ORIGINAL ARTICLE

Plasmid Mediated Quinolone Resistance Genes (*Qnr A* and *S*) in *Klebsiella pneumoniae* Isolated from ICU Hospital Acquired Infection

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ABSTRACT

Background: *Klebsiella pneumoniae causes any types of hospital-acquired infections (HAIs). Resistance to quinolone antibiotics increased rapidly in K. pneumoniae.* ***Objectives:*** *This study was designed to investigate the prevalence of quinolone resistance, detection of plasmid mediated (qnrA and qnrS) genes in K. pneumoniae causing hospital-acquired infections (HAIs) in ICUs of Mansoura Emergency Hospital. Additionally, determination of risk factors for quinolone resistance.* ***Methodology:*** *K. pneumoniae isolates were collected from different samples of patients admitted to Mansoura Emergency Hospital and presented with different infections. K. pneumoniae isolation and identification was performed by biochemical reactions. K. pneumoniae quinolone susceptibility was identified by disc diffusion method. The qnrA and qnrS were detected by PCR.* ***Results:*** *A total of 65 K. pneumoniae isolates were recovered in the studied ICUs patients´ samples. The highest resistance of K. pneumoniae isolates was to ciprofloxacin and ceftriaxone (80% and 70%, respectively). The qnrS gene was detected in 28 cases, and qnrA was detected in 2 cases. Regarding risk factors for resistance, male sex (OR=4. 9, P: 0.016), diabetes (OR: 6.9, P: 0.073) and hospital stay more than 8 days (OR: 1.99, P 0.002) were significantly associated with quinolone resistant K. pneumoniae.* ***Conclusion:*** *High rates of resistance were detected in K. pneumoniae to many classes of antibiotics. Quinolone resistant was reported among the highest one. Presence of quinolone resistance genes qnrA & qnrS could be considered among the main causes of this resistance. Diabetes mellitus, hypertension and increased hospital stay are significant risk factors for development of this resistance.*

INTRODUCTION

Klebsiella pneumoniae is a common bacterial pathogen that causes significant hospital-acquired infections including pneumonia, wound infection, urinary tract infection, and speticaemia [1]. *Klebsiella pneumoniae* is one of the ESKAPE organisms which are extremely resistant to several classes of antibiotics [2]. *K. pneumoniae* is extremely resistant to third-generation cephalosporins, aminoglycosides, fluoroquinolones, and carbapenems [3].

Quinolone antibiotics work by interfering with DNA gyrase and topoisomerase IV to prevent bacteria from synthesizing DNA. The less toxic and broader-acting fluorinated types (ciprofloxacin, gatifloxacin, norfloxacin, and moxifloxacin) are available. They are very active against *K. pneumoniae* and other *Enterobacteriaceae* [4]. Quinolone resistance is brought on by the improper overuse of quinolones [5]. Three mechanisms exist for quinolone resistance; the first is the *qnr* gene or determinant (A, B, C, D, V, S), which codes for a pentapeptide repeat unit protein that shields DNA gyrase from quinolone activity. Aminoglycoside acetyl transferase AAC (6)-Ib, which causes quinolone to be acetylated, is the second process. The third mechanism is an increase in efflux pump caused by the efflux pump genes *Qep A* and *Oqx AB*. The *qnr* gene determinants are *qnr* (A, B, C, S, D, V), with A & S being the most prevalent. Their expression is influenced by the strength of their promotor [5-6].The plasmid-mediated *qnr* gene in *K. pneumoniae* was initially described in the United States in 1994 [7-8]. Since then, qnr protein families based on DNA homology have been created, including *qnrA, qnrB, qnrS, qnrC, qnrD, and qnrVC* [9]. The risk of infection with resistant organisms increases markedly in patients admitted to intensive care units (ICU). This risk is caused by long-term ICU stays, the use of broad-spectrum antibiotics, invasive techniques, and persistent underlying conditions [5].

