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PLASMID PROFILE ANALYSIS OF SALMONELLAE ISOLATED FROM SOME MEAT PRODUCTS

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A B S T R A C T

A total of 300 random samples of luncheon, sausage and frozen minced meat (100 of each) were collected from different supermarkets at Qalyubia governorate. The samples were transferred directly to the laboratory under strict hygienic conditions and bacteriologically examined for detection and plasmid profile analysis of salmonellae. This work determined the plasmid profiles of the salmonella serovars by using ethidium bromid stained 1% agarose gel electrophoresis. In this study, plasmid analysis revealed that the isolated salmonella serotypes carry different numbers of plasmids with variable molecular weights ranged from 1233.18 to 15280.82 base pair (bp). The obtained results revealed that *S. typhi, S. typhimurium* and *S. enteritidis* contain a common molecular weight band at 7532.35 bp. and the number of plasmids ranged from 4 to 5 bands. Moreover, *S. typhi and S. typhimurium* sharing the same band at 1233.18 bp. In the current study, *S. enteritidis* and *S. typhimurium* were recognized to have common band at 1631.12 bp. The obtained results revealed that *S. typhi*, and *S. enteritidis* have 4 bands but *S. typhimurium* have 5 bands.

KEY WORDS: Luncheon, Plasmid, Salmonellae, Sausage, Minced Meat.

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1. I N T R O D U C T I O N

Inspection of food for the presence of salmonella became one of the routine work all over the world due to the low infective dose of salmonella. Methods for its detection are required to prove the presence of one cell in a defined food sample. Cultural methods for salmonella's detection involve a non selective pre-enrichment, followed by selective enrichment and plating on selective and diagnostic agars. Suspect colonies are confirmed biochemically and serologically. The complete test requires three to 4 days to obtain a negative result and up to 7 days to get a confirmed positive result [15]. At least 6 serovars of *Salmonella (abortus ovis, cholera suis, dublin, enteritidis, gallinarum/pullorum and typhimurium)* harbour a virulence plasmid, although not all isolates of these serovars do. These plasmids vary in size among the serovars. All of these plasmids contain the Salmonella plasmid virulence (spv) locus. This locus harbours 5 genes designated spv RABCD [23].The first gene spvR encodes an activator of spv ABCD, but the exact function of the encoded proteins is not fully known, these genes are induced by growth restriction, reduced nutrient supply or lowered pH and are involved in intra-macrophage survival of salmonella [20]. It was mentioned that most of the pathogenic salmonella strains carry virulence associated plasmids which are essential for systemic infections [2, 22]. Moreover, drug-resistant plasmids are of

great importance in medicine and in epidemiological studies of the microorganisms as the drug resistance of clinical isolates is mostly due to the presence of drug resistance plasmids. Some authors associate certain plasmids or genes with virulence and pathogencity of salmonellae [2, 10]. Other reports interrelate between plasmid profiling and antimicrobial drug resistance of different salmonellae [5, 10]. Studies of some salmonella outbreaks were built on plasmid profiling [5, 21]. Antimicrobial drug resistance of salmonella as major factor of salmonella outbreaks and persistence of salmoellosis was reported by many authors [3, 5].The seriousness of salmonella has stimulated interest in developing fast and specific tests for the detection of this pathogen. Methods such as immunoassays have proven to be inexpensive and require less labor and time to perform than culture type. Numerous genotyping methods have been applied for typing of salmonella. Plasmid profiling analysis supplies a quick and relatively easy method to fingerprint bacterial strains and to study their spread. Thus, plasmid profiling has played an important role in studies of zoonotic aspects of salmonellosis as well as salmonella biology [17]. Plasmid profiling provided a mean for sero-grouping of salmonellae and appears to be a useful tool for characterizing various strains from common sources in addition to the spread of such strains [12].

