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Research Article

Prevalence and Tetracycline Resistance Profile of *Salmonella typhi* from Local Milk Consumed in Yola, Adamawa State, Nigeria

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ABSTRACT

Biological contamination of food by pathogens such as *Salmonella typhi* is mostly caused by poor personal and public hygiene which threatens public health with foodborne diseases. This study sought to determine the prevalence of *S. typhi* in local milk consumed in Yola, Adamawa State, Nigeria and their tetracycline resistance profile. A total of one hundred and twenty-two milk samples were collected around the city from local milk vendors and packaged in sterile containers for analysis. *Salmonella typhi* was isolated from the milk samples on *Salmonella Shigella* agar and characterized using Polymerase Chain Reaction (PCR). *Salmonella* isolates were then tested for sensitivity against tetracycline using agar well diffusion method. Out of the 122 samples, only 3 (2.46%) were contaminated with *S. typhi*. Two out of the three 2 (67%) samples exhibited phenotypic resistance to tetracycline and only one (133%) was sensitive, implying low prevalence of *S. typhi* contamination. However, *S. typhi* is a pathogenic bacterium and its presence in food intended for human consumption is not expected. Also, the resistance exhibited by the bacteria to the most commonly used antibiotic is of public health concern.

Keywords: *Salmonella typhi, Biological contaminant, Tetracycline, Antimicrobial resistance*

INTRODUCTION

Food safety remains a global concern with Sub-Saharan Africa bearing the largest burden of food safety risks (Geresu *et al.,* 2021). World Health Organization (WHO) 2015 report on the global burden of foodborne diseases estimated that more than 600 million cases of foodborne illnesses and 420,000 deaths occur annually, with 98% of food safety burden coming from developing nations. Ghana for example records about 626 000 food poisoning prevalence, 298 100 cases of hospitalization and 90, 000 deaths yearly due to issues associated with food safety (Cudjoe *et al.,* 2022). The Nigerian health sector reported that over 50% of deaths among children occur as a result of food safety issues (Akeredolu, 2014). In Nigeria, food borne diseases remain more deadly than vector borne diseases with the government spending about US$3.6 billion yearly on food borne diseases (Deb, 2018). Cudjoe *et al.* (2022) maintained that though food regulatory bodies exist in these countries, the application of food safety standards in formal food industries remains problematic with little or no attention given to local food markets which serve as the

largest food sources in the Sub-Saharan African region. Geresu *et al*. (2021) mentioned the major sources of food safety concern in the local food markets to include improper handling of food, vending sites hygiene practices of food vendors, bad transportation and inadequate education especially at the consumption level. These have led to microbial, chemical, fecal and even debris contaminations (Cudjoe *et al.,* 2022; Stella *et al.,* 2020) which are all threats to food safety and security. Microbial contamination is one of the most common causes of food borne illness in the food chain and mostly associated with poor food hygiene practices that lead to feco-oral human-to-human transmission of pathogens (Ifiora *et al*., 2020; Geresu *et al.,* 2021).

Salmonella spp. is one of the common microbial food contaminants and the leading cause of food borne illness accounting for thousands of death globally (WHO, 2015; Adzitey *et al*., 2020; Geresu *et al.,* 2021). This is mostly seen in the animal food chain as it is commonly found as contaminants in poultry, egg and dairy products alongside *Escherichia coli* and *Campylobacter* spp (Eng *et al*., 2015; Gargano *et al*., 2021). *Salmonella* is a rod shaped Gram negative facultative anaerobe that belongs to the family Enterobacteriaceae with around 2600 serotypes identified in the *Salmonella* genus. The genus *Salmonella* is classified into two species, *Salmonella enterica* and *Salmonella bongori* (Gargano *et al*., 2021). Among all the subspecies of *Salmonella*, *S. enterica* is found predominantly in mammals and contributes approximately 99% of *Salmonella* infections in humans and warm-blooded animals (Eng *et al.,* 2015). *Salmonella enterica* serovar *typhi* which is commonly known as *Salmonella Typhi* (*S. Typhi*) is one of the commonest pathogenic food contaminants that are transmitted through food or water contaminated with feces from infected persons, persistent excretors or from chronic asymptomatic carriers who handle food as humans are the only reservoir of the pathogen (Ohanu *et al.,* 2019; Smith *et al.,* 2008). It is the aetiological agent of typhoid fever and affects all age groups and classes (Cudjoe *et al.,* 2022; Smith *et al*., 2008). Typhoid fever remains a global public health challenge with higher burden in low and middle income countries due to poverty and lack of access to safe food and water (Ohanu *et al*., 2019; Smith *et al*., 2008).