This study was aiming to determine the prevalence of quinolone resistance and to detect plasmid mediated (*qnrA* and *qnrS*) genes in *Klebsiella pneumoniae* causing hospital acquired infection in ICUs of Mansoura Emergency Hospital. Additionally, the determination of risk factors for quinolone resistance.

METHODOLOGY

This study was carried out over a period of 24 month from January 2017 to December 2018. Samples were collected from patients with hospital acquired infections (HAIs) at ICUs of Mansoura Emergency Hospital who were presented with signs and symptoms of hospital acquired infections; no pre-admission infection (proved by history and clinical examination that revealed no signs or symptoms of infection), signs and symptoms of infection developed three days or more after admission. Diagnosis of the suspected type of infection and the type of sample collected was based on the Center of Disease Prevention and Control (CDC) criteria for HAIs[10]

Samples processing and identification of *Klebsiella pneumoniae*:

During the study's duration, samples were collected from infected patients. Collected samples include endotracheal aspirates (ETA), sputum samples, blood samples, urine samples and wound swabs. The samples were then transported without delay to the Department of Medical Microbiology and Immunology at faculty of medicine at Mansoura Faculty of Medicine.

Colony morphology, Gram stained films, and biochemical reactions were used to identify the isolates. *K pneumoniae* reacted positively in citrate, voges proskauer, lysine decarboxylase and kliger iron sugar and reacted negatively in indole, methyl red and oxidase test [11].

- **Antibiotic sensitivity testing** was performed using the disc diffusion method in accordance with CLSI recommendations for *Klebsiella pneumoniae* isolates [12].
- **Molecular detection of (quinolone resistance genes *A, S*):**

DNA extraction: DNA was extracted by Qiagen DNA extraction kits (Qiagen GambH, Qiagen strasse 1) according to manufacturer instructions. Quinolone resistant genes (*qnr A* and *qnr S*) were amplified by PCR using the following primers

Gene *A* primer:-

Forward primers5′ATTTCTCACGCCAGGATTTG3′, Reverse primers 5′GATCGGCAAAGGTTAGGTCA 3′.The expected product is 516-bp [13].

Gene *S* primer

Forward primers 5′ TCGACGTGCTAACTTGCG3′, Reverse primers

5′GATCTAAACCGTCGAGTTCGG3′. The expected product is 466 bp [14].

The PCR reaction was performed according to the previously described protocol; 95°C for 5 minutes (primary denaturation), 35 cycles each cycle consist of (denaturation at 95°C for 1 minute, annealing at 56°C for 40 second and extension at 72°C for 1 minute) then final extension at 72 °C for 10 minute [15]. The PCR products were visualized by agrose gel electrophoresis[16].

Statistical analysis:

Data were evaluated statistically using SPSS version 21. The Kolmogorov-Smirnov test was initially used to determine whether the data were normal. Number and percentage were used to describe qualitative data. The Chi-square test was employed to examine associations between categorical variables, and the Fischer exact test was applied when the anticipated cell count was unknown (less than 5). The presentation of continuous variables was mean SD (standard deviation). The Student t test was used to compare the two groups. In order to anticipate the most important determinants and to account for any interactions and confounding effects, significant variables were entered into a logistic regression model using the enter statistical technique. The level of significance is set at (p-value 0.05) for all of the aforementioned statistical tests that have been conducted.

RESULTS

This study was conducted over a period of 24 months from January 2017 to December 2018, 90 samples were collected from patients with signs and symptoms of HAI in the ICUs of Mansoura Emergency Hospital. Seventy-eight samples were positive for bacterial growth. *Klebsiella pneumoniae* represented 83% from all positive samples (65 isolates). The majority of isolates were from ETA (68%) table (1). Regarding demographic data, the isolates were collected mostly from male patients (48 representing 74%) and from age group more than 40 years (54, representing 83%).

