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Epidemiology of Salmonella and *Salmonellosis*

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ABSTRACT. The prevalence of enteritis and its accompanying diarrheal and other health challenges linked to infections with *Salmonella* has continuously plagued sub Saharan Africa. In Nigeria, typhoid fever is among the major widespread diseases affecting both young and old as a result of many interrelated factors such as inadequate sanitaion, indiscriminate use of antibiotics and fecal contamination of water sources. Morbidity associated with illness due to *Salmonella* continues to increase with untold fatal consequences, often resulting in death. An accurate figure of cases is difficult to arrive at because only large outbreaks are mostly investigated whereas sporadic cases are under-reported. A vast majority of rural dwellers in Africa often resort to self medication or seek no treatment at all, hence serving as carries of this disease. Non typhoidal cases of salmonellosis account for about 1.3 billion cases with 3 million deaths annually. Given the magnitude of the economic losses incurred by African nations in the battle against salmonella and salmonellosis, this article takes a critical look at the genus *Salmonella*, its morphology, isolation, physiological and biochemical characteristics, typing methods, methods of detection, virulence factor, epidemiology and methods of spread within the environment.

1. INTRODUCTION

The study of *Salmonella* began with Eberth's first recognition of the organism in 1880, and the subsequent isolation of the bacillus responsible for human typhoid fever by Gaffky (Le Minor, 1991). Further investigations by European workers characterized the bacillus and developed a serodiagnostic test for the detection of this human disease agent (Tindall *et al*., 2005). Thereafter, D.E. Salmon isolated the bacterium then thought to be the etiological agent of hog cholera, but later disproved. The genus was named *Salmonella* by Lignieres in 1900 in honour of D.E. Salmon. Further investigations led to the isolation of other *Salmonella* species (Su and Chiu, 2006). An antigenic scheme for the classification of *Salmonella* was first proposed by White and subsequently expanded by Kauffmann into Kauffmann-White scheme, which currently includes more than 2540 serovars (Popoff and Le Minor, 2005). *Salmonella* nomenclature is very complex and scientists used different systems to refer to and communicate about this genus. Unfortunately, current usage often combines several nomenclature systems that divide the genus into species, subspecies, subgenera, groups, subgroups, and serotypes (serovars), and all these usages caused lots of confusion among researchers (Rakesh *et al*., 2009). *Salmonella* nomenclature has progressed through a succession of taxonomical and serological characteristics and on the principles of numerical taxonomy and DNA homology (Tindall *et al*., 2005). The nomenclature for the genus *Salmonella* has evolved from the initial one serotype-one species concept proposed by Kauffmann in 1966 on the basis of somatic (O), flagellar (H) and capsular (Vi) antigens. In the early development of the taxonomic scheme, biochemical reactions were used to separate *Salmonella* into subgroups and the Kauffmann-White scheme was the first attempt to systematically classify *Salmonella* using scientific parameters. The scientific development in *Salmonella* taxonomy occurred in 1973 when Crosa *et al*. (1973) demonstrated using DNA-DNA hybridization that all serotypes and sub-genera I, II, and IV of *Salmonella* and all serotypes of Arizona were related at the species level. Thus, they belonged to a single species, and the exception later described was called

Salmonella bongori, previously known as subspecies-V. Other taxonomic proposals have been made based on the clinical role of a strain and biochemical characteristics that divided the serovars into subgenera (Brenner *et al*., 2000; Ezaki *et al*., 2000). The antigenic formulae of *Salmonella* serovars are defined and maintained by the World Health Organization (WHO) Collaborating Centre for Reference and Research on *Salmonella* at the Pasteur Institute, Paris (Popoff *et al*., 2004). Presently, *Salmonella* genus consists of two species: *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* is further divided into six subspecies; *S. enterica* subsp. *enterica* (I), *S. enterica* subsp. *salamae* (II), *S. enterica* subsp. *arizonae* (Illa), *S. enterica* subsp. *diarizonae* (lllb), *S. enterica* subsp. *houtenae* (IV), and *S. enterica* subsp. *indica* (VI) (Popoff and Le Minor, 2005).

2. CHARACTERISTICS OF *SALMONELLA*

Salmonella are Gram negative, facultative anaerobic, rod shaped bacteria belonging to family *Enterobacteriaceae*. Members of this genus are motile by peritrichous flagella, except *Salmonella enterica* serovar Pullorum and *Salmonella enterica* serovar Gallinarum. *Salmonella* are 2-3 x 0.4-0.6 μm in size and they are chemoorganotrophs, with ability to metabolize nutrients by both respiratory and fermentative pathways (D'Aoust *et al*., 2001; Popoff and Le Minor, 2005). Hydrogen sulphide (H_2S) is produced by most *Salmonella* but a few serovars like *Salmonella paratyphi* A and *Salmonella choleraesuis* do not produce H_2S. Most Salmonellae are aerogenic; however, *Salmonella typhi* does not produce gas. Members of the genus have a % G+C content of 50-53. They are urease and Voges-Proskauer negative and citrate utilizing (Montville and Matthews, 2008). Most *Salmonella* do not ferment lactose and this property has been the basis for the development of numerous selective and differential media for culture and presumptive identification of *Salmonella* spp; they include xylose lysine decarboxycholate agar, *Salmonella-Shigella* agar, brilliant green agar, Hektoen enteric agar, MacConkey's agar, lysine iron agar and triple sugar iron agar. Isolation of *Salmonella* from food and environmental samples with culture method utilizes the multiple steps of pre-enrichment and enrichment on the selective and differential media in order to increase the sensitivity of the detection assay (Andrews and Hammack, 2001; Anderson and Ziprin, 2001). Isolation of Salmonella often involves pre-enrichment, a process in which the sample is first cultured in a non-selective growth medium such as buffered peptone water or lactose broth with the intent of allowing the growth of any viable bacteria, and the recovery of injured cells. Subsequently, pre-enriched samples are cultured on enrichment media to restrict the growth of undesirable bacteria. Enrichment media commonly used include tetrathionate broth, selenite cystine broth and Rappaport Vassiliadis broth. Following the enrichment period, the enriched cultures are spread onto selective and differential agar plate and then typical colonies for *Salmonella* has to be identified. Final confirmation of typical colonies is determined by series of biochemical and serological tests (Rakesh *et al*., 2009). A few *Salmonella* serovars do not exhibit the typical biochemical characteristics of the genus and these strains pose problem diagnostically because they may not easily be recovered on the commonly used differential media. About 1% of the *Salmonella* serovars submitted to Centre for Disease Control (CDC) ferment lactose; hydrogen sulphide production too was quite variable (Ziprin, 1994). *Salmonella* chrome agar medium has been described very promising for detection of both lactose positive and lactose negative *Salmonella* isolates from food samples (Dick *et al*., 2005).