Local milk is one of the major marketable food in Nigeria most especially in the northern part of the country where it serves as important source of nutritious and healthy natural food consumed as fresh, skimmed or fermented milk (Maikai and Madaki 2018; Alhaji *et al.*, 2019). The relative high rate of local milk consumption in the northern part of the country can be attributed to the abundance of cattle in that part of the country (Maikai and Madaki 2018). For cultural and individual inclinations, some people consume either fresh or fermented cow milk, while others consume both forms. The milk value chain is largely handled by the informal sector dominated by local herders residing in satellite villages. They transport it to towns and cities where they retail it at outlets, streets or local markets (Olatoye *et al*., 2016).

Milk in the informal dairy sector in Nigeria is handled under poor hygiene condition which exposes it to risk of microbial contamination, thus posing threat to public health and food safety (Uzoaga *et al.*, 2020). Microbial contamination of milk can occur within the udder, outside the udder, from the surface of equipment used for milk handling and storage, environment and the milk handler (Karshima *et al*., 2013; Soepranianondo *et al.,* 2019). Poor hygiene post pasteurization has also been reported to contribute to recontamination of milk and milk products by pathogenic organisms, thus pasteurization cannot always guarantee the safety of milk retailed in most of our local food market settings (Smith *et al.,* 2008; Adzitey *et al*., 2020). Dysentery and diarrhea are the most common food borne diseases experienced in most of Nigeria's local societies (Cudjoe *et al.,* 2022). In most of the cases, victims resort to self-prescription of antibiotics such as tetracycline, metronidazole etc (Jamiu *et al*., 2016; Cudjoe *et al.,* 2022) unless the situation gets out of control. This has resulted to the irresponsible use of tetracycline in most Nigerian communities seen today. Irresponsible antibiotic use has been reported in several literatures as drivers of antibiotic resistance which has attracted attention of relevant authorities globally (Gargano *et al*., 2021). Nevertheless, measures to curtail antibiotic misuse in most low-middle income countries such as Nigeria are still poorly implemented (Jamiu *et al*., 2016). These can result in the development of antibiotic resistance by microbes through evolutionary pressure which can easily be spread to other microorganisms both horizontally and vertically (McEwen, 2006; Lhermie *et al*., 2016; Gargano *et al*., 2021). Infection by such organisms leads to ineffective treatments and economic lost.

Microbial resistance to antibiotics is now a global issue which requires multi-sectorial action. Major drivers of this issue are misuse and abuse of antibiotics. The propagation and spread of antimicrobial resistance is however promoted by lack of clean water, sanitation, inadequate infection prevention and control (WHO, 2021). Thus, poor hygiene practice has the potential of re-contaminating processed or ready to eat foods with harmful pathogens such as *S. typhi* during retailing. This exposes local milk consumers and by extension the entire populaces to risk of typhoid fever through feco-oral human-to-human transmission of *S. typhi*.

Due to paucity of information on the incidence of *S. typhi* contamination of local milks consumed in the study area as well as their antibiotic resistance pattern, this research sought to determine the incidence of *S. typhi* contamination as well as its tetracycline resistance status in local milk consumed in the study area.

MATERIALS AND METHODS

Study location

The study was conducted in Yola, the Capital of Adamawa State. Yola, within the northeastern part of Nigeria, is located on the Benue River between latitude 9°12'30.20"N and longitude 12°28'53.26"E.

Sampling method and sample collection

Local milk retailed in the city was targeted for sampling. All local milks retailed in the state capital had equal chances of being sampled. One hundred and twenty-two (122) local milk samples were collected through simple random sampling in separate, sterile and well-labelled plastic containers. About 10 ml of milk samples were collected from each vendor in sterile sample bottles. The samples were placed in ice-cold box and transported to Chevron Biotechnology Center, Modibbo Adama University Yola, where the analysis was carried out. The samples were stored at 4℃ until the commencement of the analysis.

