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***Salmonella enterica* POSSESSING *invA* GENE ISOLATED FROM DRINKING WATER IN KAFANCHAN, KADUNA STATE, NIGERIA**

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ABSTRACT

This study aimed at screening drinking water in Kafanchan, Kaduna state for the presence of *Salmonella enterica* and subsequently, detect some virulence genes. Samples of various water sources were enriched in Selenite F broth for 18 hours and subsequently streaked on *Salmonella-Shigella* agar. Typical colorless colonies with/without dark centers were identified using Gram staining, biochemical tests, serological tests, and partial 16S rRNA sequencing. Three samples (borehole, pipe-borne and well water) were found to be contaminated with *Salmonella enterica*. The isolates were then screened for the presence of some virulence genes (invasive gene (*invA* and *spvA*) using PCR technique with specific primers. All the isolates were shown to possess the *invA* gene which is an indication of their pathogenicity. None of them showed amplicons for the *spvA* gene. The sequences have been deposited in the GenBank library with accession numbers KT737736 - KT737738. The isolation of this organism from drinking water is of public health significance, because *Salmonellosis* and typhoid fever are endemic in Nigeria. Furthermore, the risk is more amplified due to the presence of the *invA* gene which is known to be responsible for the invasion of the host cell by the pathogen. Proper attention needs to be paid to drinking water in this part of Kaduna state, Nigeria.

Key words: Salmonella enterica, drinking water, invA, Kafanchan.

INTRODUCTION

Bacterial pathogens which are transmitted through drinking water usually infect the digestive tract of infected animals and humans, from where they end up in drinking water via faecal contamination (WHO, 2011). It has been estimated that there are at least 2 billion people globally who use drinking water which has been contaminated with faeces (WHO, 2019)

Salmonella enterica is a pathogen which causes illness which could lead to death, with symptoms range from gastroenteritis to systemic infections (Lavigne and Blanc-Potard, 2008). *Salmonella* Typhi has also been implicated in large outbreaks of waterborne typhoid and is a major public health problem worldwide. (Pires *et al.*, 2012 and WHO, 2019). *Salmonella* spp have been widely isolated from raw meats, poultry and poultry products, milk and milk products, water, food and the environment (Hendriksen *et al.*, 2008; Khan *et al.*, 2009; Abakpa *et al.*, 2013; Firouzi *et al.*, 2013; Raufu *et al.*, 2014 and Smith *et al.*, 2015).

Tagoe *et al.* (2011) reported an isolation rate of 10% for *Salmonella enterica* when working with

sachet water in Cape coast metropolis of Ghana.

In another study carried out in Katsina state, Nigeria, children had the highest isolation rate of *Salmonella* Typhi. This was linked to the fact that 80% of them drank water from non-potable sources (Abdullahi *et al.*, 2014).

Several virulence genes which are target genes for PCR amplification of *Salmonella* species including *invA*, *sitC*, *spvA*, *spvB* and *spvC*, have been used to detect as well as screen for genotypic virulence in *Salmonella* isolates from environmental and food samples. This study aimed at isolating *Salmonella enterica* from drinking water sources of Kafanchan town in Kaduna state and determining the presence of some virulence genes (*invA* and *spvA*)

MATERIALS AND METHODS

A total of 87 samples of drinking water from various sources were collected from Kafanchan in Jema'a Local government area, Kaduna state, Nigeria between March 2014 and February 2015. The samples included pipeborne water, well water, boreholes, commercially sold sachet water and stream water.

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One litre (1L) of each sample was collected in sterile containers from which ten (10) milliliters of each water sample was inoculated into 10ml of double strength Selenite F broth and incubated at 37°C for 18 hours. After this period, a loopful of the enrichment culture which showed growth (turbidity and reddish colouration) was streaked on selective plating medium, Salmonella-Shigella (SS) agar, and further incubated at 37°C for 18 hours (Abakpa *et al.*, 2011). Suspected *Salmonella enterica* colonies appeared colorless with or without dark centers. The isolates were purified by re-streaking to obtain pure cultures and then they were Gram stained and stored on nutrient agar slants for further identification.

