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Characterization of Ampicillin Resistant Gene (*blaTEM-1*) Isolated from *E. coli* in Northern Jordan

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Abstract

The aim of this project is to study the molecular characteristics of *blaTEM-1* gene, and the associated ampicillin resistance mechanisms present in *E. coli* in north Jordan. In this study, 150 unrelated *Escherichia coli* bacterial samples were isolated from different clinical sources (urine, blood, pus and abscess) and tested for their susceptibility to 17 antimicrobial agents, including ampicillin. The isolates were typed by plasmid profiling, and were investigated by PCR for the presence of various resistance genes. Out of the 150 isolates, 14 strains were multi-resistant; they showed resistance to Cotrimoxazole (70%), Ampicillin (67%), nalidixic acid (51%), Cephalothin (27%), Augmentin 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 ranging from 1.9 to 21.1 Kb compared with the control *E.coli* ATCC 25922 (size range from 2 to 19.5 Kb). PCR results showed the presence of blaTEM-1 gene which was responsible for Ampicillin resistance in 5 of the 14 isolated *E. coli* strains; the gene was located on a plasmid having a size of 1190 bp. This is the first study describing the presence of the *blaTEM-1* gene in bacterial isolates in Northern Jordan. The *blaTEM-1* gene found in this plasmid showed strong correlation between genotype conferred resistance determined by PCR and antibiotic susceptibility patterns.

Keywords: *blaTEM-1 gene, antimicrobial resistance, PCR, resistant plasmid Bulinus species.*

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Introduction

Escherichia coli (E. coli) are considered one of the main causes of nosocomial infections in humans. It's also a common inhabitant of human and animal guts [1], and it's considered an indicator of fecal contamination in water and food. Pathogenic variants cause intestinal and extra intestinal infections, including gastroenteritis, urinary tract infection, meningitis, peritonitis, and septicaemia [2,3]. The widespread use of antibiotics could be associated with the selection of antibiotic resistance mechanisms in pathogenic and non-pathogenic isolates of *E.coli* [4]. Resistance to antibiotics is highly prevalent in bacterial isolates worldwide, particularly in developing countries [5-7]. The spread of resistance genes through plasmid transfer plays an important role in the dissemination of resistance genes in Gram-negative enteric pathogens [8]. Routine monitoring of antibiotic resistance tends to help in providing information on antibiotic therapy and resistance control [9]. Normal intestinal flora is a reservoir for resistance genes, the prevalence of resistance in commensal *E. coli* is reported to be a useful indicator of antibiotic resistance in bacteria in the community [10]. The emergence of multi-resistant *Escherichia coli* has been previously reported in humans and in different animal species increasing the public health concern [11]. On the other hand, the production of extended spectrum beta-lactamases (ESBLs) by Enterobacteriaceae, specifically by *E. coli*, has caused a major concern in several countries, being frequently implicated in human infections. Previous reports have described ESBL-containing *E. coli* strains in healthy animals [12-14]. Studies involving *E. coli* are of particular relevance because these species can occupy multiple niches, including human and animal hosts [15]. In addition, *E. coli* strains can exchange efficiently genetic material with pathogens such as *Salmonella, Shigella, Yersinia*, and *Vibrio species*, as well as with pathogenic *E. coli* [16,17].

Resistance to β-lactam antimicrobial agents in *E. coli* is primarily mediated by β-lactamases, which hydrolyze the β-lactam ring and thus inactivate the antibiotics [18]. Several

different β-lactamases have been described; over 200 β-lactamases have been classified into four main groups and eight subgroups according to their functional and structural characteristics [19-21]. It has been reported that TEM-, SHV- and OXA-type β-lactamases are the most predominant ones [22]. In a study done on Portuguese Cheese, 85% of the isolates identified belonged to the Enterobacteriaceae family. The presence of the *blaTEM* gene was detected in 80.9% of the tested isolates [23].

The present study aims to study the molecular characteristics of *blaTEM-1* gene, and the associated ampicillin resistance mechanisms present in *E.coli* in northern Jordan.

