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Molecular Characterization of *Salmonella enterica* Isolated From Cooked Rice Hawked in Bodinga Metropolis, Sokoto State Nigeria.

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ABSTRACT

Salmonella is a genus species of rod-shaped and Gram-negative bacteria that belong to the family known as Enterobacteriaceae. Salmonellosis is caused by Salmonella spp one of the most common and widely distributed food borne diseases, and is found in cooked rice and other food products, it is a global public health problem. The present study was conducted to characterize *Salmonella enterica*. Isolated from cooked rice hawked in Bodinga Metropolis. A total of 205 samples were collected and analysed using standard microbiological methods. The isolated Salmonella spp were identified using biochemical tests and confirmed using polymerase chain reaction (PCR). Antibiotic sensitivity test were carried out using disc diffusion method. Two samples (0.98%) were found to be positive for Salmonella spp. The two Salmonella species detected were *Salmonella enteritidis* and *Salmonella typhimurium*. The isolated Salmonella species were (100%) sensitive to ceftriaxone and ciprofloxacin. The result of PCR for the detection of invA showed that only one isolates was positive with amplification at 796 bp. In conclusion, the results of this finding showed the presence of *Salmonella enterica* contamination in the cooked rice hawked in Bodinga local government, indicating potential sources and routes of Salmonella in cooked rice hawked. Detection of invasion A gene (inv A gene) indicated poor sanitation and handling of the cooked rice in the studied area.

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1.0 INTRODUCTION

Salmonella is a genus species and rod-shaped Gram-negative bacteria belonging to the family Enterobacteriaceae. They are divided into two species as *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* were further sub divided into six subspecies that include over 2,600 serotypes. It was named after Daniel Elmer Salmon, an American veterinary surgeon (Brenner *et al.,* 2000). It does not form spore, and is always predominantly motile having cell diameters between 0.7 and 1.5 μm, lengths from 2 to 5 μm, it possess peritrichous flagella, meaning that it is found all around the cell body (Brenner *et al.,* 2000).

However, most of them are chemotrophs, meaning they usually obtain their energy from oxidation and reduction reactions by using organic sources. They are anaerobes, which is capable of generating ATP with oxygen ("aerobically") when it is available, or by using electron acceptors as well as fermentation ("anaerobically") when oxygen is not available hence they are facultatives (Brenner *et al.,* 2000). Salmonella species are intracellular pathogens of certain serotypes causing illness. It usually causes infections due to ingestion of food contaminated by animal feces, or by human feces, such as by a food-service worker at a commercial eatery (Brenner *et al.,* 2000).

These bacteria has serotypes which refers to as possession of distinct characteristics. These serotypes have been divided into two main groups typhoidal and Nontyphoidal serotypes which can be transferred from animal-to-human and from human-to-human. They usually invade only gastrointestinal tract and cause infection known as Salmonellosis, in which the symptoms of it can be resolved without antibiotics (Goldrick, 2003). However, in sub-Saharan Africa, Nontyphoidal Serotypes can be invasive and cause paratyphoid fever, which requires immediate treatment with antibiotics, whereas Typhoidal serotypes can only be transferred from human-to-human, and can cause food-borne infection, typhoid fever, and paratyphoid fever (Goldrick, 2003).

Typhoid fever is caused by Typhoidal serotypes which spread by invading the bloodstream or in addition spreading throughout the body, thereby invading organs, and secreting endotoxins (the septic form). This can lead to life-threatening hypovolemic shock and septic shock, and requires intensive care including antibiotics (Goldrick, 2003). These disease remains as a major global-health concern and is said to be responsible for an estimated 10.9 million cases, and approximately 117,000 deaths, annually (CDC, 2013). It mortality rate in the pre-antibiotic era was estimated to be 10–30% (CDC, 2013). It was reported that there are 892 cases in 38 states as a results of eating contaminated onion (CDC, 2021). However, the effective antimicrobial therapy over the past 70 years has reduced the overall mortality rate to <1% (Cosby *et al*.,2015). Moreover, the recent emergence and dissemination of multi-drug resistant strains of *S. typhi* and paratyphi has limited the effective treatment options for enteric fever (Luquez,2017). In which the World Health Organization (WHO) has recently listed fluoroquinolone-resistant Salmonella as a "priority pathogen", which has been identified as one of the families of bacteria thought to pose the greatest risk to human health through rising antimicrobial resistance (WHO, 2017). Therefore, the treatment options for these Typhoidal fever particularly in the outpatient setting are now severely limited (Muhammed *et al*.,2019). In this case, the third generation cephalosporins are commonly used in the empirical treatment of these Typhoidal fever and are a valuable treatment option in the setting of MDR and fluoroquinolone resistant isolates (Muhammed *et al.,*2019). This reports have described the emergence of extended spectrum beta-lactamase producing *S. typhi* including a strain of extensively drug resistant (XDR) *S. typhi* genotype responsible for a large typhoid outbreak (Doumith *et al.,*2016). How ever, any strains that are resistant to third-generation cephalosporins may require treatment with carbapenems, which are either unavailable or prohibitively expensive in resource limited settings (Batabyal, 2018).

