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Research Article

ANTIBIOTIC RESISTANCE PATTERN OF *E.COLI* ISOLATES FROM URINE: 3 YEAR RETROSPECTIVE STUDY

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Abstract- Background- Urinary tract infections (UTI) by *E.coli* are very common in clinical practice. In last few years antimicrobial resistance has increased that can result in treatment failure. Antibiotic sensitivity pattern may vary in different geographical locations. Aim of this study is to determine the pattern of antimicrobial resistance to *E.coli* among the UTI patients. Retrospective observational study of last three years (Jan 2017- Dec 2019) was done from microbiological record of urine samples. Information of patients with UTI caused by *E.coli* were recorded with their Antimicrobial resistance pattern. *E.coli* was 43% from the total positive culture isolates. Antibiotic resistance observed high for Nalidixic acid (88.5%), Cefotaxime (72.1%), Norfloxacin (71%), Ceftazidime (63.7%), Cotrimoxazole (58.4%) Cefoxitin (51.4%) and low resistance for Gentamicin (32.1%), Amikacin (13.4%), Piperacillin-Tazobactam (12.2%), Imipenem(5.9%) and Nitrofurantoin (5.8%). Conclusion- Due to high antimicrobial resistance to most commonly prescribed antibiotics, we recommend that Nitrofurantoin can be used as empirical treatment option in UTI due to low resistance.

Keywords- *Urinary tract infections, Antibiotic resistance, Surveillance*

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Introduction

Urinary tract infection (UTI), is one of the most common type of infections with bacterial etiology occurring in hospital and community settings. These bacterial infections may complicate and lead to chronic and recurrent infections. Among bacteria the most common agents that lead urinary tract infections are *E.coli* followed by *Klebsiella* spp, *Proteus* spp., *Staphylococcus* spp., and other members of *Enterobacteriaceae* family [1-3]. *E.coli* is the single most common etiological agent. Commonly used antibiotics for treatment of UTIs are cotrimoxazole, nitrofurantoin, ciprofloxacin and ampicillin. However, there is worldwide increase in resistance of antibiotics among urinary tract pathogens which limit the treatment options and leading to the increased cost of healthcare. Extended-spectrum beta-lactamases (ESBL) producer isolates have become endemic now in many parts of the world and complicate the situation [4-6]. The prevalence and antimicrobial resistance pattern of uropathogenic *E.coli* over the years in community and hospital setting is not completely known in India. Although now a days in many hospitals' surveillance is done and the information is produced for antibiotic stewardship programme. This information is important because it not only provides knowledge of trend of resistance pattern of organism in that hospital or geographical area, which may vary in different time but also the health status of a population. These surveillance data help the treating doctor in decisions for disease management, reduce the rate of treatment failure and the cost of cure. Therefore, in this study we recorded the trends of antibiotic resistance of uropathogenic *E.coli* in 3 years (2017-2019) and assessed whether, there is an increase in resistance to different agents in outpatients and inpatient department isolates.

Materials and Methods

The three-year retrospective study of urine samples from Jan 2017 to Dec 2019, was conducted in the Department of Microbiology at Dr. D.Y. Patil Medical college Hospital and Research Center (Dr. D.Y. Patil Vidyapeeth) Pimpri, Pune 411018.

The microbiology records of inpatients and those attending the outpatient department were retrospectively reviewed. Clinical information such as age, gender, date of sample collection and antibiotics sensitivity test results were obtained.

Urine samples received in the department of microbiology from inpatient and outpatient department with suspected Urinary tract infection in three years included in the study. Urine culture was done by a semi-quantitative method on CLED (Cysteine Lactose Electrolyte Deficient) medium. Inoculated culture plates were incubated for 18-24 hours at 37°C.Culture plate with a growth of $\geq 10^5$ CFU/mL of urine of a single organism or if mixed flora present then a predominant species was considered significant for positive culture. Isolates were identified by standard biochemical reactions. The antibiotic susceptibility testing (ABST) was carried out on the Mueller -Hinton Agar by Kirby Bauer disc diffusion method. The antibiotic discs (Hi-media) tested include Amikacin (30 µg), Gentamicin (10 µg), Norfloxacin (10 µg), Imipenem (10 µg), Meropenem (10 µg), Piperacillin/Tazobactam (100/10 µg), Ceftazidime(30 µg), Cefoxitin (30 µg), Cotrimoxazole (1.25/23.75µg), Nitrofurantoin (300 µg), Nalidixic acid (30 µg). The interpretation of results was based on the recommendations of the Clinical Laboratory Standards Institute (CLSI) [7].

