

Antibiotic Sensitivity and Sequence Amplification Patterns of Genes in Multidrug Resistant Enterobacteria Isolates from Processed Foods in some West African Countries

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Received 25 September 2010, revised 11 October 2011, accepted 12 October 2011

Abstract

Diarrhoea, dysentery and other diseases due to other enteric bacteria have reportedly been found to resist chemotherapeutic treatment in some West African communities with fatal consequences in some cases. This study was carried out to determine multidrug resistance patterns of Enterobacteria isolates from processed ready-to-eat foods. Indigenously processed food samples of different types were collected from two Francophone and two Anglophone countries in the West African sub-region during the wet and dry seasons of a sampling period of two years. Enterobacteria were isolated from the samples using standard techniques. Amplification of chromosomal DNA of the isolates using the Polymerase Chain Reaction was carried out. The results obtained were subjected to statistical analyses. All isolates showed resistance to cefuroxime (90.7%), nitrofurantoin (90.6%), augmentin (86.1%) and ampicillin (51.2%) while all were sensitive to gentamycin and ciprofloxacin. There was amplification indicating the presence of *invA* gene at a position of 240 bp. There was no amplification at all for the *spvC* gene in any of the isolates tested. Multidrug resistant enteric bacteria in these foods containing the *invA* gene could lead to infections with uncontrolled antibiotic use. The presence of enteric bacteria in the foods analyzed which provide undeniable evidence of the poor microbiological quality of these foods could form the basis of a useful databank in formulation of food-borne disease control and prevention strategies.

Key words: Enterobacteria, multidrug resistance, processed foods, isolates in West African

Introduction

Microbial diversity has been analyzed mainly by methods revealing phenotypic features (Saeger and Hale, 1993). Several approaches are available for estimating genetic variation multilocus enzyme electrophoresis (Eardly *et al.*, 1990), Restriction fragment length polymorphism (RFLP) analysis (Lu *et al.*, 1992) and Random amplified polymorphic DNA technique (Bentacor *et al.*, 2004) are commonly used. The polymerase chain reaction (PCR) is a rapid and specific nucleic acid amplification method for the detection of food-borne pathogens and a number of PCR assays have been described for the detection of bacteria in foods (Chen *et al.*, 1998; Villalobo and Torres, 1998; Lampel *et al.*, 2000; O'Sullivan *et al.*, 2000; Sails *et al.*, 2003). Failure to adequately decontaminate and preserve food may allow the growth of pathogens, resulting in diseases with significant morbidity and mortality.

Food-borne illnesses caused by pathogenic bacteria still occur at unacceptably high frequencies in industri-

alized nations and developing countries. The estimates ranged from 6 to 81 million cases of food-borne illnesses per year, with 5,000 deaths occurring annually (Lampel *et al.*, 2000). Although food safety initiatives have since been introduced to reduce contamination by food handlers and to improve sanitary conditions at the sites where foods are grown, harvested and processed, food borne illnesses attributed to these potential sources of contamination continue. This is illustrated by periodic reports of major outbreaks such as those linked to contaminated sprouts in which *Salmonella* spp. or *Escherichia coli* 0157:H7 was identified as the causative pathogen (Taormina *et al.*, 1999). Processed foods are food items that have been altered from their natural state for safety reasons and for convenience. The methods used for processing foods include canning, freezing, refrigeration, dehydration and aseptic processing (Larsson *et al.*, 2006). High quality and hygiene standards must be maintained to ensure consumer safety and failure to maintain adequate standards can have serious health consequences.

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Enterobacteriaceae have been implicated in various food-borne diseases in Europe (Bolton, 1999) and this necessitated a concise study of the presence of pathogenic enterics in different convenience foods the results of which were reported at the 144th meeting of the Society for General Microbiology (Bolton, 1999). No such study, however, has been reported for Africa and West Africa in particular.

With all the aforementioned being considered, the following aims and objectives were set for this study: carrying out sampling surveys of processed foods in the West African sub-region; effecting the isolation and identification of Enterobacteriaceae from the various processed food samples and carrying out systematic characterization of the isolates using both classical and molecular techniques.