Azithromycin is an azalide antimicrobial drugs that is widely used for the empirical treatment of uncomplicated Typhoidal fever, it is always benefitting from once daily oral dosing and good tissue penetration. It excellently work in vitro by being concentrated within phagocytic cells thereby achieving intracellular concentrations of up to 200 times greater than serum (Rajan *et al*., 2017). More so, several randomised controlled trials have demonstrated the efficacy of azithromycin in adults and children when compared with fluoroquinolones, cephalosporins and chloramphenicol which is included in the treatment of fluoroquinolone intermediate or resistant strains (CDC, 2017).

The uncommon resistance to azithromycin amongst circulating *S. typhi* strains appears to be an emerging problem. Recently study was conducted, and above 1000 isolates were collected between 2013 to 2016 in Dhaka, Bangladesh where almost 12 azithromycin-resistant *S. typhi* strains and *S. paratyphi* strain were detected (Luquez, 2017). How ever, in Nigeria, the rate of morbidity associated is due to persistent increase of Typhoidal fever, in some cases, resulting to death (Akinyemi *et al.,* 2005).

The method used for, the treatment of Thyphoidal patients has been based on the use of first-line antibiotics, such as chloramphenicol and co-trimoxazole, as well as the third-generation cephalosporin in some other countries. However, the efficacies of some of these drugs have been doubtful, due to the emergence of multidrug resistance in Typhoidal causing Salmonella strains (Akinyemi *et al.,* 2005). More over, fluoroquinolones have higher efficacy both in vitro and in vivo during the treatment of severe Salmonella-associated illnesses, while there is reported strains with reduced susceptibility to ciprofloxacin among travellers in the jungles (Akinyemi *at al.,*2005).

Azithromycin can be associated with fever-clearance times averaging 4-5 days prolonged bacteremia of up to 72-96 hours post treatment and treatment failures (WHO, 2017). There is always an ineffective treatment responses that are associated with increased in the morbidity, as well as potentially

harmful effects resulted in the prolonged treatment courses, which interrupted the treatment regimens (prompted by escalation or switching antibiotics) and increased healthcare burden (Batabyal, 2018). The controlled of human infection (CHI) models have previously been applied to study antibiotic therapy in challenges caused by *S. typhi* (CDC, 2017). Therefore, these studies will offer the advantage of accurately recording clinical treatment responses in a closely monitored experimental setting with daily collection of culture samples to accurately determine the dynamics of bacteraemia, which could be In light of the increasingly limited treatment options for enteric fever, and then, to compare treatment responses to azithromycin and ciprofloxacin in healthy volunteers that have been challenged with a full antibiotic susceptible strain of *S. typhi*, which could be served as part of the programmes for the controlled of human infection studies (Rajan *et al.,*2017 It aimed at molecular Characterization of Salmonella enterica isolated from cooked rice hawked.

3.0 MATERIAL AND METHODS

3.1 Study Area

This work was undertaken in Bodinga, Sokoto Metropolitan area of Sokoto State. It was situated in the northwestern region of Nigeria, is known for its semi-arid climate and historical significance as the seat of the Sokoto Caliphate. The state spans longitudes 3°E to 7°E and latitudes 10°N to 14°N of the Equator, featuring a landscape dominated by savannah vegetation and seasonal rivers. This research has encompasses five markets: Tashar Albasa, Tashar Yar gado, and Cikin Kasuwa, scattered throughout the metropolis.

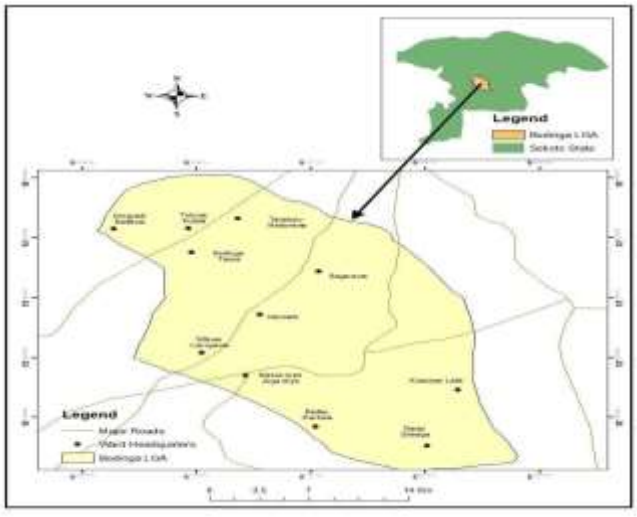


Figure 1: Map of Bodinga, Showing Bodinga as the Study Area.