Detection of ESBL(Extended spectrum β-lactamase),MBL (Metallo-β-lactamase) and AmpC Producers

Strains resistant to ceftazidime screened for Extended-spectrum beta-lactamase (ESBL) production by double disc approximation test using the discs of ceftazidime alone, ceftazidime-clavulanic and ceftazidime-tazobactam on Mueller–Hinton agar plates with lawn culture of test isolate. Figure of eight impression considered positive for ESBL producers [8,9]. While strains resistant to imipenem checked for MBL Production and ≥7mm increase in Zone of inhibition in imipenem-EDTA disc in comparison to imipenem alone on Mueller-Hinton plate with test isolate considered positive for MBL production [10].

Isolates with cefoxitin (30µg) resistance, confirmed for AmpC production using cefoxitin and cefoxitin-cloxacillin (30/200 µg) disc. A difference in the zone of inhibition of ≥ 4mm in cefoxitin-cloxacillin minus the cefoxitin disc alone considered positive for AmpC producers. Discs of cefotaxime (30µg) and cefoxitin (30µg) were placed from center to center 20mm apart on Muller Hinton agar plates with test strain. Blunting of zone of inhibition of cefotaxime disc considered as positive for inducible AmpC β-lactamase [11].

Multidrug resistant (MDR) strains were detected and considered MDR Strains if resistant to at least one agent from each of the three or more classes of drugs [8].

Results

Of the total 20,259 urine sample of patients in three years ,4013 (19.8%) were found to be culture positive. Out of total 20,259 urine samples, isolation rate of *E.coli* was 1726 (8.5%). Of the total 4013 isolates, isolation rate of *E.coli* was 1726 (43%). [Table-1] shows the number of total urine samples, positive culture isolates and *E.coli* isolates identified in three different years. In year 2019, observed increased incidence of positive urine samples from total samples received. But there was seen increased incidence of *E.coli* from positive urine samples in year of 2018. *E.coli* remained the most common isolated pathogen in all three years.

Table-1 *Sample received and number of isolates in three years*

Year	2017	2018	2019	Total
Total samples	4,743	6,534	8,982	20,259
Culture positive	770 (17.2%)	1,013 (15.5%)	2,230 (24.8%)	4,013
E.coli isolates	332 (43.1%) n-770	550 (54.2%) n-1,013	844 (37.8%) n-2,230	1726 (43%) n-4013

The basic characteristics including sex difference and age group of the study population are shown in [Table-2] which showed that 960 (55.6%) isolates caused UTI in female patient and 765 (44.3%) in male patients. In paediatric age group, 287(16.6%), in adolescent 39 (2.2%), adults 1056 (61.1%) and in elderly patients 344 (19.9%) *E.coli* isolates identified from urine in three years.

Table-2 *Sex difference and age groups of Study Population*

Sex	2017 (%)	2018 (%)	2019 (%)	Total
Male	138 (41.5%)	237 (43%)	391 (46.3%)	766 (44.3%)
Female	194 (58.4%)	313 (56.9%)	453 (53.6%)	960 (55.6%)
Total	332	550	844	1726
Age				
0-12 yrs	90 (27.1%)	89 (16.1%)	108 (12.7%)	287 (16.6%)
13-18 yrs	3(0.9%)	7 (1.2%)	29 (3.4%)	39 (2.2%)
18-65YRS	185(55.7%)	337 (61.2%)	534 (63.2%)	1056 (61.1%)
≥65yrs	54 (16.2%)	117 (21.2%)	173(20.4.%)	344 (19.9%)
Total	332	550	844	1726

The antibiotic resistance pattern distributed in three study years is shown in [Table-3]. The average high resistance was seen for Nalidixic acid (88.5%), Cefotaxime (72.1%), Norfloxacin (71%), Ceftazidime (63.7%), Cotrimoxazole (58.4%) and Cefoxitin (51.4%). The low resistance was seen for gentamicin (32.1%), amikacin (13.4%), piperacillin-tazobactam (12.2%), imipenem (5.9%) and nitrofurantoin (5.8%).

