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A large increase in the antibiotic resistance of *Klebsiella pneumoniae* causing urinary tract infections after the Coronavirus pandemic

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Abstract

Urinary tract infection (UTI) is one of the most common bacterial infections caused by several species of Enterobacteriaceae around the world. Due to the lack of accurate information on the bacterial agents of UTIs and to evaluate possible changes in their prevalence of antibiotic resistance, this descriptive cross-sectional study was conducted in the Bojnourd - Shirvan region of North Khorasan, Iran from April to March 2019 and 2021. In this regard, urine samples were collected from outpatients and inpatients referred to Imam Khomeini Shirvan Hospital, and their bacteria were isolated. The Isolates were identified using biochemical and microbiological tests, and their antibiotic susceptibility was determined using the disk diffusion method based on the standard protocols provided by the CLSIs. In the two years studied, the main infectious agents were *Escherichia coli* and *Klebsiella* sp, respectively. However, slight differences were found in the prevalence of other agents, such as Enterobacter, *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, Pseudomonas aeruginosa, Proteus mirabilis, and Citrobacter sp. A comparison of the results of the two-period points showed that the pattern of prevalence and the type of infectious agents did not show any unexpected changes; however, there was a large increase in the resistance of *Klebsiella* sp. isolates causing UTIS to three antibiotics, including ceftriaxone (CRO), imipenem (IPM) and gentamicin (GEN) after the pandemic coronavirus disease in 2021. This could have been due to the preventive and therapeutic use of antibiotics in Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) patients during this short period. A clear pattern was unfortunately found in Shirvan, Tehran, and possibly other regions. Although we noticed the pattern for just one of the bacterial agents involved in UTIs, it could be considered a real threat to general health.

1. Introduction

Over the past two decades reputable authorities, including the World Health Organization (WHO), have increasingly found indications of antibiotic resistance to infectious agents (especially hospital-resistant strains) in their annual reports and programs and consider this an actual and growing health threat, emphasizing the need to manage antimicrobial activities as it may intensify in the future (Getahun, 2020). Urinary tract infections (UTIs) are one of the most common bacterial infections, affecting an estimated 150 million people worldwide each year, particularly in

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developing countries. Its economic burden is estimated at more than $ 6 billion (Kibret, et al. 2014). These infections can be asymptomatic or symptomatic (Kumar, et al. 2013). In the asymptomatic infection, bacterial colonization is seen in the urinary tract without symptoms; however, microbial invasion and inflammation in the urinary tract are observed simultaneously with colonization in the symptomatic form (Rajni, et al. 2008). Although the frequency of UTIs varies depending on age, sex, hospitalization, or catheterization, they are the second and the most common bacterial infection in outpatient settings after respiratory infections in the United States (Ghorashi et al., 2011; Abbo, 2014; Hashemi, 2016).

While urinary tract infections are usually caused by gram-negative bacteria, *including Escherichia coli, Klebsiella, Proteus mirabilis, Pseudomonas aeruginosa*, *Acinetobacter,* and *Serratia* sp., the gram-positive bacteria *Enterococcus* and *Staphylococcus* species can be also involved. *E. coli* is responsible for 75 to 95% of asymptomatic bacteriuria, cystitis, and pyelonephritis in young women. However, a small proportion of infections are caused by other bacteria, such as Enterobacteriaceae, other gram-negative bacilli, *Staphylococcus faecalis*, and group B *streptococcus* (GBS) (Kumar, 2013). Proper treatment required an awareness of the pattern of antibiotic susceptibility and resistance of the UTI agents in each country or region.

Due to the lack of accurate information about the causes of urinary tract infections in the Bojnourd-Shirvan region of North Khorasan province, Iran, this study focuses on the distribution of UTI agents involved in these infections. The pattern of antibiotic resistance of the isolated *Klebsiella pneumoniae* was investigated in three ethnic populations, Kurdish, Persian, and Turkish, in this northeastern border region near Turkmenistan.

2. Materials and methods

2.1. Study population

In this descriptive cross-sectional study, urine culture samples were collected from inpatients and outpatients referred to Imam Khomeini in Shirvan in the Bojnourd-Shirvan region (37° 28' 29" N / 57° 19' 45" E), from April to March in 2019 (before pandemic Corona disease) and again in 2021.

