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Antimicrobial susceptibility and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) typing of Gram negative bacteria isolated from urinary tract infections in Mansoura, Egypt

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The aim of the study was to determine the resistance patterns of Gram negative bacterial isolates recovered from patients suffering from urinary tract infections (UTIs) in Mansoura university hospitals, Egypt and also to investigate their epidemiological relatedness using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique. The most prevalent etiological agents of UTIs were the Gram-negative bacilli bacteria including *Escherichia coli*, *Pseudomonas* spp. and *Klebsiella* spp. Among the isolates, *Pseudomonas* spp. showed the highest antimicrobial resistance rate and was significantly resistant to most of the antimicrobials more than other isolates. Antimicrobial susceptibility testing showed that imipenem could be considered as the drug of choice for the treatment of infections caused by multi-resistant isolates of UTIs. SDS-PAGE classified the *E .coli*, *Pseudomonas* and *Klebsiella* isolates into 5, 2 and 5 types, respectively.

Key words: Gram negative bacteria, urinary tract infections (UTIs), antimicrobial susceptibility testing, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

INTRODUCTION

Urinary tract infections (UTIs) are a serious health problem affecting millions of people each year (Naish and Hallam, 2007). They are considered to be among the most common infectious diseases affecting all age groups, from infants to the elderly (Bojić-Milicević et al., 2005). UTIs are the most common infections seen in hospitalized patients and the second most common, after respiratory tract infections, seen in the general population (Fragoulis et al., 2007). Bacteria are by far the most frequent cause of UTIs, and aerobic Gram-negative bacilli predominate

(Clarridge et al., 1987; Elgaml et al., 2013). An important task of the clinical microbiology laboratory is the performance of antimicrobial susceptibility test of significant bacterial isolates. The goals of testing are to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infections (Jorgensen and Ferraro, 2009). In almost all cases there is a need to start treatment before the final microbiological results are available. Area-specific monitoring studies aims to gain knowledge about the type of pathogens res-

Table 1. Interpretation chart for antimicrobial susceptibility pattern according to the National Committee for Clinical Laboratory Standards (NCCLS, 2000).

Antimicrobial agent	Symbols	Disc content (µg)	Inhibition zone diameter (mm)		
			R	I	S
Ampicillin	AM	10	≤11	12-13	≥14
Amoxacillin	AX	25	≤13	14-16	≥17
Cephradine	CE	30	≤14	15-17	≥18
Cefuroxime	CXM	30	≤14	15-17	≥18
Cefoperazone	CEP	75	≤15	16-20	≥21
Cefepime	FEP	30	≤14	15-17	≥18
Imipenem	IMP	10	≤13	14-15	≥16
Amikacin	AK	30	≤14	15-16	≥17
Gentamicin	CN	10	≤12	13-14	≥15
Ciprofloxacin	CIP	5	≤15	16-20	≥21
Levofloxacin	LEV	5	≤12	13-15	≥16

R: Resistant, I: intermediate, S: sensitive.

ponsible for certain infection and their resistance patterns which may help the clinician to choose the right empirical treatment (Hryniewicza et al., 2001).

On the other hand, typing techniques are useful for establishing clonal relationships between individual isolates in hospital settings which are important to recognize nosocomial transmission and guide infection control practice. One of the most commonly used typing techniques which allows a higher degree of taxonomic discrimination and is useful for epidemiological study is sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (de Freitas and Barth, 2002; Sazakli et al., 2005).

The aim of the study was to obtain data on susceptibility patterns of major pathogens isolated from both community acquired and hospital UTIs in Mansoura University Hospitals, Egypt towards different classes of antimicrobial agents used in the treatment of UTIs and also to investigate their epidemiological relatedness using SDS-PAGE technique.

MATERIALS AND METHODS

Bacterial isolates

Twenty five isolates of *Pseudomonas*, twenty five isolates of *Escherichia coli* and twenty isolates of *Klebsiella* were isolated from Mansoura University Hospitals, Dakahlia governorate, Egypt. All the bacterial isolates were obtained from urine clinical specimens. The specimens were processed immediately using standard procedures and the bacterial isolates were identified according to Barrow and Feltham (1993) and Collee et al. (1996). Thereafter, the bacterial isolates were re-identified to the species level by sequencing of their *16s-rRNA* gene according to Elgaml et al. (2013).

