How to Cite:
Shalal, S. H., Jaber, N. N., & Hussein, K. R. (2022). Molecular characterization of integrons and Genotyping of Salmonella enterica isolated from diarrheal animals and humans. *International Journal of Health Sciences*, *6*(S5), 9456–9465. https://doi.org/10.53730/ijhs.v6nS5.9603

Molecular characterization of integrons and Genotyping of *Salmonella enterica* isolated from diarrheal animals and humans

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***Abstract*---**Resistance to antibacterial drugs is a major health problem because of its impact. It is important to study the prevalence of antibiotic resistance genes in order to understand.Twenty five *Salmonella enterica* isolated from human and animal diarrhea. Traditional culture methods, biochemical methods, and molecular methods were used to characterize the samples during December to September 2020 to 2021. Disk diffusion method was used to determine antimicrobial susceptibility patterns of isolates identified as MDR by the Clinical and Laboratory Standards Institute (CLSI). The PCR amplification was used to show presence of class 1, 2 and 3 integrons. Class 1 integrons and class 3 were detected in 100% of the multidrug-resistant *Salmonella* isolates,whlie class2 detected among 40%. RAPD-PCR fingerprinting regarded successfully as a reliable, reproducible, accurate and sensitive discriminatory method to *Salmonella enterica*. The results of RAPD-PCR amplification of *Salmonella enterica* isolates revealed that 24 isolates (96.%) also showed about one to six amplification bands per isolate, except one isolates were untypeble. The most frequent band was a 1400bp band (72%), while the lowest frequent band was 400bp (8%) among an individual isolates.

Keywords---*Salmonella enterica* Multidrug Resistance, Integrons, RAPD-PCR.

Introduction

Diarrheal diseases are caused by bacterial, parasitic, or viral enteric organisms being transmitted to humans through feces contaminating food sources or water. Human feces contamination in the environment is the most common cause of human diarrhea [1,2]. There are several subspecies of *Salmonella* 99.5 percent of foodborne illness in humans and animals is caused by *Salmonella enterica subspecies enterica* (subspecies 1) [3] *S. enterica* subsp*. enterica* strains have reservoirs in domestic and wild animals, and can be infected to humans is usually by consumption of contaminated foodstuffs [4]. Antibiotics have long been regarded as one of the greatest contributions to medicine and humanity, as microorganism-killing or microorganism-inhibiting compounds or substances used to treat a wide range of infectious diseases caused by bacteria in both animals and humans in the twentieth century [5,6].In recent years due to its high impact on public health MDR strains have emerged as a result of the increased use of antimicrobials to treat Gram-negative bacterial infections [7] As a result, the acquisition of resistance genes has been viewed as a significant contributor to the antimicrobial resistance is widely distributed and spreading.whether through transfer (vertical or horizontal) with mobile genetic elements such as plasmids and transposons are used in the latter mechanism[8]. Integrons, which are mostly carried by plasmids or contained within a transposon, have a well-established and documented mechanism and role in microorganism distribution. [9,10] The presence of an integrase gene (intI) and a proximal primary recombination site (*attI*) characterizes an integrin [8, 11]. Four general classes of integrons have been identified and distinguished to date, referred to as classes 1–4. Multi-resistant integron is the name given to this type of integron (RIs), classes 1–3 Integrons can acquire the same gene cassettes through a similar recombination platform, Integration occurs through recombination sites from such integrons, which is supported by in vitro excision [12]. Antibiotic resistance gene cassettes may also be found in the remaining classes of integrons, but their global prevalence is low[13].

The RAPD-polymerase chain reaction (PCR) is thought to be a useful method for obtaining genetic information [14,15]. It is a technique that is universally applicable to any genome and is quick, simple, easy, and inexpensive. RAPD amplicon products can be analyzed by either DNA sequencing or agarose gel electrophoresis depending on the labeling of primers with a suitable fluorescent dyes. Moreover, RAPD-PCR is widely used for typing of the bacterial isolates in cases of the outbreaks due to its rapid, inexpensive, simple, and easy use [16] also it can be used to discriminate, screen, and to determine genetic relatedness among isolates [17]. The aim of the study is to detect the molecular characterization of integrons and genotyping of *Salmonella enterica* isolated from diarrheal animals and humans.

