

SHORT REPORT

High occurrence and unusual serotype diversity of non-typhoidal *Salmonella* in non-clinical niches, Angola

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Received 19 August 2016; Final revision 14 October 2016; Accepted 14 November 2016;
first published online 22 December 2016

SUMMARY

Non-typhoidal *Salmonella* is an important burden, particularly in developing countries of the African region. We report for the first time in Angola, a sub-Saharan African country with commercial/travel relationships with Europe, an unexpectedly high occurrence of *Salmonella* ($n = 12/63$, 19%) from a high diversity of sources, particularly farm and wild animals. The detection of diverse serotypes ($n = 12$), involving putative new *S. enterica* subsp. *salamae* serotypes, is also of note, reinforcing the need for a comprehensive surveillance in Angola critical to identify animal/food/environmental sources of salmonellosis with impact on animal health, local people, tourists and exported products.

Key words: Africa, Angola, non-clinical sources, non-typhoidal *Salmonella*, *Salmonella enterica* subsp. *salamae* serotypes.

Non-typhoidal *Salmonella* (NTS) is an important pathogen in developing countries of the African region, as was recently highlighted by the first WHO Initiative to Estimate the Global Burden of Foodborne Disease. In this report, 59 000 global deaths due to NTS were estimated (2010), with 32 000 deaths in the African region, and 22 000 deaths due to invasive disease, mostly in children [1]. Nevertheless, data to understand NTS epidemiology in most sub-Saharan African countries, namely related to the disease burden, serotype distribution, diversity of reservoirs and transmission routes are lacking, as recently highlighted [2, 3]. Among those countries with no NTS data is the former Portuguese African colony of Angola, a country with a high burden

of severe infectious diseases (e.g. malaria, diarrhoeal diseases) associated with an extended vulnerable population (e.g. HIV-infected adults, young children) and poor hygiene, which facilitates bacterial transmission and infection spread [4]. Of note was the last year's economic growth and close demographic, commercial or travel relationships of Angola with European countries (http://eeas.europa.eu/delegations/angola/175/angola-and-the-eu_en#Economic+%26+trade+relations). Food and live animals are among the goods traded between the European Union (EU) and Angola, with an increased trade flow in the last years (2012–2015) (http://trade.ec.europa.eu/doclib/docs/2006/sepember/tradoc_122456.pdf). Here we report a high occurrence and wide spread of unusual or putative new serotypes of antibiotic susceptible *Salmonella* in non-clinical sources of Angola and discuss the potential impact of globalization for their spread to other geographical regions.

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Table 1. Characterization of *Salmonella* isolates recovered from diverse sources in Angola

<i>Salmonella enterica</i> and subspecies	ST/eBG	Source (sample number/origin)§	Regions (communes)¶
Serotypes/PFGE type* (no. isolates)†	(no. isolates)‡		
<i>Salmonella enterica</i> subsp. <i>enterica</i> (subsp. I)			
Sundsvall/S2 (<i>n</i> = 2)	ST488 (<i>n</i> = 1)	Springboks (1/wild animal park)	Dombe Grande
Chester/S3 (<i>n</i> = 7)	ST411/eBG49 (<i>n</i> = 1)	Springboks (2/wild animal park)	Dombe Grande
		Grey monkeys (12/wild animal park)	Dombe Grande
		Cows (13/farm C)	Benguela
		Pigs (16/farm D)	Benguela
		Healthy person (20, 21)	Benguela
Poona/S4 (<i>n</i> = 2)	ST608 (<i>n</i> = 1)	Grey monkeys (11/wild animal park)	Dombe Grande
Muenster/S5 (<i>n</i> = 2)	ST321/eBG36 (<i>n</i> = 1)	Grey monkeys (11/wild animal park)	Dombe Grande
Braenderup/S9 (<i>n</i> = 2)	ST22/eBG24 (<i>n</i> = 1)	Avian feed (47/farm B)	Benguela
Cerro/S12 (<i>n</i> = 2)	ST367/eBG37 (<i>n</i> = 1)	Wastewater (59)	Benguela
<i>Salmonella enterica</i> subsp. <i>salamae</i> (subsp. II)			
9,46:z:-/S11 (<i>n</i> = 1)	ST1840 (<i>n</i> = 1)	Grey monkeys (11/wild animal park)	Dombe Grande
30:z29:z6/S7 (<i>n</i> = 1)	ST1841 (<i>n</i> = 1)	River (38)	Catumbela
4,12:l,w:z39/S8 (<i>n</i> = 1)	ST1842 (<i>n</i> = 1)	River (38)	Catumbela
50:b:z39/S6 (<i>n</i> = 1)	ST1839 (<i>n</i> = 1)	Grey monkeys (11/wild animal park)	Dombe Grande
35:z39:1,7/S1 (<i>n</i> = 1)	ST1888 (<i>n</i> = 1)	Springboks (1/wild animal park)	Dombe Grande
43:z29:e,n,x/S10 (<i>n</i> = 3)	ST1887 (<i>n</i> = 1)	Avian feed (65/farm A)	Benguela