Table 1: Number and percentage of types of positive samples collected during the period of the study

samples	No&%
Endotracheal tube	44(68%)
Urine	5(7.7%)
Blood	5(7.7%)
Sputum	2(3.1%)
wound	9 (13.8%)
Total	65

The antibiotic susceptibility testing of the collected *Klebsiella pneumoniae* isolates was done by disc diffusion method. *Klebsiella pneumoniae* isolates showed highest resistance to ciprofloxacin and ceftriaxone (80% and 70%, respectively) table (2).

Table 2: Antibiotic susceptibility pattern of isolated *Klebsiella pneumoniae*

Antibiotic	Resistance (NO& %)	Suceptibility (NO& %)
Ciprofloxacin	52(80%)	13(20%)
Amikacin	24(38%)	41(62%)
Gentamicin	24(38%)	41(62%)
Ceftriaxone	45(70%)	20(30%)
Cefipime	39(61%)	26(39%)
Imipenem	27(42%)	38(58%)
Unasyn	43(67%)	22(33%)
Tazocin	32(50%)	33(50%)
Cotrimoxazole	22(34%)	43(66%)

Regarding concomitant resistance with quinolone, the highest cross resistance was with ceftriaxone (29 isolates; 55.8%) and the least was with imipenem (9 isolates; 17.3 %), table (3).

Table 1: Concomitant resistance between quinolone and other antibiotics in isolated *Klebsiella pneumoniae*

Cross resistant antibiotic	No &%
Concomitant between quinolone &ceftriaxone	29(55.8%)
Concomitant resistance between quinolone & unasyn	27(51.9%)
Concomitant resistance between quinolone & imipenem	9(17.3 %)
Concomitant resistance between quinolone & amikacin	19(36.5%)
Concomitant between quinolone & cotrimoxazole	20(38.4%)

Regarding risk factors for quinolone resistance, male sex, diabetes and hospital stay more than 8 days were significantly associated with quinolone resistant *Klebsiella pneumoniae* table (4).

Using logistic regression analysis and adjusting confounding factors, the following were independent predictors for resistance to quinolone; male gender (OR=4.9) and long hospital stay (OR=1.99) table (5).

Table 4: Demographic and clinical data of patients infected with *Klebsiella pneumoniae* & its relation to quinolone resistance.

Demographic data	Total (n=65)		Resistance to quinolone (n=52)		Susceptible to quinolone (n=13)		Test of significance	p- value
	No	%	No	%	No	%		
Age/ years							χ^2=1.63	0.201
<40 y	12	18.5	8	15.4	4	30.8		
≥40 y	53	81.5	44	84.6	9	69.2		
Mean ± SD	43.29±5.61		43.86±5.54		41.00±5.49		t=1.67	0.100
Sex							χ^2=6.45	0.011
Male	48	73.8	42	80.8	6	46.2		
Female	17	26.2	10	19.2	7	53.8		
Diabetes							4.06	**0.044***
Yes	20	30.8	19	36.5	1	7.7		
No	45	69.2	33	63.5	12	92.3		
Hypertension							3.24	0.072
Yes	24	36.9	22	42.3	2	15.4		
No	41	63.1	30	57.7	11	84.6		
Hospital stay								
<8days	27	41.5	16	27.6	11	66.7	χ^2=12.41	0.001*
≥8days	38	58.5	36	72.4	2	33.3		
Mean ± SD	8.87±2.18	9.35±2.09	7.00±1.41	t=3.18	0.001*			

χ^2: Chi square test, t: Student t-test, * significant p <0.05, NS: non-significant

Table 5: Logistic regression analysis of independent predictors of resistance to quinolone

Independent predictor	β	P -value	OR (95%CI)
Sex Male female	1.58	0.016*	4. 9 (1.34-17. 8)
Diabetes	1.933	0.073*	6.9 (0.83-57)
Hospital stay	0.692	0.002*	1.99 (1.28-3.1)

OR: odds ratio, CI: confidence interval.

Quinolone resistance genes *qnr A&S in Klebsiella pneumoniae* isolates:

All isolated *Klebsiella pneumoniae* were screened for quinolone resistance genes *qnrA & S* by PCR. The *qnr S* gene was detected in 28 cases, 3 of them were quinolone susceptible cases and *qnrA was* detected in 2 cases. Isolates positive for quinolone resistance genes *qnr A* and *qnr S* were mostly isolated from endotracheal tube aspirates (16, 53.3%) table (6, 7).

