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Antimicrobial Resistance Pattern and Plasmid Profile of Salmonella enterica Isolated from Diarrheal Children in Thi-Qar Province/Iraq

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***Abstract*:The aims of this work were to investigate the antimicrobial pattern and plasmid profile of different antibiotic resistant Salmonella species isolated from diarrheal children in Thi-Qar province and to find a possible relationship between resistance patterns and plasmid profile. Salmonella isolates were tested against 15 commonly used antimicrobial agents using the disc diffusion method to determine the resistance Patterns while plasmid DNA was extracted using alkaline lysis method and separated by agarose gel electrophoresis. all isolates were sensitive to the Amikacin and Gentamycin whereas all isolates were resistances to erythromycin; the most prevalent pattern included resistance to Nalidixic acid (50%) , Cefixim and Cefotaxim (37.5%), and to trimethoprim-sulphamethoxazole, Amoxicillin-clavulanic acid and Ampicillin (33.3%).Furthermore, many isolates were resistant to Tetracycline and Chloramphenicol (29.1%), Ciprofloxacin and Nitrofurantion (25%) and Azithromycin (20.8%), while only 12% of isolates were resistances to Norfloxacin. Plasmid analysis of clinical isolates showed several large and small plasmids were extracted from (91.7%) of the isolates and some isolates carried one or more plasmids.**

Keywords: Salmonella enteric , antimicrobial resistance, plasmids profile.

I. Introduction

Salmonella is classified under Enterobacteriaceae the family; it is gram-negative, and known as "enteric" bacteria. Salmonella are found in the intestinal tract of animals and humans. Some serotypesof Salmonella, such as S. Typhi and S. Paratyphi are only found in humans (Quinn *et al*, 2004). Salmonella enterica is one of the most commonly detected, in terms of both numbers of human infections and severe disease; the widespread of S. enterica in humans and animals worldwide have always been a major public health concern (Yoke *et al*, 2008). Enteric pathogens are a major source of morbidity and mortality throughout the world. It has been estimated more than 3 million deaths associated with Gram-negative enteric pathogen worldwide due to diarrhea and enteric fever each year (Donnenberg, 2000). The species Salmonella enterica comprises a group of gram negative bacteria that are important pathogens for humans and domestic animals (Makela and Hormaeche, 1997; Eisenstein, 1999). Salmonella enterica cause infections called Salmonellosis, Globally, Salmonellosis causes significant morbidity and mortality, it occurs after ingestion of food or water sources that have been previously contaminated with the fecal or urinary excretions of animals that can be act as reservoirs of Salmonella (Akbermehr,2012). Salmonella enterica infection presented clinical manifestations in a number of ways depending on the host susceptibility (Crump *et al*, 2010). These are including gastroenteritis, enteric fever, bacteremia and chronic asymptomatic carriage (Andrews *et al*, 2010). Salmonella bacterium is one of the commonest causes of food poisoning worldwide. They are found in different types of food, such as egg, meat, milk and other dairy products, which serves as a source of Salmonella for humans (Winokur *et al*, 2000). The use of antibiotics in animals whether as growth promoters or for treatments of infections has contributed to resistance developing against these antibiotics (Angulo *et al*, 2004). Development of drug resistance in Salmonella spp. has become an alarming public health problem (Velge *et al*, 2005). Salmonella enterica consists of more than 2668 different serotype. It can cause the disease in both humans and animals; which are the major causative pathogens of food borne disease outbreaks and also a public health concern all over the world (Akbarmehr, 2011).The two most common serotypes in the U.S. are S.Typhimurium and S. Enteritidis. S. Typhi, the serotype that causes typhoid fever, is uncommon in the U.S. (Behravesh*et al*, 2008). But, globally, typhoid fever continues to be a significant problem, with an estimated 12-33 million cases occurring annually. (Miller and Pegues, 2005). The widespread in using of antibiotics in food animals was implicated in the increasing prevalence of antibiotic resistant NTS (Angulo *et al*, 2000). Moreover, outbreaks in developing countries have a high death rate, especially when caused by strains of bacteria that are resistant to antibiotic treatment.