Therefore, the aim of the present study was to fulfill the following items:
- Conventional method for isolation and identification of salmonellae from some meat products (luncheon-sausage-frozen packed minced meat).
- Estimation of the size and quantity of the isolated plasmids of salmonellae.
- Differential comparison between the isolated salmonella plasmids.

2. MATERIALS AND METHODS

2.1. Collection of samples

A total of 300 random samples of luncheon, sausage and frozen minced meat (100 samples of each) were collected from different supermarkets at Qalyubia governorate. The collected samples were transferred directly to the laboratory in an ice box under complete aseptic conditions. The samples were immediately examined bacteriologically for the detection of salmonellae.

2.2. Plasmid extraction and profile

Media used for plasmid extraction: was Luria – Bertani broth (L.B.)

Material used in plasmid profile:
-Solution (I) composed of Glucose (50.0mM), Tris HCI, pH 8.0 (25.0mM) and EDTA, pH 8.0 (16.0mM).
This solution was prepared in a batch of 100 ml autoclaved for 15 minutes and stored at 4ºC.
- Solution (II) composed of SDS 1 % and 1N NaOH (0.2 %), EDTA, and Sodium Dodecyl Sulfate
- Solution (III) composed of 60ml of potassium acetate (5.0M), 11.5 ml of Glacial acetic acid and 28.5 ml of distilled water.

2.3. Isolation and Identification of salmonella:

The techniques adopted were carried out according to International commission on microbiological specification for foods (ICMSF) [14] and recommended by Abd El-Aziz [1].

2.4. Extraction of plasmid of salmonella isolated [4]:

2.4.1. Harvesting of bacteria: A single bacterial colony was transferred to a sterile glass test tube containing 5ml of Luria-Bertani broth (L.B broth) and incubated for 12 hours in shacking incubator at 37°C. Then 3ml of L.B broth culture were

aliquoted in two Eppendorf tubes and centrifuged at 14.000 rpm at 4°C, the supernatant was decanted and the bacterial pellet was dried carefully.

2.4.2. Alkaline lysis:
Alkaline lysis was done by adding of 100µl of solution I to each tube with vigorous vortexing and the contents of the two tubes were transferred to one tube. Then solution II was added gently as 200µl with gentile shacking or inversions and kept on ice- bath for 5 minutes. Then solution III was added as 150µl with several gentile inversions and incubated on ice- bath for 5 minutes.

2.4.3. Precipitation by Ethanol:
Centrifugation was done at 14.000 rpm at 4°C for 5 minutes. The supernatants were carefully transferred to Eppendorf tubes. The Plasmid DNA was precipitated by adding double volumes of pre-cold ethanol and incubated for 10 minutes at -20°C then centrifuged for 5 minutes at 14.000 rpm at 4°C. The supernatant was discarded and the pellet was air dried for 5-10 minutes.

2.4.4. RNase:
The pellet of DNA was dissolved in 20µl of autoclaved double distilled water, then 1.5µl of RNase A was added then incubated at 37°C for 30 minutes and the plasmid DNA was stored at -20°C for electrophoresis.

2.4.5. Electrophoresis:
Electrophoresis was carried out using horizontal 1 % agarose (Sigma®) gel combined with ethidium bromide at concentration of 0.5µg/ml. The running buffer was Tris Acetate EDTA (TAE), and then 5µl of the prepared plasmid DNA were mixed with 1µl of 6x loading buffer. The samples were loaded into the wells of the horizontal gel electrophoresis apparatus against the standard marker (Lambda Hind III). The running was done at constant current (120 V) for one hour. The plasmid DNA was visualized by using UV transilluminators and photographed using the digital camera. The molecular weights and the electrophoritic patterns of the extracted plasmids of the recovered salmonella strains were analysed by using Image quant -TL 2005, Amersham Bio Science.

3. RESULTS AND DISCUSSION

In the present study a total of 300 random samples of luncheon, sausage and frozen minced meat (100 samples of each) were examined for salmonellae and plasmid profile analysis of the isolated strains.

