



# Antibiotic Resistance Patterns and Plasmid Profiles of *Escherichia coli* Isolates from Clinical Specimens

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## Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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## ABSTRACT

**Aim:** The primary aims of this study were to investigate antibiotic resistance patterns and plasmid profile of antibiotic resistant *Escherichia coli* isolated from clinical specimens, and to find out a possible correlation between plasmids and antibiotic sensitivity patterns.

**Methodology:** Unrelated *E. coli* strains were isolated from different clinical specimens from different hospitals and primary health care centres in the Riyadh area Saudi Arabia. Antimicrobial susceptibility of *E. coli* isolates was determined using disc diffusion method against 12 commonly used antimicrobial drugs. Plasmid DNA was extracted from lysed *E. coli* cells using Plasmid Miniprep kit, and visualised using Agarose gel electrophoresis.

**Results:** The results showed that isolated strains of *E. coli* were resistant to Cotrimoxazole (70%), Ampicillin (67%), nalidixic acid (51%), Cephalothin (27%), Agumentin and Nitrofurantoin (19%), Tetracycline and Ciprofloxacin (15%) and Gentamycin (12%). Plasmid analysis of clinical isolates showed the presence of 1 to 7 plasmids with size range of 1.9 to 21.1 Kb, as compared to control *E. coli* ATCC 25922 (size range of 2 to 19.5 Kb).

**Conclusion:** The results obtained in this study showed no direct correlation between the patterns of antibiotic resistance and plasmid profiles.

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## 1. INTRODUCTION

Plasmids are carried by bacteria as extra chromosomal, self-replicating genetic elements. They exist as double stranded, circular DNA molecule capable of self-replication. Plasmids do not usually carry genes essential for the growth of bacterial cells but the genes they carry may be expressed under stressed conditions [1]. Plasmids can be transferred laterally among bacteria of different genera and kingdoms through the conjugation process. Plasmids are capable of autonomous replication and the ability to control their copy number and ensuring stable inheritance during cell division [2]. These systems efficiently promote plasmid maintenance in the bacterial population, regardless of other selective pressure, and do not provide any apparent benefit to the bacterium hosting the plasmid. However, most of the plasmids confer positively selectable phenotypes with the presence of antimicrobial resistance genes. Plasmids are considered to be living organisms in spite of their simple structure, since they are unit elements of a continuous lineage with individual evolutionary history [3].

*Escherichia coli* which is part of the normal flora of humans and other animals and a member of the *Enterobacteraceae* family, they have been known to cause a variety of human infections including urinary infection, severe abdominal pain, hemorrhagic colitis, and haemolytic-uremic syndrome which can be fatal [4,5]. Resistance to a number of antibiotics have been reported in *Escherichia coli* such as tetracycline, nalidixic acids, cefetoxin, chloramphenicol, gentamicin, ampicillin, kanamycin and sulfamethoxazole-trimethoprim, [6-9]. This has been attributed to the widespread and misuse of antibiotics in the treatment of humans or animals, and as growth promoters in feed [10].

*Escherichia coli* strains isolated from sewage treatment plants were reported to be resistant to various antibiotics [11,12]. In Saudi Arabia, *Escherichia coli* isolated from chicken intestines were found to be resistant to many antibiotics such as ampicillin, chloramphenicol, gentamycin, tetracycline, trimethoprim and sulphamethoxazole [13]. Shehabi et al. [14] reported that plasmid profiles of representative multi resistant *E. coli* isolates from human feces and drinking water in Jordan indicated the presence of two common plasmids (49, 25 kb) in

11/12 (91.6%). Integrons with conjugative R-plasmids are common and transferable among commensal antimicrobial multi resistant *E. coli* isolated from both sources [14].

The aims of the present study were to investigate the antibiotic resistance patterns and plasmid profile of antibiotic resistant *E. coli* strains isolated from different clinical sources in Riyadh area Saudi Arabia.

## 2. MATERIALS AND METHODS

### 2.1 Bacterial Isolates

Forty unrelated *E. coli* strains were isolated from different clinical sources (Urine, Blood, Pus and Abscess) from different clinical specimens from different hospitals and primary health care centres in the Riyadh area Saudi Arabia after taking the necessary ethical considerations. The isolated bacteria were confirmed by Colony morphology, direct microscopic examination using gram stain, and biochemical tests. This study was conducted at the Medical School, Al Imam Mohammed Bin Saud Islamic University, Riyadh, Saudi Arabia.

