Brief Original Article

Similarities between *Salmonella* Enteritidis isolated from humans and captive wild animals in South Africa

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Abstract

Introduction: *Salmonella* is well recognized as an aetiological agent of gastrointestinal and diarrhoeal disease. *Salmonella enterica* serotype Enteritidis (*Salmonella* Enteritidis) is one of the commonest serotypes associated with foodborne illness. In South Africa, we compared *Salmonella* Enteritidis strains isolated from humans with gastroenteritis and strains isolated from captive wild animals, between June 2011 and July 2012.

Methodology: Bacteria were phenotypically characterized using standard microbiological techniques. Genotypic relatedness of isolates was investigated by pulsed-field gel electrophoresis (PFGE) analysis.

Results: a diversity of 27 PFGE patterns amongst 196 human non-invasive isolates was shown; two PFGE patterns predominated and accounted for 74% of all human isolates. Human isolates showed a 12% prevalence rate for nalidixic acid resistance. Animal isolates from 5 different sources were investigated. With the exception of an isolate from a ground hornbill, all animal isolates (jaguar, crocodile, lion and poultry) showed PFGE pattern matches to a human isolate. Animal isolates showed susceptibility to all antimicrobial agents tested, with the exception of nalidixic acid resistance in isolates from the lion and poultry source.

Conclusions: Our data showed similarities between *Salmonella* Enteritidis strains isolated from humans and captive wild animals, suggesting a probable common source for strains from humans and animals.

Key words: *Salmonella* Enteritidis; wild animal; pulsed-field gel electrophoresis; PFGE; PulseNet.

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Introduction

Zoonotic diseases are caused by agents that are naturally transmissible between animals and humans. The majority (~61%) of infectious organisms affecting humans are of a zoonotic nature [1]. Worldwide, foodborne zoonosis, and more especially diarrhoeal diseases, is an important cause of morbidity and mortality in humans [2,3]. *Salmonella enterica* is an important zoonotic bacterial pathogen and well recognised as an aetiological agent of gastrointestinal and diarrhoeal disease [4]. Non-typhoidal *Salmonella* (NTS) remains a primary cause of reported foodborne disease events worldwide and outbreaks involving this pathogen are commonly described [5]. Worldwide, the epidemiology of human *Salmonella* disease is dominated by only a few non-typhoidal serotypes [6]. In Africa, *Salmonella enterica* serotype Enteritidis (*Salmonella* Enteritidis) and *Salmonella* Typhimurium are the two most commonly reported serotypes of NTS [7,8]. In the developed world, NTS disease is usually a self-limiting gastroenteritis with low mortality in humans, however in sub-Saharan Africa, NTS also frequently causes invasive disease which is associated with a substantial burden of illness and death [9]. *Salmonella* Enteritidis has a particular affinity for events of foodborne illness. Surveillance for foodborne disease outbreaks in the United States of America in 2007, showed that amongst aetiological agents identified in outbreaks, *Salmonella* was the second most common bacterial agent identified, with *Salmonella* Enteritidis the most commonly identified serotype [10]. In the current study, we report on the similarities between *Salmonella* Enteritidis strains isolated from humans with gastroenteritis and strains isolated from captive wild animals in South Africa.

Methodology

Study period and source of specimens/bacterial isolates

During the study period, June 2011 to July 2012, human bacterial isolates were investigated by the Centre for Enteric Diseases (CED) of the National Institute for Communicable Diseases. One hundred and ninety-six human isolates of *Salmonella* Enteritidis were investigated. The CED is a national reference centre in South Africa for human infections involving enteric pathogens, including: *Salmonella* species, *Shigella* species, diarrhoeagenic *E. coli* and *Vibrio cholerae*. The CED participates in national laboratory-based surveillance for these pathogens. Isolates are voluntarily submitted to the CED from ~200 clinical microbiology laboratories across the country. The CED is also the coordinating laboratory for the PulseNet Africa laboratory network (http://www.pulsenetinternational.org/networks/africa/). Animal specimens were investigated by Idexx Laboratories, Johannesburg, South Africa, which provides veterinary pathology laboratory services; bacterial isolates of zoonotic interest are, on an *ad hoc* basis, forwarded to the CED for genotyping and comparison to human isolates. Five animal isolates of *Salmonella* Enteritidis were investigated.

