UJMR, Volume 1 Number 1 December, 2016 *ISSN: 2616 - 0668*

https://doi.org/10.47430/ujmr.1611.017

Date Received: 23rd Nov, 2016 **Date Accepted:** 6th Dec, 2016

A Survey of *Salmonella* Species and their Antibiotic Susceptibility Profile from Selected Wells in Katsina State, Nigeria

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Abstract

The World Health Organisation (WHO) has estimated that 1.1 billion people globally lack basic access to drinking water resources, while 2.4 billion have inadequate sanitation facilities which accounts for many water related acute and chronic diseases. The study was designed to search for the presence of *Salmonella* spp from wells in Dan-musa, Mani and Danja Local Government Areas of Katsina State. The distances of wells to pit laterines were measured at collection points. The organisms were detected and isolated by cultural methods using selective media and were subjected to series of biochemical tests. They were sero-grouped using seroquick grouping reagents. Antibiotic susceptibility testing was performed by disk diffusion method. All the distances measured were below the set standard of 30 meters by WHO and the Nigerian Environmental Protection Agency. Dan-musa Local Government Area had the highest mean coliform counts of 20.7cfu/ml, while Danja had least counts of 13.8cfu/ml. Out of the 300 well water samples collected, a total of 11 *Salmonella* strains were isolated belonging to three(3) different sero-groups (Group D, A and B). Significant statistical association ($p<0.05$) was found between the occurrence of these bacterial isolates and distance of wells to pit laterines. All the isolates were resistant to multiple classes of antibiotics, the highest resistance was to ceftazidime and cefuroxime, 100% each. However, the isolates were also sensitive to flouroquinolones, chloramphenicol and cefoxitin. The presence of these pathogenic bacteria from the well water samples analysed connote a serious health risk to consumers due to threat of disease and antibiotic resistance. The study accenuates the need to set standards for citing of wells away from pit latrines, provision of adequate potable water and improved sanitation.

Keywords: *Salmonella* species, antibiotics susceptibility, wells, Katsina, Nigeria

INTRODUCTION

Waterborne diseases due to faecal pollution of human and animal origin, are responsible for approximately 2.2 million deaths annually in children under the age of five years in developing countries (WHO, 2002a; WHO, 2002b). Most of these deaths are due to inadequate potable water supplies, poor hygiene practices and insufficient sanitation infrastructures (Sobsey, 2002; WHO, 2002a; WHO 2002b). The World Health Organization (WHO) estimated that 1.2billion of the world's population lack access to safe drinking water and these people use any source of water, usually the most convenient source, regardless of its quality (WHO, 2002a).

Safe drinking water for human consumption should be free from pathogens such as bacteria, viruses and protozoan parasites, meet the standard guidelines for taste, odour, appearance and chemical concentrations, and must be available in adequate quantities for domestic purposes (Wilkes, 2009).

Pit latrine is a common method of excreta disposal in the developing world. It is popular and widely used in urban slums as well as rural areas probably because it is the simplest, cheapest and the most efficient excreta disposal method that is within the reach of poor people.

One of the major contributing factors of groundwater pollution is pit latrine mostly located near water sources such as shallow wells and boreholes. In fact, pit latrines have been identified as the major source of contamination of wells with faecal matter (Pritchard *et al.,* 2007).

Salmonella is an enteric pathogen with a worldwide distribution that comprises a large number of serovars characterized by different host specificity and distribution. This microorganism is one of the leading causes of intestinal illness throughout the world as well as the etiological agent of more severe systemic diseases such as typhoid and paratyphoid fever. Zoonotic *Salmonellae* are commonly described as foodborne pathogens, however, drinking water as well as natural waters are known to be an important source for the transmission of these enteric microorganisms (Ashbolt, 2004).

Salmonella, just like other enteric bacteria, is spread by the fecal–oral route of contamination. This microorganism can enter the aquatic environment directly with faeces of infected humans or animals or indirectly, e.g., via sewage discharge or agricultural land run off. Overall, *Salmonella* species and subspecies can be found in a large variety of vertebrates. Beside humans, animal sources of *Salmonella* include pets, farm animals and wild animals; calves, poultry, pigs, sheep as well as wild birds (sea gull, pigeon) and reptiles can all be reservoirs of *Salmonella* (Wray & Wray, 2000). *Salmonellae* are frequently found in environmental samples. In the aquatic environment this pathogen has been repeatedly detected in various types of natural waters such as rivers, lakes, coastal waters, estuarine as well as contaminated ground water (Wilkes *et al.,* 2009).The growth of *Salmonella* in water supplies is also considered possible, due to its ability to colonize surfaces and replicate in biofilms of distribution pipe systems. However, standard disinfection procedures used in drinking water treatment processes are active against *salmonellae* (Cicmanec *et al.,* 2004).

