Emerging Problems in Infectious Diseases

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...
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.
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, imipenem, 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

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (mg/L)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td><em>S. Urbana</em></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0.5</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>0.25</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>0.06</td>
</tr>
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<td>Chloramphenicol</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Colistin</td>
<td>4</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1</td>
</tr>
<tr>
<td>Florfenicol</td>
<td>4</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>4</td>
</tr>
<tr>
<td>Nalidixic Acid</td>
<td>4</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>8</td>
</tr>
<tr>
<td>Sulphamethoxazole</td>
<td>32</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>0.05</td>
</tr>
</tbody>
</table>

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


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**Conflict of interests:** No conflict of interests is declared.