# RESEARCH NOTE

# MOLECULAR CHARACTERIZATION OF *SALMONELLA* SPP DIRECTLY FROM SNACK AND FOOD COMMONLY SOLD IN LAGOS, NIGERIA

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**Abstract.** Food borne Salmonella infection is an important cause of morbidity and mortality. A total of 200 food samples commonly sold in Lagos, Nigeria comprising raw and cooked meat as well as meat products and spoilt meat were analysed for the presence of *Salmonella* spp using REVEAL serology kit, culture methods employing RPVA (Rappaport Vassiliadis agar), SSA (Salmonella-Shigella agar) and BSA (brilliant sulphite agar) and PCR method for direct detection from samples using primer salm3/4 and ST11/ST15 sets. Using the REVEAL serology kit, 74% of the samples were positive for *Salmonella* spp, while culture methods showed only 19% to be *Salmonella* spp. The PCR method revealed that *Salmonella* spp was present in 62% and 54% of the samples using primer set salm3/4 and ST11/ST15, respectively. However, the primer set ST11/ST15 was more reliable in the identification of *Salmonella* spp directly from food samples. These tools should prove useful in the continuous monitoring and control strategies especially for ready-to-eat foods, as well as in retail meat outlets, slaughter houses, fast food restaurants for the prevention and reduction of this pathogen that is of significant importance in the food industry.

**Keywords:** *Salmonella*, food samples, PCR, Nigeria

## INTRODUCTION

Food-borne diseases are an important cause of morbidity and mortality worldwide. The worldwide incidence of nontyphoidal salmonellosis is estimated at 1.3 billion cases and 2 million deaths annually (Payment and Riley, 2002). Salmonella can be isolated from raw meat, poultry and poultry products, and milk and milk products (Goman *et al*, 2002). Salmonella outbreaks have been associated with improper cooking methods, reheating of food, and improper handling of food by food preparers (Goman *et al*, 2002). Representing 30.4% of all *Salmonella* strains isolated from humans, *Salmonella enterica* serotype Typhimurium was the

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second most commonly isolated *Salmonella* serotype in the Republic of Ireland in 2001, exceeded only by *S. enterica* serotype Enteritidis (NDSC, 2001)). Although *Salmonella* gastroenteritis is generally a self-limiting illness, severe cases may require antimicrobial therapy. Food contaminated with antibiotic-resistant bacteria can be a major threat to public health, as the antibiotic resistance determinants can be transferred to other pathogenic bacteria, potentially compromising the treatment of severe bacterial infections. The prevalence of antimicrobial resistance among food-borne pathogens has increased during recent decades (Threlfall *et al*, 2000; Chiu *et al*, 2002). This increase is attributed to the selection pressure created the use of antimicrobials in food-producing animals, in addition to the unregulated use of antibiotics in humans in developing countries (Angulo *et al*, 2000; Van den Bogaard and Stobberingh, 2000).

In developing countries poor sanitary conditions appear to be the main risk factor for the transmission of *Salmonella* spp (Todd *et al*, 2008). Majority of Nigerians live at below poverty level and consume low cost food sold in bukas, where hygiene conditions in some cases are not ideal. The possibility of transmission of these non-typhoidal salmonellosis (NTS) to the customers abound. No study has been conducted in Nigeria to look at ways to identifity NTS from food and food samples as a means to provide solutions to this problem which could be associated with transmission of NTS and subsequent antibiotic resistance patterns of *Salmonella* spp isolated from the raw food and food sold by food handlers.

Several methods have been developed for rapid diagnosis of NTS from humans, food and animal sources with the aim of creating more attention to good hygiene practices from food handlers in order to reduce or eliminate risk associated from antibiotic-resistant and pathogenic bacteria originating from food (Alvarez *et al*, 2004). Other studies expressed the need for the use of antibiotic in animal feed to be strongly regulated with the main aim of minimizing the opportunity for organisms to develop resistance (Van *et al*, 2007).

This study aimed at using PCR to directly characterize *Salmonella* spp from food samples and known diagnostic test kit with culture as a confirmatory method.

## MATERIALS AND METHODS

**Sample and sample preparation**

Two hundred meat samples were analysed for the presence of *Salmonella* spp, 50 cooked meat (CM) samples from bukas, 50 raw meat samples (RM), 50 meat products from commonly consumed snacks (MP) and 50 spoilt meat samples (SM). These samples were collected from different food vendors, restaurants, hawkers, markets in Lagos metropolis, Nigeria.

The content of one bottle (7.2 g) of REVIVE medium (Neogen, Lansing, MI) for *Salmonella* was blended with 200 ml of sterile water at 42ºC for 30 seconds and then 25 g of meat sample were added and the mixture was blended at high speed for one minute. The mixed sample was transferred to a stomacher bag and incubated at 37ºC for 3 hours. The content of one bottle (9.2 g) of Selenite Cystine was mixed with 200 ml of sterile water incubated at 42ºC for 18-24 hours.