Antimicrobial agents

Antimicrobial agents used with their respective interpretation charts are shown in Table 1.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of the isolates was done by standard disk diffusion method as described in the National Committee for Clinical Laboratory Standards (NCCLS, 2000) guidelines. Briefly, from primary isolation medium one bacterial colony was taken by flamed loop, suspended in 5 ml broth and incubated overnight at 37°C with shaking. The bacterial density was adjusted to 10^6 CFU/ml. Petri dishes with Mueller-Hinton agar (4 mm depth) were used for performing the test. Streaking of the bacterial suspension with a clinical swab was done on the entire agar surface in three different directions by rotating the plate at 60° angles after each streaking. Afterwards, Petri dishes were allowed to dry for 15-20 min at room temperature. Then, by using a flamed forceps, standard commercial paper discs of selected antibiotics were inserted to the agar plates and gently pressed down to ensure contact. The plates were incubated in inverted position at 37°C overnight. After incubation, the diameters of inhibition zones were measured to the nearest mm using a Vernier caliber. The experiments were repeated at least three times and the isolates were reported as sensitive or resistant from the respective interpretation charts (Table 1).

SDS-PAGE

Whole cell lysates of test isolates were prepared for SDS-PAGE analysis as described by Nakamura et al. (2002). Briefly, from primary isolation medium one bacterial colony was picked, suspended in 5 ml LB broth and incubated overnight at 37°C with shaking. Subsequently, the broth culture was centrifuged at 15,000 rpm for 15 min at 4°C. The sediment was resuspended in 5 ml phosphate buffer solution (PBS, pH 7.2). One milliliter of the suspension was transferred into 1.5 ml microcentrifuge tubes and centrifuged at 15,000 rpm for 15 min at 4°C. The sediment was suspended in 10 µl of 10% SDS (AppliCem) and an equal volume of loading buffer [0.125 M Tris (hydroxymethyl) aminomethane (Tris, AppliCem), 4% SDS, 10% 2-mercaptoethanol (Merck), 0.2% bromophenol blue (AppliCem); pH 6.8] was added. After vigorous shaking by vortex, the prepared samples were boiled for 10 min at 100°C, centrifuged for 1 min (15,000 rpm at 20°C) and the supernatants were stored at -20°C until use.

The SDS-PAGE was carried out by using 12% (w/v) separating and 4% (w/v) stacking gels as described by Laemmli (1970). The

Table 2. Geographical distribution and clinical sources of the bacterial isolates.

Clinical isolates	Clinical isolates	Numbers of isolates	Geographical distribution	Clinical source
Pseudomonas (25)	*Pseudomonas aurigonsae* (20)	Five isolates (number 1, 3, 12, 13 and 20)	(UNC)	
		Eight isolates (number 2, 5, 14, 21, 22, 23, 24 and 25)	(MUH)	
		Seven isolates (number 7, 8, 9, 15, 16, 18 and 19)	(PUH)	
	Pseudomonas fluorescens (3)	Three isolates (number 6, 10 and 11)	(MUH)	
	Pseudomonas putida (2)	One isolate (number 4)	(MUH)	
		One isolate (number 17)	(PUH)	
Escherichia (25)	*Escherichia coli* (25)	Two isolates (number 18 and 20)	(UNC)	Urine
		Eleven isolates (number 4, 6, 8, 9, 10, 11, 12, 15, 16, 17 and 21)	(MUH)	
		Twelve isolates (number 1, 2, 3, 5, 7, 13, 14, 19, 22, 23, 24 and 25)	(PUH)	
Klebsiella (20)	*Klebsiella pneumoniae* (18)	Sixteen isolates (number 1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 15, 16, 17, 18, 19 and 20)	(UNC)	
		Two isolates (number 12 and 13)	(PUH)	
	Klebsiella oxytoca (2)	One isolate (number 6)	(UNC)	
		One isolate (number 14)	(PUH)	

(UNC) Urology and Nephrology Center, (MUH) Mansoura University Hospital, (PUH) Pediatric University Hospital

protein concentrations of the whole cell lysates were measured according to the method of Lowry et al. (1951). Five microliters of samples were electrophoresed on 12% acrylamide (Sigma) gel for 3 h at 30 mA using a small electrophoresis chamber (Thermo EC120 Mini Gel Vertical System, USA). In each gel a molecular weight mar-marker (Sigma) was included. The gels were stained in 0.25% Coomassie Brilliant Blue R250 (Sigma) in methanol: acetic acid: distilled water (5: 1: 5) for 90 min with gentle shaking. Then the gels were destained in methanol: acetic acid: distilled water (2:3:35) overnight and visualized with the GI-5000 visualization system (Spectronics Co., USA).

