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Research Article

Molecular Profiling of Class 1 Integron Among Multidrug-Resistant *Salmonella Typhi* Serovars Isolated from Hospitals in Bauchi

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Abstract

Background: Multi-drug resistance is on increase in clinical *Salmonella typhi* serovars and it is being aided by Integron that carry cassettes of resistance genes. The genetic characterization of antimicrobial resistance genes as well as their location and diversity is important in identifying factors involved in resistance, understanding the diversity of MDR strains, identifying genetic linkages among markers, understanding potential transfer mechanisms, and developing efficient detection methods

Design and Duration: This study was carried out on clinical isolates of *Salmonella typhi* isolated from selected hospitals within Bauchi Metropolis between January 2019 and February 2020. The study involves the collection of blood and stool specimens across all ages and gender between ages 0-70 years who present with fever and diarrhoea among other symptoms of typhoid in selected hospitals within Bauchi.

Aim: The aim of this research is to evaluate the molecular profile of class 1 Integron among multidrug-resistant *Salmonella typhi* from clinical specimens in selected health facilities within Bauchi metropolis.

Materials and Methods: Biodata was obtained and Phenotypic antibiotic susceptibility patterns of the isolates were determined using the Kirby Bauer disk diffusion method and screened for Multidrug resistance. Class 1 Integron and antibiotic resistance genes were detected using polymerase chain reaction and agarose gel electrophoresis.

Results: In this study, 37(77.0%) of *S. typhi* isolates were resistant to 2 or more antimicrobial agents (Multidrug resistance). Highest resistance was observed in Oxacillin 46(95.8%), Imipenem 44(91.6%), Novobiocin 41(85.4%), Erythromycin 40(83.3%), and Ampicillin 39(81.2%). The isolates were sensitive to Ciprofloxacin 31(64.5%), Colistin Sulphate 29(60.4%), and Ceftriaxone 28(58.3%). All isolates 48(100%) were Multidrug-resistant and sensitive to Ciprofloxacin, Colistin Sulphate, Ceftriaxone, and Amikacin. Class 1 integron gene was present in all isolates subjected to molecular analysis. All four resistant genes *Tem-1, Sul-1, Gyr-A, and Cat-1,* were detected in selected Multidrug-resistant isolates for this study. Class 1 Integron genes were detected in all the six (6) isolates used for molecular study which is indicative of their high frequency in *Salmonella typhi* strains. The presence of Class 1 integron is highly associated with MDR profile.

Conclusion: In this study, prevalence of class 1 integron among multidrug-resistant *Salmonella typhi* serovars was high. This shows that class 1 integron may likely be responsible for the dissemination of antibiotic resistance. Cephalosporin and fluoroquinolones remain drugs of choice in treating typhoid fever.

Keywords: *Salmonella* Serovars; Resistance Genes; Multi-Drug Resistance; Class 1 Integron

Introduction

In recent years, the prevalence of antimicrobial resistant bacterial pathogens has become a major public health concern and increasing antimicrobial resistance in *Salmonella typhi* is a serious clinical problem worldwide. Multidrug-Resistant Typhoid Fever (MDRTF) is referred to as typhoid fever caused by *Salmonella typhi* strains which are resistant to all the four first-line recommended drugs for treatment, Chloramphenicol, Ampicillin, Cotrimoxazole, and Ciprofloxacin [1]. Despite the emergence of newer antibiotics, enteric fever has continued to be a major health problem especially in sub-Saharan Africa. *Salmonella typhi* gained resistance to antibiotics like Ampicillin, Ceftriaxone, and Cotrimoxazole, besides developing resistance to efficacious drugs like Ciprofloxacin. The emergence of multidrug resistance to the commonly used antibiotics has further complicated the treatment and management of enteric fever and this is recognized as one of the greatest challenges in the management of this disease [2] Since the mid 1980's Multidrug resistance typhoid fever has caused outbreaks in several countries in the developing world, resulting in increased morbidity and mortality, this is even more pronounced in older adults, pregnant women, infants, children below 5 years, and people who have compromised immune systems [3].