### 2.2 Antimicrobial Susceptibility

Antimicrobial susceptibility of *E. coli* isolates was determined using disc diffusion method against 12 commonly used antimicrobial drugs in Saudi Arabia, according to the guidelines of CLSI [15] on Mueller Hinton Agar plates. Control strains of *E. coli* (ATCC 25922) were used in the study. Twelve antimicrobial agents were tested. The antimicrobial agents and their disc concentration were: Ampicillin, AMP, 10 µg; Norfloxacin, NOR, 10 µg; Ciprofloxacin, CIP, 5 µg; Gentamicin, CN, 10 µg; Tobramycin, TOB, 10 µg; Tetracycline, TE, 30 µg; Sulphamethoxazole-trimethoprim (Cotrimoxazole), SXT, 25 µg., nalidixic acid, NA, 30 µg; Amikacin, AMK, 30 µg; Cephalothin, KF, 30 µg; Agumentin AUG, 30 µg and Nitrofurantoin, NIT, 300 µg.

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).

### 2.3 Plasmid Isolation

The selected bacterial strain (single colony) was grown overnight in Luria-Bertani (LB) broth at 37°C with aeration using an orbital shaker and plasmid DNA was extracted from lysed *E. coli* cells using Plasmid Miniprep kit from Promega Corporation (USA).

### 2.4 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.

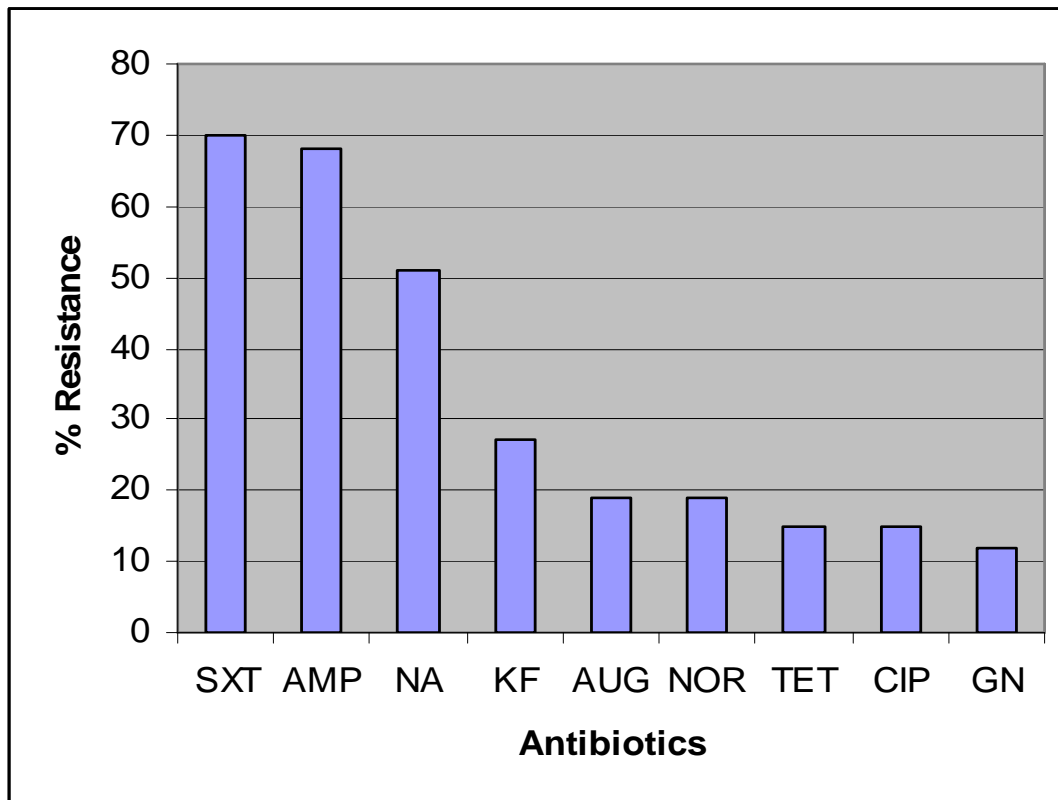
### 2.5 Statistical Analysis

The correlation of plasmids to antibiotic resistance was calculated using Microsoft Excel programme.

## 3. RESULTS AND DISCUSSION

The *E. coli* strains isolated from clinical samples showed resistance mostly to Cotrimoxazole (70%), Ampicillin (67%), nalidixic acid (51%), Cephalothin (27%), Agumentin and Nitrofurantoin (19%), Tetracycline and Ciprofloxacin (15%), and Gentamycin (12%) (Fig. 1).

Plasmid analysis of clinical isolates showed the presence of 1 to 7 plasmids per cell with size range from 1.9 to 21.1 Kb as compared with the control *E. coli* strain (ATCC 25922), where 6 plasmids were isolated with a size range of 2 to 19.5 Kb (Table 1).