Culture and identification of bacterial isolates

Bacterial isolates were identified using standard phenotypic microbiological identification and serotyping techniques, briefly described as follows. Specimens were cultured at 37°C on selective agar media and in enrichment broths (media were supplied by Diagnostic Media Products, National Health Laboratory Service, Johannesburg, South Africa and Selecta-Media, Johannesburg, South Africa); agar media included Tryptose Blood agar, MacConkey agar, Sorbitol-MacConkey agar, Xylose Lysine Deoxycholate agar and Deoxycholate Citrate agar; enrichment broths included Selenite-F Broth and Rappaport-Vassiliadis broth. Suspicious colonies cultured on agar media were further investigated and identified using the VITEK automated identification system (bioMérieux, Marcy-l'Étoile, France). Salmonella cultures were serotyped according to the White-Kauffmann-Le Minor scheme [11]. Antimicrobial susceptibility testing was performed on Mueller-Hinton agar (Diagnostic Media Products and Selecta-Media) using the Etest method (bioMérieux); the following antimicrobials were tested: ampicillin, augmentin, trimethoprim, sulfamethoxazole, chloramphenicol, nalidixic acid, ciprofloxacin, tetracycline, kanamycin, streptomycin, imipenem, ceftriaxone and ceftazidime. Minimum inhibitory concentration (MIC) breakpoint values (see footnotes in Table 1) as described by the Clinical Laboratory Standards Institute (CLSI) were used to define antimicrobial resistance [12].

Genotyping of bacteria

Genotypic relatedness of isolates was investigated by pulsed-field gel electrophoresis (PFGE) analysis of

Table 1. Summary of data for *Salmonella* Enteritidis isolated from animal species in South Africa

Type of animal species	Type or source of specimen from animal species	Symptoms of animal or reason for specimen testing	Province in which specimen was isolated	Specimen collection date	Susceptibility to antimicrobial agents tested*
Crocodile (*Crocodylus niloticus*)	Liver	Septicaemia in 6-month old hatchlings	Mpumalanga	June 2012	Susceptible to all antimicrobial agents
Lion (*Panthera leo*)	Liver	Septicaemia in a cub	Free State	February 2012	Resistant only to nalidixic acid
Jaguar (*Panthera onca*)	Faeces	Diarrhoea	Gauteng	December 2011	Susceptible to all antimicrobial agents
Poultry (*Galliformes*)	Peritoneal swab	Septicaemia and deaths in egg laying hens	Limpopo	June 2012	Resistant only to nalidixic acid
Ground Hornbill (*Bucorvus leadbeateri*)	Cloacal swab	Enteritis in a chick	Gauteng	December 2011	Susceptible to all antimicrobial agents

