First identification of *Salmonella* Urbana and *Salmonella* Ouakam in humans in Africa

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Abstract
Introduction: *Salmonella* infections are increasing worldwide, but there are few reports on *Salmonella* surveillance in African countries and other developing countries. This has made it difficult to estimate the actual burden of salmonellosis, especially in Africa. This study was conducted in a neglected Northern Region of Ghana where there are no previous data on *Salmonella* serotypes.
Methodology: Standard microbiological tests were used for isolation, identification, and serotyping. Micro-dilution was used for the antimicrobial susceptibility tests.
Results: Four serotypes of *Salmonella* were identified: *S.* Urbana, *S.* Ouakam, *S.* Senftenberg, and *S.* Stanleyville. All the serotypes were susceptible to the 20 antibiotics used in the susceptibility test. *S.* Urbana and *S.* Ouakam were identified in humans for the first time in Africa.
Conclusion: This study may serve as a baseline study for future investigations on *Salmonella* in the region and may assist public health officials to take the appropriate measures in case of a disease outbreak caused by *Salmonella* in the area. The article may also give health officials a fair idea of the resistance level of these serotypes in the region.

Key words: Salmonella serotypes; antibiotic resistance; Ghana; Africa

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Introduction

Salmonella is a major cause of morbidity and mortality in both humans and animals. The provision of good sanitation in developed countries has led to a decline in recorded infections. However, *Salmonella* remains a major public health problem in many parts of various developing countries. Two of the major factors contributing to the increased burden of diseases, especially in Africa, are unhygienic practices and inadequate knowledge about the existence of microorganisms. Some people in Africa still believe that infectious diseases are a result of curses from gods and that the only way to cure infectious diseases is to pacify those gods. The problem is further compounded by logistics and the unavailability of trained personnel and adequate financial support from governments for scientific research. Non-typhoidal *Salmonella* (NTS) is on the increase and has been reported to be the leading cause of hospitalization and death among food-borne illnesses in the United States [1]. The complications are life threatening, and early detection of *Salmonella* infection is the key to saving lives. However, there are few studies on human salmonellosis and drug resistance in Ghana and other sub-Saharan African countries, and the true burden of the disease is also unknown because of the lack of prevalence surveys in most African countries. Most of the cases go unnoticed or are misdiagnosed as other enteric diseases or malaria; these cases occasionally lead to preventable deaths. Certain studies on *Salmonella* in Ghana did not identify *Salmonella* to the serotype level, and most isolated microorganisms were not subjected to antimicrobial susceptibility testing [2]. Prevalence data on *Salmonella* in the northern region of Ghana is not readily available.

Furthermore, the outbreak or isolation of *S*. Urbana is not a frequent phenomenon worldwide as compared to other serotypes of *Salmonella*. Over the last three decades, only seven serious cases of *S*. Urbana in humans have been reported (Table 1). The principal reservoirs for NTS are the gastrointestinal tract of animals, including poultry, livestock, pets, and reptiles

Table 1. Worldwide reported cases of *Salmonella* Urbana and Ouakam

Serotype	Country	Origin	Reference
***S.* Urbana**	Ghana	Human	This work
	Finland	Human	11
	Czech Republic	Human	11
	Latvia	Human	11
	Slovakia	Human	3
	Japan	Human	16
	USA	Turkey, Bovine, Reptile, Equine, Wild animal	17
	USA	Human	17
	Thailand	Human	18
	India	Human	19
	Argentina	Human	20
	Italy	Human	21
***S.* Ouakam**	Ghana	Human	This work
	Sweden	Feed	22
	Morocco	Food	4
	Germany	Poultry	23
	UK	Poultry	24
	USA	Dairy	25
	Sweden	Feed	26
	USA	Human	17
	USA	Turkey, Chicken, Feed, Equine, Wild animal, Porcine	17
	UK	Human	25

[3]. Until now, only one reported case of *S.* Ouakam in food from Africa has been recorded [4], but there have been no reports of *S.* Ouakam from human isolates. There is little or no information about the antimicrobial resistance status of Enterobacteriaceae, especially *Salmonella*, in the northern regions of Ghana. A review of all the *S*. Urbana and *S*. Ouakam infections worldwide is presented in Table 1. In the present study, we isolated, identified, serotyped, and performed antibiotic susceptibility tests of *Salmonella* spp. on clinical faecal samples from the northern region of Ghana to determine the prevalence of *Salmonella* in patients who submitted stools for analysis in a teaching hospital.

