



## **Antibiotic Resistance Pattern of Some Pathogens Isolated From Clinical Samples**

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### **ABSTRACT**

*At present scenario, the multi drug resistant microorganisms (MDRO's) causes various life threatening infections which even leads to death of an infected individual. A broad range of microorganisms, such as Staphylococcus aureus and Pseudomonas aeruginosa as well as Enterobacteriaceae like Escherichia coli and Klebsiella pneumoniae were highly resistant to commonly used antibiotics. Resistance towards antimicrobial agents is increasing gradually, with reports of multi drug resistant isolates. The present study was conducted to determine the resistant pattern among the isolates of Escherichia coli and Klebsiella pneumoniae. In present investigation, total 87 urine samples were screened on different bacteriological media. Out of total, 66.67% samples were found positive as well as the ratio of female to male was found higher. Total 116 isolates of E. coli and 62 isolates of K. pneumoniae were found. Antimicrobial susceptibility testing was done as per the method suggested by Kirby-Bauer using disc diffusion technique. Most of the isolates (61.23%) were found resistant when tested against different antibiotics. Out of total resistant isolates, 61.46% isolates belongs to E. coli while remaining were K. pneumoniae. The result of antibiogram showed that, E. coli isolates were highly resistant to cephalothin followed by cefpodoxime, ampicillin, amikacin, amoxycylav, cefepime, ceftriaxone, gentamicin, tetracycline and ciprofloxacin while against cefoperazone/sulbactam and ceftazidime/clavulanic acid less resistance was observed. High resistance among isolates of K. pneumoniae was observed against cefpodoxime, followed by cephalothin, amikacin, ampicillin, amoxycylav, tetracycline, cefepime, ceftriaxone, gentamicin, cefperazone/sulbactam, ciprofloxacin and ceftazidime/clavulanic acid respectively. Imipenem was the most active antibiotic against all isolates of E. coli and K. pneumoniae.*

**Key Words:** *Escherichia coli, Klebsiella pneumoniae, multi drug resistance*

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### **INTRODUCTION**

An antibiotic was considered as a magic bullet when introduced firstly because they combating against different microorganisms. The discovery of antimicrobial agents had such a great impact on the rate of survival of individual from life threatening infections. Due to the over use of these life saving drugs, they lost their efficacy as well as more costly and difficult antibiotics were introduced in the market to treat simpler infections [1]. The antibiotic sensitivity pattern of microorganisms may changes form place to place and time to time therefore in clinical practice present information is much more useful on drug resistance pattern.

Emergence of resistance to different antimicrobial agents has become an important problem when considering the public health. We live in the era of multi drug resistant organisms (MDRO's) which are very much difficult and sometimes even not possible to treat [2]. Recently the problem has become very much crucial because multiple drug resistance occurs gradually in various strains. And the multiple drug resistance (MDR) property is steadily increasing.

Recently, the management of infections in hospital settings is becomes a drastic issue due to the emergence of resistant microorganisms to different antimicrobial drugs [3]. So, the aim of this study was design as such to determine the prevalence and antibiotic resistance pattern of *Escherichia coli* and *Klebsiella pneumoniae* isolated from clinical samples such as urine collected from pathological laboratories.

### **MATERIALS AND METHODS**

Collection of urine specimens

In the present study, total 87 specimens of urine were collected from different pathological laboratories of Amravati city, Maharashtra and all these specimens were analyzed in laboratory [4].

#### Method of Collection:

Aseptically, collected freshly voided midstream urine specimens in a sterile clean catch container. After collection of urine specimens, it will immediately transport to the laboratory in sterile Luria broth (LB) within 2 hours of collection for further processing. Incubate these LB broth tubes overnight at 37°C [5]. When collecting the urine specimen care should be taken to avoid infection due to positive urine specimens.