3. PHYSIOLOGY AND BIOCHEMICAL CHARACTERISTICS

The biochemical properties of *Salmonella* spp show that almost all *Salmonella* serovars do not produce indole, hydrolyze urea, nor deaminate phenylalanine or tryptophan. Most of the serovars readily reduce nitrate to nitrite and most ferment a variety of carbohydrates with the production of acid, and have been reported to be negative for Voges-Proskauer (VP) reaction. Other prominent characteristics of this genus include the ability of most serovars to produce hydrogen

sulfide (H_2S) and decarboxylate lysine, arginine and ornithine with a few exceptions (Popoff and Le Minor, 2005). Most *Salmonella* serovars utilize citrate with a few exceptions such as *Salmonella* Typhi, *Salmonella paratyphi* A and a few *Salmonella choleraesuis* serovars. Dulcitol is generally utilized by all serovars except *Salmonella enterica* subsp. *arizonae* (Illa) and *Salmonella enterica* subsp. *diarizonae* (lllb) (Popoff and Le Minor, 2005). Lactose may not be utilized by most *Salmonella* serovars, however, it has been reported that less than 1% of all *Salmonella* spp ferment lactose (Ewing, 1986). Furthermore, *Salmonella* isolation from different sources with routine selective and differential media utilizes non-lactose fermentation as a key biochemical property and commonly used differential plating media for isolation of *Salmonella* contains lactose. *Salmonella* serovars are considered resilient microorganisms that readily adapt to extreme environmental conditions. Optimum temperature for growth is in the range of 35 – 37^{o}C but some can grow at temperatures as high as 54^{o}C and as low as 2^{o}C (Gray and Fedorka-Cray, 2002). *Salmonella* grow in a pH range of 4 - 9 with the optimum being 6.5 – 7.5. They require high water activity (aw) for growth (> 0.94) but can survive at aw of < 0.2 such as in dried foods. Inhibition of growth occurs at temperatures < 7^{o}C, pH < 3.8 or aw < 0.94 (Hanes, 2003; D'Aoust and Maurer, 2007). The outer membrane (OM) of *Salmonella*, as with almost all Gram-negative bacteria, is composed of outer membrane proteins (OMPs) and lipopolysaccharides (LPS). LPS plays an essential role in maintaining the cell's structural integrity and protection from chemicals. In the host organisms, they act as endotoxins and as a pyrogen displaying a strong immune response. Structurally, they are composed of three distinct components: lipid A, core oligosaccharide and O-polysaccharide (Bell and Kyriakides, 2002; Raetz and Whitfield, 2002).

4. RAPID DETECTION METHOD FOR *SALMONELLA*

Polymerase chain reaction (PCR)

Nucleic acid (DNA or RNA) based methods have become very popular for rapid detection of pathogens. The first *in vitro* amplification of mammalian genes using the Klenow fragment of *Escherichia coli* DNA polymerase was carried out by Kary Mullis (Mullis and Faloona, 1987). This assay is now popularly known as polymerase chain reaction (PCR).

The polymerase chain reaction is a method which produces multiple copies of a target DNA. The PCR method uses a thermostable polymerase enzyme (Taq polymerase) to create multiple copies of target DNA. Detection of target DNA is achieved through the use of short sections of synthetic, single stranded DNA known as oligonucleotide primers. These primers can be designed to be specific for an individual organism, or for a group of organisms (Simon, 1999).

PCR also works by using a cycling of different temperatures. It also requires the target template DNA, primers, dNTPs and Taq polymerase (Tenover *et al*., 1997). This large number of a target DNA segment can then be detected using standard detection methods such as agarose gel electrophoresis or membrane hybridization. The ability of PCR to produce extremely large numbers of copies of a specific nucleic acid segment provides the requirements for the rapid, very sensitive and specific detection of desired microorganisms in a water sample.

PCR holds great potential for the direct detection of microbial pathogens and detection of virulence genes in water and wastewater (Malorny *et al*., 2003a; del Cerro *et al*., 2002). Specific nucleic acid primers already exist for most of the major waterborne pathogens and have been proven to be specific for these organisms. It is both highly specific and sensitive and is capable of detecting very small numbers of microorganisms in a sample. In addition, multiple primers can be used to detect different pathogens in one multiplex reaction (Ziemer and Steadham, 2003; Moganedi *et al*., 2007; El-Lathy *et al*., 2009).

PCR based methods have been found to be very sensitive for detection of *Salmonella* spp in environmental water samples and other sources (Way *et al*., l993; Pathmanathan *et al*., 2003; Aurelie *et al*., 2005). PCR based detection assays for rapid and specific detection of *Salmonella* in wastewater were compared with conventional method and reported; PCR method was comparable to the culture method (Fricker and Fricker, 1995; Simon, 1999).

