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Bacteriological Characterization of Fluoroquinolones-Resistant *Klebsiella pneumoniae* Clinical Isolates During Covid-19 Pandemic

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Abstract

The treatment of *Klebsiella pneumoniae* infections has become more difficult due to the emergence of multidrug-resistant strains. Fluoroquinolones have been widely used to treat respiratory tract infections and urinary tract infections caused by different bacteria. Therefore, this study aimed to evaluate fluoroquinolones resistance and detect ESBL producers in various clinical samples in Duhok Province, Iraq. *K. pneumoniae* was identified from different clinical samples by conventional microbiological tests. In addition, antibiotic susceptibility was detected by the Kirby-Bauer disc diffusion method. Out of 120 *K. pneumoniae* isolates, 73.3% were causing urinary tract infections, in most cases, 74.2% were among females and 25.8% among males, and among those 120 isolates, 76.7% of isolates were from outpatients, and 23.3% were inpatients. The resistance rate among all samples was 52.5%, 36.7%, 33.3%, 33.3%, ciprofloxacin, levofloxacin, norfloxacin, and ofloxacin, respectively in urine samples, the resistance to nalidixic acid was 22.5%. Among 120 samples, only 12 isolates were hypermucoviscosity. About 25% of ciprofloxacin and 17.5% of levofloxacin resistances were ESBL producers simultaneously. According to this study, *K. pneumoniae* is more susceptible to norfloxacin, ofloxacin, and nalidixic acid than ciprofloxacin and levofloxacin. Additionally, this study establishes a link between ESBL and fluoroquinolone resistance.

Keywords: *Klebsiella pneumoniae, Fluoroquinolones, ESBLs, Clinical specimens.*
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I. INTRODUCTION

Klebsiella pneumoniae is a Gram-negative bacterium and one of the most critical species that cause various infections such as urinary tract infection (UTI), pneumonia, meningitis, wound or surgical site infections, liver abscesses, and bloodstream infections (CDC, 2010; Martin and Bachman, 2018; Mahon *et al*., 2019). Fluoroquinolones (FQs) are synthetic antibacterial agents that inhibit bacterial DNA gyrase and are bactericidal that have been widely utilized for many years, to treat respiratory infections and UTIs caused by aerobic Gram-positive, Gram-negative bacteria, anaerobes, and atypical bacteria (Sharma *et al*., 2009; Shahram *et al*., 2015). In addition, FQs are commonly given to UTIs caused by extended-spectrum beta-lactamase (ESBL)-producing *K. pneumoniae* because they are broad-spectrum antibacterial medicines (Goudarzi *et al*., 2015; Wiener *et al*., 2016) as well FQs were recommended in the treatment of community-acquired pneumonia in COVID-19 patients since FQs exert antiviral activity and immune-modulatory activity as some studies determined (Metlay *et al*., 2019; Karampela *et al*., 2020 Yacouba *et al*., 2021).

With the increased use of FQs in veterinary and human medicine, developing resistance to these antibiotics became a rising problem (Hooper and Jacoby, 2015). It was previously believed that FQs resistance could be acquired only through chromosomal mutations (Kim *et al*., 2009) by several mechanisms including modifications to the target topoisomerase IV and DNA gyrase, alterations to the drug efflux, and methods of drug entry (Jacoby, 2005). However, until 1998, when for the first-time new mechanism, plasmid-mediated resistance to quinolones was documented in the *K. pneumoniae* isolates from urine samples (Martínez-Martínez and Jacoby, 1998).

Determining FQs resistance among *K. pneumoniae* is important for empirical therapy, especially among ESBL-producing clinical isolates. Moreover, up-to-date knowledge monitoring of antibiotic sensitivity patterns of bacteria globally are important (Johnson, 2015; Fuhrmeister and Jones 2019), especially after *K. pneumoniae* was recognized as an urgent threat to human health and on the top list of WHO priority pathogens listed bacteria for which proclaimed new antibiotics are urgently needed (WHO, 2017; CDC, 2019). Therefore, the current study aims to determine FQs resistant to *K. pneumoniae* among

ESBL and non-ESBL producer isolates from various clinical specimens in Duhok province, Iraq.