A sterile transfer pipette was used to dispense 5 drops of the sample broth into a REVEAL device and the test result was recorded after 15 minutes.

**Culture method**

A loopful of the sample broth was streaked on Rappaport Vassiliadis agar (Neogen, Lansing, MI) and incubated at 37ºC for 24 hours. The same process was repeated on Bismuth Sulfite agar (Neogen, Lansing, MI). Colony morphology on both types of agar was noted. A colony each was randomly taken from each of agar plates and sub-cultured on *Salmonella Shigella* agar (Oxoid, Hamshire, UK) at 37ºC for 24 hours followed by sub-culturing on Nutrient agar at 37ºC for 24 hours. Pure isolates were inoculated on slants prepared using Nutrient agar and incubated at 37ºC for 24 hours. Isolates were characterized according to Cowan (1993).

**PCR**

DNA extraction was carried out directly from the samples by boiling 1.5 ml aliquots of sample in broth for 5 minutes and the supernatant following sedimentation at 12,000*g* for 5 minutes were used as source of DNA. The concentration of extracted DNA was estimated using a Nanodrop spectrophotometer (Nanodrop; Pretoria, South Africa).

PCR was carried out using the primer set Salm3 (5′-GCTGCGCGCGAACGGCGAAG-3′) and Salm4 (5′-TCCCGCCAGAGTTCCCATT-3′) (Cocolin *et al*, 1998) and in a 25 l reaction mixture containing 1 x PCR buffer (Promega, Madison, WI), 1.5 mM magnesium chloride, 200 M of each dNTP, 20 pmol of each primer, 1.25 U *Taq* DNA polymerase (Promega, Madison, WI). The DNA was diluted to give a final concentration of between 10-200 ng/ l and 1 l of this was used in the PCR. Amplification was carried out in an Eppendorf Mastercycler Gradient (Hamburg, Germany) using the following cycling condition; 95ºC for 5 minutes, 35 cycles of 95ºC for 1 minute, 65ºC for 1 minute and 72ºC for 90 minutes and a final extension step of 72ºC for 10 minutes.

PCR amplicons were separated by 1.5% agarose gel electrophoresis at 80 volts and 50 bp DNA size markers were used for calibration.

Primer set ST11 (5′-AGC CAA CCA TTG CTA AAT TGG CGC A -3′) and Salm4 (5′-GGT AGA ATT CCA GCG GGT ACT G -3′) was also used according to the method of Croci *et al* (2004).

## RESULTS

**REVEAL method**

Out of the 200 samples analysed using the REVEAL method, 74 % were found to be positive for *Salmonella* spp. Of the 50 cooked meat (CM) samples collected, 42 (84%) samples were positive of the 50 raw meat (RM) samples collected, 41 (82%) were positive. Only 40 samples from the commonly consumed snacks (MP) and spoilt meat (SM) could be analyzed with 21 (52%) being positive.

**Culturing method**

The Rappaport Vassiliadis medium showed 42 positive for CM, 41 positive RM, 35 positive MP, and 44 positive SM. This test did not produce any false-negative result for any of the categories of meat tested. BSA detected positive results for all 50 RM, 46 CM, 35 MP and 28 SM samples.

Thus of a total of 189 presumptive *Salmonella* isolates obtained by culture of the meat samples only 37 (19%) of the food samples contained *Salmonella* spp of which *Salmonella typhimurium* accounted for 4 (8%), *S. paratyphi* A 9 (24%), *S. arizonae* 8 (22%) and *Salmonella* spp 46% after biochemical characterization (Table 1).

**PCR analysis**

PCR identification of *Salmonella* spp using the Salm3/Salm4 primer set showed that 125 (62%) was positive (presence of

Table 1
*Salmonella* isolates obtained from the various meat samples tested from Lagos, Nigeria.

| Type of meat | *S. typhi* | *S. paratyphi* A | *S. arizonae* | *Salmonella* spp | Total |
|---|---|---|---|---|---|
| Cooked meat (CM) | 3 | 2 | 1 | 9 | 15 |
| Raw meat (RM) | 1 | 6 | 7 | 5 | 19 |
| Spoilt meat (SM) | - | 1 | - | 1 | 2 |
| Meat products (MP) | - | - | - | 1 | 1 |
| Total | 4 | 9 | 8 | 16 | 37 |

389 bp band), while using the ST 11/ ST 15 primer set, 109 (54%) were positive (411 bp) (data not shown).