#### Isolation and Identification of bacteria:

From these incubated LB broth tubes, a calibrated loop delivering 0.01ml of the sample was inoculated on Mackonkey agar, nutrient agar, Cystein Lactose Electrolyte Deficient (CLED) agar and Eosin Methylene Blue (EMB) agar plates. Then, the plates were incubated at 37°C for 24 hrs. All the bacterial isolates were identified by cultural, morphological and biochemical characteristics followed by standard conventional methods [6].

#### Antibiotic Susceptibility Testing:

The pattern of antibiotic resistance among *E. coli* and *K. pneumoniae* isolates were performed as per method suggested by Bauer-Kirby [7] i.e., disc diffusion method using Mueller-Hinton agar plates. For this, take a full loop of cultured bacteria and inoculated into 2-3 ml of nutrient broth tubes and tubes were incubated at 37°C for 4-6 hrs. After incubation period is over, the tubes were taken out from the incubator and allow it steady for 5 min and after that by using the sterile cotton swab; the cultured organisms were inoculated on to the entire surface of Mueller-Hinton agar plates. The antibiotic discs were placed at a specific and proper distance in between them and the plates were incubated overnight at 37°C. The antibiotic disc were used in the present study, Amikacin (30 µg), Amoxyclav (30 µg), Ampicillin (20 µg), Cefepime (30 µg), Cefodoxime (30 µg), Cefperazone/sulbactam (20/10 µg), Cephalothin (30 µg), Ceftazidime/clavulanic acid (20/10 µg), Ceftriaxone (30 µg), Ciprofloxacin (5 µg), Gentamycin (10 µg), Imipenem (10 µg), Tetracycline (10 µg). All antibiotics were obtained from Hi Media laboratory, Mumbai, India. Quality control strain of *Escherichia coli* and *Klebsiella pneumoniae* were used in the study.

## RESULTS AND DISCUSSION

Total 87 urine specimens were collected from different pathological laboratories of Amravati city, Maharashtra. Majority of specimens were collected from female (60.91%) rather than from male (39.08%). Out of these 87 collected specimens, 58 urine specimens were found positive while remaining samples are sterile. The ratio of positive samples from female to male was higher. There are 63.79% (37) positive specimens were collected from female while 36.20% (21) were from male. Out of these 58 positive samples, 178 Gram negative bacterial isolates such as *Escherichia coli* and *Klebsiella pneumoniae* were recovered and detected on the basis of cultural, morphological and biochemical characteristics.

Out of total 178 isolates, 116 isolates were *Escherichia coli* while 62 isolates were belong to *Klebsiella pneumoniae*. From the present study it was observed that, *E. coli* which accounts for 65.16% and was the commonest bacteria isolated from urine specimen followed by *K. pneumoniae* 34.83%. Fig.1 depicts the prevalence of *E. coli* and *K. pneumoniae* isolated from urine samples.

Sr. No.	Bacteria	No. of bacterial isolate	% of bacterial isolate
1	<i>E. coli</i>	116	65.16 %
2	<i>K. pneumoniae</i>	62	34.83 %
	Total	178	-