Those that were confirmed to be Gram negative were subjected to biochemical tests such as urease, oxidase, triple sugar iron test, Indole, Methyl red Voges Proskauer test and citrate tests. The isolates that were confirmed to belong to the genus *Salmonella* were screened with the Microgen Enterobacteriaceae GN A ID kit and the Microgen *Salmonella* latex agglutination kit. The 16S rRNA gene was amplified using the polymerase chain reaction and the DNA

sequences were screened against the NCBI GenBank to find the most similar identity.

Specific primers sets (Table 1) were used to detect 16SrRNA, *invA* and *spvA* genes in selected *Salmonella enterica* isolates in separate PCR reactions. The PCR reaction mixtures of 10 µl contained 5µl of master mix (Promega, USA), 1µl of nuclease free water, 3µl of template DNA and 0.5µl of each primer mix. A tube containing all the above except the DNA template was also included to serve as a negative control.

PCR amplification included the initial denaturation at 94°C for 5m. This was followed by denaturation at 94°C for 20 seconds; annealing was done at 56, 61 and 61°C respectively for each primer for 30s and 36 cycles; and extension at 72°C for 45s. The final extension was at 72°C for 5m and then a hold temperature at 4°C. Five microlitres of the PCR product was electrophoresed in 2% agarose gel (Bioline) containing 5µl of 10 mg/ml ethidium bromide at 100V for 45m. A 1kb plus DNA marker was used as molecular size marker. DNA amplifications were examined under ultraviolet transilluminator and results documented (Del Cerro *et al.*, 2003; Das *et al.*, 2012).

Table 1: List of primers used in this study

Primer	Primer sequence (5-3)	Size(bp)	Tm(°C)	Reference
<i>invA</i> -F	ACA GTG CTC GTT ACG ACC TGA AT	224	61.0	Das <i>et al.</i> (2012)
<i>invA</i> -R	AGA CGA CTG GTA CTG ATC TAT			
<i>spvA</i> -F	GTC AGA CCC GTA AAC AGT	604	61.0	Del Cerro <i>et al.</i> (2003)
<i>spvA</i> -R	GCA CGC AGA GTA CCC GCA			
16SrRNA-F	AGA GTT TGA TCA TGG CTC AG	1500	56.0	IDT(USA)
16SrRNA-R	AAG GAG GTC ATC CAA CCG CCA			

Key: F- Forward primer, R- Reverse primer, Tm- Annealing temperature

RESULTS

Borehole, tap and well were each contaminated with *S. enteritica* with 1% each, while none was recovered from the other sources of water (Table 2).

The three isolates of *S. enteritica* were further identified as *S. Typhimurium* (2 isolates) and *S. Typhi* (1 isolate) and were shown to harbor the *InvA* gene with none of the isolates harboring *SpvA* gene (Table 3). Plate I shows the gel image of the *InvA* amplicons

Table 2: Occurrence of *Salmonella enterica* in various water sources of Kafanchan, Kaduna state

Sample type	Number(n)	N(%)
Borehole	16	1(1.15)
Tap	24	1(1.15)
Wells	38	1(1.15)
Stream	03	0(0)
Packaged water	06	0(0)
Total	87	3(3.45)

Key

n- Number of each sample type, N- Number of water samples contaminated with *Salmonella enterica*

Table 3: Source of *Salmonella enterica* isolates that possessed virulence genes

Isolate no	Identity	Source	InvA gene	SpvA gene	GenBank Accession number
115	<i>Salmonella enterica</i> ser Typhimurium	Borehole	+	-	KT737738
117	<i>Salmonella enterica</i> ser Typhi	Well water	+	-	KT737737
118	<i>Salmonella enterica</i> ser Typhimurium	Tap water	+	-	KT737736