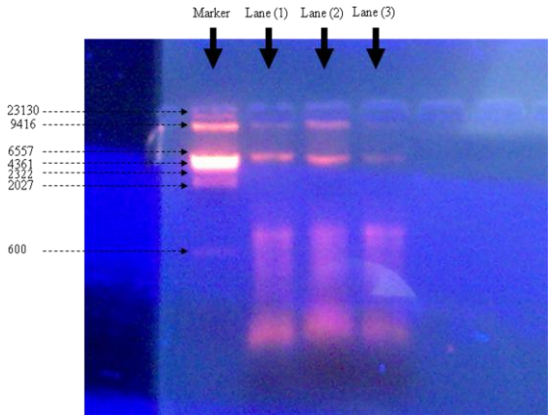

Photo 1 Ethidium bromide stained 1 % agarose gel electrophoresis showing plasmid profile of the isolated strains of salmonella.Marker = Lambda Hind III DNA marker (Promega USA cat # G 1711). The bands of the Mol. W. of the marker from top to the bottom are 23130bp, 9416bp, 6557bp, 4361bp, 2322bp, 2027bp and 600 bp. Lane (1) = *S. typhi.* Lane (2) = *S. typhimurium.* Lane (3) = *S. enteritids*

Plasmid profile analysis has been successfully used to investigate recent outbreaks of food borne diarrheal illness and has been important in identifying epidemic strains of *S. enteritidis* [23]. Virulence plasmid is important for the pathogenicity of *S.enteritidis* strains [2] and plasmid free strains might be less virulent [11].
The obtained results agree with earlier studies [6, 7] reported that *S. enteritidis* and *S. typhimurium* are the most common causes of food borne salmmonellosis in human. In this study, plasmid profile of

salmonella serovars which were isolated from some meat products (luncheon, fresh sausage and frozen minced meat) have been analyzed and the electrophoretic profiles of the plasmids of these strains have been determined by ethidium bromide stained 1% agarose gel electrophoresis and resolved into bands as shown in Photo (1) and table (1).

Table 1 Molecular weight in base pair of the recovered plasmids of the isolated salmonella strains compared to Lambda Hind III DNA marker (Promega USA).

Lanes / Bands	Marker (Mol. W.)	Lane 1 (Mol. W.)	Lane 2 (Mol. W.)	Lane 3 (Mol. W.)
1	23130.00	15031.50	15280.82	15534.29
2	9416.00	7532.35	7532.35	7532.35
3	6557.00	1527.24	1631.12	1631.12
4	4361.00	1233.18	1383.69	1295.57
5	2322.00	-	1233.18	-
6	2027.00	-	-	-
7	600.00	-	-	-

Mol. W. referred to Molecular weight (bp). Marker= Lambda Hind III DNA marker (Promega USA). Lane (1)=*S. typhi.* Lane (2)= *S. typhimurium.* Lane (3)= *S. enteritidis* .

In this study, plasmid analysis revealed that salmonella serotypes carry different numbers of plasmids with variable molecular weights ranged from 1233.18 to 15280.82 bp. as shown in table (1). These results nearly similar to former research [8] found that *Salmonella* serotypes carry plasmids with variable molecular weights ranged from 759 to 21724 bp. corresponding to the size of Hind III DNA marker. Also, it was reported that strains of salmonella often carry plasmids ranging in size from 2-150 kbp [17]. But frequencies and size distributions vary between serovars.

The result present in table (1) revealed that *S. typhi*, *S. typhiumruium* and *S. enteritidis* harboured 4, 5 and 4 plasmids, respectively. *S. enteritidis* harbored 4 plasmids as shown in table (1) and these results agreed with similar findings in previous studies [8, 9, 18].

