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Original Research Article

Determination of Tem-1 Gene in Multidrug Resistance *Salmonella* Species Isolated from Diarrheogenic Patients Attending Aminu Kano Teaching Hospital Kano (AKTH)

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Abstract: *Salmonella* is one microorganism in which resistant serotypes have emerged because of the widespread use of antibiotics. The study was aimed to determine the prevalence of TEM 1 gene in multidrug resistance *salmonella* species isolated from diarrheogenic patients attending Aminu Kano Teaching Hospital Kano. Standard microbiological techniques were employed for isolation and identification of *Salmonella*. Disc diffusion method was used for antibiotic susceptibility testing. Molecular techniques was used for detection of TEM 1 resistant gene in multidrug resistant isolates. The result showed 22 out of 170 samples were positive for *Salmonella* spp which accounted for 12.9%. Higher incidence of *Salmonella* was found among subjects of age between 21 – 30 years. *Salmonella* spp were highly resistant to ampicillin (86.4%), streptomycin and gentamicin (77.3%) each, chloramphenicol (72.7%) and augmentin (61.2%). Less resistant was recorded by ciprofloxacin (27.3%), tetracycline and erythromycin 36.4% each. Based on the activity of the antibiotics, ciprofloxacin, tetracycline and erythromycin were the most effective antibiotics while ampicillin, streptomycin and gentamicin were less effective. From the result, 8 (36.4%) isolates were resistant to three or more classes of antibiotics tested while 14 (63.6%) of the isolates were resistant to less than three antibiotics. The gel electrophoresis results for the PCR products obtained for detection of *TEM-1* gene for each of the 8 *Salmonella* isolates showed 6 (75%) out of the 8 multidrug resistant showed presence of *TEM-1* gene at 296bp. It is concluded that *TEM-1* gene was one of the gene responsible drug resistant in *Salmonella*.

Keywords: Antibiotics, Diarrhea, Resistant Gene, *Salmonella*.

INTRODUCTION

Salmonella is a member of the Enterobacteriaceae family, which is gram negative, facultative anaerobe and non-acid fast, and non-sportive and mobile (You *et al*., 2010). These intestinal organisms have more than 2500 different serotypes that are divided into three distinct species: *Salmonella typhi*, *Salmonella choleraesuis* and *Salmonella enterica* (Black *et al*., 2010). *Salmonella enterica*, the sub-type of Enterica serotype enteritidis, *Salmonella enteritidis*, is the most important cause of salmonellosis. It is one of the most important infectious diseases between humans and animals, mostly related to the consumption of meat, poultry, eggs and milk (Guerra *et al*., 2002).

Therefore, this bacterium is a food-borne pathogen. Salmonellosis can be a form of typhoid fever (typhoid), septicemia, and gastrointestinal infections (gastroenteritis). The common serotypes involved in *Salmonella gastroenteritis*, are *Salmonella typhimurium* and *Salmonella enteritidis* (Ranjbar *et al*., 2007). The onset of these sudden symptoms varies from 12 hours to

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a week, and many people recover without the need for antibiotics (Su *et al*., 2005). Due to the addition of antibiotics to animal diets, improper, excessive and arbitrary use of antibiotics and lack of proper monitoring of drug administration has led to the development of antibiotic-resistant strains (Parry, 2003). The main problem in treating infections caused by these organisms is the emergence of multidrug resistance (MDR), which often leads to prolonged hospitalization and increased therapeutic costs compared with antibiotic susceptible microbes and Finally, Drug Therapeutic Failure (DTF) (Teethaisong *et al*., 2016).

According to the Centers for Disease Control and Prevention (CDC), World Health Organization and Food and Agriculture Organization of the United Nations (2004), genus *Salmonella* is divided into two species: *Salmonella enteric*a (*S. enteric*a) and *Salmonella bongori* (*S. bongori*) (Lopez *et al*., 2012). Six subspecies (subsp) *S. enteric*a include; *S. enterica* subsp. *houtenae*, *S. enterica* subsp. *Arizonae*, *S. enterica* subsp. *enterica*, *S. enterica* subsp. *Salamae*, *S. enterica* subsp. *indica* and *S. enterica* subsp. *Diarizonae* (Gong *et al*., 2014). The largest number of reported food-borne is known by *S. enterica* subsp. *enterica* serovar typhimurium (*S. typhimurium*) in the USA (Jahid *et al*., 2015).

