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Study of Uropathogen and its Antimicrobial Resistance Pattern from Tertiary Care Rural Hospital

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ABSTRACT

Urinary tract infections are serious health problem affecting 150 million people globally in each year. The distribution of antimicrobial susceptibility data of UTI-causing microorganisms changes from time to time and from place to place. So the present study was undertaken with the aim to study the microorganisms and their antimicrobial resistance patterns of microorganisms involved in UTIs. A total of 352 clinical specimens suspected of having UTIs were processed for identification of microorganisms and their antimicrobial resistance patterns of microorganisms involved in UTIs. A total of 221(62.78%) bacterial uropathogens were isolated. *E.coli* was the dominant bacteria among all isolated uropathogens with the prevalence rate of 42.08%. The second most prevalent isolate was *S.aureus* 62(28.05%).The prevalence of UTI is more among females 143(71.5%) as compared to males 78(51.32%). Female to male ratio was 1.83:1 *E.coli* is the predominant organism in the age group of 21-40 and in 81-90 years. The prevalence of *S.aureus* and *P.aeruginosa* were higher in the extreme age groups. The percentage of uropathogens in the geriatric age group was 80.52% Ward-wise, majority of the isolates were from Surgery(69.23%) and obgyn(69.49%). Isolate wise, Higher prevalence of *E.coli* were from Medicine (38.85%) and OPD (32.73%) and that of *Staphylococcus aureus* were seen in Obgy(32.20%),ICU(24.14%) and Surgery(23.08%) and pediatric wards whereas from other wards *E.coli* was predominant. Uropathogens showed higher resistance to fluoroquinolones and lower resistance was seen to aminoglycoside. In conclusion, although *E.coli* is the predominant isolate causing UTI, the pathogen that is gaining significance is *S.aureus*.

Keywords: Urinary tract infections, uropathogens, antimicrobial resistance

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INTRODUCTION

Urinary tract infections (UTIs) are serious health problem affecting 150 million people globally in each year.[1,2] Despite the advances in and the wide spread availability of antimicrobials, UTIs continue to be the most common causes of infections in hospitalized patients, accounting for approximately 40% of the hospital acquired infections.[3,4] They are the most common bacterial infection in patients of all ages and are one of the most important causes of morbidity, occasionally becoming life threatening, forcing the general population to seek medical attention, and accounting for considerable health care costs. [5, 6]

Increasing multidrug resistance in bacterial uropathogens is an important and emerging public health problem. The Infectious Disease Society of America (IDSA) identified some microorganisms for new effective therapies. Those microorganisms were called "ESKAPE pathogens "which include *Enterococcus faecium*, *S. aureus*, *Klebsiella spp*., *Acinetobacter spp.*, *Pseudomonas spp.*, and *Enterobacter spp.* [7] The commonest bacterial agent involved in causation of UTIs is *Escherichia coli*, although the distribution of pathogens that cause UTI is changing. [8]

Mainstream pathogens associated with UTIs are known for their resilience and exhibit the property of antimicrobial tolerance. In addition, they are also known for their phenotypic and genotypic features that make them prime contenders in conferring the infection. Host factors have to be considered as they are equally responsible for the cause of the infection and make the individual vulnerable. These include age, diabetes, long term hospitalized patients and the kind of medical devices among the patients like the catheters.[9] UTI is challenging, not only because of the large number of infections that occur each year, but also because the diagnosis of UTI is not always straight forward. UTI has to be distinguished from other diseases that have a similar clinical presentation, some UTIs are asymptomatic or present with atypical signs and symptoms. The increased emergence of antimicrobial resistance in the uropathogens, probably due to the empirical administration of antibacterial therapy, even before the availability of the urine culture results, is a matter of growing concern worldwide. [10, 11] The distribution of antimicrobial susceptibility data of UTI-causing microorganisms changes from time to time and from place to place. [12] So the present study was undertaken with the aim

- To study the microorganisms involved in UTIs
- To study the antimicrobial resistance patterns of microorganisms involved in UTIs

MATERIALS AND METHOD:

The Prospective study was carried out in the department of Microbiology in tertiary care rural hospital from the period of July 2012 to December 2013. A total of 352 non-duplicate clinical

specimens suspected of having UTIs were processed according to the standard laboratory methods. The sample plates which yielded a colony count of $>10^5$ Colony Forming Units (CFUs) /ml were considered as suggestive of significant bacteriuria. The uropathogens were further identified by their morphologies and biochemical characteristics.

