Serogenotyping and emergence of extended spectrum beta lactamase genes in non-typhoidal Salmonella: first report from Saudi Arabia

---Manuscript Draft---

<table>
<thead>
<tr>
<th>Manuscript Number:</th>
<th>JMM-D-16-00305R1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Title:</td>
<td>Serogenotyping and emergence of extended spectrum beta lactamase genes in non-typhoidal Salmonella: first report from Saudi Arabia</td>
</tr>
<tr>
<td>Short Title:</td>
<td>Serogenotyping and resistance genes in Salmonella</td>
</tr>
<tr>
<td>Article Type:</td>
<td>Correspondence</td>
</tr>
<tr>
<td>Section/Category:</td>
<td>One Health</td>
</tr>
</tbody>
</table>
| Corresponding Author:   | Abiola Senok  
                         | COLLEGE OF MEDICINE ALFAISAL UNIVERSITY  
                         | RIYADH, SAUDI ARABIA |
| First Author:           | Ghada Garaween  |
| Order of Authors:       | Ghada Garaween  
                         | Ali Somily  
                         | Adeola Raji  
                         | Sascha Braun  
                         | Wael Al-Kattan  
                         | Atef Shibl  
                         | Ralf Ehrich  
                         | Abiola Senok  |
Serogenotyping and emergence of extended spectrum beta lactamase genes in non-typhoidal Salmonella: first report from Saudi Arabia

Ghada Garaween¹, Ali Somily², Adeola Raji¹, Sascha Braun³, Wael Al-Kattan¹, Atef Shibli¹, Ralf Ehricht³, Abiola Senok¹

¹College of Medicine, Alfaisal University, Riyadh Saudi Arabia
²Department of Pathology and Laboratory Medicine, College of Medicine, King Khalid University Hospital and King Saud University, Riyadh, Saudi Arabia.
³Alere Technologies GmbH Löbstedter Straße 103 -105 D-07749 Jena, Germany
⁴InfectoGnostics Research Campus, Jena, Germany

Running title: Serogenotyping and resistance genes in Salmonella

This work was presented in part at the 26th European Congress of Clinical Microbiology and Infectious Diseases (9th-12th April 2016; Amsterdam)

Word count: 1971 words

Corresponding author

Dr Abiola Senok
Department of Microbiology & Immunology
College of Medicine, Alfaisal University
P.O. Box 50927; Riyadh 11533; Saudi Arabia
Tel: +966 112157661; Fax: +966 112157651
Email: asenok@alfaisal.edu

Manuscript Including References (Word document) Garaween et al_NTSREVISED_plus
*Salmonella* are Gram negative bacteria with >50 serogroups and >2500 serotypes. Ubiquitous non-typhoidal *Salmonella* (NTS) serotypes are associated with high morbidity and mortality. The classical approach for *Salmonella* classification into O-groups based on the O-antigen has been replaced by the Kauffmann-White-Le Minor scheme in which antigenic formulas of the serovars based on the O-antigen, H1-antigen and H2-antigen are denoted (Grimont & Weill 2007; Guibourdenche *et al.* 2010). The Kauffmann-White-Le Minor scheme is now the internationally accepted gold standard for *Salmonella* nomenclature to facilitate international comparability of *Salmonella* surveillance data and for outbreak investigations (Tindall *et al.* 2005; Guibourdenche, Roggentin *et al.* 2010). Available data on the distribution of *Salmonella* serotypes in Saudi Arabia has been based on classical O-antigen detection method (Kambal 1996; Somily *et al.* 2012; Elhadi *et al.* 2013). In recent years, resistant NTS including those harbouring extended spectrum beta-lactamase (ESBL) and carbapenemase genes have emerged (Sanchez-Vargas *et al.* 2011; Somily, Sayyed *et al.* 2012; Huang *et al.* 2013). This study was carried out to characterize NTS isolates from Saudi Arabia using serogenotyping and to determine carriage of ESBL and carbapenemase genes.