2.2. Isolation sampling and identification of bacteria

The midstream of the urine samples were collected from the patient and cultured using a standard and calibrated loop in Blood agar and McConkey agar culture media. The samples showing the presence of 10^5 CFU/ml and more isolate after 48 hours of culture observation were considered positive cultures (Majumder, 2022). All isolates were identified using biochemical and microbial tests, such as oxidase, nitrate reduction, Simons Citrate, TSI reaction, Urease, Indole, motion, Methyl Red (MR), and Voges-Proskauer (VP) and lysine decarboxylase (MERCK, Germany). The identified isolates were cultured in BHI (Q-Lab / Canada) medium containing 15% glycerol and stored at minus 70 ° C for later usage.

2.3. Evaluation of antibiotic resistance

The antibiotic resistance/susceptibility was tested using the Kirby Bauer method. In this method, the Müller-Hinton agar medium (QL, Canada) and antibiotics Cefixime (CFM), ceftriaxone (CRO), gentamicin (GEN), imipenem (IPM), ampicillin-sulbactam (SAM), ceftazidime (CAZ), nitrofurantoin (NIT), ciprofloxacin (CIP), Amoxicillin (AMX), trimethoprim-sulfamethoxazole (STX) were used. The antibiotics were chosen according to the national and CLSI consensus guidelines (CLSI 2018, 2020), and the susceptibility results were interpreted considering the mentioned CLSI. The antibiotic concentrations were 5µg for CIP, 10µg for GEN and IPM, 1.25/23.75 µg for STX, 10/10 for Ampicillin-sulbactam, 300 µg for NIT, and 30 µg for the rest. In this method, after preparing Müller-Hinton agar medium, a microbial suspension equivalent to half McFarland was prepared and cultured on the plates. The antibiotic discs were then placed and incubated for 36 hours at 35 °C. Furthermore, quality control by type culture collection strains in the hospital laboratory was done. The *E. coli* ATCC25922 standard strain was used for quality control of culture media and antibiotic discs.

2.4. Ethical Issues

This study was approved by the Ethical Committee of the North Khorasan University of Medical Sciences, Bojnurd, Iran, (IR-NKUMS.REC. 1397/042).

2.5. Statistics and analysis

Excel software was used for all calculations. The results were expressed by calculating the percentage and drawing graphs. Chi-square test statistical analysis was performed using Minitab software, version 19.0. P-values < 0.05 were considered statistically significant.

3. Results and discussion

3-1. Frequency distribution of microbial agents causing urinary tract infections

A total of 3010 and 1550 patients were screened in 2019 and 2021, respectively. In 2019, 126 out of 3010 (4.18%) total performed urine cultures were UTIs positive. Accordingly, *E. coli* and *Klebsiella* sp. were the most frequently isolated bacteria of UTIs with a prevalence of 66.66% and 19.84%, respectively. Followed by *Enterobacter*, Staphylococcus *saprophyticus*, and *St. epidermidis* with a prevalence of 4.8%, 3.96%, and 1.58%. Lastly, *Staphylococcus* Coagulase-negative, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Citrobacter* sp. showed the least and same prevalence of 0.79 % (Figure 1).

Similarly, in 2021, 73 cases (4.70%) out of 1550 showed positive UTIs. Again, *E. coli* and *Klebsiella* sp. were the most frequently isolated bacteria of urinary tract infections with a prevalence of 65.75% and 20.55%, respectively. Moreover, *Pseudomonas aeruginosa*, with a prevalence of 5.48%, *Streptococcus* sp. and *Staphylococcus aureus* with 2.74%, and *Staphylococcus saprophyticus* and *Enterobacter* with 1.37% were the bacterial agents causing UTIs among the patients (Figure 1).

