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

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*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 The classical approach for *Salmonella* classification into O-groups based on the O-antigen has been replaced by the Kauffmann-White-Le Minor scheme in which antigenic formulas of the serovars based on the O-antigen, H1-antigen and H2-antigen are denoted (Grimont & Weill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 habouring 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, $C_1$, $C_2$-$C_3$, $D_1$, $E_1$, $E_4$, 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: $bla_{\text{TEM-1}}$, $bla_{\text{PSE-1}}$, $bla_{\text{OXA-1}}$, $bla_{\text{CMY}}$ and $bla_{\text{CTX-M.}}$ The members of the ESBL $bla_{\text{CTX-M}}$ 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 ($bla_{\text{TEM-1}}$ n=4; $bla_{\text{PSE-1}}$ n=2) and *Salmonella* ser. Kentucky ($bla_{\text{TEM-1}}$). The $bla_{\text{OXA-1}}$ gene was found in *Salmonella* ser. Infantis and *Salmonella* ser. Enteritidis while $bla_{\text{CMY}}$ was identified in *Salmonella* ser. Minnesota/Elbeuf (Table). One isolate (*Salmonella* ser. Handen) harboured the $bla_{\text{TEM-1}}$ gene along with the ESBL $bla_{\text{CTX-M1}}$ gene while one *Salmonella* ser. Typhimurium isolate harbored the $bla_{\text{TEM-1}}$ gene in combination both $bla_{\text{CTX-M1}}$ and $bla_{\text{CTX-M15}}$ ESBL genes (Table). One isolate (unknown serovar) haboured four ESBL genes which were all $bla_{\text{CTX-M}}$ (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 (Elmadiena *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 $bla_{\text{TEM-1}}$. This is consistent with reported high prevalence of $bla_{\text{TEM-1}}$ in NTS (Usha *et al.* 2008). Although *Salmonella* spp. harbouring ESBL $bla_{\text{CTX-M}}$ 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 $bla_{\text{CTX-M-15}}$ 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 $bla_{\text{CTX-M}}$ in NTS in Saudi Arabia and that of $bla_{\text{CTX-M2}}$, $bla_{\text{CTX-M8}}$ and $bla_{\text{CTX-M26}}$ 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 $bla_{\text{CTX-M}}$ 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.

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## REFERENCES

**Al Benwan, K., A. Al Mulla, H. Izumiya & M. J. Albert (2010).** Erythema nodosum and bilateral breast abscesses due to Salmonella enterica serotype Poona. *J Clin Microbiol* **48**, 3786-3787.

**Albert, M. J., K. Al Obaid, W. Alfouzan, A. R. Sheikh, E. Udo, H. Izumiya, D. M. Bulach & T. Seemann (2014).** Isolation of Salmonella enterica serovar Kentucky strain ST 198 and its H2S-negative variant from a patient: implications for diagnosis. *J Clin Microbiol* **52**, 4090-4093.

**Bangtrakulnonth, A., S. Pornreongwong, C. Pulsrikarn, P. Sawanpanyalert, R. S. Hendriksen, D. M. Lo Fo Wong & F. M. Aarestrup (2004).** Salmonella serovars from humans and other sources in Thailand, 1993-2002. *Emerg Infect Dis* **10**, 131-136.

**Braun, S. D., A. Ziegler, U. Methner, P. Slickers, S. Keiling, S. Monecke & R. Ehricht (2012).** Fast DNA serotyping and antimicrobial resistance gene determination of salmonella enterica with an oligonucleotide microarray-based assay. *PLoS One* **7**, e46489.

**Campos, M. J., G. Palomo, L. Hormeno, M. Ugarte, M. C. Porrero, S. Herrera-Leon, S. Vadillo, S. Piriz & A. Quesada (2013).** Co-occurrence of ACSSuT and cephalosporin resistance phenotypes is mediated by int1-associated elements in nontyphoidal Salmonella enterica from human infections in Spain. *Microb Drug Resist* **19**, 384-391.