Integrons are genetic elements that are widely known for their role in the dissemination of antibiotic resistance, particularly among Gram negative bacterial pathogens. They are similar to transposons that mediate multiple drug resistance [4]. This genetic element has the potential for acquiring "new" antibiotic resistance genes through the recombination of these genes into the integron's integration site attl. An integrase gene, intI1, a signature of class 1 integrons, mediates this recombination event. Integrons serve as a vehicle for exchange of drug resistance genes and their eventual dissemination [5]. Integrons are major players in the spread of antibiotic resistance. In resistance integrons, the functional integron platform is linked to mobile DNA elements such as transposons and/or conjugative plasmids, thus enhancing transfer between cells and species [6].

These integrons share a pool of gene cassettes, the majority of which encode resistance to antibiotics. Cassette arrays in mobile integrons are usually short, with the longest recorded array having eight cassettes [7], presumably because cassette expression is driven from a single promoter, and proximal cassettes are poorly expressed. The pool of cassettes carried by mobile integrons can confer resistance to most classes of antibiotics used in medicine and agriculture [8]. Each of the major classes of integron now found in antibiotic resistant pathogens has a similar, and recent, evolutionary history.

The existence of antibiotic resistance genes outside of integrons in some cases suggests that genes for resistance to some antibiotics existed and were disseminated by clonal proliferation in these populations for some time and that the incorporation of integrons and additional resistances into the genome in these populations occurred separately [7]. The dual existence of antibiotic resistance genes within and outside integrons limits the sensitivity of the Int-PCR test. Whereas Int-PCR had only moderate success as a diagnostic tool for detecting or ruling out some individual antibiotic resistances, its high sensitivity and specificity levels for detecting drug resistance indicate that Int-PCR can be a valuable screening tool for identifying or ruling out multidrug resistance in *Salmonella* strains. Thus, in using Int-PCR, the detection of integrons can be followed by either Sensititre (phenotypic) testing or PCR techniques for the detection of specific genes encoding antibiotic resistances associated with class 1 integrons to verify resistance patterns.

Materials and Methods

Study area

The study was carried out in selected hospitals across Bauchi metropolis. They include Abubakar Tafawa Balewa University Teaching Hospital, State Specialist Hospital, Bauchi, Infectious Disease Hospital Bayara and Comprehensive Health Centre, Tashan Babiye. The study was conducted on both Febrile and Diarrhoeic patients of all ages and sex attending these hospitals. The specimens were collected from patients diagnosed by clinicians with either fever, gastroenteritis or both. Demographic information and important Bio data was also recorded.

Bacterial isolates

A total of thirty-seven (37) Multidrug Resistant (MDR) *Salmonella typhi* isolates were recovered from 518 blood and stool samples which were collected from both inpatients and outpatients in the selected hospitals of the study area. The pure isolates were used to extract DNA and amplify the antibiotic resistance genes.

Genomic DNA extraction

Colonies of each isolate were picked and suspended in 200 µl of distilled water and mixed by vortexing. Genomic DNA was extracted by the boiling method and briefly, 1.5ml of the sample in broth was centrifuged at 10,000rpm for 5 minutes. The supernatant was discarded and the pellets were washed twice with sterile water. After this, 200µl of sterile water was added to the pellets, the pellets were vortexed to homogenize and boiled in a dry bath at 100°C for 10 minutes. This was followed by vortexing and centrifugation at 12,000 rpm for 5 minutes. The supernatant was transferred into another pre-labeled Eppendorf tube by gentle aspiration using a micropipette. The supernatant contains the DNA needed for electrophoresis assay and was stored at 20°C. The concentration and purity of the extracted DNA were estimated using a Nanodrop spectrophotometer and the integrity of the DNA was assessed by 1.2% agarose gel electrophoresis [9].

Electrophoresis of extracted *Salmonella* genomic DNA products

The extracted genomic DNA products of *Salmonella typhi* were analyzed with electrophoresis on 1.2 % agarose w/v gels stained with Ethidium Bromide and visualized by UV illumination. A current of 120 V was applied to each gel. Eight microliters of extracted genomic DNA products mixed with 3 µl of 6X loading dye were loaded onto an agarose gel. A 2000 kbp DNA ladder was used as a marker for the extracted genomic DNA products [10].