PCR does not require the culturing of microorganisms and therefore can improve detection efficiency, time and labor. It negates the requirement for indicator organisms as pathogenic microorganisms can be directly detected from a wastewater sample. PCR also has the advantage of being able to be used to determine the viability of a microorganism and thus, is not restricted by dormancy status or the ability to culture the microorganism (Simon, 1999).

The use of PCR as a routine surveillance tool in the water industry still remains a potential for the future. At present, the costs and expertise required to use these techniques remain prohibitive for most laboratories. Rapid advances, however, have recently been made in a number of these problem areas, promising the potential for viable solutions in the near future.

5. *SALMONELLA* TYPING METHODS

Biotyping

Salmonella strains in a particular serovar may be differentiated into biotypes by their utilization pattern of selected substrates such as carbohydrates and amino acids. In many serovars there are few biochemical tests in which significant numbers of strains behave differently and so the number of identifiable biotypes within a serovar can be obtained. The organisms expressing different phenotypes of a given serotype are considered a different biotype, and these differences can be associated with differences in virulence properties (Anderson and Ziprin, 2001).

Duguid *et al*. (1975) developed a scheme for biotyping to study the epidemiology of infections with *Salmonella typhimurium*. This scheme was based on the use of 15 biochemical characters. Thirty-two potential primary biotypes were defined by the combinations of positive and negative reactions shown in 5 tests (d-xylose, m-inositol, l-rhamnose, d- tartrate and m-tartrate) most discriminating in *Salmonella typhimurium*. These primary biotypes were designated by numbers (1-32) and the full biotypes were developed by an additional 10 secondary tests and finally a total of 24 primary and 184 full biotypes have been identified.

Recently, de la Torre (2005) used the biochemical kinetic data to determine strain relatedness among *Salmonella enterica* subsp. *enterica* isolates. Different biochemical tests results were used in the determination of strain relatedness among different serovars of *Salmonella enterica* subsp. *enterica* (59 *Salmonella typhimurium* strains, 25 *Salmonella typhimurium* monophasic variant strains, 25 *Salmonella anatum* strains, 12 *Salmonella tilburg* strains, 7 *Salmonella virchow* strains, 6 *Salmonella choleraesuis* strains, and l *Salmonella enterica* (4,5,l2::) (Hoszowski and Wasyl, 2001).

Serotyping

The basis of *Salmonella* serotyping depends upon the complete determination of the different antigens; somatic (O), flagellar (H) and capsular (Vi) antigens.

The aim of the serological testing procedure is to determine the complete antigenic formula of the individual *Salmonella* isolate. Commercially available polyvalent somatic antisera kits consist of mixtures of antibodies specific for major antibodies (Herikstad *et al*., 2002).

Antigen-antibody complexes are formed (agglutination) when a bacterial culture is mixed with a specific antiserum directed against bacterial surface components. The complexes are usually visible to the naked eye which allows for easy determination of O and H antigens by slide agglutination. Some cultures are monophasic and may be directly H-typed, whereas the second phase in a diphasic culture is determined after phase inversion. After full serotyping of the *Salmonella* culture the name of the serotype can be determined by using the Kauffmann-White Scheme. The serological typing of *Salmonella* has led to identification of large number of *Salmonella* serovars. Currently, Kauffmann-White scheme recognizes 2610 *Salmonella* serovars, the majority (2587) belongs to *S. enterica*, while the remaining (23 serovars) are assigned to *S. bongori* (Guibourdenche *et al*., 2010).

Somatic (O) antigens

These are heat stable antigens which are composed of phospholipid-polysaccharide complexes. Analysis of O antigens revealed polysaccharide (60%), lipid (20 to 30%), and hesomione (3.5-4.5%). The nature of terminal groups and the order in which they occur in the repeating units of the

polysaccharide chain provide the specificity to the numerous kinds of O antigens (Hu and Kpoecko, 2003).
Somatic antigens are resistant to alcohol and dilute acid. Different variants (smooth, rough) are prevalent in *Salmonella* spp and these variations affect the serological typing of *Salmonella*. In addition, smooth (S) to rough (R) variations occur in *Salmonella* (Yousef and Carlstrom, 2003).
The heat stable O antigen consist of lipopolysaccharide-protein chain exposed on the cell surface and are classified as major and minor antigens. The major category consists of antigens such as somatic factors O:4 and O:3, which are specific determinants of serogroups like B and E respectively. In contrast, minor somatic antigenic components, such as O:l2 are nondiscriminatory, as evidenced by their presence in different serogroups (D'Aoust *et al*., 2001). These are heterogeneous structures and the antigenic specificity is determined by the composition and the lineage of the O group sugars and sometimes mutation affect the sugars leading to new O antigen (Grimont and Weill, 2007).

Flagellar (H) antigens
H-antigens are heat labile proteins associated with the peritrichous flagella and can be expressed in one of two phases. These are heat labile antigens that are present in the flagella of *Salmonella* are proteineous in nature and are called flagellin (Yousef and Carlstrom, 2003). The flagellin is a keratinomyosin epiderm-fibrinogen group protein of 40 kDa in molecular weight. The amino acid content and the order in which these acids present in the flagellins determine the specificity of the different H antigens. The flagellar agglutination occurs very rapidly and the aggregates formed are loosely knit and floccular forms (Raetz and Whitfield, 2002).
The phase 1 H-antigens are specific and associated with the immunological identity of the particular serovars, whereas phase 2 antigens are non-specific antigens containing different antigenic subunit proteins which can be shared by many serovars. These homologous surface antigens are chromosomally encoded by the H1 (phase 1) and H2 (phase 2) of the vh2 locus. By convention each serotype has been denoted by an antigenic formula with the major O antigen, followed by phase l H-antigen(s), and then phase 2 H-antigen(s). The phase l H-antigens are designated by lowercase letters and then phase 2 H-antigens by Arabic numerals or some instances by components of e or z series (Brenner *et al*., 2000; Grimont and Weill, 2007).

