Molecular epidemiology analysis of *Salmonella enterica* serotype Typhi used Pulsed-Field Gel Electrophoresis (PFGE)

التحليل الوبائي الجزيئي لبكتريا *Salmonella enterica* serotype Typhi بأستخدام جهاز الترحيل الكهربائي ذا الحقل النبضي

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Abstract

Strain typing is an integral part of molecular epidemiology used to discern the clonality of *Salmonella* Typhi involved in local epidemics. The purpose of this study is to identify sporadic *Salmonella enterica* serotype Typhi by conventional and molecular methods that include characterization by Pulsed Field Gel Electrophoresis (PFGE) and to present molecular epidemiology analysis. Thirty isolstes of *Salmonella typhi* from sporadic clinical cases of typhoid fever were obtained. They represent cases from Baghdad, Basra, Babylon and Diala provinces during the period between June 2005 to July 2006. Two biotypes were obtained, 26 isolates under biotype I and four under biotype II. Two antibiogram patterns were obtained: twenty-nine isolates were susceptible to all antibiotic used while the remaining isolate was of different pattern. Plasmid profiling allowed little or no differentiation amongst these isolates. Only 4 (13.3%) isolates were found to contain plasmids which were of three patterns, the majority of strains 26 (86.7%) isolates did not show any plasmid. BOX-PCR fingerprinting has revealed 9 distinct patterns.Cluster analysis by UPGMA based dendrogram revealed six clusters with 90% similarity. Pulsed-field gel electrophoresis (PFGE) of digested chromosomal DNAs from these *Salmonella* Typhi isolates was performed using the restriction endonucleases *Xba*I (5'-TCTAGA-3') and *Spe*I (5'-ACTAGT-3'). *Xba*I-based analysis was superior to *Spe*I restriction patterns. PFGE fingerprinting with *Xba*I restriction have yielded sixteen distinct patterns. Cluster analysis by UPGMA based dendrogram revealed seven clusters with 90% similarity. PFGE fingerprinting with the comparative use of the *XbaI* and *Spe*I endonucleases have proved high discriminatory value to other molecular methods and a helpful tool for the epidemiological typing of *Salmonella* Typhi isolates.

Keywords: *Salmonella* Typhi . sporadic Typhi . BOX-PCR . PFGE .

المستخلص

تم الحصول على ثلاثين عزلة لبكتريا *Salmonella enterica* serotype Typhi من محافظات مختلفة من العراق شملت بغداد ، بابل ، البصرة، ديالى من عينات دم لمرضى يعانون من حمى التايفونيد , شخصت العزلات في مختبرات الصحةالعامة المركزي خلال الفترة الزمنية المحصورة بين حزيران 2005 و تموز 2006 . التنميط المظهري شمل على التنميط الحيوي Biotype باستخدام عدة التشخيص API 20E حيث تبين وجود نمطين من انماط الحياتية تضمن الاول26 عزلة بنسبة 86.7 % بينما الثاني تضمن 4 عزلات بنسبة 13.3%. التنميط الاخر و المعتمد على حساسية العزلات للمضادات الحياتية، اظهر أن 29 عزلة كانت حساسة لجميع مضادات الحياة المستعملة و هي amikacin, ampicillin, ceftriaxone, ciprofloxacin, gentamicin) بينما أظهرت أن عزلة (chloramphenicol, tetracycline, trimethoprim-sulfamethoxazole, nalidixic acid واحدة مفاومة ل nalidixic acid مما يتبين وجود نمطين من انماط التثبيط الحياتي و كان النمط الاول بنسبة 96.7%. التنميط الجيني شمل على المحتوى البلازميدي plasmid profile لقد تبين ان هذه الطريقة لم تظهر اختلافات بين العزلات و التي يمكن اعتمادها لغرض المقارنة ولتحديد الانماط والكشف عن مدى التشابه وبائيا بينها، و قد لوحظ وجود 4عزلات فقط تمتلك لبلازميدات و بثلاث أنماط مختلفة بينما 26 عزلة عدم امتلاكها للبلازميدات. استعملت تقنية BOX-PCR للكشف عن مدى تكرار مناطق بين الجينات ومدى اختلافها بين العزلات و قد لوحظ تكرار حزم منDNA تراوح عددها من 2-11 حزمة و بحجم جزيئي يتراوح ما بين 200-1500 زوج قاعدي و حيث تم الحصول على 9 انماط مختلفة و بينت نتائج التحليل الاحصائي ان معامل التشابه Dice Coefficient كان 0.33-1. تم تحليل البيانات اعتماداً على UPGM حيث اظهرت نتائج التحليل التجميعي , بأن نسبة التشابة

