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Typing of *Salmonella* Species: A Mini-Review

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Abstract
Nontyphoidal salmonellae are among the foremost bacterial pathogens implicated in food-borne gastroenteritis worldwide. *Salmonella* subtyping is one of the most common methods infer potential sources of human and animal *Salmonella* infection. Various typing methods for the analysis of *Salmonella* have been developed during the last decade. Because of their high discriminatory power, these methods have been increasingly used even in routine diagnostics. The description of several independent genotypic characteristics in addition to that of the phenotypic properties enables a complex and exact identification of *Salmonella* strains. This review summarizes the available *Salmonella* subtyping technique and discusses the use of conventional phenotypic and molecular typing methods for epidemiological study of *Salmonella.*

1. Introduction

Salmonellosis is an important public health problem causing substantial morbidity and mortality, and thus also has significant economic impact worldwide. The incidence of typhoid fever has decreased in recent years. Food-poisoning caused by non-typhoidal *Salmonella* strains has now reached higher proportions in many countries despite improvements in hygiene and sanitation (CDC, 2009). Nontyphoidal *salmonellae* are one of the most important food borne pathogens, leading to millions of cases of enteric diseases, thousands of hospitalizations and deaths worldwide each year (CDC, 2009; Dunkley *et al*., 2009). The strain-typing methods for bacterial pathogens play an important role in understanding infectious-disease transmission, tracking, and distribution. It has been used widely to distinguish *Salmonella* clinical isolates recovered from humans, animals and food-borne disease (Gudmundsdottir *et al*., 2003; Kubota *et al*., 2005).

Phenotypic methods are traditional typing methods for discriminating between bacteria from a single species based on phenotypes, such as serotyping, phage typing, biotype and antibiogram. Therefore, the use of molecular subtyping techniques for the genetic fingerprinting and grouping of *Salmonella* based on their genotypic characteristics is now becoming usual in laboratories (Hunter *et al*., 2005). These methods include PFGE, ribotyping, random amplified polymorphic DNA, amplified fragment length polymorphism and multisequence locus typing (Yan *et al*. 2004; Laconcha *et al*. 1998). This mini-review will focus on the use of conventional phenotypic typing methods, as well as of the most commonly applied molecular typing methods in the context of their uniqueness and potential application in *Salmonella* typing.

2. *Salmonella* typing and subtyping

2.2. Phenotypic methods for Salmonella typing

2.2.1. Serotyping

Salmonella serotyping plays an essential role in determining species and subspecies. Initial step for routine diagnosis of strains and this can be done with commercially available poly and monovalent antisera. The genus *Salmonella* has been identified and classified to have over 2500 serovars by Kaufmann-White scheme. This technique remains as a paramount for differentiating members of the *Salmonella* genus following biochemical identification. In this method, a series of antisera was used to detect different antigenic determinants such as somatic (O), capsular (Vi) and flagellar (H) antigens on the surface of bacterial cell (Tenover *et al*., 1997). The O antigen is a polysaccharide of a cell surface lipopolysaccharide and designated by numbers. In most clinical laboratories, initial serotyping is done using polyvalent O antisera to allow *Salmonella* isolates to be grouped into different O groups designated in capitalized latter. For example *Salmonella* Typhimurium belongs to group B and *Salmonella* Enteritidis to group D. Many *Salmonella* show diphasic production of flagellar antigens and each strain can spontaneously and reversibly vary between these two phases with different sets of H antigens (Brenner *et al*., 2000). Classically, serotyping was use to divide *Salmonella* into distinct species based on their serotypes, given rise to one species per serotype (Dauga *et al.,* 1998). Of the two species of *Salmonella*, *S*. enterica and *S*. bongori, over 99% of serotypes are grouped into species *S*. enterica, and nearly 60% of them belong to the subspecies enterica (subspecies I) (Brenner *et al*., 2000; Brenner & McWhorter-Murlin, 1998). Serotyping has a wide acceptance as a method to differentiate *Salmonella* strains, and it is an important tool in

public health. This traditional serotyping method has many limitations. It is based on the use of expensive antisera; also the procedure is time consuming, requires well-trained technicians, and some isolates are not typeable.