Methodology

Ninety-one faecal samples from unrelated cases were obtained in sterile bottles from the laboratory section of a teaching hospital from both in-patients and outpatients who presented stool samples for analysis in January 2010. As control, ten fresh faecal samples were collected with a transportable medium (MEUS, Como, Italy), from an open defecation zone in the Tamale metropolis, where presumably healthy people defecated. Demographic and other clinical data of patients could not be retrieved due to inadequate record keeping. This research was a collaborative project between the Biotechnology Department of the University for Development Studies in Tamale, Ghana, and the Universidad Complutense de Madrid in Spain. The faecal samples were pre-enriched in 9 mL of buffered peptone water (BPW) and incubated at 37°C for 18 hours. The incubated samples were selected on modified semi-solid Rappaport Vassiliadis (MSRV) (BioMérieux, Marcy l'Etoile, France) at 41.5°C for 48 hours. The suspected *Salmonella* colonies were further identified on both *Salmonella* identification agar (SMID2) (BioMérieux, Marcy l'Etoile France) and xylose lysine deoxycholate (XLD) agar plates (BioMérieux, Marcy l'Etoile, France). Identification was further confirmed biochemically using the BBL Enterotube II (Becton Dickinson; Franklin lakes, USA). The serotypes were first identified at the VISAVET Health Surveillance Centre

Table 2. Antimicrobial susceptibility values of the various serotypes of *Salmonella*

	MIC (mg/L)			
.	***S.* Urbana**	***S.* Ouakam**	***S.* Stanleyville**	***S.* Senftenberg**
Ampicillin	0.5	0.5	0.5	0.5
Ceftazidime	0.25	0.5	0.25	0.25
Cefotaxime	0.06	0.06	0.06	0.06
Chloramphenicol	4	4	4	4
Ciprofloxacin	0.03	0.03	0.03	0.015
Colistin	4	4	4	4
Gentamicin	1	0.5	0.5	0.5
Florfenicol	4	4	4	2
Kanamycin	4	4	4	4
Nalidixic Acid	4	4	4	4
Streptomycin	8	8	8	16
Sulphamethoxazole	32	8	64	8
Tetracycline	1	2	2	2
Trimethoprim	0.05	0.05	0.05	0.05

in Madrid, Spain, using the slide agglutination test with antisera and were reconfirmed at the Instituto de Salud Carlos III, in Madrid, Spain, using the Kaufmann-White *Salmonella* serotyping scheme published by Institut Pasteur [5] and the Edwards and Ewing's identification method [6].

Antibiotic susceptibility of the isolates was determined using both the Kirby Bauer disk diffusion method and the broth microdilution method in microtiter plates (Sensititre EUMV2, Trek Diagnostics, Inc., Westlake, USA) according to CLSI guidelines [7]. Twenty antibiotics were used for the susceptibility testing with *Escherichia coli* ATCC 25922 strain as a control. The breakpoints were determined using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [8] and the National Committee for Clinical Laboratory Standards (NCCLS) document M31-A2 [9] for clinical breakpoints.

Results

Four of the samples were positive for *Salmonella.* Three of the isolates were isolated from samples collected from a teaching hospital, and one was isolated from a sample from the open defecation zone. The carriage rate of *Salmonella* in this study was 3.9%. The serotypes identified in this study were *S.* Urbana*,* *S.* Ouakam, *S.* Stanleyville, and *S.* Senftenberg. *S.* Senftenberg was isolated from the open defecation zone, while the rest were isolated from outpatients and in-patients from the hospital.

The isolates were susceptible to all the antibiotics tested: amoxicillin-clavulanate, cefoxitin, amikacin, apramycin, imipenen, aztreonam, ciprofloxacin, sulfamethoxazole, gentamicin, ampicillin, cefotaxime, ceftazidime, tetracycline, streptomycin, trimethoprim, chloramphenicol, florfenicol, kanamycin, nalidixic acid, and colistin. The minimum inhibitory concentrations (MICs) of some of these antibiotics are shown in Table 2.