90% لستة مجاميع و وجود علاقة بين هذه المجاميع وقد تمت مناقشتها من الناحية الوبائية . اعطى التحليل الكروموسومي بواسطة جهاز الترحيل الكهربائي ذا الحقل النابض Electrophoresis (Pulsed Field Gel) توزيعا مناسبا للقطع الكروموسومية المهضومة بواسطة الانزيمين القاطعين *Xba*I و *Spe* I و ظهر الانزيم الاول تفوقا بشكل واضح حيث اعطى 16 نمطا بينما تم الحصول على 5 انماط للانزيم الثاني. اظهرت نتائج تقطيع الكروموسوم بانزيم *Xba*I احتواء الانماط لعدد من الحزم تراوحت 2-15 حزمة من DNA من مجموع العزلات بحجم جزيئي يتراوح ما بين 50-550كيلو زوج قاعدي و بينت نتائج التحليل الاحصائي ان معامل التشابه Dice Coefficient كان 0.13 -1 و تم تحليل البيانات اعتماداً على الـ UPGMA نسبة التشابة 90% لسبعة مجاميع و لوحظ وجود اختلاف جيني كبير بين عزلات ا لبكتريا مع امتلاك هذه العزلات لنفس النمط المصلي خلال مدة العزل والاماكن مختلفة وهذا يدل على وجود علاقة وبائية بين العزلات. ان تقنية الترحيل الكهربائي في الحقل النابض المعتمد على هضم الدنا بواسطة انزيمين هاضمين قد اظهرت تفوقا واضحا في التفريق بين العزلات المدروسة و هذا التفوق راجع الى دراسة المقارنة لنتائج هظم الدنا بانزيمين مختلفين. وبهذا اثبتت هذه الطريقة فاعليتها كوسيلة تنميط وبائية مقارنة بالطرق الاخرى مثل الكشف عن المحتوى البلازميدي .

الكلمات المفتاحية: حمى التيفوئيد ، جهاز الترحيل الكهربائي ذا الحقل النبضي ، التنميط الحيوي

Introduction

Typhoid fever, caused by *Salmonella enterica* serotype Typhi (*S.* Typhi) is a major public health problem, particularly in developing countries. The Annual global incidence of typhoid is 21,650,974 cases and 216, 510 deaths annually. An estimated 10,118,879 cases occur annually in Asia alone [1]. In Iraq, electricity plants, water purification and sewage treatment plants were destroyed during the 1991 war. These, coupled with deterioration of sanitation facilities due to long international sanction have led to increased cases of infectious diseases including Typhoid Fever [2]. have reported 1812 cases in 1989 while the number increased to 21356 in 2001. The number of cases after the last war (2003) is expected to be much higher with the collapse of the health care system.

In relation to effective surveillance and the development of rational control strategies for this important human disease, the availability of detailed and accurate data related to the molecular epidemiology of *S.* Typhi is crucial. However, epidemiological investigations used to determine the source and spread of *S.* Typhi have been hampered by the absence of reliable and sufficiently discriminative methods of differentiating individual strains beyond the species level [3].

Detailed isolates identification is essential for useful investigations of *S.* Typhi outbreaks. Epidemiological investigations have traditionally relied on biochemical and serological methods for the primary identification of isolates [4]. Various phenotyping methods (e.g., biotyping, phage typing and antibiotics susceptibility pattern) have been used to subtype *Salmonella* isolates. The modern typing methods are based on characterization of the genotype of the organism by analysis of plasmid and chromosomal DNA [5]. However, these phenotypic methods lack discrimination and are often complemented by the more sensitive and discriminative molecular techniques [6]. Genotyping methods such as plasmid fingerprinting, multilocus enzyme electrophoresis(MLEE), pulse-field gel electrophoresis (PFGE), Random Amplified Polymorphic DNA Analysis(RAPD), Amplified Fragment Length Polymorphism (AFLP) and Repititive-PCR based method have been developed for subtyping within *Salmonella* serotype Typhi [3,7,8,9,10].