Archived multidrug resistant NTS identified from 2007-2014 at King Khalid University Hospital Riyadh, Saudi Arabia were studied. Ethical approval was obtained from the hospital Ethics committee. Classical serogrouping based on O-antigen was routinely performed as part of the identification protocol. Serogenotyping was carried out using the Salm-SeroGenoTyping AS-1 Kit (Alere Technologies GmbH, Jena, Germany) as previously described (Braun *et al.* 2012). This assay incorporates 255 different targets to analyse O- and H-phases antigens, assigns the genotype to the antigenic formula according to the Kauffmann-White-Le Minor scheme and detects some resistance genes. Molecular genotyping for ESBL and carbapenemase genes was carried out using the Check-MDR
CT103 DNA microarray (CheckPoints BV, Wageningen, Netherlands) (Naas et al. 2010; Stuart et al. 2012).

Forty-eight NTS isolates from stool (n=32), blood (n=10), perianal abscess (n=2), and one isolate each from urine, wound, chest drain and bone marrow were studied. Isolates were mostly from male patients (n=31) and paediatric age group (n=27). With the exception of one S. enterica subsp. salamae, all isolates were S. enterica subsp. enterica. The S. enterica subsp. salamae isolate was classified as S. enterica serotype II 13:b on serogenotyping. For the S. enterica subsp. enterica, 19 serovars were identified (Table). One isolate was classified as unknown because the combination of O- and H1-antigens detected did not match any known serovar (I, 28:z38: n.d.) and the H2-phase was not detectable. The most predominant were: Salmonella ser. Enteritidis (n=16), Salmonella ser. Typhimurium (n=9), Salmonella ser. Infantis (n=4) and there were two each for Salmonella ser. Handen, and Salmonella ser. Poona (Table). The reference manual published by the WHO Collaborating Centre for Reference and Research on Salmonella (Grimont & Weill 2007) was consulted to make a comparative analysis of the antigenic formulae obtained from serogenotyping and determine the expected O-antigen serogroup. Based on this comparative analysis with the WHO reference manual, these 19 serovars were found to belong to nine serogroups namely: B, C1, C2-C3, D1, E1, E4, G, L/V (Table). There was full agreement of the antigenic formulae generated by serogenotyping with the expected formulae as denoted in the WHO reference manual. We then compared these nine serogroups with the results from classical O-antigen serogrouping which was performed routinely in the diagnostic laboratory. From the routine serogrouping performed in the diagnostic laboratory, the isolates were distributed into 8 serogroups namely: D1, B, C1, C2, E1, G, O and Salmonella ser. Typhi, with four isolates which were not assigned into any serogroup (Table). Based on comparison of the classical
serogroup data and assigned serotype based on antigenic formula we ascertained that from the classical O-antigen grouping three isolates were assigned into wrong serogroups including one isolate from Group D1 which was misclassified as *Salmonella* ser. Typhi and four isolates which were not identified (Table). The following genes associated with beta lactam resistance were identified: *bla*<sub>TEM</sub>-1, *bla*<sub>PSE</sub>-1, *bla*<sub>OXA</sub>-1, *bla*<sub>CMY</sub> and *bla*<sub>CTX-M</sub>. The members of the ESBL *bla*<sub>CTX-M</sub> family identified were CTX-M1, CTX-M2; CTXM8; CTX-M9; CTX-M15; CTX-M26. Ten isolates belonging harboured only one gene. These include *Salmonella* ser. Typhimurium (*bla*<sub>TEM</sub>-1 n=4; *bla*<sub>PSE</sub>-1 n=2) and *Salmonella* ser. Kentucky (*bla*<sub>TEM</sub>-1). The *bla*<sub>OXA</sub>-1 gene was found in *Salmonella* ser. Infantis and *Salmonella* ser. Enteritidis while *bla*<sub>CMY</sub> was identified in *Salmonella* ser. Minnesota/Elbeuf (Table). One isolate (*Salmonella* ser. Handen) harboured the *bla*<sub>TEM</sub>-1 gene along with the ESBL *bla*<sub>CTX-M1</sub> gene while one *Salmonella* ser. Typhimurium isolate harbored the *bla*<sub>TEM</sub>-1 gene in combination both *bla*<sub>CTX-M1</sub> and *bla*<sub>CTX-M15</sub> ESBL genes (Table). One isolate (unknown serovar) haboured four ESBL genes which were all *bla*<sub>CTX-M</sub> (CTX-M 2, 8, 9, 26). No isolate was found to harbour carbapenamase genes.