The Isolates were tested for Antibiotic susceptibility testing by Kirby-Bauer disc diffusion method on Mueller Hinton agar as per CLSI Approved Standard M100-S17). [13]

The antibiotics tested for *Staphylococcus aureus* were amikacin(Ak)30µg, ciprofloxacin(CIP) 5µg, gentamicin (G)10µg, calithromycin (CLR)15 µg, cefotaxime(CF) 30µg, sparfloxacin (SF)5µg, cefuroxime(CR)30µg, cefoperazone (CFP)75µg, ampiclox(ACX) 20µg, azithromycin(AZ) 15µg, cefadroxil (CD) 30 µg, vancomycin (Va) 30µg, cotrimoxazole (Cot)30µg, linezolid(Lz) 30µg.

The antibiotics tested for urinary tract infection were amikacin (An) 30µg, ofloxacin (ox) 5µg, gentamicin (G)10µg, norfloxacin (NR) 10µg,cefaclor (CFC) 30µg, ciprofloxacin (CIP)5µg, nitrofurantoin (NF) 300µg, cefoperazone (CFP)75unit, ceftriaxone (CTX) 30µg, cefuroxime(CR)30µg, cefadroxil (CD) 30µg, netilin (NET) 30µg.

The antibiotics tested for *Pseudomonas* were ceftazidime (CAZ), gentamicin (GEN)10µg,ticarcillin (Ti), piperacillin (Pi), amikacin (Ak) 30µg, cefepime (CPM), cefoperazone (CFP)75µg, ciprofloxacin (CIP)5µg,levofloxacin (Le), tobramycin (TOB), netilin (NET) 30µg, meropenem (MRP).

Antibiotic disc was obtained from Hi-media Laboratories Pvt. Ltd, Mumbai, India.

Statistical analysis was done by using standard normal test (z test). Value- Z >1.98 statistically significant.

RESULTS AND DISCUSSION:

The changing trends in the aetiopathogenesis of UTIs and the emerging resistance to the antimicrobial agents are a matter of worldwide concern. Even with the adequate precautions, preventive measures and the advances in therapy, UTIs still remain the commonest infections, both in the hospitalized patients and in the out patients. This may plausibly be due to the advancing age, increase in the immunocompromised hosts, prolonged hospitalization, inadequate personal and environmental hygiene, instrumentation (catheters), co morbidities and functional or anatomical abnormalities.[14]

Out of 352 urine samples, a total of 221(62.78%) bacterial uropathogens were isolated. No growth, insignificant growth in 131(37.21%). *Escherichia coli* were the dominant bacteria among all isolated uropathogens with the prevalence rate of 42.08%. The second most prevalent isolate was *S. aureus* 62(28.05%). (Table 1)

The prevalence of UTI in our study was found to be 62.78%. Similar were the findings of Mahesh E et al prevalence rate of 66.78 %, Adebola O et al 68.5%, Devanand P et al 53.82%, and S Patel et al 46.48%. [15-17, 10] Lower prevalence was noted by Jharna M et al (26.01%), M. Dash et al 34.5%, M.Mehta et al 36.68%.[18-20] The high prevalence may be due to genuine population susceptibility because factors like sexual intercourse, peer group influence, pregnancy, low socio-economic status.

