

ANTIMICROBIAL RESISTANCE PATTERN AND PLASMID PROFILE OF SOME SALMONELLA SPP. ISOLATED FROM CLINICAL SAMPLES IN RIYADH AREA

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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 clinical samples and to find a possible relationship between resistance patterns and plasmid profile.

Salmonella isolates were tested against 12 commonly used antimicrobial agents using the disc diffusion method to determine the resistance Patterns while plasmid DNA was extracted using Plasmid Miniprep method and separated by agarose gel electrophoresis.

All strains Isolated were sensitive to Amoxicillin, Ciprofloxacin, Kanamycin and Amikacin. Isolated strains also showed resistance to Cotrimoxazole (20%), Erythromycin (90%), Ampicillin (90%), naladixic acid (30%), Cephalothin (13%), Tetracycline (60%), Gentamycin (4%) and Chloramphenicol (10%). Plasmid analysis of clinical isolates showed the presence of 1 to 5 plasmids with size range of 1.8 to 115 MDa.

In conclusion, the number of antibiotics a *Salmonella* strain is resistant to may be directly related to the number of plasmids present in that strain. Therefore, recommended antimicrobials for the treatment of infections caused by *Salmonella spp.* should be based on a recent antibiogram tests.

Keywords: Antibiotics, *Salmonella spp.*, antimicrobial resistance, plasmids

Introduction

Salmonella is a Gram-negative bacterium belonging to the family *Enterobacteriaceae*, and known as "enteric" bacteria. **Salmonella** are found in the intestinal tract of animals and humans. Some serotypes

of **Salmonella**, such as **S. Typhi** and **S. Paratyphi** are only found in humans (Miller and Pegues, 2005). In humans, *Salmonella* are the cause of two diseases called salmonellosis: enteric fever (typhoid), resulting from bacterial invasion of the bloodstream, and acute gastroenteritis, resulting from a foodborne infection/intoxication (Foley *et al.*, 2006). *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 (Mlot, 2000). **Development of drug resistance in Salmonella spp. has become an alarming public health problem** (Velge *et al.*, 2005).

Salmonella can be grouped into more than 2,400 serotypes. (Miller and Pegues, 2005). 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. (Behraves, *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). 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* strains isolated from different clinical sources in Riyadh area Saudi Arabia.

Material and Methods

Bacterial Isolation and Identification

Hundred and five unrelated *Salmonella* strains were isolated from different clinical sources from patients of different age and sex attending hospitals in Riyadh area Saudi Arabia, (Table 2). Clinical samples (faeces) were grown on brilliant green agar and xylose-lysine-deoxycholate (XLD) agar (Defco). The isolated bacteria were confirmed by Colony morphology,

direct microscopic examination using gram stain, and biochemical tests, triple sugar iron and lysine agars, other biochemical tests used were fermentation of glucose, urease reaction, lysine decarboxylase, indole test, and H₂S production purchased from Oxoid-UK.

This study was conducted at the Medical School, Al Imam Mohammed Bin Saud Islamic University, Riyadh, Saudi Arabia.

Antimicrobial Susceptibility

Antimicrobial susceptibility of *Salmonella* isolates was determined using disc diffusion method against 12 commonly used antimicrobial drugs in Saudi Arabia, according to the guidelines of CLSI on Mueller Hinton Agar plates. Twelve antimicrobial agents were tested. The antimicrobial agents and their disc concentration were: Ampicillin, (AMP), (10µg); Norfloxacin, (NOR), (10µg); Ciprofloxacin, (CIP), (5µg); Gentamicin, (GEN), (10µg); Tobramycin, (TOB), (10µg); Tetracycline, (TE), (30µg); Sulphamethoxazole-trimethprim (Cotrimoxazole), (SXT), (25µg); nalidixic acid, (NA), (30µg); Amikacin, (AMK), (30µg); Cephalothin, (CEF), (30µg); Agumentin, (AUG), (30µg) and Nitrofurantoin, (NIT), (300µg).

The size of the area of suppressed growth (zone of inhibition) was determined by the concentration of the antibiotics present in the area and, therefore, the diameter of the inhibition zone denotes, the relative susceptibility to a particular antibiotic. The interpretation of the results as sensitive or resistant was determined according to standard charts provided by the manufactures (OXOID Limited, Basingstoke, Hampshire, England).

DNA isolation and plasmid profile

The selected bacterial strain (single colony) was grown overnight in Luria-Bertani (LB) broth at 37⁰C with aeration using an orbital shaker and plasmid DNA was extracted from lysed *E.coli* cells using Plasmid Miniprep kit from Promega Corporation (USA).