3.2 Research Design

This cross-sectional descriptive study, were conducted on hawked cooked Rice in Bodinga Sokoto Metropolis. Samples was collected from markets places: Tashar Albasa, Tashar Yarg Gado, and Cikin Kasuwa and Molecular chacterization of *Salmonella enterica*. It have been carryout using PCR methods. And the resistant were determined using Kirby Bauer disc method. The laboratory work was conducted in Microbiology laboratory. Usmanu Danfodiyo university sokoto state, Nigeria.

3.3 Sample collection

A total of 205 samples were collected from children hawking rice in Bodinga metropolis, Sokoto state. Five grams (5g) of the whole rice was collected from each individual hawking from the selected places in Bodinga metropolis: Tashar Albasa 80. Samples and Cikin Kasuwa 75, and Tashar yar gado 60 totaling to 205 samples. The samples were put in a sterile plain container with a white mouthed cover and transported in a cool box, to the Microbiology Laboratory in College of veterinary medicine, Usmanu Danfodiyo University, Sokoto for further analysis.

3.4 Preparation of Media

All media are prepared according to the manufacturer s instructions. Buffered Peptone Water (BPW) is a broth for enriching the sample and was prepared by weighing Ten grams (10g) of buffered peptone water powder and dissolved in 500ml sterile distilled water, 5ml was dispensed in a sterile test tube and autoclaved at 121°C for 15 minutes Rappaport Vassiliadis Soya (RVS); This is a differential medium, and ten points eight grams (10.8g) powder of RVS were weighed and dissolved in 400ml of sterile distilled water, 5ml was dispensed in clean test tube and sterilized using autoclave at 121°C for 15 mins. Brilliant Green Agar (BGA); This is a selective medium. Thirteen grams (13g) powder of (BGA) were weighed and dissolved in 500ml of sterile distilled water in the conical flask, and it was sterilized using autoclave at 121°C followed for 15 mins and allowed to cool at room temperature and poured on Petri dishes allowed to solidify and use. Nutrient Agar (NA). This is a general-purpose medium. Eight point four grams (8.4g) of nutrient agar were weighed and dissolved in 300ml of sterile distilled water in the conical flask. It was autoclaved at 121°C for 15mins allowed to cool and poured in the slanted Bottles and allowed to solidify in a slanted manner. Mueller Hinton Agar; Thirty-eight grams (38g) of Mueller Hinton agar powder were weighed and dissolved in 1 litre of distilled water, it was mixed and dissolved completely, and it was then sterilized by autoclaving at 121,°C for 15 mins. It was poured on the Petri dishes and allowed to solidify.

3.5 Sample Preparation and Isolation of *Salmonella* spp

Samples was processed by weighing 1g of rice from each of the child hawking and put in 10ml of sterile buffered peptone water contained in the test tube, it was then incubated at 37°C for 24 hours. One milliliter (1 ml each) from incubated buffered peptone water were inoculated in 9ml of prepared RVS and incubated at 37°C for 24hrs. After which it was streaked on brilliant green agar and incubated at 37°C for 24hrs, to ascertain the *Salmonella* spp in rice samples. Using a sterile wire loop each of the suspected *Salmonella* spp colony was picked and inoculated into the fresh nutrient agar slanted bottles and incubated at 37°C for 24hours. It was then kept in the refrigerator at 4°C for further use. Suspected *Salmonella* spp, were subjected to Gram's staining and biochemical tests: which include: Urease test, Coagulate test, citrate test, Triple Iron Sugar test, Voges Proskauer, Catalase test, indole test, mortality test and Methyl Red (MR) test.

3.6 Gram staining

It was carried out according to the method described by Oyeleke and Manga (2008). The sterile wire loop was used to pick up a colony of the bacterial isolates and emulsified on drop of normal saline dropped on a clean glass slide. It was fixed by heating the smear by passing the slide over a Bunsen flame 3 times. After fixation, the smear was covered with crystal violet for 30 seconds and then washed with sterile water. Excess water was drained off to avoid diluting the mordant, and then the iodine solution was applied for 60 seconds and washed with water. It was decolorized with 95% ethanol and then, washed with water. Lastly, flooded the slide with Safranine (counter stain) for 30 seconds, it was allowed to air dry and a drop of oil immersion was dropped, it was examined with light microscope using x100 objective.

