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Antimicrobial resistance, Virulence profiles of *Salmonella enterica* serovar Typhimurium isolated from clinical samples

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Abstract:

A total of 33 *Salmonella enterica* serovar (ser.) Typhimurium isolates were isolated from clinical samples. These isolates were subjected to testing and analyzed for antibiotic resistance and virulence genes by using simplex PCR. All isolates were sensitive to gentamycin, kanamycin, nalidixic acid, chloramphenicol, and sulfisoxazol. on other hands the isolates showed intermediate resistance to streptomycin while one isolate (# 22) showed resistance to chloramphenicol and tetracycline. For ampicillin, six isolates were resistance to the drug. 33 of the showed either intermediate or full resistance to one or two of the animicrobials tested. Most isolates were positive for teen of the virulence genes tested (*msgA, tolC, spaN, invA, ipfC, sitC,sopB, orgA, pagC* and pefA) . For *sitC*, three isolated were negative to this virulence gene .While two isolate were negative to *lpfC* . One isolate # 33 was negative to *orgA* and *spaN*. These results suggest that *S*. Typhimurium from clinical is virulent, and that capable of causing salmonellosis in humans and it may contribute to pathogenesis

Key words: Virulence genes, Antibiotic resistance, *Salmonella* Typhimurium

مقاومة الجراثيم للادوية وملامح الضراوة لبكتريا السالمونيلا نوع Typhimurium المعزولة من العينات السريرية

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الخلاصة:

تضمنت الدراسة عزل ٣٣ نوع من السالمونيلا (**Typhimurium**) من العينات السريرية. تعرض هذه العزلات لاختبار وتحليل للمقاومة وضراوة الجينات للمضادات الحيوية باستخدام البسيط **PCR**. كانت جميع العزلات حساسة للجنتاميسين، الكاناميسين، حمض النااليديكسيك، الكلورامفينيكول، **sulfisoxazol** من جهة اخرى العزلات أظهرت مقاومة متوسطة للستربتومايسين عدا عزلة واحدة (# ٢٢) وأظهر مقاومة الكلورامفينيكول والتتراسيكلين. أظهرت ستة عزلات مقاومة للامبسلين . ٣٣ عزلة أظهرت مقاومة إما متوسطة أو كاملة إلى واحد أو اثنين من **animicrobials**. وكانت معظم العزلات كانت موجبة لعشرة من جينات الضراوة (*msgA*، *tolC* ، *spaN* ، *InvA*، *ipfC* ، *sopB*، *OrgA*، *sitC pagC* , وpefA). فيما يخص ***sitC*** , كانت ثلاث عزلات سالبة لهذا الجين. بينما اثنين من العزلات كانت سالبة ل***lpfC***. وعزلة واحدة (٣٣) كانت سالبة ل *orgA* و *spaN* . وتشير هذه النتائج إلى أن سالمونيلا نوع Typhimurium هي الاكثر ضراوة والاكثر امراضية في البشر .

1. Introduction

Salmonella are recognized as major food-borne pathogens in humans worldwide usually due to the consumption of contamination food or water. A variety of foods have been implicated as vehicles transmitting salmonellosis to humans, including poultry, beef, pork,

eggs, fish and vegetables [1,2,3] .Infection by *Salmonella enterica* is significant public health concerns across the globe. *Salmonella* penetrate from the gut lumen into the epithelium of the small intestine, causing acute gastrointestinal illness such as gastroenteritis, organ focal infection, and systemic febrile infection [4]. According to FoodNet which was established in 1996 in collaboration with CDC, USDA, FDA and selected state health departments estimated that 3.6 million (39 %) food borne illness were caused by bacteria in which non-typhoid *Salmonella* has caused about 1, 0267,561 cases of food-borne illnesses, 19, 336 cases of hospitalization [5]. So far more than 2610 serovar of Salmonella enterica have been recognized from all over the world, and almost all are able to cause illness in humans and animals [6,3]. Children are prone to an infection caused by *Salmonella,* but infants, elderly, and immunocompromised people are more likely to attract severe infections.*Salmonella enterica* serovar Typhimurium (*S*. Typhimurium) is one of the main serovars causes of human gastroenteritidis according to Center for Disease Control and Prevention. Furthermore, the two most frequent serovars *S*. Typhimurium and *S*. Enteritidis. *S.* Typhimurium is one of the main serovars of *Salmonella enterica. S.* Typhimurium is predominately found in the intestinal lumen. This serovars gains its toxicity because of the large amount of lipopolysaccharides (LPS) that make up the outer membrane of the bacteria. *S.* Typhimurium is not the most dangerous type of *Salmonella*. Some symptoms caused by *S.* Typhimurium include diarrhea, abdominal cramps, vomiting, and nausea. Strain identification is important for effective investigation of source outbreaks on other hands, molecular tools are necessary for monitoring and prevention diseases. Among molecular -based techniques used recently Polymerase cycle reaction. In this study, *S.* Typhimurium isolates from clinical samples were examined for Antimicrobial resistance and PCR for virulence genes.

