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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

Index terms— ekiti state, prevalence, resistance, salmonella typhi.

1 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— Author : Department of Sciences Technology, Microbiology Unit, Federal Polytechnic, P.M.B. 53513, Ado -Ekiti, Nigeria. E-mail : ajibvijay@yahoo.com (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* have been identified, most human infections are caused by a limited number of serovars. *S. serovar typhimurium* and enteritidis are the most common causes of human Salmonellosis worldwide, although other serovars have been 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 developed countries 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, morbidity associated 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-person and also to other foods. Person-to-person spread with family groups is often associated with poor personal hygiene but there is the opportunity of airborne and surface-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) persons. However, antimicrobial drug resistance has become increasingly common in *S. typhi*, which has complicated therapy (Herikstad et al., 2002). Antimicrobial agents such as fluoroquinolone and third generation cephalosporins 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 State to conventional antimicrobial drugs.

II.

3 Materials and Methods

4 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.

5 b) Culture, Isolation and Identification of Samples

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

6 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 (trimethoprim-sulfamethoxazole), Quinolones and Fluoroquinolone (nalidixic acid, ciprofloxacin) were determined by using the broth microdilution method; susceptibility to streptomycin was measured by using the disk-diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2012).

7 d) Pulsed -Field Gel Electrophoresis

All *Salmonellae* isolates were analyzed for genetic relatedness by PFGE by using *Xba*I according to the CDC PulseNet protocol (Ribot 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.

8 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 described in Park et al. (2006). Primer pairs used for amplification of β -lactamase genes were: *bla* *cmv* (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.

10 Results and Discussion

The prevalence of *Sal typhi* in the various locations in Ekiti state over the years of 2009 -2011 is shown in table 1. 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. 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 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 *Salmonella* 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 thirtysix 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, Ampicillin 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 *Salmonella* 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 *Salmonella* 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 *Salmonella* 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.

11 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 *Salmonella* typhi because a 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 *Salmonella* 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. ¹

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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	

Figure 1: Table 1 :

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Antibiotics	Ado	Ikere	Ido	Ikole	Ifaki
	43	43	34	40	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/Sulfameth oxazole	100	100	100	100	100

Figure 2: Table 2 :

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