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Plasmid Profiles of Multidrug Resistant Clinical Salmonella Isolates From A Teaching Hospital, Jos, Nigeria

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Abstract: *The Salmonella infection is becoming a serious threat to both developed and developing nations although it is more pronounced in Asia and sub-African nations evident by the recent outbreaks in both Uganda and Tanzania this year. Thus, this study was carried out to ascertain the antibiotic profiles and plasmid profiles of the clinical Salmonella isolates. Ten serogrouped Salmonella isolates were obtained from the Medical Microbiology Department of the Jos University Teaching Hospital Jos, Nigeria. The Salmonella isolates were tested by the Kirby-Bauer disc diffusion method for drug susceptibility according to National Committee for Clinical Laboratory Standards Institute (NCCLS) guidelines. The antibiotics used were Cotrimoxazole (30µg), Streptomycin (30µg), Chloramphenicol (30µg), Sparfloxacin (10µg), Ciprofloxacin (10µg), Ofloxacin (10µg), and Pefloxacin (30µg), Amoxicillin (30µg), Amoxicillin+Calvulanic acid (30µg) and Gentamicin (10µg). The plasmid DNA extraction was carried out in Veterinary Research Institute(NVRI), Vom, Nigeria and was done using Thermo Scientific Gene Jet™ Plasmid Miniprep Kit (Promega,USA).The plasmids bands were separated by electrophoresis in agarose gel. The results showed four different resistance patterns and all the isolates were 100% susceptible to Ciprofloxacin, Pefloxacin and Chloramphenicol but high resistance against Amoxicillin+Clavulanic Acid,Amoxicillin and Ofloxacin. Seventy percent (70%) of the MDR Salmonella harbored plasmids while 30% of them conferred their resistances on their chromosomal DNA. The plasmid number ranged from 1 to 7 with varied sizes of 1500bp to above 20,000bp.There is possible distribution of plasmid related resistant bacteria within this region and Ciprofloxacin and Pefloxacin are still effective against the organisms for now.*

Keywords: *Antibiotic susceptibility, plasmid profile, multidrug resistant Salmonella.*

I. Introduction

Salmonellae which belong to the family Enterobacteriaceae are usually categorized as typhoidal and non-typhoidal base on the infections they cause .The typhoidal group is human restricted whereas non-typhoidal category is mostly animal pathogens but are zoonotic in nature and can cause food infection. There have been several reports to this effect. Salmonella serovars have been reported to be responsible for causing the highest number of bacterial food born infection in the United States [1].In developed countries non-typhoidal Salmonella(NTS) are only associated with gastrointestinal disease while in sub –African region, they constitute blood stream infections especially in malnourished and those with impaired immunity due to HIV and malaria infection .The most serotypes responsible for the invasion in humans are the invasive non- typhoidal Salmonella(iNTS) like S. Typhymurium(ST313) and S. Enteritidis [2].

Typhoid or enteric fever is an ancient disease caused by S.Typhi and has afflicted mankind causing serious havoc since human populations grew large enough to contaminate their water and food supplies [3].The burden of infection is increasing rather than reducing evident by the outbreak of typhoid in Uganda, March, 2015 where 1940 suspected cases were reported and at the beginning of the outbreak, S.Typhi was confirmed in 4 out of the 16 specimens screened [4]

One of the unanticipated features of the Salmonella genus has been the revelation that many virulence factors and high clinically significant resistance to antibiotics are attributed to plasmids. For instance, it has been reported that the transfer of antibiotic resistance by extra chromosomal R factors occurs widely among these bacteria [5] and Salmonella are said to share more than 70-80% of genes with other enteric bacteria, like E. coli [6]. In recent years there has been a report of some Salmonella isolates from UK containing virulence-resistance plasmids that were characterized which they mainly caused invasive infections in adults linked to Africa [7].Few years ago, another trend associated with multidrug resistance was the emergence of virulence-resistance (VR) plasmids which are hybrid plasmids that harbor resistance(R) and virulence (V) determinants. The appearance of these plasmids is said to be of concern because they lead to the co-selection of virulence through the use of antimicrobial agents [8, 9].Recent examples of plasmid-mediated resistance to expanded-spectrum cephalosporins have been reported showing the severity of current situation in enteric pathogens. The swapping between plasmid(s) and the bacterial chromosome and integration of resistance genes into genetic elements like

integrons contribute in acquisition and dissemination of resistance genes [10].Therefore the aim of this study was to identify the multi-drug resistant pattern of the Salmonella isolates and find out whether the multidrug-resistance was plasmid-encoded. This may provide a guide to empirical therapy.