Pulsed-field gel electrophoresis (PFGE) is one of the most common technique used to perform comparative chromosomal DNA analysis of serovar Typhi . Studies of the molecular epidemiology of *S.* Typhi have been conducted since the early nineties from last century using pulsed-field gel electrophoresis (PFGE) and have shown significant genetic diversity of *S.* Typhi isolates both from outbreaks and sporadic cases of typhoid fever [11,12,13].

Molecular typing is performed to determine whether different isolates give the same or different results for one or more tests. Epidemiologically related isolates share the same DNA profile or "fingerprint," whereas sporadic or epidemiologically unrelated isolates have distinctly different patterns. If the isolates from different patients share the same "fingerprint," they probably originated from the same clone and were transmitted from patient-to-patient by a common source or by a common mechanism [14].

In this study we used PFGE to analyze the genetic diversity of *S.* Typhi isolates originating from patients with typhoid fever from different geographic areas in Iraq.PFGE analysis was undertaken to determine whether the isolates epidemiologically were identical or different genetically.

Materials and methods

Bacterial isolates

Therty isolates obtained from the Central Public Health Laboratory (CPHL) in Baghdad and diagnosed as *Salmonella* using conventional methods during the period June 2005 - June 2006. The samples represented sporadic cases of typhoid fever referred from the following health centers in Baghdad: Baghdad Medical City Hospital, Ibn AL-Khatib Hospital for Infectious Diseases and AL-Elwyia Children Hospital. As well, samples were referred from Babylon, Basra and Diala provinces. Kuffman and white scheme was followed for serological confirmation and typing using Anti-Salmonella sera (Biomerieux).

Bacteriological assay

API 20E system(Biomerieux) was designed to evaluate a biotyping. All isolates were tested for resistance to Amikacine10 µg, Ampicillin10 µg, Trimethoprim-Sulfamethoxazole 25 µg ,Tetracycline 30µg, Gentamicin 10 µg, Chloramphenicol 30 µg, Ceftriaxone 30 µg, Nalidixic acid 30 µg, and Ciprofloxacin
5 µg. Antibiotic sensitivity tests were done using the standard Kirby-Bauer disc-diffusion method [15].

DNA extraction:

Genomic DNA was extracted from cultures of *Salmonela* Typhi using Wizard Genomic DNA Purification Kit, Promega (USA).

Plasmid DNA extraction:

Plasmid DNA was extracted by the procedure modified from the method of Kado and Liu,1980[16].

BOX-PCR:

Single Repetitive primer corresponding to the BOX-PCR element (5'CATCGGCAAGGCGACGCTGACG-3') was used according to the protocol of [17]. This primer targeted the repetitive intergenic sequence elements of *Salmonella* Typhi. The primer was supplied by Alpha – DNA Company 50 – 60 pico moles/µl. A 25µl PCR mixture contained 1µl DNA 0.05 µg/µl, 0.5µl *Taq* polymerase 5U/ µl, 1µl Primer- BOX-PCR 50 Pico moles/ µl, 1µl dNTPs(200 mM), 3µl $MgCl_2$ (25 mg/ml) ,2.5µl (10X Buffer), Xµl H_2O. Amplification cycles:Predenaturation 95°C 7min and 25 cycles of Denaturation 94°C 60 seconds, Annealing 60°C 60 seconds, Extension 72°C 60 seconds with final extension at 72°C 5min. Following the gel electrophoresis of the amplified product, the resolved bands were indicative for the number of repetitive intergenic sequences.

PFGE:

Preparation of DNA.

DNA for PFGE analysis was prepared by a modification of the method of [3]. To prepare chromosomal DNA, bacterial cells were grown overnight at 37°C in 10 ml LB-media broth .The culture turbidity should be approximately to turbidity standard tube No. 4 MacFarland. Cells were then centrifuged at 1000 rpm for 3 min, washed with (10 Mm Tris-HCl (pH 7.5), 1 M NaCl), and mixed with an equal volume of molten 2% low-melting-point agarose (promega ,USA). Agarose blocks were incubated overnight at 37°C in lysozyme solution (2 mg of lysozyme per ml in 6 mM Tris-HCl, 1 mM NaCl, 100 mM EDTA, 0.5% Brij, 0.22% deoxycholate, 0.5% Sarkosyl, and 2 µg of RNase per ml); this was followed by deproteination (in 1 mg of proteinase K per ml in 0.5 M EDTA and 0.5% Sarkosyl) at 50°C for 48 h. The cell debris and proteinase K were then removed by two washes in 5 ml of (10 mM Tris-HCl- 0.1 mM EDTA (TE buffer) containing 1 mM phenylmethylsulfonyl fluoride) (Sigma Chemical) for 2 h at room temperature. DNA plugs were then equilibrated in TE buffer for 2 h at room temperature.

