GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL 14, NO. 2, 2008: 179 - 182

RESISTANCE PATTERN OF *SALMONELLA* ISOLATES FROM FOOD, ANIMAL AND HUMAN SOURCES TO COMMON ANTIMICROBIAL AGENTS

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(Received 28, February 2007; Revision Accepted 19, October 2007)

ABSTRACT

Swab samples obtained from the floor of different poultry farms, slaughter houses and fish ponds in parts of Delta State of Nigeria were screened for the presence of *Salmonella* spp. Prevalence rates were 50.0%, 70.0% and 48.5% respectively. Samples of vegetable salad, raw beef and milk were also similarly screened and prevalence rates were 26.7%, 53.3% and 67.5% respectively. Fifty-five percent of fecal samples and 32% of urine samples obtained from patients with diarrhea and urinary tract infections were contaminated with *Salmonella* respectively. All isolates were subjected to *in-vitro* antibiotic susceptibility tests using the disc diffusion method. Ciprofloxacin appears the most effective antimicrobial agent as only 2.5% of isolates from poultry, slaughter-house and fish pond were resistant to it. Similarly, only 7.7% of isolates from food items and 5.0% of isolates from patients were resistant to it. Cephalexin followed by nalidixic acid was least effective. Sixty percent of isolates from poultry, slaughter house and fish pond, 35.9% of isolates from food sources and 37.5% from patients were resistant to cephalexin. The presence of *Salmonella* spp. in these samples is a public health risk as all strains of this organism are potentially pathogenic.

KEY WORD: Antimicrobial agents, Resistance, *Salmonella* spp.

INTRODUCTION

Salmonella infection is one of the most common food infections worldwide, causing an estimated 1.41 million cases of infections and 500 human deaths annually in the United States of America (Mead *et al., 1999*). It is however, usually difficult to evaluate the extent of infections in developing countries because of the very limited scope of studies and lack of coordinated epidemiological surveillance systems (Santos *et al.*, 2003). Common manifestations of *Salmonella* infections are diarrhea, typhoid and paratyphoid fevers. Few people also develop Reiters syndrome, a nonspecific urethritis coupled with arthritis and conjunctivitis. This syndrome can last for months, years or even lead to chronic arthritis (Osterom, 1999).

Food items such as poultry, meat and meat products are the common sources of *Salmonella* infections (D'Aoust, 1994; Bryan & Doyle, 1995 and Santos *et al.*, 2003). The pathogen can gain access to meat, at any stage during butchering which can become a source of contamination to consumers. Reports of contamination of raw milk, vegetable salad and well water with *Salmonella* spp. are also well documented in literature (Rohrback *et al.*, 1992; Amard *et al.*, 1994 and Sumath *et al.*, 2005). Studies on prevalence of *Salmonella* spp in meat and food items eaten raw will not only augment the available epidemiological data but will also be guide to treatment and mapping out preventive strategies against the infections.

MATERIALS AND METHODS

Samples collection and isolation of bacteria

Samples were collected from animal sources, raw food items and patients. Twenty swab samples each were obtained from the floor of three poultry farms and abattoirs located in Udu, Warri and Ughelli in Delta State of Nigeria. Eleven water samples each from 3 fish ponds, 40 raw milk samples and 15 samples each of vegetable salad and beef, were also collected randomly from open markets in these towns. The vegetable salad was made up of lettuce, cabbage, carrots and cucumber. Sixty faecal sample from patients with diarrhea and 22 urine samples from patients with urinary tract infection were also collected. All samples were cultured in selenite F both in screw-capped bottles and incubated at 37^{0}C for 3-5 days. Subcultures were then made onto plates of deoxychocolate citrate agar, and incubated for another 24hrs. Colonies with black centers were sub-cultured onto nutrient agar and incubated for another 24hrs. The cultures on nutrient agar plates were subjected to Gram-staining, motility, urease production, hydrogen sulfide production and citrate utilization tests. All Gram-negative, rod-shaped, motile, urease-negative isolates that produced on alkaline slant with acid butt on triple sugar Iron agar slants and are able to utilize citrate as sole source of carbon were regarded as species of the genus *Salmonella*

Antibiotic Susceptibility Testing

Antimicrobial agents susceptibility testing was conducted by using the disc-diffusion method (NCCLS, 1998). The multodicks (optudisc) used contained the following antibacterial agents: tarivid (ofloxacin)-10mg: peflaxin (pefloxacin) – 10mg: ciproflox (ciprofloxacin)-10mg; nalidixic acid-30mg: augmentin (amoxicillin-clavulanic acid)-30mg: gentamicin-10mg: Streptomycin-30mg: cephalexin-10mg: septrin (trimethoprim-sulphamethozazole)-30mg and ampicillin-30mg.