Capsular (Vi) antigens
The capsular antigens are present in *Salmonella* Typhi, *Salmonella dublin* and *Salmonella paratyphi* A. The Vi antigen could be purified by chemical method. The thermal solubilization of capsular antigen (Vi) antigen is necessary for the immunological detection of serotypes containing capsular antigens (Fluit, 2005).
More than 99% of *Salmonella* strains causing human infections belong to *Salmonella enterica* subspecies *enterica*. Although not common, cross-reactivity between O antigens of *Salmonella* and other genera of *Enterobacteriaceae* do occur. Therefore, further classification of serotypes is based on the antigenicity of the flagellar H antigens which are highly specific for *Salmonella* (Scherer and Miller, 2001). Officially recognized by the World Health Organization (WHO), the Kauffmann-White diagnostic scheme involves the primarily subdivision of *Salmonella* into serogroups and further delineated into serotypes based on the O, H and Vi antigenic formula (Popoff and LeMinor, 2005).

Phage typing
Bacteriophages are the most abundant entities on earth and have contributed a lot to the field of molecular biology and biotechnology. Many mysteries of molecular biology were solved using bacteriophages. Bacteriophages are getting enormous amount of attention due to their potential to be used as antibacterials, phage display systems, and vehicles for vaccines delivery. They have also been used for diagnostic purposes (phage typing) as well (Clark and March, 2006).
These bacterial viruses have genetic material in the form of either DNA or RNA, encapsulated by a protein coat (Clark and March, 2006). The capsid is attached to a tail which has fibers, used for

attachments to receptors on bacterial cell surface. Most of the phages have polyhedral capsid except filamentous phages (Ackerman, 1998).

Phages infect bacteria and can propagate in two possible ways; lytic life cycle and lysogenic life cycle. When phages multiply vegetatively they kill their hosts and the life cycle is referred to as lytic life cycle. On the other hand, some phages known as temperate phages can grow vegetatively and can integrate their genome into host chromosome replicating with the host for many generations (Inal, 2003). If induction to some harsh conditions like ultraviolet (UV) radiations occurs then the prophage will escape via lysis of bacteria (Inal, 2003).

The specificity of phages for bacterial cells enables them to be used for the typing of bacterial strains and the detection of pathogenic bacteria. Phage typing is also known as the use of sensitivity patterns to specific phages for precisely identifying the microbial strains. The sensitivity of the detection would be increased if the phages bound to bacteria are detected by specific antibodies. For the detection of unknown bacterial strain its lawn is provided with different phages, and if the plaque (clear zones) appears then it means that the phage has grown and lysed the bacterial cell, making it easy to identify the specific bacterial strain (Clark and March, 2006).

There are certain other methods which can be employed to detect pathogenic bacteria such as the use of phages that can deliver reporter genes (e.g. lux) specifically (Kodikara *et al*., 1991) or using green fluorescent protein (Funatsu *et al*., 2002) that would express after infection of bacteria. Similarly, phages having a fluorescent dye covalently attached to their coats can be used to detect specific adsorption (Hennes *et al*., 1995; Goodridge *et al*., 1999). The detection of some of the released components such as adenylate kinase (Corbitt *et al*., 2000) after the specific lysis of bacteria and the use of antibodies and peptides that are displayed by phages which bind to toxins and bacterial pathogens specifically can also be used, (Petrenko and Vodyanoy, 2003).

Dual phage technology is another application of phages in detection of bacteria, in which phages are used to detect the binding of antibodies to specific antigens (Sulakvelidze and Kutter, 2005). Phage amplification assay can also be used to detect pathogenic bacteria. The technique has most extensively been used for the detection of *Mycobacterium tuberculosis*, *E.coli*, *Pseudomonas*, *Salmonella*, *Listeria*, and *Campylobacter* species (Barry *et al*., 1996).

The applications of phages range from the diagnosis of the disease, through phage typing, and its prevention (phage vaccine), to the treatment (phage therapy).There is the hope that phages could be useful to humans in many ways.

6. MOLECULAR TYPING OF *SALMONELLA*

Conventional culture methods have been popularly employed in identifying and isolating microorganisms present in wastewater. Unfortunately, as a result of inconsistently expressed phenotypic traits, these classical typing approaches are often unable to discriminate between related outbreak strains.

The ability to characterize and determine the genetic relatedness among bacterial isolates involved in a waterborne outbreak is a prerequisite for epidemiological investigations. Detailed strain identification is essential for the successful epidemiological investigation of *Salmonella* outbreaks. Investigations have relied traditionally on serological and antibiogram techniques. In contrast, modern typing methods are based on characterization of the genotype of the organism. Thus, molecular typing or fingerprinting of *Salmonella* isolates is an invaluable epidemiological tool that can be used to track the source of infection and to determine the epidemiological link between isolates from different sources (Rakesh *et al*., 2009).

Some molecular typing systems can distinguish among epidemiologically unrelated isolates based on genetic variation in chromosomal DNA of a bacterial species (Swaminathan and Matar, 1993). Usually, this variability is high, and differentiation of unrelated strains can be accomplished using a variety of fingerprinting techniques.

The genotypic methods are those methods, which are based on the genetic structure of an organism and include polymorphisms in DNA restriction patterns based on cleavage of the chromosome. The digestion of the chromosomal DNA provides variable number of the DNA fragments, thus revealing

genetic variations. Genotyping methods are less subject to natural variation, though various factors may be responsible for genetic variants such as insertions or deletions of DNA into the chromosome, the gain or loss of the extra chromosomal DNA, and random mutations that may create or eliminate restriction sites (Tenover *et al*., 1997).
There is currently no gold standard typing system available for *Salmonella* fingerprinting, however, the combination of different genotyping methods such as plasmid profile analysis, ribotyping, characterization of virulence factors in *Salmonella* serovars, enterobacterial repetitive intergenic consensus sequence analysis (ERIC-PCR), random amplified polymorphic DNA (RAPD) and pulsed field gel electrophoresis methods have been evaluated for more precise subtyping of *Salmonella* serovars (Mohand *et al*., 1999; Lagatolla *et al*., 1996; Shangkuan and Lin, 1998).

