Salmonella enterica

Enteric fever caused by *Salmonella enterica* serotype Typhi (*Salmonella Typhi*) and *Salmonella enterica* serotype Paratyphi A, B or C (*Salmonella Paratyphi A, B or C*) remains a major disease burden in developing countries (Buckle *et al.*, 2012). Enteric fever is associated with poor sanitation and contaminated water and food; a faecal–oral transmissible disease. *Salmonella Paratyphi* is commonly reported throughout Asia (Arndt *et al.*, 2014). In sub-Saharan Africa, very few data exist on the prevalence and characterization of *Salmonella Paratyphi*. The lack of systematic surveillance data for *Salmonella Paratyphi* from sub-Saharan African countries makes it difficult to accurately estimate the proportion of enteric fever caused by *Salmonella Paratyphi* or accurately estimate the burden of disease (Arndt *et al.*, 2014). PubMed literature searches revealed that Nigeria reports the most comprehensive data on *Salmonella Paratyphi* from sub-Saharan Africa. Nigeria has reported that up to 34% of enteric fever cases are caused by *Salmonella Paratyphi* A (Akinremi *et al.*, 2007). Reports of *Salmonella Paratyphi* from other sub-Saharan African countries are very scanty; reports mostly just include the identification of *Salmonella Paratyphi*, with no further phenotypic or genotypic characterization of the isolates (Onyango *et al.*, 2009; Tadesse, 2014). Published South African data are equally lacking concerning the prevalence of *Salmonella Paratyphi*; we could only find a single report from 1978 on enteric fever caused by *Salmonella Paratyphi* C (Jacobs *et al.*, 1978). In the current study, we report on the phenotypic and genotypic characterization of *Salmonella Paratyphi* isolated in South Africa over the years 2003 to 2014.

Bacterial isolates were investigated by the Centre for Enteric Diseases (CED) of the National Institute for Communicable Diseases, the national reference centre in South Africa for human infections due to enteric pathogens. Isolates were voluntarily submitted to the CED through a national active laboratory-based surveillance program from ~200 clinical microbiology laboratories across the country. In addition to this, the CED audits the national laboratory information system in order to identify additional cases that were not submitted for further characterization. Bacterial isolates were identified using standard phenotypic microbiological identification and serotyping techniques, briefly described as follows. As required, bacterial colonies were identified using the VITEK 2 Compact 15 automated microbial identification system (bioMérieux). *Salmonella* isolates were serotyped according to the White–Kauffmann–Le Minor Scheme. Differentiation of *d*-tartrate-non-fermenting *Salmonella Paratyphi B* from *d*-tartrate-fermenting *Salmonella Paratyphi B* variant Java [variant *L* (+) *tartrate* (+)] was performed using a previously described PCR methodology which detects allelic variants of a gene associated with the fermentation of *d*-tartrate (Malorny *et al.*, 2003). Antimicrobial susceptibility testing was performed using the Etest method (bioMérieux); the following antimicrobials were tested: ampicillin, ceftriaxone, trimethoprim, sulfamethoxazole, chloramphenicol, ciprofloxacin, tetracycline and azithromycin. MIC breakpoint values as described by the Clinical Laboratory Standards Institute (CLSI, 2015) were used to define antimicrobial resistance. Genotypic relatedness of isolates was investigated by PFGE analysis of XbaI-digested genomic DNA on a CHEF-DR III electrophoresis system (Bio-Rad) using a PulseNet protocol (http://www.pulsenetinternational.org/protocols/). PFGE patterns were analysed using BioNumerics (version 6.5) software (Applied Maths). PFGE clusters were defined by isolates with patterns having ≥90% similarity following dendrogram analysis.

To the best of our knowledge, this is the first study describing an investigation of paratyphoid fever in a sub-Saharan African country over an extended time period. In South Africa, for the years 2003 to 2014, >27 000 cases of *Salmonella* infection were recorded by the CED; 52 cases of *Salmonella Paratyphi* were identified as compared with 1122 cases of *Salmonella Typhi*. For the *Salmonella Paratyphi* cases, 51 were from human sources, whilst one case was sourced from food. Gender and age were known for 49 patients. With regard to gender, 53% (26/49) were male and 47% (23/49) were female. Age of patients ranged from 1 month to 70 years (median 26.5 years): 12 patients were <5 years old, two patients were 5–14 years old and 35 patients were >14 years old. These age distributions were similar to those previously reported for cases of invasive non-typhoidal *Salmonella* in South Africa (Feasey *et al.*, 2010). The majority of cases (58%; 30/52) were from two provinces in South Africa, i.e. Gauteng (35%; 18/52) and Western Cape (23%; 12/52).

