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Antimicrobial Susceptibilities of Salmonellae Isolated from Food Handlers and Cattle in Lagos, Nigeria

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Abstract

Purpose: Food handlers play an important role in the transmission of typhoid bacilli and other *Salmonella* spp. This study was conducted to determine the prevalence and antimicrobial resistance patterns of *Salmonella* spp. from food handlers and cattle and compare the patterns with specimens from patients.

Methods: A total of 206 stool samples from apparently healthy food handlers from *bukkas* (cafeteria) and 100 stool samples from cattle were collected in Lagos Metropolis between June 2006 and June 2007. The prevalence and susceptibility patterns of the *S. typhi* isolates from the samples and those obtained from the stool samples of 27 patients were determined.

Results: *Salmonella* species isolated from the stool samples collected from food handlers were *S. typhi*, *S. enteritidis*, *S. choleraesuis, S. paratyphi* A and *S. arizona* with prevalence of 6.8%, 5.3%, 2.9%, 1.5% and 0.5%, respectively. *S. enteritidis* and *S. typhimurium* were isolated from 100 faecal cattle samples with prevalence of 12% and 3%, respectively. Nearly all the isolates (including the clinical isolates) were sensitive to nitrofurantoin, nalidixic acid, and ofloxacin, while all were sensitive to ciprofloxacin.

Conclusion: Food handlers working in *bukkas* (cafeteria) and cattle are possible reservoirs of *Salmonalle* species in Lagos. Other than fluoroquinolones, *Salmonalle* species exhibit high levels of resistance to most antibiotics.

Keywords: Salmonella species; Food handlers; Cattle, Antibiotic resistance; Lagos.

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Introduction

The incidence of food borne infection by Salmonellae is an important problem world-wide. Salmonellae are strongly associated with agricultural products of which more than 95% of cases of the infections are food-borne and salmonellosis accounts for approximately 30% of deaths resulting from food-borne illnesses in the United States [1]. Raw and undercooked meat, poultry, eggs and milk are the most commonly implicated vehicles of *Salmonella* spp infection [2,3] . Non-typhoidal salmonella are mostly implicated in food-borne infections and include mainly *S. enterica, S. typhimurium* and *S. enteritidis* [4]. *S. enterica* serovar *typhi* is the etiologic agent of typhoid fever and causes an estimated 16.6 million cases and 600,000 deaths worldwide each year. A syndrome similar to typhoid fever is caused by paratyphoid serotypes of *Salmonella* [5].

S. typhi is transmitted through food or water that has been contaminated with faeces from either acutely infected persons, persistent excretors or from chronic asymptomatic carriers handling food. Humans are the only host for *S. typhi*; there are no environmental reservoirs [5,6]. Typhoid fever is still a major public health problem in many developing countries [6]. It is a sporadic disease in developed countries that occurs mainly in returning travelers with occasional point-source epidemics [7]. In endemic areas, identified risk factors for the disease include eating food prepared outside the home, such as ice cream or flavored iced drinks from street vendors [8], drinking contaminated water [9], having a close contact or relative with recent typhoid fever [10], poor housing with inadequate facilities for personal hygiene [11], and recent use of antimicrobial drugs [8]. For the detection of carriers, several samples are often examined because of the irregular nature of shedding [12].

Up to 10 percent of convalescing patients with untreated typhoid fever excrete *S. typhi* in the faeces for up to three months; one to four percent becomes long-term carrier, excreting the organism for more than one year. Up to 25 percent of long-term carriers have no history of typhoid. Chronic carriage is more common among women and the elderly and in patients with cholelithiasis [13]. About five percent of patients clinically cured from typhoid remain carriers for months or even years. Antibiotics are usually ineffective on *Salmonella* carriage (even if salmonellae are susceptible to them) because the site of carriage may not allow penetration by the antibiotics [14].

In Nigeria, transmission of typhoid fever appears to occur all year round but slightly heavier in April and July [15]. Even so, not much has been reported about salmonellae (typhoidal and non-typhoidal) amongst food handlers. The purpose of the study is to carry out antimicrobial susceptibility patterns of *Salmonella* species from food handlers and cattle and compare the patterns with clinical Salmonella isolates.

Methods

Stool samples were collected from 206 apparently healthy food handlers from *bukkas* (cafeteria) and convenient 100 randomly selected cattle in Lagos Metropolis between June 2006 and June 2007. In addition, 5 ml blood samples were also collected from 27 patients confirmed through laboratory investigation to have typhoid fever. Food handlers excluded from the study include Excluded from the food handlers those with clinical signs of typhoid fever and those on antibiotics for at least two weeks prior to the study. The stool samples were collected in leak-proof containers and were promptly transported to the Molecular Biology and Biotechnology Laboratory of the Nigerian Institute of Medical Research, Yaba, Lagos (NIMR) within one hour of collection for proper processing.

For the isolation of Salmonella species, a loopful of stool sample was inoculated aseptically into a McCartney bottle

containing 9 ml of Selenite F broth and incubated at 37 ^{o}C for 24 h and then streaked onto Salmonella-Shigella agar and incubated for 24 h at 37 ^{o}C. For clinical isolates, 5 ml of patient's blood was aseptically added into 45 ml of Brain Heart Infusion (BHI) broth and aseptically transferred into medical flat containers containing BHI agar slant to form a biphasic medium and then incubated at 37 ºC. The blood culture was subcultured daily for 7 days on Salmonella-Shigella (SS) agar plate and incubated at 37 ºC for 24 h. Identification of isolates was done using Salmonella chromogenic agar and characterized using the method of Cowan [16].