Random amplified polymorphic DNA (RAPD)-PCR
Over the last decade, polymerase chain reaction has become a widespread technique for several novel genetic assays based on selective amplification of DNA (Bardakci, 2001). The popularity of PCR is primarily due to its apparent simplicity and high probability of success. Unfortunately, because of the need for DNA sequence information, PCR assays are limited in their application. The discovery that PCR with random primers can be used to amplify a set of randomly distributed loci in any genome facilitated the development of genetic markers for a variety of purposes (Williams *et al*., 1900; Welsh and McClelland, 1990).
Random Amplification of Polymorphic DNA (RAPD) is a modification of the PCR in which a single, short and arbitrary oligonucleotide primer, able to anneal and prime at multiple locations throughout the genome, can produce a spectrum of amplification products that are characteristics of the template DNA. No knowledge of the DNA sequence for the targeted gene is required, as the primers will bind somewhere in the sequence, but it is not certain exactly where. This makes the method popular for comparing the DNA of biological systems that have not had the attention of the scientific community, or in a system in which relatively few DNA sequences are compared (Senthil Kumar and Gurusubramanian, 2011).
The simplicity and applicability of the RAPD technique have captivated the interest of many scientists. Perhaps the main reason for the success is the gain of a large number of genetic markers that require small amounts of DNA without the requirement for cloning, sequencing or any other form of the molecular characterization of the genome of the species in question.
The standard RAPD technology utilises short synthetic oligonucleotides (about 10 bases long) of random sequences as primers to amplify nanogram amounts of total genomic DNA under low annealing temperatures by PCR. Amplification products are generally separated on agarose gels and stained with ethidium bromide (Bardakci, 2001).
Welsh and McClelland (1990) independently developed a similar methodology using primers about 15 nucleotides long and different amplification and electrophoretic conditions from RAPD and called it the arbitrarily primed polymerase chain reaction (AP-PCR) technique. PCR amplification with primers shorter than 10 nucleotides known as DNA amplification fingerprinting (DAF) have also been used to produce more complex DNA fingerprinting profiles (Caetano-Annoles *et al*., 1991). Although these approaches are different with respect to the length of the random primers, amplification conditions and visualization methods, they all differ from the standard PCR condition in that only a single oligonucleotide of random sequence is employed and no prior knowledge of the genome subjected to analysis is required.
At an appropriate annealing temperature during the thermal cycle, oligonucleotide primers of random sequence bind several priming sites on the complementary sequences in the template genomic DNA and produce discrete DNA products if these priming sites are within an amplifiable distance of each other. The profile of amplified DNA primarily depends on nucleotide sequence homology between the template DNA and oligonucleotide primer at the end of each amplified product.
Nucleotide variation between different sets of template DNAs will result in the presence or absence of bands because of changes in the priming sites. Recently, sequence characterized amplified regions (SCARs) analysis of RAPD polymorphisms showed that one cause of RAPD

polymorphisms is chromosomal rearrangements such as insertions/deletions. Therefore, amplification products from the same alleles in a heterozygote differ in length and will be detected as presence and absence of bands in the RAPD profile (Bardakci and Skibinski, 1999).
RAPD technique has found a wide range of applications in gene mapping (Hemmat *et al*., 1994), population genetics (Chalmers *et al*., 1992; Kambhampati *et al*., 1992), molecular evolutionary genetics (Fani *et al*., 1993; Naish *et al*., 1995), and plant and animal breeding (Russel *et al*., 1993). This is mainly due to the speed, cost and efficiency of the technique to generate large numbers of markers in a short period compared with previous methods. Therefore, RAPD technique can be performed in a moderate laboratory for most of its applications. It also has the advantage that no prior knowledge of the genome under research is necessary.
Although the RAPD method is relatively fast, cheap and easy to perform in comparison with other methods that have been used as DNA markers, the issue of reproducibility has been of much concern. In fact, ordinary PCR is also sensitive to changes in reaction conditions, but the RAPD reaction is far more sensitive than conventional PCR because of the length of a single and arbitrary primer used to amplify anonymous regions of a given genome. This reproducibility problem is usually the case for bands with lower intensity. The reason for bands with high or lower intensity is still not known. Perhaps some primers do not perfectly match the priming sequence, amplification in some cycles might not occur, and therefore bands remain faint. The chance of these kinds of bands being sensitive to reaction conditions of course would be higher than those with higher intensity amplified with primers perfectly matching the priming sites. The most important factor for reproducibility of the RAPD profile has been found to be the result of inadequately prepared template DNA. Differences between the template DNA concentration of two individual's DNA samples result in the loss or gain of some bands (Welsh and McClelland, 1994; Bardacki, 1996).
Since RAPD amplification is directed with a single, arbitrary and short oligonucleotide primer, DNA from virtually all sources is amenable to amplification. Therefore, DNA from the genome in question may include contaminant DNA from infections and parasites in the material from which the DNA has been isolated. Special care is needed in keeping the DNA to be amplified from other sources of DNA.