Restriction endonuclease digestion and PFGE.

Before restriction enzyme digestion, agarose blocks were first equilibrated for 2 h in 200 µl of the appropriate buffer. Fresh buffer (containing the enzyme at 8 to 20 U/µg of DNA) was then added, and the blocks were incubated overnight at various temperatures with gentle agitation (XbaI, 37°C; SpeI, 4°C overnight and then at 37°C for 12 h). Selection of restriction enzymes was based on the recognition site of the enzyme and the G+C content of 50 to 54% previously reported for *Salmonella* spp. [17]. The following restriction endonucleases were used: *XbaI* (5'-TCTAGA-3'), and *Spel* (5'-ACTAGT-3'.) (Promega, USA). **PFGE Pulsed field gel electrophoresis run** using the

GeneNavigator™ system (Amersham Biosciences,Sweden) according to the instructions manual for complete information on set-up and use of the system in gels of 1.3% agarose in 0.5X TBE buffer (0.1 M Tris, 0.1 M boric acid, 0.2 mM EDTA) for 28 to 30 h at 200 V at a temperature of 14°C, with ramped pulse times varying according to the enzymes used (ranging from 2 to 50 s). The gels were stained with ethidium bromide and were photographed with a UV transilluminator (Spectroline; 302 nm). The DNA size standards used were a bacteriophage lambda ladder consisting of concatemers starting at 50 kbp and increasing to approximately 1,000 kbp (Promega, USA).

Interpretation of PFGE The goal of the interpretative process is to use the typing data to predict the clinical and epidemiological relationships among isolates, by [18] shows how to interpretate PFGE patterns.These changes usually result in two to three fragment differences in banding patterns. Therefore, one of the generally accepted interpretation rules is that an isolate is closely related to an outbreak isolates when the fragment difference is around two to three fragments, possibly related when it is four to six and unrelated when the difference is seven or more .

Statistical Analysis

The Restriction Enzyme Analysis (REA) patterns generated by PFGE for various isolates were compared, and the similarities of the fragment length patterns between two strains were scored by the Dice coefficient, also known as a coefficient of similarity [3]. This coefficient, F, expresses the proportion of shared DNA fragments in two isolates and was calculated by the following formula:

$$F = 2n_{xy}/(n_x + n_y)$$

Where nx is the total number of DNA fragments from isolate X, ny is the total number of DNA fragments from isolate Y, and n is the number of DNA fragments that were identical in the two isolates. An F value of 1.0 indicates that the two isolates have identical Restriction Enzyme Analysis (REA) patterns.

Cluster analysis of PFGE *Xba*I, *Spe*I *digested chromosomal DNA* type's was performed by the Un weighted Pair Group Method with Arithmetic averages (UPGMA). UPGMA is the simplest distance method of tree construction [19]. Types with 90% similarity were likely to be related and were grouped into a cluster [6]. Discriminatory power of a typing method is its ability to distinguish between unrelated strains. The discriminatory power of each typing method was determined by calculating the discriminatory index (DI) [20]. All data analysis was performed with the aid of SPSS, (Social Package Statistical analysis) (ver. 12)[21].

Results

Demographic and basic screening

Samples were distributed as 66,7% from Baghdad, 20% from Babylon, 10% from Basra and 3.3% from Diala provinces. Based on biochemical testing, two biotypes were observed. They were differentiated by their ability to decarboxylat lysine. 26 strains 86.7% were lysine (+) whereas four 13.3% were lysine (-)

There was no geographical clustering in this respect. All isolates showed strong positive agglutination with specific somatic antigen O-group: O9, K antigen Vi and specific flagella antigen H: d within 30 seconds. All isolates were uniformly susceptible to Amikacine 10 µg, Ampicillin 10 µg, Trimethoprim-Sulfamethoxazole 25 µg ,Tetracycline 30 µg , Gentamicin 10 µg, Chloramphenicol 30 µg, Ceftriaxone 30 µg, Nalidixic acid 30 µg, and Ciprofloxacin 5 µg. Whereas only one isolate was resistant to Nalidixic acid.