Also, the REVEAL kit and the two PCR-based methods were analyzed in terms of sensitivity and specificity for the identification of *Salmonella* spp using the culture technique as the gold standard. The sensitivity, specificity, PPV and NPV for the REVEAL kit were 91.9, 34.3, 33.7 and 92.1%, for Salm 3/4 primer set 100, 34.3, 33.7 and 100% and for ST 11/ ST 15 primer set 100, 55.8, 33.5% and 100%, respectively.

## DISCUSSION

Usually bacteriological detection of *Salmonella* spp from food and environmental samples can be time consuming and expensive. It therefore becomes paramount for food industries to look for faster and cheaper means of *Salmonella* identification. It has been reported that there are low levels of *Salmonella* spp in meat from subclinical infected herds (Josefsen *et al*, 2007). The results of this study shows that *Salmonella* spp constitute an important food-borne pathogen in the environment. During sample collection, it was observed that the meat sold by food vendors were not prepared and sold under appropriate hygienic conditions. In the markets where the raw meat samples were obtained, the vendors also sold the meat under unhygienic conditions .The table surface used for meat retail had small gullies and crevices where tiny bits of meat could be retained and served as media for growth of *Salmonella* spp. The tables were rarely washed and sanitized before fresh batches of meat were received and were not covered up after daily sales. The tables could be a source of the spread of *Salmonella* to other materials by flies and by direct contact with human hands. Our results show that 19-74% of the commonly consumed snacks/food and raw meat in Lagos, Nigeria habor *Salmonella* spp using the various tests carried out.

In a previous study by Raufu *et al*, (2009), *Salmonella* spp by culture method accounted for 24.8% of raw chicken parts and feces of chicken from the northeast part of the country. This is higher than our study from the southwest region (19%) by the same method. In a related study by Fashae *et al* (2010), *Salmonella* spp was isolated from feces of chickens at a frequency of 11%. Another report from southwestern Nigeria showed the prevalence of *Salmonella* spp to be 12.8% in cooked food samples in eateries in Ado Ekiti (Oluyege *et al*, 2009), while a report from south-south Nigeria reported the prevalence of *Salmonella* spp from ready-

to-eat food to be 15.9% (Isara *et al*, 2010). The results of our study also show that there was a higher prevalence of *Salmonella* spp (83%) from raw and cooked meat than commonly consumed snacks (52%) using the REVEAL kit.

The results presented show that Rappaport Vassiliadis medium semi-solid modified agar (RPVA) was effective in detection of small number of *Salmonella* in culture samples in the presence of other bacteria. However, according to the manufacturer (Neon Corp.), this medium is suitable for isolating *Salmonella* serotypes other than *S. typhi* and *S. paratyphi* type A from stool specimens with high sensitivity and specificity. But according to the results of this research, some of the *Salmonella* spp identified by RPVA were confirmed by biochemical tests to be *S. typhimurium* and *S. paratyphi* A. These results may have been due to the different samples used, the use of RPVA without adding novobiocin to suppress the growth of other organisms as well as the possibility of other organisms cross contaminating the isolates before performing the biochemical tests. BSA also showed high sensitivity and specificity in detecting *Salmonella* species from all the categories of meat sampled. However not all the isolates were confirmed by the standard biochemical tests to be *Salmonella* spp. This is because the agar also allows the growth of other organisms such as *Shigella*, *Enterobacter* and *E. coli* which may have been misinterpreted as being *Salmonella* spp.

Although the REVEAL test was easy to perform, it was often determine the positive line after 20 minutes. This made it difficult to observe some samples as being positive or negative. The competitive growth present in the SM samples may have interfered with the test and led to weak positive results.

PCR identification of *Salmonella* spp using the Salm3/Salm4 primer set was not as reliable as ST11/ST15 in the identification of our *Salmonella* spp directly from food samples especially where reagents are not readily available or too expensive for the culturing of *Salmonella* spp from food. Both PCR methods had 100% NPV and were also most reliable in ruling out organisms that were not *Salmonella* spp. Although the ST 11/ST15 primer set did not have a high PPV or specificity, but it was highly sensitive. In a related study, Malkami (2003) isolated *Salmonella* spp from fresh, frozen meat and poultry samples in Jordan using both PCR and conventional methods of identification and showed the PCR: conventional method ratio for identification gave 87% : 81% with the PCR method having high sensitivity and specificity and detection of *Salmonella* spp within 24-36 hours compared to the 3-8 days for conventional microbiology methods. This report is similar to our study regarding the time frame for *Salmonella* spp detection, but we had lower number of *Salmonella* spp identification by culture methods giving the PCR methods a lower specificity and PPV.

There requires a prompt and accurate diagnosis of *Salmonella* spp from food especially using methods that can detect *Salmonella* spp within 24 hours namely PCR. Rappaport Vassiliadis medium semisolid modified agar (RPVA) and BSA are suitable as indicator agars for detection and isolation of *Salmonella* serotypes and can be used to replace the *Salmonella Shigella* agar in diagnostic laboratories.

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