The most common organism isolated in our study was *E. coli* 93(42.08%), followed by *Staphylococcus aureus* 62(28.05 %), *P. aeruginosa* 21(9.5%), *Klebsiella sps*, *Citobacter sps* and *Candida* 13(5.88 % respectively) *Proteus sps* 4(1.81%), *Morganella morganii* 1(0.45%), *Serratia marcescens* 1(0.45%).

Several workers reported *Escherichia coli* as the dominant bacteria among all isolated uropathogens (S Patel et al 53.38%, Devanand P et al 42.58 %.) [10, 17]

Our study revealed *S. aureus* as the second most common pathogen of UTIs with a prevalence rate of (28.05%) which is similar with the findings of Adebola Onanuga et al (33.6%) and Manikandan *et al* [16, 21] who reported the organism as the second most prevalent pathogen in UTIs. Thus, these recent findings confirm *S. aureus* as an important etiologic agent in UTIs.

Our study do not correlate with D Tambekar et al study, in which *P. aeruginosa* was reported as the second most common bacterial isolate in UTI studies in India.[22] Devanand P et al and and S Patel et al study who observed *Klebsiella* as the second most prevalent isolate (18.71% and 18.92%) respectively) [17,10]

In our study the prevalence of UTI was higher in female patients 143(71.5%) than in males 78(51.32%) thus showing a female predominance. (Table 2) Statistical significance was seen.

Our study correlates with other study findings which revealed that the frequency of UTI is greater in females as compared to males [17, 10, 23] Females are more prone to develop UTIs, probably due to their characteristic anatomical and physiological changes - short urethra, its proximity to the anus, urethral trauma during intercourse, dilatation of the urethra and the stasis of urine during pregnancy. [3, 11]

Isolates wise the prevalence *of Proteus sps, Citrobacter sps, M. morganii* and *Candida sps* is higher in males as compared to females. S R Mirsoleymani, reported the incidence of Klebsiella, as the second most common pathogen, was significantly higher in males. [24]

The prevalence of uropathogens - age wise was also studied. (Table No 3) In age group below 1 year, we observed the prevalence of *Staphylococcus aureus* and *Ps. aeruginosa* to the tune of 30% and 10% whereas none of our isolates belonged to *E.coli*. But the large number needs to be studied to prove its aetiopathogenesis. In age group of 1-20 yrs

Staphylococcus aureus were dominant, but *E.coli* is the predominant organism in the age group of 21-40 years and in 81-90 years (34.78% and 38.46% respectively). In our study we noted the prevalence of *Staphylococcus aureus* and *P. aeruginosa* were higher in the extreme age groups. Research studies have explored the role of *S. aureus* in association with urinary tract infection and subsequent bacteremia. [25]

In our study we observed the Statistical significance in percentage of uropathogens isolated from the geriatric age group was 80.52% (62/77) as compared to that of younger group (adult and children) 57.82% (159/275)

Similar were the finding of Syed M A et al, in the age-wise analysis, portrayed an increased prevalence in the 61-80 years age group (39.53%).[23] The increased vulnerability in the geriatric population maybe due to their age related physiological and immunological changes and other co morbidities. [3]

Ward –wise, majority of the isolates were from the Surgery (69.23%) and obgyn (69.49%). From orthopedic ward, higher prevalence was of *P. aeruginosa* (33.33%) followed by *Klebsiella sps* (16.67%) and from Surgery *Staphylococcus aureus* (23.08%) followed by *P. aeruginosa* (11.54%) and *Klebsiella sps.*(3.85%) whereas from other wards *E.coli* was predominant.