Agarose gel electrophoresis of plasmid DNA

Electrophoresis was carried out in a horizontal gel apparatus (Scie-Plas limited, Southam, Warwickshire, United Kingdom). Electrophoresis was conducted in agarose (0.8%) gel (Fisher Biotech, New Jersey, USA) and stained with ethidium bromide. The approximate molecular mass of plasmids (in megadaltons) was determined by comparing with Lambda DNA *Hind III* digest (Promega-USA) as a standard marker.

Results

Antibacterial Susceptibility:

Salmonella strains isolated in this study were sensitive to Amoxicillin, Ciprofloxacin, Kanamycin and Amikacin. On the other hand the isolated strains showed resistance in varying percentage against the following antibiotics, Cotrimoxazole (20%), Erythromycin (90%), Ampicillin (90%), naladixic acid (30%), Cephalothin (13%), Tetracycline (60%), Gentamycin (4%) and Chloramphenicol (10%). (Figure 1), (Table 2).

DNA isolation and plasmid profile

Plasmid analysis of clinical isolates showed the presence of 1 to 5 plasmids per cell with size range from 1.8 to 115 MDa (Table 1).

Table 1 Plasmid characterization isolated from *Salmonella* strains, showing numbers and sizes and resistant antibiotics.

No. of Isolates	Number of Plasmids Isolated	Size of Plasmid (MDa)	Resistant Antibiotics
10	1	83	Ampicillin, Erythromycin,
11	3	95, 7.5, 3.7	Ampicillin, Erythromycin, Cephalothin, Nalidixic acid
18	3	90.0, 7.0, 3.95	Ampicillin, Erythromycin, Cephalothin, Tetracyclin
11	3	101, 4.2, 1.93	Ampicillin, Erythromycin, Cephalothin, Tetracyclin,
20	3	101, 7.5, 4.2	Ampicillin, Erythromycin, Cephalothin, Tetracyclin,
5	2	115, 1.9	Ampicillin, Erythromycin, Cephalothin, Tetracyclin, Co-trimoxazole, chloramphenicol, Nalidixic acid
7	2	115, 1.9	Ampicillin, Erythromycin, Cephalothin, Tetracyclin, Co-trimoxazole, Nalidixic acid
8	3	89, 7.0, 3.9	Ampicillin, Erythromycin, Cephalothin, Tetracyclin, Co-trimoxazole, Nalidixic acid
9	4	115, 53, 22, 4.2	Ampicillin, Erythromycin, Cephalothin, Co-trimoxazole, Nalidixic acid
6	5	101, 6.0, 7.5, 3.95, 1.8	Ampicillin, Erythromycin, Cephalothin, Tetracyclin, Co-trimoxazole, Chloramphenicol, Gentamycin, Nalidixic acid

Table 2 Age groups, sex of patients and antimicrobial susceptibility of isolated salmonella spp.

Age group (years)	Male	Female	Susceptible antibiotics (%)
1 - 10	25	22	Amoxicillin (100%), Ciprofloxacin (100%), Amikacin (100%), Kanamycin (100%), Gentamicin (96%), Cephalothin (87%) Co-trimoxazole (80%) Nalidixic acid (30%) and Ampicillin (10%).
10 - 20	15	13	Amoxicillin (100%), Ciprofloxacin (100%), Amikacin (100%), Kanamycin (100%) Co-trimoxazole (80%) Nalidixic acid (30%) and Ampicillin (10%). Tetracycline (40%), Nalidixic acid (30%) and Ampicillin (10%).
20 - 50	6	5	Amoxicillin (100%), Ciprofloxacin (100%), Amikacin (100%), Kanamycin (100%), Chloramphenicol, Tetracycline (40%), Nalidixic acid (30%) and Ampicillin (10%).
50 - 70	10	9	Amoxicillin (100%), Ciprofloxacin (100%), Amikacin (100%), Kanamycin (100%), Chloramphenicol, Nalidixic acid (30%), Erythromycin (10%) and Ampicillin (10%).
Total	56 (53%)	49 (47%)	