Chemicals and reagents

Salmonella Shigella agar (SS agar) (TITAN BIOTECH LTD, India), nutrient broth (lifesave-Biotech, USA), DNA extraction kit (Quick DNA Fungal/Bacterial Miniprep Kit, Zymo Research Inqaba Biotech West Africa ltd), master mix CSL-JADNA Cleaver Scientific Ltd. UK, primer (both forward and reverse) were obtained from Inqaba Biotechchemical industries Ltd, ethanol (analytical grade JHD Gunsgdong Guandgua chemical factory Co.Ltd. Shatou, Guondghuo China), multi-purpose agarose (CS Cleaver Scientific Ltd. UK). All other chemicals and reagents used were of analytical grade.

Selective plating

Isolation of *Salmonella typhi* was carried out on *Salmonella Shigella* agar (SS agar) as described by Karshima *et al.* (2013). The agar was prepared according to manufacturer's guide. A sterile swab from milk samples were streaked onto *Salmonella Shigella* agar (SS agar) and incubated for 24 hours at 37 ^{O}C. Colorless colonies with black center or completely black colony (denoting non lactose fermenters and hydrogen sulfide production) were considered presumptive *S. typhi* colony. *Salmonella typhi* presumptive colonies were then sub-cultured on SS agar and incubated for 16 hours at 37°C. Presumptive *Salmonella typhi* colonies were then sub-cultured in nutrient broth and incubated at 37°C for 24 hours. The isolates were then kept in refrigerator pending molecular characterization.

Molecular characterization of *Salmonella typhi*

DNA of the isolated *Salmonella typhi* was extracted using DNA extraction kit (Zymo Research Inqaba Biotech West Africa ltd) as described in the manufacturers guide. The extracted DNA was subjected to polymerase chain reaction in other to amplify a specific fimbrial protein gene fragment (GJS36_23500, 453-bp) typical for *Salmonella typhi* using the primer (F- 5'AGCGCTCTGGTTCTGTCTTC3' and R-5'CGTACCCGCTGTGGTATCAA3'). The reaction mixture contained 12.5µl 2X PCR master-mix, 5.5µl nuclease free water, 1µl of each of the forward and reverse primers and 5µl of the DNA template which made up a total PCR reaction volume of 25µl. The reaction condition of the thermocyler (Select Cycler II, SELECT BIOPRODUCTS) was set at 94°C initial denaturation temperature for 30sec., followed by 30 cycles of denaturing temperature of 94°C for 30sec., annealing temperature of 60°C for 30sec., and extension temperature at 68°C for 1min., followed by a final extension at 68°C for 5min. The reaction product was then confirmed using gel electrophoresis (IBI MP-1015 multipurpose Shelta scientific). Universal Kapa DNA ladder (Thermo Scientific, (EU) Lithuania) was included on the gel as a molecular size standard and the separated products were photographed under UV transilluminator.

Tetracycline susceptibility test

Isolated *Salmonella typhi* were subjected to susceptibility testing using the Kirby-Bauer method with little modifications. Briefly, petri dishes (90mm X 15mm) containing solidified nutrient agar was seeded with 0.5 McFarland concentration of the PCR identified *Salmonella typhi* under aseptic technique. Using micropipette tip, holes of 3mm was bore on the plates and 100µl of 30µg tetracycline was added into the wells. The plates were then incubated at 37^{o} C for 18 hours and observed for zone of inhibition. The susceptibility status was classified based on zone of inhibition diameter with ≤ 11mm, 12-14mm, and ≥15mm as resistant, intermediate and sensitive respectively as described by national committee for clinical laboratory standard (CLSI, 2020; Geresu *et al.,* 2021).

Statistical analysis

Data were captured and sorted into Microsoft Excel® and analyzed for Simple descriptive statistics such as frequency and percentage using SPSS version 20.

RESULTS

Out of the one hundred and twenty-two local milk samples screened for presence of *S. typhi*, only 3/122 (2.46%) milk

samples had presumptive *S. typhi* base on colony characteristics (Table 1).