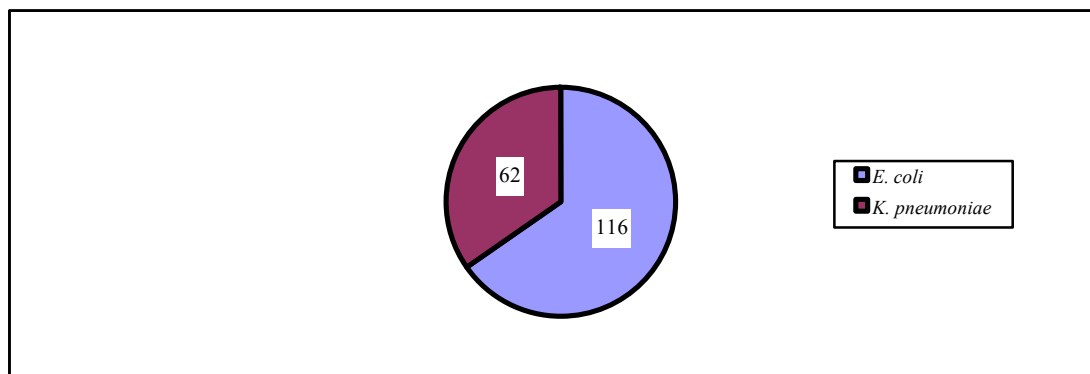


Figure 1. Prevalence of *E. coli* and *K. pneumoniae* isolated from urine specimens

Gender	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>
Male (%)	27 (23.27%)	16 (25.80%)
Female (%)	89 (76.72%)	46 (74.19%)
Total	116	62

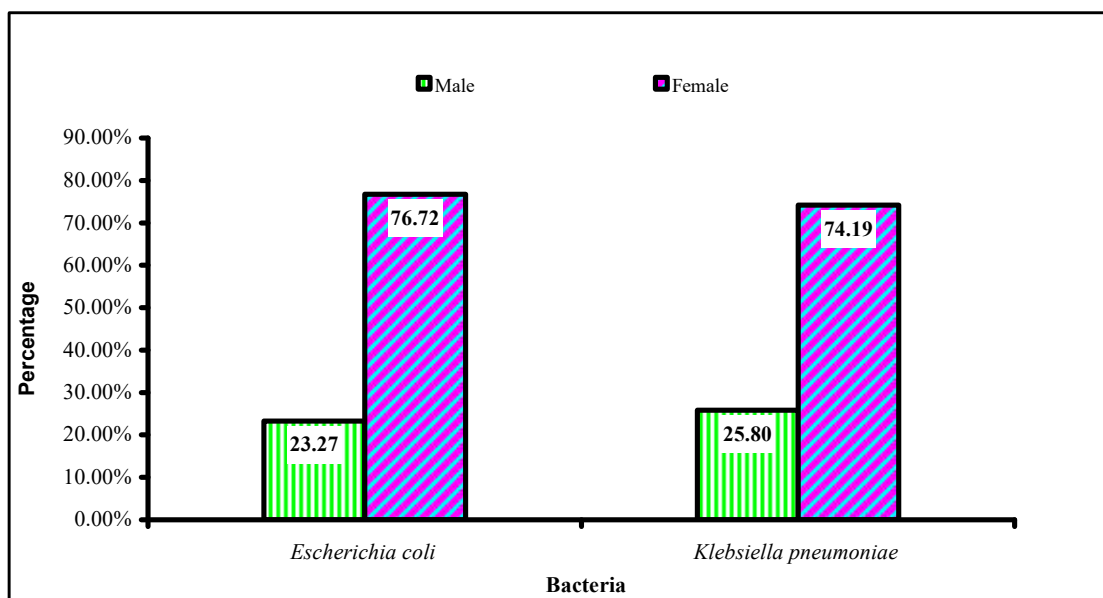


Figure 2. Gender wise percent distribution of *E. coli* and *K. pneumoniae*

The antibiogram of isolates of *E. coli* and *K. pneumoniae* was carried out according to the Bauer-Kirby method [7]. Disc diffusion method was used to determine the rate of resistance towards 13 different antibiotics. Interpretation of result was done on the basis of zone of inhibition.

All 178 isolates were tested against a panel of 13 different antibiotics and the result showed that, 61.23% (109/178) isolates were found to be resistant. Out of total 109 resistant isolates, 61.46% (67/109) isolates were belongs to *E. coli* while 38.53% (42/109) isolates were *K. pneumoniae*.