Table-3 *Antibiotic resistance pattern of E.coli over the three years*

Antibiotics	2017	2018	2019	Total
	n-332	n-550	n-844	n-1726
Nitrofurantoin	15 (4.5%)	33 (6%)	53 (6.2%)	101 (5.8%)
Nalidixic acid	322 (96.9%)	495 (90%)	711 (84.2%)	1528 (88.5%)
Norfloxacin	251 (75.6%)	399 (72.5%)	576 (68.2%)	1226 (71%)
Ceftazidime	243 (73.1%)	399 (72.5%)	459 (54.3%)	1101 (63.7%)
Cotrimoxazole	215 (64.7%)	326 (59.2%)	467 (55.3%)	1008 (58.4%)
Amikacin	31 (9.3%)	88 (16%)	114 (13.5%)	233 (13.4%)
Gentamicin	111 (33.4%)	179 (32.5%)	265 (31.3%)	555 (32.1%)
Cefoxitin	135 (40.6%)	347 (63%)	406 (48.1%)	888 (51.4%)
Cefotaxime	245 (73.7%)	410 (74.5%)	591 (70%)	1246 (72.1%)
Imipenem	3 (0.9%)	39 (7%)	61(7.2%)	103 (5.9%)
Piperacillin/ Tazobactam	33(9.9%)	62(11.2%)	116 (13.7%)	211 (12.2%)

Analysis of antibiotic resistance pattern over three years showed resistance to Nitrofurantoin, Amikacin, Cefoxitin, Imipenem and Piperacillin-Tazobazctam increased over three years. Resistance to gentamicin and cefotaxime was steady during three years. Decreasing resistance was recorded to nalidixic acid, norfloxacin, cotrimoxazole and ceftazidime.

[Table-4], shows the resistance pattern in OPD and IPD patients in these three years. Out of the 1726 *E.coli* isolates, 540(31.2%) culture isolates causing UTI obtained from the patients attending the outpatient department (OPD)and 1,186(68.7%) from the inpatients department (IPD).Among OPD and IPD isolates resistance to all antibiotics tested was higher in the IPD isolates as compared to OPD except for Nitrofurantoin where resistance in OPD isolates was higher than IPD.

Table-4 *Antibiotic Resistance trend of E.coli isolates in OPD and IPD patients over the three years*

Antibiotics	2017		2018		2019	
	IPD	OPD	IPD	OPD	IPD	OPD
Nitrofurantoin	4.10%	5.50%	5.70%	6.70%	6.20%	6.30%
Nalidixic acid	88.80%	78.80%	90.50%	88.50%	84.80%	83.30%
Norfloxacin	80.10%	63.30%	76.30%	62.40%	71%	63.10%
Ceftazidime	80.50%	53.30%	75%	65.70%	58.10%	47.50%
Cotrimoxazole	67.70%	56.60%	63.30%	48.30%	59.10%	48.50%
Amikacin	9.90%	7.70%	20.30%	8%	15.60%	9.60%
Gentamicin	35.90%	26.60%	34.60%	26.80%	32.50%	29.20%
Cefoxitin	45.80%	26.60%	65%	57.70%	52.40%	40.10%
Cefotaxime	80.90%	54.40%	77.30%	67.10%	75.60%	59.80%
Imipenem	1.20%	0%	8.40%	3.30%	9.30%	3.30%
Piperacillin/ Tazobactam	12.20%	3.30%	13.70%	4.60%	17.60%	6.60%

[Table-5] shows the number of ESBL, MBL and AmpC producers over the three years with percentage of more than 70%, 30% and 20% respectively over the period of three years.

Table-5 *Isolation rate ESBL, MBL and AmpC Producers over the three years*

ESBL	Total (%)
2017	211/285 (74.0%)
2018	318/399(79.6%)
2019	355/459 (77.3%)
MBL	
2017	1/3 33.3%
2018	18/39 (46.1%)
2019	43/61 (70.4%)
AmpC	
2017	30/135 (22.2%)
2018	169/347 (48.7%)
2019	144/406 (35.4%)

Over the period of three years, total MDR isolates were 996 (57.7%) from 1726 *E.coli* isolates. Percentage of MDR *E.coli* identified was 60.5% (201/332) in 2017, 63.2% (348/550) in 2018, 52.9% (447/884) after using above mentioned class of antibiotics .

