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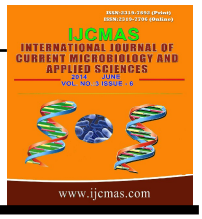

Original Research Article

Determination of Antibiotic resistance and Molecular characterization of *Salmonella sp* isolated from Poultry samples

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Keywords

Salmonella sp, Antibiotic Resistance, RAPD, Molecular Markers

A B S T R A C T

The contamination of food products with *Salmonella* generates serious health and economic consequences, which have stimulated numerous studies designed to investigate the survival and transmission of these organisms in farm animals and the environment. The present study aims at isolating *Salmonella sp* and determining the resistant patterns to different antibiotics and variations in the genetic diversity. Blood samples were collected from different poultry outlets and plated on to Brilliant Green Bile Agar and isolated pink colonies were sub cultured onto nutrient agar medium and maintained. Salmonella were identified based on their morphological and biochemical characterization. Antibiotic resistance was determined by preparing lawn cultures on MHA plates and placing standard antibiotic discs to check for zones of inhibition. DNA was isolated for molecular characterization and subjected to PCR amplification using random primers. In the present study of the 48 poultry samples tested for Salmonella 17 were positive, which were further subjected to antibiotic sensitivity. Most of the isolates were found resistant to the antibiotics used in the study. The isolated DNA when subjected to RAPD using different primers yielded multiple bands for OPA-8 and OPA-20. Phylogenetic variation were determined.

Introduction

Salmonella infections occur worldwide in both developed and developing countries and are a major contributor to morbidity and economic costs. The contamination of food products with *Salmonella* generates serious health and economic consequences, which have stimulated numerous studies designed to investigate the survival capacity and the transmission routes of these organisms in different farm animals and the environment (Sow *et al*, 2000, Winfield *et al.*, 2003). *Salmonella* is found worldwide in both cold-blooded and warm-blooded animals, and in the environment. They cause illnesses such as typhoid fever, paratyphoid fever, and food poisoning. *Salmonella* infections are zoonotic and can be transferred between humans and other animals. Many infections are due to ingestion of contaminated food (Ryan 2004). *Salmonella* can colonize and cause disease in a variety of animals. Within this genus, more than 2,500 serovars have been

described (Popoff *et al.,* 2003, Graziani *et al.,* 2008). Although all serovars may be regarded as potential human pathogens, the majority of infections are caused by a very limited number of serovars. Since these pathogens are transmitted primarily through contaminated food or water, the presence of strains in animals and ultimately in raw meat products has important public health implications (Butaye *et al.,* 2006).

The antibiotic of choice for many years was chloramphenicol, but like many pathogens in today's world, chloramphenicol- resistant strains have emerged (Scallan *et al.,* 2011). Additionally, many strains have developed resistance to ampicillin and trimethoprim/sulfamethoxazole, which are considered appropriate alternatives to chloramphenicol. Indeed, resistance to each of these '¢rst line' antibiotics is often plasmid encoded and strains harboring a plasmid encoding resistance to all three antibiotics have been isolated in Southeast Asia (Helms *et al.,* 2002). In many cases, plasmids responsible for the resistance belong to the incompatibility complex group IncHI. Patients harboring these multidrug-resistant strains have been successfully treated with fluoroquinolones, but drug resistance to fluoroquinolones has emerged. Spread of antibiotic resistances through agro-food chain remains an actual question for both researchers and public health operators.

To investigate the origins and the relationships among different isolates, more precise methods than serotyping are needed. Methods used for the subtyping of *Salmonella* include antibiotic susceptibility, pulsed-field gel electrophoresis typing (PFGE), phage typing, plasmid profiling, ribotyping, and randomly amplified polymorphic DNA analysis (Antoine et al., 2008). The molecular typing methods such as the REP-PCR (Weigel *et al.,* 2004, Rasschaert *et al.,* 2005) and RAPD-PCR methods can be used for the differentiation and characterization of *Salmonella* and to trace the clonality of strains (Versalovic *et al.,* 1991).

Among molecular techniques, PFGE is currently considered one of the most reliable typing procedures. This method is a well established and highly effective epidemiological tool for the molecular analysis of large fragments generated by restriction endonuclease digestion of genomic DNA. Very recently, the use of PFGE with endonuclease *Xba*I has been widely recognized as a sensitive means of fingerprinting *Salmonella* serovars and it has become a reference method (Michael *et al.,* Harbottle *et al.,* Herrero *et al.,* 2006).

Materials and Methods

Sample collection

A total of 15 blood samples were collected in sterile Ziploc packs from different poultry retail shops. They were stored at 4°C for further use.

