Original Research Article

Bacteriological and Molecular study of Aminoglycoside resistance among *klebsiella pneumoniae* strains isolated from urinary tract infections in pediatric cases in Egypt

Abstract

Aim of the work:The aim of this study was to identify aminoglycoside resistance among *Klebsiella pneumoniae* strains isolated from cases of urinary tract infections in pediatrics and to evaluate the presence of 16S rRNA methylase genes including armA and rmtB genes in the isolated strains. **Materials and Methods:** Ninety-eight *Klebsiella pneumonia* strains were isolated and identified by conventional microbiological methods, 65.3% isolated from cases with cystitis and 34.7% from cases with pyelonephritis. Antimicrobial susceptibility testing was done using disc diffusion test. Presence of 16S rRNA methylase genes including armA and rmtB genes were detected by polymerase chain reaction (PCR). **Results**: The overall aminoglycoside resistance was 48%. Individually, the rate of resistance against Amikacin and Gentamycin were 22%, 26% respectively. Also, 73.5% were multidrug-resistant (MDR), 20.4% were extensive drug resistant (XDR) and 61.2% strains were extended-spectrum β-lactamases (ESBL) producers. PCR amplification of 16S rRNA methylase genes revealed that armA was the most prevalent gene detected in 30 isolates; while rmtB was detected in only 17 cases. **Conclusion:** This finding highlights the alarmingly increase in multidrug-resistant *klebsiella pneumonia* isolates with high frequency of 16S rRNA methylase genes. Consequently, following a wise antibiotic policy is critically necessary to limit the potential spread of resistant genes.

Key words: *Urinary tract infection, Aminoglycosides, Klebsiella pneumonia*

Introduction

Urinary tract infection (UTI) is a very common disorder in children. Although most patients have a good prognosis, UTI can cause significant morbidity, including renal scarring, hypertension and end-stage renal disease **(1).** Early and aggressive antibiotic therapy (within 72 hours of presentation) is important to prevent damage of the kidney. Delayed therapy has been associated with increased severity of infection and greater chance of renal damage (2). Aminoglycosides are broadly preferred antimicrobial agents in the treatment of UTIs. However, several studies have mentioned a steady increase in the rate of uropathogen resistance to aminoglycosides due to the widespread and unwise use of these antibiotics, and this will lead to a reduction in therapeutic possibilities of UTIs (3).

WHO estimates of the global antibiotic resistance in 2014 listed *Escherichia coli, Klebsiella pneumoniae*, and *Staphylococcus aureus* as the three agents of greatest concern, associated with both hospital and community-acquired infections (4).

Due to abuse of antimicrobials, especially β-lactams or aminoglycosides, resistant strains are steadily growing with decreasing the efficacy of these antimicrobial agents. So, the emergence and prevalence of *K. pneumonia* strains that are resistant to wide scale of antimicrobial agents has been reported in many studies (5). Moreover, the mechanisms of resistance to aminoglycosides also include enzymatic modification of this drug, modification of the ribosomal target and decreased intracellular antibiotic accumulation by alterations of the outer membrane permeability, decreased inner membrane transport or active efflux (6). Among them, the production of aminoglycoside-modifying enzymes

is the most common mechanism of resistance to aminoglycosides. Alteration of 16S rRNA by these enzymes reduces binding to aminoglycosides, leading to high-level resistance to aminoglycosides, including arbekacin, amikacin and, kanamycin (7) Recently, seven 16S rRNA methylase genes have been identified (*arm*A, *rmt*A, *rmt*B, *rmt*C, *rmt*D, *rmt*E and *npm*A) (8).

The aim of this study was to identify aminoglycoside resistance among *Klebsiella pneumoniae* strains isolated from cases of urinary tract infections in pediatrics and to evaluate the presence of 16S rRNA methylase genes including armA and rmtB genes in the isolated strains.

Materials and Methods

Study population

This cross-sectional descriptive-analytic study was carried out on 98 non-duplicate *Klebsiella pneumonia* strains obtained from pediatric patients with UTIs in the Pediatrics Department at Tanta University Hospitals, Egypt during a period of one year from September 2017 to September 2018. Ethical approval for this study was provided by ethics and research committee, Faculty of Medicine, Tanta University. An informed consent was taken from the parents of all patients participated in the present study.