Discussion

In this study, isolation rate of *E.coli* was 43% from the culture positive isolates in three years with yearly isolation of 43.1%, 54.2% and 37.8% respectively. In similar other study by Agrawal, *et al* [12] conducted in Raipur Chhattisgarh, *E.coli* was the most common uropathogen (36%). In a study of Shanmugapriya, *et al.* [13] conducted in a tertiary care hospital of south India, 70% of the UTI caused by *E.coli* among the studied subjects. All these studies showed that *E.coli* is the predominant pathogen causing UTI. In this study, 55.6% of *E.coli* isolates identified in female patients and 44.3% in male patients. In a review article of Reza Mortazavi-Tabatabaei SA [14] after collecting data from different databases, found 65.37% female and 34.63% male patients with *E.coli* UTI. It is already known that UTI is more common in females due to the short urethra, close alignment of urethra to the anus and changes in hormonal levels. But these studies help to see the change in gender trend if any and reiterated the same findings. Isolation of *E.coli* in different age groups in this study observed was 16.6% in pediatric, 2.2% in adolescents ,61.1% in adults and 19.9% in elderly patients in three years. In a study of Veronica, *et al*. [5] they found 51% of *E.coli* in adults and 49% in elderly patients cause of UTI.

In a study of Alanazi, *et al* [1] they identified 28.7% in pediatrics, 2% in adolescents, 47.52% in adults and 21.78% in elderly patients. In our study, isolation of *E.coli* in adults was higher while in adolescents and elderly it was inagreement with these studies.

Antibiotic resistance pattern of three years in this study shows the average high resistance to Nalidixic acid (88.5%), Cefotaxime (72.1%), Norfloxacin (71%), Ceftazidime (63.7%) , Cotrimoxazole (58.4%) ,Cefoxitin (51.4%) and low resistance for Gentamicin (32.1%), Amikacin (13.4%), Piperacillin-Tazobactam (12.2%), Imipenem(5.9%), Nitrofurantoin (5.8%).In a study by Kulkarni, *et al* [15] conducted from 2012-2015 in a tertiary care hospital of Karnataka , sensitivity observed was-Imipenem (96.71%), Nitrofurantion (92.41%), Amikacin (90.89%), Piperacillin-Tazobactum (80.76%), Gentamicin (59.24%) and Norfloxacin (53.67%). In 2017-2018 study of Bangladesh by Acherjya, *et al* [16] recorded maximum antimicrobial resistance for Cotrimoxazole (95.0%), followed by Ceftazidime (75.7%), Gentamicin (70.3%), Amikacin (69.0%), Imipenem (58.9 %,), Ciprofloxacin (57.3%), Cefotaxime (37.4%), (35.2%), Nitrofurantion (4.7%).

In a 5 year study of study of Prasada, *et al* [2] they observed that antibiotic resistance of uropathogenic *E.coli* increased to Cephalosporins, Cotrimoxazole, Piperacillin Tazobactam, carbapenems, reduced for Norfloxacin and low for the Nitrofurantoin. Nitrofurantoin resistance is lowest in all studies and the reason is mostly the less use of this drug in patients preferably in hospitals. In this study, Carbepenem showed low (5.9%) resistance with increasing pattern similar to a study by Prasada, *et al* which showed increased resistance of this drug over the years. Increase in the resistance pattern to Carbapenems is worrisome as it is the last choice for the management of multidrug-resistant *E.coli*. If possible, should be given after ABST pattern only.

Comparison of OPD and IPD isolates resistance to antibiotics showed high resistance in the IPD isolates as compared to OPD except for nitrofurantoin and similar results shown by Singhal, *et al* in their study [17]. High resistance rates in IPD patients are known because of increased selection pressure in the hospital environment and spread of resistant strains through cross infection if correct infection control preventive measures are not taken. But the point for concern is that resistance in OPD isolates is also high including for fluoroquinolones which is commonly prescribed by doctors in OPD Patients. Percentage of ESBL, MBL, AmpC isolates in this study was more than 70% ,30% and 20% respectively. In a study by Jena, *et al* over a period 0f 7 months in 2012, 61.84% of isolates identified as ESBL producers and 9.21% OF MBL [18]. In a study of Nepal, *et al* over a period of 8 months in 2015, 82.6% of *E.coli* isolates found ESBL producers [19]. In a study of Shrestha, *et al* [20] done in a period of 1 year from 2018-2019, 44.55% were ESBL Producers and 39.60% OF MBL Producers. This explain the increased rate of MBL producers. In a study by Ingti, *et al* [21], 47.4% isolates were confirmed to be AmpC producers, which is found to be similar with our study. In this study MDR *E.coli* isolates were more than 50%. In Alqasim *et al*., (2018) [22] study,67% of all *E.coli* isolates were the MDR strains. This explain the importance of Antibiotic sensitivity testing for UTI and need of antibiotic policy for OPD and IPD patients for empirical treatment according to the available data.

