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STUDY OF ANTIBIOTIC RESISTANCE PATTERN AND VIRULENCE FACTORS OF ISOLATED UROPATHOGENS

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INTRODUCTION:

Urinary tract infection (UTI) implies multiplication of pathogen in the urinary tract and presence of more than 10^5 organisms/ml of midstream of urine sample. UTI occurs due to entry of bacteria in the urinary tract via urethra to bladder and then multiplication in the tract. The most common cause of urinary tract infection is Gram negative bacteria that belong to the family Enterobacteriacae. Members of this family include *Escherichia coli, Klebsiella, Pseudomonas, Enterobacter* and *Proteus.* Other causative agents of UTI are Gram positive organisms such as *Staphylococcus spp, Streptococcus spp* and yeast like *Candida.* Urinary tract is among the most common sites of bacterial infection both in community based and hospitalized patients **[5].** Despite rapid diagnosis and treatment there is reported 5% risk of long term damage owing to recurrence and consequent renal scarring with its complications. *E. coli* is the most common cause of urinary tract infection.

Despite the consistent spectrum of causative organisms, their resistance profiles have evolved significantly, posing challenges in treatment and management.

Abnormal urinary tract Eg: obstruction, calculi, vesico-ureteric reflux, neurological abnormality, in-dwelling catheter, chronic prostatitis, cystic kidney, analgesic nephropathy, renal scarring, Impaired renal function [**10].** Associated disorder which impairs defense mechanism (Eg: Diabetes mellitus, Immunosuppressive therapy). A considerable amount of risk factors such as hospitalization, underlying medical diseases, recent use of antibiotics, diabetes, and immune deficiency, indwelling catheter, urinary obstruction and congenital abnormalities will lead to this conflicting problem. Naturally, age, gender, general and sexual hygiene are other contributors to the case of uncomplicated UTI which occurs more commonly in young sexually active women.

Women are more prone to UTI than men because, in females, the urethra is much shorter and closer to the anus [**16].** As a women's estrogen levels decrease with menopause her risk of urinary tract infections increase due to the loss of protective vaginal flora.

It is estimated that about 35% of healthy women suffer symptoms of UTI at some stages in their life. The incidence of UTI is greater in women as compared to men which may be either due to anatomical predisposition or urothelial mucosal adherence to the mucopolyssacharide lining or other host factors. Women have a shorter urethra than men. In male the secretions from the prostate gland provide a better barrier against this type of infection [9, 15, **25].** Bacteria enter the tract via urethra to bladder and multiply in the urinary tract. Bacteria can easily migrate across the perineum to the urethra. Bacterial invasion can result in acute cystitis, the most common type of UTI. A more rare condition is Urethritis, a condition in which only the urethra is inflamed. When bacteria from the bladder ascend to the kidneys via the ureter, they can cause a more serious infection called Pyelonephritis.

Demonstration of virulence factors

The pathogenic potential of Uropathogens is thought to be dependent on the presence of virulence factors (VF's). Urovirulence factors of uropathogens analysed by molecular methods are useful markers for detection of uropathogenic strains. Some virulence factors such as capsule, motility, heamagglutination, cell surface hydrophobicity, urea hydrolysis, afimbrial adhesion, serum resistance, attachment to uroepithelial cells, biofilm formation play an important role in the pathogenicity of uropathogens by overcoming host defense mechanisms to cause disease. These virulence factors are located on large plasmids and/or in particular regions, called "Pathogenecity islands" (PAI's), on the chromosome [2, 7, **31].**

Recent studies have highlighted the interplay between virulence and antibiotic resistance. For instance, a study demonstrated that uropathogenic *E. coli* isolates from kidney transplant recipients exhibited higher resistance rates and possessed multiple virulence factors, including fimH and PAI genes 41.