For the 52 cases of *Salmonella Paratyphi*, 43 viable isolates were available for further analysis and further serotyped as *Salmonella Paratyphi* A (20/43; 47%), *Salmonella Paratyphi* B (19/43; 44%) and *Salmonella Paratyphi* C (4/43; 9%) (Table 1). Of the 19 isolates identified as *Salmonella Paratyphi* B, 74% (14/19) were found to be *d*-tartrate-fermenting *Salmonella Paratyphi* B variant Java [variant *L* (+) *tartrate* (+)]; a similar *Salmonella Paratyphi* B distribution was reported in a US study (Gupta *et al.*, 2008). For *Salmonella Paratyphi* A, most isolates (17/20; 85%) were cultured from invasive specimens (blood cultures) as compared with the fewer 3/20; 15%) non-invasive stool specimens. This was in contrast to
Table 1. Summary of numbers of viable *Salmonella* Paratyphi isolates

<table>
<thead>
<tr>
<th>Serotype</th>
<th>No. of isolates over years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paratyphi A</td>
<td>5</td>
</tr>
<tr>
<td>Paratyphi B</td>
<td>1</td>
</tr>
<tr>
<td>Paratyphi B variant Java</td>
<td>2</td>
</tr>
<tr>
<td>Paratyphi C</td>
<td>0</td>
</tr>
</tbody>
</table>

*Salmonella* Paratyphi B, where most human isolates (17/18; 94%) were cultured from stool specimens. This was not unexpected as most *Salmonella* Paratyphi B isolates were identified as variant Java, a variant known to typically cause a *Salmonella* gastroenteritis instead of bacteraemic enteric fever (Prager et al., 2003).

In South Africa, the number of *Salmonella* Paratyphi isolates identified by the CED gradually increased over the period from 2003 to 2014 (Table 1); *Salmonella* Paratyphi A and *Salmonella* Paratyphi B variant Java were the most commonly identified. It was difficult to compare and contrast our data against data from other countries, as published data from other countries are limited. Overall, *Salmonella* Paratyphi accounted for a small proportion (52/1174; 4.4%) of *Salmonella* isolates cultured from stool specimens. This was not unexpected as most *Salmonella* Paratyphi B isolates were identified as variant Java, a variant known to typically cause a *Salmonella* gastroenteritis instead of bacteraemic enteric fever (Prager et al., 2003).

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In conclusion, *Salmonella* Paratyphi is rarely reported in South Africa. Within this group, *Salmonella* Paratyphi A and *Salmonella* Paratyphi B variant Java were the most commonly identified isolates. Azithromycin can be recommended as a first-line therapy for paratyphoid fever in South Africa. However, we must remain alert and mindful, as *Salmonella* Paratyphi A treatment failures with azithromycin have been reported with isolate MICs going as high as 64–256 μg ml⁻¹ (Molloy et al., 2010).

**PFGE patterns were obtained for 42 isolates; they showed a large diversity of 28 different PFGE patterns (Fig. 1), suggesting that PFGE analysis would be a fitting methodology for investigating outbreaks of paratyphoid fever in the African setting.** For *Salmonella* Paratyphi A isolates, most (17/20) clustered together (cluster A; Fig. 1a), with 11/20 isolates having an indistinguishable pattern (pattern 1; Fig. 1a). Pattern 1 was compared against the PulseNet USA (http://www.cdc.gov/pulsenet/) database and found to be the most common *Salmonella* Paratyphi A pattern in the US database, with some isolates having evidence of patient travel history to India, Pakistan, Bangladesh, Cambodia and Vietnam (J. Concepción-Acevedo, personal communication). For *Salmonella* Paratyphi B variant Java isolates, most (9/15) clustered together (cluster B; Fig. 1b).

Within this cluster, patterns 2 and 3 were compared against the PulseNet USA database. Patterns 2 and 3 were the second and twelfth most common *Salmonella* Paratyphi B patterns in the US database, respectively; there was no travel history information associated with these patterns (J. Concepción-Acevedo, personal communication). Clustering of isolates is indicative of isolates with genetic similarity and clonality. Similar to our findings, other molecular investigations have also found that isolates of a particular paratyphoid serotype mostly cluster together. A study from Malaysia showed how PFGE analysis could clearly distinguish *Salmonella* Paratyphi B variant Java isolates from *d*-tartrate-non-fermenting *Salmonella* Paratyphi B isolates (Goh et al., 2003). Using PFGE analysis, a study from China reported genetic similarity of *Salmonella* Paratyphi A isolates (Gu et al., 2015).

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\textsuperscript{1}Centre for Enteric Diseases, National Institute for Communicable Diseases, Division in the National Health Laboratory Service, Johannesburg, South Africa

\textsuperscript{2}Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Correspondence: Anthony Smith
anthonys@nicd.ac.za

Abbreviation: CED, Centre for Enteric Diseases.


Fig. 1. Dendrograms of PFGE patterns following \textit{XbaI} digestion of genomic DNA: (a) \textit{Salmonella} Paratyphi A isolates, (b) \textit{Salmonella} Paratyphi B isolates and (c) \textit{Salmonella} Paratyphi C isolates.