Salmonella causes economic burden, significant morbidity and mortality in human and animal populations (Vikram *et al*., 2011). In 2013, *Salmonella* was one of the most clinically important foodborne known in the world (Jahid *et al*., 2015) and from 2008 to 2009, *Salmonella* infections were reported in over 46 states in the USA (Vikram *et al*., 2011). Basically this bacterial species on host range is limited including domestic fowl, livestock, humans, rodents, reptiles, and birds. *S. typhimurium* infection causes nausea, diarrhea (mostly self-limiting) and vomiting. In addition, the clinical sign is characterized by headache, fever and abdominal pain. The emergence of this infection agent has been reported in developing countries (Sharifzadeh *et al*., 2014).

Some gram-negative bacilli such as *salmonella* and *Escherichia coli* (*E. coli*) strains from particular types produce beta-lactamases. The Enterobacteriaceae family produces beta-lactamases, which are encoded by plasmids. For the first time, *TEM-1* was isolated from *E. coli* from a blood culture (Temonera) in Greece (Medeiros, 1984). One of the first reported beta-lactamases is *TEM-1*, which is encoded by plasmids. Almost 80 variants of the *TEM-1* penicillinases showed activities against extended-spectrum cephalosporins (Mulvey and Boyd, 2009). Today, reports indicate the prevalence of *TEM-1* beta-lactamase in certain parts of the world, suggesting that this type of beta-lactamase is a global problem (Black *et al*., 2010). The aim of the study was to determine the prevalence of TEM 1 gene in multidrug resistance *salmonella* species isolated from diarrheogenic patients attending Aminu Kano Teaching Hospital Kano (AKTH).

MATERIALS AND METHODS

Study Area

The study was conducted at Microbiology Laboratory of Aminu Kano Teaching Hospital Kano. Kano State is one of the states located in Northern Nigeria. It is geographically coordinated at 11^0 3’ N and 8^0 3’ E latitude and longitude respectively. It shares borders with Kaduna state to the west, Bauchi state to the South, Jigawa state to the East, Katsina state to the North. It has a total area of 20,131km2 (7,777sqm) and population of 13,405,300 (NPC, 2016).

Ethical Approval

An approval for the study (Reference Number: AKTH/MAC/SUB/12A/P-3/VI/1974) was obtained from Research and Ethic committee of Aminu Kano Teaching Hospital Kano. The aim of the study was explained clearly to the clients and informed consent obtained before commencing the study.

Sample Size Determination

The sample size was obtained using the following formula: $N = \frac{Z^2pq}{d^2}$. The last prevalence found of patients attending Murtala Muhammad General Hospital, Kano, Nigeria was 12.0% (Nas *et al.,* 2021). From the above formula $N = \frac{Z^2pq}{D^2}$

Where Z = 1.96, p = 12%, d = 0.05 % and q = 1 - p = 1 - 0.12 = 0.88

$N = \frac{(1.96)^2\ (0.12\times0.88)}{(0.05)^2}$

$N = \frac{3.842\ \times0.1056}{0.0025}$

Therefore, $N = \frac{0.4056}{0.0025} = 162.3$

Therefore N is approximately equals to 162, which is the minimum number of samples for the study. Therefore, a total of 8 subjects accounted for 5% of the minimum number of subjects were added for attrition, making a total of 170 samples.

Samples Collection

This study involved 170 patients attending Aminu Kano Teaching Hospital Kano for treatment of diarrhea. A sterile universal container was used for sample collection. Diarrhea samples were obtained from the patients and were processed for isolation and identification of *Salmonella* spp according to the standard microbiological techniques (Cheesbrough, 2012). All the samples collected were immediately transferred under aseptic conditions. The specimens were transported to the Laboratory of the Department of Microbiology, Aliko Dangote University of Science and Technology, Wudil for further processing.

Isolation and Identification of Bacteria

Isolation of bacteria was conducted according to the method describe by Cheesbrough (2012). During the process, a sterile wire loop was dipped into the faecal sample of the patients and streaked onto the surface of *Salmonella*-*Shigella* agar plates and incubated aerobically at 37^0C for 24 hours. After incubation, bacterial growth was observed for colony appearance and morphology. Each colony was re-inoculated into freshly prepared agar plates until a pure colony was obtained. For identification, each pure colony was Gram stained and subjected to further biochemical tests such as indole, methyl-red, Voges Proskauer, citrate utilization and motility test as described by Cheesbrough (2012).