Experimental

Material and Methods

Sampling. Description of sampling sites: The convenience food samples were collected from two Anglo-phone and two Francophone countries in the West African sub-region. These are Accra (Long. 0° 09'W, Lat. 0° 00'N) the capital of Ghana and Aflao (Long. 0° 46'E, Lat. 6° 11'N) a town on the eastern border of Ghana; Lome the Togolese capital (Long. 1° 13'E, Lat. 6° 07'N) and La Kondji (Long 1° 40'E, Lat. 6° 18'N), which is a border town east of Lome in Togo. This was followed by the capital of the Republic of Benin, Cotonou (Long. 2° 26'E, Lat. 6° 21'N) and Seme (Long. 2° 37'E, Lat. 6° 22'N) an eastern border town between the Republic of Benin and Nigeria (Fig. 1). The fourth country was Nigeria, in which sampling was carried out in some towns. The towns from which sampling was carried out in Nigeria were Port-Harcourt, Yenagoa, Abakaliki, Enugu, Ibadan, Ado-Ekiti, Ikeja, Yola, Gombe, Abuja, Makurdi, Jos, Sokoto and Birnin-Kebbi (Fig. 1).



Fig. 1. Map of West Africa showing the four countries where sampling was carried out.

- Dotted spot represent sampling site.

Table I
Sampling sites and foods sampled

Country	Sampling site	Food sample
BENIN	Cotonou	Fried Chicken, Sausage Sauce Achaba
	Seme	Fish pie Bun
GHANA	Accra	Puff-puff, Bun Fried Chicken
	Aflao	Egg roll Bun
NIGERIA	Port Harcourt, Yenagoa	Puff-puff Bun Egg roll
	Abakaliki, Enugu	Puff-puff Egg roll
	Ibadan, Ado-Ekiti, Ikeja	Puff-puff, Egg roll Bun, Meat pie
	Yola, Gombe	Puff-puff, Bun Fish pie, Dried fish
	Abuja, Makurdi, Jos	Egg roll Fish pie Bun
	Sokoto, Birnin-Kebbi	Fish pie Meat pie Egg roll
	TOGO	Lome
	La Kondji	Puff-puff Chin-chin Pie

Yola, Gombe, Abuja, Makurdi, Jos, Sokoto and Birnin-Kebbi (Fig. 1).

Description of some food samples: The convenience food samples collected included puff-puff, egg rolls, buns, fried chicken, fish pie *etc* (Table I).

Sampling procedures. The food samples were collected during the West African rainy (wet) season *i.e.* between April and October and harmattan (dry) season *i.e.* between November and March for two years 2005 and 2006. Table I shows the sampling sites and the foods sampled. Convenience food samples were collected in clean polyethylene bags and labeled accordingly. The polyethylene bags with the samples were transported aseptically in insulated rectangular, plastic food flasks (Kenchuang Co., Xhenkong, China (30 cm × 17.5 cm × 30 cm) filled with ice chips to the Microbial Physiology and Biochemistry laboratory, Department of Botany and Microbiology, University of Ibadan, Ibadan,

Oyo State, where they were unloaded and refrigerated immediately. Whenever possible, 100 g was obtained for each sample unit. The average time of travel from sampling sites outside Nigeria (Ghana through Lome and Cotonou to Ibadan) was thirteen and a half (13.5) hours. Average travel time within Nigeria was eight and a half hours

Isolation and characterization of isolates. Sample treatment to obtain isolates: Twenty-five grams of each food sample was homogenised with nine times that weight or volume of buffered peptone water (Andrews and Hammack, 2002). The different food samples were treated separately. Isolation from the egg roll was from the flour covering, the boiled egg and a combination of both. The chicken was treated by sampling from the skin and the flesh separately. Meat-pie and fish-pie samples were treated in the same manner. Each sample was mixed to ensure homogeneity. A portion (1 g) of the resulting homogenate was then transferred into sterile test tubes and serially-diluted ten-fold according to the methods described by Pollack *et al.* (2002). One (1.0) millilitre aliquot of the diluents were then plated out for incubation to get pure cultures and further work was carried out on the isolates (American Public Health Association, 1992; Andrews and Jacobson, 2003). The media for isolation were Nutrient Agar and Broth (Lab M), MacConkey Agar and Broth (Fluka); Salmonella-Shigella Agar (Lab M); Bismuth Sulphite Agar (Fluka); EMB Agar (Fluka). All were prepared according to the manufacturers' instructions. Pure cultures of isolates were stored on Nutrient agar slants at 4°C. The organisms were sub-cultured onto fresh slants every three months (90 days).