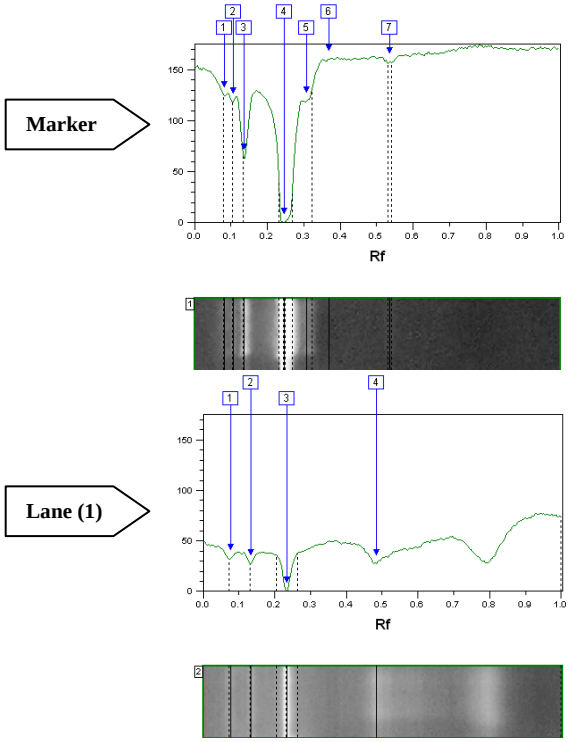

Fig 1 Electrophoretic pattern of plasmid profile of *S. typhi* compared to Lambda Hind III DNA marker and analyzed by using Image quant-TL 2005, Amersham Bio Science.

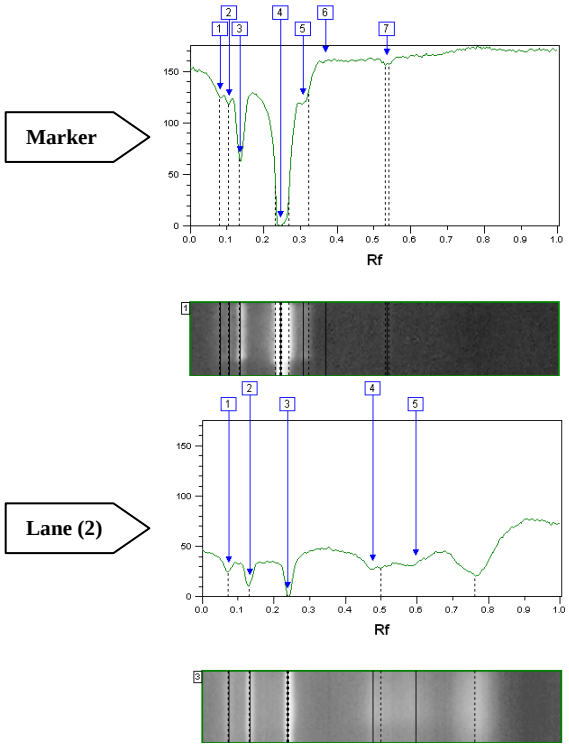

Fig 2 Electrophoretic pattern of plasmid profile of *S. typhimurium* compared to Lambda Hind III DNA marker and analyzed by using Image quant-TL 2005, Amersham Bio Science.

The results present in table (1) revealed that *S.tyhimurium* harbored 5 plasmids;

nearly similar earlier reports [16] found that *S. typhimurium* harbored 6 plasmids. Additionally, higher plasmids of *S. typhimurium*, 10 plasmids, have been recorded [18]. A low number of plasmids of *S. typhimurium*, 2 plasmids, were reported previously [8, 9, 13]. The current study found that 1.6 Kb plasmid could be recovered in *S. typhimurium*, and this agreed with early work [16].