Isolate wise, higher prevalence of *E.coli* were from Medicine unit (38.85%) and OPD (32.73%) and that of *Staphylococcus aureus* isolates were seen in pediatric wards (48%), Obgy (32.20%), ICU (24.14%) and Surgery (23.08%) (Table 4)

A study by Syed M A et al, reported a majority of the culture positive isolates were obtained from the surgical departments (38.34%), followed by those from the medical departments (33.33%) and the intensive care units (25.82%). [23]

Several studies signify *S. aureus* as a prime competitor of *E. coli* during the course of infection and Research studies have provided sufficient amount of evidences to support the colonization of *S. aureus* among pregnant women and its influence on the neonate and neonates born to mother with *S. aureus* colonization and vulnerable to infection. [26, 27] However some research studies feel that other pathogens of *Staphylococcal* genus are mistaken to be *S. aureus* but the other species of the genus are capable of causing the infection. [28] *S. aureus* colonization among women during pregnancy enhances the rate of morbidity and mortality. [29]

The extent of antimicrobial resistance shown by the pathogens towards the commonly employed drugs is an issue of global concern. The indiscriminate, inadequate and the irrational usage of antimicrobials has further contributed to the emergence of resistant strains,

which may turn out to be a leading cause for the morbidity and mortality in the developing countries.

In our study the antimicrobial resistance pattern of isolates were studied. In *E.coli*, we noted higher resistance to fluoroquinolones (ciprofloxacin 90.32%, norfloxacin 81.72%, ofloxacin 82.80%) and cephalosporins (cefuroxime 93.55% and ceftriaxone 80.65%). Lower resistance was seen to aminoglycoside (amikacin 43.01%, gentamicin 50.53%, netilin 45.16%). (Table 5)This reduced susceptibility might be due to using antibiotics without restriction. In several studies it has been shown that the highly prescribing habits of the physicians are the driving factor for the antibiotic resistance for this group of antibiotic. **(30)** The other possible explanation behind this situation is that the 3rd generation cephalosporin has been in use for a long period and must have been abused and over time organisms have developed resistant mechanisms due to changing their mode of action. Similar were the findings of J Mandal et al, the antimicrobial resistance patterns in *E.coli* was ceftriaxone 60.5%, ceptazidime 57.1%, gentamicin 59.6%, nitrofurantoin 26.9%, meropenem 9.8%, ciprofloxacin 73.08%, amikacin 23.2%.**18**

Resistance rates for ciprofloxacin against uncomplicated UTI pathogens were reported as 0-14.7 per cent in the ECO-SENS Project, 2.5 per cent in the USA and 1.2 per cent in outpatients in Canada**.** (30)

Higher antimicrobial resistance pattern resistance in S. aureus was observed to fluoroquinolones (ciprofloxacin 80.65%) and sparfloxacin(75.81%), ampiclox (75.81%)and gentamicin (70.96%) and lower resistance to calithromycin 51.61%, cotrimoxazole 40.32%, amikacin 35.48%, vancomycin 8.06% and linezolid no resistance. (Table 6)

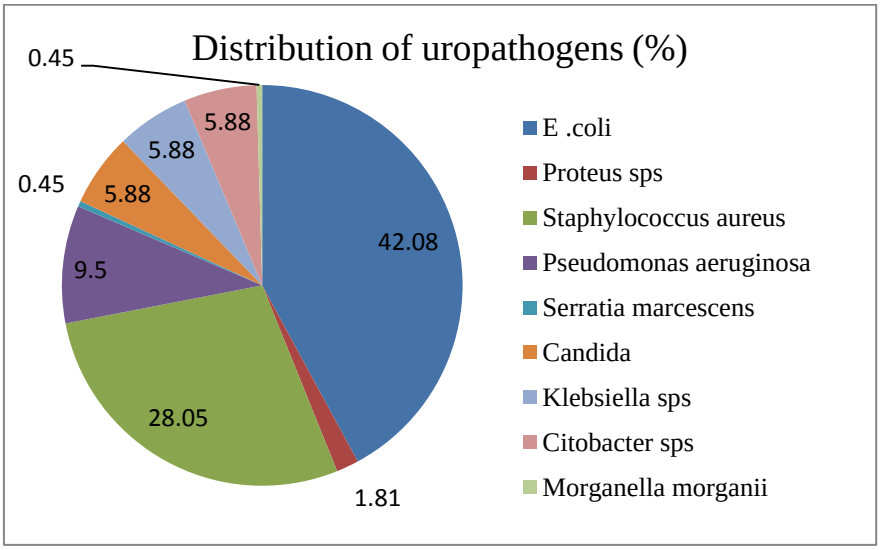

Chart 1: Distribution of uropathogens

The above table depicts *Escherichia coli* were the dominant bacteria among all isolated uropathogens with the prevalence rate of 42.08%. The second most prevalent isolate was *S. aureus* 62(28.05%).

