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RESEARCH ARTICLE

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COMPARATIVE ANALYSIS OF ANTIBIOTIC RESISTANCE PATTERN OF *KLEBSIELLA SPECIES* AFTER COVID-19 PANDEMIC

Palwasha[1], Hasnain Hassan[2], Farah Salim[3], Attaullah[4], Syed Muhammad Shadab khan[5], Shaukat Ali[6], Muhammad Sohail[7], Muhammad Jawad Ullah[8*]

[1]City University of Science and Information Technology, Peshawar, Pakistan
[2] City University of Science and Information Technology, Peshawar, Pakistan
[3] Peshawar Medical College, Peshawar, Pakistan
[4] Shaheed Benazir Bhutto University, Sheringle (Dir Upper) Pakistan
[5] City University of Science and Information Technology, Peshawar, Pakistan
[6] City University of Science and Information Technology, Peshawar, Pakistan
[7] Department of Allied Health Sciences Iqra National University, Peshawar, Pakistan
[8*] Department of Allied Health Sciences Iqra National University, Peshawar, Pakistan

***Corresponding Author:** Muhammad Jawad ullah,
* Department of Allied Health Sciences Iqra National University Peshawar, Email (jawadkhalil3132@gmail.com)

Abstract

The *Klebsiella spp* are Gram negative, encapsulated, non-motile, facultative, anaerobic, lactosefermenting bacteria. The antibiotic resistance is already very high and due to COVID-19 it is further increases to the alarming level, which could cause serious problem on the health care system of the country. The misuse of antibiotics, self-prescribed medication, and wide use of antibiotics due to COVID-19 and shortage of drugs are some of the most common causes for the development of antibiotic resistance in Pakistan. A total of 65 drinking water samples were collected from different areas of district Peshawar Pakistan and were cultured on MacConkey Agar media for selective isolation of Gram-negative lactose fermented *Klebsiella spp*. Initially morphological identification was done in order to identify the *Klebsiella spp* among the isolated lactose fermented bacteria (*E.coli, Citrobacter and Enterobacter*). We performed Gram staining for the conformation of Gram positive or Gram negative bacteria. We noticed that all the samples were Gram negative bacteria, in which 32 samples shaped rod form and red in color, which means they were *Klebsiella spp.* Different biochemical tests (Catalase test, Citrate test and motility test) were performed for the detection of

Klebsiella spp. All the 32 samples were positive for catalase and citrate and were non-motile. Disc diffusion test was performed for the determining the antibiotic resistant pattern of *Klebsiella spp* isolates. Thirteen different antibiotics were used; Ampicillin, Gentamicin, Amikacin, Cefotaxime, Polymyxin B, Tobramycin, Ciprofloxacin, Ceftazidime, Imipenem, Ceftriaxone, Meropenem, Levofloxacin and Ofloxacin. Total of 72% showed resistant to all of the mentioned antibiotics. 16% (1291-
samples showed sensitivity to Meropenem. 12% samples showed sensitivity to Gentamicin, Amikacin, Cefotaxime, Imipenem, Meropenem, levofloxacin and Ofloxacin. The current study isolated the *Klebsiella spp* from drinking water samples and determined the antibiotic resistance pattern after COVID-19. Molecular level study could help us to evaluate those genes which are

Vol.31 No.4 (2024): JPTCP making the bacteria resistant. Samples should be collected from different cities of Pakistan to check
the resistivity pattern of *Klebsiella spp* across Pakistan.

1. INTRODUCTION

Klebsiella spp belong to the family Enterobacteriaceae and are non-motile, capsulate, Gram-negative bacilli. *Klebsiella pneumoniae* is a commensal bacterium found in the gastrointestinal and respiratory tracts, and on the skin of healthy individuals. It is also ubiquitous in the environment. It is an opportunistic pathogen capable of causing a wide range of community-acquired and nosocomial infections, such as urinary tract infections (UTIs), respiratory tract infections and infections of wounds and soft tissue(1). It has in recent years become one of the world's leading causes of nosocomial infections, with an increasing mortality rate, particularly in immunocompromised individuals, neonates and the elderly. It is also increasingly implicated in severe community-acquired infections such as pneumonia and meningitis(2). *Klebsiella spp* grow well on an ordinary medium (nutrient agar) and on MacConkey agar they produce pink mucoid colonies. The bacterium contains a capsule, making it deadly. It is also resistant to the environment, the effects of disinfectants, and many antibiotics. It has a complicated antigenic structure, is composed of somatic and capsular antigens, endotoxin, and some strains are capable of producing exotoxin. These microbes have been linked to sepsis, meningitis, conjunctivitis, pneumonia, acute intestinal infections, and urogenital infections. The *Klebsiella spp* infections can also develop as a secondary infection against the background of infection caused by viruses, which can also lead to an increase in the rate of deaths(3). *Klebsiella spp* can grow at temperature ranging between to 35°C to 37°C while their ideal pH level is about 7.2.The species are aerobic but facultative anaerobic. It can be grown easily and shows good growth within 24 hours (4). The antibiotic resistance is the ability of bacteria to resist the effects of antibiotic like azithromycin and ciprofloxacin, which either kill bacteria or inhibit the growth of bacteria. It is very difficult to treat bacterial infections, caused by those bacteria which are antibiotic resistance. According to a world health organization (WHO) research from 2019, AMR is responsible for the deaths of 700,000 people, while it is estimated the by the year 2050 the figure will have risen to 20 million(5). The antibiotic use is unnecessarily high in Pakistan and as a result of prolonged exposure to the antibiotics the bacteria developing resistance to them. In Pakistan, multidrug resistance (MDR) is on the rise, and a research found that 77.5% of all screened isolates were resistant to three or more of the tested antibiotics. Various publications on the rise of antibiotic resistance in Pakistan attest to the rapid rise of antibiotic resistance or possibly the emergence of MDR in the entire country (6). Following the COVID-19 pandemic, a new antimicrobial resistance (AMR) pandemic has been active. It is thought that the COVID-19 pandemic is hastening the emergence of antimicrobial resistance because of the widespread usage of antibiotics. One of the top 10 most serious health issue facing humanity, according to the world health organization (WHO) is the antibiotic resistance. The antibiotic resistance is already very high in Pakistan and the COVID-19 pandemic brought it to an alarming high