Table 6: Distribution of resistance gene in different clinical samples

Type of Samples	No&% Genes of resistance *(qnr A &S)*
Endotracheal aspirates	16(53.3%)
Urine	3(10%)
Blood	2(6.7%)
Sputum	2(6.7%)
Wound samples	7(23.7%)

Table 7: Quinolone resistance gene *qnr S* and *qnr A* in quinolone resistant and susceptible *Klebsiella pneumoniae* isolates.

Resistance genes	Total (n=65)		Resistance to quinolone (n=52)		Susceptible to quinolone (n=13)		χ^2	p- value
	No	%	No	%	No	%		
Quinolone resistance gene S								
positive	28	43.1	25	48.07	3	23.1	2.65	0.103
Negative	37	56.9	27	51.9	10	76.9		
Quinolone resistance gene A								
positive	2	3.1	2	3.8	0	0.0	FET	1
Negative	63	96.9	50	96.2	13	100.0		

FET: Fischer exact test

DISCUSSION

Klebsiella belongs to Gram negative *Enterobacteriaceae*. It lives in soil and surface water and is a typical gut commensal [17]. Numerous infections caused by *Klebsiella pneumoniae*, including cystitis, pyelonephritis, meningitis, cerebral abscess, pneumonia, lung abscess, endophthalmitis, and liver abscess [18]. Over the past few years, resistance among *Klebsiella pneumoniae* has significantly increased [19].

Due to a number of factors, such as extensive and excessive use of broad spectrum antibiotics, extended hospital stays, the critical condition of the patients, and frequently used invasive procedures; intensive care units are main location for the development of antibacterial resistance [20]. This cross-sectional study was designed to identify the prevalence of quinolone resistant *Klebsiella pneumoniae* among patients with hospital acquired infections in the ICUs of Mansoura Emergency hospital. In addition to the detection of quinolone resistance genes (*qnr A* and *S*) in these quinolone resistant *Klebsiella pneumoniae* isolates

Sixty five isolates were identified as *Klebsiella pneumoniae* representing about (83%) from the total positive samples. This result is higher than that of *Sekowska et al* previous study [21] in which *Klebsiella pneumoniae* accounted for 64% of all infections. It is much higher than that of ELBoumari et al [22] (22%). In terms of infection samples, respiratory tract samples including ETA 54 (69.2%) and sputum 2 (2.58%) were the higher positive for *Klebsiella pneumoniae*. This is consistent with earlier results [23]. However, other research showed that UTI was the most common *K. pneumoniae* infection [24]. This discrepancy in the results can be attributed to the variations in infection rates and types across different intensive care units (ICUs) in various geographic locations, where the execution of infection control programs necessitates enough resources and medical staff compliance.

According to the current study, numerous different classes of antibiotics had significant rates of resistance in *K. pneumoniae*. For instance, imipenem was (42%), piperacillin-tazobactam (50%), ampicillin-sulbactam (67%), and (70%) to 3rd generation cephalosporins. Different previous studies (25, 26) reported high rates of resistance in *Klebsiella* pneumoniae causing infections in ICUs. For example, 84% resistance to third-generation cephalosporins, 44.7% resistance to imipenem [25], and 68.4% resistance to ampicillin-sulbactam [26]. However, earlier reports indicated reduced resistance to carbapenems, only compromising 9.62 percent [26]. Lower resistance level was detected to aminoglycoside (38%). This result is lower than that of *Cachón et al,* [26] (46%) for gentamicin but higher than the amikacin resistance (14%). Also, our result is lower than that of *Russo et al,* [27] who described 45% resistance rate to gentamicin and 99% to amikacin. The extensive usage of imipenem and the infrequent use of aminoglycoside as empirical therapy in the ICUs of Mansoura Emergency hospitals may be the cause of the

high prevalence of resistance found in our study to imipenem and lower resistance rate to aminoglycoside. As a result, the practice of using empirical antibiotics should be changed in light of the widespread resistance to third generation cephalosporins and carbpenems.