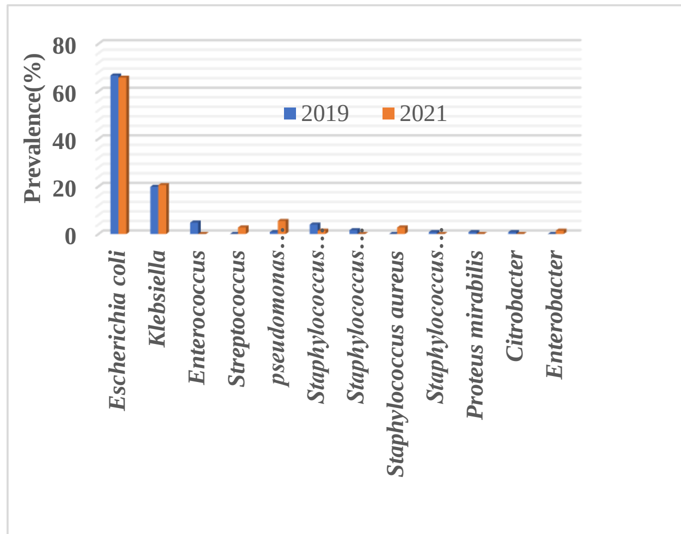

Figure 1. The prevalence of bacterial agents causing urinary tract infections in The Bojnourd-Shirvan region during 2019 and 2021.

In this and several previous studies in Iran, Greece, and elsewhere, *E. coli* has been reported as the most common cause of UTIs (Hashemi 2016; Tajbakhsh, 2015; Barari, 2013; Haghi, 2007; Mantadakis, 2011,). However, the second and third agents of UTI varied from *K.pneumoniae and Enterococcus* to *K.pneumoniae* and *Proteus spp.* in Tajbakhsh et al.'s study of patients referred to the Shahrekord Hospital (2015), to *Klebsiella* and *Pseudomonas* in Barari et al.'s study in Babol province, Iran (2013), or *Klebsiella*, and *Staphylococcus* in another study (Haghi, 2007).

3.2. Relationship between sex and number of patients with *Klebsiella* isolates

In this study, men/women and Out-patients/In-patients ratios showed 60%/40% and 40%/60% of the UTI population, respectively. In 2021, the prevalence of UTI in males was clearly higher compared with females, compared to more females in 2019. While the prevalence of urinary tract infection is usually higher in women, the unusually increased prevalence of male *Klebsiella* infection in this study was due to the significant number of referrals of male NICU patients to this hospital. This was due to male infants being evaluated for urinary tract infections caused by jaundice, as compared to female infants who are less likely to have jaundice and to undergo urine tests. Furthermore, the prevalence of *Klebsiella* UTI is also higher (46.7%) in the 15 and under age group (more in newborns), and 26.6% in the 61-75 age group (two sensitive groups) compared with other groups, as described in Table 1. The results showed any significant correlations between variables ($p > 0.05$). Similar results were observed in our previous study conducted in the Ilam province in southwestern Iran (Hashemi, 2016).

Table 1. Characteristics of UTI patients with Klebsiella in 2019 and 2021.

Variable	Category	Percent		*p*-value	
Time	-	2019	2021	2019	2021
Sex	Man	32%	60%	0.62	0.13
	Woman	68%	40%		
Source	Inpatients	28%	60%	0.23	0.52
	Outpatients	72%	40%		
Age Group (years)	0 -15	44%	46.7%	(Method Limitation)	(Method Limitation)
	16- 30	16%	0		
	31- 45	20%	6.3%		
	46- 60	4%	20.4%		
	61-75	12%	26.6%		
	76 -91	4%	0		

3.3. Antibiotic resistance of *Klebsiella pneumoniae* isolates

Due to the importance of using the evaluation results, the antibiotic resistance of *Klebsiella pneumoniae* isolates to common antibiotics used in the hospital was also evaluated. Overall, *K.pneumoniae* showed slightly more than 30% antibiotic resistance to amoxicillin (AMX:96%) and trimethoprim-sulfamethoxazole (STX:37.5%), and 12-24% to others in 2019 (Figure 2). The spread of the Coronavirus in 2019, which led to fewer patients going to the hospital, as well as the establishment of another hospital in Shirvan City, the number of patients decreased from 3010 in 2019 to 1550 in 2021 (Table 1). However, the prevalence of urinary infections caused by *Klebsiella* (4.7%) compared to 2019 (4.18%) did not show any significant change. Although there was no change in the prevalence of nitrofurantoin (NIT) and ciprofloxacin (CIP), a high increase in resistance to ceftriaxone (CRO), imipenem (IPM), and Gentamicin (GEN) was revealed. Furthermore, two newly used antibiotics, ampicillin-sulbactam (SAM) and ceftazidime (CAZ), showed more than 40% resistance (Figure 2, Table 2). Accordingly, high antimicrobial resistance was against the 3rd and 4th generation cephalosporins and other antibiotics, and the most effective antibiotic (CIP) showed 15.38% resistance (Figure 2).