II. Materials And Methods

A. Study settings, subjects and specimen collection

This cross-sectional study was conducted in Duhok province from September 2021 to January 2022. One hundred twenty *K. pneumoniae* isolates were obtained from 950 clinical specimens, including urine, sputum, pus, blood, high vaginal swab, vaginal discharge, bronchial lavage, pleural fluid, and wound. The current study enrolled of both sexes, 14 years and older that were not taking antibiotics in the past three days. Inpatients were from different departments (CCU, ICU, Surgical wards, medical wards, Covid-19 ward), while outpatients were from public and private hospitals and clinics from Duhok, Zakho, Akre, Summel, and Bardarash cities at Duhok province.

B. Ethical consideration

The ethics committee approved the study proposal and informed consent of the Duhok Polytechnic University and Duhok General Health Directorate, Kurdistan Region, Iraq.

C. Sample processing and identification of K. pneumoniae

All bacterial cultures were from patients attending to some different microbiology labs in Duhok province, then isolates for final identification were attending to the microbiology lab at Azadi teaching hospital. Primarily inoculated on blood and MacConkey agar (NEOGEN, USA). *Klebsiella* species were confirmed by morphological and biochemical tests such as IMViC tests (Alpha Chemika, India), motility test (by wet mount and culturing on SIM Media), and urease test (UK SMI, 2018; Mahon *et a*l., 2019).

D. Comparison between manual identification and the VITEK® 2 compact system

About 20 samples of *K. pneumoniae* identified with manual identification and Kirby-Bauer disc diffusion technique were compared with VITEK® 2 Compact system identification and antibiotic resistance detection (biomerieux, France).

E. String Test

To detect the hyper-mucoviscosity of *K. pneumoniae*, the string test was done on MacConkey agar as described by (Hagiya *et al.*, 2014).

F. Antibiotic susceptibility test

All *K. pneumoniae* isolates were subjected to antibiotic susceptibility tests through the Kirby-Bauer disc diffusion technique using the CLSI recommendations (Hudzicki, 2009; CLSI, 2021). Fourteen antibiotics were used in this study: nalidixic acid (NA, 30 µg), norfloxacin (NOR, 10 µg), ofloxacin (OFX, 5 µg), ciprofloxacin (CP, 10µg), levofloxacin (LOM, 5µg), cefotaxime (CTX, 30 µg), ceftazidime (CAZ, 30 µg), amikacin (AK, 10 µg), gentamicin (GM, 10 µg), neomycin (N-10 µg), (IPM, 10 µg), aztreonam (ATM, 30 µg), nitrofurantoin (NF, 300 µg), amoxicillin/clavulanic acid (AMC, 20+10 µg). The test preparation, inoculation, storing of antibiotic discs, and reading of the result were done according to the manufacturer's recommendations (bioanalyses, Turkey) (CLSI, 2021; EUCAST, 2021).

G. Phenotypic detection of extended-spectrum β-lactamase production

Phonotypical detection of extended-spectrum beta-lactamase (ESBL) was performed by double disc synergy test (DDST) using four antibiotics: amoxicillin/clavulanic acid (AMC, 20+10 µg) cefotaxime (30 µg), ceftazidime (30 µg) and aztreonam (ATM, 30 µg), as described by (Drieux *et al*., 2008

H. Statistical analysis

Data Analysis of the study sample was described using means, standard deviations, frequencies, and percentages. Data were analysed using SPSS v23 (SPSSInc, Chicago, IL, USA).

III. Results

During the current study, 120 isolates of *K. pneumoniae* were obtained from patients living in different areas at Duhok province; 76.7% were outpatients, and 23.3% inpatients. Regarding sex and ages, 74.2% were women, and 25.8% were male. The age group 31 to 40 years were the most common had been infected with *K. pneumoniae* and P-value is 0.004 Table 1.

Table 1: Age and sex-related distribution of *K. pneumoniae* isolates.

Age in years	Sex		Total No. (%)	P. Value
	Male No. (%)	Female No. (%)		
14-20	4 (3.3)	20 (16.7)	24 (20.0)	0.004
21-30	4 (3.3)	18 (15.0)	22 (18.3)	
31-40	4 (3.3)	23 (19.2)	27 (22.5)	
41-50	5 (4.2)	16 (13.3)	21 (17.5)	
51-60	8 (6.7)	9 (7.5)	17 (14.2)	
61-70	4 (3.3)	3 (2.5)	7 (5.8)	
>70	2 (1.7)	0 (0.0)	2 (1.7)	
Total	31(25.8)	89 (74.2)	120 (100)	

Concerning the clinical specimen's profile, the highest number of isolates was from urine samples, 73.3% isolates, while the lowest isolates were encountered from pleural fluid and bronchial lavage. The P-value is 0.004. Table 2.

