Resistant *Salmonella* Strains Obtained from a Large Produce Growing Region in Nigeria

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***Abstract*—**In Nigeria, as in many developing countries, typhoid fever is an endemic disease and drug resistance to Salmonella its causative agent is on the increase. We examined Salmonella strains obtained from a large produce region in the country for antibiotic resistance to eight commonly used antibiotics. Results showed 84.2% of isolates obtained were resistant to two or more antibiotics.

***Keywords*—** Irrigation, produce, Salmonella, antimicrobial resistance.

I. Introduction

FOOD borne illness caused by Salmonella spp is a worldwide problem [3]. Consistent contamination with irrigation waters has been shown to be common routes of contamination in produce related Salmonella outbreaks [4]. Typhoid fever caused by S.typhi or S.paratyphi is a major health problem with global incidence of 21 million cases and 200,000 (1-4% death worldwide) deaths per year [1]. Salmonella has become a major threat to the society due to the disease severity and recurrence of disease through carrier state [7]. Data relating to Salmonella prevalence in surface and drinking water in developing countries are quite rare. Nevertheless, data on water- borne outbreaks as well as case control studies investigating the risk factors for endemic typhoid fever confirmed the relevance of water as source for the transmission of this disease [4]. Most commonly used antibiotics for the treatment of typhoid fever are fluoroquinolone such as ciprofloxacin, ofloxacin and pefloxacin and third generation cephalosporins such as ceftriaxone [5].

Some variants of Salmonella have developed multidrug resistance as an integral part of their genome, hence are likely to retain their drug-resistant genes even when antimicrobial drugs are no longer used, making the pathogen more virulent and potent [5]. Hence the emergence and global spread of antimicrobial resistant Salmonella strains have necessitated the need to study the resistance patterns of Salmonella strains obtained from the largest produce region in Nigeria.

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II. Materials And Methods

Samples were collected in the rainy (May-October, 2010) and dry (November-March, 2011) seasons, samples included salad vegetables, irrigation water, soil and manure.

III. Isolation and identification of Salmonella

Salmonella was isolated from samples using selenite F broth enrichment and isolation on Salmonella –Shigella agar [6]. Biochemical characterization and serological identification of Salmonella was done. Antimicrobial susceptibility testing was performed by the disc diffusion method [2]. Salmonella isolates obtained in this study were further characterized by polymerase chain reaction (PCR) to assay for Salmonella spp invasive (invA) gene and Salmonella enterotoxin (stn) gene. Quinolone resistance determining region (QRDR) and multidrug resistance plasmid gene (pCT) were also detected by PCR. Escherichia coli ATCC 25922 was used as a negative control.

IV. Results

Of the two hundred and ten samples (one hundred and forty-four salad vegetables, twenty seven irrigation water and thirty nine soil/manure) analyzed, a prevalence rate of 9.0% of Salmonella spp was obtained (Table 1). Salmonella was most detected from the irrigation water with a prevalence rate of 11.1%. Three different Salmonella serotypes were distributed in samples; Salmonella typhi (S.tyhpi), Salmonella Paratyphi (S.paratyphi) and Salmonella typhimurium (S.typhimurium). With S. typhi having the highest prevalence of 68.4% (Table 2).

The multidrug resistance (MDR) patterns of the Nineteen Salmonella isolates revealed that sixteen (84.2%) were resistant to two or more antibiotics, viz; Six (31.6%) were resistant to three antibiotics, one (2.0%) was resistant to four antibiotics, two (10.5%) were resistant to five antibiotics, two (10.5%) to six and seven antibiotics respectively. The remaining three (15.8%) showed resistance to only one antibiotics, cephalothin. Higher numbers of resistant isolates were identified among isolates from vegetables and soil/manure, four isolates from vegetables showed resistance to five antibiotics, while three isolates from soil/manure were resistant to four antibiotics. Polymerase chain reaction confirmed the presence of the following genes in all Salmonella isolates obtained; Salmonella invasive (invA) and

enterotoxin (stn) genes, Quionolone resistance determining region (QRDR) genes and multidrug resistance plasmid (pCT). (Figure 1 and 2).

TABLE I
DISTRIBUTION OF SALMONELLA IN SAMPLES OBTAINED FROM IRRIGATION SITES IN THE STUDY.

Sample type	Number tested	Number positive	% Percentage in samples
Vegetables	144	15	10.4
Water	27	3	11.1
Soil/manure	39	1	2.6
Total	210	19	9.0

TABLE II
DISTRIBUTION OF SALMONELLA SEROTYPES IN SAMPLES IN THE STUDY.

Sample type	No	*S.t*	*S.para*	*S.tyhpimurium*
Vegetable	15	11	1	3
Soil/manure	1	1	-	1
Water	3	1	-	1
Total	19	13(68.4%)	1(5.3%)	5(26.3%)

No= Number assayed, *S.t= Salmonella typhi, S.para= Salmonella paratyphi,and S.tyhphimurium*

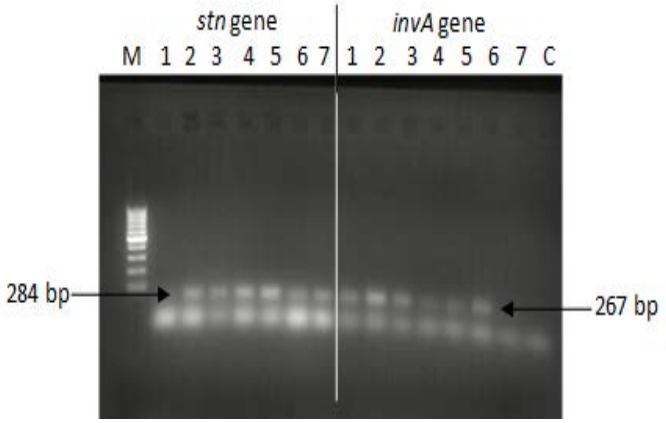

Fig. 1 Lane M= DNA marker, *Salmonella* enterotoxin (*stn* gene)= lane 1-7, invasive (*invA* gene) = lane 1-7. Lane C= negative control *E .coli* ATCC 25922.

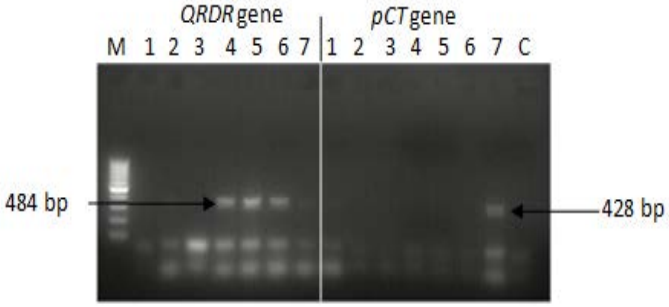

Fig. 2 Quinolone resistance determining region (QRDR) in Salmonella: Lane 1-7, Lane M= 100bp Marker lane, Lane 1-7 (left) = multidrug resistance plasmid, C= control; PCR product- DNA.

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