The bacterial colonies were differentiated first on the basis of colonial morphology followed by microscopic examination after Gram staining. Gram staining followed the procedure of Pollack *et al.*, 2002. Biochemical tests were carried out to characterize the isolates as described by Olutiola *et al.* (1991) and Pollack *et al.* (2002).

Antibiotic sensitivity testing. A 0.1 ml overnight actively growing broth culture containing 1×10^6 cfu/ml of each bacterial isolate was introduced into a Petri dish and 20 ml of molten agar added. The antibiotic sensitivity discs (HJ04/P, Abtek Biologicals Ltd.) consisting of 10 different antibiotics namely nitrofurantoin (100 µg), augmentin (30 µg), norfloxacin (10 µg), tetracycline (50 µg), gentamycin (10 µg), ciprofloxacin (5 µg), chloramphenicol (10 µg), ampicillin (25 µg), nalidixic acid (30 µg), and cefuroxime (20 µg) were placed on the solidified agar surface. The plates were left overnight at 37°C. The relative susceptibility of each isolate to each antibiotic was shown by a clear zone of inhibition. Susceptible strains showed zone diameters of 22 to 24 mm (Bauer *et al.*, 1966)

Polymerase chain reaction. Preparation of purified chromosomal DNA: Organisms were subcultured from slants onto fresh Mueller Hinton agar plates and incubated overnight. One hundred (100) µl of solution A (50 mmol Tris, pH 8.0, 5 mmol EDTA and 50 mmol NaCl-DNA wash buffer) was pipetted into eppendorf tubes and overnight cultures of bacterial isolates were harvested into the tubes. The tubes were mixed vigorously by vortexing and stored on ice. Washed cells were suspended in 400 µl of solution B (50 mmol Tris pH 8.0 and 25% Sucrose) containing 1 mg/ml lysozyme (Fisher BioReagents). The mixture was vortexed and then incubated on ice for 20 min. After incubation, 50 µl of solution C (5% SDS, 50 mmol Tris) was added and the tubes mixed by inverting the tubes rapidly. Two hundred (200) µl of solution D (10 mmol Tris, 1 mmol EDTA, pH 8.0) and 50 µl of 2 mg/ml proteinase K (promega) were added and the tubes mixed by inverting the tubes. The lysates were incubated at 56°C for 1 h in a water bath. The DNA mixture was extracted with 500 µl of phenol/chloroform/isoamyl alcohol (25:24:1) added and centrifuged at 14,000×g for 15 min. The supernatants were transferred to fresh tubes and the step repeated. Five hundred (500) µl of chloroform was added and centrifuged at 14,000×g for another 15 min. The supernatants were transferred into fresh tubes. The nucleic acids were precipitated by adding 100 µl of solution E (3 M Sodium acetate and pH adjusted to 6.0 with glacial acetic acid) and 200 µl of absolute ethanol. The tubes were vortexed and allowed to precipitate overnight on ice. After thawing, the tubes were centrifuged at 14,000×g for 30 min and the supernatant discarded. The precipitated DNA was washed twice in 70% ethanol and centrifuged at 14,000×g for 5 min at 4°C. The precipitates were dried under vacuum and re-dissolved in sterile distilled water.

Polymerase chain reaction procedure: The PCR mixture consisted of 5 µl of 5×PCR amplification buffer (promega); 1.5 mmol (1.5 µl) MgCl₂ (Roche); 200 µmol (0.5 µl) deoxyribonucleoside triphosphates (dNTP mix, promega); 1.25 U (0.25 µl) *Taq* polymerase (promega); 1 µl each of primer pair (Mission Biotech); 2 µl of DNA and distilled water was added to make a total volume of 25 µl. The mixture was subjected to 30 PCR cycles in a programmable thermal cycler (Techne TC 312, Barloworld Scientific Ltd, UK). The parameters for the amplification cycles were as follows: prior to the first cycle, the PCR mixture was incubated for 1 min at 94°C. Denaturation for 30 s at 94°C; annealing of primers for 30 s at 56°C and primer extension for 2 min at 72°C. After the last cycle, the mixture was incubated for 10 min at 72°C (Chiu and Ou, 1996). The primer sequences, their corresponding genes positions and actual length of amplification product in base pairs are listed in Table II (Chiu and Ou, 1996). Gel