3.7 Biochemical Tests

3.7.1 Urease Test

Salmonella isolates were inoculated in 3 ml slanted urea agar base containing 40% of urea salts and incubated at 37°C for 24hrs. The development of pink color showed the presence of urease, while absence of pink color indicated negative result (Oyeleke and Manga,2008).

3.7.2 Indole Production

Five ml of sterile peptone water was inoculated with a loopful culture of isolates and incubated at 37°C for 48 hrs. After incubation 2ml of Kovac's reagents was added to the medium. Development of a red/pink layer on top of the medium indicated positive result while absence of red/pink indicated negative result (Oyeleke and Manga, 2008).

3.7.3 Coagulase Test

A drop of normal saline was dropped on a clean slide. Using a sterile loop, a colony of the test organism was emulsified in the saline to form a smooth suspension. And a one drop of plasma was dropped to the suspension. It was mixed gently with the loop and bObserve for clumping within 10–15 seconds. Visible clumping/agglutination indicated positive and non visible of clumping was Negative.

3.7.4 Methyl Red Test

The test was performed by inoculating the test organism in 5 ml sterile glucose phosphate peptone water (GPPW) and incubated for 24hrs. After overnight incubation at 37 °C, a drop of methyl red solution was added. The appearance of a bright red color indicates acid production which is regarded as positive test while absence bright red color indicates negative result (Oyeleke and Manga, 2008).

3.7.5 Motility Test

The suspected colony of *Salmonella* was touched using a straight needle, it was stabbed once to a depth of only half inch to the middle of the tube. It was incubated at 37°C and examined. The development of visible stab line with cloudness were observed, the growth was confined to the stab line, with sharply defined margins and the surround medium were left clearly transparent, the result is positive. And visible stab line with clear medium indicated negative result (Oyelake and Manga, 2008).

3.7.6. Triple Sugar Iron (TSI)

In this procedure, a straight inoculating needle was used to pickup an isolated colony. It was then inoculated into the TSI slant by first stabbing the butt down to the bottom, and there by withdrawing the needle, and then used it to streak the surface of the slant. A loosely fitting closure were used to permit access of air. The result was then read after incubation at 37°C for 18 to 24 hour to check for fermentation of sugars and H_2S production (Oyeleke and Manga, 2008).

3.7..7 Voges Proskauer Test

In this test, the organism was inoculated into MR-VP broth. It was then incubated at 35–37°C for 24–48 hours. After incubation, about 1 mL of broth were transferred into a clean test tube. And 0.6 mL of VP Reagent A (α-naphthol) 0.2 mL of VP Reagent B (KOH) were added it was then Shaked and the tube was gently exposed it to oxygen. The tube was allowed to stand for 15–30 minutes. The development of pink/red color showed positive and No color change or copper/brown color showed Negative (Liao *et al.,* 2024).

3.7.8. Citrate Test

The method of the performance of a citrate test, a pre-made Simmons citrate agar slant were obtained and then streaked on the surface of the agar with a bacterial culture using a sterile inoculating needle. It was then be incubated in the tube at 35-37°C for 24 to 48 hours,

and then check for a positive result by observing a change from green to blue on the slant, indicating growth and citrate utilization, or a negative result by seeing no growth and no color change (Oyeleke and Manga, 2008).

3.7.9 Catalase Test

In the procedure for the performance of a catalase test, small sample of a pure bacterial culture was placed on a clean slide or in a test tube, and then a few drops of 3% hydrogen peroxide (H_2O_2) was added. An immediate, rapid formation of bubbles was observed which indicates a positive catalase test, as the enzyme converts H_2O_2 into water and oxygen gas. If no bubbles appear immediately, the culture were catalase negative (Oyeleke and Manga, 2008).

3.8 Antibiotics Sensitivity Test

3.8.1 Disc diffusion Method

This method is an agar diffusion method or the disk diffusion method introduced by Kirby baeur. The procedure followed is that a filter disk impregnated with antibiotics used were obtained from Abtek Biological Ltd, UK with the following: Ampicillin (10 µg), Ceftriaxone (30 µg), Ciprofloxacin (15 µg), Chloramphenicol (30 µg), Co-trimoxazole (25 µg) and Azithromycin at (15 µg), was applied to the surface of an agar plate inoculated with test bacterium at 0.5 McFarland standard, and the plate was incubated at 37°C for 24-48 hours (CLSI,2020). The effectiveness of a particular antibiotic was shown by the presence of zone of inhibition. The diameters of the zone of inhibition (ZOI) were measured in mm with a ruler and interpreted according to CLSI (2020).