Antibiotic sensitivity pattern

Antibiotic resistance among uropathogens has been escalating, complicating treatment strategies. Recent surveillance data indicate high resistance rates, with *E. coli* exhibiting over 50% resistance to several antibiotics, and rates exceeding 80% for ampicillin, nalidixic acid, and piperacillin. Aminoglycosides (amikacin, netilmicin) and carbapenems (imipenem) retain relatively lower resistance rates, making them valuable options in treatment regimens. Mechanisms of resistance include enzymatic inactivation (β-lactamases), target site modification, and efflux pumps. A study at Mbarara Regional Referral Hospital reported that only 7.5% of *E. coli* isolates were fully susceptible to all antibiotics tested, with the majority exhibiting resistance to at least three antibiotics [28, 42]. With the associated economic burden and patient morbidity, UTIs contribute to the inappropriate and excessive use of antimicrobial agents and lead to the development of antibiotic-resistant organisms, thereby creating a potential reservoir of resistant pathogens. Despite the wide availability of clinically useful antibiotics and semisynthetic analogues, a continuing search for new anti-infective agents remains indispensable because some of the major antibacterial agents have considerable drawbacks in terms of limited antimicrobial spectrum or serious side effects [27, **43].**

Bacterial mechanisms of antibiotic resistance

Several mechanisms have evolved in bacteria which confer them with antibiotic resistance. These mechanisms can chemically modify the antibiotic, render it inactive through physical removal from the cell, or modify target site so that it is not recognized by the antibiotic (Todar's, 2004). The most common mode is enzymatic inactivation of the antibiotic. An existing cellular enzyme is modified to react with the antibiotic in such a way that it no longer affects the microorganisms. An alternative strategy utilized by many bacteria is the alteration of the antibiotic target site. Reduced uptake and active efflux of antibiotic from the cell is also seen as antibiotic resistance. These and other mechanisms are shown in the figure and accompanying table below.

Antibiotic	Method of resistance
Chloramphenicol	reduced uptake into cell
Tetracycline	active efflux from the cell
β-lactams, Eryth omycin, Lincomycin	eliminates or reduces binding of antibiotic to cell target
β-lactams, Aminoglycosides, Chloramphenicol	enzymatic cleavage or modification to inactivate antibiotic molecule
Sulfonamides, Trimethoprim	metabolic bypass of inhibited reaction
Sulfonamides, Trimethoprim	overproduction of antibiotic target (titration)

MATERIALS AND METHODS

1. Isolation and identification:

Isolation:

Twenty-five isolates which cause Urinary Tract Infection (UTI) were collected from Joshi Hospital, Pune. These clinical isolates were isolated by streaking on MacConkey's agar (MA) and Luria agar (LA).

Identification:

These isolates were biochemically characterized by performing various types of tests such as IMViC (Indole, Methyl red, Voges –Proskauer, Citrate utilization test), Triple Sugar Iron (TSI) test, Oxidase test, Sugar fermentation test, Urease production test, Nitrate reduction test, and growth on 1% dettol agar. Biochemical characterization and identification of these isolates was done on the basis of Bergey's Manual of Determinative Bacteriology (9^{th} edition).

2. Determination of Virulence Factors:

a. Motility:

Motility was checked by conventional Hanging Drop technique.

b. Cell Surface Hydrophobicity:

Cell-surface Hydrophobicity was determined by the Salt Aggregation Test. Suspensions of isolates were made in 0.2 M phosphate buffer saline, (PBS pH 6.8) after growing on MacConkey's agar (MA) medium and cell density was adjusted to $O.D_{540}$ = 0.3. Suspensions were mixed with different concentrations of ammonium sulphate (1M/1.4M/2M) solutions for checking their hydrophobic property.

40µl of 0.2M PBS (pH-6.8) was added to 3 cavity slides.

40µl 1M/ 1.4M/ 2M concentrations of ammonium sulphate were added to these cavity slides. 40µl of suspension of isolates was added to each slide.

The clumps formed in different concentrations of Ammonium Sulphate were observed under binocular microscope at 45X magnification. Isolates were considered positive if they were able to aggregate in 1.4M concentration of Ammonium Sulphate.

3. Urea Hydrolysis:

This test was carried out on Christensen's Urea agar. Suspension of freshly grown isolates was made. These suspensions were stabbed and streaked on Christensen's Urea agar butts and slants. These tubes were incubated at 37^0 C for 24 hrs. Then tubes were observed for the colour change.

4. Antibiotic Sensitivity Pattern:

The antibiotic sensitivity pattern of these isolates was determined by using 12 different antibiotics (obtained from Dynamicro Ltd.) (Which are Amikacin, Ofloxacin, Gentamicin, Norfloxacin, cefaclor, Ciprofloxacin, Nitrofurantoin, Cefoperazone, Nalidixic acid, Cefuroxime, Cefadroxil, and Netilmicin on polydiscs). This test was performed according to CLSI (Clinical and Laboratory Standards Institute) guidelines. The test was carried out on Mueller Hinton (MH) agar. The isolates were accordingly classified as Sensitive, Intermediate or Resistant prepared and diluted to 5%, 10 %, 15%, 20%, and 25% w/v in Luria broth (LB).