Genetic analysis

Plasmid Profile

Fig. (1) shows Plasmid DNA band was detected in four strains 13.3% while the rest 86.7% were plasmid-free. Each of the strains contained either two, three or four plasmids. The four strains were from Baghdad city.

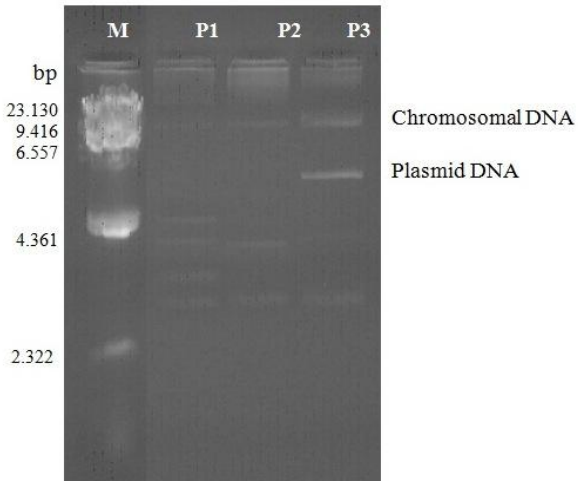

Fig. (1): Agarose gel electrophoresis of extracted plasmids. Lane M: λ DNA cut with *Hind*III; Lanes P1-P3 patterns of *Salmonella* serotype Typhi plasmid DNA (0.8 % agrose, 60 v., 1-2 hr.)

BOX – PCR Fingerprinting

The presence of 2-10 bands of DNA have a size ranging from 200 – 1500 bp Fig.(2). It was possible to differentiate the isolates into nine distinct patterns where pattern B1 contains 11 isolates, B2 with two isolates, B3 with three strains, B4 with one strain, B5 with two strains, B6 with three strains, B7 with one strains, B8 with six strains and B9 with one strain. A bands with 1250bp, 1000bp and 400bp were identical for all except for pattern B2 where the 400bp band is absent. Genetic diversity and relatedness among these strains were also evaluated by the Dice coefficient value, which ranged from 0.33– 1.0. Fig.3 showed that the percentage similarity between the different patterns seven DNA types could be grouped into six clusters at similarity of ≥90%, while the rest were of ≤90% similarity. Cluster (1) was the major cluster with 11 isolates. The suitability of BOX-element primer PCR fingerprinting as a discriminatory power was designated by statistical analysis *D* 0.567.

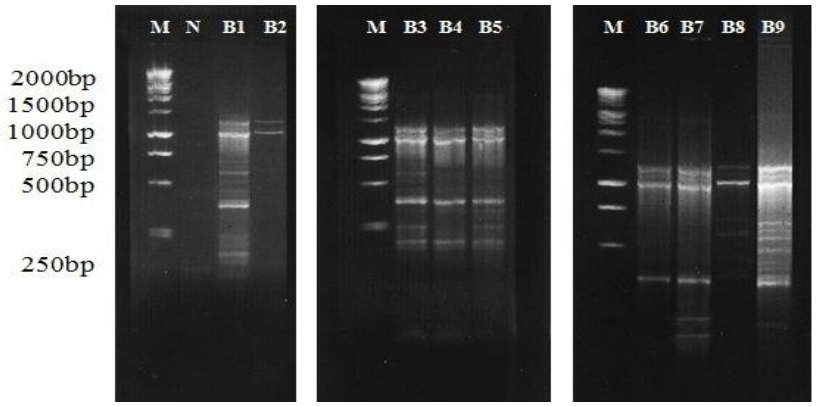

Fig.(2): BOX– PCR reaction products.Lanes: M1, Kb DNA Ladder (Promiga); B1-B9, *Salmonella*Typhi. BOX - PCR products were separated on 1.5% agaros gel Electrophoresis.