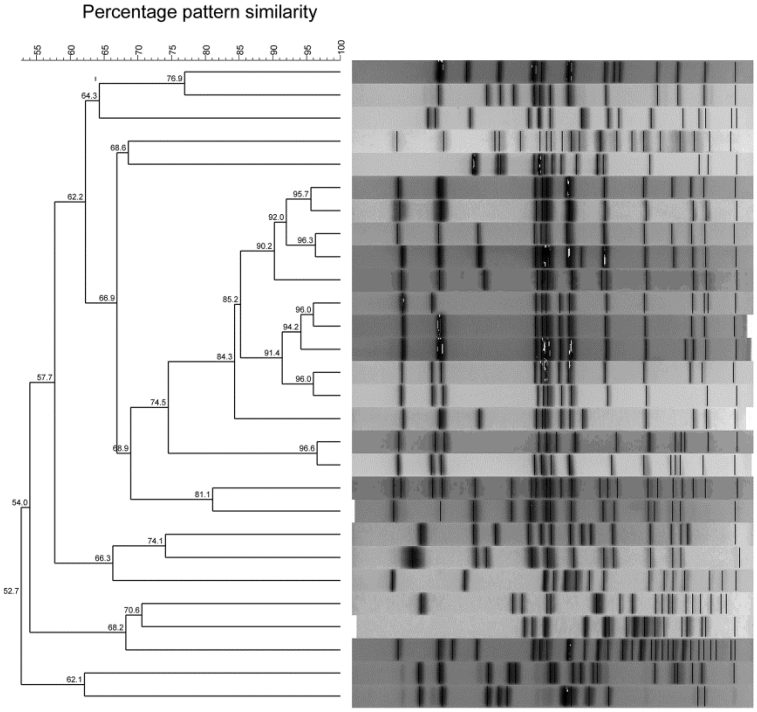
* Isolates were determined to be resistant to antimicrobial agents at the following MIC breakpoints: ampicillin, ≥16 µg/ml; augmentin, ≥16 µg/ml; trimethoprim, ≥16 µg/ml; sulfamethoxazole, ≥512 µg/ml; chloramphenicol, ≥16 µg/ml; nalidixic acid, ≥32 µg/ml; ciprofloxacin, ≥2 µg/ml; tetracycline, ≥8 µg/ml; kanamycin, ≥32 µg/ml; streptomycin, ≥64 µg/ml; imipenem, ≥8 µg/ml; ceftriaxone, ≥2 µg/ml; ceftazidime, ≥8 µg/ml.

*Xba*I digested genomic DNA on a Bio-Rad CHEF-DR III electrophoresis system (Bio-Rad Laboratories, Hercules, USA) using a PulseNet protocol [13]. PFGE patterns were captured into the PulseNet Africa database and analyzed using BioNumerics (version 6.5) Software (Applied Maths, Sint-Martens-Latem, Belgium) with dendrograms of the patterns created using the unweighted pair group method with arithmetic averages, with analysis of banding patterns incorporating the Dice-coefficient at an optimization setting of 1.5% and a position tolerance setting of 1.5%.

Results and Discussion

Salmonella Enteritidis and *Salmonella* Typhimurium are the two most commonly reported serotypes of NTS in humans in South Africa [14]. CED surveillance data for the years 2009-2011 showed that *Salmonella* Enteritidis and *Salmonella* Typhimurium accounted for 55% (3500/6345) of all *Salmonella* isolates tested; *Salmonella* Enteritidis alone accounted for 25% (1578/6345). Our human *Salmonella* Enteritidis PFGE database was established in June 2011 and includes only non-invasive (mostly from stool) isolates. For the period June 2011 to July 2012; 196 human non-invasive isolates were analyzed and they showed 27 unique patterns (Figure 1). Two predominant patterns (pattern 6 and pattern 14) were found to exist and accounted for 74% of all human isolates; pattern 6 was represented by 89 human isolates, while pattern 14 represented 55 human isolates. *Salmonella* Enteritidis is often described as a highly clonal serotype and as a result, some studies have reported how commonly used subtyping methodologies (including PFGE) may sometimes lack the discriminatory power to partition *Salmonella* Enteritidis strains into epidemiologically meaningful clusters. Our current study showed a good diversity of 27 PFGE patterns amongst 196 human isolates tested, suggesting that PFGE may still be a useful method for differentiating strains of *Salmonella* Enteritidis, at least within the African setting. Furthermore, our 196 human non-invasive isolates showed a fairly low prevalence of resistance to antimicrobial agents, with 49% of isolates showing full susceptibility to all antimicrobials tested. Fewer than 2% of isolates showed resistance to ampicillin, augmentin, trimethoprim, chloramphenicol, ciprofloxacin, kanamycin, streptomycin, imipenem, ceftriaxone or ceftazidime. The following three antimicrobials were associated with higher prevalence of resistance in isolates: 43% for sulfamethoxazole, 12% for nalidixic acid and 4% for tetracycline. Nalidixic acid-resistant isolates all showed ciprofloxacin susceptibility (MICs, 0.125-0.25 μg/ml).