Detection and sequencing of Class 1 Integron

The presence of class 1 integron gene was evaluated using primers (5'-CS F) GGC ATC CAA GCA GCA AGC (3'-CS R) AAG CAG ACT TGA CCT GAT and mapping of gene cassettes harbored in class 1 integron was performed as described by [11]. Gene cassette arrays was confirmed by direct sequencing of the amplified products and the resulting sequences were aligned and confirmed using the Genbank database and BLAST program (http:/blast.ncbi.nlm.nih.gov/Blast.cgi).

Resistant genes profiling

Salmonella typhi isolates that show MDR were used for resistant genes profiling. The resistance genes, Tem- 1, Sul-1, Gyr-A, and Cat-1, that code for p-lactamases, protein synthesis, DNA gyrase and integron abilities, respectively, to the commonly used antibiotics in the case study subjects, were investigated. DNA analysis and multiplex PCR was carried out with synthesized primers (Table 1) as described by [12].

Primers	Sequences 5´-3´	Amplicon Size (bp)	Reference
Gyr A – F	– TACCGTCATGTTATCCACGA-	343	(This study).
- R	-GTACTTTACGCCATGAACGT-		
Sul 2 – F	-TCAACATAACCTCGGACAGT-	316	(This study).
-R	-GATGAAGTCAGCTCCACCT-		
Tem 1 – F	-GCACGAGTGGGTTACATCGA-	793	(This study).
-R	-GGTCCTCCGATCGTTGTCAG-		
Cat P – F	-CCTGCCACTCATCGCAGT-	508	(This study).
-R	-CCACCGTTGATATATCCG-		

Table 1: Oligonucleotide Sequence primers used for the amplification of resistance genes in the study.

Results and Discussion

Typhoid fever is among the most endemic diseases in the tropics, it is associated with poverty and development with significant morbidity and mortality [13]. *Salmonella typhi* is more prevalent in developing countries than in developed regions. There has been a significant decrease in cases of typhoid fever in developed countries. The differences in the pattern of typhoid fever in developed and developing countries may be as a result of the unavailability of portable drinking waters and cultural habits in Africa. In recent years, emergence of ever-increasing number of antibiotic-resistant microbial strains has become a severe health threat to human-kind and one of the biggest challenges to global drug discovery programs [14].

In this study, the distribution of *S. typhi* among patients in selected Hospitals from the study area according to clinical diagnosis (Table 2), indicated a total of 518 specimens were collected among which 48 of the specimens were positive for *S. typhi.* Specimens collected from patients who presented with Fever was 308 out of which 18(5.8%) were positive for *S. typhi*. Patients with diarrhoea

were 189 with 26(13.7%) positive. Patients with symptoms of Fever and diarrhoea were 21 with 4 (19.0%) of positive specimens. Generally, Patients with typhoid fever typically present with fever characterized by high temperature rise, diarrhoea and sometimes both.

Cases	No. (%) of Specimens Collected	No. (%) of Isolates Positive	No. (%) of Isolates Negative
Fever	308	18 (5.8)	290 (94.1)
Diarrhoea	189	26 (13.7)	163 (86.2)
Fever and Diarrhoea	21	04 (19.0)	17 (80.9)
Total	518	48 (9.27)	470 (90.7)

Table 2: Distribution of *Salmonella typhi* among patients in the study area according to clinical diagnosis.

With the emergence of resistance towards traditional antibiotics, fluoroqinolone and extended-spectrum cephalosporins have been introduced as the antimicrobial agents of choice in treating MDR *S. typhi* [15]. However, reports shown an increase in the number of cases with typhoid *Salmonella* developing resistance towards fluoroquinolone. In countries with a higher incidence of MDR isolates, *S. paratyphi* displays a higher level of resistance towards fluoroquinolone compared to *S. typhi* [16].