RESULTS

The prevalence of *Salmonella* spp. varied a great deal in all sources screened. Prevalence in samples obtained from food sources was highest (55.7%) where prevalence rates ranged from 26.7% in samples of vegetable salad to 67.5% in raw milk (Table 1). About 55% of all samples obtained from animal/fish rearing environments were also contaminated with *Salmonella* spp. (Table 1). In this group, prevalence ranged from 48.5% in fish ponds to 70.0% in slaughter houses. *Salmonella* spp. was isolated in over half (55%) of stool samples from patients with diarrhea and 31.8% f urine samples of patients with urinary tract infections (Table 1).

Tables 2, 3 and 4 show the resistance patterns of

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Salmonella isolates to various antimicrobial agents. Ciprofloxacin appeared to be the most effective antibiotic as only 2.5% of isolates from animals' sources were resistant to it. All isolates from poultry, fishponds, vegetables, and urine were sensitive to it. Isolates from animal sources were most resistant to ceephalexin (60.0%) and nalidixic acid (45.0%) while these from food sources were most resistant to nalidixic acid (46.1%) and ampicillin (38.5%). Patient isolates were most resistance to gentamicin (40%) and nalidixic acid (37.5%) and cephalexin (37.5%).

Table 1: Percentage prevalence of *Salmonella* spp. from animal sources, food and patients

Sources	Number of sample collected	No. of positive sample (%)
Animal sources		
Poultry floor	20	10(50.0)
Slaughter house floor	20	14(70.0)
Fish pond	33	16(48.5)
Sub-total	73	40(54.8)
Food sources		
Raw milk	40	27(67.5)
Vegetable Salad	15	4(26.7)
Beef meat	15	8(53.3)
Sub-total	70	39(55.71)
Patients		
Faeces	60	33(55.0)
Urine	22	7(31.8)
Sub-total	82	40(48.78)
Total	222	119(53.6)

Table 2: Antibiotic Resistance of *Salmonella* Isolates from Animal Sources

Sources Isolates	No. of Isolates	No. (%) of resistant isolates									
		CPX	REF	NA	PN	OFX	AU	CN	S	CEP	SXT
Poultry floor	10	0(0.0)	2(20.0)	4(40.0)	3(30.0)	1(10.0)	2(20.0)	2(20.0)	4(40.0)	7(70.0)	3(30.0
Slaughter House	14	1(7.1)	3(21.4)	8(57.1)	0(0.0)	1(7.1)	1(7.1)	4(28.6)	5(35.7)	8(57.1)	3(21.4)
Fish pond	16	0(0.0)	2(12.5)	6(37.5)	9(56.3)	2(12.5)	2(12.5)	3(18.8)	4(25.0)	9(56.3)	1(6.3)
Total	40	1(2.5)	7(17.5)	18(45.0)	12(30.0)	4(10.0)	5(12.5)	9(22.5)	13(32.5)	21(60.0)	7(17.5)

KEY: CPX (Ciprofloxacin), PEF (Pefloxacin); NA (Nalidixic acid); PN (Ampicillin); OFX (Ofloxacin); ALL (Amoxicillin-clavulanic acid); CN (Gentamicin); S (Streptomycin); CEP (Cephalexin) and SXT (Trimethoprim-sulhamethozazole).