Figure 1. PFGE patterns (*Xba*I digestion) for *Salmonella* Enteritidis isolated in South Africa



Pattern number	Number of human isolates with pattern	Animal isolate with pattern
1	2	Jaguar
2	0	Ground hornbill
3	1	
4	1	
5	1	
6	89	Crocodile
7	1	
8	12	
9	1	
10	2	
11	1	
12	13	
13	1	
14	55	Lion
15	2	Poultry
16	1	
17	1	
18	2	
19	1	
20	1	
21	1	
22	1	
23	1	
24	1	
25	1	
26	1	
27	1	
28	1	

For the period June 2011 to July 2012, Idexx Laboratories in Johannesburg received ~8000 animal specimens for culture, of which 69 *Salmonella enterica* isolates were identified. Six isolates were serotyped as *Salmonella* Enteritidis; five isolates were available for further analysis. These five isolates were from different animal sources (Table 1) and included isolates from a jaguar (*Panthera onca*), a ground hornbill (*Bucorvus leadbeateri*), a crocodile (*Crocodylus niloticus*), a lion (*Panthera leo*), and a poultry source (*Galliformes*). There is no formalised surveillance system in place to investigate animal isolates; the CED receives animal isolates on an *ad hoc* basis; this explains the small number of animal isolates investigated. Besides nalidixic acid resistance in isolates from the lion and poultry source, all animal isolates showed susceptibility to all antimicrobials tested. With the exception of the isolate from the ground hornbill, all animal isolates showed PFGE pattern matches to a human isolate (Figure 1). The PFGE pattern from the crocodile isolate matched PFGE pattern 6, the most commonly identified PFGE pattern from human isolates. PFGE patterns of isolates from the lion (pattern 14) and the poultry source (pattern 15) were extremely similar (96% pattern similarity) and only differed by a single band; both these animal isolates also showed nalidixic acid resistance. PFGE pattern 14 is notably the second most commonly identified PFGE pattern associated with human isolates. For human isolates, of those showing PFGE pattern 14, 47% (26/55) showed nalidixic acid resistance; while of those showing PFGE pattern 15, 100% (2/2) showed nalidixic acid resistance.

Published data describing *Salmonella* isolated from captive wild animals is very limited, while data describing comparison of animal *Salmonella* isolates to human isolates is even more difficult to source; we were unable to find any data comparing genotypic data for *Salmonella* isolated from humans and captive wild animals. Amongst the limited published data on *Salmonella* isolated from captive wild animals, data on reptiles seems to be amongst the most commonly reported [15-18], including reports of *Salmonella* Enteritidis isolated from captive crocodiles [16]. Among animal species, *Salmonella* Enteritidis infections are largely associated with poultry. In South Africa, a study of *Salmonella* isolations from animal species showed that 96% of all *Salmonella* Enteritidis isolations originated from poultry [19]. Investigation of *Salmonella* Enteritidis outbreaks and sporadic cases regularly show that when human infections are identified as foodborne, then the most common sources of infection are poultry and poultry derivatives [20,21]. The diet of captive wild animals commonly includes poultry products such as chicken. Chicken products are widely available and a very affordable food source, especially for a carnivore diet which includes large volumes of meat. In South Africa, chicken meat is arguably the most affordable meat product for human consumption and as a result is a primary food source for humans. So, chicken meat and related chicken by-products may be a probable common link and may be a source of related strains of *Salmonella* Enteritidis isolated from humans and captive wild animals.

In conclusion, we show similarities between *Salmonella* Enteritidis strains isolated from humans with gastroenteritis and strains isolated from captive wild animals in South Africa, suggesting a probable common source for strains from humans and animals. A limitation of this study was the few animal strains investigated; this limitation can be overcome by establishing stronger links and networking between veterinary and human microbiology laboratories.

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