3.9 DNA Extraction

The genomic DNA was extracted using Qiagen DNA-easy kit following manufacturers instruction. Before the procedure, all samples and reagents were equilibrated to room temperature. Buffer AW1 and Buffer AW2 was prepared according to the manufacturer's instructions by adding 25 ml and 30 ml of absolute ethanol respectively. Two hundred microlitre (200 µl) of Lysis Buffer (AL) was transferred into a 1.5 ml micro-centrifuge tube. And 200 µl bacterial suspension was added to the Buffer AL in the 1.5 ml micro=centrifuge tube, it was mixed by vortexing for 15 seconds, it was incubated at room temperature for 20 mins. And the tube was centrifuged to remove drops in the lid. And again, 400 µl absolute ethanol was added to the sample, and mix by vortexing for 15 mins. After mixing, tubes were again briefly centrifuged to remove drops from in the lid. Carefully, 500 µl of the solution was added to the QIAamp Mini column (in a 2 ml collection tube) without wetting the rim. The cap was carefully closed and allowed to centrifuged at 8000 rpm for 1 min. The Mini column of QIAamp was transferred to a clean 2 ml collection tube, and the tube was discarded. Carefully, the QIAamp Mini column was opened and the remaining solution in the microtube and was added to the spin column in a 2 ml collection tube, it was closed and centrifuged at 8000 rpm for 1 min and the flow through was discarded. Again, 500 µl Buffer AW1 was added to the spin column and centrifuge at 8000 rpm for 1 min, and the flow through was discarded. And also, 500 µl Buffer AW2 was added, it was centrifuged at full speed (14,000 rpm) for 3 min and the flow through was discarded. The tubes were spun dried by centrifuging again for 3 minutes without adding any reagent. The QIAamp Mini column was placed in a clean nuclease free 1.5 ml microcentrifuge tube and the old collection tube containing the filtrate was discarded. Carefully, 60 µl Buffer AE (Elusion Buffer) equilibrated to room temperature was added directly to the silica membrane of the spin column, it was closed and incubated at room temperature for 5 min and centrifuged at 8000 rpm for 1 minute. The spin column was discarded and the microtube containing the DNA was caped and stored at -200 C until required.

3.10 Amplification of invasion A gene (invA)

PCR targeting invA gene was performed using primers previously designed by Rahn *et al.,* (2001). The 25 µL PCR mix contained the following component was prepared containing the following: master mix 12.5µL (Qiagen), 2.5µL of coral load, 1 µL of 10mM each of forward and Reverse primer (Table 1), 3 µL of nuclease free water and 5 µL of DNA template. The tubes were carefully mixed and transferred to applied bio system 9700 programmed with the following cycling conditions Initial denaturation at (94°C for 3 mins) followed by 40 cycles of denaturation at (94°C for 30 seconds), annealing specific to invA gene at (55°C for 1 min) and the extension at (72°C for 1 min), followed by final extension at 72°C for 10 mins. The PCR products obtained were kept at 4°C until required.

Table 1: **Sequence of forward and reverse invA gene primers**

Primer Name.	Sequences	Tm	Expected Amplicon Size
invA-Forward	CGG TGG TTT TAA GCG TAC TCT	**603**	769-bp
invA-Reverse	CGA ATA TGC TCC ACA AGG TTA	**574**	

Source: Rahn *et al,. (*2001)

3.11 Gel electrophoresis

In the analysis of the amplified products, the submarine agarose gel electrophoresis was performed as described by Sambrook and Russell (2001). Agarose gel (1.5%) were prepared by dissolving 0.75 gm agarose in 50 ml 1XTris- Borate EDTA (TBE) buffer and was subjected to heat until it completely dissolved and appeared as a clear transparent solution. The agarose solution was allowed to cool to 50°C and then 10µL of ethidium bromide (0.5µg/ml) dye was added to it. How ever, the gel was poured into the gel casting tray held

within the gel casting gates and the comb was placed into the slots on the tray in such a manner that a gap of 0.5 mm was left between the tips of comb teeth and the floor of casting tray and the wells were completely sealed by the agarose. The gel was allowed to solidify for 30 mins and then the comb was gently removed, and the casted gel was submerged carefully long with the running tray into the electrophoresis tank containing 1X TBE buffer. A total volume of 8 µl amplicon was transferred on a clean Para film and mixed evenly with 2 µl of 6X gel loading dye (Thermo scientific) and loaded carefully into the wells of agarose gel. And determine the size of the amplified PCR product 100 bp DNA ladder were loaded in one well. It was performed at 70 V for 1 hour and the mobility were monitored from the migration of the dye in the gel. After appropriate migration, the Agarose gel was visualized under UV trans-illuminator and the results documented.