Trimethoprim/sulfamethoxazole, ampicillin, or amoxicillin, are the best choices when treatment is needed (Miller and Pegues, 2005). Ceftriaxone, cefotaxime, or flouroquinolones are effective options for antimicrobial-resistant strains, although fluoroquinolones are not approved for persons less than 18 years of age. The specific antibiotic chosen depends on the susceptibility of the bacteria and the response to treatment. The selection of effective antibiotics is critical for the treatment of invasive Salmonella infections, but has become more difficult as antibiotic resistance has increased. The present study aims to investigate the antibiotic resistance patterns and plasmid profile of antibiotic resistant Salmonella isolates isolated from isolated from diarrheal children in Thi-Qar province/Iraq.

II. Material and Methods

Bacterial Isolation and Identification

A total of 300 stool samples were collected from children (1day-13years) suffering from diarrhea during the period from June 2015 to December 2015. All specimens were screened for the presence of Salmonella enterica by culturing on buffered peptone water, tetrathionate broth, XLD, S.S.Agar, brilliant green agar and Nutrient agar. The specimens were identified by biochemical tests, confirmed by API 20E system. The suspected Salmonella genus was sent to the Central Public Health Laboratories (National Center of Salmonella in Baghdad) "for serotyping diagnosis" and molecular identification by using invA gene.

Antimicrobial Susceptibility

All isolates Salmonella isolates in this study were tested for resistance to 15 antimicrobials on Mueller-Hinton agar (Difco Laboratories, Detroit, MI) by a disk agar diffusion method (Khan *et al*, 2006). The following antimicrobials were used: Amikacin (30 mg), Amoxicillin-clavulanic acid (30 mg), Tetracycline (30 mg) Norfloxacin(30mg) ,Erythromycin(15 mg), Ciprofloxacin(5mg) Chloramphenicol(30 mg) ,Cefotaxim(30 mg), Gentamicin(10 mg) ,Azithromycin(15 mg) ,Ampicillin(10 mg) ,Nalidixic acid(30 mg) ,Cefixim(5 mg) ,Trimethoprim /sulphamethoxazole(25 mg) ,Nitrofurantion(300 mg). The Sensitivity and resistance were determined by the criteria of the Clinical and Laboratory Standard Institute (CLSI, 2010).

Plasmid profiling

Plasmid DNA of the strains was isolated by using the alkaline lysis method following the protocol of (Ponce *et al*. (2008). 1.5 ml from overnight cultures of bacterial growth in Nutrient broth was centrifuged at 12,000 x g for 1 min. The pellet was resuspended in 1 ml of SET buffer (20% sucrose, 50 mM EDTA, and 50 mM TriseHCl, pH 7.6), centrifuged for 1 min at 12,000 x g and resuspended in 150 ml of SET buffer. Cells were lysed by mixingwith 350 ml lysis buffer (1% SDS and 0.2 M NaOH) and incubated for 30 min in ice. Then, 250 ml of acetate buffer (3.0 M sodium acetate, pH 4.8) was added. Tubes were mixed by inversion and incubated for 20 min in ice. After centrifugation at 12,000 x g at 4◦C, 700 ml of the upper aqueous phase was transferred to a clean tube and DNA was precipitated by one volume of isopropyl alcohol. The pellets were washed with 1 ml ethanol and dissolved in 50 ml of TE buffer (50 mM Tris, 1 mM EDTA, pH 8.0).

Agarose gel electrophoresis of plasmid DNA

The plasmids were separated on 1.0% agarose gels in 1X TBE buffer at 64 V for 2 h. The DNA ladder (1kb) from (bioneer, Korea) was used as a molecular marker.

III. Results

Bacterial Isolation and Identification

A total of 300 samples of stool from children suffering from diarrhea have been collected and tested only 24 samples are given growth for Salmonella enterica this is about 8 % of diarrheal children as in figure (1).