PCR test carried out to confirm the presumptive *Salmonella typhi* isolates base on the presence of specific fimbrial protein gene fragment (GJS36_23500, 453-bp) revealed that all the three isolates 3/3(100%) harbor the specific gene characteristic of *S. typhi* (Figure 1.).

Out of the three molecularly confirmed *S. typhi* isolates, two 2/3 (67%) exhibited phenotypic resistance to tetracycline with 0.00mm zone of inhibition (Figure 2.).

Table 1. Prevalence of *Salmonella typhi* in Local Milk Samples

Total number of samples examined	Number of presumptive *S. typhi* positive observed (%)	Number of *S. typhi* negative observed (%)
122	3 (2.46%)	119 (97.54%)

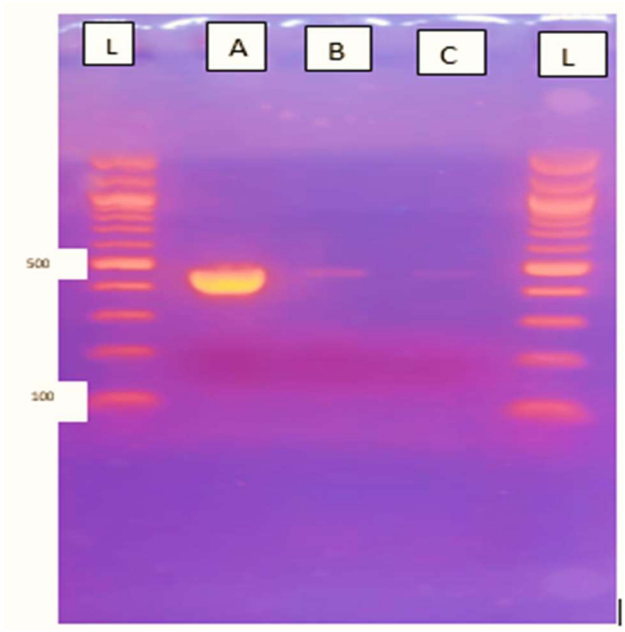

Figure 1. PCR Product Electrophoresis of Fimbrial Protein Gene Fragment (GJS36_23500, 453-bp).

Lane L is 100bp marker; lanes A-C are positive samples with bands equivalent to 453bp

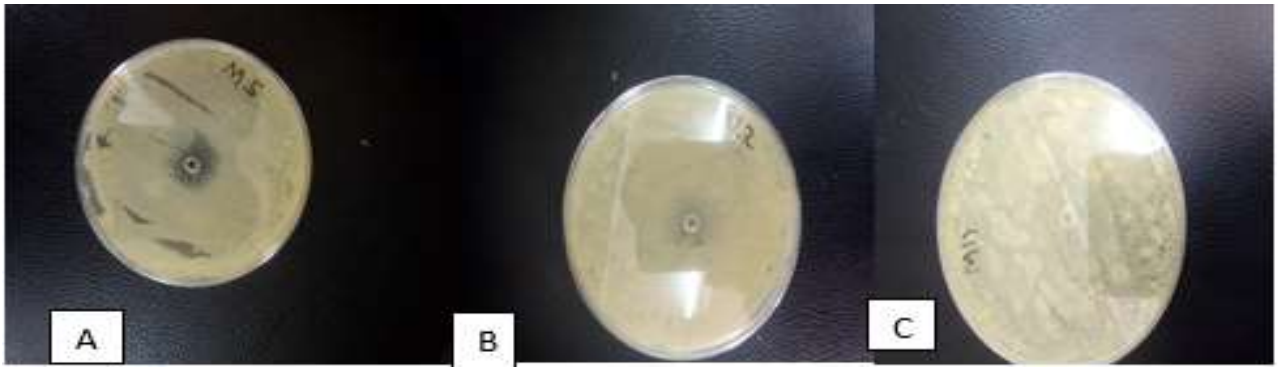

Figure 2. Phenotypic Tetracycline Resistance Result of the *S. typhi* Isolates.

A; tetracycline sensitive, B and C tetracycline resistant

DISCUSSION

Salmonella typhi is a pathogenic organism that is considered as a contaminant in food intended for human consumption, and food samples are normally screened for this pathogen by regulatory agencies such as National Agency for Food and Drug Administration and Control (NAFDAC) in Nigeria.