3.12 Statistical Analysis

The data were analyzed using SPSS Microsoft words to calculate t-test and determine the significance difference between location and prevalence of Salmonella isolated which was consideration at $p < 0.05$

4.0 RESULTS AND DISCUSSION

4.1 Occurrence of Salmonella spp in Cooked Rice Hawked in Bodinga Metropolis

Salmonella spp was isolated from rice hawked in Bodinga metropolis. Out of 205 samples of rice collected 2 (0.98%) was found to be contaminated with Salmonella spp. Two hundred three (203) samples were positive for Enterobacter and Proteus spp having 183 (98%) and 20 (9.76) respectively Table. 2. The statistical analysis using results showed no significance difference between the 3 location.

4.2 Morphological and Biochemical Characteristics of Salmonella spp and other Bacteria Isolated from Cooked Rice Hawked in Bodinga Metropolis

The colonial morphology of Salmonella spp isolated from cooked rice hawked in Bodinga metropolis was pinkish whitish and red colonies on brilliant green agar, in which the target bacteria (Salmonella) reacted gram negatively and the shapes of the cells were rods and arranged singly. The biochemical characteristics of Salmonella spp isolated from Rice hawked in Bodinga Metropolis Sokoto State, was found to be negative in urease and indole, while motility and Methyl red test (MR) are positive Table 3.

4.3 Frequency of occurrence of the bacteria identified

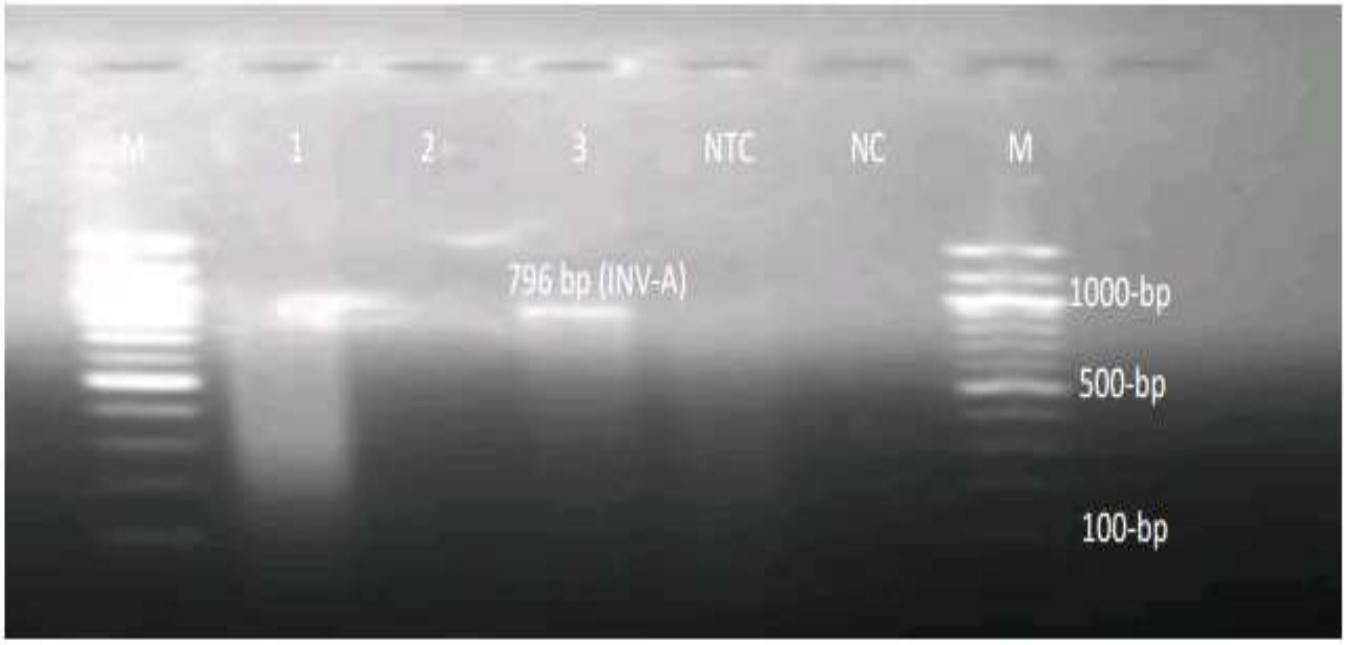
The frequency of occurrence was less in Salmonella which was found to be 2 (0.98%), Proteus spp 10% and Enterobacter which had the highest frequency occurrence at 89%. Table

4.4 Antibiogram of Salmonella spp Isolated

The result of sensitivity and resistance pattern of Salmonella spp to antibiotics tested are presented in Table 5. Salmonella spp were found to be 100% sensitive to Ciprofloxacin and ceftriaxone. However, it was observed that the isolates were resistance to Ampicillin, Cotrimoxazole, Chloramphenicol and Azithromycin at 100% each.