7. PATHOGENICITY AND VIRULENCE

The nature of pathogenicity of an organism lies in the virulence genes or virulence factors. However, these terms are still not strictly defined (Wassenaar and Gastraa, 2001). The possible virulence factors of *Salmonella* have been understood with the gain in the knowledge on the molecular mechanism behind the pathogenicity of *Salmonella*. Recently, the involvement of effector proteins in the survival and replication of *Salmonella* in host cells has been elucidated. The majority of virulence genes of *Salmonella* are clustered in a region distributed over the chromosome, called *Salmonella* Pathogenicity Islands (SPI) (Groisman and Ochman, 1996; Marcus *et al*., 2000). Until recently, five SPIs (SPI 1-5) have been identified on the *Salmonella* chromosome at centisome 63, 31, 82, 92 and 25 cs, respectively (Blanc-Portard and Groisman, 1997; Hayward and Koronakis, 2002).

Each SPI was responsible in various cellular activities towards the virulence factor of the organism (Wong *et al*., 1998; Wood *et al*., 1998). On completion of genome sequence of *Salmonella* Typhi strain CT 18, five more regions were identified and designated as SPI-6, SPI-7, SPI-8, SPI-9 and SPI-10.

SPI-6 encodes for *saf* and *tcf* fimbrial operon and SPI-7 encodes for Vi biosyntheis genes and also for the IV fimbrial operon (Parkhill *et al*., 2001; Pickard *et al*., 2003). The 6.8 kb large SPI-8 encodes for genes conferring resistance to bacteriocin, SPI-9 for type 1 secretion system, whereas SPI-10 encode for *sef* fimbrial operon (Galan *et al*., 1992; Parkhill *et al*., 2001). The flagella mediated bacterial motility accelerates but is not required for *Salmonella enteritidis* invasion in differentiated Caco-cells (van Asten *et al*., 2004).

Salmonella virulence factors were also detected in virulence plasmids in certain *Salmonella* serovars namely *Salmonella abortusovis*, *Salmonella cholerasuis*, *Salmonella dublin*, *Salmonella*

enteritidis, *Salmonella gallinarum*, *Salmonella pullorum* and *Salmonella typhimurium*, although not all isolates of these serotypes carry the virulence plasmid (Rotgar and Casadesus, 1999). All plasmids contain the 7.8 kb *Salmonella* plasmid virulence (*spv*) locus. This locus harbored five genes designated spv RABCD and expressions of *spv* genes which may play a role in the multiplication of intracellular *Salmonella* (Chu *et al*., 2001). The results showed that *spv*B together with *spv*C conferred virulence to *Salmonella typhimurium* when administered subcutaneously to mice (Matsui *et al*., 2001).

Salmonella Typhi CT 18 exhibited a 106 kb large cryptic plasmid with some homology to a virulence plasmid of *Yersinia pestis*. However, the majority of *Salmonella typhi* tested did not harbor this plasmid. Cryptic plasmid has also been reported for *Salmonella paratyphi* C, *Salmonella derby*, and *Salmonella copenhagan*, *Salmonella durban*, *Salmonella give* and *Salmonella infantis* (Rotgar and Casadesus, 1999). Hybridization analysis has shown a few other serotypes such as *Salmonella johannesburg*, *Salmonella kottbus* and *Salmonella newport* found to bear the virulence plasmids.

Salmonella produces both endotoxin and exotoxin and virulence due to these toxins are well documented. The endotoxin, lipid portion (lipid A) of the outer lipopolysaccharide (LPS) membrane of *Salmonella* elicits a variety of *in vitro* and *in vivo* biological responses. The best studied exotoxin of *Salmonella* was the heat labile *Salmonella* enterotoxin (*stn*) of approximately 29 kDa encoded by *stn* gene (Prager *et al*., 1995; Portillo, 2000). A study on 90 kDa heat labile enterotoxins of *Salmonella typhimurium* was also reported by Rahman and Sharma (1995). The role of fimbriae and the flagella of *Salmonella* have been well identified in the attachment and movement of the organism but their roles in pathogenesis are still not properly understood (Folkesson *et al*., 1999; Edwards *et al*., 2000; Portillo, 2000).

Characterization of different virulence factors in *Salmonella* serotypes have been carried out by amplifying different gene sequences responsible for specific phenotypic properties. The amplification of *inv*A gene by PCR indicates the presence of invasion gene in *Salmonella* serovars. A PCR based study demonstrated that *stn* gene was present in all *Salmonella enterica* serovars, whereas it was absent in *Salmonella bongori* (Prager *et al*., 1995). The cumulative effects of virulence by these genes were found to be responsible for invasion to the epithelial cells of intestine and thereafter leading to gastrointestinal disorder. PCR assays for several virulence (*inv*, *him*) and functional (*iro*B, *fim*Y) genes were developed for detection of *Salmonella* in the environment, in food or faeces samples (Bej *et al*., 1994; Baumler *et al*., 1998; Yeh *et al*., 2002; Malorny *et al*., 2003a). The *fli*C gene also has been successfully used for molecular typing studies on *Salmonella*, based on high variability of the central region (Dauga *et al*., 1998).

8. SALMONELLA; RESERVOIRS AND EPIDEMIOLOGY

The primary reservoir of *Salmonella* is the intestinal tract of birds and animals, particularly of poultry and swine. The organisms are excreted in faeces from which they may be transmitted by insects and other creatures to a large number of places such as water, soils and kitchen surfaces. There are host adaptations patterns among serovars, namely; highly host adaptive, less host adaptive and non-host adaptive (Ecuyer *et al*., 1996). Human host adaptive serovars include *Salmonella typhi* (causative agent of typhoid fever); in contrast, the highly host adaptive chicken pathogens viz., *Salmonella pullorum* and *Salmonella gallinarum* are not human pathogen. There is no report of *Salmonella typhi* host range extending beyond human beings. Hence, isolation of *Salmonella typhi* from food or water must be indication of contamination from human beings. Other *Salmonella* serovars are found to be host adapted animal pathogens and sources of zoonotic infections (Ziprin and Hume, 2001).