Materials and Methods

Bacterial isolates

The period of the study was extending started from December 2020 to September 2021 . The total collected samples for each type were 200 samples were the diarrheal samples were collected from two sources the patients humans (Different age ranges of both males and females) from Mohammed Al-Mosawi Hospital, Bnit –Huda Hospital and Public Health Laboratory and the animal samples (cow ,sheep and Goat) from veterinary hospital and field. The samples examined for detection *Salmonella enterica* in Al-Nassiriyah city,South of Iraq.All the strains were confirmed to be *S. enterica* by standard biochemical and Api-20E at Department of Public Health Laboratories of the Technical Institute, Southern Technical University which that processed the specimens At the Biomedical Science Laboratory, By plating the cultures on selective media, the purity of the strains was confirmed (XLD and SSA)

Antimicrobial sensitivity testing

The susceptibility of *S.enterica* isolates to antimicrobial agents was tested, and the disk diffusion method, as described in the Clinical and Laboratory Standards Institute (CLSI) guidelines, was used to identify MDR strains [18]. The antibiotics were selected according to CLSI standard and previous studies in this field as follows: Amikacin (10 µg), Tetracyclin (10 µg), Ampicilin (25 µg), ceftazidime (30 µg), cefteriaxon (10 µg) , imipenem (10 µg), gentamicin (10 µg),b ciprofloxacin (10 µg), Levoflxacin (5 µg) and Trimethoprim (10 µg) (Mast Companies, UK)

Bacterial DNA extraction

DNA of bacteria stored broth were extracted by using Bosphore® Bacterial DNA Extraction Spin Kit (Anatolia, Turkish). These method is based on the silica membrane column separation, they involve extraction of nucleic acids by removing and purifying the bacterial nucleic acids.

Detection and characterization of class 1, 2 and 3 integrons.

Amplification of integrase genes using intI1, intI2, and intI3 specific primers was used to look for class 1, 2, and 3 integrons in MDR *S. enterica* (Table 1). The PCR reactions were prepared in a thermaocycler (Eppendorf master cycler®, MA) in a total volume of 25 L, and amplification was performed as the following protocol was used for PCR according [19].The amplified products were electrophoresed on 1.2% agarose gel and after staining with ethidium bromide (0.5 mg/ml) visualized in gel document system

Detecion RAPD PCR

PCR master mix (abm , canada), contained 12.5 µL of640 CGTGGGGGCCT3 µLof (0.3 µM) of primer, 9.5µL of distilled water, and 2.0 µL (100 ng) of template DNA. All reaction mixtures were put in the thermocyclcer (Master Cycler® ep Realplex Eppendorf, USA). An initial denaturation step at 94°C for 2 minutes, 45 cycles at

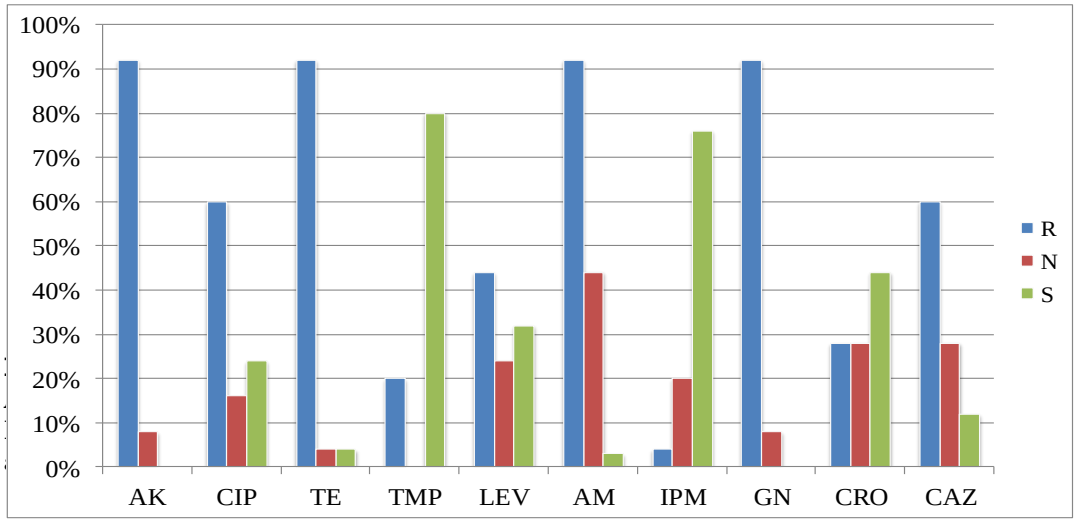
94°C for 1 minute, 40°C for 1 minute, 72°C for 5 minutes, and a final extension step at 72°C for 8 minutes were used for PCR cycling.