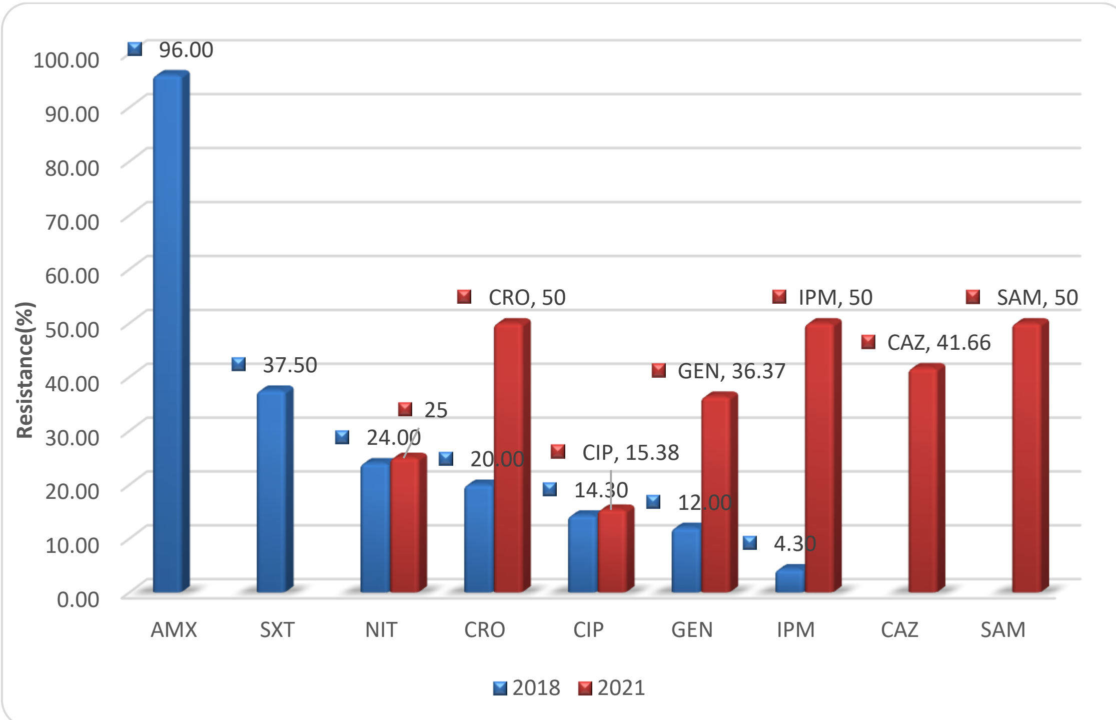

Figure 2. Antibiotic resistance pattern of *Klebsiella pneumoniae* isolates. Amoxicillin (AMX), trimethoprim-sulfamethoxazole (STX), Ceftriaxone (CRO), Gentamicin (GEN), Imipenem (IPM), Ampicillin-sulbactam (SAM), Ceftazidime (CAZ), Nitrofurantoin (NIT), and Ciprofloxacin (CIP).

Similarly, high resistance rates to these antibiotics were also reported in other recent studies (Table 2). Mirnejadet et al. reported 40.15%, 40%, 31%, 33.2%, and 20.3% antibiotic resistances of 202 *Klebsiella* isolated from clinical samples in Shahrekord, Iran (including 48.5% of urine samples), against aztreonam, amikacin, ciprofloxacin, gentamicin, and meropenem, respectively (Mirnejhad et al, 2013). In 2013, Amraei et al. evaluated 120 isolated K.*pneumoniae* from patients admitted to Imam Hossein, Taleghani, and Mofid Children's Hospitals in Tehran, Iran. The five least antibiotic resistance were related to cefotaxime (64%), piperacillin (60%), gentamicin (42%), meropenem, and imipenem (23%) (Amraei, 2014).