Pseudomonas aeruginosa showed an increased resistance towards the the flouroquinolones (levofloxacin 90.48%, ciprofloxacin 85.71%) and decreased resistance to aminoglycoside (amikacin 47.61%, gentamicin 57.14%) meropenem 38.09%. (Table 7) Our study correlates with the finding of J.Mandal et al amikacin 41.9%, gentamicin 57.4%, meropenem 47.6% except for ciprofloxacin which was lower 58.8%.[18]

Table 1: Gender wise distribution of Uropathogens

Gender	*S.aureus*	*E.coli*	*Klebsiella sps*	*Citrobacter sps*	*Pseudo sps*	*Proteus sps*	*Serratia sps*	*M. morganii*	*Candida sps*	Total
Male n= 152	18 (11.84)	33 (21.71)	4(2.63)	6 (3.95)	6(3.95)	3(1.97)	0	1(66)	7 (4.61)	78 (51.32%)
Female n=200	44 (22)	60 (30)	9(4.5)	7 (3.5)	15 (7.5)	1 (0.5)	1(0.5)	0	6 (3)	143 (71.5%)

The above table depicts the prevalence of UTI is more among females 143 (71.5%) as compared to males 78 (51.32%). Isolates wise the prevalence of *Proteus sps, Citrobacter sps, M. morganii* and *Candida sps* is higher in males as compared to females.

Table 2: Age wise distribution of Uropathogens

Age	Total isolates	*S. aureus*	*E.coli*	*Klebsiela sps*	*Citobacter sps*	*Pseudo sps*	*Proteus sps*	*Serratia sps*	*M. morganii*	*Candida sps*	Total
>1	10	3(30)	0	0	0	1(10)	0	0	0	0	4(40%)
1 to 20	61	14(22.95)	10(16.39)	1(1.64)	2(3.28)	3(4.92)	1(1.64)	1(1.64)	0	2(3.28)	34(55.74%)
21-40	115	14(12.17)	40(34.78)	4(3.45)	4(3.45)	3(2.60)	3(2.60)	0	0	2(1.74)	70(60.87%)
41-60	89	15(16.85)	21(23.60)	2(2.25)	4(4.49)	5(5.62)	0	0	0	4(4.49)	51(57.30%)
61-80	64	15(23.44)	17(26.56)	6(9.38)	3(4.69)	7(10.94)	0	0	1(1.56)	3(4.69)	52(81.25%)
81-90	13	1(7.69)	5(38.46)	0	0	2(15.38)	0	0	0	2(15.38)	10(76.92%)
	352	62	93	13	13	21	4	1	1	13	221

In the above table it is noted the prevalence of *Staphylococcus aureus* and *P. aeruginosa* were higher in the extreme age groups. *E.coli* is the predominant organism in the age group of 21-40 years 40(34.78%) and in 81-90 years 5(38.46%)