Sr. No.	Antibiotics	No. of resistant isolates	% of resistant isolates (n=67)
1	Amikacin	52	77.61%
2	Amoxyclav	47	70.14%
3	Ampicillin	56	83.58%
4	Cefepime	42	62.68%
5	Cefodoxime	62	92.53%
6	CFS	29	43.28%

7	Cephalothin	63	94.02%
8	CAC	26	38.80%
9	Ceftriaxone	39	58.20%
10	Ciprofloxacin	32	47.76%
11	Gentamicin	36	53.73%
12	Imipenem	00	00.00%
13	Tetracycline	35	52.23%

Note: CFS:- Cefperazone/sulbactam, CAC:- Ceftazidime/clavulanic acid

Based on the result of antibiogram it was observed that, majority of *E. coli* isolates were found resistant to some family members of drug cephalosporin i.e., cephalothin (63), cefpodoxime (62), cefepime (42) and ceftriaxone (39) also high resistance was observed towards ampicillin (56), amikacin (52), amoxycylav (47), gentamicin (36), tetracycline (35) and ciprofloxacin (32). While less number of isolates were found resistant towards ceferazone/sulbactam (29) and ceftazidime/clavulanic acid (26). The reason behind this property is the presence of extended spectrum  $\beta$ -lactamase (ESBL) inhibitor. The study revealed that, *E. coli* isolates were highly resistant

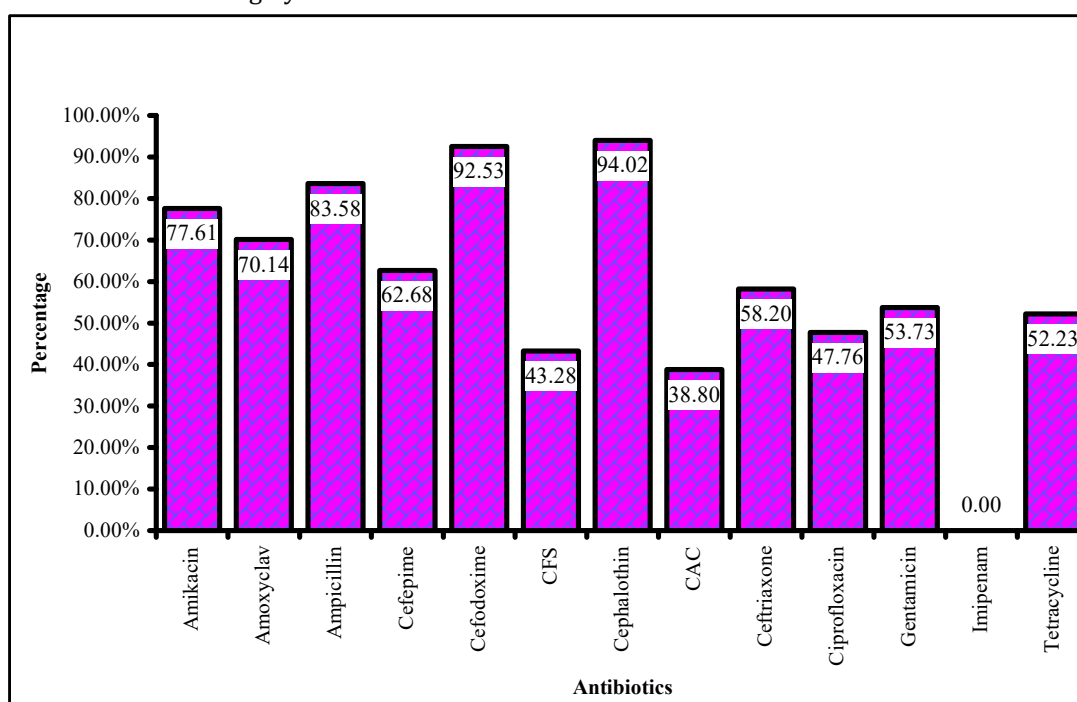
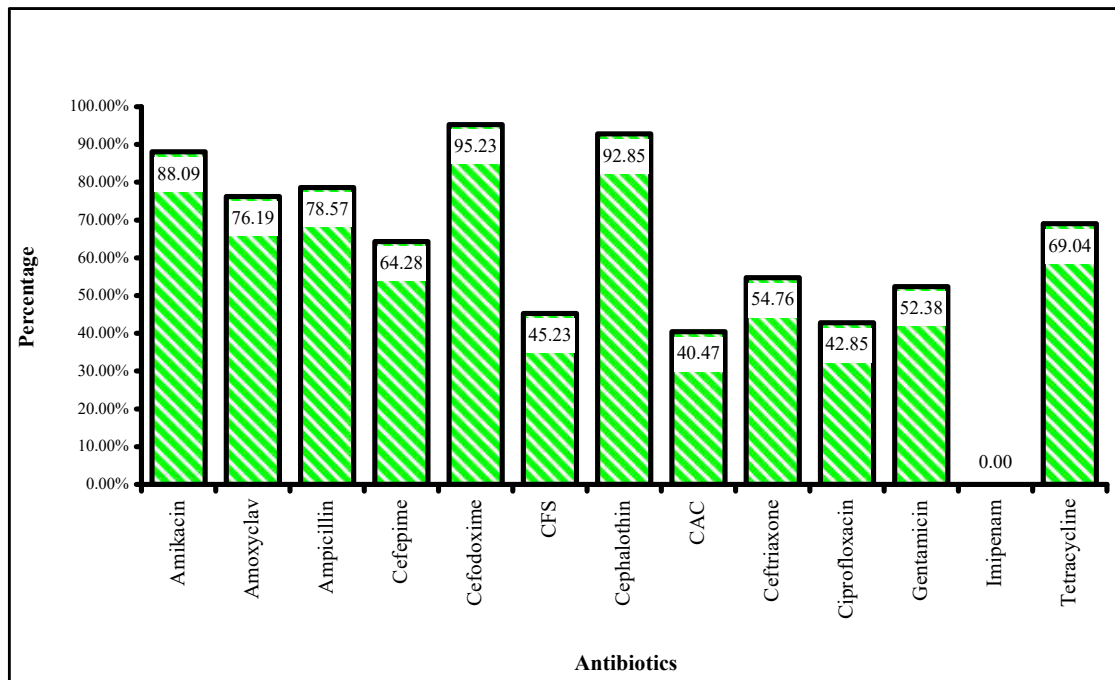