Based on the results of a study conducted by Kiani-Abri on 136 *Klebsiella pneumoniae* isolated from patients referred to Shahrekord teaching hospitals in 2013, the resistances of isolates from urine, blood, wound, sputum to cotrimoxazole, cephalothin, tetracycline, cefotaxime, and gentamicin were 58.1%, 52.9%, 47.8%, 39.7%, and 36.8%, to nitrofurantoin and chloramphenicol 25.7%, amikacin 21.3%, norfloxacin 11.8%, nalidixic acid 19.9%, and ciprofloxacin and imipenem 9.6% (Table 2) (Kiani-Abari, 2015). In 2015, *K. pneumoniae* isolated in Shushtar hospitals, Khuzestan (Southwest of Iran), were reported as resistant to ceftazidime (30%), ceftriaxone (43.5%), aztreonam (50%), gentamicin (30%), amikacin (43.5%), and ciprofloxacin (23.5%) (Zadeh, 2021).

A comparison of the lowest values reported regarding the antibiotic resistance of pathogenic *Klebsiella* isolates, mainly related to urinary infections, provides some interesting points (Table 2). Although at first glance, the antibiotic resistance patterns of different regions are different, the similarities can also be observed

Table 2. Comparison of antimicrobial resistance patterns of *K. pneumoniae* isolates, considering the least resistances.

Sample source/Year	Antibiotics and the corresponding resistance (%)					Reference
Shirvan Iran. 2019	Nitrofurantoin 24	Ceftriaxone 20	Ciprofloxacin 14.30	Imipenem 15	Gentamicin 12	This study
Shirvan Iran. 2021	Imipenem 55	Ceftriaxone 50	Gentamicin 36.37	Nitrofurantoin 25	Ciprofloxacin 15.38	This study
Shahrekord, Iran. 2012	Azternam, 40.15	Amikacin 40	Ciprofloxacin 31	Gentamicin 33.2	Meropenem 20.3	Mirnejad, 2013
Shahrekord, Iran. 2013	Cotrimaxazole 58.1 Cephalothin 52.9	Cefotaxime 39.7	Gentamicin 36.8	Nitrofurantoin and Chloramphenicol 25.7	Ciprofloxacin and Imipenem 9.6	Kiani-Abri, 2015
Tehran, Iran. 2014	Cefotaxime 64	Piperacillin 60	Gentamicin 42	Meropenem 23	Imipenem 23	Amrae, 2014
Khuzestan, Iran. 2015	Aztreonam 50	Ceftriaxone, Amikacin 43.5	Ceftazidime, 30	Gentamicin 30	Ciprofloxacin 23.5	Zadeh, 2021
Ankara, Turkey 2014 - 2016	Nitrofurantoin 49.1	Co-trimoxazole 30.7	Ceftazidime, 21.4 Gentamicin 16.5	Ciprofloxacin 7.1	Imipenem: No resistance Ceftriaxone, 1.1	Gunduz, 2018
Europe, 2018 260n-UTI from 18 Countries	Ampicillin-sulbactam,56.2	Ciprofloxacin 40.2/ 83.6(ESBL) Ceftriaxone, 41.5,	Ceftazidime, 39.2	Gentamicin 26.5	Meropenem 7.7/ 17.9(ESBL) Amikacin, 10	Critchley, 2020
Addis Ababa, Ethiopia. 2019	Cefazolin 98 Cefotaxime 97	Gentamicin 72	Ciprofloxacin 37, Piperacillin 37	Meropenem 24.3 Imipenem 12.1	Amikacin 3	Awoke, 2021
Tehran, Iran. 2020 477n	Aztreonam 58.8	Meropenem, Imipenem, 67.7%, 67.7%	Gentamicin, 78.8	Ciprofloxacin, 84.4,	Cefepime, 86.6 Ceftriaxone, 87.7,	Sanikhani, R., 2021