To our knowledge, the data presented represents the first description of *Salmonella* serotyping data from Saudi Arabia using antigenic formulae in line with the Kauffmann-White-Le Minor classification scheme. Based on serogenotyping data, 19 *Salmonella* serotypes were identified with full agreement between the antigenic formulae generated by serogenotyping and the formulae in the WHO reference manual. This approach now enables comparability of data from Saudi Arabia with international data. The top two predominant serotypes (*Salmonella* ser. Enteritidis and *Salmonella* ser. Typhimurium) are similar to those reported in studies from Spain, USA and Taiwan (Lauderdale *et al.* 2006; Campos *et al.* 2013; Huang, Wang *et al.* 2013). This is in contrast to Thailand where *Salmonella* ser.
Weltevreden was the commonest serotype in humans followed by *Salmonella* ser. Enteritidis (Bangtrakulnonth *et al.* 2004). Several of the serotypes we have found in this study were identified in a recent report from Sudan (Elmadien *et al.* 2013), which is not surprising as there are close links and population movements between both countries. We identified two *Salmonella* ser. Poona isolates and this represents the second description of this serotype in the Arabian Gulf region (Al Benwan *et al.* 2010). The emergence of multidrug resistant *Salmonella* ser. Kentucky in returning travelers from Egypt, Kenya and Tanzania has been described (Majtan *et al.* 2006; Weill *et al.* 2006). Although *Salmonella* ser. Kentucky was described from Kuwait (Albert *et al.* 2014), to our knowledge, our finding represents the first description from Saudi Arabia. Furthermore, based on data from serogenotyping, we identified a possibly new *Salmonella* serotype as the combination of O- and H1-antigens detected did not match any known serovar (I; 28:z38: n.d.) in the WHO database (Grimont & Weill 2007).

The commonest beta lactam resistance gene was *bla*TEM-1. This is consistent with reported high prevalence of *bla*TEM-1 in NTS (Usha *et al.* 2008). Although *Salmonella* spp. harbouring ESBL *bla*CTX-M genes have been described in other parts of the world, there is limited data from the Arabian Gulf region and no report from Saudi Arabia. Although *bla*CTX-M15 in *Salmonella* isolates from Kuwait and United Arab Emirates has been reported (Rotimi *et al.* 2008), to our knowledge our findings represent the first description of *bla*CTX-M in NTS in Saudi Arabia and that of *bla*CTX-M2, *bla*CTX-M8 and *bla*CTX-M26 in *Salmonella* isolates in the Arabian Gulf region. We speculate that acquisition of these genes is occurring in *Salmonella* isolates circulating in our setting. It is even more worrying that we identified a potentially “new” serovar of *Salmonella* which harboured four *bla*CTX-M genes as this suggests the emergence of increasingly resistant NTS isolates. A limitation of our work is that it is a
single-center study and multicentre studies for the molecular epidemiological mapping of NTS in Saudi Arabia are needed.