Taxonomically the genus *Salmonella* comprises two species namely *S.bongori* and *S.enterica*. The species *S.enterica* is further differentiated in to six subspecies (*Enterica*, *Salamae, Arizonae, Diarizonae, Indica* and *Houtenae*) among which the *S.enterica* subspecies Enterica is mainly associated to human and other warm blooded vertebrates. On the basis of the clinical syndromes caused, *Salmonella* are divided in to two distinct groups namely the typhoidal and non- typhoidal *Salmonella* serovars (Ashbolt, 2004).

Enteric fevers, typhoid and paratyphoid fever are severe, contagious systemic diseases caused by the infection of the serovarsTyphi and Paratyphi. Even though not common in developed countries, enteric fevers remain an important and persistent health problem in less industrialized nations. Overall, in 2003 an annual incidence of approximately 17 million cases of typhoid and paratyphoid fevers was reported worldwide (Cicmanec *et al.,* 2004).

Aim

The aim of this research work is to search for *Salmonella* species from well water samples and determine their antibiotic susceptibility profile in some selected Local Governments of Katsina State.

Objectives

1. To determine the total coliform counts of the well water samples.
2. To isolate and identify *Salmonella* species biochemically and serologically.
3. To determine the antibiotic susceptibility profile of the *Salmonella* isolates.

Materials and Methods

Study Area

The study was carried out in Katsina State, one of the 36 states of Nigeria. Katsina state is situated at 12.99^{o} North latitude, 7.6^{o} East longitude and 464m elevation above the sea level with two distinct season's wet and dry seasons. Three (3) Local Governments were selected, namely: Mani, Dan-musa and Danja.

Sample Collection

Three hundred (300) well water samples were collected in sterile 60ml plastic containers. The collected sample were labeled and transported to the laboratory of the Department of Microbiology Ahmadu Bello University, Zaria in ice packs and analysed within 6 hours.

Wells were observed for the presence or absence of well casings, covers and distance from the latrine or soak away.

Sample Analysis

All the media used in this study were prepared and sterilized according to manufacturer's instructions. The media used included; Selanite broth, Salmonella-Shigella agar, Nutrient agar and Muellar Hinton agar (Oxoid UK).

One (1ml) of the sample was pre-enriched into 9mls selenite broth and was incubated at 37^0C for 24 hours, it was then sub-cultured onto prepared SSA plates and further incubated at 37^0C for 24hours. Cream coloured colonies and some with black centres were gram stained and stored on nutrient agar slants for further characterization by employing biochemical tests.

Biochemical identification

The biochemical tests employed were; Indole, Triple sugar Iron (TSI), Sugar fermentation, Oxidase, Urease, Motility and Citrate utilization test (Chessbrough, 2006).

Serological identification and Sero-grouping of Salmonella Isolates

Suspected *Salmonella* isolates were serologically confirmed using Oxoid Rapid *Salmonella* Antibody agglutination. Confirmed *Salmonella* isolates were further sero-grouped using *Salmonella* sero-quick grouping kit (Copenhagen, Denmark).

Antibacterial Susceptibility Testing

Each of the isolates were subjected to antibacterial susceptibility testing using the Bauer-Kirby method that has been standardized and evaluated by the methods of Clinical and Laboratory Standards Institute (CLSI 2012). The susceptibility testing was carried out using Mueller Hinton agar. The organisms were tested in vitro for susceptibility to the following commonly used antibacterial drugs:

Ceftaxidime (30µg), Cefotaxime (30µg), Chloramphenicol (30µg), Cefixime (5µg), Ofloxacin (5g), Ciprofloxacin (5µg), Tetracyclin (30µg) and Nitrofurantoin (300ng).

RESULTS

The mean coliform counts of the well water samples obtained from the three (3) study locations showed that Dan-musa Local Government Area had the highest mean coliform counts of 20.7cfu/ml, followed by Mani with 19.7cfu/ml and Danja Local Government Area had the least counts of 13.8cfu/ml respectively (Table 1).

Table 1: Mean coliform count of the well water samples obtained from the (3) study locations in Katsina State.

Location	No. of Samples	Mean coliform count (cfu/ml)	SEM
Danmusa	100	20.7	0.0012
Mani	100	19.7	0.0134
Danja	100	13.8	0.0428
Total	300	54.2	0.0574

Key: CFU=Colony forming unit, SEM= Standard error mean.