among them. Overall, the lowest resistance range of 20% to 40% of most antibiotics used in the treatment process is worrying news. Although it is not possible to compare the uniformity of the reported results due to the relatively different consumption patterns of antibiotics in different regions and countries, a comparison can be made regarding the three antibiotics gentamicin, imipenem, and ciprofloxacin (Table 1). Accordingly, *Klebsiella* isolates showed resistance to gentamicin (GEN) in a range of 30-42% between 2013 and 2015 in Iran. However, there was lower resistance (16.5%) in the isolates collected during 2014-2016 in a hospital in Istanbul, Turkey (Gunduz, 2018), and two very high resistances, one 72% resistance in Addis Baba, Ethiopia hospital isolates in 2019 (Awoke, 2021) and the other 78.8% resistance in isolates from Tehran Children's Hospital, Iran to gentamicin (GEN) in 2020 (Table 2).

Regarding antibiotic resistance of *Klebsiella* isolates to ciprofloxacin (CIP), the lowest value of most reports was in the range of 23-43%, with reported lower resistance values (7.1% in Turkey and 9.6% in Iran) and very high resistance value (84.4% in Ethiopia) to this antibiotic (Table 2). Similar to this pattern, regarding antibiotic resistance to imipenem, the lowest resistance was reported in the range of 9.6-37. Although the absence of resistance in Turkish samples and the low resistance (9.6%) of Shahrekord isolates in 2013 were positive statistics, the high percentage of resistance of Tehran isolates in 2020 to imipenem and penipenem is very worrying (Table 2). Overall, this increasing trend of antibiotic resistance as a threat to public health and a challenge for treatment has encouraged researchers to address new research areas. Among them, the potential of using phage against pathogenic bacteria is a strategy that has recently received attention. In this regard, the isolation and determination of the characteristics of a novel bacteriophage against carbapenemase-producing *K.pneumoniae* strains isolated from Ventilator-associated pneumoniae (VAP) infection of COVID-19 patients have been reported (Mohammadi, 2023).

4. Conclusion

In general, there is a difference in the resistance pattern of *Klebsiella* isolates from urinary tract infections depending on the geographical area, although similarities can be seen depending on the extent of the area. A study conducted in the Shirvan region, Iran, showed that the antibiotics ampicillin, cefazolin, cephalothin, and cotrimoxazole (with a resistance percentage between 40 to 80%) have practically lost their effectiveness in this area. Furthermore, a very high and unexpected increase in resistance was revealed against ceftriaxone (CRO), imipenem (IPM), and gentamicin (GEN) during the Corna pandemic in Shirvan and Tehran during 2019- 2021 . This more than a hundred percent increase in the antibiotic resistance of *Klebsiella sp.* (a primary cause of urinary tract infections) could have occurred due to the preventive and therapeutic use of antibiotics in Coronavirus patients during this short period. This unfortunately clear pattern was evident in Shirvan, Tehran, and possibly other regions and can be considered a real threat to the general health, even though it is just one of the bacterial agents involved in UTIs.

Extensive bacterial diversity involved in urinary tract infections and a wide range of resistance to various antibiotics as well as multi-resistance manifestations of their isolates emphasize the potential of urinary tract infections as a threat to community health and can lead to a rise in mortality, especially in vulnerable groups such as the elderly and hospitalized patients. This study concluded that the Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or COVID-19, its resulting increase in hospitalized patients, including patients admitted to the ICU, and the use of antibiotics to prevent secondary infections or therapeutic aspects and lifestyle changes in the community have lead to a possible change the profiles of the antibiotic resistance of the bacterial infections in the recent post-coronary period. The pattern of biodiversity of infectious agents and their degree of resistance to change requires planning and implementation of timely monitoring research for intelligent management of treatments. In addition, knowing the pattern of distribution of bacteria in any area and the pattern of their antibiotic resistance and the use of this information in the correct prescription of antibiotics can lead to more efficient and scientific treatment.

Conflict of interest

The authors declare that they have no competing interests.

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Ethical approval

This study was approved by the Ethical Committee of the North Khorasan University of Medical Sciences, Bojnurd, Iran, (IR-NKUMS.REC. 1397/042).

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