RESULTS AND DISCUSSION:

1. Prevalence

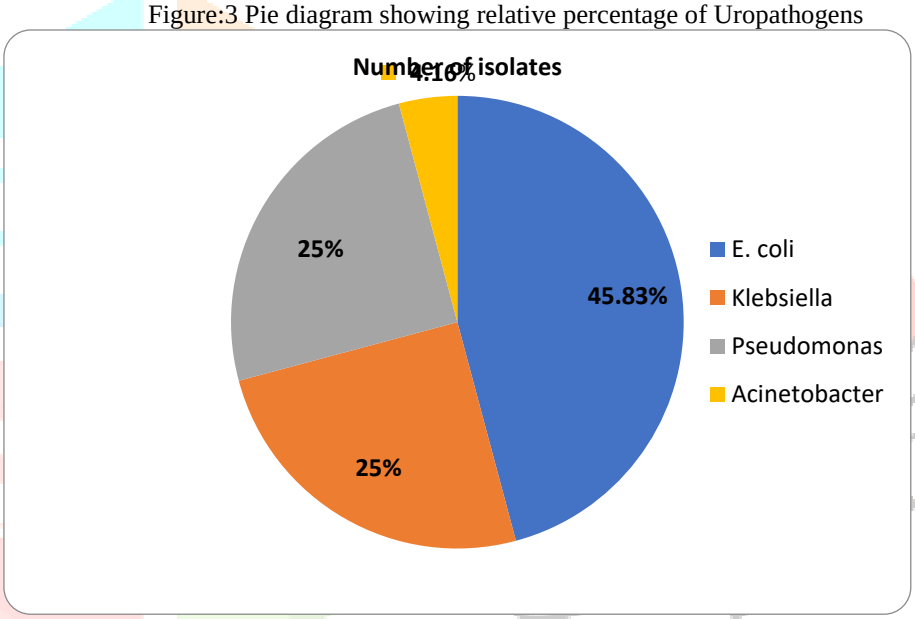
Bacterial pathogens causing Urinary tract Infection:

Total 24 clinical isolates were obtained from Joshi Hospital, Pune and were found to belong to the following genera:

Table:5 Genus wise distribution of Uropathogens

Genus	Number of isolates	Relative percentage
E. coli	11	45.83%
Klebsiella	6	25%
Pseudomonas	6	25%
Acinetobacter	1	4.16%
Total	24	100%

Figure:3 Pie diagram showing relative percentage of Uropathogens



Note: The total number of isolates is very less for the statistical analysis but in order to get an overview the calculations have been done.

Inference:

Among the total (24) clinical isolates obtained from Joshi hospital *E-coli* was in higher percentage (45.83%).

Identification of uropathogens:

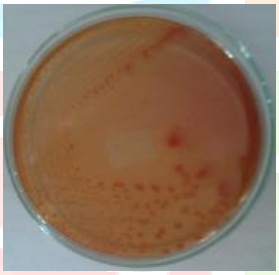
Uropathogens were identified biochemically and characterized.

Table: 3 List of identified uropathogens

Key: I : Indole test; VP : Voges Proskeur test; MR : Methyl Red; C: Citrate test; + : Positive test; - : Negative test

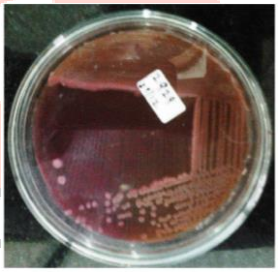
Genus	Colony morphology on MacConkey's agar	Gram character	Motility	Biochemical characteristics			
				I	MR	VP	C
Escherichia coli	Lactose fermenting, Pink color colonies with serrated margin. Some were mucoid and circular with smooth margin.	Gram negative Short rods.	All strains motile.	+	+	-	-
Klebsiella	Lactose fermenting, pink color, mucoid and smooth circular colonies	Gram negative Short rods.	Non-motile.	-	-	+	+
Proteus	Lactose non-fermenting, colorless colonies with swarming growth.	Gram negative Short rods.	Motile	Glucose and xylose were fermented with acid and gas production. Gelatinase test positive, Citrate test positive.			
Pseudomonas	Lactose non fermenting colonies (on MA) Some with bluish –green diffusible pigment and some with brown pigment on Luria Bertani agar plate.	Gram negative Short rods.	Motile	Oxidase test positive, Gelatinase test positive, Growth on 1% dettol agar plate. Growth on 1% dettol agar plate.			