Isolation of *Salmonella sp*

The samples were labeled and swabbed on nutrient agar using sterile cotton swabs. The plates were incubated at 37 °C overnight. Gram negative colonies were streaked on to Brilliant Green Bile Agar which is selective for Salmonella sps to check for characteristic growth of pink colonies. The pure cultures were cultured on nutrient agar slants and subjected to biochemical test as per Bergye's manual of determinative Bacteriology.

Antibiotic sensitivity test

Kirby-Bauer antibiotic testing method (Disc diffusion method) was followed to test for antibiotic sensitivity of the isolated strains.

100 µl of the broth culture was swabbed using a sterile swab to form a bacterial lawn onto the Muller Hinton Agar plates and the plates were allowed to stand still for diffusion for approximately 30 minutes. Antibiotic Disc Dispenser was used to dispense disks containing specific antibiotics onto the plate. 4 antibiotics namely Amoxicillin, Ampicillin, Tetracycline and Gentamycin were used. Using a flame-sterilized forceps, gently press each disc to the agar to ensure that the disc is attached to the agar. Plates are incubated overnight at 37°C and the zone of inhibition was measured.

DNA Isolation

The isolated bacterial samples were cultured on LB broth and incubated for 24-48 hours. The culture was centrifuged at 10,000 RPM for 10 minutes, after removing the supernatant the pellet was dissolved in 1 ml extraction buffer (100 mM Tris, 10 mM EDTA and 250 mM NaCl, pH= 8.0 and 1% Sodium Dodecyl Sulfate) and incubated in dry bath at 60^0 C for 30 min. After centrifugation at 6,000 rpm for 5 min, supernatant was treated with equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) and centrifuged at 8,000 rpm for 5 min.

DNA was precipitated using equal volume of chilled isopropanol. The DNA samples were tested qualitatively on 0.8% agarose gel and quantified by using a Nanodrop spectrophotometer

PCR

A set of 10 decamer oligonucleotides was used in the present study as single primers (OPA series) in the Polymerase Chain Reaction. The polymerase chain reaction was carried out in a final volume of 25 µl containing 100 ng DNA, 3 U of Taq DNA polymerase (Chromous Biotech, Bangalore), 2.5 mM each dNTPs (Chromous Biotech, Bangalore) and 100 pmol of primers (Eurofins Genomics, Bangalore). The DNA amplification was performed in the Corbett RG 6000 thermo cycler using the following conditions: complete denaturation (94°C for 5 min), followed by 35 cycles of amplification (94°C for 45 sec, 34°C for 1 min and 72°C for 1 min) and the final elongation step (72°C for 5 min). Total volume of the amplified product (25µl) of each sample was subjected to electrophoresis on 2.0 % agarose gel containing ethidium bromide in 1x TBE buffer at 120 V for 1 h.

Data Analysis

Total volume of the amplified product (25µl) of each sample was subjected to electrophoresis on 1.5 % agarose gel containing ethidium bromide in 1xTAE buffer at 100V for 1 hr. Finally, the DNA bands were observed on a Gel Doc system and the photographs were captured. The RAPD profiles were analyzed based on the presence or absence of individual RAPD bands. The genetic distance was calculated by the coefficient of Frequency similarity. The matrix of genetic distance was used for grouping the bacterial samples based on the dendrogram constructed by UPGMA (Unweighed Pair Group Method with Arithmetic averages) cluster analysis to produce a phylogenetic tree.

Results and Discussion

Isolates that were suspected to be *Salmonella sp*. appeared as pink colored colonies on Brilliant Green Bile agar. 7 such isolates were selected, further purified and maintained on Nutrient Agar slants for further use (figure-1). The selected isolates were found to be Gram negative rods on Gram's staining. Based on the colony, cell morphology and biochemical characterization as per Bergye's manual of determinative Bacteriology the isolates were identified as *Salomonella sp.*

DNA was isolated from the selected strains using phenol: chloroform method and was further quantified using NanoDrop Spectophotometer**.** The qualitative analysis of DNA was done by agarose gel electrophoresis (figure-2). The obtained DNA showed sharp single bands on 0.8% agarose gel.

The samples were amplified with ten arbitary primers the two (OPA-8 and OPA-20) (figure- 3 and 5) which showed the maximum clear banding pattern was used further for dendogram analysis Figure- 4 and 6). By observing the banding pattern, the no. of polymorphic and monomorphic bands were calculated. A total of 30 bands were obtained using prime OP A8. Among which, 30 % were monomorphic, 50 % were polymorphic and 20 % unique.

Similarly for primer OP A20 observing the banding pattern, the no. of polymorphic and monomorphic bands were calculated. A total of 40 bands were obtained using primer OP A20. Among which, 35 % were monomorphic, 55 % were polymorphic and 10 % unique.