Determination of Multidrug Resistance

The *Salmonella* spp isolates were subjected to antibiotic susceptibility testing using the agar disk diffusion method, as described by Bauer *et al*., (1996). Mueller-Hinton agar plates were inoculated with an overnight culture of each isolate by streak plating. The standard antibiotic sensitivity discs were aseptically placed at equidistance on the plates and allowed to stand for an hour. The plates were incubated at 37°C for 24 hours. Sensitivity pattern of the isolates to sparfloxacin (30 µg), streptomycin (30 µg), augmentin (10 µg), perfloxacin (30 µg), amoxacillin (30 µg), Chloramphenicol (30 µg), Gentamicin (10 µg), tavavid (30 µg), Ciprofloxacin (10 µg) and Septrin (30 µg), produced by Abtek pharmaceutical limited, were determined. Isolates were divided into two groups based on the zone of inhibition produced by the antibiotic disc; susceptible, and resistant according to the Clinical and Laboratory Standards Institute guideline; performance standards for antimicrobial susceptibility testing (CLSI, 2010). Isolates that were observed to be resistant to at least four different classes of antibiotics were classified as being multidrug resistant (Ezekiel *et al.,* 2011).

Molecular Characterization of resistance gene of the Isolates

Specific primers for the TEM 1 resistance gene (F: CGCCGCATACACTATTCTCAGAATGA, R: ACGCTCACCGGCTCCAGATTTAT) designed using NCBI Primer-BLAST was used in the study as adopted by Ali *et al*., (2025).

RESULTS

Age and Gender Distribution of the Study Subjects

A total of 170 stool samples of the study were examined in the study after meeting the inclusion criteria. Interviews based on questionnaire were conducted with the respondent. From the total of 170 subjects as indicated in Table 1, 92 were males (54.1%) while 78 which accounted for 45.9% were female. Based on the age of the subjects, age category 11 – 20 years has the highest frequency 58 (34.1%) followed by 0 – 10 year with total frequency of 47 (27.6%) while least frequency 8 (4.7%) was recorded by subjects with age category 51 – 60 years.

Table 1: Age and Gender Distribution of the Study Subjects

Characteristics	Number (n)	Percentage (%)	*P* value
Gender			
Male	92	54.1	0.20740*
Female	78	45.9	
Total	**170**	**100**	
Age (Years)			
0 – 10	47	27.6	0.0001**
11 – 20	58	34.1	
21 – 30	23	13.6	
31 – 40	20	11.8	
41 – 50	14	8.2	
51 - 60	8	4.7	
Total	**170**	**100**	

Key: * Result is not significant at $p<0.005$**;** **Result is significant at $p<0.005$

Identification of Salmonella spp

The result for identification of *Salmonella* spp is presented in Table 2. The isolate was identified based on Gram staining, cultural characteristics and biochemical test. The result showed that the isolate is Gram negative bacteria, positive for MR test but negative for indole, VP and citrate utilization test. The isolate cannot ferment lactose and produce transparent colourless colony in both MacConkey and *Salmonella-Shigella* agar.

Table 2: Identification of Salmonella spp

Isolates	Morphology	Biochemical Test						
		GS	MR	IN	VP	CIT	MOT	LF
Salmonella spp	Colourless colony with dark patches at the centre on SSA	-	+	-	-	-	+	-

Key; SSA= *Salmonella Shigella* agar, GS = Gram staining, MR = Methyl-red, IN – Indole test, VP – Voges Proskauer, CIT = Citrate utilization, MOT = motility, LF = Lactose fermentation

Prevalence of Salmonella spp

The Prevalence of *Salmonella* in the stool sample of the participants is presented in Table 3. The result showed 22 out of 170 samples were positive for *Salmonella* spp which accounted for 12.9%. Higher incidence of *Salmonella* was found among subjects of age between 21 – 30 years.

Table 3: Distribution of Isolates based on Age Group of the Study Subjects

Age (Years)	Samples (n)	Positive samples	Percentage (%)	*P*-value
0 – 10	47	2	1.18	0.32051*
11 – 20	58	4	2.35	
21 – 30	23	7	4.12	
31 – 40	20	5	2.94	
41 – 50	14	3	1.76	
51 – 60	8	1	0.59	
Total	**170**	**22**	**12.94**	

Key: * = There is no statistical significant difference in the number of *Salmonella* isolated based on the age of the patients in the study. Hence, the result is not significant at $p<0.05$.

Antibiotic Susceptibility of the Isolates

The antibiotics susceptibility of the isolates to some commonly used antibiotics is presented in Table 4. The results showed that *Salmonella* spp were highly resistant to ampicillin (86.4%), streptomycin and gentamicin (77.3%) each, chloramphenicol (72.7%) and augmentin (61.2%). Less resistant was recorded by ciprofloxacin (27.3%), tetracycline and erythromycin 36.4% each. Based on the activity of the antibiotics, ciprofloxacin, tetracycline and erythromycin were the most effective antibiotics while ampicillin, streptomycin and gentamicin were less effective.