2.2.2. Phage-typing

This typing method has proven to be epidemiologically valuable in strains differentiation within a particular *Salmonella* serotype. In this subtyping approach, *Salmonella* strains are separated into different phage types based on their reactivity against a set of serovars specific typing phages. This technique has been developed for some relevant serovars (Oslen *et al*., 1993). This method is cheap and does not require specific equipment. It requires great experience in interpretation of results and is not always fully reproducible between laboratories (Ross and Heuzenroeder, 2005). Phage typing represents a valuable method in characterizing isolates that exhibit less common phage reaction pattern. This method is inadequate in the investigation of the same pahge-types (Hopkins et al., 2011). Despites the drawback, phage typing still represents a valuable technique for an initial evaluation of the potential relatedness among isolates, in the source studies investigating *S*. Enteritidis and *S*. Typhimurium.

2.2.3. Antimicrobial resistance typing

This typing technique determines the profile of resistance of a microbial strain towards a panel of an antimicrobial agent. Antimicrobial susceptibility testing is usually carried out to determine which antibiotic is effective in treating bacterial infection in vivo. It has been quite commonly used in the past as subtyping method to determine correlation between isolates. Nowadays, antimicrobial resistance typing is less used frequently for this specific purpose. This technique is cheap and does not require specific equipment and reagents like phage-typing. Antibiogram has poor discriminatory power because antimicrobial resistance is under selective pressure and often is associated with mobile genetic elements and strains which are epidemiologically related may have different antimicrobial susceptibility due to loss of plasmids or the acquisition of new genetic material (Davies, 1997; Tenover et al., 1997).

2.3. Genotypic methods for Salmonella typing

2.3.1. Pulsed field gel electrophoresis

In 1984, Schwartz and Cantor came up with the idea and principles of developing PFGE, which has become most famous and frequently used molecular typing tool in molecular epidemiology due to its highly discriminatory nature (Schwartz & Cantor, 1984). It has also been used in genetic and epidemiological analyses of more than 98 different pathogens comprising both gram negative and gram positive bacteria and other fungal organism (Goering, 1998). PFGE is a molecular typing method, which was adopted more than a decade ago for fingerprinting strains in an outbreak situation and is easy to perform (Garaizar *et al.,* 2000; Murase *et al.,* 2004). Many reports indicate that PFGE is widely used in tracking the source of *Salmonella* infections for different serovars and are well discriminatory in nature (Best *et al.,* 2009; Dionisirt *et al.,* 2006). PFGE typing is more time and labor-intensive, with lack of sensitivity for different serovars (Kariuki *et al.*, 2010). Also, with procedures and standardization of the protocols, it is necessary to ensure analysis reproducibility. The CDC has established a PulseNet network which helps in facilitating inter laboratory comparisons of different PFGE patterns obtained in different regions or countries of a given isolate, through a set of a programmed database (Pierre *et al.,* 2011). Historically, PFGE is one of the earliest molecular DNA subtyping systems, showing the pattern of fingerprinting for *Salmonella* strains which is suitable as an epidemiological tool for investigating outbreaks. Moreover, it is considered as a gold standard for molecular typing of *Salmonella* and many other bacterial pathogens (Pierre *et al.,* 2011).

2.3.2. Ribotyping

This is a genotyping method that can clarify and classify bacteria on the basis of differences in rRNA. This method is known as rRNA gene restriction pattern determination or ribotyping. It involves digestion of bacterial DNA by a common restriction endonuclease and many fragments are generated. After gel electrophoresis and transfer of the fragments to a membrane, they are probed with a region of the rRNA operon to reveal the patterns of rRNA genes. By using 16S plus 23S rRNA as a probe, a seventeen simpler pattern is formed (Grimont *et al*., 1986). It results in profiles consisting of only a small number of bands, which are quite simple to interpret. For bacterial typing, ribotyping has a relatively low discriminatory power and for that reason, it has not been commonly used for the outbreak epidemiological investigation (Olsen, 2000). Moreover, due to its complexity and technicality, it is not a preferred strain typing method for bacterial pathogens (Tenover *et al.,* 1997). This technique has low discrimination and may not be good for surveillance studies or epidemiological findings (Riley, 2004; Aarts *et al.,* 2001).