Table 1 Primers used for PCR amplification for detection of class 1, 2 and 3 integrons

Gene	Primer	Sequence (5′-3′)	Amplification product (bp)	Reference
Intl1	Intl1-F Intl1-R	TCTCGGGTAACATCAAGG AGGAGATCCGAAGACCTC	254	19
Intl2	Intl2-F Intl2-R	CACGGATATGCGACAAAAAGG TGTAGCAAACGAGTGACGAAATG	788	19
Intl3	Intl3-F Intl3-R	AGTGGGTGGCGAATGAGTG TGTTCTTGTATCGGCAGGTG	600	19

Result

Four hundred samples were collected from diarrheal humans and animals. The results showed 13 (6.5%) of samples from humans and 12 (6%) from animals (cattle,sheep and goat) were *Salmonella enterica*. *S.enterica* isolates showed a varied levels of resistances to antibiotics: Ampicillin 23/25 (92%); Gentamycin 23/25 (92%), tetracyclin 23/25 92%% and Amikacin 23/25 (92%) while all isolates were sensitive to Imipenem 12/25 (76%) and Trimethoprim20/25 (80%). Because the isolates were completely resistant to equal amounts of antibiotics, the present study showed an increase in the incidence of multiple drug resistance (MDR) or. more than one antibiotic in three antimicrobial categories (equal or greater than three).One isolates resistances to 9 antibiotic (90%) considered as multi-drug resistant while three isolates resistances to 8 antibiotic (80%) five isolates were resistant to 7 antibiotics (Figure 1).



Detection and characterization of integrons

All 25 isolates were confirmed to be *S.enterica* through the amplification of the intI1, intI2 and intI3 gene tested for all *S. enterica* isolates with molecular weight approximately (254, 788, 600 bp) gene obseved class 1,2 and 3 integrons were detected (100%) in isolates while class 2 integrons (44%) of *S .enterica* isolates, which showed to carry intI1 and intI3 genes respectively. The intI2 gene was identified in only 11 isolates in Figure (2)