2. Materials and methods

2.1 Bacterial strains

Thirty three isolates of *Salmonella enterica* serovar Typhimurium were selected for this study. These strains were of clinical origin and were obtained from the Arkansas Department of Health (USA).

2.2. Antimicrobial susceptibility testing by disk diffusion

All of the *S.* Typhimurium isolates used in this study were tested for resistance to eight antibiotics on Mueller-Hinton agar (Difco Laboratories, Detroit, MI) by a disk diffusion method [7]. The antibiotics that were used: kanamycin (30 µg), streptomycin (10 µg), tetracycline (30 µg), nalidixic acid (30 µg), sulfisoxazol (25 µg), ampicillin (10 µg), chloramphenicol (30µg) and gentamycin (10 µg). Susceptibility and resistance were determined in accordance with the criteria of the Clinical and Laboratory Standards Institute [8]. *Escherichia coli* ATCC 25922 was used for quality control, because it is susceptible to all of these antibiotics.

2.3 PCR detection of virulence genes

S. Typhimurium isolates were screened for 10 virulence genes by the simplex PCR method using single set primers [9]. Primers used for this study are listed in Table 1. The template DNA from the isolates was extracted from overnight cultures by using the DNeasy ® Blood and Tissue kit (Qiagen, Valencia, CA, USA). The PCR reaction mixture with a final volume of 10 µl, contained 2 µl of template DNA, 5 µl of GoTaq Green Master Mix (Promega), 1 µl of each needed forward and reverse primers, and 1 µL of distilled water. PCR cycle conditions were as follows: 5 min for the initial denaturation at 95 °C; 30 cycles of 40 s at 94 °C, 60 s at 66.5 °C, and 90 s at 72 °C, and a final extension of 10 min at 72 °C. The PCR products were visualised by electrophoresis on 1.2 % agarose gels in 1 × TAE buffer at 50 V for 85 min.

Table 1: Primers used in PCR for detection of virulence genes in *S.* Typhimurium

Gene	Sequence of Nucleotides	size (bp)	Function of gene
pagC	F- CGCCTTTTCCGTGGGGTATGC R- GAAGCCGTTTATTTTTGTAGAGGAGATGTT	454	survival within macrophage
msgA	F- GCCAGGCGCACGCGAAATCATCC R- GCGACCAGCCACATATCAGCCTCTTCAAAC	189	survival within macrophage
invA	F- CTGGCGGTGGGTTTTGTTGTCTTCTCTATT R- AGTTTCTCCCCCTCTTCATGCGTTACCC	1070	Host recognition /invasion
spaN	F- AAAAGCCGTGGAATCCGTTAGTGAAGT R- CAGCGCTGGGGATTACCGTTTTG	504	Entry into nonphagocytic cells
orgA	F- TTTTTGGCAATGCATCAGGGAACA R- GGCGAAAGCGGGGACGGTATT	255	Host recognition /invasion
sitC	F- CAGTATATGCTCAACGCGATGTGGGTCTCC R- CGGGGCGAAAATAAAGGCTGTGATGAAC	768	Iron acquisition
lpfC	F- GCCCCGCCTGAAGCCTGTGTTGC R- AGGTCGCCGCTGTTTGAGGTTGGATA	641	Host recognition /invasion
sopB	F- CGGACCGGCCAGCAACAAAACAAGAAGAAG R- TAGTGATGCCCGTTATGCGTGAGTGTATT	220	Host recognition /invasion
pefA	F- GCGCCGCTCAGCCGAACCAG R- GCAGCAGAAGCCCAGGAAACAGTG	157	Host recognition /invasion
tolC	F- TACCCAGGCGCAAAAAGAGGCTATC RCCGCGTTATCCAGGTTGTTGC	161	Host recognition /invasion