Table 2: Distribution of *K. pneumoniae* from various clinical specimens between both sexes.

Samples	Sex		Total No. (%)
	Male No. (%)	Female No. (%)	
Urine	13 (10.8)	75 (62.5)	24 (20.0)
Sputum	8 (6.7)	3 (2.5)	22 (18.3)
Pus	4 (3.3)	4 (3.3)	27 (22.5)
High vaginal swab	0 (0.0)	4 (3.3)	21 (17.5)
Blood	3 (2.5)	0 (0.0)	17 (14.2)
Wound	2 (1.7)	1 (0.8)	7 (5.8)
Pleural fluid	0 (0.0)	1 (0.8)	2 (1.7)
Bronchial lavage	1 (0.8)	0 (0.0)	120 (100)
Vaginal Discharge	0 (0.0)	1 (0.8)	24 (20.0)
Total	31 (25.8)	89 (74.2)	120 (100.0)

Antibiotic susceptibility patterns toward FQs antibiotics showed that 52.5%, 36.7%, 33.3%, and 33.3% of isolates expressed a resistance rate to ciprofloxacin, levofloxacin, norfloxacin, and ofloxacin, respectively. Meanwhile, out of 88 urine isolates, 22.5% were resistant to nalidixic acid.

Cephalosporin class showed high resistance compared with other antibiotics such as monobactam, carbapenem, and aminoglycoside class; for example, 45%, 55.8%, and 49.2% were resistance rates of amoxicillin/clavulanic acid, cefotaxime and ceftazidime, respectively, while monobactam and carbapenem expressed less resistance 39.2% and 29.25, respectively table 3.

Table 3: Antibiotic susceptibility patterns of *K. pneumoniae.*

Antibiotics	Sensitive No. (%)	Intermediate No. (%)	Resistance No. (%)	Not applicable No. (%)
Ciprofloxacin	43 (35.8)	14 (11.7)	63 (52.5)	0 (0)
Levofloxacin	61 (50.8)	15 (12.5)	44 (36.7)	0 (0)
Norfloxacin	77 (64.2)	3 (2.5)	40 (33.3)	0 (0)
Ofloxacin	75 (62.5)	5 (4.2)	40 (33.3)	0 (0)
Nalidixic acid	49 (40.8)	12 (10.0)	27 (22.5)	32 (26.7)
Amoxicillin/ clavulanic acid	51 (42.5)	15 (12.5)	54 (45.0)	0 (0)
Cefotaxime	50 (41.7)	3 (2.5)	67 (55.8)	0 (0)
Ceftazidime	51 (42.5)	10 (8.3)	59 (49.2)	0 (0)
Aztreonam	60 (50.0)	13 (10.8)	47 (39.2)	0 (0)
Imipenem	80 (66.7)	5 (4.2)	35 (29.2)	0 (0)
Amikacin	61 (50.8)	19 (15.8)	40 (33.3)	0 (0)
Gentamicin	83 (69.2)	1 (0.8)	36 (30.0)	0 (0)
Neomycin	2 (1.7)	1 (0.8)	0 (0.0)	117 (97.5)
Nitrofurantoin	59 (49.2)	1 (0.8)	28 (23.3)	32 (26.7)

Regarding the combination of ESBL-producing and fluoroquinolone resistance, the highest ESBL-producers were seen among ciprofloxacin resistance isolates, statically considered significant table 4.

Table 4: Relationship between fluoroquinolones resistance and ESBL producers.

Antibiotics	ESBL- Positive No. (%)	P. value
Ciprofloxacin	30 (25.0)	0.010
Levofloxacin	21 (17.5)	0.019
Norfloxacin	19 (15.8)	0.126
Ofloxacin	19 (15.8)	0.116
Nalidixic acid	14 (15.9)	0.086

Some isolates expressed hypermucoviscosity (HMV) phenotypic colony morphology characteristics that were detected by the String test; only 12.5% urine isolates and 14.3% pus isolates showed these colony features as in Table 5.