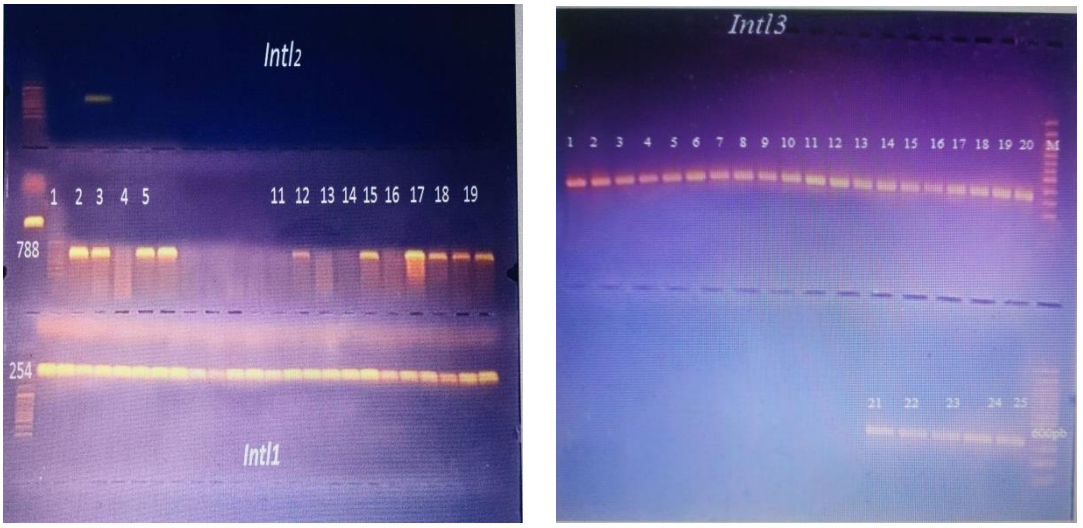


Figure (2): Agarose gel electrophoresis reveals the intl1-intl2- PCR products of (A): Figure (B)Agarose gel electrophoresis reveals the intl3- PCR products of Land100 Lane M: 100-1500 bp DNA Ladder. Lanes 1- 24 : *Salmonella enterica* 1500pb DNA ladder LanM 1-25 Salmonella enterica

Out of 25 *S.enterica* isolates, 24 could be typed one was found to be untypable by RAPD. There were 12 clusters formed from 24 *S. enterica* isolates (PRAPD1 to RAPD12) The isolates had distinct banding patterns, indicating that they were genetically distinct. Among the 25 isolates of *S.enterica,* 24 isolates can be classified by RAPD, while 1 Found unprintable. 24 isolates were collected from *S. enterica* in 12 groups (PRAPD1 to RAPD12) isolates showed Unique banding patterns that indicate heredity Where it was noted that the samples (7,8,9) in the bands are very similar, despite the difference in the source addition the samples (21,22,23) very similar in bands but different in soures isolated The highest percentage appeared in size 1400, while the lowest percentage appeared in size 400 in Figure (3)

Figure (3) Agarose gel electrophoresis reveals the RAPD- PCR products of : Lane M: 100 -1500 bp DNA Ladder. Lanes 1- 23 :24-25 *Salmonella enterica*

Discussion

The rate of *S. enteritidis* isolation or prevalence was 8.5 percent in the south and east of Iran, which was similar to our findings [20]. Anthor study in Iraq was consistent with other studies in Basra the percentage isolates *Salmonella enterica* 37/100 (37%) from humans [21] correspond with the current study in (35) Al-Diwaniyah isolated *Salmomella enterica* from 30 samples from stool and blood 12(40%) while this study is similar to a study in Nasiriyah by Hanan [22] with percentage 13%.

Antibiotic resistance was caused by fals, such as use of antibiotics in animal feed, the randme use of antibiotics without regard for doctors' advice, as well antibiotics in animal feed and microorganisms develop resistance to antibiotics. The current study corresponds with Hanan [22] which that all of the isolates tested positive for ampicillin and cefazolin resistance; the most common pattern was resistance to antibiotics inculd gentamycin, ciprofloxacin, amikacin, while differet of that nitrofurantoin, imipenem, and erapenem resistance (36.36, 36.36 and 18.1% respectively) was found in all isolates. Al-Atabi study showed that complete resistance to Amoxicillin+Clavulanic acid and Ampicillin [23]. Antibiotic sensitivity results showed that *Salmonella* spp. samples had increased sensitivity to Gentamicin and Ciprofloxacin., Trimthoprime sensitivity was moderate, but the results showed complete resistance to amoxicillin and piperacillin in these studies, which differed from the current study. As a result of these findings, it is possible to consider that Trimethoprim and imipenem as the better treatment against *Salmonella* spp.

Plasmids, transposons, and the recently discovered integrons are examples of mobile genetic elements which are capable of horizontal or vertical transfer of antibiotic resistance genes. Integrons have been implicated in the evolution and spread of multidrug resistance in Gram-negative bacteria and there is a strong link between the two between PCR detection of integron genes and dividual resistance phenotypes that correspond Results indicate that *Salmonella* isolates

from diarrhal humans and animals multiple genes specifying identical resistance phenotypes and integrin class1and class3 (100%) while integrin class3 (44%).