Methods

Bacterial isolates

One hundred and fifty unrelated *E. coli* bacterial samples were isolated from different clinical sources (urine, blood, pus and abscess) from Princess Rahma Hospital, Princess Basma Hospital, Yarmouk Clinic and King Abdullah Hospital in Irbid area in North Jordan. The isolated bacteria were confirmed by direct microscopic examination based on Gram stain, and standard biochemical tests [24].

Antimicrobial susceptibility test

The antibiotic sensitivity testing of bacterial isolates was analysed according to Kirby-Bauer standard single disc diffusion method [25] and the clinical laboratory standard institute [26] on Mueller Hinton Agar plates. Seventeen antimicrobial drugs, including ampicillin, were tested (Table 1). 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 manufacturers. The bacterial strain *E.coli* ATCC 25922 was used as control.

Table 1: *The antibiotics and the concentrations used for Sensitivity testing of E. coli samples, following the disc method.*

Antibiotic	Symbol	Concentration (µg)
Amikacin	AMK	30
Ampicillin	AMP	10
Augmentin	AUG	30
Azteronam	AZT	30
Cefamandole	CMD	30
Cefotaxime	CTX	30
Cefprozil	CPR	30
Ceftazidime	CAZ	30
Ceftizoxime	ZOX	30
Ceftriaxone	CTR	30
Cephalothin	KF	30
Cotrimoxazole	SXT	25
Imipenem	IMP	10
Gentamycin	GN	30
Nalidexic acid	NA	30
Nitrofurantoin	NIT	300
Norfloxacin	NOR	10
Piperacillin	PIP	100
Rifampicin	RIF	5
Teicoplanin	TCP	30
Tetracycline	TET	30
Tobramycin	TOB	10

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. The plasmid DNA was then extracted from lysed *E.coli* cells using a Plasmid Miniprep kit from Promega Corporation (USA).

Detection for the presence of *blaTEM-1* β-lactamases causing resistance to Ampicillin by PCR

PCR analysis was used to detect the presence of *blaTEM-1* gene (Promega-USA), with the primers: 5' – TTC TTG AAG ACG AAA GGG C- 3' (size 19 b) and 5' - ATG GTG AGT GGA ACG AAA AC- 3' (size 20 b). Primers and amplification conditions for *blaTEM* were described previously [27]; the size of the amplified product was 1190bp. Amplified DNA products were resolved by conventional electrophoresis through horizontal 0.8% agarose gel (Scie-Plas limited, Southam, Warwickshire, United Kingdom) containing ethidium bromide with 0.5X Tris-Borate-EDTA buffer at 150v; the results were visualized and photographed under a UV light. The approximate size of plasmids (in bases) was determined by comparing them to a standard marker Lambda DNA Hind III digest (Promega-USA). 16S rRNA primers were used as internal control for all PCRs [28]. The following primers were used 5'- GGA GGA AGG TGG GGA TGA CG- 3' (size 20 b) and 5'- ATG GTG TGA CGG GCG GTG TG – 3' (size 20 b); the size of the amplified product was 241 bp. PCR was performed on a Gene Amp PCR system 9700 (Perkin-Elmer Crop/ Applied Biosystems Division). Amplification conditions were 30 cycles of 94°C for 60 seconds, 60°C for 60 seconds, and 72°C for 60 seconds, with a final extension of 72°C for 5 minutes.

Results

Antimicrobial susceptibility test

All isolates were from human origin (urine, blood, pus and abscesses). The *E. coli* strains isolated from the clinical samples showed resistance mostly to Cotrimoxazole (70%), Ampicillin (67%), Nalidixic acid (51%), Cephalothin (27%), Augmentin and Nitrofurantoin (19%), Tetracycline and Ciprofloxacin (15%), and Gentamycin (12%) (Figure 1).

Plasmid profile

Plasmid analysis of the 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 which was found to contain 6 plasmids with sizes ranging from 2.0 to 19.5 bp (Table 2 and Figures 2 and 3).