All patients included in the present study were subjected to full medical history and clinical examination. Demographic data like age and sex, underlying risk factors, history of urinary tract infection, site of infection and antibiotic usage have been reported earlier.

Klebsiella pneumoniae strains were identified by standard routine microbiological methods described by **Cheesbrough 2006** (9). and then confirmed using the biochemical tests "API 20 E test strips" (bioMe´rieux, Marcy l'Etoile, France).

Detection of virulence factors:

Detection of hemolysin production

Haemolysin production by *Klebsiella pneumoniae* was detected by determining the presence of zone of lysis around each colony on 5% sheep blood agar plate (oxoid,UK) (10)

Detection of biofilm producers

Biofilm production was detected for *Klebsiella pneumoniae* by using Congo red agar method (CRA). After inoculation of the organisms on CRA, based on the intensity of color change of CRA medium which is directly proportional to the amount of biofilm produced by the organisms, the biofilm producing organisms were classified into three categories as strong biofilm producers, moderate biofilm producers and weak biofilm producers *(11)*

Antimicrobial susceptibility testing:

A) Kirby-Bauer disc diffusion method

Antibiotic susceptibility testing for *Klebsiella pneumoniae* isolates was performed on Muller-Hinton agar (Oxoid, UK) by Kirby-Bauer disc diffusion method using the Clinical and Laboratory Standards Institute guidelines *(CLSI 2016) (12).* The size of inhibition zone around each antimicrobial disk was interpreted as sensitive, intermediate or resistant according to the CLSI criteria. In this study, the following groups of antibiotics had been tested: aminoglycosides [Amikacin (30μg), Gentamicin (10μg)], cephalosporins

[Cefazolin (30μg), Cefotaxime (30μg), Cefoxitin (30 μg), Ceftriaxone (30μg), Ceftazidime (30μg) Cefepime (30 μg)], aminopenicillin [Ampicillin (10μg)], carbapenems [Imipenem (10μg)], sulfonamides [Trimethoprim / Sulfamethoxazole (1.25/23.75 μg)], β-lactam/β-lactamase inhibitor [Amoxicillin / Clavulanic acid (20/10μg)] and nitrofuran [Nitrofurantoin (300 μg)]. Also, Nalidixic acid (30μg), Ciprofloxacin (5 μg), Norfloxacin (10μg), and Levofloxacin (5 μg) were used to detect quinolone and fluoroquinolone resistance.

American Type Culture Collection (ATCC) strain (*E. coli* ATCC 25922) was used as a quality control strain in antimicrobial susceptibility testing.

The isolates resistant to Aminoglycoside were stored at -70°C in brain heart infusion containing 20% glycerol for molecular study.

B) Phenotypic tests for detection of Extended-Spectrum β-Lactamases(ESBL) producing isolates:

1. Screening test of ESBL by disk diffusion method

All the *Klebsiella pneumoniae* isolates that were resistant to third generation cephalosporins (exhibiting the growth inhibition zone of < 22 mm for Ceftazidime, < 27 mm for Cefotaxime or Aztreonam, and < 25 mm for Ceftriaxone by disc diffusion test) were considered a potential ESBL producer according to CLSI guidelines (12).

2.   Phenotypic confirmatory test for ESBL (Combined Disc Method)

In order to confirm the presence ESBL-producing strains, the combined disc test was carried out on Mueller-Hinton agar with disks, containing cefotaxime (30 μg), and ceftazidime (30

μg) placed respectively to a distance of 20 mm from combined disks of cefotaxime/clavulanic acid (30.10 μg), and ceftazidime/clavulanic acid (30.10 μg). A difference of ≥ 5 mm between the inhibition zone diameters of either of the cephalosporin disks (cefotaxime, and ceftazidime) and their respected cephalosporin-clavulanate disk is considered to be phenotypic confirmation of ESBLs production *(CLSI 2016) (12).*

Klebsiella pneumoniae ATCC 700603 was used as a positive control for ESBL test.

C) Determination of an approximate MIC-value for aminoglycosides using Epsilometer test (E- test).