Quinolones antibiotic are widely used, which increase the incidence of resistance [28]. The current study found that *K. pneumoniae* has a high resistance rate to quinolones (80%). This result is consistent with earlier studies where resistance was ranged from 88% [29] to 94% [23]. According to other studies, resistance was slightly lower; (60%) [30]. On contrast, other studies such as *Peymani et al.* [5] and *Shahraki et al.* [31] reported that quinolone resistance was 34% and 18%, respectively. This increase in quinolone resistance may be partially explained by the high levels of plasmid mediated 3rd generation cephalosporin and aminoglycoside resistance that may also carry plasmid-mediated quinolone resistance *qnr* genes[7]. In our locality, the widespread use of quinolones as an empirical therapy for the management of community-acquired illnesses may also be a contributing factor.

There is widespread quinolone resistance caused by plasmids in the *Enterobacteriaceae* family, including *K. pneumoniae* [7]. The *qnr* gene family, which physically protects DNA gyrase and topoisomerase IV from quinolones' inhibitory effect, are among these genes [8]. Our study aimed to identify the prevalence of *qnrA* and *qnrS* in *K. pneumoniae* isolated from patients in the ICUs at the Mansoura Emergency Hospital and to identify potential risk factors for the emergence of quinolone resistance.

In our study, PCR was used to identify the plasmid-mediated quinolone resistance genes *qnrA* and *qnrS*. Among *K. pneumoniae* isolates, *qnr S* was more prevalent than *qnrA*. Twenty-eight K. *pneumoniae* isolates out of a total of 65 isolates were found to be positive for the quinolone resistance gene *qnrS* (43%). Three of these *qnrS* positive isolates were quinolone sensitive, while the remaining twenty-five were quinolone resistant. The *qnrA* gene was detected in two isolates of *K. pneumoniae* (3%).

In most studies, *qnrS* is higher than *qnrA*, but the prevalence percentage varies. Similar to our findings, *qnrS* prevalence among *K. pneumoniae* was 37% in China [32]. However, earlier research revealed a higher result (78%) [33]. On the other hand, some research indicates low levels of *qnrS*, such as 16% in Japan [34], and 2% in Kenya [35]. Regarding *qnrA*, our study reported that it was present in 3% of *K. pneumoniae* isolates, which is consistent with the previous findings [36], which found that 10% of the isolates had quinolone-resistant *qnrA*. Other studies indicated a higher prevalence of *qnrA* up to 69% [37].On contrary, several reports in Nigeria [30] and Pakistan [38] did not find that gene at all. This discrepancy in results can be brought about by variations in the geographic distribution of *qnr* genes, varied antibiotic policies, variable quinolone consumption rates, as well as various sample kinds and detection techniques.

Our study reported that older age (over 40), the presence of comorbidities (such as hypertension or diabetes), and prolonged hospital stays (greater than 8 days) were significant risk factors for developing quinolone resistance. These results are consistent with those of *Injac et al.* [23], who established that an extended hospital stay and advanced age are major risk factors for the development of resistant *Klebsiella*, and with *Peymani et al.* [5], who found that invasive procedures, the use of broad-spectrum antibiotics in the past, and an extended hospital stay all increase resistance.

CONCLUSION

Klebsiella pneumoniae represents an important hospital acquired infection causing pathogen. High rates of resistance were detected in *K. pneumoniae* to many classes of antibiotics. Quinolone resistant was reported among the highest one. Presence of quinolone resistance genes *qnrA* & *qnrS* can be considered among the main causes of this resistance. Diabetes mellitus, hypertension and increase hospital stay are significant risk factors for development of this resistance.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

This study was approved by the ethical committee in the Faculty of Medicine, Mansoura University. Informed consent was received from each participant in the study.

Conflict of interest: No conflict of interest.

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