Table 2: Percentage Distribution of Presumptive *Salmonella* isolates in the well water obtained from the Study Areas in Katsina State

Location	Number	No. (%) *Salmonella* spp.
Danja	100	9 (9.0)
Dan-musa	100	12 (12.0)
Mani	100	22 (22.0)
Total	300	43 (14.3)

Table 3: Serological identification of *Salmonella* isolates

	No. (%)	Observation	Inference
	11 (25.6)	Agglutination	Positive
	32 (74.2)	No Agglutination	Negative
Total	43 (100)		

Key: No. (%) Number and percentage of isolates

Table 4 shows results for sero-grouping of *Salmonella* isolates, 5(45.4%) were found to belong to sero-group D.(*S.*Typhi), 2(18.2%) belongs to sero-group A and B(ParatyphiA and Typhimurium) respectively, and lastly 2(18.2%) isolates were non-typable.

Table 4: Sero-Grouping of *Salmonella* Isolates

Salmonella sero-groups	No. positive (%)
Group A (S. paratyphi A)	2 (18.2)
Group B (S. Typhimurium)	2 (18.2)
Group D (S. typhi)	5 (45.4)
Non-typable	2 (18.2)

Table 5 shows results of the measured distances of wells positive for *Salmonella* spp. from pit latrines, the distances were between 3-16m, which were all below the limit set by WHO (2006) and that of Nigerian Environmental Protection Agency of 30m each and 15.24m of the United State Environmental Protection Agency(USEPA). Most of the wells were uncovered while some were partially covered with woods or old rusted roofing sheets.

Table 5: Distance between pit laterines and wells positive for S*almonella* Species In Dan-musa, Danja and Mani Local Government Areas of Katsina State

Location	Well Identity	Distance (m)
Dan-musa	Dm3	3
	Dm53	12
	Dm75	6
	Dm82	4
Mani	Mn6	7
	Mn22	6
	Mn67	8
	Mn69	6
Danja	Dj11	4
	Dj66	7
	Dj67	13

Keywords: Mn=Mani, Dj = Danja, Dm = Dan-musa

Table 6 shows the association between occurrence of the *Salmonella* serovars isolated and distance of wells from pit laterines. Significant association was found ($p<0.05$), the strength of the association was further determined (odds ratio). The odds ratio for the three(3) sero-groups were determined, but that of *S.* Typhi was the strongest (2.908), this shows that *S.* Typhi is approximately three (3) times more likely to be found in wells with close proximity to pit laterines than the other two (2)sero-groups.

Table 6: Association between the *Salmonella* serovars isolated and distance of wells rom pit laterines in Dan-musa, Danja and Mani Local Government Areas of Katsina State

Distance (m)	*Salmonella* Serovars				Total
	S. Typhi	*S.* Paratyphi A	*S.* Typhimurium	Non typable	
1 - 5	1	1	0	0	2
6 – 10	4	1	1	0	6
11 – 15	0	0	0	2	2
>15	0	0	1	0	1
Total	**5 (45.4%)**	**2 (18.2%)**	**2 (18.2%)**	**2 (18.2%)**	**11 (100)**

(χ^2=27.343, df= 12, p= 0.007; OR= 2.908, 95% CI= 1.130-7.481)

Figure 1 shows the percentage antibiotic resistance. The 11 *Salmonella* isolates were tested against eight (8) different antibacterial drugs. *S.* Typhi is the serotype with the highest percentage resistance of 62.5% among the strains isolated; the isolates were resistant to five (5) different antimicrobial drugs, while *S.*Paratyphi A and *S.* Typhimurium had the least percentage antimicrobial resistance of 37.5%.