II. Methodology

2.1 Antimicrobial Susceptibility

The clinical Salmonella isolates (sero-group ranging from A to D and Poly O) were collected from Medical Microbiology Department of Jos University Teaching Hospital (JUTH), Plateau State, Nigeria. Each of the isolates was emulsified and the suspension adjusted to 0.5 McFarland Standard which is approximately equivalent to $1x10^8$cfu/ml.The Salmonella isolates were tested by the Kirby-Bauer disc diffusion method for drug susceptibility according to National Committee for Clinical Laboratory Standards Institute (NCCLS) guidelines [11]. The antibiotics used were Cotrimoxazole (30µg), Streptomycin (30µg), Chloramphenicol (30µg), Sparfloxacin (10µg), Ciprofloxacin (10µg), Ofloxacin (10µg), and Pefloxacin (30µg), Amoxicillin (30µg), Amoxicillin+Calvulanic acid (30µg) and Gentamicin (10µg).The diameters of the zones of inhibition were measured diagonally and the average measurement was taken. The diameters of zones of inhibition exhibited by the Salmonella isolates of less than (<) 14mm was considered as resistant,14mm-18mm (moderate) and greater than 18mm as susceptible. E.coli ATCC25922 (as standard reference strain) was used as a quality control for the disk diffusion.

2.2 Plasmid DNA Extraction and Profiles

The plasmid DNA extraction was carried out in Veterinary Research Institute,Vom,Nigeria and was done using Thermo Scientific Gene Jet™ Plasmid Miniprep Kit (Promega,USA) alongside alkaline lysis solution..The standard DNA molecular weight marker used in this research was Thermo Scientific O'Gene Ruler 1 Kb Plus DNA Ladder,ready to use.The plasmids bands were separated by electrophoresis in agarose gel [12].

III. Results

The results obtained show that zones of inhibition varied from 00mm to 28mm with four different resistance patterns as R type-AuOfxS, R type-AmAuOfx, R type-AmAuS and R type-SxtAmAuOfxS with R type- AmAuS the most common (Table 1).Table 2 shows that the isolates exhibited resistance against Amoxicillin+Clavulanic Acid,Amoxicillin and Ofloxacin.However they were 100% susceptible to Ciprofloxacin,Sparfloxacin and Chloramphenicol. Plasmids profiling showed that 7 of the isolates harboured plasmid DNA while 3 had none. The plasmid number ranged from 1 to 7 with molecular weight of 1500bp to greater than 20,000bp and plasmids with high molecular weight were found in serogroup C, D and Poly O (Table3, Fig.1).

Table 1.Susceptibility of Clinical Salmonella Isolates (Serogroups) to Antibiotics

Isolate	Antibiotics/Zones of diameter (mm)										
i.d no.	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S	MDR Pattern
1	15	26	25	26	16	00	19	26	00	00	Au,Ofx,S
2	26	26	20	26	00	00	15	24	00	00	Am,Au,Ofx,S
3	26	26	23	26	00	00	21	26	00	20	Am,Au,Ofx
4	24	25	14	28	00	00	26	26	00	23	Am,Au,Ofx
5	23	23	23	26	00	00	23	23	00	23	Am,Au,Ofx
6	18	26	25	26	00	00	20	26	17	00	Am,Au,S
7	00	23	24	26	00	00	18	26	00	00	Sxt, Am,Au Ofx,S
8	17	21	26	26	00	00	19	26	23	00	Am,Au,S
9	18	26	26	26	00	00	18	26	20	00	Am,Au,S
10	18	20	24	26	00	00	22	26	20	00	Am,Au,S
ATCC 29522	20	26	26	26	00	00	18	26	16	16	Control

SXT (Cotrimoxazole), CH (Chloramphenicol), S (Streptomycin), PEF, (Pefloxacin), SP (Sparfloxacin), OFX (Ofloxacin), AM (Amoxicillin), AU (Amoxicillin + Clavulanic Acid), CN (Gentamicin), CPX (Ciprofloxacin), MDR (Multidrug Resistance), i.d no. (Identification number), <14mm (resistance), 14mm-18mm (moderate), >18mm (susceptible)