From here, it was explained that all isolated samples carried the Class1 integrons have been reported as the most common and widespread especially in clinical settings and are found in many Gram-negative bacteria and a small number of Gram-positive bacteria which are one of the main contributors to the problem of antibiotic resistance dissemination [24,25] Class 2 intergroups are similar with first integrons, and class 2 integron integrase (intI2), which is 40% identical to the intI1 gene but they only appear in bacterial genomes on a sporadic basis. Class 2 integrons are less common, but they have been found in *Salmonella enterica, E. coli, Acinetobacter baumannii,* and other bacteria [26,27,28,29 and 30]. Transposon (Tn402) has class 3 integrons, but they are oriented in the opposite direction as class 1 integrons. The class 3 integrase gene is 60% similar to the class 1 integrase gene. This explain the similarity result in class1 and class3. The prevalence of class 1 integrons in Salmonella spp. isolated from various poultry species was investigated in an Egyptian study was as high as 100%. Integrons have been identified as important risk factors in the spread of antibiotic resistance genes across various classes of antibiotics, according to resear[30]. We confirmed that class 1 and class 3 are the most common types of integron in this study.

To learn more about the relationship between the resistance gene pool and its mobilization via transposons and integrons.The genotyping was done by RAP-PCR so as to find out genetic relatedness among isolates. RAPD techniques were used to preduce DNA fingerprints from *Salmonella enterica* isolates, they showed greater discriminatory power in distinguishing closely related strains of *Salmonella* and produced more complex banding patterns. More human and animal isolates, on the other hand, showed similar banding patterns. Almost certainly the same clone is circulating in these places as the above described are sharing the same building block in the hospital and the chances of cross contamination increases The majority of patients and animals in our hospital were infected with different clades of organisms, as revealed by RAPD-typing there by demonstrating clonal diversity among isolates transmission of genes. Since the samples came from a variety of species and ages, the differences could be due to a variety of factors contributing to the emergence of varying proportions of *Salmonella* as well as other environmental factors in addition to geographical location, the type of feed and medicines used [22] In some studies, *Salmonella* outbreaks are common isolation or a high prevalence of a problem could indicate a serious issue in the loss of hygiene in hospitals and animal herds, in addition to environmental pollution in Iraq and Nasiriyah in particular

Conclusions

This study confirms the role directe contacte animal and contamination enverоment addtion hospatiles reservoir of multidrugresistant *Salmonella* highlights the importance of continuing to monitor food-borne zoonotic bacterial pathogens throughout the food chain.

الخلاصه
تعتبر مقاومة الأدوية المضادة للبكتيريا مشكلة صحية كبيرة بسبب تأثيرها. من المهم دراسة انتشار الجينات المقاومة للمضادات الحيوية .تم الحصول على خمسة وعشرين عينه من السالمونيلا المعوية المعزولة من الإسهال البشري والحيواني. تم استخدام طرق الاستزراع التقليدية والطرق البيوكيميائية والطرق الجزيئية لتوصيف العينات خلال الفترة من ديسمبر إلى سبتمبر 2020 إلى 2021. تم استخدام طريقة الانتشار القرصي لتحديد أنماط الحساسية للمضادات الميكروبية للعزلات التي تم تحديدها على PCR). تم استخدام تضخيم تفاعل البوليميراز المتسلسل (CLSI من قبل معهد المعايير السريرية والمخبرية (MDRأنها لإظهار وجود الإنتكرونات من الفئة 1 و 2 و 3. تم الكشف عن تكامل الفئة 1 والفئة 3 في 100٪ من عزلات السالمونيلا المقاومة للأدوية المتعددة ، بينما تم الكشف عن الفئة 2 بين 40٪. ايضا تم استخدام طريقة تمييزية موثوقة وقابلة للتكرار ودقيقة لعزلات RAPD-PCR حيث أظهرت نتائج تضخيم RAPD-PCRوحساسة ضد السالمونيلا المعوية. وهي طريقه السالمونيلا المعوية أن 24 عزلة (96.٪) أظهرت أيضًا حوالي واحد إلى ستة نطاقات تضخيم لكل عزلة ، باستثناء عزلة واحدة هي 400 bp) ، بينما كان النطاق الأدنى المتكرر 72% bpكانت غير نمطية. كان النطاق الأكثر شيوعًا هو النطاق 1400 8 %) بين العزلات الفردية

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