Figure 3. Resistance pattern of *E. coli* isolates tested against 13 different antibiotics

Sr. No.	Antibiotics	No. of resistant isolates	% of resistant isolates (n=42)
1	Amikacin	37	88.09%
2	Amoxycylav	32	76.19%
3	Ampicillin	33	78.57%
4	Cefepime	27	64.28%
5	Cefodoxime	40	95.23%
6	CFS	19	45.23%
7	Cephalothin	39	92.85%
8	CAC	17	40.47%
9	Ceftriaxone	23	54.76%
10	Ciprofloxacin	18	42.85%
11	Gentamicin	22	52.38%
12	Imipenam	00	00.00%
13	Tetracycline	29	69.04%

Note: CFS:- Cefperazone/sulbactam, CAC:- Ceftazidime/clavulanic acid



**Figure 4. Resistance pattern of *K. pneumoniae* isolates tested against 13 different antibiotics**

According to results, in case of *K. pneumoniae*, high resistance rate was observed against cefpodoxime (40), cephalothin (39), amikacin (37), ampicillin (33), amoxyclav (32), tetracycline (29), cefepime (27), ceftriaxone (23), gentamicin (22) as well as some less resistant isolates was observed against ceftazidime/clavulanic acid (17) respectively.

There was only one antibiotic which was efficient against both resistant isolates i.e., imipenem. All isolates of *E. coli* and *K. pneumoniae* was found 100% susceptible to imipenem and hence in present it is a drug of choice but in near future due to the over use of this drug there might be the emergence of resistance to this drug as well. During the study, it was observed that some isolates of *E. coli* and *K. pneumoniae* showed the zone size which is in the range of intermediate towards imipenem, hence in near future these isolates can emerge resistance towards the imipenem.