Antimicrobial Agent (µg)	Number of isolates and zone of inhibition (mm) against antibiotics									
	Std (mm)	ST01	ST02	ST03	ST04	ST05	ST06	ST07	ST08	ST09	% MDR
Ampicillin (10)	15	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(100)
Augmentin (30)	15	(0.0)	(12.2)	(0.0)	(15.2)	(17.3)	(16.1)	(14.0)	(0.0)	(0.0)	(33.3)
Amikacin (30)	13	(10.0)	(0.0)	(12.2)	(13.3)	(0.0)	(12.2)	(11.2)	(0.0)	(11.0)	(11.1)
Ceftriaxone (30)	24	(0.0)	(15.0)	(17.0)	(32.5)	(24.6)	(33.0)	(19.3)	(0.0)	(27.4)	(44.4)
Cefuroxime (30)	24	(23.0)	(0.0)	(32.3)	(0.0)	(0.0)	(28.3)	(26.0)	(12.2)	(0.0)	(33.3)
Cefotaxime (30)	20	(0.0)	(10.0)	(24.7)	(22.3)	(13.2)	(0.0)	(27.1)	(0.0)	(21.1)	(44.4)
Cephalothin (5)	15	(17.0)	(0.0)	(18.2)	(13.3)	(0.0)	(12.2)	(20.2)	(0.0)	(11.0)	(33.3)
Chloramphenicol (5)	21	(20.0)	(0.0)	(12.2)	(13.3)	(0.0)	(25.2)	(11.2)	(21.0)	(22.0)	(33.3)
Ciprofloxacin (5)	17	(30.2)	(32.3)	(22.1)	(16.0)	(28.0)	(21.5)	(23.2)	(19.3)	(25.0)	(88.8)
Cotrimoxazole (25)	16	(25.0)	(22.0)	(10.5)	(14.0)	(11.0)	(23.2)	(9.5)	(21.0)	(18.0)	(55.5)
Colistin Sulphate (25)	14	(15.0)	(13.0)	(0.0)	(21.3)	(12.0)	(22.5)	(12.5)	(17.0)	(11.0)	(44.4)
Erythromycin (25)	17	(0.0)	(0.0)	(0.0)	(13.3)	(0.0)	(10.2)	(0.0)	(0.0)	(0.0)	(100)
Fusidic acid (5)	30	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(100)
Gentamycin (10)	17	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(12.2)	(10.0)	(0.0)	(11.0)	(100)
Imipenem (10)	17	(0.0)	(21.0)	(20.2)	(19.3)	(21.5)	(14.0)	(20.2)	(18.0)	(11.0)	(66.6)
Novobiocin (5)	16	(7.0)	(0.0)	(11.0)	(5.3)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(100)
Oxacillin (5)	15	(2.0)	(0.0)	(0.0)	(0.0)	(0.0)	(1.2)	(11.2)	(0.0)	(5.0)	(100)
Methicillin (5)	15	(0.0)	(0.0)	(7.0)	(0.0)	(0.0)	(11.2)	(0.0)	(10.7)	(0.0)	(100)
Tetracycline (30)	30	(8.0)	(0.0)	(12.2)	(13.3)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(100)

Table 3: Multidrug-resistant pattern of selected isolates from this study.

CLSI, 2018; ST = *Salmonella typhi;* MDR = Multi Drug Resistant.

It was observed in the present study that all isolates selected for further molecular studies were multidrug resistant to more than 10 out of the twenty antibiotics tested (Table 3). Isolates ST02, ST05 and ST07 were resistant to more antibiotics while isolates ST04, ST06 AND ST07 were resistant to least number of antibiotics. All isolates (100%) were Multidrug resistant and sensitive to Ciprofloxacin, Colistin Sulphate, Ceftriaxone and Amikacin.The emergence of *Salmonella* with antimicrobial resistance is mainly promoted by the use of antibiotics in animal feeds to promote the growth of food animals, and in veterinary medicine to treat bacterial infections in those animals [17]. This poses a high risk of zoonotic disease with the transmission of MDR *Salmonella* strains from animals to humans via ingestion of food or water contaminated with the animals' faeces, direct contact, or the consumption of infected food animals.