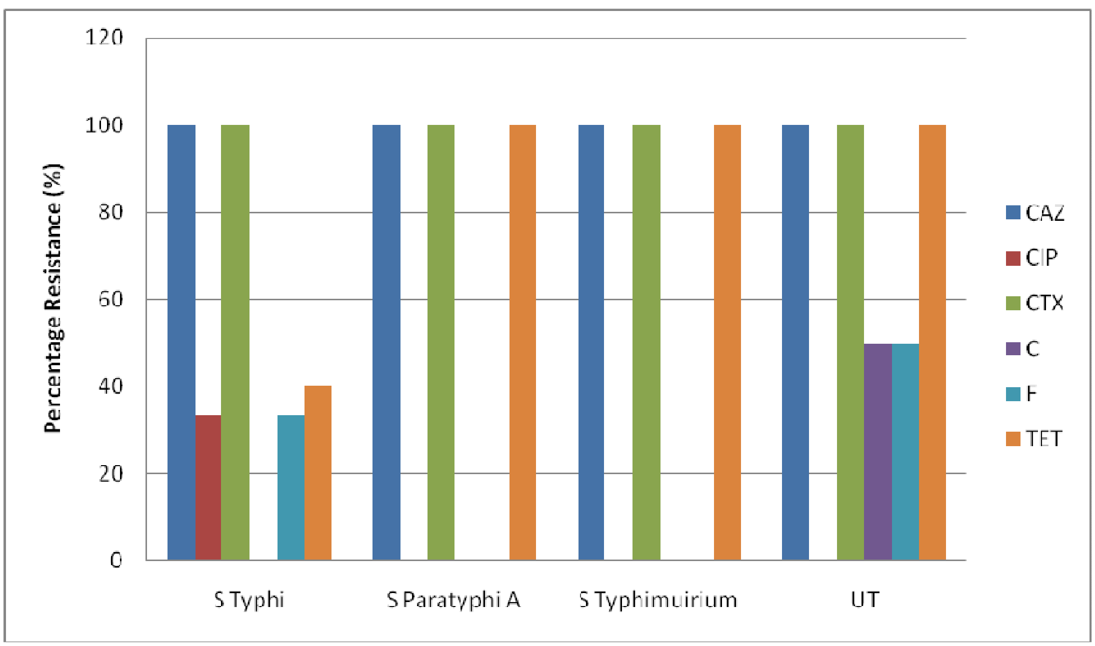

Figure 1: Percentage Antibiotic Resistance of *Salmonella* isolates
Key: CAZ= Ceftazidime, CIP= Ciprofloxacin, CTX= Cefotaxime, C= Chlorampenicol, F= Nitrofurantoin, TET= Tetracycline.

Table 7 shows the multi-drug resistant pattern of *Salmonella* seovars with the resistant phenotypes. Multiple antibiotic resistance (MAR) were also determined for the *Salmonella*serovars (Fig 1). Five resistant phenotypes were obtained with varying combinations of 3,4 and 5. The predominant MAR phenotypes were CAZ,CTX,TET having 38.0% and CAZ,CIP,TET,CTX having 50.0% and CAZ,F,CIP,TET,CTX 67%.

Table 7: Multidrug Resistance Pattern of *Salmonella* serovars showing the resistant isolates

Organisms	No. of resistant Isolate	Resistant pattern
S. Typhi	1	CAZ, TE, CTX
	2	CAZ,CIP,TE,CTX
	1	CAZ,TE,CTX
	1	CAZ,F,CIP,TE,CTX
S. Paratyphi A	2	CAZ, TE,CTX
S. Typhimurium	2	CAZ, TE,CTX

KEY: CIP=Ciprofloxacin, TE=Tetracycline, CTX=Cefotaxime, CAZ=Ceftazidime,F=Nitrofurantoin.