2.3.3. Randomly amplified polymorphic DNA

This technique was first described by Williams *et al.,* (1990) and Welsh and MacCelland (1990). It is a type of PCR with a random DNA segment amplification. This method is based on the use of short random sequence primers (8-12 nucleotides), there by proceeds with PCR using large template of genomic DNA. The RAPD is not as traditional PCR techniques, because it does not need any specific knowledge of the DNA sequence of the target pathogens. The 10-mer primers are identical and for amplification to occur at the DNA segment, it is dependent on positions that are complementary to the primers sequence. A number of studies have reported success in using RAPD assays to differentiate *Salmonella* serovars or to distinguish strains within single serotypes. Such results have been obtained for *Salmonella* Typhimurium, *Salmonella* Enteritidis and *Salmonella* Panama (Carraminana *et al.,* 1997; Betancor *et al.,* 2003; Soto *et al.,* 2001). The advantage of RAPD is that, it is simple to perform, widely applicable and does not require previous knowledge of the genome. For a first-screen typing method, RAPD is generally considered to be an excellent choice (Williams *et al.,* 1999). The main demerit of RAPD analysis is low reproducibility and difficulties occur in relating the different patterns.

2.3.4. Restriction fragment length polymorphisms

In this technique, extracted chromosomal DNA of the bacteria is digested with a restriction enzyme and the fragments are separated by agarose gel electrophoresis. The separated DNA fragments are then transferred (blotted) onto a nitrocellulose or nylon membrane (Southern, 1975). The membrane bound nucleic acids is then hybridized to one or more labeled probes homologous to the examining genes. The probes are labeled with a number of detectable moieties, including enzyme-colorimetric substrates or enzyme-chemiluminescent substrates. The differences in the number and sizes of the fragments detected by hybridization are known as RFLPs. This technique was first used to study the diversity of *Salmonella* strains Dublin, Enteritidis and Typhimurium, using two randomly cloned chromosomal fragments of serovars Enteritidis. The strains were grouped together by antibiograms, plasmid profiling and phage typing also showed similar hybridization patterns by RFLP (Tompkins *et al.,* 1989). The discriminatory power of this technique is proportional to the identical numbers of targeted genetic elements in the bacterial genome and their distribution among the restriction fragments after electrophoresis.

2.3.5. Plasmid fingerprinting

Plasmid fingerprinting provides a rapid and dependable means of identifying bacterial isolates of the same strain. The stability, wide distribution, and diverse nature and size of extra-chromosomal elements make it suitable for virtually all bacterial genera (Tenover, 1985). Plasmid fingerprinting was the first molecular techniques to be used as a microbial typing tool (Taylor *et al*., 1982). A plasmid is an extra-chromosomal DNA molecules separate from the chromosomal DNA. In many instances, it is circular, double-stranded and usually found in bacteria. It is particularly important, since most of the plasmids harbor virulence and antimicrobial resistance properties in *Salmonella*. The plasmids found in *Salmonella* differ in size 2-200kb with different functionalities (Porwollik & McClelland, 2003; Rychlik et al., 2006). This because plasmids are not stable and may lose or acquired by strains and some organisms contains few or no plasmids, this technique is not useful for subtyping *Salmonella*

3. Conclusion

Salmonella consists of a variety of serotypes that have a wide host range due to their versatile pathogenic potentials. *Salmonella* source attribution through microbial subtyping requires a collectection of isolates representative of the real exposure of humans to the micro-organism. Non-human isolates are compared to the human ones by using discriminative subtyping techniques in order to identify overlapping profiles between them. Traditional methods of strain identification based on serotyping and phage typing have been an underlying basis of *Salmonella* epidemiology many decades ago. Genotypic typing methods are capable of providing detailed strain characteristics which are far beyond the capacities of phenotypic typing methods. Such molecular-based characteristics have proved to be very helpful in epidemiological studies of *Salmonella* strains. In many studies, the results demonstrate that the use of combination methods enables easier discrimination among strains, mostly when trying to separate isolates that appear clonal by single typing method. The combinational approach has proved to be essentially valuable in many public health settings where the determination of the origin of *Salmonella* is required for intervention measures. It would be advisable to use highly discriminatory subtyping method and, when possible, multiple methods in parallel to get a clear picture of the differences between the strains. Another critical issue is the implementation of effective national and international networks combining laboratories that cover the populations of interest and regularly share epidemiological information of the subtyping data.

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