Figure: 4 Isolation of Uropathogens on MacConkey's agar



JH- 7 Pseudomonas

JH-1 Klebsiella



JH-21 *E.coli*

2. Virulence factors:

a) Motility:

Inference:

All the uropathogens *(E.coli* and *Pseudomonas*) were found to be motile except the *Klebsiella* isolates.

b) Cell surface hydrophobicity:

Cultures were positive which showed clumping in 1.4M ammonium sulphate showing presence of cell surface hydrophobicity. *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were taken as controls and were found to be negative for cell surface hydrophobicity test.

Table: 5 Cell surface hydrophobicity test of uropathogens

Key: "-" indicates no formation of clumps + and ++ indicates formation of clumps.

Isolate	1M Ammonium sulphate	1.4M Ammonium sulphate	2M Ammonium sulphate
JH-1 *(Klebsiella)*	+	+	+
JH-5 *(Klebsiella)*	+	+	+
JH -25 *(Klebsiella)*	+	+	+
JH-2 *(Pseudomonas)*	++	+	+
JH-7 *(Pseudomonas)*	+	+	+
JH-10 *(Pseudomonas)*	+	+	+

JH -17 *(Pseudomonas)*	++	-	-
JH-3 *(E. coli)*	-	-	-
JH-4 *(E. coli)*	+	+	-
JH-14 *(E. coli)*	-	-	-
JH -16 *(E. coli)*	+	+	-
JH -21 *(E. coli)*	+	+	+
JH -23 *(E. coli)*	+	+	+
JH -24 *(E. coli)*	+	+	+
E. coli ATCC 25922	-	-	-
P. aeruginosa ATCC 27853	-	-	-

Inference:

Out of 14 clinical isolates tested, 12 clinical isolates (72%) were positive for cell surface hydrophobicity test while JH-3 *E. coli* and JH-14 *E. coli* showed negative cell surface hydrophobicity test.

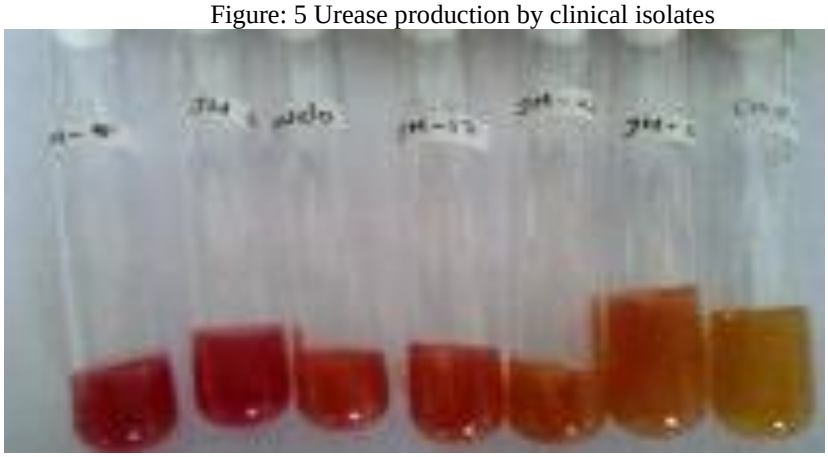
c) Urease production:

Table: 6 Urease production of uropathogens

Key**:** '+' indicates slight color change; '++' indicates intermediate color change; '+++' indicates relatively intense color change.

Isolate	**Urease production (change in color in Christensen's urea agar)**
JH-1 *(Klebsiella)*	+++
JH-5 *(Klebsiella)*	+++
JH -25 *(Klebsiella)*	+++
JH-2 *(Pseudomonas)*	++
JH-7 *(Pseudomonas)*	++
JH-10 *(Pseudomonas)*	++
JH -17 *(Pseudomonas)*	++
JH-3 *(E. coli)*	+
JH-4 *(E. coli)*	+
JH-14 *(E. coli)*	+
JH -16 *(E. coli)*	+
JH -21 *(E. coli)*	+
JH -23 *(E. coli)*	+
JH -24 *(E. coli)*	+

Figure: 5 Urease production by clinical isolates



Inference:

All the isolates of *Klebsiella* (JH-1, JH-5 and JH-25) showed significantly intense color change while *Pseudomonas* (JH-2, JH-7, JH-10, JH-17) showed intermediate color change. All the *E.coli* (JH-3, JH-4, JH-14, JH-16, JH-21, JH-23, JH-24) isolates showed slightly color change.