The majority of the gene cassettes located within these plasmids consist of resistant genes that confer the antimicrobial resistant property of the serotype against traditional antibiotics such as Chloramphenicol, Tetracycline, Amoxicillin, Fluoroquinolone and Streptomycin [18]. Some serotype of *Salmonella* has begun to develop resistance towards broad spectrum cephalosporin as a result of mutated genes that encode for extended - spectrum Beta lactamase, hydrolyzing antibiotics with Beta-lactam rings such as Cephalosporin and Cephamycins [19].

Class 1 integrons were detected in all six (6) *Salmonella* isolates used for molecular studies (Figure 1) which is indicative of their high frequency of occurrence in *Salmonella* strains. The presence of class 1 integron group is highly associated with resistance to antibiotics as all the isolates with the class 1 integrons had multidrug resistance pattern. A strong association of class 1 integrons with identified resistance to specific antibiotics was demonstrated and attributed in part to the existence of resistance genes within these integrons. The existence of antibiotic resistance genes outside of integrons in some study suggests that genes for resistance to some antibiotics existed and were spread by clonal proliferation in these populations for some time and that the incorporation of integrons and additional resistances into the genome in these populations occurred independently. The dual existence of antibiotic resistance genes within and outside integrons limits the sensitivity of the Int-PCR test.

Int-PCR can be a valuable screening tool for identifying or ruling out multidrug resistance in *Salmonella* strains. Thus, in using Int-PCR, the detection of integrons can be followed by either Sensititre (phenotypic) testing or PCR techniques for the detection of specific genes encoding antibiotic resistances associated with class 1 integrons to verify resistance patterns. Thus, in epidemiological surveys, the use of Int-PCR can provide time and cost savings compared to running extensive antibiotic resistance panels on a large number of samples [20].

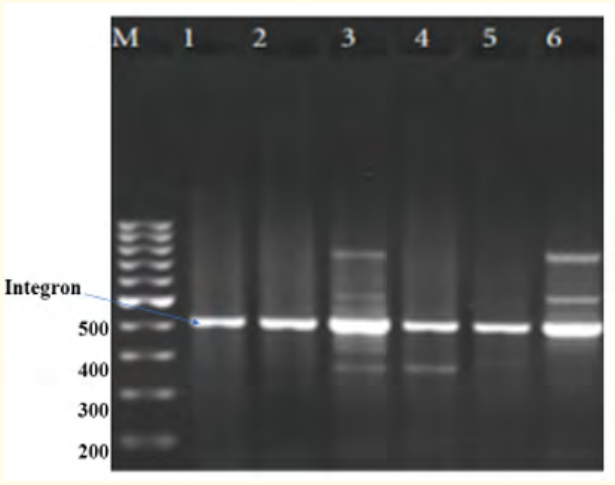

Figure 1: Agarose gel electrophoresis of Class 1 Integron genes with bands in lane 1 – 6.

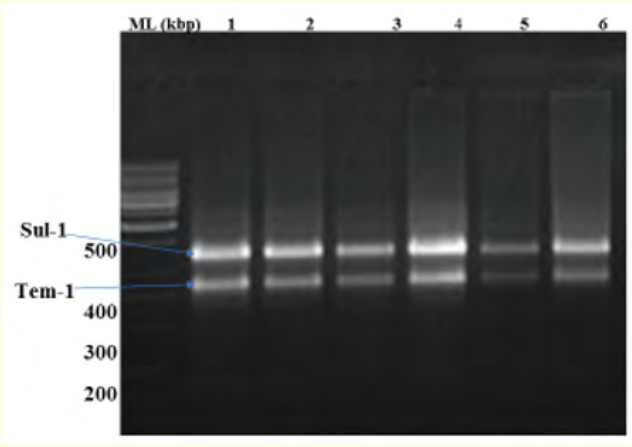

Figure 2: Multiplex PCR gel electrophoresis profile for Sul-1 and Tem-1 genes in MDR *Salmonella typhi* used in the study.

During this study, *Salmonella typhi* isolates that showed MDR were used for resistant genes profiling. The resistance genes, Tem- 1, Sul-1, Gyr-A, and Cat-1, that codes for B-lactamases, protein synthesis, DNA gyrase and integron abilities, respectively, to the commonly used antibiotics in the case study subjects were investigated. DNA analysis and multiplex PCR was carried out with synthesized primers. All four resistance genes where detected and found in selected isolates for his study (Figure 2, 3 and 4).