Table 4: Antibiotic Susceptibility Testing outcomes of the Isolate (n = 22)

Antibiotics	Conc. (µg/ml)	No. Sensitive isolates (%)	No. Resistant isolates (%)
Ampicillin	30	3 (13.6)	19 (86.4)
Augmentin	10	7 (31.8)	15 (61.2)
Nalidixic acid	30	9 (40.9)	13 (59.1)
Erythromycin	10	14 (63.6)	8 (36.4)
Gentamicin	20	5 (22.7)	17 (77.3)
Chloramphenicol	10	6 (27.3)	16 (72.7)
Streptomycin	30	5 (22.7)	17 (77.3)
Norfloxacin	30	10 (45.5)	12 (54.5)
Tetracycline	10	14 (63.6)	8 (36.4)
Ciprofloxacin	10	16 (72.7)	6 (27.3)

Distribution of Multi Drug Resistant (MDR) Isolates from Study Subjects

Table 5 represents the distribution of multidrug resistant and multidrug resistant *Salmonella* isolates. The result showed that 8 (36.4%) isolates were resistant to three or more classes of antibiotics tested while 14 (63.6%) of the isolates were resistant to less than three antibiotics.

Table 5: Distribution of Multi-drug resistant Isolates

Isolates	No. identified	Percentage (%)	P-value
MDR	8	36.4	0.20083*
NMDR	14	63.6	
Total	**22**	**100**	

Key: Result is not significant, MDR=Multi-drug resistant, NMDR=Non-Multi-drug resistant

Molecular Detection of Resistance Genes

The figure 1 represent the gel electrophoresis results for the PCR products obtained for detection of *TEM-1* gene for each of the 8 *Salmonella* isolates. Lane M is 1000 bp DNA ladder; lanes 1 - 8 are positive samples while lane 9 is negative control.

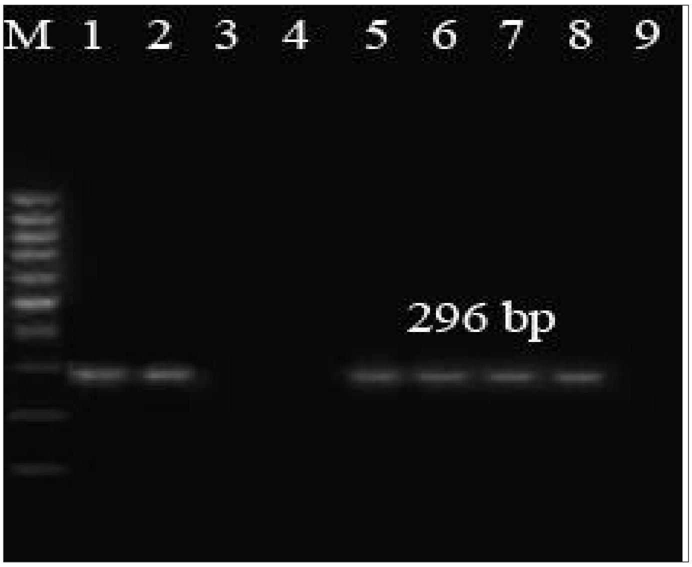

Figure 1: Gel electrophoresis for detection of *TEM-1* gene

DISCUSSION

Salmonella is an important pathogenic bacterium predominantly found in animals and human. *Salmonella* is known with more than 2500 serovars of *enterica* worldwide (Torpdahl *et al*., 2013). Finding of the present study revealed that cases of diarrhea were mostly found among subjects of 11 – 20 years and number of cases decreased in older subjects with less than 1% (result is not significant at $p<0.05$). This finding agrees with that of Nwaoha *et al*. (2017). A decrease in number of cases among older subjects might be due to fact that the immune system of older ones get stronger in resistance against agents of diarrhea as can be seen in the work of Gascon *et al*. (2000) symptoms of a diarrhea in older subjects may also be lighter compared to younger ones, because they could easily be given over-the-counter-drugs for treatment.

The result of the present study showed 22 out of 170 samples were positive for *Salmonella* spp which accounted for 12.9%. Higher incidence of *Salmonella* was found among subjects of age between 21 – 30 years. Several studies on isolates associated with diarrhea indicated *Salmonella* as one of the major cause of gastrointestinal infection. Similar study was conducted by Ali *et al*. (2023) on enteric bacterial pathogen in stools of residents of urban and rural regions of Nigeria, the results shows the most frequently encountered pathogens in rural area are *E. coli* (28%), followed by *Salmonella* (16%), *Shigella* (14%), *Aeromonas* (9%) and *Campylobacter* (8%). Similarly, the result of this research was inconformity with the study conducted by Kim *et al*., (2015) on enteric bacteria isolated from diarrhea patients in Korea which reveals that *Escherichia coli* was the most prevalent isolated accounted for 22%, this is followed by *Clostridium* 14% and *Salmonella* 13.5%. On the other hand, study conducted by Lopez *et al*., (1996) on Enteropathogenic agents isolated in persistent diarrhea, the result shows that *Salmonella* was the most frequently isolated bacteria, which have similar distribution with *Escherichia coli* and followed by *Shigella.* All the above studies justify the finding of the present study.

