

Brief Communication

Molecular typing of *Salmonella* spp isolated from food handlers and animals in Nigeria

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Abstract: A total of 61 isolates of *Salmonella* spp (made up of 26 clinical isolates and 20 food handler and 15 animal isolates) were typed by RAPD-PCR for the purpose of screening for epidemiologically related isolates. The RAPD -PCR typing method used comprised six primers namely 787, 797, 784, 1254, RAPD 1 and RAPD 2 but 784 and 1254 did not produce discriminatory patterns and so were dropped. From the 61 strains, RAPD fingerprinting with primers RAPD 1, 2 produced 22 and 24 fingerprint patterns respectively. RAPD fingerprinting with primers 787, 797 produced 17, 11 fingerprinting patterns respectively. Combinations of the two RAPD 1 and 2 primers increased the discrimination of *Salmonella* strains to 32 patterns rather than the other primers used. Primer 797 was the least discriminatory. This study showed that the RAPD 1 and 2 primers would be useful for epidemiological typing of the *Salmonella* spp in Nigeria.

Keywords: *Salmonella*, food handlers, animals, RAPD-PCR

Introduction

Food-borne diseases are an important cause of morbidity and mortality worldwide [1]. *Salmonella* spp can be isolated from raw meat and poultry products as well as from milk and milk based products [2]. Worldwide the incidence of nontyphoidal salmonellosis is estimated at 1.3 billion cases and 3 million deaths annually [3]. Although *Salmonella* gastroenteritis is generally a self-limiting illness, severe cases may require antimicrobial therapy. Food contamination 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.

In addition, *Salmonella enterica* Typhi is the causative agent of typhoid fever and this organism has been responsible for about 200,000 deaths out of an annual occurrence of 21.6 million cases [4]. Typhoid fever is

transmitted by food and water contaminated by the faeces and urine of patients and carriers. Polluted water is the most common source of typhoid. In developed countries sporadic typhoid fever is generally reported amongst travellers returning from an area of endemicity, whereas in developing countries particularly in Africa and South East Asia, this disease may be associated with high incidences of morbidity and mortality [5-6]. In Africa, about 4.36 million cases occur out of an estimated population of 427 million [7]. In Nigeria, following the report by Smith *et al.* [8] food handlers that were apparently healthy carriers of *S. Typhi* accounted for up to 6% of cases.

Also, In developing countries poor sanitary conditions appear to be the main risk factor for the transmission of *Salmonella* spp. *Salmonella enterica* serovar Typhimurium (group D) and *S. enterica* serovar Enteritidis (groupB) have been reported to account for 79 - 95% of all bacteraemic non-typhoidal *Salmonella* infections in sub-Saharan Africa and also food

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borne outbreaks [9-10].

Various typing techniques have been employed for the identification of *Salmonella* to screen for common source outbreak or origins and relationships amongst different isolates. More precise molecular typing methods have been employed such as REP-PCR, RAPD-PCR, PFGE, ERIC-PCR for identification of *Salmonella* spp [10–13].

Majority of Nigerians live at below poverty level and patronize low cost foods such as those sold in bukas, however the hygiene conditions of those bukas in some cases are not ideal. The possibility of transmission of *S. enterica* serovar Typhi and non-typhoidal salmonellosis (NTS) to the customers abound. No study has been done in Nigeria to look at ways to identify the NTS from food samples, as well as *S. enterica* serovar Typhi from food handlers using molecular techniques as a means to proffer solutions to the problem that could be associated with transmission of NTS and subsequent antibiotic resistant patterns of *Salmonella* spp isolated from these raw foods and foods sold by food handlers.

The aim of the study is the identification of methods that would allow prompt detection and faster diagnosis of *Salmonella* spp isolated from the food handlers as well as the commonly consumed meat in our environment and compare the isolates with clinical samples of *Salmonella* spp from patients presenting with febrile illness.

Materials and methods

A total of 61 *Salmonella* spp (made up of 26 clinical isolates and 20 food handler and 15 animal isolates) were subjected to PCR –RAPD analysis after total DNA extraction by the SDS lysis method of Marmur [14] and boiling method of Holmes and Quigley [15]. The purity of the DNA was checked using a nanodrop spectrophotometer.

The 26 clinical *Salmonella* spp comprised *S. Typhi* (15), *S. Paratyphi* (1), *S. choleraesuis* (9) and *S. Enteritidis* (1). While the food handler isolates comprised *S. Typhi* (13), *S. Paratyphi* (3), and *S. Choleraesuis* (4). The animal isolates comprised *S. Typhimurium* (3) and *S. Enteritidis* (12).