All confirmed aminoglycosides resistant strains based on their non-susceptibility to gentamycin and amikacin using the agar disk diffusion method were further subjected to the Epsilometer test (E test; bioMe´rieux, Marcy l'Etoile, France) on Muller-Hinton agar to determine the minimum inhibitory concentration (MIC) values of amikacin and gentamycin. The MIC breakpoints for the resistant strains are (≥16, ≥2) mg/ml for Amikacin and gentamycin respectively according to the CLSI 2016 guidelines.

Molecular Study (Detection of 16S rRNA methylase genes by conventional polymerase chain reaction):

1) DNA extraction

DNA was extracted from *Klebsiella pneumonia* isolates that found to be resistant to at least one of the tested aminoglycosides in the disk diffusion method by using a DNA extraction kit (QIAprep Spin Miniprep Kit, Qiagen, Germany). Before DNA extraction, the aminoglycoside resistant *Klebsiella pneumonia* strains were cultured in Luria-Bertani

broth (Sigma-Aldrich, USA) at 37°C for 18 hours. Then the DNA extraction was performed according to the manufacturer's instructions.

2) Polymerase chain reaction amplification

A polymerase chain reaction (PCR) assay was performed on a thermocycler, Gene Amp® PCR System 9700 - Applied Biosystems), to screen the presence 16S rRNA methylase genes ; armA and rmtB in aminoglycoside resistant *K.pneumoniae* strains was detected. The primers used for amplification of armA, and rmtB genes were supplied by (Invitrogen Thermo Fisher Scientific, USA) as shown in **Table 1**.

The reaction mixtures were prepared in a total volume of 25μl (24μl of PCR master mix plus 1μl of template DNA) including 5ng of genomic DNA, 2.0U of Taq DNA polymerase, 10mM dNTP mix at a final concentration of 0.2mM, 50mM MgCl2 at a final concentration of 1.5mM, 1μM of each primer, and 10X PCR buffer in final concentration of 1X. A tube containing PCR reaction without any DNA template was used as negative control.

Thermocycling conditions were as follows: initial denaturation at 95 ºC for 5 min; 30 cycles of 94 ºC for 45 s, optimized annealing temperature (51ºC for *arm A and rmt B*) for 45 s and 72 ºC for 45 s; followed by a final extension at 72 ºC for 7 min.

Amplifications were performed using the following program: initial denaturation at 95 for 5 min followed by 35 cycles of 45 sec at 94 ºC, 45 sec at annealing temperature (51ºC for *arm A and rmt B*), 45 sec at 72ºC and a final extension period of 7 min at 72 ºC.

3) Agarose gel electrophoresis

PCR products were analyzed by electrophoresis on (2%) agarose gel at 100 V for 30 min and stained with ethidium bromide. DNA bands were visualized by Gel Documentation system (BioRad Gel Doc^{TM} EZ Imager, USA).

Statistical Analysis:

In the present study, statistical analyses of data were carried out using the Statistical Package for the Social Sciences (SPSS), version 20 (Armonk, NY: IBM Corp). The Kolmogorov-Smirnov test was used to test normal distribution of variables. Quantitative variables were described in the form of mean ± SD, while categorical data were presented as the number and percentage. The significance of the difference between groups for quantitative variables was determined by the student's t-test. Categorical variables were compared using the chi-square (χ2) test. The probability (P) values of ≤0.05 were considered statistically significant, P-values ≤ 0.001 were considered highly significant indicated *, while P> 0.05 was considered statistically not significant.

Results

From 98 studied cases, 24 (24.5%) were male and 74 (75.5%) were female; the mean age was 7.8 ± 7.55 years with an age range of 2 to 15 years. Among those, 64 (65.3%), and 34 (34.7%) were identified to have cystitis and pyelonephritis, respectively. Patients were divided according to the source of infection into two groups: **group 1** (n=56) patients with community-acquired UTIs, and **group 2** (n=42) patients who suffer from hospital-acquired UTIs. Patient characteristics are summarized in **Table 2**. According to the obtained results, No gender difference was observed among two patients groups while patients suffering from hospital-acquired UTIs displayed a significant elder age (P <

0.001). Also, the prevalence of pyelonephritis is significantly higher in patients with hospital acquired UTIs compared to patients with community-acquired UTIs (P < 0.001).

Detection of virulence factors

As regard to the virulence factors of *k.pnemoniae,* 30 out of 98 UPEC strains showed hemolysis on sheep blood agar. However, there was no statistically significant difference between hemolysin producing community-acquired and hospital acquired UPEC (P= 0.206).