Acknowledgements: No funding support was obtained for this work
REFERENCES


<table>
<thead>
<tr>
<th>Serovar based on Salmonella serogenotyping</th>
<th>No. of isolates</th>
<th>O Antigen</th>
<th>H1 Antigen</th>
<th>H2 Antigen</th>
<th>Serogroup based on comparison of antigenic formulae with WHO Reference*</th>
<th>Serogroup based on classical O-antigen grouping**</th>
<th>Beta-lactam resistance genes***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agona</td>
<td>1</td>
<td>4</td>
<td>f,g,s</td>
<td>[1,2]</td>
<td>B</td>
<td>Not identified</td>
<td>-</td>
</tr>
<tr>
<td>Anatum</td>
<td>1</td>
<td>3,{10}{15}{15,34}</td>
<td>e,h</td>
<td>1,6</td>
<td>E1</td>
<td>Not identified</td>
<td>-</td>
</tr>
<tr>
<td>Blockley</td>
<td>1</td>
<td>6,8</td>
<td>k</td>
<td>1,5</td>
<td>C2-C3</td>
<td>C1</td>
<td>-</td>
</tr>
<tr>
<td>Braenderup</td>
<td>1</td>
<td>6,7,14</td>
<td>e,h</td>
<td>e,n,z15</td>
<td>C1</td>
<td>D1</td>
<td>-</td>
</tr>
<tr>
<td>Enteritidis</td>
<td>16</td>
<td>1,9,12</td>
<td>g,m</td>
<td>-</td>
<td>D1</td>
<td>D1 (1 isolate was identified as <em>S. typhi</em>)</td>
<td><em>bla</em>OXA-1 (n=1)</td>
</tr>
<tr>
<td>Hadar</td>
<td>1</td>
<td>6,8</td>
<td>z10</td>
<td>e,n,x</td>
<td>C2-C3</td>
<td>C2</td>
<td>-</td>
</tr>
<tr>
<td><em>Handen</em></td>
<td>2</td>
<td>1,13,23</td>
<td>d</td>
<td>1,2</td>
<td>G</td>
<td>Not identified</td>
<td><em>bla</em>TEM-1 &amp; <em>bla</em>CTX-M1 (n=1)</td>
</tr>
<tr>
<td><em>Infantis</em></td>
<td>4</td>
<td>6,7,14</td>
<td>r</td>
<td>1,5</td>
<td>C1</td>
<td>O</td>
<td><em>bla</em>OXA-1 (n=1)</td>
</tr>
<tr>
<td><em>Kentucky</em></td>
<td>1</td>
<td>8</td>
<td>i</td>
<td>z6</td>
<td>C2-C3</td>
<td>C2</td>
<td><em>bla</em>TEM-1 (n=1)</td>
</tr>
<tr>
<td><em>Minnesota/Elbeuf</em></td>
<td>1</td>
<td>21/44</td>
<td>b</td>
<td>e,n,x</td>
<td>L/V</td>
<td>Not identified</td>
<td><em>bla</em>CMY (n=1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>----</td>
<td>-----</td>
<td>-----</td>
<td>----</td>
<td>-----</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>Newport</td>
<td>1</td>
<td>6,8,20</td>
<td>e,h</td>
<td>1,2</td>
<td>C2-C3</td>
<td>C2</td>
<td></td>
</tr>
<tr>
<td>Ouakam</td>
<td>1</td>
<td>9,46</td>
<td>z29</td>
<td>-</td>
<td>D2</td>
<td>D1</td>
<td></td>
</tr>
<tr>
<td>Poona</td>
<td>2</td>
<td>1,13,22</td>
<td>z</td>
<td>1,6</td>
<td>G</td>
<td>G</td>
<td></td>
</tr>
<tr>
<td>Senftenberg</td>
<td>1</td>
<td>1,3,19</td>
<td>g,s,t</td>
<td>-</td>
<td>E4</td>
<td>E1</td>
<td></td>
</tr>
<tr>
<td>Souza/Madjorio</td>
<td>1</td>
<td>1,13,19</td>
<td>d</td>
<td>e,n,x/</td>
<td>e,n,z15</td>
<td>E1</td>
<td></td>
</tr>
<tr>
<td>Tarshyne</td>
<td>1</td>
<td>9,12</td>
<td>d</td>
<td>1,6</td>
<td>D1</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>Tunis</td>
<td>1</td>
<td>3,{10},{15}</td>
<td>y</td>
<td>z6</td>
<td>G</td>
<td>E1</td>
<td></td>
</tr>
</tbody>
</table>
| Typhimurium   | 9  | 1,4,[5],12 | i   | 1,2 | B     | B    | blaTEM-1 (n=4)  
                  |    |        |     |     |       |      | blapSE-1 (n=2)  
                  |    |        |     |     |       |      | blaTEM-1,  
                  |    |        |     |     |       |      | blactX-M1 &  
                  |    |        |     |     |       |      | blactX-M15  
                  |    |        |     |     |       |      | (n=1)  
| Unknown       | 1  | 28    | z38 | n.d | -     | Not identified  
                  |    |        |     |     |       |      | blactX-M2,  
                  |    |        |     |     |       |      | blactX-M8,  
                  |    |        |     |     |       |      | blactX-M26 &  
                  |    |        |     |     |       |      | blactX-M9  
                  |    |        |     |     |       |      | (n=1)  

* comparative analysis with the WHO reference manual, these 19 serovars were found to belong to nine serogroups namely: B, C1, C2-C3, D1, E1, E4, G, L/V
Classical O-antigen serogrouping identified 8 serogroups and four isolates were not assigned into any serogroup. Comparison of the classical serogrouping data and assigned serogroup based on antigenic formula showed that classical serogrouping wrongly assigned three isolates.

***Number of isolates with the resistance gene is shown in brackets