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Comparison of Different Typing Methods in Salmonella Strains

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Abstract: *Salmonella* serotypes are the most important cause of food poisoning due to different foodstuffs. In this study, three methods including serotyping, antimicrobial resistance pattern and plasmid profile analysis (PPA) were determined and compared. 50 isolates of *Salmonella* obtained from food products sending to veterinary organization of Tehran province and Pasteur veterinary laboratory were studied. Serotyping was performed using Mast Diagnostic kit. Antimicrobial resistance pattern was carried out by Kirby and Bauer disk diffusion method according to the NCCLS criteria. Plasmids were extracted by using Takara kit (Takara, Japan) and electrophoresed on 1% agarose gel. 50 *Salmonella* isolates belonged to 11 serotypes and showed six different antimicrobial resistance patterns. These strains contained one to four plasmids and indicated four different plasmid profiles. All the serotypes were carrying a 3.036 MD plasmid and all of them were resistant to Ampicillin and Streptomycin. Serotyping distinguished more strains in compare with the two other methods. Since the determined serotypes contained few plasmids, PPA could not apply for typing efficiently. Moreover, PPA showed that all the strains contained a 3.036 MD plasmid, which can be considered as a common characteristic of *Salmonella* strains isolated from foodstuffs in the studying area.

Key words: Salmonella · Antisera · Multi-drug resistance · Resistotyping · PPA

INTRODUCTION

Among Gram negative bacteria causing enteritis due to food products, the most important organisms belong to the members of *Salmonella* genus. These organisms are spread widely in nature and their major sources are human and foods. Food poisoning due to *Salmonella* initiates with eating foods which include specific serotypes of this genus in sufficient amount. The main habitat of *Salmonella* serotypes is animal's bowel such as birds, reptiles, farm animals and human. These organisms are excreted along with feces and contaminate water and soil. When waters or food products which are contaminated by insects or other intermediates, are consumed by human or animals, these organisms are spread again by feces via continuing of this cycle. Repetition of this cycle by international exchanges of animal products and animal feeding stuffs is the major cause of global spreading of *Salmonella*.[1-3]. An epidemiological tool in analysis of *Salmonella* infections outbreak, identification of the source of infection and distinguishing *Salmonella* strains causing infection is typing of *Salmonella* isolates. Generally, bacterial typing is performed by various methods such as Biotyping, Serotyping, Phagetyping, Resistotyping, Bacteriocintyping and Genotyping. In addition, molecular techniques such as Plasmid Profile Analysis (PPA) are used as valuable tools in epidemiological studies of gram negative bacteria. PPA is utilized in investigation of outbreaks of infectious diseases, identification of infection sources, epidemic strains of bacteria and epidemic plasmids. Also it is applied for typing of bacteria [4]. In Iran most of epidemiological studies on *Salmonella* infections has been limited to serotyping and antimicrobial resistance pattern. So this study was performed in order to determine and compare serotyping, antimicrobial resistance pattern and PPA of *Salmonella* strains isolated from food products.

MATERIALS AND METHODS

Bacterial Strains: 50 *Salmonella* strains were isolated from foodstuffs (meat, chicken, fish,...) which were sent to veterinary organization of Tehran province and Pasteur veterinary laboratory .

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Bacteriology: Preliminary bacteriological and biochemical assays were performed for all samples of food products. Firstly primary suspension was prepared from each sample, pre-enrichment was done in non-selective liquid medium and Lactose broth, buffered peptone water was used for this purpose. Then enrichment was performed in selective liquid broth including Selenit F broth and Tetrathionate novobiocin broth. After that culture on solid selective media including Edel and Kampelmacher, MacConkey Agar and *Salmonella – Shigella* Agar was performed.

TSI, SIM, IMViC and Urea test were carried out as biochemical tests [4-6].

Serological Experiments: Final identification of the isolated *Salmonella* strains was performed by agglutination test using the Mast Diagnostic kit (Mast Group Ltd. Merseyside, UK). Using monovalent and polyvalent antisera existing in this kit and Kauffmann – White table, group and serological variety of bacteria was recognized[6].