Table 3: Ward wise distribution of Uropathogens

Ward	Total	*S.aureus*	*E.coli*	*Klebsiella sps*	*Citrobacter Sps*	*Pseudo sps*	*Proteus sps*	*Serratia sps*	*M. morganii*	*Candida sps*	Total
OPD	55	6(10.91)	18 (32.73)	2(3.64)	2 (3.64)	0	0	0	0	0	28 (50.91%)
Medicine	139	12 (8.63)	54(38.85)	2 (1.44)	4 (2.88)	6(4.32)	3(2.16)	0	1(0.72)	7(5.04)	89(64.02%)
ICU	29	7 (24.14)	3 (10.34)	2(6.90)	2 (6.90)	3(10.34)	1(3.45)	0	0	1(3.45)	19 (65.51%)
Obgy	59	19(32.20)	9 (15.25)	5 (8.47)	3 (5.08)	3(5.08)	0	1(1.69)	0	1(1.69)	41 (69.49%)
Ortho	6	0	0	1(16.67)	0	2(33.33)	0	0	0	0	3(50%)
Pediatrics	25	12(48)	0	0	1(4)	1(4)	0	0	0	1(4)	15(60%)
Surgery	26	6(23.08)	6 (23.08)	1(3.85)	1 (3.85)	3 (11.54)	0	0	0	1(3.85)	18 (69.23%)
TB	13	0	3 (23.08)	0	0	3(23.08)	0	0	0	2(66.67)	8 (61.54%)
	352	62	93	13	13	21	4	1	1	13	221

The above depicts majority of the isolates were from the Surgery (69.23%) and obgyn (69.49%). Higher prevalence of *Staphylococcus aureus* isolates were seen in Obgy and pediatric wards whereas from other wards *E.coli* was predominant.

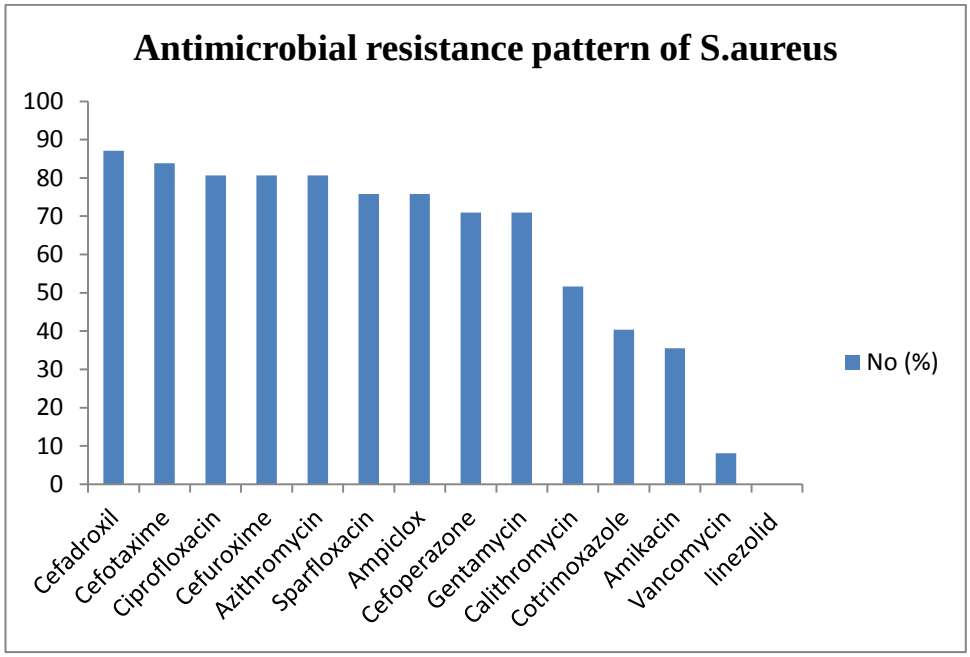

Chart 2: Antimicrobial resistance pattern of S. aureus

The above table depicts lower resistance to calithromycin 51.61%, cotrimoxazole 40.32%, amikacin 35.48%, vancomycin 8.06% and linezolid no resistance.