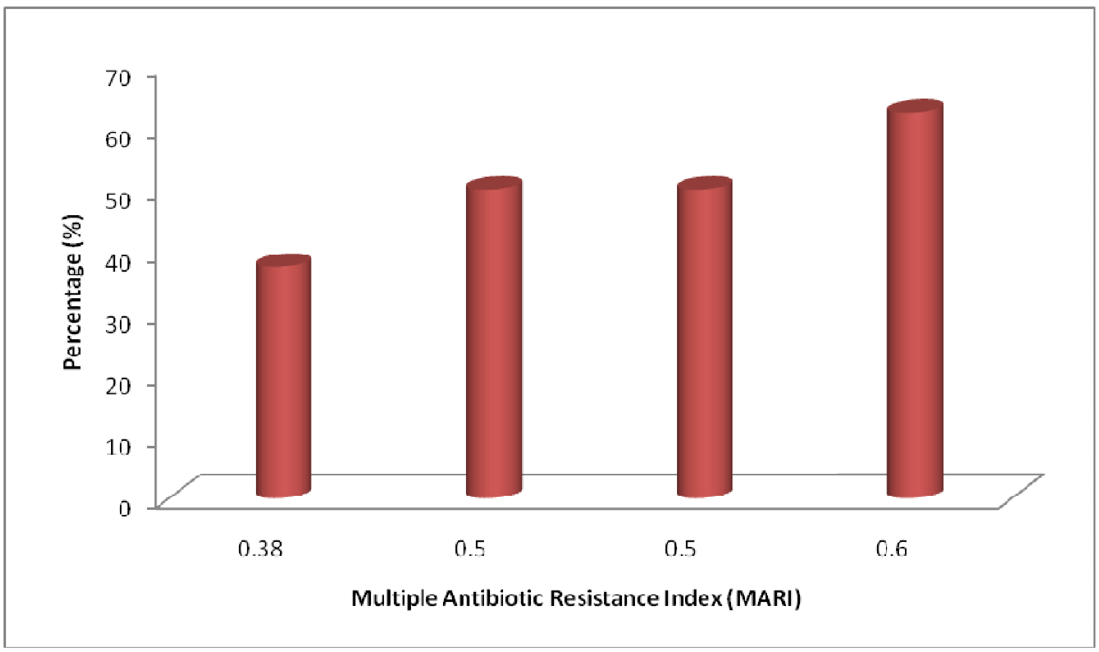

Figure 2 Multiple Antibiotic Resistance Indices and the Percentage of *Salmonella* serovars isolated from well water sample in Dan-musa, Mani and Danja Local Government Areas of Katsina State.

DISCUSSION

Groundwater, particularly private and public hand-dug wells supply drinking water for more than half of the Nigerian population. High coliform counts of 20.7 cfu/ml obtained in this study indicated that the population of people obtaining drinking water from these wells are at risk of water borne diseases. The result showed that none of the water samples met the WHO (2008) guidelines for drinking water quality, which states that the coliform count in drinking water both piped and unpiped should be 0 cfu/ml and 10cfu/ml (NAFDAC, 2007).

It was generally observed that wells located close to pit latrines are the wells positive for the presence of these pathogenic bacteria.

This finding corroborates that of Sudgen (2006) who stated that the greater the distance between the latrine and the water point, the lower the risk of contamination. Also, if the time taken for a pathogen to be transferred to the water point is large, the pathogen would have died off and that the water would no longer be a threat to public health.

The high prevalence of antibiotic resistant bacteria in Nigeria and other developing countries has been associated with several factors including indiscriminate use due to unregulated access of non-professionals to different classes of antimicrobial over-the-counter (Okeke *et al.,* 1999). Tetracycline which has the highest resistance in this study is one of the most commonly available for use as growth promoter and routine chemoprophylaxis for livestock in Nigeria. They are readily available in different dosage forms and in combination with other antibiotics and vitamins (Jonathan and Afe, 2012).

All the serotypes isolated were found to be resistant to more than one (1) class of the tested antibiotics. The most common resistance was to ceftazidime, cefotaxime and tetracycline (100%) each, while resistance to ciprofloxacin was only observed in *S.typhi* (33.3%). However, resistance to fluoroquinolones (ofloxacin was not encountered while reduced susceptibility of *S.typhi* isolates to ciprofloxacin was observed (66.7%). However, all the serotypes were 100% sensitive to third generation cephalosporins (cefixime).

All the serotypes were susceptible to fluoroquinolones except *S.* Typhi which shows reduced susceptibility of 66.7% to ciprofloxacin. The high susceptibility of the serovars to fluoroquinolones and cefoxitin as recorded in this study may be due to the high cost of the antibiotics and they are injectables (Akinyemi *et al.,* 2007). This is similar to report byAbdullahi *et al.* (2013).

Likewise, susceptibility to third generation cephalosporins as found in this research work is in agreement with the findings of Farhan *et al*., (2012) in Karachi, Pakistan.

The multiple antibiotic resistance index (MARI) was calculated and the isolates all showed MARI of >0.38. An observation of MARI $\geq$0.38 depicts the source as high risk source, and that, the antibiotics are in constant abuse, also, the act is bringing about high selective pressure (Suresh *et al.*, 2000).

CONCLUSION

The present results is a warning signal to all stakeholders in community health in the study location to direct action at protecting drinking water from faecal contamination and limiting persistence of antimicrobials in groundwater.

RECOMMENDATIONS

(i) Prompt well water quality assessment should be undertaken by Katsina State rural water and sanitation agency (KT-RUWASSA)

(ii) The public health workers should ensure that the distance of pit latrine to shallow wells in the study areas meet the recommended distance of 30 m by WHO(2007).

(iii) Katsina State Government should ensure adequate and efficient public water supply through the provision of pipe borne water.

(iv) Public enlightenment campaign on the effect of contaminated water and the danger of citing shallow wells close to pit latrines should be embarked upon by the government and the media (print and electronic), schools, religious bodies e.t.c

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