and this might have major consequences for the nation's health care system. The wide use of antibiotics, self-prescribed medications, misuse of antibiotics, shortage of drugs, and overuse of antibiotics in COVID-19 pandemic are the most common reason for the development of antibiotic resistance in Pakistan (7). Various antibiotics, including moxifloxacin, cephalosporins, quinolones, carbapenems, tigecycline, linezolid ceftriaxone and azithromycin, were utilized to treat patients during the pandemic. Vancomycin, Amikacin, Azithromycin, Rapamycin, and doxycycline are further antibiotic therapies used for the treatment of COVID-19 disease (8). The antibiotics used to treat patients in the ward, before admission to the ICU, included levofloxacin, ceftriaxone/cefotaxime plus macrolide, imipenem, fluoroquinolone, meropenem, ceftriaxone/cefotaxime, co-amoxiclav plus macrolide, piperacillin/tazobactam, co-amoxiclav, Ceftazidime, Co-amoxiclav plus quinolone and Ceftriaxone (9). The azithromycin, ceftriaxone, piperacillin-tazobactam, amoxicillin-clavulanic acid, ciprofloxacin and meropenem were the most commonly used antibiotics. Azithromycin, one of the most often given antibiotics, is used by COVID-19 hospitalized patients in Pakistani hospitals (10). In West Africa *Klebsiella* resistance to ciprofloxacin was higher than in other regions. Resistance to the penicillin, ampicillin and trimethoprim was generally high in all regions. *Klebsiella* was 38.0% resistant to Gentamicin. *K. pneumoniae* was 34.2% and 46.7% resistant to either ceftriaxone or cefotaxime (11). Another study showed that 92.3% *Klebsiella* was resistant to ampicillin. *Klebsiella* was resistant to ceftazidime and ceftriaxone (46.2% and 53.8%) respectively (12). In Nepal *K. pneumonia* was 100% sensitive to amikacin, gentamicin, meropenem, and imipenem while it was 100% resistant to ampicillin, cefotaxime, and ceftazidime. Followed by *S. aureus* resistant to Gentamicin (90%) and ofoxacin (90%). CoNS strains resistant to Ampicillin and ciprofloxacin (83%, each) (13). In Sri Lanka *Klebsiella pneumoniae* have high resistance rates for norfloxacin 48.5%, cotrimoxazole 43.6%, co-amoxiclav 59%, nalidixic acid 28% and ciprofloxacin 41%. Amikacin and imipenem showed 100% sensitivity to all *Klebsiella* while nitrofurantoin and gentamicin had 91.5% and 89.7% sensitivity respectively (14). Another study showed that that *Klebsiella* was highly sensitive to meropenem, imipenem (93%), amikacin (73%), ciprofloxacin (74%), Amoxicillin/Clavulanate (36.36%), Cefotaxime (27.27%), Ceftazidime (9%), Ceftriaxone (54.5%), Chloramphenicol (18.1%), Clindamycin (9%) and Vancomycin (9%) (15). A study conducted in Iran reported that *K. pneumoniae* showed different levels of resistance to antibiotics gentamicin (55.7%), ciprofloxacin (57.7%), meropenem (80.4%), tobramycin (61.4%), imipenem (50%) and cefepimeaztreonam- piperacillin/tazobactam (70%) (16). Another study showed that 5% to 50% enhanced antimicrobial resistivity in *K. pneumonia* for polymyxin B as compared to before covid-19 pandemic (17). Another study showed that during Covid-19 pandemic *K. pneumonia* was highly resistance to colistin. *K. pneumonia* was 93%, 93.5% and 100% resistant to cefazolin, ceftazidime and Ampicillin respectively. 73.5% resistance was presented by *K. pneumonia* to trimethoprim/sulfamethoxazole(18).

2. MATERIALS AND METHODS

2.1. Duration of study

The present study was conducted in City University of Science and Information Technology, from January 2023 to June 2023. This research methodology was composed of three major phases; collection of samples from different areas of District Peshawar, isolation and identification of *Klebsiella spp* and the comparative analysis of antibiotic resistance pattern of *Klebsiella spp* after COVID-19 pandemic.