4.5 Molecular Characteristics of Salmonella Isolated

Molecular identification was done using conventional PCR for the detection of invA gene. Hundred base pairs (100-bp) DNA ladder was used as a marker, in which only one isolate was amplified with 796-bp (Fig. 2).



Keys M= DNA ladder (100-bp), Biolabs UK, 1 to 3= Sample well, NTC= No template control, NC= Negative control

Figure 2: Molecular amplification of inv A gene of *Salmonella* spp

Table 2: Prevalence of Salmonella spp isolated from Rice hawked in Bodinga metropolis according to sampling site

Sampling site	Number of negative samples n (%)	Number of positive samples n (%)	Total
Tashar Albasa	67(100)	0 (0)	67(100)
Tashar Yargado	68 (98.6)	01(1.4)	69 (100)
Cikin Kasuwa	68 (100)	01(1.4)	69 (100)
Total	**203 (99.02)**	**2 (0.98)**	**205 (100)**

Table 3: Morphological and Biochemical characteristics of Salmonella enterica isolated from cooked rice hawked in Bodinga Metropolis

Code	Gram RxN	Morp	Mot.	MR	Urea.	Indol.	Cit.	TSI	Cit.	H2S	Oxi.	Vp	Bacteria isolated
HCR21-20	Rods	Pinkish colonies	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve	Salmonella enterica
HCR55-130	Rods	Red pinkish	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ves	-ve	-ve	Proteus spp
HCR130-205	Rods	Reds colonies	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve	Enterobacter spp

Keys; +=positive, -=Negative, Mot =motility, RxN= Reaction, MR= Methyl red, Urea= urease, Indol= Indole, TSI= Triple sugar iron, Cit= Citrate, Cat= Catalase, H2S= Hydrogen sulphide, Oxi= Oxidase, VP= Voges Proskauer, HCR= Hawked Cooked Rice.

Table 4: Frequency of occurrence of the Bacteria Identified

Bacteria	Frequency	Percentage (%)
Salmonella spp	2	0.98
Proteus spp	20	9.76
Enterobacter spp	183	89
Total	**205**	**100**

Key,: spp= species, %= percentage

Table 5: Sensitivity and resistance pattern of *Salmonella* spp to antibiotics tested (n=2)

Antibiotics (µg)		Zone of inhibition measured in (mm)		
		Sensitive (%)	Resistant (%)	Total (%)
Ampicillin	(10 µg)	0(0)	2(100)	2(100)
Ceftriaxone	(30 µg)	2(100)	0(0)	2(100)
Ciprofloxacin	(15 µg)	2(100)	0(0)	2(100)
Chloramphenicol	(30 µg)	0(0)	2(100)	2(100)
Cotrimoxazole	(25 µg)	0(0)	2(100)	2(100)
Azithromycin	(15 µg)	0(0)	2(100)	2(100)

Key: ug=Microgram, %=Percentage, n=Number, mm=minimeter, ssp = species

4.0 DISCUSSION

Salmonellosis is a worldwide public health issue that causes foodborne infection and other illnesses. Foodborne infection can occur at any point in the food chain, including production, processing, distribution, and retail marketing (Silvia *et al*., 2005). There is significant efforts put in place to eradicate typhoid, nevertheless Salmonella food poisoning persisted as a major public health issue globally (Antunesa *et al*., 2003). The use of antimicrobial drugs in food animals is a probable explanation for the rising prevalence of antimicrobial-resistant Salmonella since the majority of Salmonella infections are contracted through the ingestion of infected foods and foods at food cooking point and during preparation (Antunesa *et al.,* 2003).

Out of the 205 samples of cooked rice that were gathered, 2 (0.98%) were determined to be positive for Salmonella, according to this study conducted. Therefore, the overall prevalence of 0.98% in the 205 samples that were analyzed is lower than what is found in several recent studies conducted in Jos and surrounding (Bata shalangwa *et al.,* 2016, Ogun *et al*., 2019), which reported prevalences of 11% in raw beef, 14.1% from commercial broiler chicken, and 15.4% in raw chicken and meals and cooked foods.