Figure.1 Colonies showing pink colour on BGB Agar plates

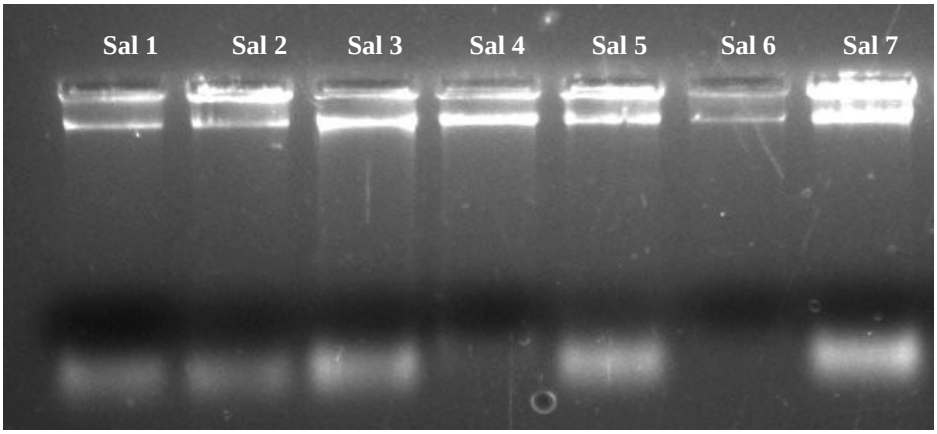

Figure.2 DNA bands on Agarose gel

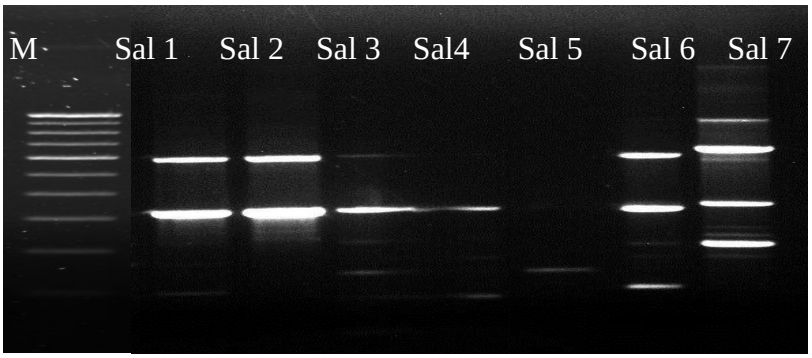

Figure.3 DNA fingerprinting result OPA-8 primer

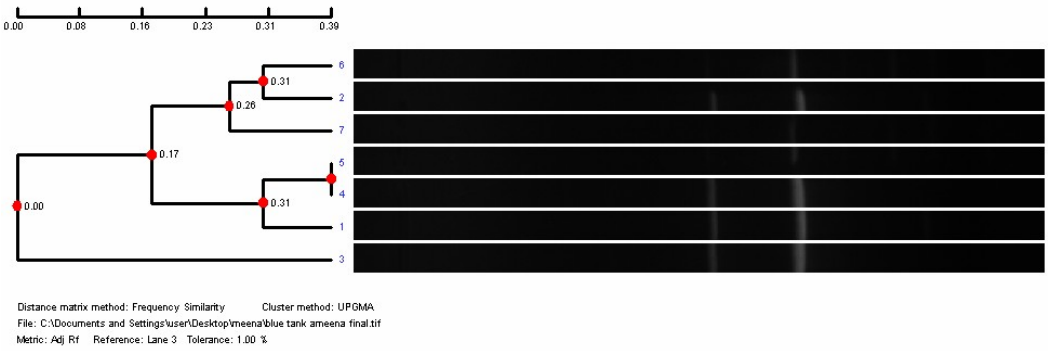

Figure.4 Dendrogram analysis for OPA-8 primer

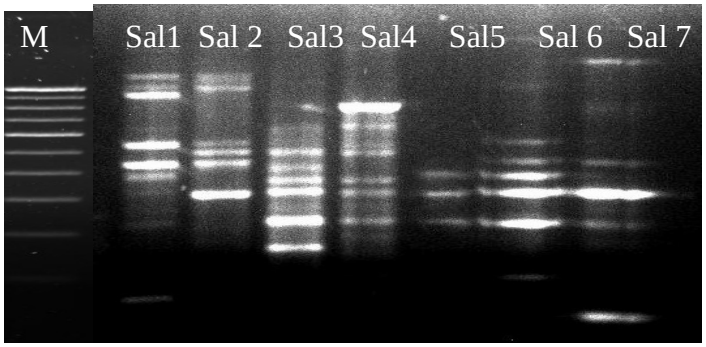

Figure.5 DNA fingerprinting result OPA-20 primer

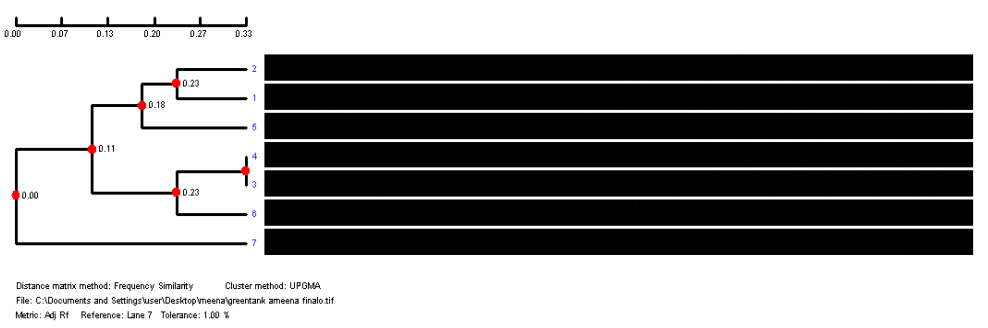

Figure.6 Dendrogram analysis for OPA-20 primer

The UPGMA dendrogram was constructed based on the similarity matrix indicating the diversity among *Salmonella sp*. isolated from various poultry shops. Dendrogram obtained using primer OPA-8 RAPD data, strain S4 and S5 showed 100 percent similarity and strain S1 is closely related to them. The dendrogram obtained using primer OPA-20 RAPD data, strain 3 and strain 4 showed 100 percent similarity and strain S6 is closely related to them. The degree of polymorphism observed gives us an understanding about the genetic variability among the different *Salmonella* strains.

References

Antoine ST, Annaelle K, Anne B 2008. Epidemiological analysis of *Salmonella* enterica from beef sampled in the Microbiol 123: 191-197.

Butaye P, Michael G, Schwartz S, Barett T, Brisabois A, White D 2006. The clonal spread of multidrug-resistant non-typhi*Salmonella* serotypes. J Microbes Infect 8: 1891–1897.

Graziani C, Busani L, Luzzi I 2008. Antimicrobial resistance in *Salmonella enterica* serovar Typhimurium from human and animal sources in Italy. J Vet Microbiol 414–418.

Harbottle H, White D, McDermott P, Walker R, Zhao S 2006. Comparison of multilocus sequence typing, pulsed-field gel electrophoresis, and antimicrobial susceptibility typing for characterization of *Salmonella* enterica serotype Newport isolates. J ClinMicrobiol 44: 2449–2457.

Helms, M.P., Vastrup, P., Gerner-Smidt, P., Molbak, K., 2002. Excess mortality associatedwith antimicrobial drug-resistant Salmonella Typhimurium. Emerg.Infect. Dis. 8, 490-495.