Table II
Primer sequences and positions of genes

Primer	Sequence	Gene	Corresponding positions	Length of product (base pairs)
SPVC-1	ACTCCTTGCACAACCAAATGCGGA	<i>spvC</i>	505–528	571
SPVC-2	TGTCCTTCTGCATTTTCGCCACCATCA	<i>spvC</i>	1052–1075	
INVA-1	ACAGTGCTCGTTTACGACCTGAAT	<i>invA</i>	104–127	244
INVA-2	AGACGACTGGTACTGATCGATAAT	<i>invA</i>	324–347	

electrophoresis was carried out. 25 µl of the amplified DNA samples were mixed with bromophenol blue and loaded onto the different wells in the electrophoretic tank. *Salmonella typhi* DNA (NIMR, Lagos, Nigeria) was used as the positive control and sterile distilled water was the negative control. It was allowed to run at 120 V for 1 h 30 min. The amplified DNA fragments in the gel were visualized and the pictures taken with an ultraviolet transilluminator (Hoefler macrovue UV-25, Amersham Biosciences, UK).

Results

Forty three organisms were identified from all the samples. The isolated organisms fall into the following Genera: *Citrobacter*, *Edwardsiella*, *Enterobacter*, *Escherichia coli*, *Klebsiella*, *Proteus*, *Salmonella*, *Serratia* and *Shigella*. The percentage occurrence relative to country is shown in Table III. Eleven point six (11.6) percent of the isolated organisms were from the Republic of Benin and Togo, while 9.3% were isolated from Ghana. Nigeria had the highest percentage of isolated organisms with 67.5%.

Figure 2 shows the patterns of resistance and sensitivity of the isolated organisms to the antibiotics tested.

Table III
Percentage occurrence of Enterobacteria isolates from processed foods relative to country

Country	Number of isolates	Occurrence (%)
Republic of Benin	5	11.6
Ghana	4	9.3
Nigeria	29	67.5
Togo	5	11.6
Total	43	100

All the organisms showed resistance of above 50% to four antibiotics, nitrofurantoin – 90.6%, augmentin – 86.1%, ampicillin – 51.2% and cefuroxime – 90.7%; while gentamycin and ciprofloxacin had 0% resistance (Fig. 2). Ninety seven point seven (97.7) percent of the organisms were sensitive to both tetracycline and chloramphenicol while 95.3% and 79.1% were sensitive to norfloxacin and nalidixic acid respectively (Fig. 2). Table IV shows the prevalence of the antibiotic resistant strains of the isolated organisms. Both strains of *Salmonella* and three out of four *E. coli* strains were resistant to nitrofurantoin. All strains (100%) of *Enterobacter*, *Proteus*, *Salmonella* and *Serratia* were resistant to augmentin. *Enterobacter* (8.3%) and *Citrobacter* (16.7%) were resistant to norfloxacin. Only one strain of *E. coli*

Table IV
Percentage prevalence of antibiotic-resistant strains of Enterobacteria isolates from processed food samples