Salmonella choleraesuis is a pathogen of swine but sometime causes severe systemic infections in humans (Ziprin, 1994; Wang *et al*., 1996). Similarly, *Salmonella dublin* may cause septicemia in cattle and can be transmitted to humans from milk and milk products (Reher *et al*., 1995). *Salmonella enteritidis* and *Salmonella senftenberg* are host adapted to chicken and turkey respectively. Some *Salmonella* serovars are not host adapted and also tend to be less virulent than

the host-adapted serotypes, but they are found to be responsible for an overwhelming number (90%) of incidents of human salmonellosis (Webber, 1996; Hunter, 1997).

Typhoid cases are stable with low numbers in developed countries, but non typhoidal salmonellosis has increased worldwide. Typhoid fever usually causes mortality in 5 to 30% of typhoid-infected individuals in the developing world. The World Health Organization (WHO) estimates 16 to 17 million cases occur annually, resulting in about 600,000 deaths. The mortality rates differ from region to region, but can be as high as 5 to 7% despite the use of appropriate antibiotic treatment (Scherer and Miller, 2001).

In Nigeria, typhoid fever is among the major widespread diseases affecting both young children and young adults as a result of many interrelated factors such as inadequate facilities for processing human wastes and indiscriminate use of antibiotics. Morbidity associated with illness due to *Salmonella* continues to be on the increase, in some cases resulting in death (Talabi, 1994; Akinyemi *et al*., 2005). A more accurate figure of salmonellosis is difficult to determine because normally only large outbreaks are investigated whereas sporadic cases are under-reported (Scherer and Miller, 2001; Parry, 2006). On the other hand, non typhoidal cases account for 1.3 billion cases with 3 million deaths (Hanes, 2003; Hu and Kopecko, 2003).

The infectious dose of *Salmonella* depends upon the serovar, bacteria strain, growth condition and host susceptibility. On the other hand, host factors controlling susceptibility to infection include; the condition of the intestinal tract, age and underlying illnesses or immune deficiencies. The infectious dose of *Salmonella* is broad, varying from $1\text{-}10^9$ cfu/g. However, single-food-source outbreaks indicate that as little as 1 to 10 cells can cause salmonellosis with more susceptibility to infection by YOPI (Young, Old, Pregnant and Immunocompromised) groups (Yousef and Carlstrom, 2003; Bhunia, 2008).

Information about incidence and serovars distribution of *Salmonella* in domestic animal populations is essential for understanding the relationships within and among reservoirs of *Salmonella* in animals and humans that are ultimately responsible for zoonotic disease transmission (Gast, 1997). *Salmonella* infection is usually acquired by the oral route, mainly by ingesting contaminated food or drink. *Salmonella* can be transmitted directly from human to human or from animal to human without the presence of contaminated food or water, but this is not a common mode of transmission.

9. MECHANISM OF SPREAD

Transmission of *Salmonella* to humans traditionally has been attributed to contaminated animal-product foods, but epidemiological studies have demonstrated that cases are sporadic and may more likely involve environmental sources than previously thought. It has been suggested that contaminated soils, sediments and water as well as wildlife may play a significant role in *Salmonella* transmission (Schutze *et al*., 1998). Moreover, geographic clusters of cases in which no verifiable food source have been determined, such as those recently caused by *S. javiana* in the southeastern US which do not follow the same geographic patterns as cases which have been linked to a known food source but rather mimic amphibian distribution patterns (Srikantiah, 2004). *S. typhi*, which is only transmitted from human to human, is most common in developing nations where access to safe drinking water may be limited and waste disposal and treatment may be inadequate (Velema *et al*., 1997).

First, environment contaminated with *Salmonella* serves as the infection source because *Salmonella* can survive in the environment for a long time. Subsequently, *Salmonella* is transmitted to vectors such as rats, flies and birds where *Salmonella* can be shed in their faeces for weeks and even months. Following the direct transmission, moving animals such as swines, cows and chickens act as the important risk factor for infection. These animal reservoirs are infected orally because *Salmonella* normally originates from the contaminated environment and also contaminated feed. Human get infected after eating food or drinking water that is contaminated with *Salmonella* through animal reservoirs. However, *Salmonella typhi* and *Salmonella paratyphi* A do not have animal reservoir, therefore infection can occur by eating improperly handled food by infected

individuals (Newell *et al*., 2010). Besides, transmission of *Salmonella* to the food processing plants and equipments for food preparation are also of great importance. Once carried by vectors or transferred to food, consumption by human can result in the risk of salmonellosis. The *Salmonella* cells can attach to food contact surfaces such as plastic cutting board which may develop into biofilm once attached and hence cause cross-contamination. Consequently, *Salmonella* can enter the food chain at any point from livestock feed, through food manufacturing, processing and retailing as well as catering and food preparation in the home (Wong *et al*., 2002).

Spread of *Salmonella* may be facilitated in water storage tanks in a building, from wild animal feces or even from carcasses. Poor sanitation, improper sewage disposal and lack of clean water system cause the transmission of typhoid fever. In areas where typhoid fever is endemic, water from lakes or rivers which are used for public consumption and are sometimes contaminated by raw sewage are the main sources of infection. The consumption of unboiled water during 1997 typhoid outbreak in Dushanbe, Tajikistan caused 2200 cases of illness and 95 deaths (Penteado and Leitão, 2004; Bordini *et al*., 2007).

Salmonella contamination of fresh produce could be due to the entry of *Salmonella* through scar tissues, entrapment during embryogenesis of produce, natural uptake through root systems and transfer onto edible plant tissues during slicing. The human health risk is increased further by *Salmonella* preference to grow on fresh produce during retail display at ambient temperature. In 2000, cantaloupe from Mexico resulted in a *Salmonella* Poona outbreak in USA (Penteado and Leitão, 2004; Bordini *et al*., 2007). The table below shows the prevalence of predominant *Salmonella* serotypes from different sources (Table 1).