Screening of the milk samples based on morphological characteristics revealed three isolates (3/122) as presumptive *Salmonella typhi.* Also, a more reliable confirmatory assay carried out using PCR method revealed that all the three presumptive *S. typhi* colonies were *S. typhi* based on the

presence of unique fimbrial protein gene fragment (GJS36_23500, 453-bp) (Figure 1.). Molecular methods of characterization (PCR) is a more specific method of identifying and characterizing microorganisms compared with microbiological methods such as biochemical tests, micro and macroscopic examination etc The reason for the specificity of the method hinges to its ability to identify specific genes unique to organisms of interest (Bastam *et al.,* 2021). Generally, detection of such pathogenic microorganisms in food is associated with contamination from processing water, food processing by infected people or carriers, and poor personal and public hygiene (Olufunke *et al.,* 2014). The 2.46% *S. typhi* prevalence obtained in present study is far lower than 25% reported by Maduka *et al.* (2013) in similar study carried out in Maiduguri, Borno State of Nigeria, 20% reported by Olufunke *et al.* (2014) in similar study carried out in Osun State of Nigeria. Findings of similar studies carried out in other countries including a study carried out in Iran by Bastam *et al*. (2021) reported 51.4%, 57.1% and 50% *S. typhi* prevalence in raw milk, pasteurized milk and cheese respectively using real time qPCR which are far higher than the findings in the present study. However, similar studies have been carried out on general *Salmonella* spp in various foods in different parts of the country. Such works include those of Liamngee *et al*. (2013) who reported the detection of *S. typhi* in Soyabean milk sold in Makurdi metropolis, Oloso *et al.* (2019) who reported 23% *Salmonella* spp. prevalence in broiler value chain in Nigeria, Karshima *et al.* (2013) and Esonu *et al.* (2021) who reported *Salmonella* spp prevalence in local milk as 6.4% and 6.7% respectively in Kanam, Plateau State and Zaria Kaduna State all in Nigeria.

The low prevalence of *S. typhi* reported in this study can be attributed to relatively good hygiene practice that reduced the tendencies of fecal contamination of the milk or absence of typhoid outbreak as at the time of the study. Nevertheless, possible microbial contamination by pathogens of other serovar species and genera cannot be ruled out by the findings of this present study as this study specifically focused on *Salmonella enterica* serovar *typhi* commonly known as *Salmonella Typhi* (*S. Typhi*). However, even a low prevalence as reported in this study poses public health issues as *Salmonella* is not expected to be found in ready-to-eat food and food substances intended for human consumption (Esonu *et al*., 2021). Consumption of such milk can lead to poisoning or infection which can be subsequently spread in the community through human to human feco-oral route leading to Typhoid outbreak. Also, the tendencies of cross contamination at retailing outlets cannot be ruled out as vendors normally exchange materials at their local milk retail outlets. Although incidence of microbial food poisoning is poorly documented in Nigeria, a few have been documented in a review work published by Cudjoe *et al.* (2022). The review mentioned an incidence of food poisoning in Ibadan, Oyo State as a result of consumption of incorrectly preserved sandwiches said to have been contaminated with *Salmonella*. It also documented that food poisoning occurred in Ambrose Alli University and other surrounding environments as a result of *staphylococcal aureus* poisoning. Thus, microbial contamination of food is of great concern in public health which requires maximum attention of responsible regulatory authorities.

Activities carried out post pasteurization are critical to the biological safety of the local milk. When the prevalence of *S.typhi* (2.46%) reported in the present study is compared with the prevalence of general *Salmonella* spp (0.00%) reported in the work of Mhone *et al*. (2012) who collected the milk sample directly from farm, it can be observed that the prevalence of the general *Salmonella* spp is still lower than that of serovar specific *S. typhi* isolated from same local milk sample collected at retailing points. From this analogy, it is empirical to give credence to previous findings that unhygienic food handling; processing, transport and storage are key factors that predispose foods to contamination by pathogenic organisms.