Antimicrobial Resistance Pattern: All isolates were routinely tested by the single disk diffusion method described by Kirby and Bauer according to the NCCLS criteria against the following antibiotics. Ampicillin (Am), Streptomycin (S), Ciprofloxacin (Cp), Furazolidone (Fu), Gentamicin (Gm), Chloramphenicol (C), Cefazolin (Cz), Tetracycline (Te) and Trimethoprim – Sulphamethoxazole (SxT) (Padtanteb, Iran). Diameter of the inhibition zones were interpreted based on the NCCLS subcommittee's recommendations [6-8].

Plasmid Analysis: Extraction of plasmid was carried by using Takara kit (Takara, Japan). Extracted plasmids were separated by electrophoresis on a 1% Agarose gel (Roche, Germany) by using TBE buffer (10X stock solution per liter: Tris base 108g, Boric acid 55g, Na_2 EDTA-$2H_2O$ 9.3g) and loading buffer (Bromophenol blue 0.7%, SDS 7% and Glycerol 33%). DNA fragments were visualized by examination in UV light. The sizes of the fragments were determined by running the samples with 100 bp marker [9].

RESULTS AND DISCUSSION

From samples sent to veterinary organization of Tehran province and Pasteur laboratory, 50 strains of *Salmonella* were isolated. All isolated and identified bacteria possessed the morphological and biochemical

Table 1: Antimicrobial resistance patterns of different isolated *Salmonella* strains

No. of resistance Patterns	Resistance patterns
1(4%)	S, Am, Cp, C, Fu, Gm, Te, Cz, SxT
2(16%)	S, Am, C, Fu, Gm, Cz, Te, SxT
3(10%)	S, Am, Fu, Gm, Cz, Te
4(28%)	S, Am, Gm, Cz, Te
5(20%)	S, Am, Te
6(22%)	S, Am

Table 2: Plasmid profiles in *Salmonella* serotypes

No. of plasmid profile	Weights of bands (MD)	Size of bands (kb)
1	3.036	4.6
2	3.036-3.762	4.6-5.7
3	3.036-3.762-4.356	4.6-5.7-6.6
4	3.036-3.762-4.356-4.81	4.6-5.7-6.6-7.3

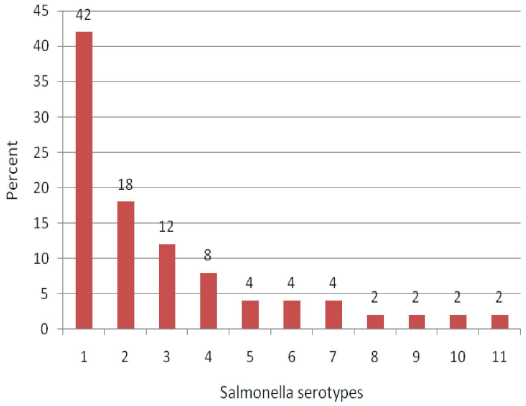

Fig. 1: Percent of Salmonella serotypes
1- S.Durban, 2- S. Nigeria, 3- S.Thompson, 4- S.II C1(z6,z), 5- S.Uno, 6-S.Newport, 7-S.Enteritidis, 8-S.IIC1(z42,gt), 9-S.IIC2(5,1,z29), 10- S.IIC1(-,z29), 11- S.IImjimwema

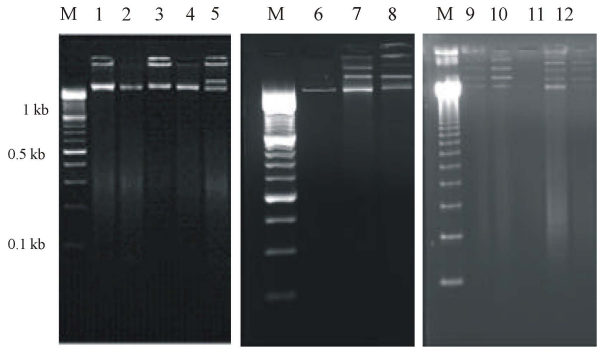

Fig. 2: Four different plasmid profiles, M[100 bp(0.1kb) Marker]-1,2,3,4,6 (one band)-5 (2 band) -7,8,(3 band) 9,10,11,12, (4 band)