2.2. Study design

The study design of the current research was quantitative and cross-sectional study.

2.3. Study Duration
The duration of the current research was 6 months from January 2023 to June 2023.

2.4. Sample size
A total of 65 samples (calculated by open epi) (tape water) were collected in the study from different areas of District Peshawar.

2.5. Exclusion and Inclusion Criteria
In this study the filter water and boil water was excluded and drinking water (Tape water) was included.

2.6. Sample collection
Samples of drinking water were collected in sterile bottles (the pH, temperature of H_2O samples were checked) from different areas of District Peshawar. The samples were transferred to CUSIT Medical Laboratory of Department of Allied Health Science, City University of Science and Information Technology following standard microbiological protocol.

2.7. Sampling Processing
After samples collection, the MacConkey agar media was used for the isolation of bacterial isolates from drinking water samples. MacConkey agar media is a selective and differential culture medium for Gram-negative bacteria (*Klebsiella spp*).

2.8. Identification of Bacterial Isolates
The bacterial isolates were Gram stained to differentiate Gram-positive and Gram-negative bacteria. Further identification was done by using different biochemical tests such as citrate, catalase and motility test.

2.9. Antibiotics Sensitivity of Bacterial Isolates
For antibiotic profiling, Muller Hinton Agar (MHA) media was prepared, autoclaved and poured into the plates. By using sterile cotton swabs a bacterial lawn was prepared on MHA agar petri plates and were allowed to dry. The disc diffusion method was used. According to CLSI-2022 guidelines, different antibiotics i.e., Ampicillin, Gentamicin, Amikacin, Cefotaxime, Polymyxin B, Tobramycin, Ciprofloxacin, Ceftazidime, Imipenem, Ceftriaxone, Meropenem, Levofloxacin and Ofloxacin were used. After this the plates were incubated at 37°C for 24hrs. After incubation timing, the zones around each antibiotic disc were measured in mm.

3. RESULTS
In this study, a total of 65 drinking water samples were collected from the District of Peshawar by using sterile bottles. Out of 65(100%) drinking water samples, 32 (49%) samples were *Klebsiella spp* isolates, while 33 (51%) were other Gram-negative bacterial isolates e.g. *E coli, Citrobacter and Enterobacter*.

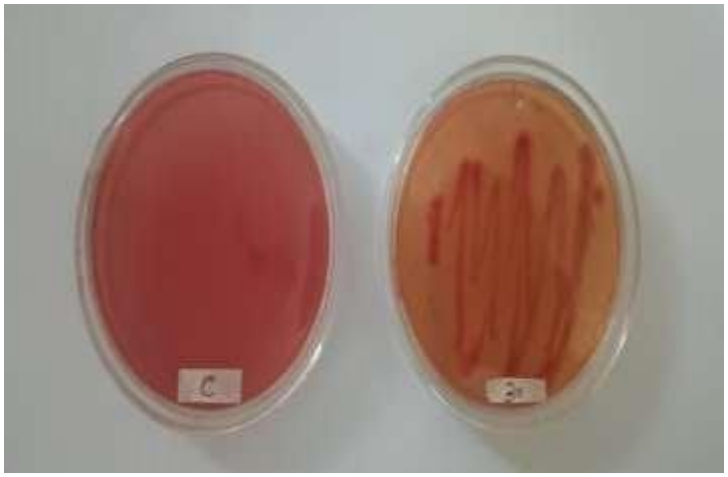

Figure 1: Growth of *Klebsiella spp* with pink colour colonies on MacConkey Agar medium.

Initially morphological identification was done in order to identify the *Klebsiella spp* among the isolated lactose fermented bacteria (*E.coli, Citrobacter and Enterobacter*). We performed Gram staining for the conformation of Gram positive or Gram negative bacteria. We noticed that all the samples were Gram negative bacteria, in which 32 samples shaped rod form and red in color, which means they were *Klebsiella spp.* Different biochemical tests (Catalase test, Citrate test and motility test) were performed for the detection of *Klebsiella spp*. All the 32 samples were positive for catalase and citrate and were non-motile