Table 1: Prevalence of *Salmonella* species from different sources

Country	Sample/source	Prevalence	Predominant serotypes	Reference
Iran	Chicken	86/190 (45.3%)	Thompson	Dallal *et al*., 2010
	Beef	38/189 (20.1%)		
Brazil	Poultry carcass	0/127 (0%)	Enteritidis	Freitas *et al*., 2010
	Poultry viscera	2/73 (2.7%)		
Spain	Pig	43/804 (5.3%)	Anatum,	Gomez-Laguna *et al*., 2010
	Herd	22/67 (32.8%)	Typhimurium	
China	Chicken	276/515 (53.6%)	Enteritidis,	Yang *et al*., 2010
	Pork	28/91 (30.8%)	Typhimurium,	
	Beef	13/78 (16.7%)	Shubra, Indiana,	
	Lamb	16/80 (20%)	Derby, Djugu	
Morocco	Slaughter house	75/105 (71%)	Infantis, Bredeney,	Bouchrif *et al*., 2009
	Sea food	10/105 (9.5%)	Blokley, Typhimurium, Mbandaka, Branderup II, Kiambu	
Bangladesh	Chick egg	4/80 (5%)	Typhimurium	Hasan *et al*., 2009
Republic of Ireland	Retail pork	13/500 (2.6%)	Typhimurium	Prendergast *et al*., 2009
Turkey	Chicken part	1/168 (0.6%)	Typhimurium	Centinkaya *et al*., 2008
	Minced meat	0/45 (0%)	Infantis	
	Ready-to-eat-salad	0/100 (0%)		
	Raw vegetable	0/78 (0%)		
	Raw milk	0/25 (0%)		
Iran	Raw poultry	24/134 (17.9%)	Enteritidis,	Jalali *et al*., 2008
	Cooked poultry	3/56 (5.4%)	Baibouknown	
	Raw meat	8/101 (7.9%)		
	Cooked meat	2/118 (1.7%)		
	Turkey	1/3 (33.3%)		
	Quail	2/5 (40%)		
	Vegetable	3/38 (7.9%)		
Lithuania	Faeces	28/85 (32.9%)	Enteritidis,	Pieskus *et al*., 2008
	Caecum	12/52 (23.1%)	Typhimurium	
	Dust	5/34 (14.7%)		
	Water	1/10 (10%)		
Turkey	Tulum cheese	6/250 (2.4%)		Colak *et al*., 2007

Vietnam	Pork	32/50 (64%)	London, Havana,	Van *et al*., 2007
	Beef	31/50 (62%)	Anatum, Hadar,	
	Chicken	16/30 (53.3%)	Albany,	
	Shell fish	9/50 (18%)	Typhimurium	
Malaysia	Street food	12/129 (9.3%)	Biafra, Braenderup,	Tunung *et al*., 2007
	Fried chicken	1/18 (5.6%)	Weltevreden	
	Kerabu jantung	3/5 (60.0%)		
	pisang	6/9 (66.7%)		
	Sambal fish	2/5 (40%)		
	Mix vegetable			
Brazil	Chicken abattoir	29/288 (10.1%)	Enteritidis, Typhimurium	Cortez *et al*., 2006
South India	Egg	38/492 (7.7%)	Enteritidis	Suresh *et al*., 2006
Jordan	Chicken, meat	25/93 (26.9%)	Enteritidis, Typhimurium	Malkawi and Gharaibeh, 2004
Malaysia	Selom	16/43 (37.2%)	Weltevreden,	Salleh *et al*., 2003
	Pegaga	8/26 (30.8%)	Agona,	
	Kangkong	8/25 (32%)	Senftenberg,	
	Kesum	8/18 (44.4%)	Albany	
Albania	Chicken meat sample	30/461 (6.5%)	Enteritidis	Beli *et al*., 2001
India	Fish	104/730 (14.3%)	Weltevreden,	Hatha and
	Crustacean	48/276 (17.4%)	Typhi, Paratyphi B, Mgulani, Typhimurium	Lakshamanaperumalsamy, 1997
Malaysia	Retail poultry	158/445 (35.5%)	Enteritidis,	Rusul *et al*., 1996
	Litter	8/40 (20.0%)	Muenchen,	
	Poultry farm	2/10 (20.0%)	Kentucky, Blockley	
Malaysia	Chicken portion	13/33 (39.4%)	Blockley,	Arumugaswamy *et al*., 1995
	Chicken liver	6/17 (35.3%)	Enteritidis,	
	Chicken gizzard	8/18 (44.4%)	Chincol,	
	Cooked meat, chicken	4/28 (14.3%)	Muenchen, Agona	
	Vegetable	14/60 (23.3%)		
	Safay	4/16 (25%)		
	Prawn	2/19 (10.5%)		
	Oriental shrimp paste			

(Source: Pui *et al*., 2011)

10. CONCLUSION

The prevalence of diseases caused by salmonella has indeed assumed a public health dimension, salmonellosis is one of the leading cause of diarrhea diseases globally and is directly associated with poor water hygiene and availability coupled with contamination of food. In Nigeria, Typhoid fever caused by *Salmonella spp* is a major cause of death, second only to malaria. Current clinical diagnosis of typhoid fever using widal test relying on the antigen-antibody agglutination is often not reliable and hence leading to false test results. Given the high incidence of diseases caused by Salmonella, it is necessary to understand wholly this highly virulent and pathogenic organism, its epidemiology and pattern of spread with a view to promoting better and early detection which certainly would spare mankind the stress and burden of these diseases. Molecular techniques which will guarantee quick and reliable diagnosis should also be introduced and adopted by hospitals, diagnostic laboratories and other health professionals as a means for better health service delivery. It is also recommended that *Salmonella* research institutes and organizations be established as a means of broadening general understanding of this organism and the various diseases it causes.

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