Similar SDS-PAGE patterns were considered as one pattern. Accordingly, the tested isolates were successfully classified into different SDS-PAGE patterns.

RESULTS AND DISCUSSION

Bacterial isolates

The overall species distribution is shown in Table 2.

Antimicrobial susceptibility testing

Pseudomonas isolates showed the highest antimicrobial resistance rate and was significantly resistant to most of the antimicrobials than other isolates. All *Pseudomonas* isolates (100%) were resistant to ampicillin, amoxicillin, cephradine, cefuroxime and cefoperazone, 96% were resistant to cefepime, 28% were resistant to imipenem, 48% were resistant to amikacin, 68% were resistant to

gentamicin, 52% were resistant to both ciprofloxacin and levofloxacin (Table 3).

These results are in accordance with study in Iran conducted by Farajnia et al. (2009) showing that, *Pseudomonas* isolates possessing the highest antibiotic resistance rate was significantly resistant to most of the antimicrobials. Moreover these resistance profiles were higher than that reported in El-Astal (2004) study which showed 14.8% resistance for ceftriaxone, 95.4% for amoxicillin, 26.1% for gentamicin, 8.3% for amikacin and 17.8 % for ciprofloxacin. Also in Minia, Egypt, Gad et al. (2008) found that, *Pseudomonas* urinary tract infection isolates were 100% resistant to ampicillin and amoxicillin and were highly resistance to both quinolones and aminoglycosides antibiotics, where 95% of the isolates were resistant to azithromycin.

Regarding *Escherichia* isolates, 96% of the isolates were resistant to both ampicillin and amoxicillin, 92% were resistant to cephradine, 32% were resistant to cefuroxime, 20% were resistant to both cefoperazone and cefepime, 24% were resistant to gentamicin, 36% were resistant to both ciprofloxacin and levofloxacin. On the other hand, 100% of isolates were sensitive to both amikacin and imipenem (Table 4).

These results are in accordance with Zhao et al. (2009) who reported that, resistance rate of *E. coli* isolates to ampicillin was greater than 90% and all the strains were multidrug resistant. And by comparison of these results with that reported by Raka et al. (2004); they reported lower resistance to amoxicillin, ampicillin, ciprofloxacin and gentamicin than that obtained in the present study

Table 3. Antimicrobial sensitivity patterns of the isolated *Pseudomonas* species.

Isolate number	β-Lactams							Aminoglycosides		Quinolones		Number of antimicrobial agents isolates resistant to (N)
	AM	AX	CE	CXM	CEP	FEP	IMP	AK	CN	CIP	LEV	
1	R	R	R	R	R	R	S	S	S	R	R	8
2	R	R	R	R	R	R	S	S	R	R	R	9
3	R	R	R	R	R	R	S	R	R	R	R	10
4	R	R	R	R	R	R	S	S	R	S	S	7
5	R	R	R	R	R	R	S	R	R	S	S	8
6	R	R	R	R	R	R	R	S	R	R	R	10
7	R	R	R	R	R	R	S	S	S	R	R	8
8	R	R	R	R	R	R	S	S	S	S	S	6
9	R	R	R	R	R	R	R	R	R	S	S	9
10	R	R	R	R	R	R	R	R	R	S	S	9
11	R	R	R	R	R	R	S	R	R	S	S	8
12	R	R	R	R	R	R	S	S	S	S	S	6
13	R	R	R	R	R	R	S	S	R	R	R	9
14	R	R	R	R	R	R	S	R	R	R	R	10
15	R	R	R	R	R	R	R	S	S	R	R	9
16	R	R	R	R	R	R	S	R	R	R	R	10
17	R	R	R	R	R	R	S	R	R	R	R	10
18	R	R	R	R	R	R	S	R	R	R	R	10
19	R	R	R	R	R	R	R	R	R	S	S	9
20	R	R	R	R	R	R	S	S	S	S	S	6
21	R	R	R	R	R	R	R	R	R	S	S	9
22	R	R	R	R	R	R	R	R	R	S	S	9
23	R	R	R	R	R	R	S	S	R	R	R	9
24	R	R	R	R	R	R	S	S	S	R	R	8
25	R	R	R	R	R	S	S	S	S	S	S	5

AM: Ampicillin, AX: Amoxicillin, CE: Cephradine, CXM: Cefuroxime, CEP: Cefoperazone, FEP: Cefepime, IPM: Imipenem, AK: Amikacin, CN: Gentamicin, CIP: Ciprofloxacin, LEV: Levofloxacin, S: sensitive, R: resistant.