2. Antibiotic sensitivity pattern:

a) Multi disc rings UTI (Dynamicro labs Pvt. Ltd):

Table: 7 Standard antibiotic sensitivity table

ANTI-MICROBIAL	DISC CONTENT	ZONE DIAMETER (in mm)		
		RESISTANT	INTERMEDIATE	SUSCEPTIBLE
Amikacin (AN)	30mcg	≤ 14	15-16	≥ 17
Ofloxacin (OX)	5mcg	≤ 12	13-15	≥ 16
Gentamycin (G)	10mcg	≤ 12	13-14	≥ 15
Norfloxacin (NR)	10mcg	≤ 12	13-16	≥ 17
Cefaclor (CFC)	30mcg	≤ 14	15-17	≥ 18
Ciprofloxacin (CIP)	5mcg	≤ 15	16-20	≥ 21
Nitrofuantoin (NF)	300mcg	≤ 14	15-16	≥ 17
Cefoperazone (CFP)	75mcg	≤ 15	16-20	≥ 21
Nalidixic acid (NA)	30mcg	≤ 13	14-18	≥ 19
Cefuroxime(CR)	30mcg	≤ 14	15-17	≥ 18
Cefadroxil (CD)	30mcg	≤ 14	15-17	≥ 18
Netilmicin (NET)	30mcg	≤ 12	13-14	≥ 15

Table: 8 Antibiotic sensitivity patterns of uropathogens

Key: R =indicates resistant isolates; I =indicates intermediates isolates; S= indicates sensitive isolates

Antibiotics / **Cultures**	**AN**	**OX**	**G**	**NR**	**CFC**	**CIP**	**NF**	**CFP**	**NA**	**CR**	**CD**	**NET**
E. coli												
JH-3	S	S	R	S	R	R	S	R	R	R	R	S
JH-4	R	R	S	R	R	R	S	R	S	R	R	S
JH-14	S	R	R	R	R	R	S	R	R	R	R	S
JH-21	R	R	S	R	I	R	S	S	R	R	S	S
JH-23	R	R	R	R	R	R	S	R	R	R	R	R
JH-24	I	I	S	R	S	R	S	S	R	I	S	S
JH-28	S	R	R	R	R	R	R	R	R	R	R	R
JH-30	S	R	S	R	R	R	I	S	R	S	R	S
JH-32	S	R	R	R	R	R	S	R	R	R	R	R
E. coli ATCC 25922	S	S	S	I	R	S	S	S	I	S	I	S
Klebsiella												
JH-1	I	S	R	R	R	R	I	R	R	R	R	S
JH-5	S	R	R	R	R	R	R	I	R	R	R	S
JH-25	R	R	R	R	R	R	S	R	R	R	R	R
JH-26	S	S	S	S	R	S	R	S	R	R	R	S
JH-29	S	S	S	S	R	S	R	S	R	S	R	S
JH-31	S	S	S	S	R	S	R	S	S	S	R	S
Pseudomonas												
	R	R	R	R	R	R	R	R	R	R	R	R
	S	S	S	S	R	S	R	I	R	R	R	S
	S	S	S	S	R	S	R	S	R	R	R	S
	R	I	S	R	R	R	S	R	R	R	R	S

	S	S	S	I	I	S	R	S	R	R	R	S
	S	S	I	S	I	S	R	S	R	R	R	S

Inference:

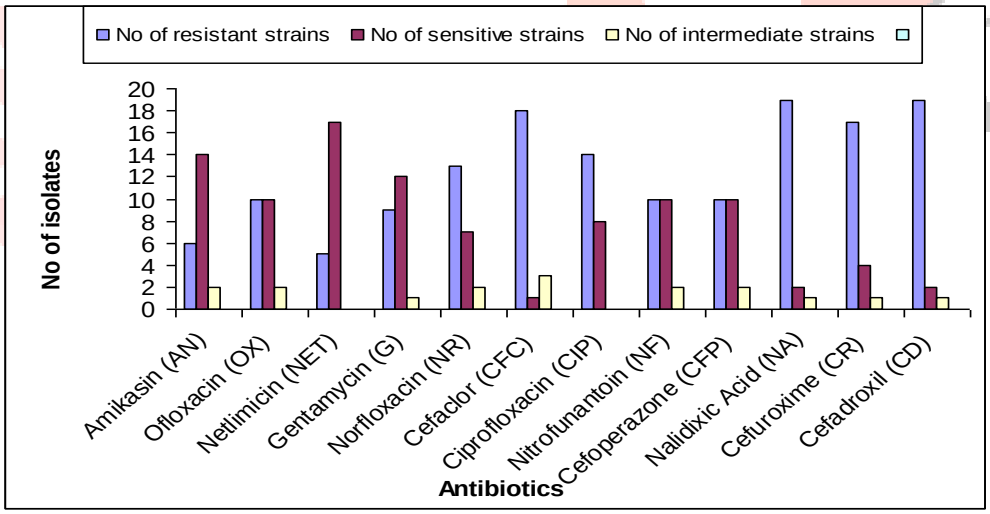
Out of 9 isolates of *E.coli* JH-23 was resistant to 11 antibiotics while amongst the 5 isolates of *Pseudomonas* JH-2 was resistant to all (12) antibiotics.

In case of *Klebsiella* isolates, JH-25 was resistant to 11 antibiotics.

Table: 9 Percentage of sensitive and resistant strains of Uropathogens

Antibiotic	**No of resistant strains**	**No of intermediate strains**	**% resistant strains**	**% of sensitive strains**
Amikasin (AN)	6	2	42.85%	63.36%
Ofloxacin (OX)	10	2	45.45%	45.45%
Netilimicin (NET)	5	0	22.72%	77.27%
Gentamycin (G)	9	1	40.90%	54.54%
Norfloxacin (NR)	13	2	59.09%	31.81%
Cefaclor (CFC)	18	3	81.81%	4.45%
Ciprofloxacin (CIP)	14	0	63.36%	36.36%
Nitrofunantoin (NF)	10	2	45.45%	45.45%
Cefoperazone (CFP)	10	2	45.45%	45.45%
Nalidixic Acid (NA)	19	1	86.36%	9.09%
Cefuroxime (CR)	17	1	77.27%	18.18
Cefadroxil (CD)	19	1	86.36%	9.09%

Figure: 6 Number of resistant, sensitive and intermediate isolates of Uropathogens

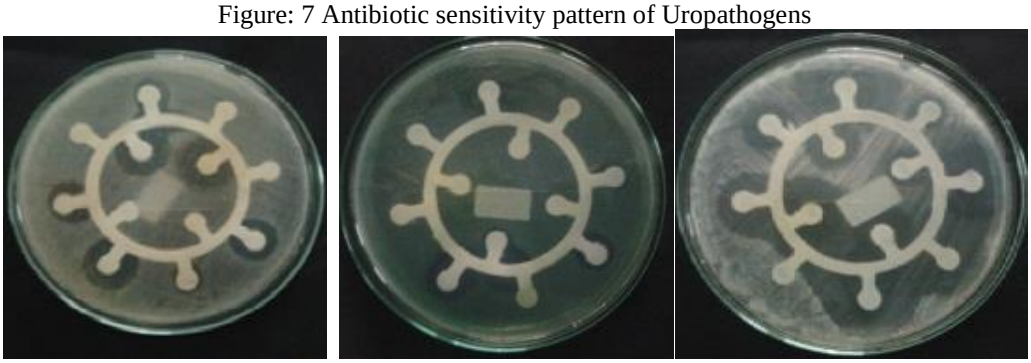


Inference:

Out of 20 clinical isolates tested, 86.36% of clinical isolates were resistant to Nalidixic acid and Cefadroxil.

Out of 20 clinical isolates tested, 77.27% of clinical isolates were sensitive to Netilmicin and 63.36% were sensitive to Amikacin.

Figure: 7 Antibiotic sensitivity pattern of Uropathogens



JH-5 *Klebsiella* ***JH-10 Pseudomonas*** **JH-4 *E. coli***

Table: 10 Percentage of resistant strains of Uropathogens

Name of antibiotic	*E.coli*	*Pseudomonas*	*Klebsiella*
Amikasin (AN)	33.33%	40%	16.66%
Ofloxacin (OX)	77.77%	20%	33.33%
Netilmicin (NET)	33.33%	20%	16.66%
Gentamycin (G)	55.55%	20%	50%
Norfloxacin (NR)	88.88%	40%	50%
Cefaclor (CFC)	88.88%	80%	100%
Ciprofloxacin (CIP)	100%	40%	50%
Nitrofunantoin (NF)	11.11%	80%	66.66%
Cefoperazone (CFP)	66.66%	40%	33.33%
Nalidixic Acid (NA)	88.88%	100%	83.33%
Cefuroxime (CR)	77.77%	100%	66.66%
Cefadroxil (CD)	77.77%	100%	100%