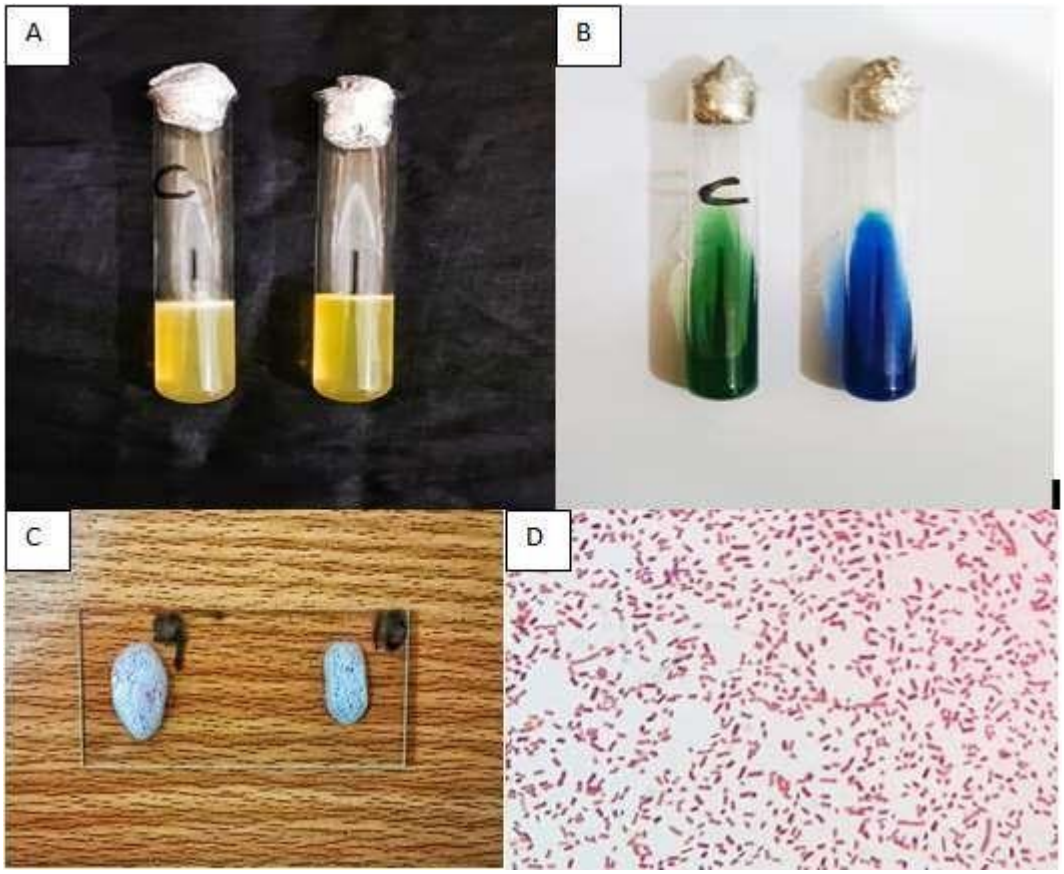

Figure 2. Show the (A) motility negative, (B) citrate test, (C) Catalase and (D) Gram staining

Table 1: Biochemical tests

S.No	Catalase	Citrate	Motility	Gram-staining
1	Positive	Positive	Negative	Gram-Negative
2	Positive	Positive	Negative	Gram-Negative
3	Positive	Positive	Negative	Gram-Negative
4	Positive	Positive	Negative	Gram-Negative
5	Positive	Positive	Negative	Gram-Negative
6	Positive	Positive	Negative	Gram-Negative
7	Positive	Positive	Negative	Gram-Negative
8	Positive	Positive	Negative	Gram-Negative
9	Positive	Positive	Negative	Gram-Negative
10	Positive	Positive	Negative	Gram-Negative
11	Positive	Positive	Negative	Gram-Negative
12	Positive	Positive	Negative	Gram-Negative
13	Positive	Positive	Negative	Gram-Negative
14	Positive	Positive	Negative	Gram-Negative
15	Positive	Positive	Negative	Gram-Negative
16	Positive	Positive	Negative	Gram-Negative
17	Positive	Positive	Negative	Gram-Negative
18	Positive	Positive	Negative	Gram-Negative
19	Positive	Positive	Negative	Gram-Negative
20	Positive	Positive	Negative	Gram-Negative
21	Positive	Positive	Negative	Gram-Negative
22	Positive	Positive	Negative	Gram-Negative
23	Positive	Positive	Negative	Gram-Negative
24	Positive	Positive	Negative	Gram-Negative
25	Positive	Positive	Negative	Gram-Negative
26	Positive	Positive	Negative	Gram-Negative
27	Positive	Positive	Negative	Gram-Negative
28	Positive	Positive	Negative	Gram-Negative
29	Positive	Positive	Negative	Gram-Negative
30	Positive	Positives	Negative	Gram-Negative
31	Positive	Positive	Negative	Gram-Negative
32	Positive	Positive	Negative	Gram-Negative

Disc diffusion test was performed for the determining the antibiotic resistant pattern of *Klebsiella spp* isolates. Thirteen different antibiotics were used; Ampicillin, Gentamicin, Amikacin, Cefotaxime, Polymyxin B, Tobramycin, Ciprofloxacin, Ceftazidime, Imipenem, Ceftriaxone, Meropenem, Levofloxacin and Ofloxacin.

Figure 3: *Klebsiella spp* show the sensitivity to the antibiotics in Muller Hinton agar media.