* PFGE types are designated by 'S and number'.

† All the isolates were susceptible to ampicillin, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline and trimethoprim.

‡ ST, Sequence type; eBG, eBurstGroup (<http://mlst.warwick.ac.uk/mlst/dbs/Senterica>). Number of isolates analysed by MLST. Serotypes and STs described for the first time in this study are presented in bold.

§ Details of the type and number of samples for each source collected: healthy volunteers (*n* = 18/rectal swabs); food-producing animals and their environments [rectal swab pools of healthy chickens (*n* = 5/13 animals), cows (*n* = 3/9 animals) and pigs (*n* = 2/6 animals); corn feed (*n* = 3); floor/walls swabs (*n* = 2); water (*n* = 3)]; wild animal park [grey monkeys (*n* = 2/fresh faeces); springboks (*n* = 3/fresh faeces); water (*n* = 2)]; aquatic environments [rivers/lagoon (*n* = 3); drinking water (*n* = 7 treated and *n* = 5 untreated)]; and waste water [urban sewer lines (*n* = 3); treatment station (*n* = 2)].

¶ Samples were collected from Benguela Province, which comprises nine municipalities (Benguela, Lobito, Bocoio, Balombo, Ganda, Cubal, Caimbambo, Baía Farta, Chongorói), divided into >30 communes [6].

As part of a surveillance project on the occurrence of clinically relevant Gram-negative and Gram-positive bacteria [5, 6], we screened for the presence of *Salmonella* by standard methods [7] in a total of 63 samples recovered from different communes and sources from Benguela Province (including the second biggest city of Angola, Benguela): healthy volunteers, food-producing animals and their environments, wild animals and aquatic environments (Table 1). *Salmonella*-suspected colonies were identified by biochemical tests, slide agglutination and *invA* gene-PCR [7]. Antibiotic susceptibility was determined following European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (http://www.eucast.org/clinical_breakpoints/) and clonal relatedness by *Xba*I PFGE [7] and MLST (<http://mlst.warwick.ac.uk/mlst/dbs/Senterica>). Serotyping was performed at the National Centre of *Salmonella* (INSA, Lisbon) or the World Health Organization (WHO) Collaborating

Centre for Reference and Research on *Salmonella* (Institute Pasteur, Paris).

A high percentage [19%, 95% confidence interval (CI) 9.3–28.7, *n* = 12/63] of samples from different origins carried *Salmonella*. NTS occurrence in healthy humans (11%, 95% CI 0.0–25.5, *n* = 2/18, non-related individuals) was higher than other studies (e.g. 1%, Senegal; 2.4%, Guinea-Bissau) [8]. Despite differences in methodology and different sample sizes, these results suggest that asymptomatic carriers may be an important source of NTS in Angola. The dispersion of NTS in aquatic environments (10%, 95% CI 0.0–23.2, *n* = 2/20), farm (22%, 95% CI 2.9–41.1, *n* = 4/18) and wild animals (57%, 95% CI 20.3–93.7, *n* = 4/7) (Table 1) also suggest an extended diversity of NTS reservoirs and possible transmission routes in this region, as described in a few studies performed in African countries [9, 10]. This previously unreported epidemiological NTS scenario is of relevance for local