Table 2. Percentage (%) Susceptibility and Resistance of Salmonella Isolates to Individual Antibiotics

Antibiotics	No. of Iso.Res.	% Resistance	No. of Isolate susceptible	% susceptible	% Moderate
SXT	1	10	4	40	50
CH	0	0	10	100	0
SP	0	0	10	100	0
CPX	0	0	10	100	0
AM	9	90	0	0	0
AU	10	100	0	0	10
CN	0	0	7	70	30
PEF	0	0	10	100	0
OFX	6	60	3	30	10
S	7	70	3	30	0

SXT (Cotrimoxazole), CH (Chloramphenicol), S (Streptomycin), PEF, (Pefloxacin), SP (Sparfloxacin), OFX (Ofloxacin), AM (Amoxicillin), AU (Amoxicillin + Clavulanic Acid), CN (Gentamicin), CPX (Ciprofloxacin), Iso.Res.(Isolate Resstant)

Table 3. Plasmids number and sizes of multidrug resistant clinical Salmonella isolates

Isolate i.d No.	Serogroup	No. of plasmids	Sizes (bp)
1	C	2	3000, 7000
2	A	2	3000, 400
3	C	4	1500, 2000,3000,10000
4	C	2	4000, 5000
5	D	1	2000
6	Poly O	7	2000, 2500, 3000, 4000, 5000, 2000, >20000
7	B	0	-
8	D	4	1500, 2000, 20000, >20000
9	D	0	-
10	D	0	-

i.d No. (Identification number)

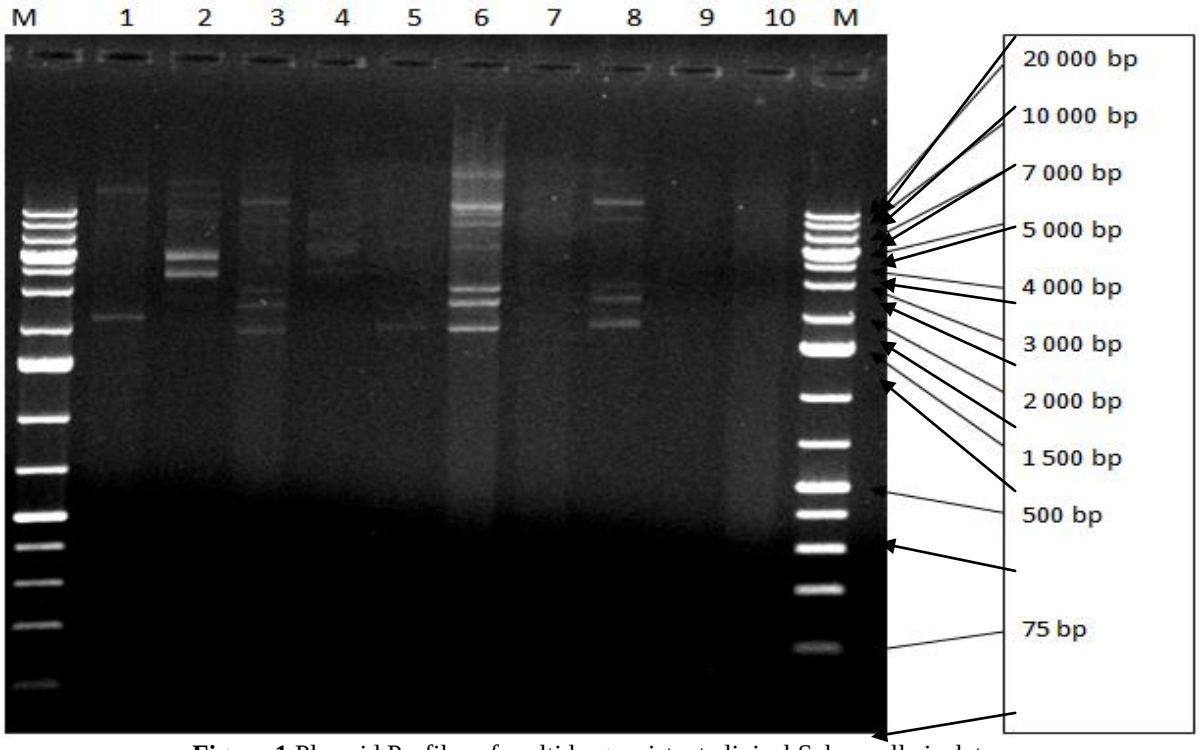

Figure 1. Plasmid Profiles of multidrug resistant clinical Salmonella isolates

M (Ladder), 1-10 (isolates identification number)