However, the results of this study showed that cooked rice samples taken from the Bodinga metropolis in Sokoto state contained distinct bacterial species, including *Salmonella* spp, *Proteus* spp, and *Enterobacter* spp. This investigation was compared to a study conducted on food sold on university campuses in southwestern Nigeria, which identified bacterial isolates belonging to six genera in this investigation. The bacterial isolates' distribution across the different food samples. The most frequently identified bacterial isolate was *E. coli,* at 29.49%, followed by *Klebsiella* spp. (25.64%) and *Proteus* spp. (17.59%), while *Salmonella* and *Pseudomonas* spp were found in 12.82% and 11.54% respectively, and Enterobacter at 2.5% which was the least common organism. Once more, the current study was contrasted to one that found a total of 51 enterobacterial pathogens in stool samples. This study's total prevalence of Salmonella was 8.11% (six Salmonella serotypes), which is consistent with the 13.50% incidence rate found by Abdullahi, 2010 in Kano State. Several investigations in Nigeria have discovered diverse prevalence rates of *Salmonella* spp in seemingly healthy animals, foods, and animal products: 6.4% in chicken, 14.1% in raw meat, 39% in seemingly healthy slaughtered animals and 2% in raw beef and food, respectively (Akeem *et al.,* 2019, Jarere *et al.,* 2015).

This study investigation observed drug resistance in two *Salmonella* spp recovered, and were (100%) sensitive to Ciprofloxacin and Ceftrixone, and were found to be (100%) resistant to ampicillin, co-

trimoxazole, Chloramphenicol and azithromycin. It was in similar to Bahnass *et al.*,(2015) which reported from the study carried out that Salmonella strains were susceptible to ciprofloxacin (Cajetan *et al.,* 2013). Its in comparison to previous reports by Molla *et al.,* (2006) at the central part of Ethiopia where it is isolated among sheep and goat meat, and foods sold at the road sites. Akinyemi *et al.,* (2005) how ever in Nigeria, it was gotten from human isolates by Zelalem *et al.,* (2011), in Addis Ababa also the Salmonella isolates of were isolated from from dairy farms. The effectiveness of such drugs like ciprofloxacin could be because of that they are not widely used in countries like Ethiopia and African countries (Zelalem *et al.,*2011). In addition to this, the effectiveness of this drug due to the fact that it is not well distributed to all societies and not simply prescribed rather than it is used as drug of choice in antibiotic resistant individuals. Moreover, ciprofloxacin is not commonly used to treat animals in that country (Zelalem *et al*., 2011).

The resistance of Salmonella spp to these drugs is emerging to be a significant public health problem, and are considered to be one of classes of drugs of choice used in the treatment of invasive non-typhoidal infections in situations where ampicillin is clinically ineffective (Rahn *et al.,* 2001). Several research has shown that there is a linear correlation of resistance to beta-lactam antimicrobials with the lactamase level over some time and resistance to beta-lactam can be attained by escalating enzyme levels (Hamilton *et al.,* 2012). Hence, the protracted use or misuse of these drugs may have selected for resistance over a long period time (Hamilton *et al*., 2012).

On this present study the Salmonella spp isolated were characterized using molecular method of detection (PCR) in which invA gene was detected from single isolate. One isolates was found to be negative after amplification, which may be as a result of mutations in primer binding site (Rahaman, 2002) Or it may be that the second sample is not truly Salmonella.

The invA gene is essential for full virulence in Salmonella and is thought to trigger internalization required for invasion of deeper tissue (Rahn *et al.,* 2001). This, finding closely agrees with other studies that reported the detection of this gene in almost all *Salmonella* spp (Rahn *et al.,* 2001)and amplified using conventional PCR assay using invA primers specific for Salmonella (Rahn *et al.,* 2001). Amplification of the invA gene is now recognized as an international standard procedure for the detection of the Salmonella genus Rahn *et al.,* 2001).

5.0 CONCLUSION

In conclusion, this research was carried out to characterized Salmonella enterica isolated from cooked rice hawked in Bodinga Metropolis, and species of Salmonella harboring invasion A gene were seen.. And was tested against antibiotics and was found to be sensitive and resistant. This shows contamination, which could be a potential source of Salmonellosis in the studied area.

6.0 RECOMMENDATION

It was recommended that, hygienic practices and sanitary measures should be employed in cooking rice hawked by the children on the road sites, to reduce the level of contamination by microorganisms and build a healthier society. However, sequencing and other molecular diagnostics should be used in detection of Salmonella and resistance genes.

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