Table 5: HMV phenotype of *K. pneumoniae.*

Type of sample	No. of isolates	No. of Positive String test (%)
Urine	88	11 (12.5)
Pus	8	1 (0.83)
Blood	3	0
High vaginal swab	4	0
Pleural fluid	0	0
Bronchial lavage	0	0
Sputum-tip	11	0
Wound	3	0
Vaginal Discharge	1	0
Total	120	12 (10.0)

IV. DISCUSSION

K. pneumoniae is a significant multi-drug resistant gram-negative pathogen that causes different infectious diseases from UTI to pneumonia (Wang *et al*., 2020; Stojowska-Swędrzyńska *et al*., 2021). In the current study, 120 *K. pneumoniae* isolates have been collected from various clinical specimens with a peak incidence of 73.3% in urine. This data is in accordance with two studies done in Duhok city by Naqid *et al*., (2020) where they found that out of 130 isolates, 66.2% were from urine, and the second study showed that out of 281 isolates, 47% were belong urine (Mohammed *et al*., 2019). Another study in Iraq by Hasan *et al*., (2021) in Kirkuk city reported urine sample were 45% over than other clinical specimens. Similar results mentioned from studies done in Iran by Shahraki, (2014) they found that out of 184 samples, 132 were urine, and Eftekhar and Seyedpour, (2015) found that urine comprised the highest one, 35 out of 79 clinical samples. This might be because UTI occur more than in other systems seeking investigation and follow-up. Most isolates of clinical specimens in this study were from female patients, which is identical to these studies (Ghadiri *et al*., 2019; Mohammed *et al*., 2019; Naqid *et al*., 2020; Abd Al-Hamed and Abd Al-Mayah, 2021). The reason is back to the female lower urinary tract anatomy and its proximity to the reproductive organs (Czajkowski *et al*., 2021). This research found that *K. pneumoniae* had the highest prevalence among 21-40 years of the female age group, which is near to the study by Mohammed *et al*., (2019) and by Hasan *et al*., (2021) in Kirkuk city revealed that the high-risk age group was among 10-35 years and 36-55 years, respectively. The latter study is also near to our result because the author did not allocate ages as has been allocated in this study.

A study done by Abdulrazzaq and Faisal (2022) found that VITEK® 2 compact system cannot totally rely to diagnosis *Enterococcus spp*. that need 16S rRNA gene sequencing for conformation. While *K. pneumoniae* is easy growth in the lab and has distinguishable characteristics (Mahon *et al*., 2019) that are easy to identify by biochemical tests, however, VITEK® 2 compact system was used for double-checking and samples were identical in their results with Manual identification and antibiotic susceptibility test.

Some urine isolates showed HMV colony features which are considered virulence factors for attachment in the urinary system, and some studies classified *K. pneumoniae* into two pathotypes, classical *K. pneumoniae* (cKp) and Hypervirulent *K. pneumoniae* (hvKp) (Mike *et al*., 2021). The hvKp considers more commonly in the community (Russo, 2019); some studies consider HMV (positive string test) as a hvKp (Shon *et al*., 2013; Choby., 2020). On another side, studies show that not all hvKp strains are HMV, and some cKp strains possess this characteristic; therefore, the string test alone is not sufficient (Russo and Marr, 2019; Liu *et al*., 2019).

In this study, ciprofloxacin had the highest resistance rate 52.5% among all five FQs used during the study. This percentage matches the study in Iraq by Mohammed *et al*., (2019), who found 55.9% of the clinical isolates were resistant to ciprofloxacin. Similarly, 50% of ciprofloxacin-resistant *K. pneumoniae* were isolated from various clinical specimens in Iraq Al-Diwaniyah hospitals (Abd Al-Hamed *et*

al., 2021). This is also reported 66.9% resistance rate to ciprofloxacin (Aminul *et al*. 2021). In contrast, other research found less resistance to ciprofloxacin, in Duhok city which was 22.3 % Naqid *et al*., (2020), in India was 19.6% Priyadarshini *et al*., (2019), in the south-east of Iran was 18.4% Shahram *et al*., (2015), and in western Iran was 27.65% Malek Jamshidi *et al*., (2019). However, these low percentages of ciprofloxacin resistance could be back to little use of this antibiotic in that time and place, especially in the first study, where the resistance rates of ceftriaxone and cefepime were 65.4% and 60.8% respectively Naqid *et al*., (2020) which interpret Beta-lactams antibiotics were more used than FQs, and another two studies in Iran show Nalidixic acid is more resistant than ciprofloxacin, may nalidixic acid is more prescribed and used than other FQs in Iran.