Key

+ = Gene detected, - = Gene not detected, Ser- serovar

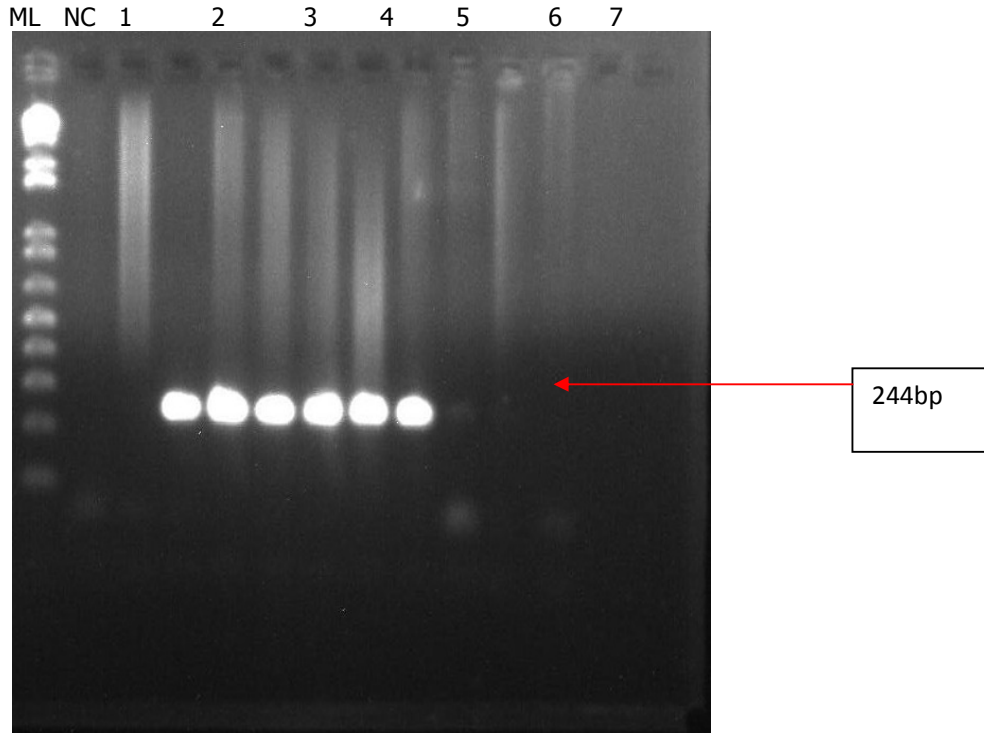


Plate I: Gel Image of invA Amplicons in *Salmonella* spp Isolates

Key: ML- 1 Kb plus molecular ladder, NC-negative control, Lane 1- *Escherichia coli*, Lanes 2-4: *Salmonella* spp isolates from Kafanchan, Lanes 5-7: positive for the invA gene

DISCUSSION

The isolation of these organisms from water sources is of public health concern as it shows that there is a potential risk to consumers especially those who consume the water without any further treatment. A survey of Kaduna state showed that there is improper sewage disposal in most of the parts, indiscriminate defecation and improper siting of wells and boreholes close to latrines and soak-aways. This agrees with the findings of Olukosi *et al.* (2008) where it was observed that about 36% of wells sampled during a study carried out in Zaria metropolis of Kaduna state were situated in environments with very poor hygienic conditions. Data relating to *Salmonella* prevalence in surface and drinking water in developing countries are quite rare. Nevertheless, data on water- borne outbreaks as

well as case control studies investigating the risk factors for endemic typhoid fever confirmed the relevance of water as source for the transmission of this disease (Abakpa *et al.*, 2013).

The invA gene was detected in all three of the *Salmonella* isolates that were tested. On the contrary, none of them showed the presence of the spvA gene. The 100% detection rate of invA gene by PCR suggests that the gene is conserved among *S. enterica* isolates (Murugkar *et al.*, 2003). Furthermore, it is a predominant virulence gene necessary for the serovars of *S. enterica* to express virulence in the host (Das *et al.*, 2012). This gene is essential for full virulence in *Salmonella* spp (Mezal *et al.*, 2013). This gene has also been amplified in other studies (Abakpa *et al.*, 2015; Smith *et al.*, 2015).

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This further confirms that the isolates obtained in this study are potentially pathogenic as amplification of *invA* gene is recognized as an international standard procedure for the detection of *Salmonella* genus (Amini *et al.*, 2010). However, none of the *Salmonella* isolates in this study produced amplicons for the *spvA* gene. *SpvA* is one of the most important virulence genes in the *spv* operon, but it is however not present in all *Salmonella* isolates. The sequences of the isolates from this study have been successfully deposited in the GenBank of the NCBI and they have been

assigned accession numbers. It is recommended that Kaduna state inhabitants that live in the area that were studied should be advised not to consume water only after proper treatment such as boiling at 100°C for at least a minute. Sources of drinking water such as boreholes, wells should be constructed properly and should also be situated far away from livestock and domestic animals, gutters, latrines, soak-aways and any other sources of contamination. The *invA* gene can also be used as a rapid test for screening for the presence of *Salmonella* in food, water and human samples.

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