Table 2: Results of antibiotic sensitivity test in mm

S. No	S. Code	AMP	CN	AK	CTX	PB	TOB	CIP	CAZ	IMP	CRO	MEM	Lev	OFX
1	1	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R	13 R	0 R	0 R
2	2.1	0 R	0 R	0 R	10 R	0 R	0 R	0 R	0 R	14 R	0 R	0 R	0 R	0 R
3	2.2	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R
4	3	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R	14 R	0 R	0 R
5	4	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R
6	5	0 R	0 R	8 R	0 R	4 R	0 R	0 R	0 R	0 R	0 R	16 R	0 R	0 R
7	6	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R
8	7	0 R	0 R	6 R	0 R	10 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R	0 R
9	8.1	0 R	8 R	12 R	0 R	6 R	0 R	0 R	0 R	0 R	0 R	17 R	0 R	0 R
10	8.2	0 R	10 R	10 R	0 R	4 R	0 R	0 R	0 R	12 R	0 R	0 R	8 R	0 R
11	8.3	0 10	2 0 4	2 0 16	12 0 0	0 4 R	R R R	R R R	R R R	R R R	R R R	R R R	R R R	R R R
12	9	0 R	12 R	4 R	0 R	0 R	0 R	0 R	0 R	14 R	0 R	0 R	8 R	0 R	0 R
13	10.1	0 10	4 0 0	0 0 14	0 23	0 0 R	R R R	R R R	R R R	R S R	R			
14	10.2	0 R	8 R			6 R 0 R	0 R 4		0 R 6		0 R	R 0 R R	0 R 0	R 0 R
15	10.3	0 8	0 0 2	0 0 0 0	0 8 0	R R R	R R R	R R R	R R R	R R R	R R R	R R R	R R R	R R R

16	11	0 R	0 R	0 R	0 R R S	0	0	R	0 R		0 R		0 R	0 R	23	0 R	0 R
17	12.1	0 R	0 R	18 S	0 R	10 R	0 R	0 R		24 S	23 S	0 R	29 S	14 R	0 R		
	18	0 R	12.2 R	0 19	0 12 16	0 24	24 0	28	17		0 R		S R	R S R	S S R	S S	
19	20.1	0 R	20	19	25	10	12	0	0	38	17	33	35	39	S S S R R R R S R S S S		
20	20.2	0 R	0 R	20 S	22 R	10 R	16 S	0 R		23 S	26 S	17 R	23 S	14 R	0 R		
21	21.1	0	8	0	0	4	12	0	12	0	6	0	8	R R R R R R R R R R R R R			
22	21.2	0 R	10 R	12 R	0 R	4 R	6 R	0 R		10 R	12 R	0 R	0 R	0 R	0 R		
23	21.3	0	6	0	4	8	0	0	16	0	0	0	0	R R R R R R R R R R R R R			
24	21.4	0 R	8 R	10 R	10 R	0 R	4 R	0 R		0 R	10 R	6 R	0 R	0 R	0 R		
25	21.5	0 R	10	8 2 R R	0 R R	6 R	10 R	0 R		16 R	18 R	14 R	24 R	4 S			
26	25.1	0 R	0 R	4 R	0 R	0 R	0 R	0 R		0 R	0 R	0 R	12 R	0 R	0 R		
27	26.1	0	0	0	0	0	0	0	0	0	0	0	0	R R R R R R R R R R R R R			
28	26.2	0 R	0 R	0 R	0 R	0 R	0 R	0 R		0 R	0 R	0 R	0 R	0 R	0 R		
29	26.3	0 R	10 R	8 R	0 R	6 R	10 R	0 R		16 R	18 R	14 R	24 S	4 R	2 R		
30	26.4	0 R	10 R	0 R	0 R	0 R	8 R	0 R		0 R	6 R	0 R	0 R	0 R	0 R		
31	26.7	0	8	6	0	0	0	0	12	4	0	0	0	R R R R R R R R R R R R R			
32	26.8	0 R	10 R	8 R	0 R	6 R	10 R	0 R		12 R	16 R	0 R	33 S	9 R	2 R		

Ampicillin (AMP), Gentamicin (CN), Amikacin (AK), Cefotaxime (CTX), Polymyxin B (PB), Tobramycin (TOB), Ciprofloxacin (CIP), Ceftazidime (CAZ), Imipenem (IMP), Ceftriaxone (CRO), Meropenem (MEM), Levofloxacin (Lev), Ofloxacin (OFX).

Samples 1, 2.1, 2.2, 3, 4, 5, 6, 7, 8.1, 8.2, 8.3, 9, 10.2, 10.3, 21.1, 21.2, 21.3, 21.4, 25.1, 26.1, 26.2, 26.4 and 26.7 were resistant to Ampicillin, Gentamicin, Amikacin, Cefotaxime, Polymyxin B, Tobramycin, Ciprofloxacin, Ceftazidime, Imipenem, Ceftriaxone, Meropenem, Levofloxacin and Ofloxacin. Samples 10.1, 11, 21.5, 26.3 and 26.8 were resistant to all of the above antibiotics while these were sensitive to meropenem.