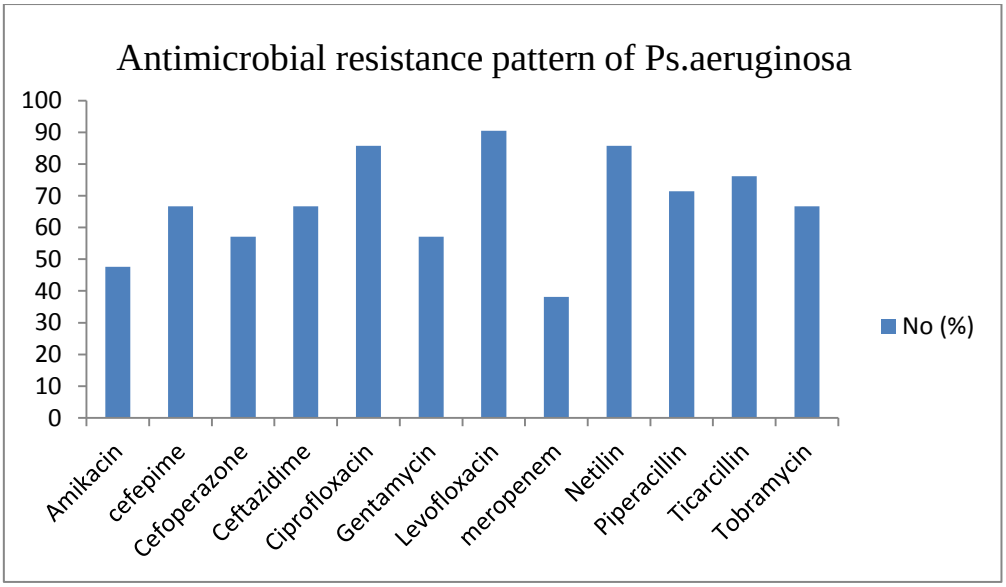

Chart 3: Antimicrobial resistance pattern of Ps.aeruginosa

The above it is observed *Pseudomonas aeruginosa* showed an increased resistance towards the the flouroquinolones (Levofloxacin 90.48%, Ciprofloxacin 85.71%) and decreased resistance to aminoglycoside (amikacin 47.61%, gentamicin 57.14%) meropenem 38.09%.

Table 4: Antimicrobial resistance pattern of member of Enterobacteriaceae

Antibiotic tested	*E.coli*	*Citrobacter sps*	*Klebsiella sps*	*Proteus sps*	*S.marcescens*	*M. morganii*
Ciprofloxacin	90.32	76.92	84.62	75	100	100
Gentamicin	81.72	76.15	76.92	50	0	100
Netilin	45.16	53.85	76.92	25	0	0
Norfloxacin	81.72	92.31	100	75	100	0
Ceftriaxone	80.65	84.62	84.62	75	100	100
Amikacin	43.01	38.46	46.15	25	0	0
cefaclor	69.89	61.54	84.62	50	100	100
Nitrofurantoin	89.25	84.62	76.92	75	0	0
Ofloxacin	82.8	76.92	92.31	75	100	0
Cefuroxime	93.55	61.54	84.62	100	100	100
Cefadroxil	90.32	92.31	76.92	75	0	100
Cefoperazone	54.84	76.15	92.31	50	0	0

The above table depicts higher resistance to fluoroquinolones (ciprofloxacin 90.32%, norfloxacin 81.72%, ofloxacin 82.80%) and cephalosporins (cefuroxime 93.55% and ceftriaxone 80.65%). Lower resistance was seen to aminoglycoside (amikacin 43.01%, gentamicin 50.53%, netilin 45.16%).

CONCLUSION:

Although *Escherichia coli* have been reported as the predominant isolate causing urinary tract infection, the involvement of other pathogens cannot be denied. The pathogen that is gaining significance in its association with UTI is *S. aureus*. And the development of resistance of the pathogens towards the antimicrobial agents is a global issue.

- We suggest that empirical antibiotic selection should be based on knowledge of the local prevalence of bacterial organisms and antibiotic sensitivities rather than on universal or even national guidelines.
- judicious use of antimicrobial agents
- Development of antimicrobial policy that will guide the prescription, sale, and use of antibiotics through regular surveillance of resistant organisms in our environments.

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