characteristics of *Salmonella*. By using Mast diagnostic kit, eleven serotypes of *Salmonella* were identified. The highest percent belonged to *S. Durban* (42%), followed by *S.Nigeria* (18%) and *S.Thompson* (12%) (Figure 1). All fifty isolated strains were resistant to Am and S and the highest rate of sensivity was against Cp, followed by C and SxT. Resistance to three or more antibiotics (Multiple Drug Resistance = MDR) was shown in 58% of the examined strains. 50 *Salmonella* isolates showed six different patterns of resistance which the most common of them was S/Am/Gm/Cz/Te (28%) (Table 1).*Salmonella* serotypes contained one to four plasmids of 3.036, 3.762, 4.356 and 4.81 MD (Table 2). 50 *Salmonella* serotypes showed four different plasmid profile. All serotypes contained a 3.036 MD plasmid and all these serotypes were resistant to Am and S (Figure 2).

Members of genus *Salmonella* are the most important cause of gastroenteritis due to foodstuffs [4, 10-14]. In our study, the 50 isolates of *Salmonella* isolated from different foodstuffs were belonging eleven different serotypes. *Salmonella* serotypes showed high percentage of resistance to the tested antibiotics. They showed six different patterns of resistance. The most common resistance pattern was S/Am/Gm/Cz/Te (28%). Resistance patterns of *Salmonella* strains isolated in our study is compatible with the results of other investigation in different countries [3,4,10-13, 15,16].

Considering that phenotypic characteristics such as biochemical patterns, existence of antigens in the surface of cells and antibiotic resistance patterns intend to modify with any change in the growth conditions, using of these characteristics for epidemiological studies of diseases due to heterogen species is of low value. Today, applying molecular methods such as PPA is necessary for epidemiological studies of heterogen species.

Schuman *et al*. (1985) introduced pasteurized milk, contaminated by *Salmonella typhimurium* as the most important cause of the largest epidemic of *Salmonellosis* until that time in United States. This strain contained four plasmids of 6,10,98,158 kb [17]. Halvo *et al*. (2003) in Norway applied PPA for molecular analysis of *Salmonella* serotypes isolated from fish feed factories and fish feed ingredients and also finding the source of infection [2]. Shelley *et al*. (2002) in Pensilvania determined plasmid pattern of *Salmonella Newport* isolated from animals. From 44 studying serotypes, 37 carried only a 140 kb plasmid and another 11 strains showed three plasmid profiles consisting of two 3-band patterns and one 2-band pattern. Existence of 140 kb plasmid in the majority of the strains indicated epidemiological relationship between them [15]. Baggesen *et al*. (2000) in Denmark compared plasmid profile of *Salmonella typhimurium*, phage-type DT104 isolated from Denmark, Europe and United States. All the strains contained a 95 kb plasmid, which indicated the common source of this strain in different parts of the world [16]. Nanna *et al*, (2002) in Finland determined plasmid profile of *Salmonella agona* isolated from animals, foodstuffs and human. 35% of serotypes were carrying plasmids larger than 20 kb and 15% of them plasmids with six different profiles [12]. *Salmonella* serotypes in our study contained one to four plasmids and showed four different plasmid profiles. All the serotypes carried a 3.036 MD plasmid.

In plasmid profile analysis the number and size of plasmids is a way for better identification of bacterial strains. Application of plasmid pattern for strains containing multiple plasmids is more useful. When the number of plasmids was fewer than three, the power of plasmid pattern for distinction of strains decreased. In our study, the number of plasmids in isolated strains was few and PPA could not apply for typing of them but all the serotypes contained a 3.036 MD plasmid, which indicates epidemiological relationship between them. Moreover, all the strains were resistant against Ampicillin and Streptomycin. Therefore, this plasmid may carry the genes of resistance to these two antibiotics.

In this study we compared three methods of Serotyping, determining of antibiotic resistance pattern and plasmid profile analysis, for *Salmonella* strains isolated from foodstuffs. Serotyping distinguished more strains and PPA could not apply for typing efficiently, because the strains contained few plasmids. But plasmid profile analysis indicated that all the serotypes contained a 3.036 MD plasmid, which can be considered as a common characteristic of *Salmonella* serotypes isolated from foodstuffs in this area.

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