Table 3: Antibiotic Resistance of *Salmonella* Isolates from Food Sources

Sources Isolates	No. of Isolates	No. (%) of resistant isolates									
		CPX	REF	NA	PN	OFX	AU	CN	S	CEP	SXT
Raw milk (Nunu)	27	2(7.4)	3(11.1)	13(48.1)	12(44.4)	3(11.1)	3(11.1)	8(29.6)	5(18.5)	10(37.0)	6(22.0)
Vegetable Salad	4	0(0.0)	0(0.0)	1(25.0)	1(25.0)	0(0.0)	0(0.0)	2(50.0)	2(50.0)	1(25.0)	1(25.0)
Beef meat	8	1(12.5)	2(25.0	4(50.0)	2(25.0)	0(0.0)	0(0.0)	3(37.5)	3(37.5)	3(37.5)	1(12.5)
Total	39	3(7.7)	5(12.5)	18(46.1)	15(38.5)	3(7.7)	3(7.7)	13(33.3)	10(25.6)	14(35.9)	8(20.5)

KEY: CPX (Ciprofloxacin), PEF (Pefloxacin); NA (Nalidixic acid); PN (Ampicillin); OFX (Ofloxacin); ALL (Amoxicillin-clavulanic acid); CN (Gentamicin); S (Streptomycin); CEP (Cephalexin) and SXT (Trimethoprim-sulhamethozazole)

Table 4: Antibiotic Resistance of *Salmonella* Isolates from patients

Sources Isolates	No. of Isolates	No. (%) of resistant isolates									
		CPX	REF	NA	PN	OFX	AU	CN	S	CEP	SXT
Faces	33	2(6.1)	3(9.1)	12(36.4)	7(21.2)	3(9.1)	9(27.3)	13(39.4)	8(24.2)	10(30.3)	7(21.2)
Urine	7	0(0.0)	1(14.3)	3(42.9)	3(42.9)	0(0.0)	0(0.0)	3(42.9)	1(14.3)	5(71.4)	0(0.0)
Total	40	2(5.0)	4(10.0)	15(37.5)	10(25.0)	3(7.5)	9(22.5)	16(40.0)	9(22.5)	15(37.5)	7(17.5)

KEY: CPX (Ciprofloxacin), PEF (Pefloxacin); NA (Nalidixic acid); PN (Ampicillin); OFX (Ofloxacin); ALL (Amoxicillin-clavulanic acid); CN (Gentamicin); S (Streptomycin); CEP (Cephalexin) and SXT (Trimethoprim-sulhamethozazole).

DISCUSSION

Salmonella infections are considered one of the most widespread food-borne zoonoses in industrialized as well as developing countries though incidence varies between countries (Osterom, 1999). Farm animals (Mims *et al.*, 2004) food items such as poultry meats (Bryan and Doyle, 1995), meat and meat products etc have been implicated as sources of infection.

The 70% and 50% prevalence rates of slaughter houses and poultries respectively, obtained in this survey are quite high. Current data on microbial contamination of floors of slaughter houses are rare. Wray (2001) reported prevalence rates of 6.2% to 23% and 1.4% to 11.2% in caecal contents of pigs and water used in slaughter houses respectively in parts of Europe. Rates of 15 to 20% have been reported in poultries in Britain (Morgan, 1980).

The high prevalence rates recorded are probably due to the low sanitary practices in the slaughter houses and poultries sampled. Cows were slaughtered, washed, eviscerated, skinned and processed on the floor. Butchers and retailers walked between carcasses as they transacted their businesses. Contamination of meat products with *Salmonella* has decreased significantly as a result of Hazard Analysis and Critical Control point (HACCP) systems, which establish performance standards at slaughter-houses and processing plants in the United States and other developed countries (Funk and Gebreyes, 2004). Such standards which includes antimicrobial interventions for the removal contamination from carcasses even when put in place in this locality, are never adhered to. Data on prevalence of *Salmonella* in fish ponds is scare as previous studies are mainly restricted to prevalence in fish, shrimps or amphibians.

The percentage of milk and vegetable salad samples contaminated with *Salmonella* were also higher (Table 1) than previous reports (Rohback *et al.*, 1992 and Thi *et al.*, 2005). Sources of milk contamination include the dairy workers, animals and equipment. The contaminating microorganisms in meat are reported to derive from the animals' pre-slaughter environment and may be of faecal, soil, water or feed origin. Other sources of carcass contamination include the animals' hide, gastrointestinal tract, respiratory tract, slaughter-house personnel and equipment. Sources of contamination of vegetables include the farm environment, post-harvest exposure to contaminated surfaces and handlers.