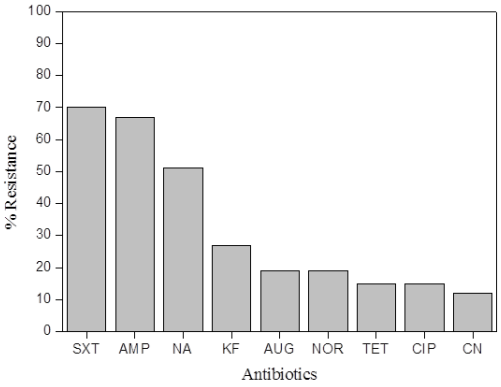

Figure 1: *Percentage resistance to the antibiotics used against E. coli bacteria in this study following the susceptibility test.*

Table 2: *Plasmid characterization. The isolated plasmids from E.coli strains, showing numbers, sizes and resistance to antibiotics.*

Isolate No.	Clinical Source	Hospital	Number of Plasmids Isolated	Size of Plasmid (Kb)	Resistant Antibiotics
1	Urine	Princess Basma	5	21.0, 15.0, 5.0, 4.2. 3.5	-
2	Swab	Princess Basma	5	21.0, 14.5, 5.0, 3.5. 2.0	AMP
3	Pus	Princess Basma	2	21.0, 3.5	TCP, TET, IMP
4	Abscess	Princess Basma	4	21.0, 17.0, 9.0, 4.3	TET, TCP, AUG, IMP, PIP, RIF
5	Urine	Yarmouk	4	21.5, 19.0, 4.3, 2.0	AMP, CIP, NA, NOR, CXM, KF
6	Swab	Yarmouk	7	21.0, 7.5, 4.0, 4.2, 3.0, 2.5, 1.9	AMP, SXT, NA, CXM
7	Urine	Princess Rahma	4	21.0, 15.0, 5.0, 1.9	AUG, NOR, CAZ, SXT, AMP, NIT, AMK, AZT, CTR, PIP, TOB, GN, NA
8	Abscess	Princess Rahma	3	21.0, 18.0, 4.2	AMP, NA, SXT, KF
9	Blood	Princess Rahma	1	21.0	AMP, SXT, KF, NA, NOR
10	Swab	Princess Rahma	1	21.0	SXT, NA, AMP, NIT, KF
11	Urine	King Abdullah	3	21.2, 15.0, 2.0	TET, GN, AMP, NOR, NA
12	Swab	King Abdullah	4	21.0, 19.5, 9.0, 2.0	-
13	Pus	King Abdullah	2	21.0, 4.6	CIP, TET, NA, GN, AMP, NOR
14	ATCC 25922	ATCC	6	19.5, 16.0, 5.0, 4.9, 4.2, 2.0	-

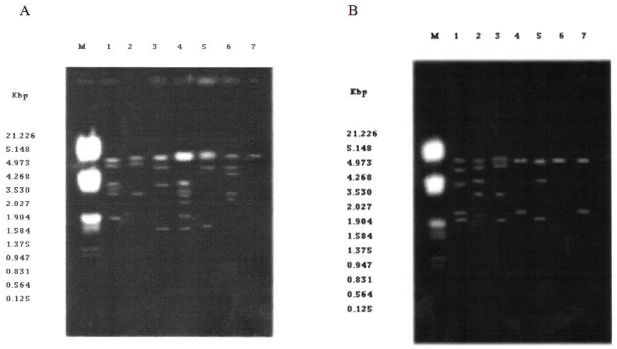

Figure 2: *Agarose gel electrophoresis showing the plasmids isolated from E. coli in all the samples. M: Lambda DNA EcoR1 + HindIII Markers. (A); Lane 1: E. coli ATCC 25922, Lane 2: E. coli isolated from Abscess (princess Rahma Hospital), Lane 3: E. coli isolated from Urine (princess Rahma Hospital), Lane 4: E. coli isolated from Swab (Yarmouk clinic), Lane 5: E. coli isolated from Urine (King Abdullah Hospital), Lane 6: E. coli isolated from Urine (Princess Basma Hospital), Lane 7: E. coli isolated from Blood (princess Rahma Hospital). (B); Lane 1: E. coli isolated from Swab (Princess Basma Hospital), Lane 2: E. coli isolated from Abscess (princess Basma Hospital), Lane 3: E. coli isolated from Urine (Yarmouk clinic), Lane 4: E. coli isolated from Pus (Princess Basma Hospital), Lane 5: E. coli isolated from Swab (King Abdullah Hospital), Lane 6: E. coli isolated from Swab (Princess Rahma Hospital), Lane 7: E. coli isolated from Pus (King Abdullah Hospital).*