The disc diffusion method of NCCLS [5] was employed for antimicrobial susceptibility testing. Briefly, overnight cultures of each isolate were adjusted to 0.5, McFarland turbidity standard and using sterile swabs, the test organisms were inoculated onto Mueller Hinton agar. The test organisms were inoculated onto Mueller Hinton agar after which sterile forceps were used to distribute the antibiotic discs on the inoculated plates. After proper diffusion of the antibiotic into the agar, the plates were now incubated at 37 ºC for 18 h and the zones of inhibition were measured. The antimicrobial discs used include amoxicillin (25 µg), cotrimoxazole (25 µg), nitrofurantoin (300 µg), gentamicin (10 µg), nalidixic acid (30 µg), ofloxacin (30 µg), co-amoxyclav (Augumentin™, 30 µg) tetracycline (30 µg), ciprofloxacin (5 µg) and ampicillin (10 µg).

Prior to the commencement of this study, ethical approval was obtained from the Ethical Committee in NIMR. Participation of food handlers was voluntary and verbal consent was obtained from each subject.

Results

Salmonella species were isolated from 17% of the stool samples obtained from the food handlers while 15% of the faeces from the cattle had the organisms. The organisms isolated from the stool from food handlers were *S. typhi* (6.8%), *S. enteritidis* (5.3), *S. choleraesuis* (2.9%), *S. paratyphi* A (1.5%) and *S. arizona* (0.5%). The isolates from the cattle faeces were *S. enteritidis* (12%) and *S. typhimurium* (3%). The susceptibility of the organisms from food handlers to ofloxacin (96%) and chloramphenicol was 96% and 27.6%, respectively. The organisms were completely resistant to tetracycline, ampicillin and amoxicillin. All the clinical isolates were sensitive to ofloxacin but 92.6% of them were resistant to tetracycline and amoxicillin, 59% were resistant to chloramphenicol and a large number were equally resistant to tetracycline, cotrimoxazole, amoxicillin and clavulanic acid combination. Nearly all the isolates were sensitive to nitrofurantoin, nalidixic acid, ofloxacin, while all were sensitive to ciprofloxacin. However, the food handler isolates were more resistant to chloramphenicol than the clinical isolates.

Five common antibiotic resistance patterns were identified amongst the food handlers and cattle isolates as $Gen^{r}Nal^{r}Tet^{r}Amx^{r}Ap^{r}Cot^{r}$, $Aug^{r}Tet^{r}Amx^{r}Cot^{r}Ap^{r}Chl^{r}$, $Gen^{r}Aug^{r}Tet^{r}Amx^{r}Ap^{r}Chl^{r}$, $Aug^{r}Tet^{r}Amx^{r}Ap^{r}Chl^{r}$ and $Aug^{r}Tet^{r}Amx^{r}Ap^{r}$. The commonest resistance patterns amongst the clinical isolates were $Gen^{r}Aug^{r}Tet^{r}Amx^{r}Ap^{r}Cot^{r}$ and $Nal^{r}Aug^{r}Tet^{r}Amx^{r}Ap^{r}$, (*Abbreviations:* Gen: Gentamicin, Nal: nalidixic acid, Tet: tetracycline, Amx: amoxycillin, Ap: ampicillin, Cot: cotrimoxazole, Chl: chloramphenicol, Aug: Augumentin™ - amoxicillin and clavulanic acid combination).

Discussion

Typhoid and paratyphoid fevers are of major public health concern due to the emergence of resistance to antibiotics currently being used for therapy. In recent times, the emergence of resistance to fluoroquinolones, the presently recommended first line of therapy, has become a major concern [17]. In our study, the susceptibility of the isolates of Salmonellae species from humans and cattle to the fluroquinilones has been reported. However, the resistance of many

antibiotics to the organisms observed in our study confirms an earlier report by Olukoya *et al* [18] and the report made by Chukwuani *et al* [19] that *Salmonella* species in recent years, have become progressively more resistant to clinically useful antibiotics including chloramphenicol, ampicillin, amoxicillin and cotrimoxazole). Uncontrolled use of several antibiotics in the form of across-the-counter purchase of drugs and streets hawking still existing in various parts of Nigeria that could be contributing to the high resistance of the organisms to many of the antibiotics. There have been reports linking antimicrobial resistance of human isolates with agricultural use of antibiotics [20].

Most *bukkas* in Lagos are located in environments that are not very hygienic. This implies that food handlers and cattle meat (which are often not properly treated before ending up in the *bukkas*) could be possible source of infection of Salmonellae to many of the customers that patronize the *bukkas*. The high resistance of the food handler isolates as compared to the clinical isolates is very worrisome due to the fact that the food handlers who never knew they harbored *Salmonella* species especially *S. typhi* stand the chance of infecting more people who come into the *bukkas* (local restaurant) with the highly resistant *Salmonella* species and hence further spread of these resistant strains may occur. Although fluoroquinolones still remain the drug of choice for *Salmonella* species in Nigeria, a previous report by Hohmann [4] indicated that quinolones do not predictably or consistently shorten carriage of *Salmonella* organisms in faeces and so it would not be recommended to treat food handlers or health care workers with antibiotics for non-typhoidal infections as opposed to typhoidal carriage where management of chronic carriage could involve long term use of oral antibiotics and consideration of cholecystectomy. Therefore an antibiotic therapy is not routinely recommended for the empiric treatment of mild to moderate presumed or proven *Salmonella* gastroenteritis in healthy individuals.

Conclusion

Many food handlers working in *bukkas* (cafeteria) and cattle are possible reservoirs of Salmonellae and could transmit the organisms to the customers that patronize those local restaurants, considering the poor sanitary conditions of many of these eating places in Lagos. Although the Salmonella isolates are susceptible to the fluoroquinolones, many commonly used antibiotics are resistant.

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