The prevalence of *Salmonella* in clinical samples varies in different parts of the world. Castro *et al.* (2002) isolated *Salmonella* spp. from 45.9% of stocl and 2.8% of urine samples from patients with diarrhoea and urinary tract infections respectively in Brazil while Obi *et al.* (2003) isolates *Salmonella* spp. in 14.7% of fecal samples of patients with diarrhoea in South Africa. These values are lower than the 55% and 31.1% obtained from stools and urine samples respectively in this survey. This is an agreement with the fact that the incidence of enteric infections is influenced by personal and community sanitary practices as well as provision of potable water supply.

Salmonella Enteritidis infection is considered to be a self-limiting disease, requiring no antibiotic therapy, except in immunocompromised patients and young children where organism becomes invasive, causing septicaemia (Mims *et al.*, 2004). For such cases, chloramphenicol, ampicillin, gentamicin, amoxicillin, cotrimoxazole or mecillinam were the drugs of choice (Turnbull, 1979). There are reports of increasing resistance to most of these and even some new generation antibiotics such as pefloxacin (Malorny *et al.*, 1999; Edrdrem *et al.*, 2005; Johnson *et al.*, 2005). In this survey, isolates from environment where animals are slaughtered or reared were most resistant to cephalexin (60%), nalidixic (45.0%) and least resistant to ciprofloxacin (2.5%). The resistance of *Salmonella* isolates from various food sources to ampicillin, streptomycin and tetracycline (Table 3) are similar to other reports from Canada (Johnson *et al.*, 2005). Percentage of isolates resistant to ciprofloxacin was however lower than the 15% reported in Canada (Johnson *et al.*, 2005). The percentage of isolates from raw meat resistant to ciprofloxacin (7.7%) is also lower than the 49.9% reported by Malony *et al.* (1999) in Germany. This may be partly due to the wide scale use of flouroquinolones for supplementing animal feeds in areas where these surveys were conducted which could result in development of large scale resistance in isolates.

Isolates from fecal and urine samples of patients were most resistant to gentamicin, nalidixic acid and cephalexin but least resistant to ciprofloxacin (Table 4). These results are different from the reports of **Cabrera *et al.* (2004)** in Spain and Erdrem *et al.*(2005) in Turkey where clinical isolates of *Salmonella* were most resistant to ampicillin, tetracycline, amoxillin-clavulanic acid and nalidixic acid. The reasons for these differences are not clear. However, over 20% of clinical isolates from this survey were also resistant to ampicillin and augmentin (amoxicillin-clavulanic acid). The percentage of ciprofloxacin resistant isolates from clinical samples (5.0%) is much lower than that reported by Chin *et al.* (2002) in Taiwan. The fluoroquinolones (ofloxacin, pefloxacin and ciprofloxacin) are more expensive and comparatively less abused than the older generations antibiotics such as ampicillin and nalidixic acid. This may be partly responsible for the lower percentage of resistant isolates.

Isolates from all three sources were most resistant to the same set of antibiotics, i.e. nalidixic acid, cephalexin and gentamicin, in addition, to isolates from patients. The observed differences in the percentages of isolates resistant to these antibiotics from the three sources are not statistically significant (Turkey Kramer, P = 0.05; for nalidixic acid, cephalexin, and gentamicin). This is similar to the findings of Rouahi *et al* (2000) in Morocco where multi-resistance to different antibiotics did not differ significantly between *Salmonella enteritidis* strains from food and patients. It is also similar to that of Guncagul *et al.* (2004) who reported that there wee no identical patterns of antibiotic resistance in *Salmonella enteritidis* isolates from chicken meat, chicken intestines and humans.

Resistant strains *Salmonella* were common in the animal rearing/processing environment, food items and patients screened. The observed resistance of some strains to the fluoroquinolones, which are amongst the drugs of choice for treating enteric infections, is a source of concern to all. Strategies aimed at checking the spread of these isolates should be developed.

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