Antibiotic	Enterobacteria isolates/percentage prevalence								
	<i>Citrobacter</i> n=6	<i>Edwardsiella</i> n=2	<i>Enterobacter</i> n=12	<i>E. coli</i> n=4	<i>Klebsiella</i> n=4	<i>Proteus</i> n=4	<i>Salmonella</i> n=2	<i>Shigella</i> n=7	<i>Serratia</i> n=2
Nitrofurantoin	6 (100)	2 (100)	11 (91.7)	3 (75)	2 (50)	4 (100)	2 (100)	6 (85.7)	2 (100)
Augmentin	4 (66.7)	2 (100)	12 (100)	3 (75)	2 (50)	4 (100)	2 (100)	6 (85.7)	2 (100)
Norfloxacin	1 (16.7)	– (0)	1 (8.3)	– (0)	– (0)	– (0)	– (0)	– (0)	– (0)
Tetracycline	– (0)	– (0)	– (0)	1 (25)	– (0)	– (0)	– (0)	– (0)	– (0)
Gentamycin	– (0)	– (0)	– (0)	– (0)	– (0)	– (0)	– (0)	– (0)	– (0)
Ciprofloxacin	– (0)	– (0)	– (0)	– (0)	– (0)	– (0)	– (0)	– (0)	– (0)
Chloramphenicol	– (0)	– (0)	1 (8.3)	– (0)	– (0)	– (0)	– (0)	– (0)	– (0)
Ampicillin	3 (50)	2 (100)	6 (50)	3 (75)	– (0)	2 (50)	1 (50)	5 (71.4)	– (0)
Nalidixic Acid	3 (50)	– (0)	2 (16.7)	1 (25)	– (0)	2 (50)	– (0)	1 (14.3)	– (0)
Cefuroxime	6 (100)	2 (100)	12 (100)	3 (75)	3 (75)	4 (100)	1 (50)	6 (85.7)	2 (100)

n = number of isolates tested; figures in parentheses represent percentage prevalence

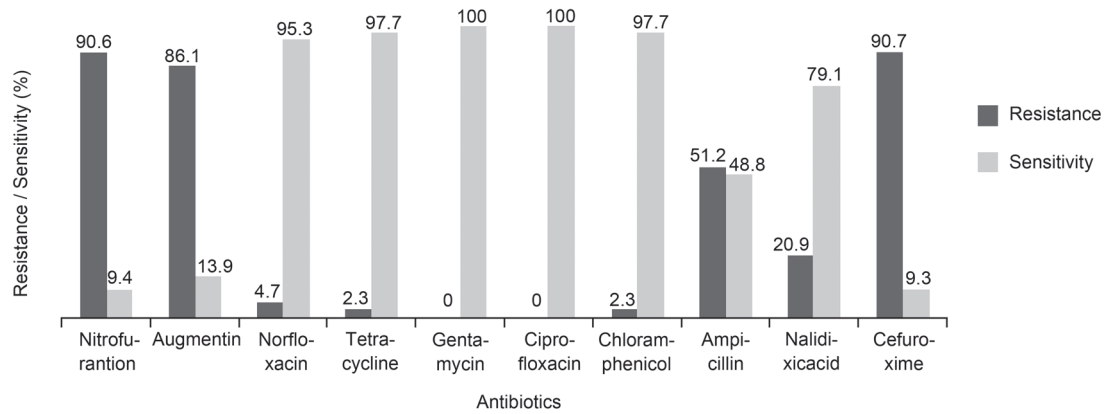


Fig. 2. Percentage sensitivity and resistance of Enterobacteria isolates.

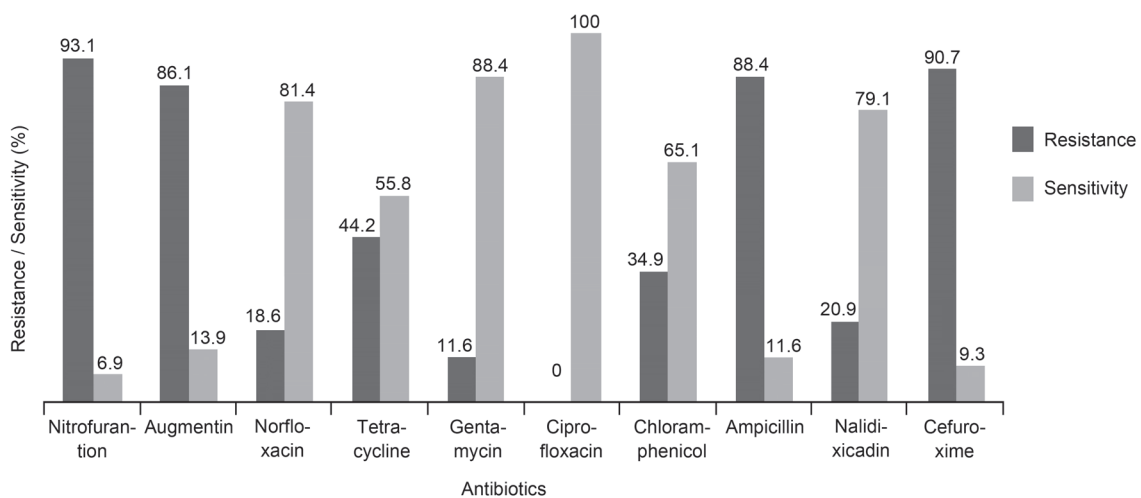


Fig. 3. Percentage sensitivity and resistance of Enterobacteria isolates after plasmid curing.