For RAPD-PCR analysis, the parameters in each PCR reaction was optimized in order to maximise discriminatory power of the reaction for typing the *Salmonella* spp. A set of five primers were analysed in this study. The primers include : RAPD 1: 5' TGA GCA TAG ACC TCA 3'; RAPD 2: 5' CCC GTC AGC A 3'; 787: 5' AAC GCG CAA C 3'; 797: 5' AGC GTC ACT C 3', 784: 5'-GCG GAA ATA G 3' and Primer 1254 (5'-CCG CAG CCA A-3').

The reaction for primer 1 and 2 was prepared using 25 ul per tube, containing 20 ng DNA of each strain, x1 PCR buffer, 2mM Magnesium Chloride, 200µM of each dNTP, 20pmol of primer and 1U Taq DNA polymerase. Amplification was carried out in an Eppendorf Mastercycler gradient using the following cycling parameters. An initial denaturation was performed at 94°C for 5 minutes and 40 cycles of 94°C for 1 minute, 40°C for 1 minute and 72°C for 2 minutes. This was followed by a final extension of 72°C for 7 minutes.

The reaction for the primers 787 and 797 were as follows: The following buffer concentration was used: x1 PCR buffer, 2mM Magnesium Chloride, 200µM of each dNTP, 60pmol of primer and 1U Taq DNA polymerase.

The cycling parameter used was an initial denaturation at 94°C for 5 minutes and 30 cycles of 94°C for 1 minute, 36°C for 1 minute and 72°C for 2 minutes. This was followed by a final extension of 72°C for 5 minutes.

Lastly, the reaction for the primer 784 was x1 PCR buffer, 2mM Magnesium Chloride, 200µM of each dNTP, 40pmol of primer and 1U Taq DNA polymerase.

Amplification was carried out using the following cycling parameters. An initial denaturation was performed at 94°C for 5 minutes and 30 cycles of 94°C for 1 minute, 34°C for 1 minute and 72°C for 2 minutes. This was followed by a final extension of 72°C for 5 minutes.

The RAPD-PCR was repeated at least twice.

Results

Using the primers 1254 and 784, there was no amplification bands in most isolates.

The RAPD1 primer differentiated the 26 clinical

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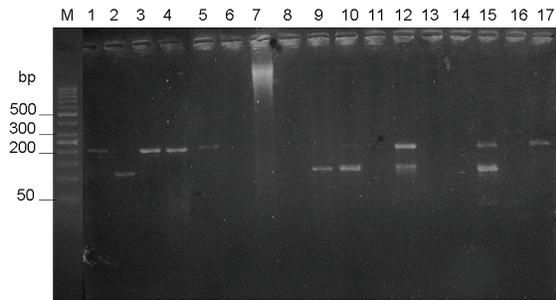


Figure 1. Lanes 1 – 4: animal *Salmonella* spp, M: λ -Hind III molecular weight marker, Lanes 5-17 food handler isolates

isolates into 6 profiles, 15 animals isolates had 7 profiles while, 20 food handler isolates had 9 profiles i.e. a total of 61 isolates were differentiated into 22 RAPD profiles. **Figure 1** shows typical RAPD patterns obtained with this

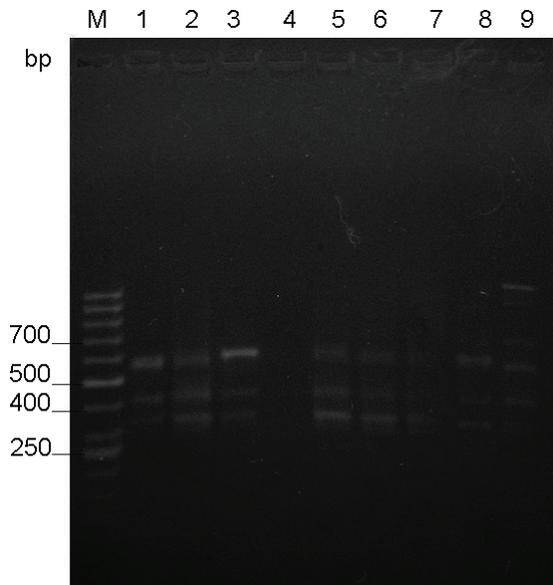


Figure 2. Lane M: 100 bp molecular weight marker, Lanes 1-9 food handler *Salmonella* spp.

primer.

Amplification with primer RAPD2 divided the 26 clinical isolates into 5 profiles, 15 animal isolates showed 7 profiles, while 20 food handler isolates had 12 profiles i.e. a total of 61 isolates were differentiated into 24 RAPD profiles. **Figure 2 & 3** shows typical RAPD patterns obtained with this primer.