**Elhadi, N., R. Aljindan & M. Aljeldah (2013).** Prevalence of nontyphoidal Salmonella serogroups and their antimicrobial resistance patterns in a university teaching hospital in Eastern Province of Saudi Arabia. *Infect Drug Resist* **6**, 199-205.

**Elmadiena, M. M. A. N., A. A. El Hussein, C. A. Muckle, L. Cole, E. Wilkie, K. Mistry & A. Perets (2013).** Antimicrobial susceptibility and multi-drug resistance of Salmonella enterica subspecies enterica serovars in Sudan. *TropicalAnimal health and production* **45**, 1113-1118.

**Grimont, P. A. & F. X. Weill (2007).** Antigenic formulae of the Salmonella serovars. *WHO Collaborating Centre for Reference and Research on Salmonella*, Paris France, Institut Pasteur.

**Guibourdenche, M., P. Roggentin, M. Mikoleit, P. I. Fields, J. Bockemuhl, P. A. Grimont & F. X. Weill (2010).** Supplement 2003-2007 (No. 47) to the White-Kauffmann-Le Minor scheme. *Res Microbiol* **161**, 26-29.

**Huang, J., M. Wang, H. Ding, M. Ye, F. Hu, Q. Guo, X. Xu & M. Wang (2013).** New Delhi metallo-beta-lactamase-1 in carbapenem-resistant Salmonella strain, China. *Emerg Infect Dis* **19**, 2049-2051.

**Kambal, A. M. (1996).** Antimicrobial susceptibility and serogroups of Salmonella isolates from Riyadh, Saudi Arabia. *Int J Antimicrob Agents* **7**, 265-269.

**Lauderdale, T. L., F. M. Aarestrup, P. C. Chen, J. F. Lai, H. Y. Wang, Y. R. Shiau, I. W. Huang, C. L. Hung & T. hospitals (2006).** Multidrug resistance among different serotypes of clinical Salmonella isolates in Taiwan. *Diagn Microbiol Infect Dis* **55**, 149-155.

**Majtan, V., T. Majtan, J. Majtan, M. Szaboova & L. Majtanova (2006).** Salmonella enterica serovar Kentucky: antimicrobial resistance and molecular analysis of clinical isolates from the Slovak Republic. *Jpn J Infect Dis* **59**, 358-362.

**Naas, T., G. Cuzon, H. Truong, S. Bernabeu & P. Nordmann (2010).** Evaluation of a DNA microarray, the check-points ESBL/KPC array, for rapid detection of TEM, SHV, and CTX-M extended-spectrum beta-lactamases and KPC carbapenemases. *Antimicrob Agents Chemother* **54**, 3086-3092.

**Rotimi, V. O., W. Jamal, T. Pal, A. Sovenned & M. J. Albert (2008).** Emergence of CTX-M-15 type extended-spectrum beta-lactamase-producing Salmonella spp. in Kuwait and the United Arab Emirates. *J Med Microbiol* **57**, 881-886.

**Sanchez-Vargas, F. M., M. A. Abu-El-Haija & O. G. Gomez-Duarte (2011).** Salmonella infections: an update on epidemiology, management, and prevention. *Travel Med Infect Dis* **9**, 263-277.

**Somily, A. M., S. B. Sayyed, H. A. Habib, A. S. Al-Khattaf, F. E. Al Otabi, Z. Shakoor & A. M. Kambal (2012).** Salmonella isolates serotypes and susceptibility to commonly used drugs at a tertiary care hospital in Riyadh, Saudi Arabia. *J Infect Dev Ctries* **6**, 478-482.

**Stuart, J. C., G. Voets, J. Scharringa, A. C. Fluit & M. A. Leverstein-Van Hall (2012).** Detection of carbapenemase-producing Enterobacteriaceae with a commercial DNA microarray. *J Med Microbiol* **61**, 809-812.

**Tindall, B. J., P. A. Grimont, G. M. Garrity & J. P. Euzeby (2005).** Nomenclature and taxonomy of the genus Salmonella. *Int J Syst Evol Microbiol* **55**, 521-524.