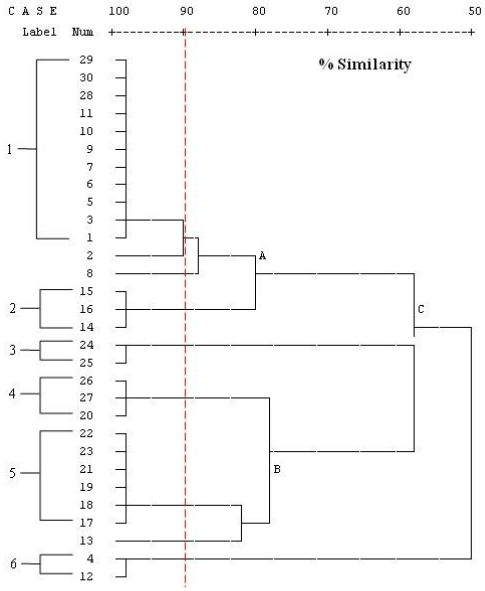

Fig.(3): Dendrogram showing the percentage of genetic similarity between the BOX-PCR Fingerprinting patterns obtained from 30 sporadic *Salmonella* Typhi, using the unweighted pair group arithmetic means method (UPGMA).

PFGE patterns

PFGE analysis have revealed the presence of sixteen distinct cleavage patterns with *Xba*I, yielded between 2-15 bands ranging in size from 50-550 kb Fig. (4). This indicates high genetic diversity. Genomic groups and relatedness was analyzed by the Dice coefficient value, which ranged from 0.13–1.0 Fig.(5). he percentage similarity between the different patterns thirteen DNA types could be grouped into seven clusters of similarity ≥ 90%, while the rest were of ≤ 90%. Cluster I was the major cluster containing 8 strains followed by cluster VI and V. The analysis investigated the suitability of PFGE discriminatory power as designated by statistical analysis *D* 0.986.

Four cleavage patterns were observed by digestion with the enzyme *Spe*I, yielded between 11-15 fragment ranging in size from 50- 550 kbp Fig.(6) .Genetic diversity and relatedness were significant as revealed by the Dice coefficient value, which ranged from 0.74– 1.0 Fig.(7). Four DNA types could be grouped into four clusters of similarity ≥90%. There were no differences between clusters.

In summary, group S1 contained 12 isolates, S2 contained 8 strains, S3 contained 4 strains and group S4 contained 6 strains. No geographical preference was observed. The comparative use of the two restriction endonucleases in PFGE analysis significantly increased the discriminatory value of this technique to *D* 0.986 as strains that exhibited the same fragment pattern with one enzyme occasionally differed in their fragment patterns obtained with the other enzyme.

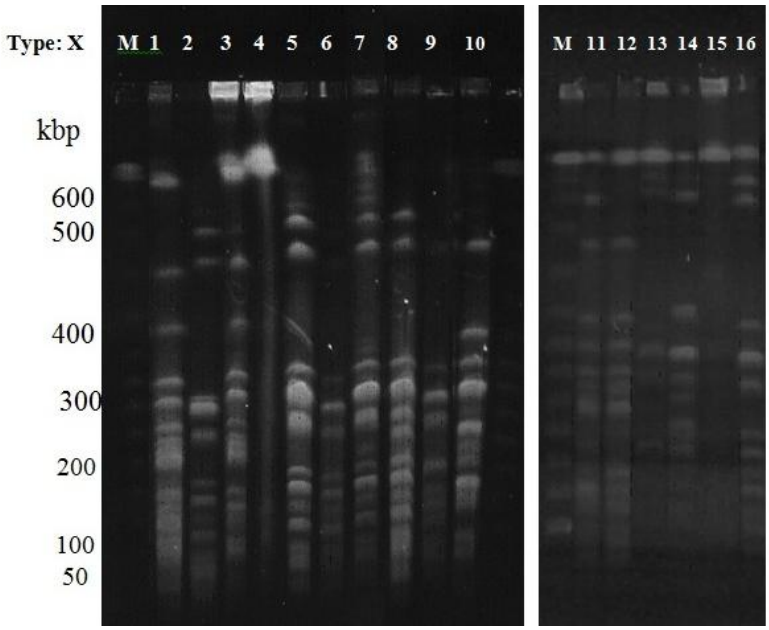

Fig. (4): PFGE analysis of *Salmonella* Typhi using restriction enzymes *Xba*I. Lanes:M, DNA size standers 1000 Kbp (Promega-Marker Lambda Ladders). Lanes:1-16 patterns of S. Typhi. PFGE was performed with ramped pulsed time 40s,22h, 14°C ,200v,100a