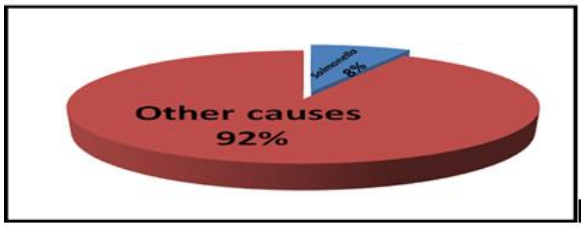

Figure (1): The occurrence of *Salmonellaenterica* isolated from children diarrheal

A. **Molecular diagnosis of S. enterica isolates**

B. *A total of 24 isolates of Salmonella enterica which identified by conventional biochemical test, API 20- E and serological test were subjected to DNA extraction and PCR assay for presence of invA gene. A positive results have seen in 24(100%) of isolates subjected to PCR assay as inFigure (2).*

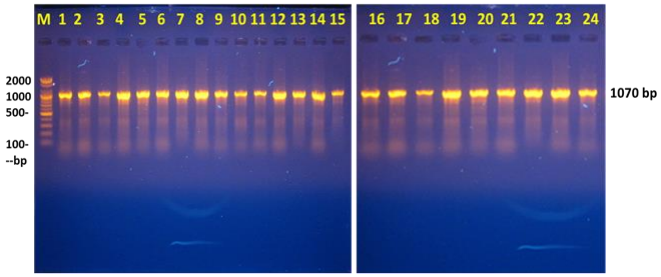

Figure (2): Conventional PCR for detection of invA 1070bp genes, M: MarkerDNA ladder (100-2000) bp, run on 1.2% Agarose gel; at 50V for 85 min

Antibacterial Susceptibility

The antimicrobial susceptibility test for 24 Salmonella enterica isolates showed that all isolates were sensitive to the Amikacin and Gentamycin while all isolates were resistances to erythromycin ,12/24 isolates were resistance to nalidixic acid , 9/24 isolates were resistances to Cefixim and cefotaxim, 8/24 isolates were resistances to Trimethoprim-sulphamethoxazole ,Amoxycav and Ampicillin , 7/24 isolates were resistances to tetracycline and chloramphenicol, 6/24 isolates were resistance to

Ciprofloxacin and Nitrofurantion, 5/24 isolates were resistances to azithromycin , in addition to 3/24 isolates were resistances to Norfloxacin as in table(1)

Table (1): Percentage of antibiotics resistance by *Salmonellaenterica* against 15 types of antibiotics according to CLSI, 2010 (n=24)

Antimicrobial Agents	Salmonella enterica					
	No. R	% of R	No. I	% of I	No. S	% of S
Amikacin (AK)	-	-	-	-	24	100%
Gentamycin (CN)	-	-	-	-	24	100%
Norfloxacin(NOR)	3	12.5%	-	-	21	87.5%
Azithromycin(AZM)	5	20.8%	9	37.5%	10	41.7%
Ciprofloxacin(CIP)	6	25%	15	62.5%	3	12.5%
Nitrofurantion(NIT)	6	25%	6	25%	12	50%
Tetracycline(TE)	7	29%	2	8.3%	15	62.5%
Chloramphenicol(C)	7	29.1%	1	4.1%	16	66.6%
Ampicillin(AMP)	8	33.3%	13	54.1%	3	12.5%
Trimethoprim-sulphamethoxazole (SXT)	8	33.3%	2	8.3%	14	58.3%
Amoxicillin- clavulanic acid (AMC)	8	33.3%	1	4.1%	15	62.5%
Cefotaxim (CTX)	9	37.5%	9	37.5%	6	25%
Cefixim(CFM)	9	37.5%	14	58.3%	1	4.1%
Nalidixic acid (NA)	12	50%	9	37.5%	3	12.5%
Erythromycin(E)	24	100 %	-	-	-	0%

Plasmid profile

Several large and small plasmids were extracted from Salmonella isolates. Out of twenty two (91.7%) of these isolates carried one or more plasmids ; five isolates carried small plasmids of size 1,000, 1,600, 4000 and 8000 bp, However, ten isolates carried more than 10,200 bp as in figure (3

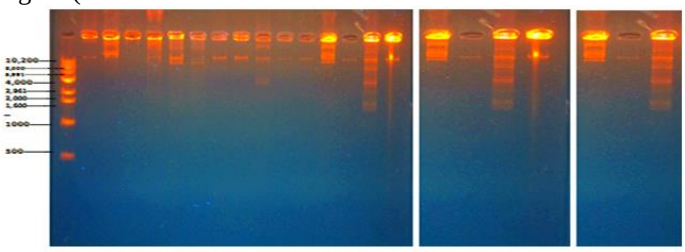

Figure (3): Gel electrophoresis of plasmids DNA of isolates using 1%agarose for , 2 hours at 64 V M: Marker DNA ladder (500-10200bp)