Table 3: Showing percentage of antibiotic susceptibility of *Klebsiella spp*

Name of antibiotics	Percentage of intermediate resistance (%)	Percentage of resistance (%)	sensitivity (%)
Ampicillin	0%	0%	100%
Gentamicin	3.1%	0%	96.9%
Amikacin	12.5%	0%	87.5%
Cefotaxime	3.1%	0%	96.9%
Polymyxin B	0%	0%	100%
Tobramycin	6.25%	0%	93.8%
Ciprofloxacin	0%	0%	100%
Ceftazidime	9.37%	0%	90.6%
Imipenem	12.5%	0%	87.5%
Ceftriaxone	0%	0%	100%
Meropenem	28.1%	0%	78.9%
Levofloxacin	6.25%	0%	93.8%
Ofloxacin	3.1%	0%	96.9%

Sample 12.1 was resistant to Ampicillin, Gentamicin, Cefotaxime, polymyxin B, Tobramycin, Ciprofloxacin, Ceftriaxone, levofloxacin and Ofloxacin. While it was sensitive to Amikacin, Ceftazidime, Imipenem and Meropenem. Sample 12.2 was resistant to Ampicillin, Gentamicin, Cefotaxime, Polymyxin B, Ciprofloxacin, Ceftriaxone and Ofloxacin while it was sensitive to Amikacin, Tobramycin, Ceftazidime, Imipenem, Meropenem and Levofloxacin. Sample 20.1 was resistant to Ampicillin, Polymyxin B, Tobramycin, Ciprofloxacin, Ceftazidime, and Ceftriaxone. While it was sensitive to Gentamicin, Amikacin, Cefotaxime, Imipenem, Meropenem, levofloxacin and Ofloxacin. Sample 20.2 was resistant to Ampicillin, Gentamicin, Cefotaxime, Polymyxin B, Ciprofloxacin, Ceftriaxone, Levofloxacin and Ofloxacin. While it was sensitive to Amikacin, Tobramycin, Ceftazidime, Imipenem and Meropenem.

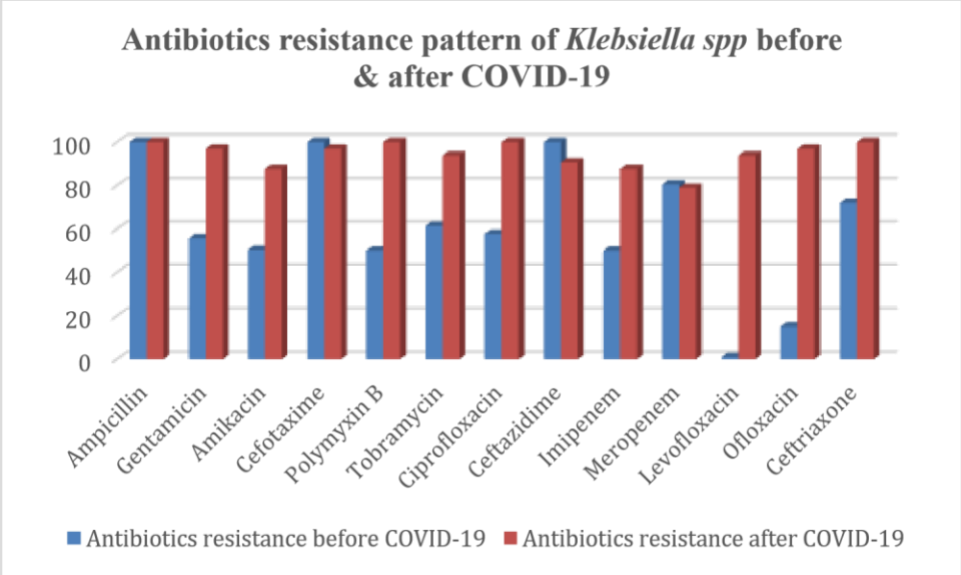

Figure.4: shows the antibiotic resistance before and after COVID-19

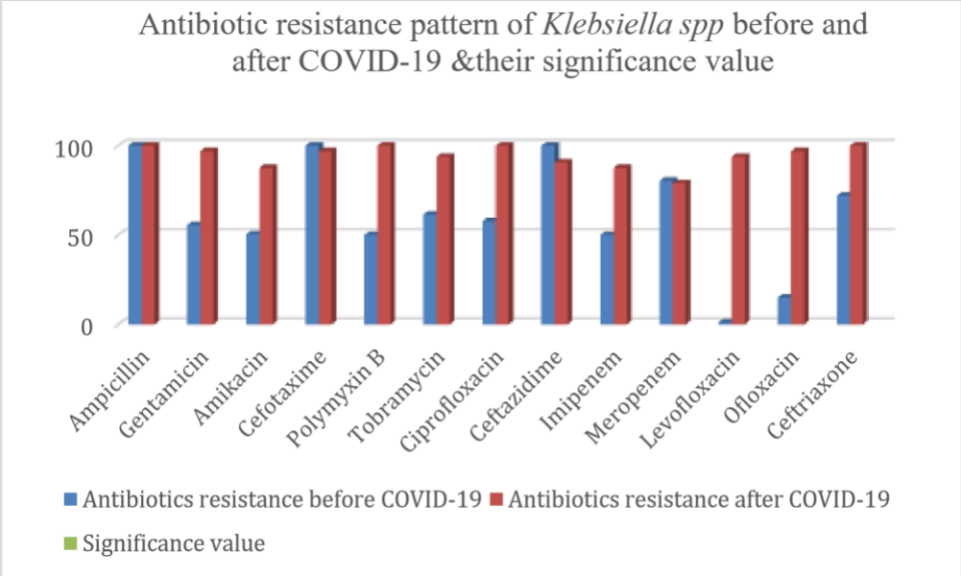

Figure 5 : shows significant value of all the mentioned antibiotics before and after COVID-19