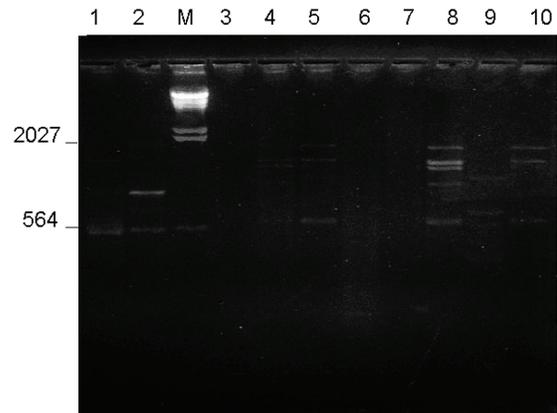


Figure 3. Lanes: 1-2 food handler *Salmonella* spp, M , λ -Hind III molecular weight marker; lanes 3-10, *Salmonella* food handler isolates.

The primer 787 identified 26 clinical isolates with 2 profiles, 15 animal isolates showed 5 profiles, 20 food handler isolates showed 10 profiles i.e. a total of 61 isolates were differentiated into 17 RAPD profiles.

The amplifications with primer 797 divided the 61 isolates in to the following distinct RAPD profiles: 26 clinical isolates showed 6 profiles, 15 animal isolates showed 2 profiles, 20 food handler isolates showed 3 profiles i.e. a total of 61 isolates were differentiated into 11 RAPD profiles. A combination of the RAPD 1 and RAPD2 profiles gave 32 RAPD patterns. A combination of 787 and 797 gave 18 RAPD profiles.

Discussion

From our study the use of the primer 784 and 1254 did not produce any discrimination amongst the isolates as a lot of isolates did not show any amplification band even after four PCR repeats. This result is different from that obtained by Tekeli *et al.* [16] and Quintaes *et al.* [17]; using primers 1254 and 784 respectively for *S. enterica* serotype Enteritidis and *S. enterica* serotype Typhi. In fact primer 784 by Quintaes *et al.* [17] gave the highest discrimination amongst their human *S. Typhi* isolates from Brazil.

From our study the best primers that showed high discrimination amongst our isolates were

RAPD 1 and RAPD 2 whose combination gave 32 RAPD patterns amongst 61 isolates. A comparison of three RAPD primers for 57 strains of *Salmonella* spp gave 42, 51 and 54 fingerprint patterns, however Authors suggested that a combination of two of the RAPD and ERIC fingerprinting would be most useful for differentiation of field isolated *Salmonella* spp and epidemiologic studies [12]. Another study from Morocco confirmed a combination of the ERIC typing and PFGE produced the best discrimination amongst the *Salmonella* isolates from human and food sources [18]. Although our other primers did not produce much discrimination, all the primers were generally able to discriminate amongst the *S. Typhi* isolates. None of the RAPD patterns between the clinical isolates and food handler isolates were similar. This shows a high degree of clonality amongst our *Salmonella* spp in Nigeria, as it was obvious that the isolates obtained were from separate set of samples. This is expected as the clinical strains (from febrile patients) were identified in a previous study by Smith *et al.* [19] and also in 2007 while the food handler and animal isolates were obtained from a study in 2008.

Our study using primer 787 showed that it had low typeability especially amongst our clinical isolates while in contrast primer 797 although the least in terms of typeability gave the best discrimination patterns amongst our clinical isolates and could be found useful in epidemiological typing of our clinical isolates as a whole.

Interestingly, from our study some of the food handler and animal isolates shared the same RAPD patterns (**Figure 1**), which is indicative of the fact that the food handlers could have been infected from the animal sources as samples were collected from sites used by food handlers.

The clinical isolates showed the lowest typeability amongst all the isolates using the four different primers. Most studies have carried out several typing methods (including RAPD-PCR) for *Salmonella* spp [12, 16-18], and have confirmed severally that a combination of methods were useful to achieve the greatest degree of genetic diversity and for epidemiological purposes. Apart from RAPD typing of *Salmonella* spp recent methods used for genetic mechanism of antibiotic resistance include microarray and RFLP [20]. In Nigeria, there has been no known documentation of

molecular typing techniques for *Salmonella* spp only RAPD-PCR of *S. Typhi* by Smith *et al.* [19]; and plasmid profile analysis by Akinyemi *et al.* [21].

In conclusion, It has been shown from our study that RAPD-PCR offers high discrimination amongst the *Salmonella* spp in our environment; this is in combination with RAPD 1 and 2. Our clinical isolates of *Salmonella enterica* serovar Typhi species had unique fingerprints and did not have the same RAPD patterns with others, which goes to show the high degree of clonality amongst out *S. Typhi* strains. RAPD-PCR is therefore a good technique for epidemiological typing of our isolates in Nigeria, moreso it is cheap and reproducible.

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Abbreviations: REP-PCR: Repetitive Extragenic Palindromic-PCR; RAPD-PCR: Randomly Amplified Polymorphic DNA-PCR; PFGE: Pulsed-field gel electrophoresis; ERIC-PCR: Enterobacterial Repetitive Intergenic Consensus -PCR; RFLP: Restriction Fragment Length Polymorphism.

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