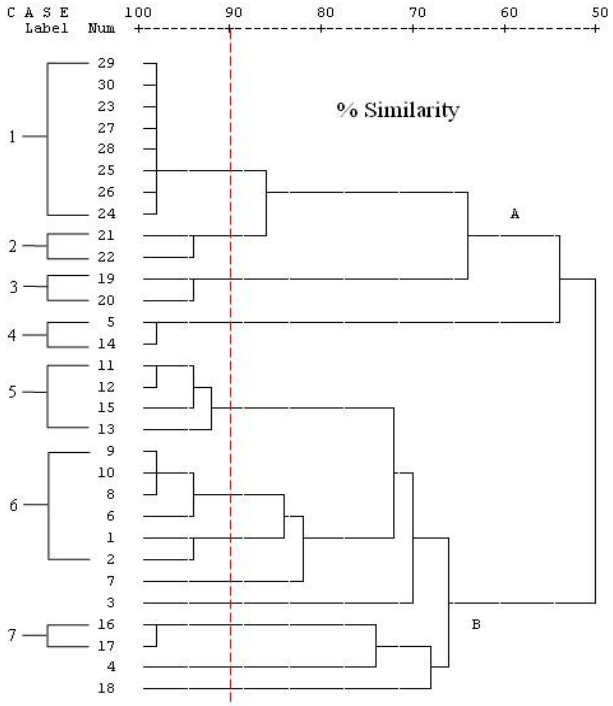

Fig.(5): Dendrogram showing the percentage of genetic similarity between the 16 *Xba*I digestion PFGE patterns obtained from 30 sporadic *Salmonella* Typhi , using the unweighted pair group arithmetic means method(UPGMA).

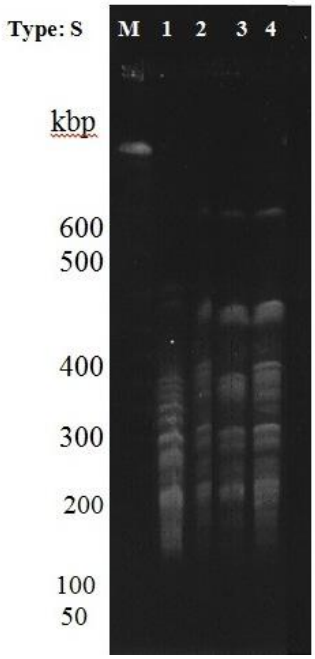

Fig.(6): PFGE analysis of *Salmonella* serotype Typhi using restriction enzymes *Spe*I. Lanes:M, DNA size standers 1000 Kbp (Promega-Marker Lambda Ladders). Lanes:1- 4 patterns of *Salmonella* serotype Typhi . PFGE was performed with ramped pulsed time 50s,22h, 14°C ,200v,100a

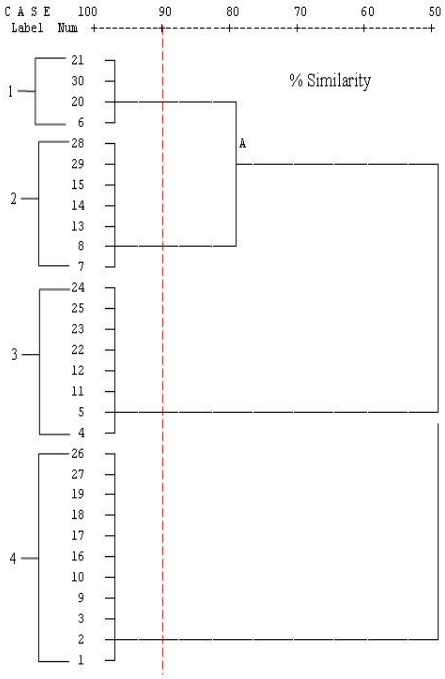

Fig.(7): Dendrogram showing the percentage of genetic similarity between the 4 *Spe*I digestion PFGE patterns obtained from 30 sporadic *Salmonella* serotype Typhi , using the unweighted pair group method with arithmetic averages (UPGMA).

Discussion

Typhoid fever is one of the major health problems in developing countries, fostered by many interrelated factors, including increased urbanization, inadequate supplies of clean water, antibiotic resistance, the variable efficacies of vaccine preparations, and the increased regional movement of large number of migrant workers. It is thus obvious that there is an urgent need for effective epidemiological surveillance. Therefore, specific and reliable epidemiological markers for *S*. Typhi are required [7].

Salmonlla Typhi is a serovar in which the majority of strains are plasmid free [22]. There was similar observation from this study. These findings can be explained by the facts that the plasmids were not associated with antimicrobial resistance as well as their instability in *Salmonella* Typhi rather than an inherent barrier to the entry or establishment of foreign plasmid DNA.