Inference:

The isolates of *E.coli* were 100% resistant to Ciprofloxacin (CIP) while isolates of *Klebsiella* were 100% resistant to Cefaclor (CFC) and Cefadroxil (CD) while the isolates of *Pseudomonas*, were 100% resistant to Nalidixic Acid (NA), Cefuroxime (CR), Cefadroxil (CD).

DISCUSSION

Prevalence:

There are three major routes by which uropathogens can invade and spread within the urinary tract, including the ascending, hematogenous and lymphatic pathways (Fig. 1) [36]. The ascending route is the most common pathway of UTI in females. In the initial step of the ascending route, the uropathogen can colonize the periurethral area and migrate up the urethra to colonize the bladder.Escherichia coli were predominant amongst the clinical isolates obtained. In many known references across the world it is mentioned that they predominate upto 60-80% in organism causing UTI. We obtained total 24 (45.83%) isolates of *E. coli* causing UTI. Here due to small number of isolates, the statistical analysis was difficult [8,17,20,25,36]

The next abundant were *Pseudomonas* and *Klebsiella* with the share of 25%. The other isolate obtained was *Acinetobacter.*

Colony Morphology Differences:

Colony morphology is a useful preliminary criterion in differentiating bacterial genera and even isolates within the same species. On **MacConkey's agar**, *E. coli* typically forms **pink colonies** due to lactose fermentation (Madigan et al., 2018). The mucoid appearance observed in isolates JH-14 and JH-3 may be attributed to **exopolysaccharide production**, which enhances biofilm formation and virulence [45]

Klebsiella spp. are well known for their **prominent capsule**, which explains the **highly mucoid and large colonies** observed for isolates JH-5 and JH-29. This capsule contributes to their pathogenic potential and resistance to host immune defenses [46]

Pseudomonas spp. exhibit distinct pigmentation, which can aid in identification. The **bluish-green pigment (pyocyanin)** produced by isolate JH-7 is characteristic of *Pseudomonas aeruginosa*, while the **brown diffusible pigment (pyomelanin)** produced by JH-10 has been associated with oxidative stress protection and persistence in clinical environments [47]

Acinetobacter spp. typically form **small, smooth, circular colonies** on culture media. The pinpoint colonies with entire margins observed in isolate JH-6 are consistent with previous descriptions [48]

Thus, colony morphology differences observed in the isolates reflect **species-specific traits** as well as **strain-level variations** linked to virulence, biofilm formation, and pigment production.

Virulence Factors:

As noted by Morin M.D. Hopkins W.J. (2002), there are certain virulence factors which play a role in pathogenesis. The pathogens of UTI shows a unique pattern of invasion due to their virulence factors. UTI based on distinctive properties of organism is known as virulence factors [10, 19, 29, 32].

Virulence factors of UPEC includes the ability to adhere to uroepithelial cells

Motility:

The motility results provide further insight into the potential pathogenicity of these isolates. Motility, particularly flagella-mediated motility, is an important virulence factor in urinary tract infections (UTIs). It enables bacteria such as *E. coli* and *Pseudomonas* to **ascend the urinary tract**, facilitating colonization of the bladder and kidneys [49]

In contrast, *Klebsiella* spp. are **non-motile** due to the absence of flagella [50]. Their pathogenesis in UTIs relies more heavily on **capsule formation, adhesins, and biofilm production** rather than motility. The observation that all other isolates were motile supports the hypothesis that motility may be a contributing factor to **ascending urinary tract infections** caused by these organisms.

Cell Surface Hydrophobicity:

The perquisite for the bacterial colonization or infection is the adherence of the organism to the host cell. Bacterial adhesion to host cells is also governed by non specific interactions such as cell surface hydrophobicity. Hydrophobicity plays a vital role in the adherence of microbes to a wide variety of surfaces such as skin, soft tissue, wounds and endothelial cells and facilitates biofilm formation due to bacterial adhesion.