In the hemolysin producers UPEC strains, there was a statistically significant difference between the prevalence of k.pneumoniae strains causing pyelonephritis 64.7% (22/30) and those causing cystitis 12.5% (8/30) (P < 0.001) **(Table 3)**.

Antimicrobial susceptibility testing

Antimicrobial testing of isolated *Klebsiella pneumoniae* revealed that all *Klebsiella pneumoniae* isolates were resistant to ampicillin and cefazolin. The majority of the isolates were non susceptible to Trimethoprim/sulfamethoxazole (87/98, 88.8%), ceftriaxone (78/98, 79.6%), amoxicillin/clavulanate (74/98, 75.5%), cefotaxime (73/98, 74.49%), and Ceftazidime (63/98, 64.3%)**.** However, the tested isolates exhibited sensitivity rates to amikacin (76/98, 77.55), imipenem (75/98, 76.53)**,** nitrofurantoin (74/98, 75.5) and gentamicin (70/98, 71.43)**.**

Besides that, the overall resistance rate towards fluoroquinolones and quinolones was (66/98, 67.3%). The frequencies of resistance to quinolones were as follows: Levofloxacin (64.29%), Ciprofloxacin (71.43%), Norfloxacin (82.65%), and Nalidixic acid (91.84%) **(Table 4)**.

Antimicrobial resistance pattern of isolated *Klebsiella pneumoniae* indicated that 6 (6.1%) of isolates were sensitive, 72(73.5%) were MDR, 20 (20.4%) were XDR, and 60 (61.2%) were ESBL producer (**Table 5**).

By comparing the antimicrobial susceptibility of community-acquired (group I) and hospital-acquired (group II) UPEC strains, the present study indicated that there was a statistically significant difference in resistance pattern of UPEC strains isolated from community and hospital-acquired UTI (**Table 6**).

Results of molecular study

Screening of aminoglycoside-resistant isolates for 16S rRNA methylase genes revealed that armA was the most prevalent detected gene observed in 30 isolates while rmtB *genes* were detected 18 tested isolates.

Correlation between MIC for aminoglycosides and Genetic Determinants of Resistance

In the current study, *Klebsiella pneumoniae* isolates harbored armA showed a significantly elevated MIC for Amikacin and gentamycin when compared with those lacking this gene which explains the importance of this determinant in increasing MIC value **(Table 7).**

Table 1: Primers used for detection of the presence of armA, rmtB genes in minoglycoside-resistant strains of *Klebsiella pneumoniae*

Resistance genes	Primers	Amplicon size (bp)
armA	5'- ATTTCTCACGCCAGGATTTG-3′	516

	5'- GATCGGCAAAGGTTAGGTCA-3′	
rmtB	5'- GATCGTGAAAGCCAGAAAGG-3′ 5'- ACGATGCCTGGTAGTTGTCC-3′	469

Table 2: Demographics and clinical characteristics of patients infected with *Klebsiella pneumoniae* in both groups.

Groups Variables	Uropathogenic *K.pneumoniae* isolates		Total UTIS patients **N=98**	**P-Value**
	Patients with CA *K.pneumoniae* **(n=56)**	Patients with HA *K.pneumoniae* **(n= 42)**		
Sex				
Male	14(25.0%)	10(23.8%)	24(24.5)	p = 0.892
Female	42(75.0%)	32(76.2%)	74 (75.5)	
Age (y)				
Min. –Max.	2.0 – 15.0	2.0–15.0	2.0–15.0	ᵗp<0.001*
Mean± SD	7.80 ± 7.55	7.80 ± 7.55	7.80 ± 7.55	
Site of infection: Cystits Pyelonephritis	50(89.3%) 6(10.7%)	14(33.3%) 28(66.7%)		p<0.001*

p: p values for **Chi square test,** ᵗp: p values for Student t-test *: Statistically significant at p ≤ 0.05and highly significant at p ≤ 0.001* Group 1 Community acquired UTI (CAUTI) Group 2 Hospital acquired UTI (HAUTI).

Table 3: Distribution of hemolysin producers among studied *klebsiella pneumoniae* strains (n=98).