IV. Discussion

Plasmids generally represent diverse category of extra-chromosomal generic elements which carry resistance genes .It is evident from the result obtained that 70% of the multidrug resistant (MDR) Salmonella strains is as a result of the plasmids the organisms harbored. That is, plasmid-bearing multidrug resistance accounted for a great percentage of Salmonella in this study area as it has been documented elsewhere [13] .This finding is in the same vein with earlier report of 60.86% of multidrug resistant Salmonella isolates to harbor plasmids[14]. Also, the report that 85.7% of the MDR Salmonella was due to the plasmids the organisms possessed [15] is closely related to this present study. The probable absence of plasmids in 3 MDR Salmonella strains (although with limitation because of lack of PCR analysis since) suggests that their multidrug resistance phenomenon is not plasmid related but could be chromosomally related. That is the resistance ability is conferred on their chromosomal DNA [16].

The plasmid sizes of 1.5 to > 20Kb (15000 to >20,000bp) seen here is at variance with that of past report[15] who documented sizes of 4.7-5.7Kb but it is closely related to the finding of an earlier work [17].There is indication that some of the MDR Salmonella possessed small size plasmids while others were of bigger size. The higher molecular weight plasmids are said to be plasmids that confer resistance to multiple antibiotics(R-plasmids) and most of them are conjugative besides storage of genetic information, they contribute to spread of genes in bacterial population [18].On the other hand some of the low molecular weight plasmids are known to increase resistance to phage infection due to the presence of restriction modification systems [18].

The possession of 7 plasmids by one of the MDR Salmonella isolates is a bit strange to normal range of 2-6 plasmids for non-typhoidal Salmonella and again much higher than the lower range normally expressed by typhoidal Salmonella [19], therefore it is of medical importance. The plasmids also revealed that bacterial isolates with the same resistance antibiotic profile can differ in their plasmid profiles. This may explain a diversity in plasmid contents of bacterial isolates and the role of different plasmids in the resistance to a particular antibiotic[19].The MDR Salmonella strains were seen to carry plasmids with encoded resistance to Amoxicillin,
Amoxicillin+Calvulanicacid,Ofloxacin,Septrin;Amoxicillin,Amoxicillin+CalvulanicAcid,Ofloxacin;Amoxicillin,Amoxicillin+Clavulanic Acid, Septrin and other MDR Salmonella strains harbored plasmids encoded for Cotrimoxazole, Amoxicillin, Amoxicillin+Calvulanic acid, Ofloxacin and Septrin. Most of these antibiotics are primary antibiotics which were formerly first line antibiotics in treating Salmonella infections. More challenging is the occurrence of plasmid encoding resistance to Ofloxacin being a secondary generation antibiotic is a confirmation of advancement of the salmonellae exhibiting resistance to current antimicrobial agents which may present a tendency of bacteria challenging the efficacy of even newer drugs if not check mate. The presence of plasmid encoded multidrug resistance(PEMDR) in these Salmonella strains is probably a suitable adaptation to changing antibiotic environment overtime[20].Earlier reports had also shown that the resistance of gastro enteric Salmonella and Shigella strains to antimicrobial agents was largely due to production of extended spectrum β – lactamases(ESβLs) encoded on plasmids as well as on the chromosome[21,22,23].This could explain the multidrug resistance characteristics of the 3 non-plasmid and plasmids harbored isolates.

In conclusion, study evidence shows there is a high likelihood that plasmids are responsible for the high incidences of MDR.The antibiotics that are still effective here are Ciprofloxacin, Pefloxacin , Sparfloxacin and Chloramphenicol although the use of Chloramphenicol is normally discouraged because of its adverse side effects. There is need for periodic antibiotic profiling of these organisms to avoid further emergence of more plasmid related resistance.

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