4. DISCUSSION

Klebsiella spp belong to the family Enterobacteriaceae and are non-motile, capsulate, Gram-negative bacilli. *Klebsiella pneumoniae* is a commensal bacterium found in the gastrointestinal and respiratory tracts, and on the skin of healthy individuals. It is also ubiquitous in the environment. It is an opportunistic pathogen capable of causing a wide range of community-acquired and nosocomial infections, such as urinary tract infections (UTIs), respiratory tract infections and infections of wounds and soft tissue(1). It has in recent years become one of the world's leading causes of nosocomial infections, with an increasing mortality rate, particularly in immunocompromised individuals, neonates and the elderly. It is also increasingly implicated in severe community-acquired infections such as pneumonia and meningitis(2). *Klebsiella spp* grow well on an ordinary medium (nutrient agar) and on MacConkey agar they produce pink mucoid colonies. The bacterium contains a capsule, making it deadly. It is also resistant to the environment, the effects of disinfectants, and many antibiotics. It has a complicated antigenic structure, is composed of somatic and capsular antigens, endotoxin, and some strains are capable of producing exotoxin. These microbes have been linked to sepsis, meningitis, conjunctivitis, pneumonia, acute intestinal infections, and urogenital infections. The *Klebsiella spp* infections can also develop as a secondary infection against the background of infection caused by viruses, which can also lead to an increase in the rate of deaths(3). *Klebsiella spp* can grow at temperature ranging between to 35°C to 37°C while their ideal pH level is about 7.2.The species are aerobic but facultative anaerobic. It can be grown easily and shows good growth within 24 hours (4). The antibiotic resistance is the ability of bacteria to resist the effects of antibiotic like azithromycin and ciprofloxacin, which either kill bacteria or inhibit the growth of bacteria. It is very difficult to treat bacterial infections, caused by those bacteria which are antibiotic resistance. According to a world health organization (WHO) research from 2019, AMR is responsible for the deaths of 700,000 people, while it is estimated the by the year 2050 the figure will have risen to 20 million(5). The antibiotic use is unnecessarily high in Pakistan and as a result of prolonged exposure to the antibiotics the bacteria developing resistance to them. In Pakistan, multidrug resistance (MDR) is on the rise, and a research found that 77.5% of all screened isolates were resistant to three or more of the tested antibiotics. Various publications on the rise of antibiotic resistance in Pakistan attest to the rapid rise of antibiotic resistance or possibly the emergence of MDR in the entire country (6). Following the COVID-19 pandemic, a new antimicrobial resistance (AMR) pandemic has been active. It is thought that the COVID-19 pandemic is hastening the emergence of antimicrobial resistance because of the widespread usage of antibiotics. One of the top 10 most serious health issue facing

humanity, according to the world health organization (WHO) is the antibiotic resistance. The antibiotic resistance is already very high in Pakistan and the COVID-19 pandemic brought it to an alarming high and this might have major consequences for the nation's health care system. The wide use of antibiotics, self-prescribed medications, misuse of antibiotics, shortage of drugs, and overuse of antibiotics in COVID-19 pandemic are the most common reason for the development of antibiotic resistance in Pakistan (7). Various antibiotics, including moxifloxacin, cephalosporins, quinolones, carbapenems, tigecycline, linezolid ceftriaxone and azithromycin, were utilized to treat patients during the pandemic. Vancomycin, Amikacin, Azithromycin, Rapamycin, and doxycycline are further antibiotic therapies used for the treatment of COVID-19 disease (8). The antibiotics used to treat patients in the ward, before admission to the ICU, included levofloxacin, ceftriaxone/cefotaxime plus macrolide, imipenem, fluoroquinolone, meropenem, ceftriaxone/cefotaxime, co-amoxiclav plus macrolide, piperacillin/tazobactam, co-amoxiclav, Ceftazidime, Co-amoxiclav plus quinolone and Ceftriaxone (9). The azithromycin, ceftriaxone, piperacillin-tazobactam, amoxicillin-clavulanic acid, ciprofloxacin and meropenem were the most commonly used antibiotics. Azithromycin, one of the most often given antibiotics, is used by COVID-19 hospitalized patients in Pakistani hospitals (10). The current study aimed to isolate and identify phenotypically characteristics of *Klebsiella spp* from water samples (tap water) collected from different areas of district Peshawar Pakistan. The results showed that *Klebsiella spp* was isolated and identified phenotypically in 32 samples (out of 65 samples). The antibiotic resistance pattern of *Klebsiella spp* was also determined against different antibiotics such as Ampicillin, Gentamicin, Amikacin, Cefotaxime, Polymyxin B, Tobramycin, Ciprofloxacin, Ceftazidime, Imipenem, Ceftriaxone, Meropenem, levofloxacin and Ofloxacin.