Table 4. Antimicrobial sensitivity patterns of the isolated *Escherichia* species.

Isolate number	β-Lactams							Aminoglycosides		Quinolones		Number of antimicrobial agents isolates resistant to
	AM	AX	CE	CXM	CEP	FEP	IMP	AK	CN	CIP	LEV	
1	R	R	R	S	R	R	S	S	S	S	S	5
2	R	S	R	S	S	S	S	S	S	S	S	2
3	R	R	R	S	S	S	S	S	S	S	S	3
4	R	R	R	R	S	S	S	S	S	S	S	4
5	S	R	S	S	S	S	S	S	S	S	S	1
6	R	R	R	R	R	R	S	S	R	R	R	9
7	R	R	R	S	S	S	S	S	S	S	S	3
8	R	R	R	S	S	S	S	S	R	S	S	4
9	R	R	R	R	R	R	S	S	R	R	R	9
10	R	R	R	R	S	S	S	S	R	R	R	7
11	R	R	S	S	S	S	S	S	S	R	R	4
12	R	R	R	R	R	R	S	S	R	R	R	9
13	R	R	R	S	S	S	S	S	S	S	S	3
14	R	R	R	S	S	S	S	S	S	R	R	5
15	R	R	R	R	S	S	S	S	S	S	S	4

Table 4. Contd.

16	R	R	R	S	S	S	S	S	R	R	R	6
17	R	R	R	S	S	S	S	S	S	R	R	5
18	R	R	R	R	S	S	S	S	S	S	S	4
19	R	R	R	S	S	S	S	S	S	S	S	3
20	R	R	R	S	S	S	S	S	S	S	S	3
21	R	R	R	S	S	S	S	S	S	S	S	3
22	R	R	R	S	S	S	S	S	S	S	S	3
23	R	R	R	S	S	S	S	S	S	S	S	3
24	R	R	R	R	R	R	S	S	S	R	R	8
25	R	R	R	S	S	S	S	S	S	S	S	3

AM: Ampicillin, AX: Amoxicillin, CE: Cephradine, CXM: Cefuroxime, CEP: Cefoperazone, FEP: Cefepime, IPM: Imipenem, AK: Amikacin, CN: Gentamicin, CIP: Ciprofloxacin, LEV: Levofloxacin, S: sensitive, R: resistant.

where 43% of isolates were resistant to amoxicillin, 41% to ampicillin, 1% to ciprofloxacin and 9.7% to gentamicin and higher resistance to amikacin than that obtained in the present study where 4.9% of isolates were resistant. Also, the results of the present study are higher than that recorded in a study in Iran conducted by Farajnia et al. (2009) showing the rates of resistance of *E. coli* isolates as the predominant cause of UTI, to a panel of antibiotics, including penicillins, cephalosporins, quinolones and aminoglycosides, which are routinely used to treat UTI infections. Moreover, these percentages of resistance of *E. coli* isolates are lower than that obtained by Akram et al. (2007) who reported different sensitivity patterns of some antimicrobial agents against *E. coli* isolates isolated from urinary tract infections in India. They reported that 65% of *E. coli* isolates were resistant to ceftazidime, 56% to cefotaxime, 55% to ceftriaxone, 64% to gentamicin, 73% to tobramicin, 51% to amikacin and 69% to ciprofloxacin and norfloxacin.

Regarding *Klebsiella* isolates, 100% of isolates were resistant to both ampicillin and amoxicillin, 90% were resistant to cephradine, 70% were resistant to cefuroxime, 60% were resistant to both cefoperazone and cefepime, 25% were resistant to amikacin, 40% were resistant to gentamicin, 20% were resistant to ciprofloxacin, 15% were resistant to levofloxacin. On the other hand, 100% of isolates were sensitive to imipenem (Table 5).