was resistant to tetracycline and 1 strain of *Enterobacter* was resistant to chloramphenicol. *Klebsiella* and *Serratia* were the only two organisms whose strains were sensitive to ampicillin, while all strains of *Klebsiella*, *Salmonella* and *Serratia* were sensitive to nalidixic acid. All organisms isolated were sensitive to gentamycin and ciprofloxacin.

Based on the knowledge of the presence of plasmids (results not shown here), the isolates were cured and the percentage resistance and sensitivity patterns after curing were studied (Fig. 3). Ninety three point one percent (93.1%) of the organisms became resistant to nitrofurantoin. The resistance and sensitivity to augmentin, ciprofloxacin, nalidixic acid and cefuroxime remained the same as before curing was carried out. Antibiotic resistance after curing increased from 4.7% to 18.6% in norfloxacin, from 2.3% to 44.2% for tetracycline, from 2.3% to 34.9% for chloramphenicol and from 51.2% to 88.4% for ampicillin (Fig. 4). All the organisms were totally sensitive to Gentamycin before curing, but after curing, sensitivity to the antibiotic reduced to 88.4% (Fig. 3).

PCR amplification products of *invA* and *spvC* genes. Figure 4 shows the result of the agarose gel elec-

trophoresis of PCR-amplified *invA* gene from some of the Enterobacteria isolates. Lane 1 shows the molecular weight marker and the amplified DNAs at different base

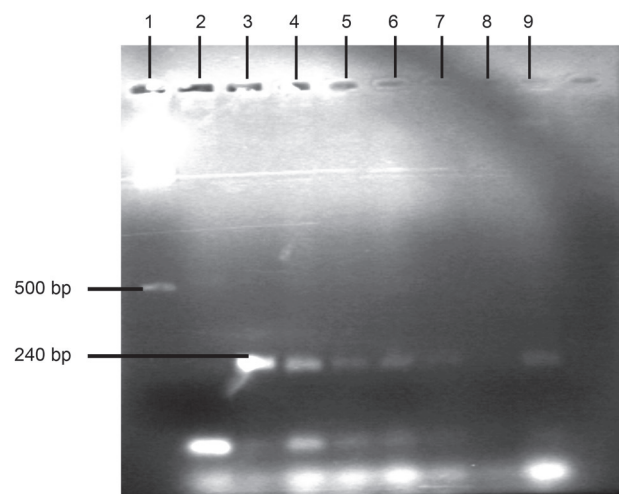


Fig. 4. Electrophoretic pattern on agarose gel of PCR-amplified *invA* gene from some Enterobacteria isolates.

Lane 1: λ Hind – III molecular weight marker; Lane 2: PCR negative control (sterile distilled water); Lane 3: PCR positive control (*Salmonella typhi* DNA (NIMR)); Lane 4: *Salmonella* sp.; Lane 5: *Shigella* sp.; Lane 6: *Salmonella* sp.; Lane 7: *Shigella* sp.; Lane 9: *Enterobacter* sp.

pairs, the lowest band being 500 bp. Distilled water was used as the negative control and there was no amplification hence no band appeared (Lane 2). A *Salmonella typhi* DNA was used as the positive control. The appearance of the band shows that there was amplification of the chromosomal DNA (Lane 3) hence the presence of the *invA* gene. Lanes 4 to 9 contained DNAs from *Salmonella* (lanes 4 and 6), *Shigella* (lanes 5, 7 and 8) and *Enterobacter* (lane 9). There was amplification in lanes 4, 5, 6, 7 and 9 indicating the presence of *invA* gene. Amplification was absent in lane 8. The expected corresponding positions should be 243 bp. The result here shows a migrated position of 240 bp. There was no amplification of the *spvC* gene at all indicating that the gene being screened for was not present in any of the isolates tested.

