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Prevalence of Resistance among *Salmonella Typhi* Isolates in Ekiti- State, Southwestern Nigeria 2009-2011

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Abstract - The prevalence of Sal typhi in the various locations in Ekiti state over the years of 2009 -2011 was investigated. The highest number of the isolates from 50 samples was found at Ikere-Ekiti, Ikole- Ekiti and Ifaki Ekiti at a total number of 131 respectively, this was followed by Ado- Ekiti with an isolate number of 130. The lowest number of isolate was recorded at Ido Ekiti with 78 isolates in the year 2009 and this increased in both 2010 and 2011. The susceptibility patterns reported in 2009 in the different locations showed that resistance was indicated in streptomycin, chloramphenicol, cefepime, nalidixic acid tetracycline and trimethoprim-sulfamethoxazole. This same trend of resistance was repeated in 2010 and 2011. The notable change was a significant increase in the resistance to ampicillin from 20% to 100% in Ado Ekiti and from 39% to 100% at Ikole Ekiti and also Gentamycin from 18% at Ikere-Ekiti in 2009, to 86% and 84% in 2010 and 2011 respectively. The difference in demographic factors in the different locations could be a convincing factor in the prevalence of Sal typhi. With PFGE, a total of thirty-five 35 (79%) patterns in the locations were observed and thirty-six 36(72%) of each isolates had the 3 most common patterns. The isolates with these patterns were found to show high resistance to Streptomycin, Chloramphenicol, Cefepime, Ampicilin and nalidixic acid (SCCAN). All isolates that are resistant to the antibiotics mentioned earlier contain qnrB2, 35(70%) isolates contained blaCMY-2'; 39 (78%) isolates contained blaCMY-23' the mechanism for extended-spectrum cephalosporin, Aminoglycosides and Quinolone resistance. The genes that code for this resistance have proven to be remarkably mobile and widely distributed within and between species.

Keywords : *ekiti state, prevalence, resistance, salmonella typhi.*

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I. Introduction

During the past decade, multidrug-resistant (MDR) *Salmonella typhi* have increased substantially in most part of the world especially in developing countries. The prevalence of this bacterium increased from 1% of human isolates in 1998 to 21% in 2003. *S. typhi* is resistant to at least chloramphenicol, streptomycin, sulfamethoxazole, cefoxitin, tetracycline, amoxicillin-clavulanic acid, and ampicillin. This phenotype exhibits decreased susceptibility to Ceftriaxone (CDC, 2006), a critically important antimicrobial agent for treating invasive Salmonellosis in children (Guerrant *et al.,* 2001). Members of the bacterial genus *Salmonella* are among the major pathogens that cause Salmonellosis in humans. Most human *Salmonella* infections are thought to be associated with food borne transmission from contaminated animal-derived meat and dairy products (Voetsch *et al*., 2004). Human infections are commonly caused by ingestion of food that has been contaminated by animal feces (Herikstad *et al.,* 2002). Although >2,500 serovars of *S. typhi*havebeen identified, most human infections are caused by a limited number of serovars. *S.* serovar *typhimurium*and *enteritidis* are the most common causes of humanSalmonellosis worldwide, although other serovars havebeen reported to be more prevalent in some regions(Hald*et al.,* 2007).Adequate sanitary measures have led to a decrease in cases of typhoidal Salmonella infections in developedcountries such as the USA where the incidence is low. However, in these countries, non-typhoidal *Salmonellosis* is common, and most of these cases are associated with outbreaks from contaminated meat, dairy products or by cross-contamination from foods contaminated with *Salmonella* (,Glynn,*et al.,* 2005). In Nigeria especially in Ekiti state, morbidityassociated with illness due to *Salmonella* continues to be on the increase and in some cases, resulting in death (Ajibade *et al.*, 2010).