The antibiotics susceptibility of the isolates to some commonly used antibiotics in the present study showed that *Salmonella* spp was highly resistant to ampicillin, streptomycin and gentamicin, chloramphenicol and augmentin. Less resistant was recorded by ciprofloxacin (27.3%), tetracycline and erythromycin 36.4% each. Based on the activity of the antibiotics, ciprofloxacin, tetracycline and erythromycin were the most effective antibiotics while ampicillin, streptomycin and gentamicin were less effective. The result of the present study was in conformity with the finding of Karambu *et al*., (2013) who reported sensitivity to Nalidixic acid and ciprofloxacin and resistivity to ampicillin by enteric bacteria isolated from diarrhea infected children in Kenya. Similar resistance to amoxicillin by enteric bacteria associated with diarrhea in children was revealed in a study done in Abuja, Nigeria by Ifeanyi *et al*., (2015). Mandomando *et al*., (2007) reported 85% isolates were sensitive to Tetracycline and 62% isolates of *Salmonella* spp. were resistant to Ampicillin. According to Patel *et al*., (2008) 10% of the *Salmonella* isolates were resistant to Ampicillin and 7.1% were resistant to Nalidixic acid. The resistance to ampicillin in this finding is much lower than our study. This may be due to empirical use of these antibiotics and development of resistance (Obi *et al*., 2007).

The result showed that 36.4% of the isolates were resistant to three or more classes of antibiotics tested while 63.6% of the isolates were resistant to less than three antibiotics. This finding was higher than that that found in Bangladesh (Sharif *et al*., 2022) who reported 15% and in Iran (Mirjafari *et al*., 2016) who reported less than 30% MDR among enteric bacteria associated with diarrhea in children. The prevalence of

MDR strains of pathogenic *Salmonella* might be a significant cause of severe and prolonged health outcomes among subjects with diarrhea. The rapid and uncontrolled increase in use of antibiotics as human treatment and animal feed and treatment options has significantly contributed to the rise and spread of MDR, which is supported by the previous studies (Sharif *et al*., 2023).

The gel electrophoresis results for the PCR products obtained for detection of *TEM-1* gene for each of the 8 *Salmonella* isolates showed 6 (75%) out of the 8 multidrug resistant showed presence of *TEM-1* gene at 296bp. *TEM-1* gene is **o**ne of the first beta-lactamases which is plasmid encoded. In addition to *Salmonella*, other bacteria such as *Pseudomonas aeruginosa* also have the ability to produce this enzyme. Many reports indicated that the prevalence of *TEM-1* beta-lactamase is a global problem, especially in many parts of the world (Shebani *et al*., 2010). Finding of this study agrees with the finding of Doosti *et al*., (2015) who reported a prevalence rate of 62% for *TEM-1* gene and a high drug resistance in *Klebsiella pneumoniae* isolated from cockroaches from hospitals which is somewhat similar to the present study's results.

CONCLUSION

The result of the present study showed 22 out of 170 samples were positive for *Salmonella* spp which accounted for 12.9%. Higher incidence of *Salmonella* was found among subjects of age between 21 – 30 years. The antibiotics susceptibility of the isolates to some commonly used antibiotics in the present study showed that *Salmonella* spp was highly resistant to ampicillin, streptomycin and gentamicin, chloramphenicol and augmentin. Less resistant was recorded by ciprofloxacin (27.3%), tetracycline and erythromycin 36.4% each. Based on the activity of the antibiotics, ciprofloxacin, tetracycline and erythromycin were the most effective antibiotics while ampicillin, streptomycin and gentamicin were less effective. The result showed that 36.4% of the isolates were resistant to three or more classes of antibiotics tested while 63.6% of the isolates were resistant to less than three antibiotics. The gel electrophoresis results for the PCR products obtained for detection of *TEM-1* gene for each of the 8 *Salmonella* isolates showed 6 (75%) out of the 8 multidrug resistant showed presence of *TEM-1* gene at 296bp. It is recommended, that there is need to improve early diagnosis and empirical treatment of wound infections.

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