3. Results

Antimicrobial susceptibility testing of study isolates showed that all 33 isolates were sensitive to gentamycin, kanamycin, nalidixic acid, chloramphenicol, and sulfisoxazol. Most of the isolates showed intermediate resistance to streptomycin while one isolate (# 22) showed resistance to chloramphenicol and tetracycline Table 2. For ampicillin, six isolates were resistance to the drug. 33 of the showed either intermediate or full resistance to one or two of the animicrobials tested. (Table 2). In this study, the size of zone of inhibition of every antibiotic disc was measured in millimeter and while those zones of inhibition compared with zone diameter interpretive standards from CLSI [8]. (Table 2).

Table 2: Antibiotic resistance patterns of isolates

ID	AM 10	C -30	GM-10	K-30	NA-30	SXT-25	S-10	TE-30
1	S	S	S	S	S	S	I	S
2	S	S	S	S	S	S	I	S
3	S	S	S	S	S	S	I	S
4	R	S	S	S	S	S	I	S
5	R	S	S	S	S	S	I	S
6	S	S	S	S	S	S	I	S
7	S	S	S	S	S	S	I	S
8	R	S	S	S	S	S	I	S
9	S	S	S	S	S	S	I	S
10	S	S	S	S	S	S	I	S
11	S	S	S	S	S	S	I	S
12	S	S	S	S	S	S	I	S
13	S	S	S	S	S	S	I	S
14	R	S	S	S	S	S	I	S
15	S	S	S	S	S	S	I	S
16	S	S	S	S	S	S	I	S
17	S	S	S	S	S	S	S	S
18	S	S	S	S	S	S	I	S
19	S	S	S	S	S	S	I	S
20	S	S	S	S	S	S	I	S
21	S	S	S	S	S	S	I	S
22	S	R	S	S	S	S	I	R
23	S	S	S	S	S	S	I	S
24	S	S	S	S	S	S	S	S
25	S	S	S	S	S	S	S	S
26	S	S	S	S	S	S	I	S
27	S	S	S	S	S	S	S	S
28	S	S	S	S	S	S	I	S
29	S	S	S	S	S	S	I	S
30	S	S	S	S	S	S	S	S
31	R	S	S	S	S	S	S	S
32	S	S	S	S	S	S	S	S
33	R	S	S	S	S	S	I	S

S. Typhimurium were screened for teen virulence genes (*msgA, tolC, spaN, invA, ipfC, sitC,sopB, orgA, pagC* and *pefA*) by using simplex PCR, most thirty three isolates were positive for teen of the virulent genes tested. For *sitC*, three isolated were negative to this virulence gene (Fig.1).While two isolate were negative to *lpfC*(Fig. 2).

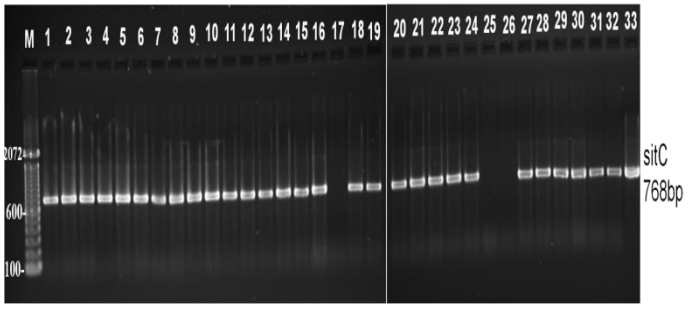

Figure 1. Agarose gel electrophoresis of *sitC.* PCR products amplified from *S.* Typhimurium. M: MW marker (1 kb ladder); 1 to 33 Lanes: *S.* Typhimurium isolates.