Although, the primary cause of Salmonellosis is consumption of contaminated foods, there is the potential for secondary spread, from person-to-personand also to other foods. Person- to-person spread with family groups is often associated with poor personal hygiene but there is the opportunity of air-borne andsurface–to-surface spread within the toilet and the bathroom, especially during the diarrhea phase (Ajibade*et al.,* 2010; Ajibade, 2012). Despite improved public health, serious infections with *Salmonella* remain a major clinical and public health concern in Nigeria and worldwide.*S. enterica* are a leading cause of food borne disease worldwide (Hald *et al.,* 2007). In the United States, as many as 1.4 million cases of *S.enterica*-associated disease occur annually (Humphery, 2000; Voetsch *et al*., 2004; Marie *et al.,* 2012) Whileusually self-limiting, Salmonellosis may require antimicrobial drug treatment in infants, the elderly or immunocompromised

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persons. However, antimicrobialdrug resistance has become increasingly common in *S. typhi*, which has complicated therapy (Herikstad *et al.,* 2002). Antimicrobial agents such as fluoroquinolone and third-generation cephalosporin's are commonly used to treat severe human *Salmonella* infections (Lee *et al.,* 2009). Resistance to these and other antimicrobial drugs, as well as multidrug resistance, has increased over the last several decades, probably as a consequence of antimicrobial agents in use at intensive animal husbandry and medicine (WHO, 2002). Antimicrobial resistant strains of *Salmonella species* are now widespread all over the world. In developed countries, it is becoming more and more accepted that a majority of resistant strains are of zoonotic origin and have acquired their resistance in an animal host before being transmitted to human through the food chain (Helms *et al*., 2004). The emergence of antimicrobial drug resistance is a matter of concern. This work intends to determine the prevalence of resistance of *Sal typhi* isolated from major towns in Ekiti Stateto conventional antimicrobial drugs.

II. Materials and Methods

a) Collection of Sample

Stool samples were collected from patients who visited hospital in Ado Ekiti, Ido Ekiti, Ikere Ekiti, Ikole Ekiti, Ifaki Ekiti between February 2009 and September 2011. Fifty (50) samples each were collected from the hospital.

b) Culture, Isolation and Identification of Samples

Samples were cultured on Salmonella-Shigella agar, incubated at 37°C for 24hrs. Smooth colonies withblack centre were sub cultured on to MacConkey agar (Oxoid), incubated at 37°C for 18-24hr. Pale colonieswere isolated and confirmatory biochemical test (Gram's reaction, urease reaction, citrate utilization andmotility) were conducted. *Salmonella enterica* was identified by using standard biochemical tests andcommercial typing antiserum (Statens Serum Institute, Copenhagen, Denmark) according to the manufacturer's instructions.

c) Antimicrobial Susceptibility Profile

The susceptibility profile of samples from which *Sal typhi* (44) was isolated was determined. Minimum inhibitory concentration (MIC's) of ten (10) antimicrobial drugs: **Phenols** (chloramphenicol), **Penicillins** (Ampicillin), **Cephalosporin (**cefepime, cefotaxime), **Tetracycline** (tetracycline), **Aminoglycosides** (Gentamycin, streptomycin), **Sulfonamides** (trimethoprin-sulfamethoxazole), **Quinolones and Fluoroquinolone** (nalixidic acid, ciprofloxacin) were determined by usingthe broth-microdilution method; susceptibility to streptomycin was measured by using the disk-diffusionmethod as recommended by the Clinical and Laboratory Standards Institute(CLSI,2012).

d) Pulsed – Field Gel Electrophoresis

All *Salmonellae* isolates were analyzed for genetic relatedness by PFGE by using *Xba*1 according to the CDC PulseNet protocol (Ribopt *et al.,* 2006). Electrophoresis was performed with a CHEF-DR111 system in the Science Technology Research Lab, Federal Polytechnic, Ado- Ekiti by using 1% Seakem agarose in 0.5x Tris-borate-EDTA at 180V. Running conditions consisted of 1 phase from 2.2 to 63.8s for a run of 22h.