## DISCUSSION

The present study showed that *E. coli* and *K. pneumoniae* were the commonest pathogen isolated from urine specimens. The present data showed that strains of *E. coli* stand for 65.16% while strains of *K. pneumoniae* represent 34.83% which was less in number as compared to *E. coli*. Similar study was carried out by some other researchers around the world [4, 8, 9 and 10].

Majority of isolates were recovered from specimens collected from females rather than from males. Similar study was carried out by [4]. Study carried out by [11 and 12] reported that *E. coli* and *K. pneumoniae* was frequently found in urine samples.

This study demonstrated the resistance pattern among the isolates of *E. coli* and *K. pneumoniae* recovered from urine cultures. For this, urine specimens were collected from different pathological laboratories of Amravati city, India. The study showed that, majority of organisms (75.84%) was isolated from samples collected from females. This study correlates with earlier study carried out by [13].

Our data confirmed that *E. coli* and *K. pneumoniae* isolates are highly resistant to ampicillin (83.58% and 73.57%) respectively. These findings support previous findings of [14, 15 and 16] as they reported that, these isolates being more resistant to ampicillin. Present study revealed that, high rate of resistance was observed among both isolates against amikacin and gentamicin. Lower resistance rate or dissimilar results towards amikacin and gentamicin among both isolates were reported by [4]. In case of gentamicin, both isolates were found very highly resistant [17].

In the present study, strains of *E. coli* and *K. pneumoniae* found tremendously resistance towards amoxyclav. Dissimilar results were observed by [18]. From the study it was observed that, cefpodoxime and cephalothin was not as such effective drug against these isolates. As compared to these drugs slightly lower resistance was seen against cefepime. In case of ceftazidime/clavulanic acid much lower resistance was seen because there was a combination of sulbactam and clavulanic acid i.e., a  $\beta$ -lactamase inhibitor. Our data demonstrated that near about 45% of both genera were resistant to

ciprofloxacin while most of the isolates were also resistant to tetracycline and ceftriaxone. Similar findings were reported by [4].

In our study, none of the single isolate was found resistant to imipenem i.e., all isolates were highly susceptible. Near about similar results were reported by [4], all isolates of *E. coli* and *K. pneumoniae* were susceptible to imipenem when studied. Some other researches also reported similar findings when both isolates tested against imipenem [19, 20 and 21].

## CONCLUSION

From the present study it was concluded that, all isolates were resistant to most of the antibiotics used. The antibiotics like ceftazidime/sulbactam and ceftazidime/clavulanic acid possesses the slightly higher efficacy when compared to others. Among the other antibiotics, imipenem was highly efficient drug against these isolates. Despite this efficiency, there was a general increase in the resistance pattern of isolates towards commonly used antibiotics and would be a great trouble to treat the bacterial infection. Now a day's, from the study it was seen that *E. coli* and *K. pneumoniae* isolates were becoming less susceptible to aminoglycosides, cephalosporins and other  $\beta$ -lactam antibiotics. To avoid the burden of resistant bacteria in community, it is significantly important to have stringent antibiotic policies as well as surveillance programmes for multi drug resistant organisms and infection control procedures are need to be implemented regularly. Routine monitoring of antibiotic sensitivity pattern of bacteria such as *E. coli* and *K. pneumoniae* is very much useful and the findings of the study willingly made accessible to the clinicians and medical practioners so as to treat bacterial infection by prescribing alternative drug using the data.

Attention should be triggered and the solutions designed by constant hard work by clinician, microbiologist, pharmacist, medical practioners and community to greatly endorse this overcoming serious situation. Along with, regular hand washing is the easiest way to minimize the burden and spread of resistant organism should be encouraged.

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