These results are higher than that reported by Farajnia et al. (2009) study, where 91.1% of *Klebsiella* spp. were resistant to ampicillin, 1.3% were resistant to amikacin, 19% were resistant to gentamycin and 1.3% were resistant to ciprofloxacin. In addition, the results are higher than the results of Barisic et al. (2003) who reported that 93.6% of *Klebsiella* isolates were resistant to amoxicillin, 32.7% to ampicillin, 28% to cefuroxime and 14% to norfloxacin. Moreover, by comparing these results with that reported by Raka et al. (2004), lower resistance to amoxicillin, ampicillin and ciprofloxacin was recorded, where 76% of isolates were resistant to amoxicillin, 49% were resistant to ampicillin, 8% were resistant to ciprofloxacin. In contrast, higher resistance to aminoglycosides was recorded where

54% of isolates were resistant to gentamicin and 51% were resistant to amikacin.

From the results of the sensitivity patterns of all isolates it was clear that there are many differences of antibiotic sensitivity test between different studies. These differences may be attributed to the fact that resistance rates vary from country to country (Gales et al., 2001).

Moreover, imipenem was the most efficient antimicrobial agents among all isolates, where all *Escherichia* and *Klebsiella* isolates (100%) and 72% of *Pseudomonas* isolates were sensitive to this antimicrobial agent. This increase in the resistance rate of *Pseudomonas* isolates to imipenem is due to its extensive use and this is in accordance with study conducted by Troillet et al. (1997). They reported parallel increase in resistance to imipenem with its use among Gram-negative bacilli and particularly *Pseudomonas*.

SDS-PAGE typing

SDS-PAGE is currently one of the most commonly used techniques for the characterization and analysis of proteins and it has been used as a taxonomic tool for identification of various bacterial species and yielding valuable information on the similarity and dissimilarity amongst bacterial cultures (Chung, 1987). The polyacrylamide gel electrophoresis (PAGE) of proteins analysis has been used widely in typing of many bacterial strains. Protein patterns offer considerable potential for typing bacterial strains of clinical interest, especially for species with other typing methods are not available (Holmes et al., 1991; Malik et al., 2003).

In the present study, protein profiles were very similar and characteristic among the isolates of each group of microorganisms and several isolates exhibited characteristic proteins that may be useful markers for epidemiological investigation.

SDS-PAGE of total cell protein extracts of 25 tested *Pseudomonas* isolates produced characteristic patterns containing about 32 discrete bands with molecular weights

Table 5. Antimicrobial sensitivity patterns of the isolated *Klebsiella* species.

Isolate number	β-Lactams							Aminoglycosides		Quinolones		Number of antimicrobial agents isolates resistant to
	AM	AX	CE	CXM	CEP	FEP	IMP	AK	CN	CIP	LEV	
1	R	R	R	R	R	R	S	R	R	R	R	10
2	R	R	R	R	S	S	S	S	S	S	S	4
3	R	R	R	R	R	R	S	R	R	S	S	8
4	R	R	R	R	R	R	S	R	R	S	S	8
5	R	R	R	R	R	R	S	S	S	S	S	6
6	R	R	R	R	R	R	S	R	R	S	S	8
7	R	R	R	S	S	S	S	S	S	S	S	3
8	R	R	R	R	R	R	S	S	S	S	S	6
9	R	R	R	R	R	R	S	S	R	R	R	9
10	R	R	R	R	R	R	S	S	R	S	S	7
11	R	R	R	S	S	S	S	S	S	S	S	3
12	R	R	R	S	S	S	S	S	S	S	S	3
13	R	R	R	S	S	S	S	S	S	S	S	3
14	R	R	S	S	S	S	S	S	S	S	S	2
15	R	R	R	R	R	R	S	S	S	S	S	6
16	R	R	R	R	R	R	S	S	R	R	S	8
17	R	R	R	R	R	R	S	S	S	S	S	6
18	R	R	S	S	S	S	S	S	S	S	S	2
19	R	R	R	R	S	S	S	S	S	S	S	4
20	R	R	R	R	R	R	S	R	R	R	R	10