e) PCR Detection of Antimicrobial Drug Resistance Genes

Presence of *qnr* genes was determined by using PCR with primers QP1 and QP2 for *qnrA,* FQ1 and FQ2 for *qnrB,* and 5'-ATGGAAACCTACAATCATAC-3' and 5'-AAAAACACCTCGACTTAAGT-3' for *qnrS*. The *qnrB* allele was determined by amplification and sequencing with primers FQ1 and FQ2. Screening for *aac(6')-1b-cr* was performed as as described in Park *et al.* (2006). Primer pairs used for amplification of **β-**lactamase genes were: bla_{cmy} (5'-ATGATGAAA AAATCGTTATGC-3') and (5'TTGCAGCTTTTCAAG AATGCGC-3'), bla_{OXA-1} (5'AATGGCACCAGATTCAACTT-3') and (5'-CTTGGCTTTTATGCTTGATG-3'); and bla_{SHV} (5'-GGTTATGCGTTATATTCGCC-3') and (5'-TTAGCGTT GCCAGTGCTC-3'). bla_{CTX-M} genes were screened by using a multiplex PCR assay (Woodford *et al.,*2006).

III. Results and Discussion

The prevalence of *Saltyphi* in the various locations in Ekiti state over the years of 2009 - 2011 is shown in table 1. The highestnumber of the isolates from 50 samples was found at Ikere-Ekiti, Ikole- Ekiti and Ifaki Ekiti at a total number of 131 respectively, this was followed by Ado- Ekiti with an isolate number of 130. The lowest number of isolate was recorded at Ido Ekiti with 78 isolates. The lowest number of isolates was recorded in the year 2009 and this increased in both 2010 and 2011.The susceptibility patterns reported in 2009 in the different locations are shown in table 2. It was observed that resistance was indicated in streptomycin, chloramphenicol, cefepime, nalidixic acid tetracycline and trimethoprim-sulfamethoxazole. This same trend of resistance was repeated in 2010 and 2011 (tables 3 and 4). The notable change was a significant increase in the resistance to ampicillin from 20% to 100% in Ado Ekiti and from 39% to 100% at Ikole Ekiti and also Gentamycin from 18% at Ikere –Ekiti in 2009, to 86% and 84% in 2010 and 2011 respectively.

The difference in demographic factors in the different locations could be a convincing factor in the prevalence of *Sal typhi* in the towns. Ado-Ekiti been a capital city with high urbanization is prone to a lot of cross contamination due to its congestion and over-crowding condition. Ido Ekiti for example, is not over crowded and has a medical center situated there-in. This has a meaningful influence on the environmental

condition and the inhabitants especially in the access to good medical condition.

The increasing prevalence of multidrug-resistant Salmonella *sp* is a global health concern. In Ekiti State, colonization of infection with *Saltyphi* has been a reportable condition for > 20 years. From 2009 to 2011, the agents for which a major change in susceptibility was observed were Tetracycline, Trimethoprim-sulfamethoxazole, nalidixic acid, ampicillin, cefotaxime and streptomycin. With PFGE, a total of thirty-five 35 (79%) patterns in the locations were observed and thirty-six 36(72%) of eachisolates had the 3 most common patterns. The isolates with these patterns were found to show high resistance to Streptomycin, Chloramphenicol, Cefepime, Ampicilin and nalidixic acid (SCCAN). All isolates that are resistant to the antibiotics mentioned earlier contain *qnrB2*, 35(70%) isolates contained $bla_{CMY-2'}$; 39 (78%) isolates contained $bla_{CMY-23'}$ the mechanism for extended-spectrum cephalosporin, Aminoglycosides and Quinolone resistance. The genes that code for this resistance have proven to be remarkably mobile and widely distributed within and between species. Integrons are widely distributed among *Saltyphi* and are potentially capable of transmitting drug resistance (Mary *et al.,* 2009). These findings correspond to that of Karen *et al.* (2010) where it was reported that most Salmonella isolates in South Africa are resistant to at least ampicillin (A), Chloramphenicol (C), Streptomycin(S), Sulfonamide (Su) and Tetracycline (T) (ACSSuT). They have been described as been found in a multidrug resistance (MDR) region that is located on the chromosome in a region termed *Salmonella* genome island 1 (SGI1) (Boyd *et al.,* 2001).The spread of resistance has repeatedly been shown to be associated with antimicrobial drug use, which stresses the importance of the prudent use of these drugs; a notion reinforced by the observation that resistance can be revised (Marie *et al.,* 2012). Plasmid mediated Fluoroquinolone resistance have been reported in South Africa to have implications for cons transference of resistance to the major antimicrobial agents used to treat typhoid fever (Karen *et* al.,2010). Multidrug resistance is evidenced in this study and this can be ascribed to the wide spread use of antibiotics both inside and outside of medicine, selling of the antibiotics over the counter without prescription, misuse and over use of antibiotics by doctors as well as patients and inappropriate prescription. The emergence of *Sal typhi* strains that are resistant to commonly used antibiotics is important to clinicians, microbiologists and those responsible for the control of communicable diseases.