and international salmonellosis control strategies. In fact, Angola is currently an important economic and trade partner for the EU, benefiting from free access to EU markets for all Angolan products, and an emigration and tourism destination for Europeans (http://eeas.europa.eu/delegations/angola/175/angola-and-the-eu_en#Economic+%26+trade+relations; http://trade.ec.europa.eu/doclib/docs/2006/september/tradoc_122456.pdf). Recent studies showed that illegal importation of food animal products (including bushmeat – meat of wild animals and livestock meat) from Africa to Europe through airport passenger's luggage, including from Angola, was commonly verified [11–13]. In addition, some of those illegal food items were contaminated with foodborne pathogens such as NTS, suggesting a potential public health risk by a neglected route of transmission [12, 13]. International travel to countries in the region of sub-Saharan Africa was also considered one of the most relevant risk factors for Danish travellers being hospitalized with NTS bacteraemia [14]. Based on these findings it is of utmost importance the implementation in Angola of an integrated surveillance system of NTS in non-clinical contexts (including farm-to-fork) and clinical settings, complemented by effective strategies for the control of foodborne pathogens.

In this study, a high diversity of NTS serotypes ($n = 12$, 12 PFGE types/12 STs) was observed. Of note, was the unusually high prevalence of *Salmonella enterica* subsp. *salamae* serotypes ($n = 6$), corresponding to potentially new ones and/or assigned to new STs (Table 1). In developed European countries such as the UK some serotypes of this subspecies have been recognized to cause disease, occasionally severe infections [15]. Some cases have been associated with reptile meat consumption and/or travel to African countries, but the sources and transmission routes of those serotypes are still not well-understood worldwide [15, 16]. The lack of global data together with our identification of diverse potential sources (e.g. wildlife and farm animals) of *S. enterica* subsp. *salamae* spread in Benguela province (Table 1) emphasize the need to clarify the role of this subspecies as a cause of human salmonellosis in Angola as well as in other countries, including developed ones to which Angola has commercial or travel relationships. Moreover, less-frequent human-related *S. enterica* subsp. *enterica* serotypes distributed in different non-clinical sources, including wildlife and farm animals, are here described for the first time for Angola. Of note is a *S. Chester* clone disseminated in diverse animal species and healthy persons from

distant Benguela communes (Table 1), suggesting the occurrence of multi-niche adaptive features in these strains that might facilitate their association with salmonellosis in this region. The NTS serotypes detected in this study are not commonly associated with antibiotic resistance which might explain the antibiotic susceptibility of all isolates, despite the presence of multidrug-resistant bacteria of other species in the same samples [5, 6].

In conclusion, this study evidenced a high occurrence of NTS in Angola involving multiple non-clinical sources, including healthy humans, aquatic environments, farm and wild animals. Our data also suggest that Angola seems to be a hotspot of *Salmonella enterica* diversity involving potentially new serotypes. The detection of *Salmonella enterica* subsp. *salamae* serotypes from a high diversity of sources is also of note, suggesting potential reservoirs and transmission routes globally unknown for this subspecies. Insights on NTS epidemiology in Angola provided by this study reinforces the need for a comprehensive NTS surveillance in this country critical for developing public health strategies to prevent salmonellosis at both local and international levels.

ACKNOWLEDGEMENTS

The authors are grateful for financial support from FCT/MEC (Fundação para a Ciência e a Tecnologia/Ministério da Educação e Ciência) through national funds and co-financed by FEDER, under the Partnership Agreement PT2020 (grant no. UID/MULTI/04378/2013 – POCI/01/0145/FERDER/007728) and from project NORTE-01-0145-FEDER-000011 – Qualidade e Segurança Alimentar — uma abordagem (nano) tecnológica. J.C. is supported by a Ph.D. fellowship from Fundação para a Ciência e a Tecnologia (grant no. SFRH/BD/93091/2013). J.M. was supported by a Ph.D. fellowship from Fundação para a Ciência e a Tecnologia (grant no. SFRH/BD/77518/2011). T.G.R. is supported by a Ph.D. fellowship from Fundação para a Ciência e a Tecnologia (grant no. SFRH/BD/75752/2011).

The authors thank Hélder Gonçalves, Erineia Gonçalves and Walter Bonfim, for support in sample recruitment and Larissa Margalho for laboratory technical support. We are also very grateful to Jorge Machado (INSA, Lisbon, Portugal) and to François-Xavier Weill (Institute Pasteur, Paris, France) for *Salmonella* serotyping.

DECLARATION OF INTEREST

None.

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