The hypothesis that adherence is prerequisite to pathogenecity or colonization (biofilm formation). In our study, The *E.coli* isolates (JH-23, JH-4) and *Klebsiella* isolates (JH-25 and JH-28) were highly mucoid. These may help to escape host defense mechanisms or pathogenesis. The mucoid nature is also helpful in gaining resistance to various antibiotics. Due to this mucoid nature there is restriction to entry of antibiotics inside the cell and thus multi drug resistant. Cell surface Hydrophobicity was tested by using different concentrations (1M/1.4M/ 2M) of Ammonium sulphate. They were considered hydrophobic as they formed clumps at a 1.4M concentration of ammonium sulphate. Out of 14 isolates tested, 12 isolates were showed cell surface Hydrophobicity. JH -3 *E.coli* and JH-14 *E.coli* showed negative cell surface Hydrophobicity.

Urease production:

Urease production was tested in 14 isolates on Christensen's urea agar. All 14 isolates of uropathogens were showed urease production. The *Klebsiella* isolates (Jh-1, JH-5 and JH-25) showed intense red color formation. *Pseudomonas* isolates (JH-2, JH-7, JH-10 and JH-17) were showed intermediate color change.

In Bergey's manual of determinative Bacteriology (9th edition) it is mentioned that the genus *E.coli* does not produce urease but here the isolates of *E.coli* were produced it. They showed slight colour change in Christensen's urea agar. The urease production may lead to renal calculi composed of struvite, which contains of magnesium, Ammonium phosphate or apatite which consist of calcium phosphate. Occasionally, these stones completely fill the renal pelvis forming "Staghorn calculi".

Antibiotic Sensitivity Test:

Urinary tract infections (UTIs) remain one of the most common bacterial infections globally, with *Escherichia coli*, *Klebsiella spp.*, *Pseudomonas spp.*, and *Acinetobacter spp.* as the predominant etiological agents. Although this spectrum has remained stable over decades, the **antimicrobial resistance (AMR) patterns** of these organisms have shifted significantly. In the present study, we observed that 86.3% of isolates were resistant to Nalidixic acid and Cefadroxil, while higher susceptibility was retained for aminoglycosides such as Netilmicin (77.3%) and Amikacin (63.3%). These findings mirror global surveillance data, which consistently report a decline in fluoroquinolone and cephalosporin efficacy, with aminoglycosides and carbapenems retaining relative effectiveness against MDR uropathogens [51].

The mechanisms underlying resistance in these pathogens are **multifactorial**. Enzymatic inactivation, particularly through β-lactamase production in Enterobacterales, remains the most prevalent [53]. Other mechanisms include **target site alterations** (such as gyrA mutations mediating fluoroquinolone resistance), reduced membrane permeability, and the development of **efflux pump systems** that expel antibiotics from bacterial cells. The ability of pathogens to simultaneously harbor multiple resistance mechanisms complicates treatment outcomes[54].

Our findings further highlight the interplay between **virulence and AMR**. A strong association was noted between the **mucoid phenotype, cell surface hydrophobicity, and resistance**. For example, *E. coli* JH-23 and *Klebsiella* JH-25 were resistant to 11 out of 12 antibiotics and displayed mucoidy, hydrophobicity, and urease production. Similarly, *Pseudomonas* JH-2 exhibited pan-resistance along with hydrophobicity and urease activity. The mucoid phenotype enhances biofilm formation, creating physical and chemical barriers that reduce antibiotic penetration and promote horizontal gene transfer of resistance determinants [NPJ Biofilms and Microbiomes, 2024]. Recent reports support these findings, showing that **hypermucoviscous *K. pneumoniae* and mucoid *E. coli*** frequently exhibit MDR profiles, with capsule production linked to immune evasion and antibiotic tolerance [54, 56]

The convergence of virulence and resistance is a critical concern. The co-expression of traits such as capsule formation, urease production, and hydrophobicity with MDR profiles enhances bacterial survival in the urinary tract, thereby increasing persistence and recurrence of infection [57]. This underscores the limitation of traditional antibiotic stewardship alone. Novel adjunctive strategies targeting **virulence factors**—including capsule depolymerases, biofilm-disrupting enzymes, and efflux pump inhibitors—are increasingly being explored to complement existing antimicrobials [58]

In conclusion, our study confirms that **uropathogens displaying virulence determinants are also more likely to be multidrug resistant**, reflecting a global trend of intertwined pathogenicity and resistance. Addressing this dual threat requires not only prudent antibiotic use but also innovative anti-virulence approaches to improve treatment outcomes in UTIs.

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