Discussion

Even though the numbers of faecal samples involved in this work were limited, the isolation of exotic *Salmonella* spp. is of great public concern in the northern region of Ghana. The access to potable water in this region is a major problem that the residents face, especially in the dry season, in both rural and urban areas. Uncovered dugout wells are common in the region. The wells serve as a source of drinking water for people and animals, especially free-range animals such as cattle, goats, sheep, poultry, and reptiles as well. These wells can also be the sources of infections with exotic *Salmonella* spp. Only 2% of the rural population in Ghana had access to piped water within their homes, yards, or plots, and this percentage has remained constant over the past five years [10]; the situation in the northern regions is the worst. We suggest that water sources, especially the untreated ones, should be examined to trace the source of infections of people with exotic *Salmonella* spp. since it is highly possible that the infected people might drink or use infected water.

There have been no reported cases of infection due to *S*. Urbana in Ghana or in Africa. Recently, there was a report of severe infections caused by *S*. Urbana in Finland, Czech Republic, and Latvia [11]. The *S*. Ouakam serotype is rarely isolated from either humans or food, and this serotype has never been isolated from humans in Africa. The only report on *S*. Ouakam was from Morocco, where it was isolated from food [12]. Isolates were also susceptible to all the antibiotics used in that study, similar to our study, where the four isolates were susceptible to the 20 antibiotics used in the susceptibility testing. There have been no reports of *S*. Ouakam from humans in Ghana or Africa. Most of the reported cases of *S*. Stanleyville and *S*. Senftenberg isolation in humans occurred in Africa, so these seem to be endemic in Africa. *S*. Stanleyville and *S*. Senftenberg have been previously isolated in other West African countries [13,14]; however, they have never been reported in Ghana. There are few reports of the isolation of *Salmonella* in Ghana, probably due to the lack of facilities and logistics in the region for performing these tests.

Presently, it could be said that there is little risk of these isolates resulting in serious infections or superbugs in the region due to the sensitivity of the isolates to all the antibiotics used in this study (Table 2). A study on the susceptibility of other serotypes in northern Ghana in 2008 also reported susceptibility of all their *Salmonella* isolates to all antibiotics used [15]. Similarly, most of the *Salmonella* spp. isolated from a neighbouring country, Burkina Faso, were also susceptible to most of the antimicrobials tested [13]. However, these susceptible strains can acquire genetic elements in the long term through intra-species and inter-species horizontal gene transfer via mobile genetic elements, and become resistant to clinically relevant antibiotics.

Conclusion

To the best of our knowledge, this study has identified for the first time the presence of *S*. Urbana and *S*. Ouakam in humans in Africa. We have also reported for the first time the presence of *S*. Stanleyville in Ghana, and *S*. Senftenberg in northern Ghana. We recommend that more attention be paid to the deplorable state of the water supply system in the northern region of Ghana. Regular antibiotic resistance susceptibility surveillance to evaluate and monitor infectious diseases in the region may help identify the resistance pattern.

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References

1. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM (2011) Foodborne illness acquired in the United States--major pathogens. Emerg Infect Dis 17: 7-15.
2. Saba CKS, González-Zorn B (2012) Microbial Food safety in Ghana: A Meta-Analysis. J Infect Dev Ctries 6: 828-835.
3. Kocianová H, Litvová S, Stefkovicová M, Gavacová D, Rovný I, Glosová L, Hudecková H (2010) Exotic pets as a potential source of *Salmonella.* Epidemiol Mikrobiol Imunol 59: 9-12.
4. Ammari S, Laglaoui A, En-Nanei L, Bertrand S, Wildemauwe C, Barrijal S, Abid M (2009) Isolation, drug resistance and molecular characterization of *Salmonella* isolates in northern Morocco. J Infect Dev Ctries 3: 41-49.
5. Grimont PAD, François-Xavier W (2007**)** Antigenic formulae of the *Salmonella* serovars. WHO Collaborating Centre for Reference and Research on *Salmonella*. Institut Pasteur, Paris, France.
6. Ewing W H (1986) Edwards and Ewing’s identification of Enterobacteriaceae, 4th ed., p.181-340. Elsevier Science Publishing Co., Inc., New York, N.Y.
7. Clinical and Laboratory Standards Institute (2009) Performance standards for antimicrobial susceptibility testing, 19th ed. Approved standard M100-S19. CLSI, Wayne, PA.
8. European Committee on Antimicrobial Susceptibility Testing (2010) Available: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/EUCAST_QC_tables_1.3.pdf. Accessed on 13 July 2010.
9. National Committee for Clinical Laboratory Standards (2002) Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals-2nd Edition: Approved Standard M31-A2. NCCLS, Wayne, PA, USA
10. DHS (2003) Ghana DHS-Final Report. Demographic and Health Survey, Maryland.