AM: Ampicillin, AX: Amoxicillin, CE: Cephradine, CXM: Cefuroxime, CEP: Cefoperazone, FEP: Cefepime, IPM: Imipenem, AK: Amikacin, CN: Gentamicin, CIP: Ciprofloxacin, LEV: Levofloxacin, S: sensitive, R: resistant.
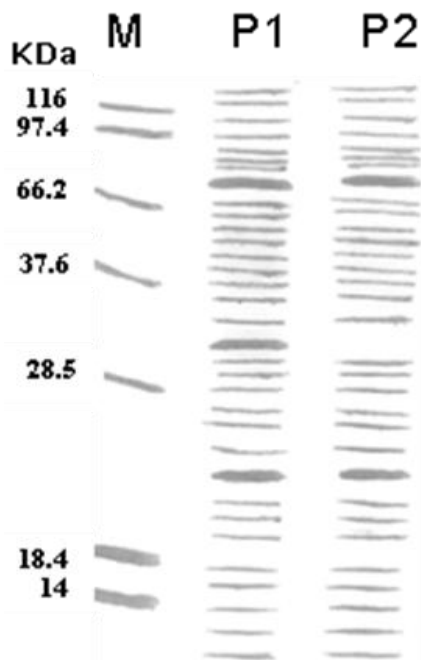
Figure 1. Schematic representation of different total cell protein patterns of *Pseudomonas* isolates. Lane M is molecular weight marker. Lanes P1 and P2 are patterns No. 1 and 2, respectively.

in the range from 14.4 to 116 KDa estimated by polyacrylamide gel electrophoresis. The patterns among all tested isolates were nearly the same; however, few differences were observed. In the present study, two different total cell protein patterns were detected by SDS-PAGE (Figure 1). The first pattern was represented by 21 isolates (No. 1, 2, 3, 6, 7, 8, 9, 10, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 and 25) and the second pattern was represented by 4 isolates (No. 4, 5, 11 and 13). The difference between the two patterns is that the second pattern lacks a band at about 33.05 KDa. The results figure out the sensitivity of SDS-PAGE as a powerful tool allowing a higher degree of taxonomic discrimination and for typing and subtyping of microorganisms even at the subspecies level, where out of 20 *P. aeruginosa* species identified by *16s-rRNA* sequencing 18 belonged to the first pattern (No. 1, 2, 3, 7, 8, 9, 12, 14, 15, 16, 18, 19, 20, 21, 22, 23, 24 and 25) and 2 belonged to the second pattern (No. 5 and 13). Also out of 3 *P. fluorescens* species identified by *16s-rRNA* sequencing two belonged to the first pattern (No. 6 and 10) and one belonged to the second pattern (No. 11). Moreover, out of two *P. putida* species identified by *16s-rRNA* sequencing one belonged to the first pattern (No. 17) and was belonged to the second pattern (No. 4).

SDS-PAGE of total cell protein extracts of 25 tested *E. coli* isolates produced patterns containing about 30 discrete
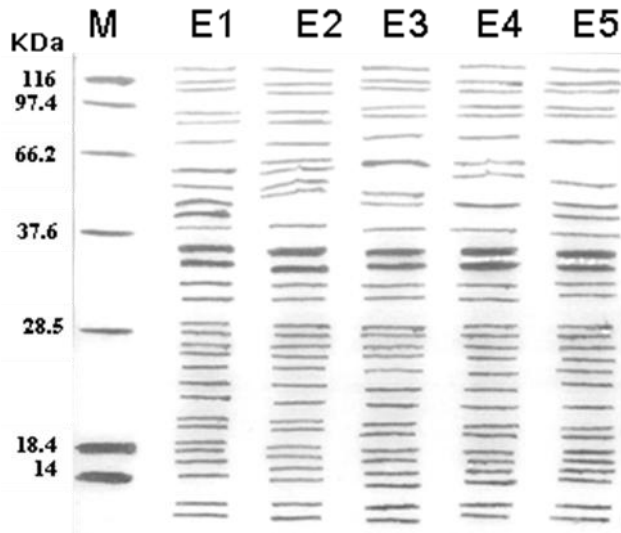
Figure 2. Schematic representation of different total cell protein patterns of *E. coli* isolates. Lane M is molecular weight marker. Lanes E1 to E5 are patterns No. 1, 2, 3, 4 and 5, respectively.
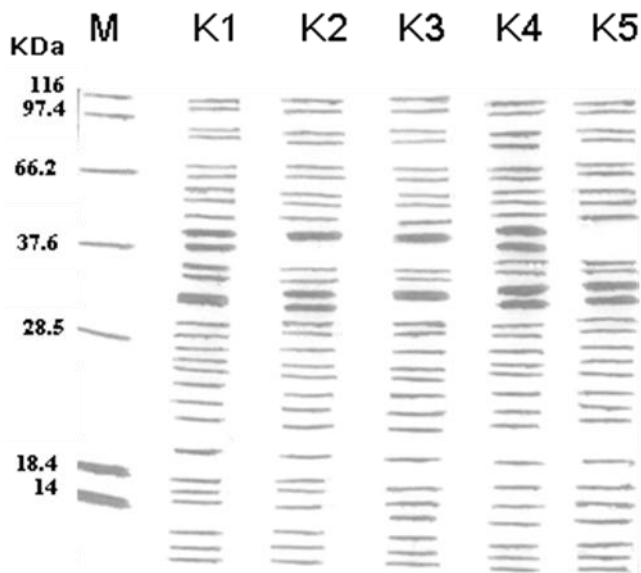