Discussion

The presence of enteric bacteria in processed/ ready-to-eat foods provides undeniable evidence of the poor microbiological quality of the foods in the different countries. These observations are concurrent with those earlier obtained by Lewis *et al.* (2005). In the said work, it was shown that the presence of these bacteria, especially *Salmonella*, in the water samples analyzed was due to improper disinfection and cross contamination from hands, packaging *etc.* The mean bacterial count was the highest in Nigeria, followed by the Republic of Benin in year 2005 as compared with year 2006 during which the mean bacterial count was the highest in Togo, although there were no significant differences in the mean counts over the two years in all countries.

The results of this work conformed those reported by Vigano *et al.* (2007), who studied the microbiological quality of ready-to-eat foods in Pemba Island, United Republic of Tanzania. Of 300 food samples analyzed, 58% coliforms were found in household meals and 98% in milk samples. *E. coli* was the most frequently isolated species. *Salmonella* spp. was found exclusively in cow milk. The boiled or fried foodstuffs were faecally contaminated and the contamination was likely to have occurred after preparation and before consumption. Kaneko *et al.* (1999) reported isolating *Listeria* spp. from two samples of raw vegetables and five kinds of cooked foods. The isolation rates for coliforms were 6.1–50% for intact vegetables and 50–66.7% for fresh products. The aerobic count and coliform values showed that the raw vegetables cut for salad were the most heavily contaminated among the six kinds of ready-to-eat foods examined. Room temperature, high water activity and a pH of > 4.6 provides an environment conducive to pathogen growth.

The use of DNA amplification to generate genomically distinct groups of products has begun to play an

increasingly important role in the differentiation of bacterial species, serotypes and strains (Jensen and Hubner, 1996). Bacterial plasmids are known to confer a wide variety of phenotypic modifications and genetic flexibility upon their hosts by carrying genes that may code for toxin production, iron sequestration, adhesiveness and serum resistance (Novick, 1982; 1987). The existence of such plasmids in *Salmonella* species (Holmberg *et al.*, 1984) and Enterobacteria (Lamb *et al.*, 1984) has been well documented and comparative plasmid DNA analysis has been shown to be useful for tracing the source of outbreaks of salmonellosis (Olsvik *et al.*, 1985; Bakeri *et al.*, 2003).

In many African countries, antibiotics can be bought over the counter, and it is well known that the natural bacterial gastrointestinal flora of our human population could act as a reservoir for the dissemination of resistance-conferring plasmids (R plasmids). This event could also contribute to the high level of antimicrobial resistance in *Salmonella* spp. isolated from nosocomial infections (Orman *et al.*, 2002). In contrast to other opportunistic pathogens, *Salmonella* spp. are causative agents of zoonotic rather than nosocomial infections. In this regard, widespread use of antimicrobial agents in domestic animals of economic importance may have also contributed to increased levels of resistance in *Salmonella* spp. (Orman *et al.*, 2002).

In order to select an appropriate set of primers, one must consider the type of samples to be analysed and what non-*Salmonella* strains were included in specificity determinations (Ziemer and Steadham, 2003). A multiplex PCR primer set targeting *invA* gene and virulence plasmid *spvC* gene of the virulence plasmid (*invA/spvC*) (Chiu and Ou, 1996) was used in this study. The oligonucleotide primer for this PCR were synthesized according to the sequences of the *spvC* and *invA* genes shown to be unique for the *Salmonella* genus and located in the virulence plasmid and chromosome of *S. typhimurium*, respectively. *invA* is a gene located on the chromosome and is essential for *Salmonella* spp. to enter epithelial cells. *spvC* is a virulence related gene on plasmid required for survival within host cells. (Bhatta *et al.*, 2007). There are nearly 2000 *Salmonella* serovars, and those tested thus far all seem to contain *inv* genes, which enable the bacteria to invade cells. Also, there are seven *Salmonella* serovars known to contain the virulence plasmid that carries the *spv* genes: Typhimurium, Choleraesuis, Dublin, Enteritidis, Gallinarium-Pullorum (Gulig *et al.*, 1993), Abortusovis (Guiney *et al.*, 1995) and Sendai.