**Usha, G., M. Chunderika, M. Prashini, S. A. Willem & E. S. Yusuf (2008).** Characterization of extended-spectrum beta-lactamases in Salmonella spp. at a tertiary hospital in Durban, South Africa. *Diagn Microbiol Infect Dis* **62**, 86-91.

**Weill, F. X., S. Bertrand, F. Guesnier, S. Baucheron, A. Cloeckaert & P. A. Grimont (2006).** Ciprofloxacin-resistant Salmonella Kentucky in travelers. *Emerg Infect Dis* **12**, 1611-1612.

201 Table: Distribution of Serovars identified by sero-genotyping with carriage of resistance genes
202

| **Serovar based on Salmonella serogenotyping** | **No. of isolates** | **O Antigen** | **H1 Antigen** | **H2 Antigen** | **Serogroup based on comparison of antigenic formulae with WHO Reference*** | **Serogroup based on classical O-antigen grouping**** | **Beta-lactam resistance genes***** |
|---|---|---|---|---|---|---|---|
| Agona | 1 | 4 | f,g,s | [1,2] | B | Not identified | - |
| Anatum | 1 | 3,{10}{15}{15,34} | e,h | 1,6 | E1 | Not identified | - |
| Blockley | 1 | 6,8 | k | 1,5 | C2-C3 | C1 | - |
| Braenderup | 1 | 6,7,14 | e,h | e,n,z15 | C1 | D1 | - |
| Enteritidis | 16 | 1,9,12 | g,m | - | D1 | D1 (1 isolate was identified as *S. typhi*) | $bla_{\text{OXA-1}}$ (n=1) |
| Hadar | 1 | 6,8 | z10 | e,n,x | C2-C3 | C2 | - |
| *Handen* | 2 | 1,13,23 | d | 1,2 | G | Not identified | $bla_{\text{TEM-1}}$ & $bla_{\text{CTX-M1}}$ (n=1) |
| *Infantis* | 4 | 6,7,14 | r | 1,5 | C1 | O | $bla_{\text{OXA-1}}$ (n=1) |
| *Kentucky* | 1 | 8 | i | z6 | C2-C3 | C2 | $bla_{\text{TEM-1}}$ (n=1) |
| *Minnesota/Elbeuf* | 1 | 21/44 | b | e,n,x | L/V | Not identified | $bla_{\text{CMY}}$ (n=1) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Newport* | 1 | 6,8,20 | e,h | 1,2 | C2-C3 | C2 | - |
| *Ouakam* | 1 | 9,46 | z29 | - | D2 | D1 | - |
| *Poona* | 2 | 1,13,22 | z | 1,6 | G | G | - |
| *Senftenberg* | 1 | 1,3,19 | g,s,t | - | E4 | E1 | - |
| *Souza/Madjorio* | 1 | 1,13,19 | d | e,n,x/ e,n,z15 | E1 | E1 | - |
| *Tarshyne* | 1 | 9,12 | d | 1,6 | D1 | B | - |
| *Tunis* | 1 | 3,{10}{15} | y | z6 | G | E1 | - |
| Typhimurium | 9 | 1,4,[5],12 | i | 1,2 | B | B | *bla*$_{\text{TEM-1}}$ (n=4) *bla*$_{\text{PSE-1}}$ (n=2) *bla*$_{\text{TEM-1}}$, *bla*$_{\text{CTX-M1}}$ & *bla*$_{\text{CTX-M15}}$ ( n=1) |
| Unknown | 1 | 28 | z38 | n.d | - | Not identified | *bla*$_{\text{CTX-M2}}$, *bla*$_{\text{CTX-M8}}$, *bla*$_{\text{CTX-M26}}$ & *bla*$_{\text{CTX-M9}}$ (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

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207 ** Classical O-antigen serogrouping identified 8 serogroups and four isolates were not assigned into any serogroup. Comparison of the classical

208 serogrouping data and assigned serogroup based on antigenic formula showed that classical serogrouping wrongly assigned three isolates

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

210