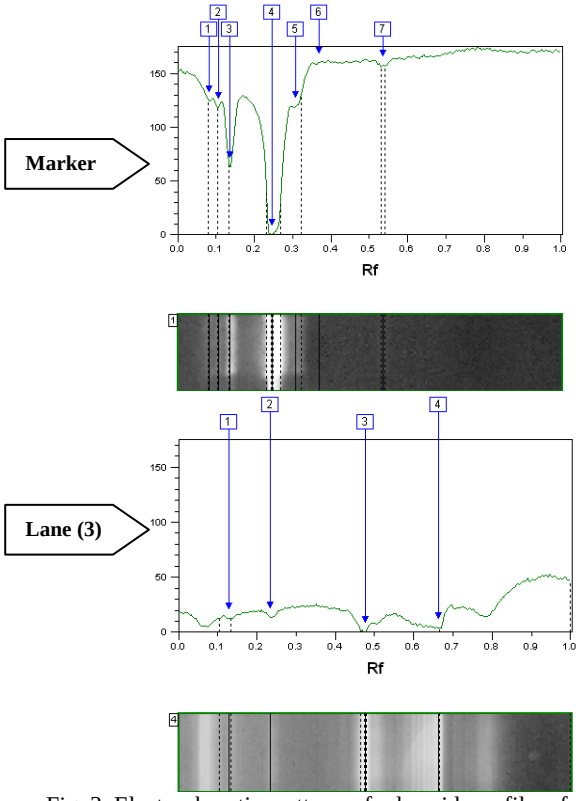

Fig 3 Electrophoretic pattern of plasmid profile of *S. enteritidis* compared to Lambda Hind III DNA marker and analyzed by using Image quant-TL 2005, Amersham Bio Science.

The results obtained in table (1) revealed that *S. typhi, S. typhimurium and S. enteritidis* contain a common molecular mass band at 7532.35 bp. and the number of plasmids ranged from 4-5 bands. The current study found that *S. typhi* and *S. typhimurium* sharing the same band at 1233.18 bp as shown in photo (1) and table (1).

The results obtained in table (1) revealed that *S. enteritidis* and *S. typhimurium* sharing the same band at 1631.12 bp. It was clear that the plasmid profile obtained provided a mean for serogrouping of salmonellae and appears to be a useful tool for characterizing of various strains from common sources in addition to the spread of such strains, similar to early reports [12].

4. CONCLUSION

In conclusion plasmid profiling analysis supplies a quick and relatively easy method to fingerprint bacterial strains and to study their spread. Thus, plasmid profiling has played an important role in studies of zoonotic aspects of salmonellosis as well as salmonella biology [17].

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تحليل الصفة البلازميدية لميكروبات السالمونيلا المعزولة من بعض منتجات اللحوم

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الملخص العربى

تم جمع عدد ثلاثمائة عينة عشوائية من اللانشون والسجق الطازج واللحوم المفرومة المجمدة (100 عينة من كل نوع) من بعض السوبر ماركت المختلفة بمحافظة القليوبية حيث تم إرسال هذه العينات على مباشرة إلي المعمل لفحصها بكتريولوجياً وتحليل الصفة البلازميدية لميكروبات السالمونيلا المعزولة باستخدام الأجاروز جيل الكتروفوريسز. تم عزل وتصنيف بعض ميكروبات السالمونيلا من السجق واللحوم المفرومة وتحليل الصفة البلازميدية لهذه المعزولات ، وأثبت التحليل البلازميدي للعترات المعزولة وجود اختلافات في الوزن الجزيئي يتراوح بين 1233.18 إلي 15280.82 قاعدة نيتروجينية، كما أثبت التحليل البلازميدي للعترات المعزولة تواجد عدد من البلازميدات في كل عترة. أثبتت النتائج أيضا أن سالمونيلا تيفي وسالمونيلا انترتيدس تحتوي علي أربع بلازميدات في حين أن سالمونيلا تيفيموريم تحتوي علي خمس بلازميدات، كما أثبت التحليل البلازميدي أيضا أن هذه المعزولات مشتركة في الوزن الجزيئي 7532.35 قاعدة نيتروجينية. وجد أن سالمونيلا تيفيموريم وسالمونيلا انتريتيدس مشتركتان في الوزن الجزيئي 1631.12 قاعدة نيتروجينية.