Because use of antimicrobial agents contributes to increasing resistance and facilitates transmission of multidrug-resistant Salmonellae, promoting guidelines aimed at improving appropriate use of antimicrobial agents may help prevent transmission of multidrug-resistant *Sal typhi* infections in the environment. In addition, the avoidance of unnecessary antibiotics usage and good hygiene at all stages in food production chain and adequate treatment of sewage, supply and drinking of portable water is of paramount importance in reducing the incidence and preventing multi-drug resistance.

IV. Conclusion

In conclusion, this study underscores the magnitude of the problem of antimicrobial drug resistance in low resource settings and the urgent need for surveillance and control of this phenomenon is necessary. The state has seen a persistently high incidence of multidrug-resistant *Sal typhi* because ma vigilant screening and decolonization program has not been implemented. From this work it was observed that for the past three (3) years, growth of multidrug resistant strain of *Sal typhi* has been exponential, rural and metropolitan rates apparently stabilized. The findings of this study can be used to assist in the direction of policy and interventions; to conduct other analyzes like the evaluation of economic cost of these diseases while attributing it to various environmental conditions. Endemic persistence of the bacterium and the measures that should be undertaken to control or eradicate it from the state are likely to remain topical subjects.

Table 1 : Incidence of *Salmonella sp* in the different towns between the years; 2009-2011

Locations	2009	2010	2011	Total(no)
Ado- Ekiti	43(86)	41(82)	46(92)	130
Ikere- Ekiti	43(86)	47(94)	41(82)	131
Ido –Ekiti	30(60)	27(54)	21(42)	78
Ikole-Ekiti	40(80)	46(92)	45(90)	131
Ifaki -Ekiti	41(82)	45(90)	47(94)	131
Total	197	206	200	

Table 2 : Susceptibility pattern of *Salmonella sp* reported in different locations in 2009(% resistance)

Antibiotics	Ado 43	Ikere 43	Ido 34	Ikole 40	Ifaki 41
Streptomycin	100	100	100	100	100
Chloramphenicol	100	100	100	100	100
Cefepime	20	79	78	98	87
Cefotaxime	100	100	100	100	100
Ampicillin	20	79	94	39	94
Nalidixic acid	100	100	100	100	100
Gentamycin	81	18	76	94	78
Ciprofloxacin	35	15	93	90	78
Tetracycline	100	100	100	100	100
Trimeth/Sulfamethoxazole	100	100	100	100	100

Table 3 : Susceptibility pattern of *Salmonella sp* reported in different locations in 2010(% resistance)

Antibiotics	Ado 41	Ikere 47	Ido 27	Ikole 46	Ifaki 45
Streptomycin	100	100	100	100	100
Chloramphenicol	100	100	100	100	100
Cefepime	20	92	89	98	39
Cefotaxime	100	100	100	100	100
Ampicillin	99	93	99	76	90
Nalidixic acid	100	100	100	100	100
Gentamycin	99	68	86	74	78
Ciprofloxacin	33	15	73	70	88
Tetracycline	100	100	100	100	100
Trimeth/Sulfameth oxazole	100	100	100	100	100

Table 4 : Susceptibility pattern of *Salmonella sp* reported in different locations in 2011(% resistance)

Antibiotics	Ado 41	Ikere 47	Ido 27	Ikole 46	Ifaki 45
Streptomycin	100	100	100	100	100
Chloramphenicol	100	100	100	100	100
Cefepime	20	92	89	98	39
Cefotaxime	100	100	100	100	100
Ampicillin	100	100	100	100	100
Nalidixic acid	100	100	100	100	100
Gentamycin	96	86	84	70	76
Ciprofloxacin	43	54	67	69	67
Tetracycline	100	100	100	100	100
Trimeth/Sulfameth oxazole	100	100	100	100	100

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