A number of other PCR primers targeting the *invA* gene for detection of *Salmonella* have been published (Rahn *et al.*, 1992, Stone *et al.*, 1994, Kumar *et al.*, 2002). Kumar *et al.* (2002) targeted the *invA* gene at 881 bp and the gene was amplified in all the *Salmonella* serovars tested.

Results from this study show that the three *Salmonella* strains tested (two isolated from this work and the positive control) contain the *invA* gene together with two *Shigella* species and one *Enterobacter* sp. One *Shigella* sp. failed to show any amplification band. None of the bacteria tested contained the *spvC* gene thus showing that they did not contain the virulence plasmid. This result is in line with the results of Bhatta *et al.* (2007) who explained that the *spvC* gene is a virulence gene on the plasmid required for survival within the host cell, and that the acquisition of this plasmid may be due to horizontal gene transfer. In the specificity testing of these primers by Chiu and Ou (1996), all non-*Salmonella* organisms isolated tested failed to produce any band, whereas all the *Salmonella* isolates tested invariably produced the *invA* amplicon. This shows that the *invA* gene is conserved in the *Salmonella* gene. However, Ziemer and Steadham (2003) tested these primers on *Salmonella* and some faecal and gut-associated bacteria. They reported that the use of the primer sets for *invA* gene used by Chiu and Ou (1996) resulted in amplification though non-specific with other faecal and gut-associated bacteria. It may be inferred that the *invA* is conserved in these other gut-associated bacteria.

Studies have provided evidence that the virulence plasmid plays a significant role in human disease (Guiney *et al.*, 1995) because except for Gallinarium-Pullorum which is specific for fowl, the other species are frequently seen as etiologic agents of enteritis in humans. Typhimurium is the most common serovars isolated from diarrhoeal patients, and Choleraesuis, Dublin and Enteritis are often isolated from patients with bacteremia (Guiney *et al.*, 1995; Chiu and Ou, 1996). Chiu and Ou (1996) reported that PCR with the two primers would produce one or two DNA fragments, depending on the *Salmonella* strains present. One band would be derived from the *invA* gene, and the other band would be derived from the *spvC* gene if a virulence plasmid was present. Therefore, the appearance of at least one band, or two if there was a virulence plasmid, would indicate a positive result. Chiu and Ou (1996) amplified the *invA* primer at 244-bp fragment within the conserved *invA* gene sequence of *Salmonella* spp. The amplified fragment gotten in this work was 240-bp which is very close to the corresponding 244-bp described by Chiu and Ou (1996).

In conclusion, the development and discovery of antibiotics for the treatment of bacterial infections was one of the most significant medical achievements of the twentieth century. Unfortunately, many of the existing antibacterial agents are under threat from the widespread emergence of bacterial resistance. Furthermore, the pace of emergence of antibiotic-resistant bacteria has outstripped the discovery of new antibiotics. New agents are therefore urgently needed to counter the

threats posed by antibiotic-resistant organisms. Pathways and targets for existing antibiotics can further be exploited for the discovery of novel agents. PCR assays have been reported and observed to be as sensitive as conventional culture methods but significantly reduced the time for detection. The use of sensitive, quantitative methods for the detection of food-borne pathogens during food processing could be used to determine points in the food production process where contamination occurs and where controls could be introduced to reduce or eliminate Enterobacteria from ready-to-eat retail food products, thereby reducing the risk to the consumer. This study reveals that the efforts being made by food standards regulatory bodies are inadequate to meet the RTE food quality. Establishment of a systematic and regular mechanism for surveillance and monitoring is urgently needed to minimize the risks attached to consuming these foods.

West Africa is becoming a tourist destination for people from developed countries. These findings could be useful in formulation of food-borne disease control and prevention strategies locally and internationally, particularly antibiotic-treatment strategies.

Acknowledgement

The authors gratefully acknowledge Dr. S. I. Smith for the use of the PCR equipment at the Genetics Division, Nigerian Institute of Medical Research, Yaba, Lagos State, Nigeria.

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