney (Lancet laboratories, Johannesburg, Gauteng); Marthinus Senekal (PathCare laboratories, Cape Town, Western Cape); Cheryl Cohen, Mireille Cheyip, Linda de Gouveia, John Frean, Nelesh Govender, Karen Keddy, Kerrigan McCarthy, Susan Meiring, Elizabeth Prentice, Vanessa Quan, Koshika Soma, Anne von Gottberg (National Institute for Communicable Diseases, Johannesburg, Gauteng).

References

1. Hanes D. Nontyphoid (2003) *Salmonella*. In Miliotis MD, Bier JW, editors. International handbook of foodborne pathogens. New York: Marcel Dekker, Inc. 135-149.
2. Ikumapayi UN, Antonio M, Sonne-Hansen J, Biney E, Enwere G, Okoko B, Oluwalana C, Vaughan A, Zaman SM, Greenwood BM, Cutts FT, Adegbola RA (2007) Molecular epidemiology of community-acquired invasive non-typhoidal *Salmonella* among children aged 2-29 months in rural Gambia and discovery of a new serovar, *Salmonella enterica* Dingiri. J Med Microbiol l56: 1479-1484.
3. Weinstein RA (1998) Nosocomial infection update. Emerg Infect Dis 4: 416-420.
4. Wenzel RP, Edmond MB (2001) The impact of hospital-acquired bloodstream infections. Emerg Infect Dis 7: 174-177.
5. Kankwatira AM, Mwafulirwa GA, Gordon MA (2004) Non-typhoidal *Salmonella* bacteraemia--an under-recognized feature of AIDS in African adults. Trop Doct 34: 198-200.
6. Kruger T, Szabo D, Keddy KH, Deeley K, Marsh J W, Hujer AM, Bonomo RA, and Paterson D L (2004) Infections with nontyphoidal *Salmonella* species producing TEM-63 or a novel TEM enzyme, TEM-131, in South Africa. Antimicrob Agents Chemother 48: 4263-4270.
7. Govinden U, Mocktar C, Moodley P, Sturm AW, Essack SY (2006) CTX-M-37 in *Salmonella enterica* serotype Isangi from Durban, South Africa. Int J Antimicrob Agents 28: 288-291.
8. Edelstein M, Pimkin M, Dmitrachenko T, Semenov V, Kozlova N, Gladin D, Baraniak A, Stratchounski L (2004) Multiple outbreaks of nosocomial salmonellosis in Russia and Belarus caused by a single clone of *Salmonella enterica* serovar Typhimurium producing an extended-spectrum beta-lactamase. Antimicrob Agents Chemother 48: 2808-2815.
9. Carattoli A, Tosini F, Giles WP, Rupp ME, Hinrichs S H, Angulo FJ, Barrett TJ, Fe PD (2002) Characterization of plasmids carrying CMY-2 from expanded-spectrum cephalosporin-resistant *Salmonella* strains isolated in the United States between 1996 and 1998. Antimicrob Agents Chemother 46: 1269-1272.
10. Mammina C, Cannova L, Massa S, Goffredo E, Nastasi A (2002) Drug resistances in *Salmonella* isolates from animal foods, Italy 1998-2000. Epidemiol Infect 129: 155-161.
11. Navarro F, Perez-Trallero E, Marimon JM, Aliaga R, Gomariz M, and Mirelis B (2001) CMY-2-producing *Salmonella enterica*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis* and *Escherichia coli* strains isolated in Spain (October 1999-December 2000). J Antimicrob Chemother 48: 383-389.
12. Miriagou V, Filip R, Coman G, Tzouvelekis LS (2002) Expanded-spectrum cephalosporin-resistant *Salmonella* strains in Romania. J Clin Microbiol 40: 4334-4336.
13. Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, Barrett TJ (2006) Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. Foodborne Pathog Dis 3: 59-67.
14. Bouallegue-Godet O, Ben Salem Y, Fabre L, Demartin M, Grimont PA, Mzoughi R, Weill FX (2005) Nosocomial outbreak caused by *Salmonella enterica* serotype Livingstone producing CTX-M-27 extended-spectrum beta-lactamase in a neonatal unit in Sousse, Tunisia. J Clin Microbiol 43: 1037-1044.
15. Olesen B, Neimann J, Bottiger B, Ethelberg S, Schiellerup P, Jensen C, Helms M, Scheutz F, Olsen KE, Krogfelt K, Petersen E, Molbak K, Gerner-Smidt P (2005) Etiology of diarrhea in young children in Denmark: a case-control study. J Clin Microbiol 43: 3636-3641.
16. National Department of Health, South Africa (2007) National HIV and syphilis seroprevalence survey 2006.Website accessed 1 April 2009.
17. Fernandez Guerrero ML, Ramos JM, Nunez A, Nunez A, de Gorgolas M (1997) Focal infections due to non-typhi *Salmonella* in patients with AIDS: report of 10 cases and review. Clin Infect Dis 25: 690-697.
18. Casin I, Breuil J, Brisabois A, Moury F, Grimont F, Collatz E (1999) Multidrug-resistant human and animal *Salmonella typhimurium* isolates in France belong predominantly to a DT104 clone with the chromosome- and integron-encoded beta-lactamase PSE-1. J Infect Dis 179: 1173-1182.
19. Helms M, Ethelberg S, Molbak K (2005) International *Salmonella* Typhimurium DT104 infections, 1992-2001. Emerg Infect Dis 11: 859-867.

Corresponding Author

Dr Karen Keddy
Enteric Diseases Reference Unit
National Institute for Communicable Diseases
Private Bag X4
Sandringham, 2131, South Africa
Tel: +27-11-3866269, Fax: +27-11-5550433,
E.mail: karenk@nicd.ac.za

Conflict of interest: No conflict of interest is declared.