IV. Discussion

One of the most important bacterial enteric pathogens is Salmonella enterica; it is a bacterium that causes Salmonellosis, and attendant public health problem (Amini *et al*, 2010). In recent years, a dramatic increase in antibiotic resistance among Salmonella and other enteric bacteria have been observed in several countries, especially developing countries (Foley *et al*, 2006).In the present study, the question regarding the relationship between antibiotic resistance patterns and the plasmid profile in Salmonella strains isolated from diarrheal children was addressed. Plasmids are extrachromosomal, replicable DNA molecule that may contain resistance gene. They have been a major factor in the spreading antibiotic resistance between bacteria (Peighambari *et al*, 2010). Plasmid profiling is a technique of isolation of plasmids present in a bacterial cell followed by electrophoresis to obtain plasmid counts and sizes; Plasmid profiling has proved to be useful for characterization and differentiation between Salmonella serovars (Olsen *et al*, 1994).In our study appeared several large and small plasmids were extracted from Salmonella isolates out of 22/24 (91.7%) of this isolates carried one or more plasmids. 5/24 isolates carried small plasmids of size 1,000, 1,600, 4000 and 8000 bp. However, 10/24 isolates carried plasmids more than 10,200 bp in addition to carried more than one plasmid. These results agree with the study of (Habeeb and Al-Shawii, 2009) in Baghdad they found that most Salmonella isolated from patients carrying plasmids, additionally to (Bosco *et al*, 2012) have reported the presence one to five plasmids in 67 isolates in Uganda. Whereas, (Rychlik *et al*, 2006) reported that plasmids of S. enterica is varies in sizes that range from 2 to more than 200 kb. In another study by (Mezal *et al*, 2014) showed that all isolates from clinical and food samples carried one or more large plasmids. Salmonella evolved into a pathogen by acquiring pathogenicity determinants through horizontal gene transfer; similarly, it developed antibiotic resistance by virtue of mobile DNA elements (Lee *et al*, 1994; Mirza *et al*, 2000) reported that antimicrobial resistance was transferable from Salmonella spp. to Escherichia coli as well as between other members of the intestinal normal flora. Plasmids are a major mechanism for the spread of antibiotic resistant genes in bacterial populations (Smalla *et al*, 2000). Conjugation occurs by F-plasmids that can transfer genes encoded for multiple resistance and mobilize other nonconjugative plasmids to host cells (Saxena *et al*, 1984). Multiple resistance genes are harbored on R-plasmids some of which are conjugative (Elwell and Falkows, 1980). Escherichia coli have been reported to transfer the antibiotic resistant genes to enteric pathogens such as Salmonella spp. and Proteus spp. and normal flora bacteria, and can be transferred between bacteria of the same or different genera (Platt *et al*, 1986). Fifteen antibiotics belong eight classes of antibiotic were used in present study in the antimicrobial susceptibility test to 24 Salmonella enterica isolates. all isolates were sensitive to the Amikacin and Gentamycin whereas all isolates were resistances to erythromycin; the most prevalent pattern included resistance to Nalidixic acid (50%) , Cefixim and Cefotaxim (37.5%), and to trimethoprim-sulphamethoxazole, Amoxicillin- clavulanic acid and Ampicillin (33.3%).Furthermore, many isolates were resistant to Tetracycline and Chloramphenicol (29.1%), Ciprofloxacin and Nitrofurantion (25%) and Azithromycin (20.8%), while only 12% of isolates were resistances to Norfloxacin . Several large and small plasmids were extracted from Salmonella isolates in our study and out of 91.7% of this isolates carried one or more plasmids; these plasmids may be responsible about the resistance to antibiotic. This research demonstrated that the horizontal transfer of antibiotic resistance plasmids can occur among Salmonella isolates via conjugation and also revealed the emergence of multidrug-resistant Salmonella

isolates as a significant health problem; this consistent with (Akbarmehr, 2012; Jaran, 2015).

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