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Antibiotic resistance and molecular characterization of seafood isolates of nontyphoidal *Salmonella* by PFGE

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Abstract

Emergence of multidrug resistant nontyphoidal *Salmonella* is a major health concern worldwide due to the predominant occurrence of *Salmonella enterica* sub-species *enterica* serovar Typhimurium phage type 104 (DT104) conferring resistance to ampicillin, chloramphenicol, streptomycin, sulphonamide and tetracycline. Apart from antibiotic resistance, the identification and genotypic characterization of pathogens is essential for epidemiological surveillance and outbreak investigations. In this study 39 isolates of *Salmonella* obtained from seafood samples were examined for their susceptibility to various antibiotics and subjected to PFGE analysis using the restriction enzyme *Xba*1. The highest percentage resistance was for erythromycin (100%) followed by nalidixic acid (15.38%), co-trimoxazole (15.38%), chloramphenicol (12.82%), ampicillin (12.82%) and tetracycline (10.25%). Six (15.38%) of the 39 isolates were multidrug resistant. The *Xba*I digested chromosomal DNA generated 7 clusters suggesting the presence of diverse *Salmonella* strains in seafood. The Discriminatory Index for PFGE obtained by *Xba*I restriction enzyme was 0.91. The PFGE has been found highly discriminatory for subtyping *S.* Weltevreden and *S.* Newport. The *Xba*I PFGE was not only discriminatory but could also distinguish multidrug-resistant strains from the sensitive ones as the two groups they belonged to different pulsotypes. The study also demonstrated multiple clones of *S.* Weltevreden, *S.* Newport and *S.* Oslo present in seafood from the south west coast of India. Genetic diversity among the similar seafood sources suggests the presence of different clones of *Salmonella* which further increases the risk of seafood being a potential source of highly pathogenic bacteria like *Salmonella*.

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Keywords:Salmonella; pulse field gel electrophoresis; antibiotic resistance

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1. Introduction

Emergence of multidrug resistance nontyphoidal *Salmonella* isolates is a major health concern worldwide with the most predominant occurrence of *Salmonella* Typhimurium phage type 104 (DT104) resistant to ampicillin, chloramphenicol, streptomycin, sulphonamide and tetracycline. Agricultural use of antibiotic increases the risk of development of antibiotic resistant zoonotic pathogens such as *Salmonella*[1]. In the recent years emergence of fluoroquinolone resistant *Salmonella* and extended spectrum β-lactam producing strains are of particular concern. Apart from antibiotic resistance the identification and genotypic characterization of pathogens are essential for epidemiological surveillance and outbreak investigations. The molecular subtyping of bacterial strains has become an essential component of outbreak investigations augmenting the identification and trace back of clusters suspected to originate from foods, the environment or nosocomial sources[2]. Epidemiological analysis of *Salmonella* from humans and animals by arbitrarily primed PCR, plasmid profiles and pulse-field gel electrophoresis (PFGE) has been well documented[3]. Pulse-field gel electrophoresis is a technique used for the separation of large DNA molecules by applying to a gel matrix an electric field that periodically changes direction. It is currently considered the gold standard method for subtyping foodborne pathogens[4]. Pulse-field gel electrophoresis (PFGE) has significantly reduced the amount of time required to complete investigations by greatly improving the accuracy of pinpointing sources of foodborne outbreaks due to bacterial pathogens. Providing the evidence of molecular genetic relatedness of two or more strains to the outbreak investigators is the primary epidemiological application of PFGE[5]. It also serves as a basis in the PulseNet national subtyping network for foodborne diseases and has entrenched itself as a powerful tool in the molecular epidemiologic linking of strains during trace back of clusters obtained from different sources. Many studies have been investigated the clonal diversity between or within the different serovars of *Salmonella* by using this typing technique[6,7]. But the PFGE studies on seafood associated nontyphoidal *Salmonella* is limited. Currently PFGE data are considered reliable and a sensitive way to detect differences between closely related strains, so that isolates with indistinguishable PFGE profiles can be classified as epidemiologically linked with a high degree of confidence[4].

2. Methodology

In this study 39 isolates of *Salmonella* obtained from seafood samples were examined for their susceptibility to various antibiotics and subjected to PFGE analysis using the restriction enzyme *Xba*1.Pulse-field gel electrophoresis was performed according to the Standardized Laboratory Protocol for Molecular Subtyping of *Salmonella* by PFGE (Pulse-Net, CDC, Atlanta, USA) (CDC 2012) with slight modifications. Briefly, *Salmonella* cells grown in 5ml LB broth was pelletized and transferred to 2 ml of Cell Suspension Buffer and the concentration of cell suspensions adjusted to 0.8-1.0 OD in a spectrophotometer. 200µl of the cell suspension is then mixed with 20mg/mL of proteinase K and 200µl of 2 % clean cut agarose (Bio Rad, CA, USA) and pipetted into disposable plug moulds (CHEF-DR II, Bio-Rad , CA, USA). After solidification the plugs were removed from the moulds and lysed by using cell lysis buffer. The samples were incubated in a 54 °C shaking water bath for 1.5-3 h with constant vigorous agitation (150-175 rpm). The tubes were removed from the water bath and the lysis buffer was discarded. The plugs were then washed two times with 10-15 ml of sterile ultrapure water (pre-heated to 54 °C) in a 54 °C water bath for 10-15 min with constant agitation. This was followed by four washes with 10-15 ml of sterile 1X TE buffer, pre-heated to 54 °C as described above. After the last wash, 5 ml of sterile 1X TE buffer (room temperature) was added to each tube to serve as storage media for the plugs. The plugs were restricted immediately or stored in 1X TE buffer at 4 °C until use. DNA was then digested with 40 U of restriction enzyme Xba1 (5'-TCTAGA-3') / plug size (Fermentas, U.S.A.) at 37 ºC. The restriction fragments were separated by electrophoresis in 0.5× TBE buffer, for 19 h at 14 ºC in a CHEF Mapper system (Bio-Rad, U.S.A.) using pulsed times of 2.2 to 63.8 s. PFGE data were analyzed using Gel Compar software (BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium)). The relation between the strains was scored by the Dice coefficient of similarity, and strains were clustered by the hierarchical clustering inter-strain similarities based on the unweighted pair group method with arithmetic averages (UPGMA).

3. Results