11. Rimhanen-Finne R, Lukinmaa S, Martelius T, Rossow H, Karpiskova R, Dedicova D, Galajeva J, Bormane A, Siitonen A, Kuusi M (2010) Cases of *Salmonella* Urbana in Finland, the Czech Republic and Latvia, January-February 2010. Euro Surveill 15: pii=19511.
12. Ammari S, Laglaoui A, En-Nanei L, Bertrand S, Wildemauwe C, Barrijal S, Abid M (2009) Isolation, drug resistance and molecular characterization of *Salmonella* isolates in northern Morocco. J Infect Dev Ctries 3: 41-49.
13. Kagambèga A, Haukka K, Siitonen A, Traoré AS, Barro N (2011) Prevalence of Salmonella enterica and the hygienic indicator Escherichia coli in raw meat at markets in Ouagadougou, Burkina Faso. J Food Prot 74: 1547-1551.
14. Tennant SM, Diallo S, Levy H, Livio S, Sow SO, Tapia M, Fields PI, Mikoleit M, Tamboura B, Kotloff KL, Nataro JP, Galen JE, Levine MM (2010) Identification by PCR of non-typhoidal Salmonella enterica serovars associated with invasive infections among febrile patients in Mali. PLoS Negl Trop Dis 9: 4.
15. Djie-Maletz A, Reither K, Danour S, Anyidoho L, Saad E, Danikuu F, Ziniel P, Weitzel T, Wagner J, Bienzle U, Stark K, Seidu-Korkor A, Mockenhaupt FP, Ignatius R (2008) High rate of resistance to locally used antibiotics among enteric bacteria from children in Northern Ghana. J Antimicrob Chemother 61: 1315-1318.
16. Minami K, Yanagawa T, Okuda M, Suzuki H, Tamura A, Izumi G, Yoshikawa N (2004) Cerebrospinal fluid cytokines in *Salmonella* Urbana encephalopathy. Tohoku J Exp Med 203: 129-132.
17. Center for Disease Control and Prevention Annual Reports. Available at: http://www.cdc.gov/.
18. Sirinavin S, Hotrakitya S, Suprasongsin C, Wannaying B, Pakeecheep S, Vorachit M (1991) An outbreak of *Salmonella* Urbana infection in neonatal nurseries. J Hosp Infect 18: 231-238.
19. Devi S, Murray CJ (1991) *Salmonella* carriage rate amongst school children--a three-year study. Southeast Asian J Trop Med Public Health 22: 357-361.
20. Curi de Montbrun SE, Ciccarelli AS, de Ampuero S, Fernández RA, Benito MA (1981) Infections due to several species of *Salmonella* in Mendoza, Argentina. Rev Argent Microbiol 13: 23-30.
21. Fortina G, Farinetti F, Comazzi G (1975) *Salmonella* serotypes isolated at the Ospedale Maggiore at Novara during the years 1966-1973 in sporadic cases of enteropathy. G Batteriol Virol Immunol 68: 95-100.
22. Wierup M, Häggblom P (2010) An assessment of soybeans and other vegetable proteins as source of *Salmonella* contamination in pig production. Acta Vet Scand 44: 181-197.
23. Schulz J, Lücking G, Jeroen D, Hartung J (2009) Prevalence of *Salmonella* in Germany battery cages and alternative housing system. 14TH International Congress of the International Society for Animal Hygiene (ISAH- Congress 2009): Sustainable animal husbandry: prevention is better than cure. Vechta, Germany. 699-702.
24. Wales A, Bresin M, Carter B, Sayer R, Davis R (2007) A longitudinal study of environmental *Salmonella* contamination in caged and free ranged layer flock. Av Pathol 36: 187-197.
25. Edington TS, Callaway TR, Anderson RC, Nisbet DJ (2008) Prevalence of multidrug-resistance *Salmonella* on commercial dairies using a single heifer facility. J Food Prot 71: 27-34.
26. Boqvist S, Hansson I, Nord Bjerselius U, Hamilton C, Wahlström H, Noll B, Tysen E, Engvall A (2003) *Salmonella* isolated from animals and feed production in Sweden between 1993 and 1997. Acta Vet Scand 44: 181-197.
27. Dryden MS, Gabb RJ, Wright SK (1996) Empirical treatment of severe acute community-acquired gastroenteritis with ciprofloxacin. Clin Infect Dis 22: 1019-1025.

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