Groups	Hemolysin producer k.pneumoniaeStrains (n=30) (30.6%)	p-value
Group I CA *K.pneumoniae* strains (n=56)	20(35.71%)	0.206
Group II HA *K.pneumoniae* strains (n=42)	10(23.81%)	
K.pneumoniae causing Upper UTI (n=64)	8(12.5%)	<0.001*
K.pneumoniae causing Lower UTI(n=34)	22(64.7%)	

p: p value for Chi square test

: Statistically significant at p ≤ 0.05 and highly significant at p ≤ 0.001

Table 4: Antibiotic susceptibility of isolated *K.Pneumoniae* by disc diffusion method

Antimicrobial Class	Antimicrobial agents (μg)	Symbol	Sensitive (mm)(N)%	Intermediate (mm)(N)%	Resistance (mm)(N)%
Aminoglycosides	Amikacin(30μg)	AK	76 (77.55)	-	22 (22.45)
	Gentamicin (10μg)	CN	70 (71.43)	2 (2.04)	26 (26.53)
Folate pathway Inhibitors	Trimethoprim/ sulfamethoxazole (25μg)	SXT	9 (9.9)	2 (2.02)	87 (88.77)
Nitrofuran	Nitrofurantoin (300μg)	F	74 (75.5)	3 (3.1)	21(21.4)
Aminopenicillin	Ampicillin (10μg)	AMP	-	-	98 (100.0)
Cephalosporins	Cefazolin (30μg)	CZ	_	-	98 (100.0)
	Cefotaxime (30μg)	CTX	25(25.51)	-	73 (74.49)
	Ceftazidime (30 μg)	CAZ	30 (30.6)	5 (5.1)	63 (64.3)
	Ceftriaxone(30μg)	CRO	20 (20.40)	-	78 (79.60)
	Cefoxitin (30g)	FOX	35 (35.71)		63 (64.3)
	Cefepime	FEP	37(37.76)		61(62.24)
β-lactam/β-lactamase inhibitor	Amoxicillin / clavulanic acid (20/10μg).	AMC	24 (18.7)	-	74 (75.5)
Carbapenems	Imipenem (10μg)	IPM	75 (76.53)	3(3.06)	20(20.41)
Quinolones	Nalidixic acid(30μg)	NAL	8(8.16%)	0	90(91.84%)

	Norfloxacin(10µg)	NOR	17(17.35%)	0	81(82.65%)
	Ciprofloxacin(5 µg)	CIP	25(25.51%)	3(3.06%)	70 (71.43)
	Levofloxacin (5 µg)	LEV	30(30.61%)	5(5.1%)	63(64.29%)

Table 5: Drug resistance pattern of isolated *K.pneumoniae* isolates

Drugs resistance pattern	*K.pneumoniae* (n=98)	
	N	%
Sensitive	6	6.1
MDR	72	73.5
XDR	20	20.4
ESBL	60	61.2
Aminoglycoside sresistance	48	48.3

Table 6: Resistance of community and hospital-acquired *K.pneumoniae* strains to the antimicrobial agents.

Antibiotic	*Group I* CA *K.pneumoniae* (n=56)	*Group 2* HA *K.pneumoniae* (n=42)	p.value
Nalidixic acid	48(85.71%)	42(100%)	^{FE}p= 0.010*
Norfloxacin	41(73.21%)	40(95.24%)	0.004*
Ciprofloxacin	32(57.14%)	38(90.47%)	0.002*
Levofloxacin	30(53.6%)	33(78.57%)	0.009*
Amikacin	3(5.35%)	19(45.24%)	<0.001*
Gentamicin	6(10.71%)	20(47.62%)	<0.001*
Trimethoprim/ sulfamethoxazole	45(80.35%)	42(100%)	^{FE}p= 0.002*
Nitrofurantoin	6(10.71%)	15(35.71%)	0.003*
Ampicillin	56(100%)	42(100%)	-
Cefazolin	56(100%)	42(100%)	-
Cefotaxime	31(55.36%)	42(100%)	<0.001*
Ceftazidime	21(37.5%)	42(100%)	<0.001*
Ceftriaxone	36(64.28%)	42(100%)	<0.001*
Cefoxitin	21(37.5%)	42(100%)	<0.001*
Cefepime	19(33.93%)	42(100%)	<0.001*
Amoxicillin / clavulanic acid	32(57.14%)	42(100%)	<0.001*
Imipenem	5(8.93%)	15(35.71%)	0.001*