Samples 1, 2.1, 2.2, 3, 4, 5, 6, 7, 8.1, 8.2, 8.3, 9, 10.2, 10.3, 21.1, 21.2, 21.3, 21.4, 25.1, 26.1, 26.2, 26.4 and 26.7 were resistant to Ampicillin, Gentamicin, Amikacin, Cefotaxime, Polymyxin B, Tobramycin, Ciprofloxacin, Ceftazidime, Imipenem, Ceftriaxone, Meropenem, levofloxacin and Ofloxacin. Similar results were observed in previous studies (11, 12, 16-19) in which *Klebsiella spp* were resistant to the above mentioned antibiotics. In current study Samples 10.1, 11, 21.5, 26.3 and 26.8 were resistant to all of the above antibiotics while they were sensitive to meropenem. Similar results were observed in previous studies (13, 15, 20) in which *Klebsiella spp* were 100% sensitive to meropenem. Sample 12.1 was resistant to Ampicillin, Gentamicin, Cefotaxime, polymyxin B, Tobramycin, Ciprofloxacin, Ceftriaxone, levofloxacin and Ofloxacin. While it was sensitive to Amikacin, Ceftazidime, Imipenem and Meropenem. Sample 12.2 was resistant to Ampicillin, Gentamicin, Cefotaxime, Polymyxin B, Ciprofloxacin, Ceftriaxone and Ofloxacin while it was sensitive to Amikacin, Tobramycin, Ceftazidime, Imipenem, Meropenem and Levofloxacin. Sample 20.1 was resistant to Ampicillin, Polymyxin B, Tobramycin, Ciprofloxacin, Ceftazidime, and Ceftriaxone. While it was sensitive to Gentamicin, Amikacin, Cefotaxime, Imipenem, Meropenem, levofloxacin and Ofloxacin. Sample 20.2 was resistant to Ampicillin, Gentamicin, Cefotaxime, Polymyxin B, Ciprofloxacin, Ceftriaxone, Levofloxacin and Ofloxacin. While it was sensitive to Amikacin, Tobramycin, Ceftazidime, Imipenem and Meropenem.

In contrast the previous study showed that *Klebsiella spp* was highly sensitive to meropenem, imipenem (93%), amikacin (73%), ciprofloxacin (74%), Cefotaxime (27.27%), Ceftazidime (9%), Ceftriaxone (54.5%) (15). According to a study conducted in Bangladesh (2021) the *Klebsiella pneumoniae* isolates showed resistance towards meropenem ceftriaxone, amikacin, ceftazidime and gentamicin (44.7%, 72.8%, 50.3%, 75.3% and 60.3% respectively) (19). Another study showed that during COVID-19 pandemic the *K. pneumonia* was 93%, 93.5% and 100% resistant to cefazolin, ceftazidime and Ampicillin respectively (18). The current research showed the emergence of *Klebsiella spp* in the drinking water sources of different areas of the district Peshawar Pakistan.

Klebsiella spp showed high level resistance to Ampicillin, Gentamicin, Amikacin, Cefotaxime, Polymyxin B, Tobramycin, Ciprofloxacin, Ceftazidime, Ceftriaxone, levofloxacin and Ofloxacin while Imipenem and Meropenem were found to be effective against *Klebsiella spp.*

5. CONCLUSION

The current study was aimed to detect and isolate the *Klebsiella spp* from drinking water (tape water) of different areas of district Peshawar Pakistan and to determine the antibiotic resistance pattern of the *Klebsiella spp* after COVID-19 pandemic. In our research out of 32 samples the 23 samples were resistant to Ampicillin, Gentamicin, Amikacin, Cefotaxime, Polymyxin B, Tobramycin, Ciprofloxacin, Ceftazidime, Imipenem, Ceftriaxone, Meropenem, levofloxacin and Ofloxacin. Only 5 samples were resistant to all of the above antibiotics while they were sensitive to Meropenem. Sample 12.1 was resistant to Ampicillin, Gentamicin, Cefotaxime, polymyxin B, Tobramycin, Ciprofloxacin, Ceftriaxone, levofloxacin and Ofloxacin. While it was sensitive to Amikacin, Ceftazidime, Imipenem and Meropenem. Sample 12.2 was resistant to Ampicillin, Gentamicin, Cefotaxime, Polymyxin B, Ciprofloxacin, Ceftriaxone and Ofloxacin while it was sensitive to Amikacin, Tobramycin, Ceftazidime, Imipenem, Meropenem and Levofloxacin. Sample 20.1 was resistant to Ampicillin, Polymyxin B, Tobramycin, Ciprofloxacin, Ceftazidime, and Ceftriaxone. While it was sensitive to Gentamicin, Amikacin, Cefotaxime, Imipenem, Meropenem, levofloxacin and Ofloxacin. Sample 20.2 was resistant to Ampicillin, Gentamicin, Cefotaxime, Polymyxin B, Ciprofloxacin, Ceftriaxone, Levofloxacin and Ofloxacin. While it was sensitive to Amikacin, Tobramycin, Ceftazidime, Imipenem and Meropenem.

Limitations

1. Current study had a small sample size due to deficiency of resources and time. More accurate results can be obtained if the sample size is kept larger.
2. Samples were collected only from district Peshawar Pakistan, future studies can be conducted in other cities of Pakistan.
3. No study was conducted at molecular level.

Recommendations

1. Large sample size for future studies.
2. Molecular level study could help us to evaluate those genes which are making the bacteria resistant.

6. References

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