Because of potency, safety profile, broad-spectrum, and bioavailability, FQs have been widely used. However, due to the overuse of these drugs in human and veterinary medicine, FQs-resistant strains have been growing steadily (Correia *et al.*, 2017). However, during the Covid-19 pandemic, FQs were widely used among patients in our setting. In the current study, 36.7% was levofloxacin resistant rate this is due to levofloxacin is newer and most potent than ciprofloxacin, is more sensitive; this finding matches the study which done in Kurdistan Region, Iraq, by Khalid *et al*., (2013) revealed that 52.4% of *K pneumoniae* isolates were causing pneumonia and 12.3% of them were levofloxacin resistance. Abd Al-Hamed and Abd Al-Mayah (2021) also observed similar data. In the current study, norfloxacin and ofloxacin were less resistant than ciprofloxacin and levofloxacin, 35.8% and 37.5%, respectively, to Malek Jamshidi *et al*. (2019). 27.65% of both isolates were resistant for norfloxacin and ofloxacin.

At the same time nalidixic acid was used only for urine samples due to its poor activity against Gram-positive organisms. Therefore, they are only used for Gram-negative bacteria. Out of 88 isolates, 22.5% of urine isolates expressed nalidixic acid resistance in our data. Although, as shown, these three FQs antibiotics' resistance is less than ciprofloxacin and levofloxacin, it could be because they are less prescribed and less used in our community.

Back to the relationship between ESBLs-producing capability and FQs resistance, these two groups of antibiotics are the most often utilized antimicrobial agents against *K. pneumoniae*. Unfortunately, the recent misuse of these antibiotics has resulted in multi-drug resistance bacteria among *K. pneumoniae* isolates (Goudarzi *et al*., 2015). Simultaneously, with ciprofloxacin resistance, resistance to β-lactam antibiotics has grown widespread among clinical isolates of *K. pneumoniae* (Paterson *et al*., 2000). The current study detected a significant relationship between ESBL producers and FQs resistance statically and biologically. For example, out of 63 ciprofloxacin resistance, 30 were ESBL producers. A study found that out of 452 *K. pneumoniae*, 5.5% were resistant to ciprofloxacin in vitro. ESBL production was detected in 60% of those 25 ciprofloxacin-resistant isolates (Paterson *et al*., 2000). However, our result matches most studies that stated resistance to FQs is now common in many ESBL-producer Gram-negative bacteria, including *K. pneumoniae* (Crémet *et al*., 2011; Al-Marzooq *et al*., 2014; Alheib *et al*., 2015; Goudarzi *et al*., 2015). Moreover, studies related to molecular aspects show that chromosomal mutations cause inhibition of FQs and that plasmid-mediated quinolone resistance (*PMQR*) genes confer low resistance. Studies revealed that ESBL producers have an association with *PMQR* genes. The link between ESBL (VEB-1) and QnrA1 determinants were found in a study (Poirel *et al*., 2007). Another study found a high prevalence of qnr genes among ESBL producer strains (Bouchakour *et al*., 2010). This data is alarming and requires constant reforming of antibiotic prescribing stewardship policy in combination with infection control monitoring programs in our hospitals and line of veterinary medicine.

V. Conclusion

This study revealed that *K. pneumoniae* isolates were more causing UTI cases among female sex from middle ages in this setting area of study. In addition, some of the urine isolates exhibited HMV, which is considered a virulence factor for attachment in the urinary system. Generally, all clinical isolates showed more susceptibility to fluoroquinolones such as norfloxacin, ofloxacin, and nalidixic acid than ciprofloxacin and levofloxacin. Cephalosporins showed high resistance compared with other antibiotics such as monobactam, carbapenem, and aminoglycoside. In addition, this study showed there is a relationship between ESBL-producing capability and FQs resistance.

Conflict Of Interests

There are no conflicts of interest associated with this publication.

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