Figure 3. Schematic representation of different total cell protein patterns of *Klebsiella* isolates. Lane M is molecular weight marker. Lanes K1 to K5 are patterns No. 1, 2, 3, 4 and 5, respectively.

bands with molecular weights in the range fof 14.4 to 116 KDa estimated by polyacrylamide gel electrophoresis. The patterns among all tested isolates were nearly the same; however, there were few differences observed. In the present study, 5 different total cell protein patterns were detected by SDS-PAGE (Figure 2). The first pattern was represented by 10 isolates (No. 1, 5, 8, 9, 10, 14, 17, 18, 21 and 25), the second pattern was represented by 4

isolates (No. 2, 11, 19 and 24), the third pattern was represented by one isolate (No. 3), the fourth pattern was represented by 7 isolates (No. 4, 7, 12, 13, 16, 22 and 23) and the fifth pattern was represented by 3 isolates (No. 6, 15 and 20). From the results, it was clear that total cell protein patterns of *E. coli* isolates are very characteristic showing 2 adjacent characteristic heavy bands among all isolates at about 35 and 34.5 KDa. The main difference between the five total cell protein patterns were the bands between 66.2 and 37.6 KDa.

SDS-PAGE of total cell protein extracts of 20 tested *Klebsiella* isolates produced patterns containing about 30 discrete bands with molecular weights in the range of 14.4 to 116 KDa estimated by polyacrylamide gel electrophoresis. The patterns among all tested isolates were nearly the same; however, there were few differences observed. In the present study, 5 different total cell protein patterns were detected by SDS-PAGE (Figure 3). The first pattern was represented by 6 isolates (No. 1, 6, 7, 10, 15 and 16), the second pattern was represented by 3 isolates (No. 3, 9 and 18), the third pattern was represented by 3 isolates (No. 2, 4 and 19), the fourth pattern was represented by 3 isolates (No. 8, 11 and 17) and the fifth pattern was represented by 5 isolates (No. 5, 12, 13, 14 and 20). The main difference between the five total cell protein patterns were the bands at about 39 and 33.1 KDa. With *Pseudomonas* isolates, these results figure out the sensitivity of SDS-PAGE as a powerful tool allowing a higher degree of taxonomic discrimination and for typing and subtyping of microorganisms even at the subspecies level, where out of 18 *K. pneumonia* species identified by *16s-rRNA* sequencing 5 belonged to the first pattern (No. 1, 7, 10, 15 and 16), 3 belonged to the second pattern (No. 3, 9 and 18), 3 belonged to the third pattern (No. 2, 4 and 19), three belonged to the fourth pattern (No. 8, 11 and 17) and 4 belonged to the fifth pattern (No. 5, 12, 13 and 20). Also out of two *Klebsiella oxytoca* species identified by *16s-rRNA* sequencing, one was belonged to the first pattern (No. 6) and one belonged to the fifth pattern (No. 14).

Conclusion

This study shows:

1. The predominance of Gram-negative bacilli as the principal causative pathogens of UTIs.
2. The predominance of *E. coli* as the principal causative pathogen of UTIs, *P. aeruginosa* over other *Pseudomonas* species and *K. pneumoniae* over other *Klebsiella* species as an epidemiological marker.
3. Antimicrobial susceptibility testing showed that imipenem could be considered as the drug of choice for treatment infections caused by multi-resistant isolates of UTIs.
4. The sensitivity of SDS-PAGE as a powerful tool allo-wing a higher degree of taxonomic discrimination and for

typing and subtyping of microorganisms even at the subspecies level.

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