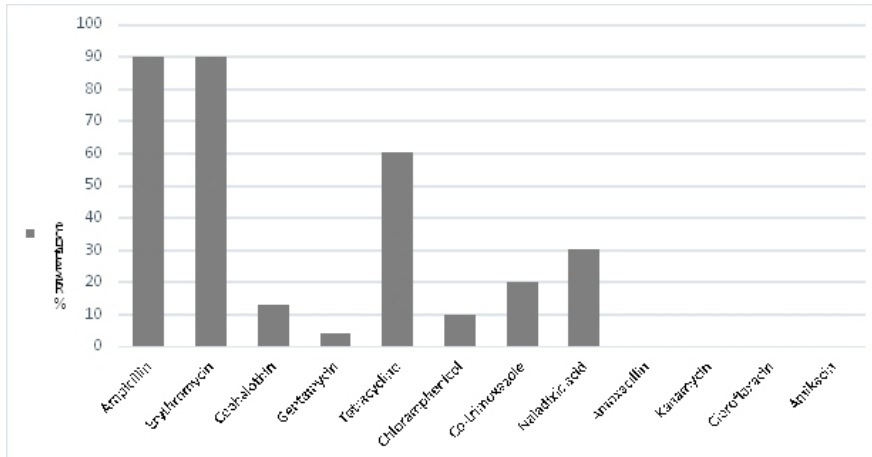


Figure 1 Percentage resistance of antibiotics used against isolated *Salmonella* spp.

Discussion

Salmonella strains are among the most common causes of human bacterial gastroenteritis worldwide (Molt, 2000). In recent years, a dramatic increase in antibiotic resistance among salmonella and other enteric bacteria have been observed in several countries, especially developing countries (Fica *et al.*, 1996; 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 clinical samples was addressed. The results obtained in this study showed that most of the strains isolated were sensitive to Amoxicillin, Ciprofloxacin, Kanamycin and Amikacin, despite the fact that these strains harbored plasmids. The isolated strains showed resistance to different types of antibiotic namely Cotrimoxazole (20%), Erythromycin (90%), Ampicillin (90%), naladixic acid (30%), Cephalothin (13%), Tetracycline (60%), Gentamycin (4%) and Chloramphenicol (10%), these resistant strains showed varying plasmid profiles ranging from one to five plasmid per cell.

Resistance to these antibiotics could be predictable especially to tetracycline because of the high use of tetracycline as an antimicrobial in animal husbandry and in human medicine. The results observed in this study are in agreement with that found by Delicato, *et al.* (2004).

Plasmid profiling of antibiotic resistant *Salmonella* strains revealed that the isolates contained various size R-plasmids, 1 to 5 plasmids per cell with size range from 1.8 to 115 MDa.

Similar finding reported by Al-Bahry (2000), in his study on plasmid DNA analysis of 28 *Salmonella* strains showed that the size of the plasmid DNA ranged from 3.1 kb to 32 kb. And a study by Son *et al.*, (2003) on isolates from fish revealed a similar size range of R plasmids (3 to 63.4 kb). Aja *et al.*, (2002) in their study of *Vibrio* strains isolated from cultured

shrimps reported that some strains were resistant to four antibiotics, others were resistant to two antibiotics and all contained one plasmid of 21.2 kb. They suggested that resistance to antibiotics could be encoded in some strains in plasmids and in others in the chromosomes.

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 non-conjugative 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* has been reported to transfer the antibiotic resistant genes to enteric pathogens such as *Salmonella* spp and *Proteus* spp and normal flora bacteria (Platt et al., 1986). Plasmids are infectious. They can be transferred between bacteria of the same or different genera. Usually all functions required for plasmid transfer, including synthesis of pili, are encoded by genes on the plasmid. Thus, after transfer to a second host, these genes may enable a newly formed trans-conjugant to become a donor in another round of conjugation. This process may be repeated several times.

The results also showed that the number of plasmids found per cell is proportional to the number of antibiotics the isolated strains are resistant to. Ten isolates harbored one plasmid were resistant to two antibiotics where on the other hand six isolated harbored five plasmids were resistant to eight different antibiotics (table 1). This suggests that further research is needed, including DNA sequencing of isolated plasmids in order to confirm dependence between antibiotic resistance of *Salmonella* strains and the presence of plasmids.

Conclusion

We conclude in this study that antibiotic resistance to *Salmonella* isolate is important and may present problems in treating cases of gastroenteritis caused by *Salmonella*. Therefore, more attention should be paid when antibiotics are prescribed and recommended antimicrobials for the treatment of infections caused by *Salmonella* spp. should be based on a recent antibiogram tests.

The collection of more bacterial isolates from various sources and the addition of other tools for genetic analysis should provide more information on the dynamics of the introduction and spread of antibiotic resistant bacteria in nature.

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Names of commercial manufacturers are provided for identification only and inclusion does not imply endorsement by the authors.

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