**Fig. 1. Percentage resistance to antibiotics used against *E. coli* bacteria in this study**  
SXT: Sulphamethoxazole-trimethprim (Cotrimoxazole); AMP: Ampicillin; NA: Nalidixic acid; KF: Cephalothin;  
AUG: Augmentin; NOR: Norfloxacin; TET: Tetracycline; CIP: Ciprofloxacin; GN: Gentamicin

**Table 1. Plasmid characterization isolated from *E. coli* strains, showing numbers and sizes and pattern of resistant antibiotics**

Isolate no.	Clinical source	Number of plasmids isolated	Size of plasmid (Kb)	Resistant antibiotics
1	Urine	5	21.0, 15.0, 5.0, 4.2, 3.5	-
2	Swab	5	21.0, 14.5, 5.0, 3.5, 2.0	AMP
3	Pus	2	21.0, 3.5	TCP, TET, IMP
4	Abscess	4	21.0, 17.0, 9.0, 4.3	TET, TCP, AUG, IMP, PIP, RIF
5	Urine	4	21.5, 19.0, 4.3, 2.0	AMP, CIP, NA, NOR, CXM, KF
6	Swab	7	21.0, 7.5, 4.0, 4.2, 3.0, 2.5, 1.9	AMP, SXT, NA, CXM
7	Urine	4	21.0, 15.0, 5.0, 1.9	AUG, NOR, SXT, AMP, TET, KF, CIP, GN, NA
8	Abscess	3	21.0, 18.0, 4.2	AMP, NA, SXT, KF
9	Blood	1	21.0	AMP, SXT, KF, NA, NOR
10	Swab	1	21.0	SXT, NA, AMP, NIT, KF
11	Urine	3	21.2, 15.0, 2.0	TET, GN, AMP, NOR, NA
12	Swab	4	21.0, 19.5, 9.0, 2.0	-
13	Pus	2	21.0, 4.6	CIP, TET, NA, GN, AMP, NOR
14	ATCC 25922	6	19.5, 16.0, 5.0, 4.9, 4.2, 2.0	-

The antimicrobial susceptibility was performed on 40 *E. coli* clinical isolates from different clinical sources (urine, blood, pus and abscess) from different hospitals in the Riyadh area. We were able to identify multidrug-resistant (MDR) *E. coli*, which according to a study reported by Sahm et al. [16] in 2001 stating that any bacteria showing resistance to more than three different classes of antibiotics is considered multidrug-resistant bacteria. In this study, the isolated strains displayed an MDR phenotype against a number of antimicrobial agents: Cotrimoxazole (70%), Ampicillin (67%), nalidixic acid (51%), Cephalothin (27%), Agumentin and Nitrofurantoin (19%), Tetracycline and Ciprofloxacin (15%), and Gentamycin (12%). The MDR phenotype of *E. coli* is of great clinical significance because trimethoprim/sulfamethoxazole, ampicillin and ciprofloxacin are among the antibiotics recommended by WHO for the treatment of bacillary dysentery [17]. Therefore, the selection of recommended antimicrobials for the treatment of infections caused by *E. coli* should be based on recent susceptibility tests.

Plasmid profiling of antibiotic resistant *Escherichia coli* isolates revealed that the isolates contained various size R-plasmids, 1 to 7 plasmids per cell with size range from 1.9 to

21.1 Kb. Al-Bahry [18] reported in his study, plasmid DNA analysis of 28 *Salmonella* strains showed that the size of the plasmid DNA ranged from 3.1 kb to 32 kb. A study by Son et al. [19] on isolates from fish revealed a similar size range of R plasmids (3 to 63.4 kb). Aja et al. [20] 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. [21] 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 [22]. Conjugation occurs by F-plasmids that can transfer genes encoded for multiple resistance and mobilize other non-conjugative plasmids to host cells [23]. Multiple resistance genes are harbored on R-plasmids some of which are conjugative [24]. *Escherichia coli* has been reported to transfer the antibiotic resistant genes to enteric pathogenic and normal flora bacteria such as *Salmonella* spp and *Proteus* spp [25].

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.

Our results showed that most isolates have resistance to 4 or more of the antibiotics used regardless of the size and number of plasmid isolated. The multi-drug resistant strains isolated in this study may be the results plasmid transfer from other multi drug resistant bacteria in nature or bacteria found in the intestine of other humans or animals.

The number and size of plasmids isolated from the *E. coli* strains used in this study did not show any correlation to antibiotic resistance ( $r = -0.38944$ ). This could be explained by the fact that plasmids may be randomly distributed in the isolated *E. coli*, or the resistance genes could be located on either the plasmids or chromosomes or the fact that the genes conferring resistance may be found in more than one plasmid with various size which is in agreement with the studies done by Alam et al. and Tabatabaei and Nasirian [26,27].

#### 4. CONCLUSION

The isolated *E. coli* stains showed resistance for 1 to 9 different antibiotics, Cotrimoxazole being the most resistant antibiotic found in this study (70%). The isolated strains found to contain various numbers of plasmids (1 to 7) with different sizes (1.9 to 21.1 Kb). No direct correlation was found between the number of plasmids isolated from *E. coli* strains and the number of antibiotics found to be resistant to these strains.

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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#### COMPETING INTERESTS

Author has declared that no competing interests exist.

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