FEp: p value for Fisher Exact for Chi square test
*: Statistically significant at $p \leq 0.05$

Table 7: MIC Values for amikacin and gentamycin

Antibiotic	E test MIC range for Amikacin and gentamycin(µg/ml)		^TP value
	Aminoglycoside Sensitive strains	Aminoglycoside Resistant strains	
Amikacin	2.04±0.72	108.27±63.12	<0.001*
Gentamycin	0.12 ± 0.15	19.87±10.79	<0.001*

^TP: p value for **T test** for comparing between the two groups

: Statistically significant at $p \leq 0.05$ and highly significant at $p \leq 0.001$
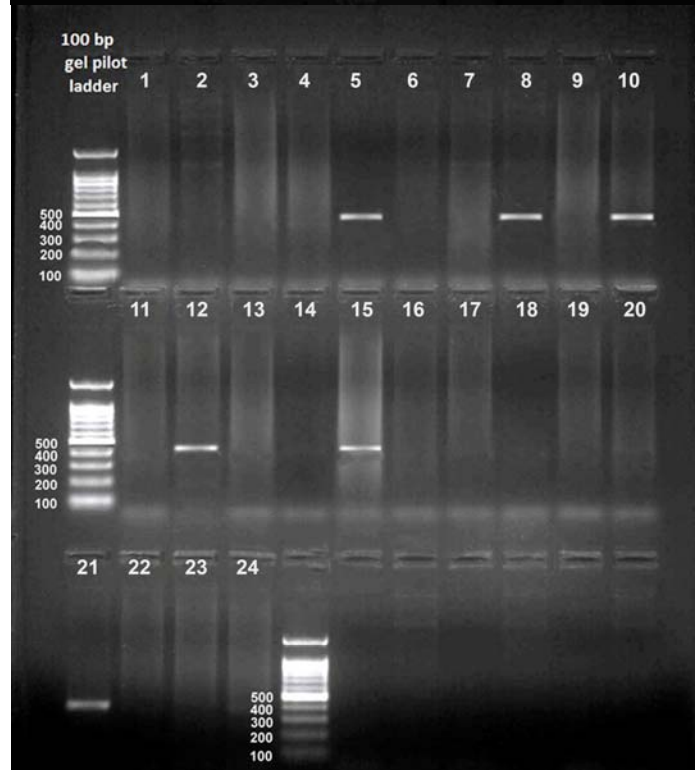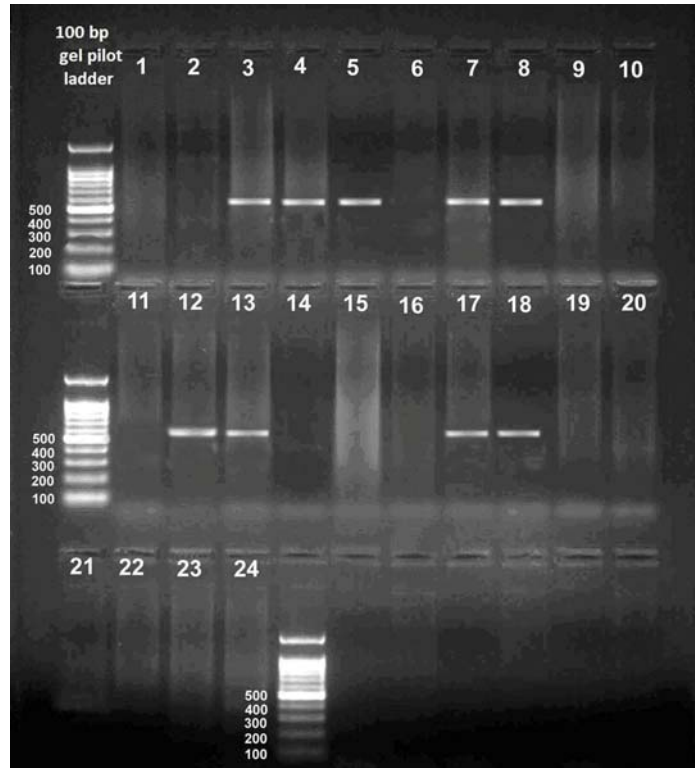
Figure 1: An **Agar gel** electrophoresis 2% stained with ethidium bromide showing the **PCR product of 16S methylase genes;** armA at 516 bp and rmtB 469 bp

Discussion

Multidrug-resistant *Klebsiella pneumoniae* isolates seem to be the emergent cause of serious infections in the developing countries worldwide. Increasing resistance in *K.pneumoniae* strains is of great concern as it is considered one of the most common pathogens causing both community & hospital-acquired UTIs (13). Management of UTIs in Egypt usually based on the empirical therapy; physicians generally imitate treatment without performing antibiotic susceptibility testing.