When two sequences are located near enough to each other (e.g., within a few kilobases), then the DNA fragment between those sites (referred to as an "interrepeat") is effectively amplified.The number and size of the generated interrepeat fragments can vary from strain to isolates [23]. Repeated genes also provide mechanisms to enhance bacterial virulence [24]. Using Box-PCR, the isolates were distributed into nine distinct patterns. Since the patterns may refer to evolutionary convergence of the same genotype, the repeated recovery of isolates with the same or nearly the same fingerprint or genotype suggests that these isolates are epidemiologically related. We have distinguished six related clusters. This indicates possible transmission of epidemic isolates via carriers moving from area to another specially worker. Variability of patterns indicates that more than one source is present, where simultaneous circulation of indigenous and imported isolate occurrence can be elucidated.

Salmonella Typhi genome has undergone major rearrangement not seen in other *Salmonella* spp. These reorganizations, produced by homologous recombination's between *rrn* operons, could be responsible for the high discrimination index detected in this serotype when molecular methods such as ribotyping and PFGE are used [25]. In this study, digestion of *Salmonella* Typhi DNA with *Xba*I for PFGE was superior over digestion with *Spe*I restriction enzym, the former was able to differentiate strains to sixteen patterns, while *Spe*I differentiate them to only four patterns. This is an indication of considerable diversity among strains associated with sporadic cases. Considerable genetic diversity has been found for *Salmonella* Typhi worldwide. [26] reported 48 *Xba*I digestions (PFGE) Patterns among 60 strains of *Salmonella* Typhi from Malaysia, 30 patterns among 30 from Indonesian and 9 patterns among 10 *Salmonella* Typhi from Thailand. [7] reported 41 PFGE patterns from 55 unrelated patients of sporadic cases. [27] reported 3o different patterns using *Xba*I digestions, from 65 isolates of *Salmonella* Typhi from south East Turkey.

Although PFGE is “a golden method" for genetic diversity analysis, recent point mutation, deletion, insertion and losing or acquiring plasmids within a subtype might account for minor differences. These changes usually result in two to three fragment differences in PFGE banding patterns. Therefore, a generally accepted interpretation rule is that an isolate is “closely” related to an outbreak isolate when the fragment difference is around two to three fragments. They are “possibly” related when four to six fragment difference and “unrelated” when the difference is seven or more[18]. In addition, identical *Salmonella* Typhi strains can be found in different areas, and even in different countries. Genetic relatedness measurment by statistical analysis of *Xba*l digestion results has revealed genetic similarity (coefficients value 0.97) based on one to two bands differences on gel. The differences between strains that gave a Dice coefficients value 0.25 to 0.47 seven or more bands difference on gel indicated genetic unrelatedness. There were seven distinct clusters, represent 26 isolates out of the total number (30 isolates). The first cluster contained eight strains, seven of which were from Baghdad and one from Diala province (north east of Baghdad) while the sixth cluster contains five isolates from Baghdad and one from Babylon (south west). Sharing of identical or closely related PFGE pattern implies mobility and movement of the isolates. The identity of isolates isolated from these regions suggests an epidemiological linkage. Thus, recirculation of certain isolates from sporadic cases is possible, although the considerable degree of genetic plasticity of *Salmonella* Typhi genome may make it difficult to trace the infectious strains.

The discriminatory power of PFGE is (*D* 0.978). It has been found that it was s superior method over BOX –PCR (*D* 0.568), and differentiate more than other techniques. Such observation agrees with the results of [28]. Because PFGE profiles of isolates reflect differences present over the entire chromosome, the information they provide differs from, and yet complements, those obtained from BOX-PCR. However, the diversity of types revealed by PFGE among *Salmonellae*, have already been classified to the subspecies and serotype levels, respectively. The effectiveness of PFGE as a tool for strain discrimination in *Salmonellae* will depend on intra serotype genetic diversity. The comparative PFGE analysis with two different endonucleases distinctly increased the discriminatory value of this method. Moreover, the use of two enzymes as this study did, provided a way to circumvent difficulties that might arise from the presence of protection systems in *Salmonell* Typhi against cleavage by a particular restriction endonuclease.The study of molecular epidemiology of Typhoid fever is important to understand the mode of spread of serovar Typhi strains in order to implement rational strategies for the prevention and control [29].

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