Detection of *Salmonella* typhi in ready-to-eat foods such as milk is unacceptable and is of public health concern. Although the detection in the present study is very low, adequate measures by responsible authorities should be put in place to eliminate it completely as recommended by the food regulatory bodies such as NAFDAC, FDA, etc. The presence of Salmonella spp. in ready to eat foods such as milk is an important source of public health hazard as Salmonella can cause diseases that are usually classified into typhoidal and non-typhoidal depending on the serovar that causes the infection (Olufunke *et al*., 2014). *Salmonella* enterica serovar typhi is the aetiologic agent of typhoid fever. Typhoid fever is an acute and sometimes life-threatening systemic febrile illness and causes an estimated 16.6 million cases and 600,000 deaths worldwide each year (Ohanu *et al.,* 2019). This disease remains one of the commonest infectious diseases in developing nations as a result of poor hygiene practices that facilitate the transmission of the causative agent.

The presence of *S. typhi* in food samples as reported in this study 67%(2/3) confirms earlier reports of Olufenke *et al.* (2014) on the emergence of antimicrobial resistant *S. typhi* in Africa and the report in the review of Pui *et at*., (2011) mentioned that the resistance of *Salmonella* strains to one or more antibiotics is on the increase across the globe.

Salmonella is one of the leading bacteria causing food borne diseases worldwide, and its resistance to tetracycline has been widely reported (Pavelquesi *et al*., 2021). In a study carried out by Adzitey *et al*. (2020) in Ghana, 86% of *Salmonella* spp isolated form local milk samples were tetracycline resistant. Olufunke *et al.* (2014) reported 90.4% and Enosu *et al.* (2021) reported 33% in similar studies in Nigeria. Tetracycline is one of the most commonly used antibiotics in both public and veterinary heath sector in Nigeria (Uzoaga *et al*., 2020). Thus, the detection of tetracycline resistant *S. typhi* is not unexpected as drivers of antimicrobial resistance (misuse and abuse of antibiotics) are common in the society where agents of propagation and spread of antimicrobial resistant organisms such as lack of clean water, sanitation, inadequate infection prevention and control exist. Studies on tetracycline resistance of other bacteria isolated from milk have also been documented. Olufemi and Olatoye (2010), recorded a high level of tetracycline resistance of 91.4% among isolates of *E. coli* O157:H7. In Malaysia, Alhaj *et al*. (2007) also observed high resistance of *E. coli* to tetracycline (81.4 %) while Shitandi (2001) obtained high resistance to penicillin (72%) and to tetracycline (57.9%) by the same organism. That high prevalence recorded in those studies is reflection of the levels of tetracycline misuse and abuse in both veterinary and public health sector. The high tendencies of transferring antimicrobial resistant *Salmonella* strains to humans through the food chain poses a public health threat which can result into higher morbidity and mortality, as well as increased cost of treatment. It can also result to cross resistance to other antibiotics with similar mechanism of action (Geresu *et al*., 2021). For these reasons, it is imperative for the relevant health care authorities to look into possible means of curtailing such tendencies to avoid degeneration of the situation.

According to WHO (2021), the continuous detection of antimicrobial resistant organisms in food, environment, hospital etc is of great concern. This can lead to spread of resistant genes across other species or non-pathogenic organisms that can result to emergence of new pathogens. It also raises cost of treatment which increases the socio-economic hardship of people. Antimicrobial resistance also places the entire globe on the risk of running out of effective antimicrobials. Thus, combatting the development and spread of antimicrobial resistance requires a multi-sectorial and multinational approach. Therefore, all stakeholders need to revisit the previous effort and strategy employed in the fight against antimicrobial resistance.

CONCLUSION

This study found low prevalence of *Salmonella typhi* contamination in the local milk consumed in the study area. However, *S. typhi* is a pathogenic bacterium that is not expected to be present in food intended for human consumption. Also, the study discovered that the *S. typhi* isolates were resistant to tetracycline which is of great public health concern particularly for it being the most commonly patronized antibiotic for diarrhea or dysentery by the populace. Relevant authorities are advised to give the necessary attention to food safety particularly in the local ready-to-eat food markets in our communities to avoid preventable food borne disease outbreak and spread of antibiotic resistance among pathogens.

AUTHORS' CONTRIBUTIONS

The study was designed by MIB and AL, JNJ supervised the laboratory work, IAH, HAZ and MBA handled the practical work. All authors contributed to development of the manuscript and approved it.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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