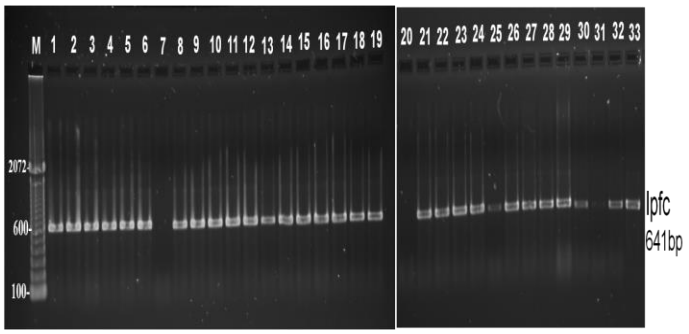

Figure 2. Agarose gel electrophoresis of *IpfC.* PCR products amplified from *S.* Typhimurium. M: MW marker (1 kb ladder); 1 to 33 Lanes: *S.* Typhimurium isolates.

One isolate # 33 was negative to *orgA* and *spaN*(Fig.3 and Fig. 4). These results suggest that *S*. Typhimurium from clinical is virulent, and that capable of causing salmonellosis in humans and it may contribute to pathogenesis.

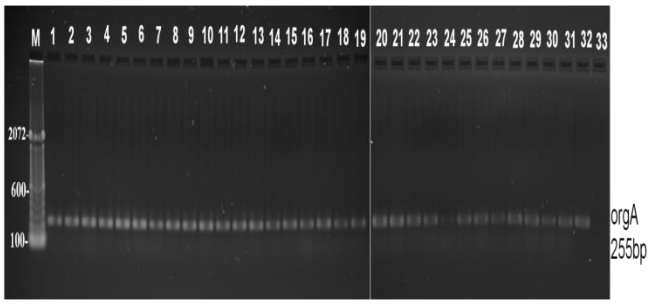

Figure 3. Agarose gel electrophoresis of *orgA.* PCR products amplified from *S.* Typhimurium. M: MW marker (1 kb ladder); 1 to 33 Lanes: *S.* Typhimurium isolates.

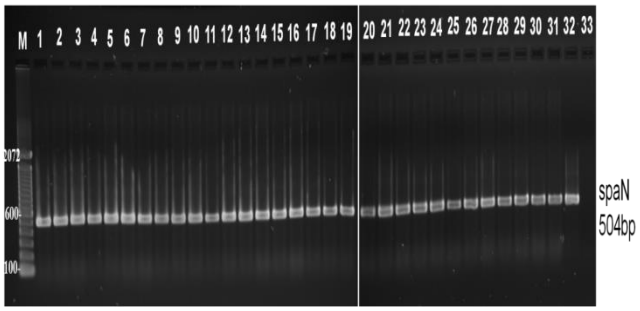

Figure 4. Agrose gel electrophoresis of *spaN.* PCR products amplified from *S.* Typhimurium. M: MW marker (1 kb ladder); 1 to 33 Lanes: *S.* Typhimurium isolates.

4. Discussion

Salmonellosis is one of the most common food borne illnesses. FDA estimated that non- typhoidal *Salmonella* caused total 1,412,498 cases of illness. Every year, there have been approximately 42,000 cases of *Salmonella* infections reported. Though this number is large itself, this is only the reported amount. There are many milder cases of Salmonellosis that are not reported, because they are not that severe. *Salmonella* infections are usually caused by the consumption of fecal contaminated water and food [5]. Our study shows that *S.* Typhimurium isolated from clinical carried the teen virulence genes, which might play an important role in invasion and survival in the host [9]. Recently, Akiyama *et al*. (2011) and Mezal *et al*. (2013) [10,3] indicated that the same virulence genes were percent in *S.* Enteritidis and *S.* Saintpaul isolated from clinical isolates, which are capable of causing human infections. In this study *S.* Typhimurium isolates examined for antimicrobials resistance, all 33 isolates were sensitive to gentamycin, kanamycin, nalidixic acid, chloramphenicol, and sulfisoxazol. Seven isolates were resistant to ampicillin, chloramphenicol and tetracycline.Lower rates of resistance in this study are in agreement with Yang (2002) [11] that have reported a low prevalence of antimicrobial resistance among *S.* Typhimurium from sources in South Korea. Futher investigations with bigger samples size are needed to identify the source and cause of drug resistance. Conclusion: *Salmonella* Typhimurium can present virulence genes (*msgA, tolC, spaN, invA, ipfC, sitC,sopB, orgA, pagC* and p*efA*) related to invasion and survival within macrophage, but that have low prevalence of antimicrobial resistance among.

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