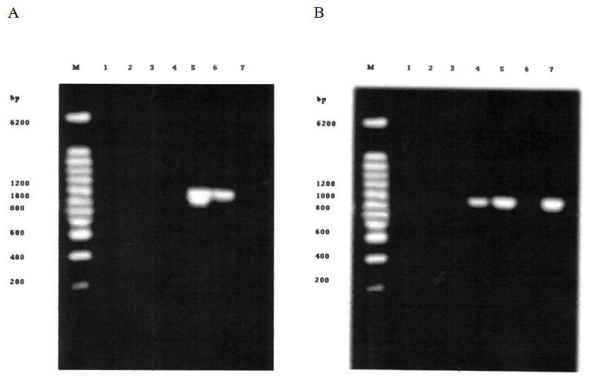

Figure 3: *Agarose gel electrophoresis of the PCR product after amplification using primers for blaTEM-1 gene resistant to Ampicillin in E. coli isolated from the samples. M: 200 bp DNA Step ladder. (A); Lane 1: E. coli ATCC 25922, Lane 2: E. coli isolated from Abscess (princess Rahma Hospital), Lane 3: E. coli isolated from Urine (princess Rahma Hospital). Lane 4: E. coli isolated from Swab (Yarmouk clinic), Lane 5: E. coli isolated from Urine (King Abdullah Hospital), Lane 6: E. coli isolated from Urine (Princess Basma Hospital), Lane 7: E. coli isolated from Blood (princess Rahma Hospital). (B); Lane 1: E. coli isolated from Swab (Princess Basma Hospital), Lane 2: E. coli isolated from Abscess (princess Basma Hospital), Lane 3: E. coli isolated from Urine (Yarmouk clinic), Lane 4: E. coli isolated from Pus (Princess Basma Hospital). Lane 5: E. coli isolated from Swab (King Abdullah Hospital), Lane 6: E. coli isolated from Swab (Princess Rahma Hospital), Lane 7: E. coli isolated from Pus (King Abdullah Hospital).*

Discussion

E.coli infection is considered one of the most common nosocomial infections in Jordanian hospitals. In a study performed at the Jordan University hospital showed that *E. coli* isolated from infected urine accounted for 32.4% of the total isolates and was resistant to Ampicillin [29], in a different investigation in other hospitals in northern Jordan, *E. coli* isolated from the pediatric patients showed a high resistance rate against ampicillin (84% approximately) [30]. In the current study, the antimicrobial susceptibility test was performed on 156 *E. coli* clinical isolates from different clinical sources (urine, blood, pus and abscess) from different hospitals in the north of Jordan. We were able to identify 14 multidrug-resistant (MDR) *E. coli* isolates, which according to a study conducted by Sahm and his group, stating that any bacteria showing resistance to more than three different antibiotics was considered a multidrug-resistant bacteria [31]. 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%), Augmentin 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 the WHO for the treatment of bacillary dysentery [32]. Therefore, the selection of recommended antimicrobials for the treatment of infections caused by *E. coli* should be based on recent susceptibility tests.

Conclusion

Clinical isolates of *E. coli* from different sources of infection were investigated for the presence of β-lactamase-encoding genes. PCR and DNA plasmid profiling showed that the ampicillin resistance was due to the presence of a *TEM-1 β-lactamase* gene. *TEM-1* is the most commonly encountered β-lactamase in Gram-negative bacteria, and up to 90 % of ampicillin resistance in *E. coli* is due to the production of *TEM-118.* This is the first study to be conducted in northern Jordan that discusses the association between the presence of *TEM β-lactamase* gene and ampicillin resistance by *E.coli*.

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