Recently, several studies have highlighted a crucial modification in the antimicrobial resistance pattern of uropathogens (14,15)

In this cross-sectional study, rates of resistance on 98 isolates of *K.pneumoniae* to ampicillin, cefazolin, Trimethoprim/sulfamethoxazole, Amoxicillin/clavulanic, ceftriaxone, cefotaxime, Cefoxitin, Ceftazidime, and Cefepime were 100%, 100%, 88.7%, 75.5%, 79.6%, 74.49%, 64.3%, 64.3%,and 62.24% respectively.

The results of the present study demonstrate high resistance rates towards the most empirically used antimicrobial agents (ampicillin & cefazolin) which may be explained by the overuse of these antibiotics as a self-medication. The majority of patients did not seek medical care in hospital except after failure of these therapies as well as over-prescription of these antimicrobial agents by private pharmacies and clinics (16).

Similar high resistance rate for Trimethoprim/sulfamethoxazole and ampicillin among *K.pnumoniae* isolates were reported by the previous studies (17,18).

In the current study, the resistance rate of extended-spectrum beta-lactamases is considerably higher than previous studies in Tehran performed by *Maleki et al, 2017* and in Mongolia performed by *Munkhdelger et al, 2017 (18,19).*

Also, this study indicated that the -majority of *K.pneumoniae* isolates were susceptible to amikacin (77.55%), imipenem (76.53%), nitrofurantoin (75.5%) and gentamicin (71.43%). These results are somewhat similar to the previous studies conducted in Nepal *(Neupane et al, 2016),* Tehran *(Maleki et al, 2017),* and Mongolia *(Munkhdelger et al, 2017) (20,19)*

Aminoglycosides considered one of the appropriate choices in treatment of complicated UTIs. Therefore, development of aminoglycoside resistance among clinical *K.pneumoniae* isolates is a serious health issue in healthcare facilities. Additionally, co-existence of ESBL and AMPc in *K.pneumoniae* strains isolated from urinary tract infection make a challenge for UTIs treatment. Furthermore, multidrug-resistant *K.pneumoniae* harboring different virulence factors has an ability to adhere and persist within the urinary tract *(Jacoby et al, 2014) (14)*

In this study, the rate of quinolones resistance including nalidixic acid, norfloxacin, ciprofloxacin, and levofloxacin was 91.8%, 82.7%, 71.4%, and 64.2% respectively which is significantly higher than that detected previously *(17,18)*

In this study, 72 out of 98 isolated *k.pneumoniae* (73.5%) were multidrug resistant (MDR) which is far higher than the 22.8% reported previously in Egypt (20).

The results of this study revealed that PCR amplification of 16S rRNA methylase genes showed that armA was the most prevalent gene detected in 30 isolates (30.6%); while

rmtB was detected in only 17 cases (17.3%). This results is somewhat different from the results of a study performed by Caiqian et al, 2015 who reported that 16S rRNA methylase genes were identified with positive rates of armA and rmtB of 11.1% and 6.2%, respectively (21).

Previous studies reported that 16S rRNA methylases first appeared in *K.pneumoniae* in 2003 *(22).* *rmtB* was first identified in *S. marcescens* from Japan in 2004, and was subsequently found in *K. pneumoniae* and *E. coli* isolates from Taiwan, Korea and Belgium *(Yan et al,2004 and Bogaerts et al, 2007*) .To date, the 16S rRNA methylase genes were prevalent globally **(23,24).**

Conclusion

This finding highlights the alarmingly increase in multidrug-resistant *klebsiella pneumonia* isolates with high frequency of 16S rRNA methylase genes. Consequently, following a wise antibiotic policy is critically necessary to limit the potential spread of resistant genes.

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