Herrero A, Rodicio M, Gonzalez-Hevia M, Mendoza M 2006. Molecular epidemiology of emergent multidrug-resistant *Salmonella* enterica serotype Typhimurium strains carrying the virulence resistance plasmid pUO-

StVR2. J AntimicrobChemother 57: 39–45.

Michael GB, Cardoso M, Schawarz S 2006. Molecular analysis of *Salmonella* enterica subsp. Enterica serovar Agona isolated from slaughter pigs. J Vet Microbiol 112: 43-52.

Popoff MY, Bockemuhl J, Gheesling L 2003. Supplement to the Kauffmann-White scheme. J Res Microbiol 154: 173-174.

Rasschaert G, Houf K, Imberechts H, Grijspeerdt K, Zutter L, Heyndrickx M 2005. Comparison of five repetitive-sequence-based PCR typing methods for molecular discrimination of *Salmonella* enterica isolates. J ClinMicrobiol 43: 3615–3623.

Ryan KJ, Ray CG (editors) 2004. *Sherris Medical Microbiology* (4th ed.). McGraw Hill. pp. 362–8. ISBN 0-8385-8529-9

Scallan E, Griffin PM, Angulo FJ,Tauxe RV, Hoekstra RM. 2011. Foodborne illness acquired in the United States--unspecified agents. Emerg Infect Dis. 2011 Jan; 17(1):16-22.

Sow AI., Seydi M., Thiaw M., Ndour C, Soumare C, Cisse M., Badiane S, Samb A 2000. Les salmonelloses au centrehospitalieruniversitaire de Fann à Dakar: aspects bactériologiques. J Med Mal Infect 30: 657–660.

Versalovic J, Koeuth T, Lupski J 1991. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. J Nucleic Acids Res 19: 6823–6931.

Weigel RM, Qiao B, Teferedegne B, Suh D, Barber D, Isaacson R, White B 2004. Comparison of pulsed field gel electrophoresis and repetitive sequence polymerase chain reaction as genotyping methods for detection of genetic diversity and inferring transmission of *Salmonella*. J Vet Microbiol 100: 205–217.

Winfield, MD, Groisman, E 2003. Role of non-host environments in the lifestyle of *Salmonella* and *Escherichia coli.* J Appl Environ Microbiol 69: 3687–3694.