The detection of resistance genes in MDR *Salmonella typhi* can be from myriads of environmental and human factors. The resistance mechanisms in *Salmonella* is mostly plasmid, but can occur in mutated chromosomal DNA of the organism. Multiplex PCR targeting associated genes based on resistance (MDR) genes with aligned standard references oligonucleotides was investigated in the *Salmonella* isolates The detection of the resistance genes in this study is generally from the plasmid. The multidrug resistance has been increasingly reported, threatening treatment success for patients with severe infections. The presence of resistance genes Tem-1, Sul-1 and Cat-A, with aligned base pairs is a shows a widespread profile within the population. This shows spread of MDR strains that need to be curbed in the study area and Nigeria in general.

The confirmed representatives of *Salmonella* isolates profile show resultant dangers of antibiotic resistance. The masking of Gyr-A and Cat-1 gene in the multiplex PCR analysis was observed using Multiplex PCR, but found to be present in subsequent single PCR runs, after optimization of annealing temperature at 55°C and 57°C (Figure 3 and 4). The PCR reaction was performed in 15pl volume containing 2 pl of DNA template, 1 pl of each forward and reverse primer,7.5pl of PCR master mix and 4.5pl molecular grade water [21].

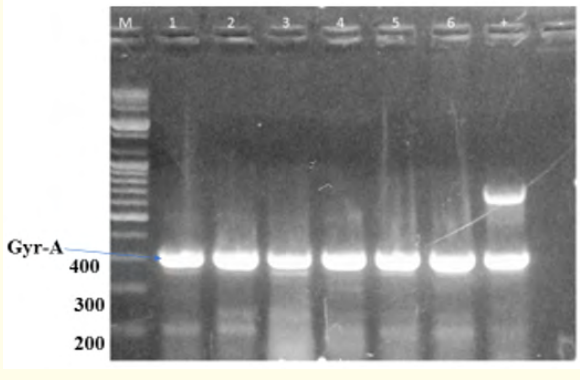

Figure 3: Agarose gel electrophoresis profile for Gyr-A resistance gene in MDR *Salmonella typhi* used in the study.

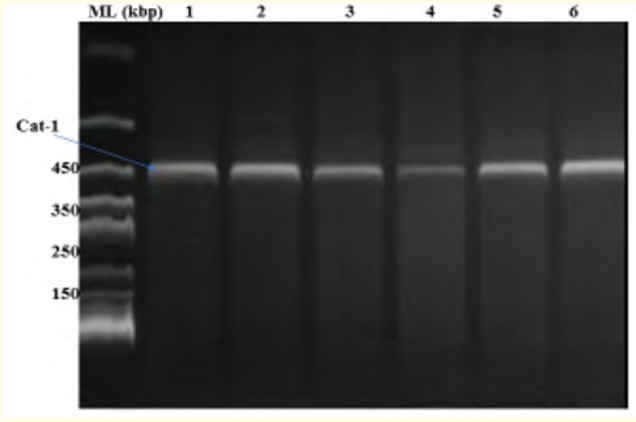

Figure 4: Agarose gel electrophoresis profile of Cat-1 gene in MDR *Salmonella typhi* isolates used in the study.

Conclusion

It was found that Class 1 Integron genes was harboured by all the six (6) isolates in this study, which confirmed the high frequency of occurrence in *Salmonella typhi* strains. The presence of Class 1 Integron is highly associated with MDR as all the isolates showed resistance to more than 2 antibiotics. The resistance genes, Tem-1, Sul-1, Gyr-A and Cat-1 were detected in all the MDR bacteria. This study established that the emergence of multidrug resistant strains of *Salmonella* has added to the urgent need for the development of more effective control measures.

Consent and Ethical Approval

Ethical approval was obtained from the Government of Bauchi State, Ministry of Health research and ethics committee, with written informed consent also sought from all patients prior to specimen and data collection.

Competing Interests

Authors have declared that no competing interests exist.

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