Conclusion

Urinary tract infection is the most frequently encountered infection and *E.coli* is the predominant pathogen of UTI affecting people of all age groups. High rates of antibiotic resistance have left few options for the treatment. Antimicrobial resistance pattern surveillance studies are necessary to provide effective empiric therapy. In this study, we observed high antibiotic resistance against *E.coli* for most antibiotics even with extended generation of Cephalosporins, Norfloxacin and Cotrimoxazole.

Application of research: Study recommend to use antibiotics with less resistance for empirical treatment like Nitrofurantoin and definitive therapy after sensitivity test report to rationalise the use of antibiotics. This study has limitations of lack of resistance rate for all antibiotics used in UTI and for this further surveillance studies and Indian databases for research are required

Research Category: Medical Microbiology

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References

[1] Alanazi M.Q., Alqahtani F.Y., Aleanizy F.S. (2018) Annals of Clinical Microbiology and Antimicrobials,17(3), 2-7.
[2] Prasada S., Bhat A., Bhat S., Mulki1 S.S., Tulasidas S. (2019) Infection and Drug Resistance,12, 1439-1443.
[3] Sabir S., Anjum A.A., Ijaz T., Ali M.M., Khan Mur R., Nawaz M. (2014) Pakistan Journal of Medical Sciences, 30(2), 389-392.
[4] Fasugba O., Mitchell B.G., Mnatzaganian G., Das A., Collignon P., Gardner A. (2016) PLOS One, 11(10), 1-14.
[5] Veronicaa Z., Angelab H., Stephanab H., Andreasc K., Benediktab H. (2019) Swiss Medical Weekly, 149, w20110.
[6] Lee D.S., Ju Lee S, Choe H.S. (2018) BioMed Research International, 7656752, 1-14.
[7] CLSI document M100-S25, Wayne, PA, USA, CLSI; 2015. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing.
[8] Gupta N., Gandham N.R., Jadhav S., Misra R.N., Angadi K., Vyawahare C. (2018) International Journal of Microbiology Research,10 (6),1283-1286.
[9] Kansal R., Pandey A., Asthana A.K. (2009) Indian Journal of Pathology and Microbiology, 52,456-7.
[10] Saha R., Jain S., Kaur I.R. (2010) Journal of the Indian Medical Association, 108, 344-348.
[11] Mohd Khari F.I., Karunakaran R., Rosli R. and Tay S.T. (2016) PLoS One, 11(3), 1-12.
[12] Agrawal M., Ramteke M., Hishikar R., Sherwani N. (2018) International Journal of Research in Pharmacology & Pharmacotherapeutics, 7(3) ,271-279.
[13] Shanmugapriya S., Saravanan T., Janani K. (2017) International Journal of Basic and Clinical Pharmacololgy , 6(6), 1445-1450.
[14] Mortazavi-Tabatabaei S.A., Ghaderkhani J., Nazari A., Sayehmiri K., Sayehmiri F., Pakzad I. (2019) International Journal of PreventiveMedicicne, 10,169.
[15] Kulkarni S.R., Peerapur B.V., Sailesh K.S. (2017) Journal of Natural Science Bioliology and Medicine, 8, 176-180.
[16] Acherjya G.K., Tarafder K., Ghose R., Islam D.U., Ali M., Akhtar N., Chakrabortty R., Sutradhar S.R. (2018) American Journal of Internal Medicine, 6(5), 132-137.

[17] Singhal A., Sharma R., Jain M., Vyas L. (2014) Annals of Medical and Health Sciences Research, 4(1), 51–56
[18] Jena J., Debata N.K., Subudhi E. (2013) Indian Journal of Medical Microbiology, 31,420-421.
[19] Nepal K., Pant N.D., Neupane B., Belbase A., Baidhya R., Shrestha R.K., Lekhak B., Bhatta D.R., Jha B. (2017) Annals of Clinical Microbiology and Antimicrobials, 16,62.
[20] Shrestha B.K., Rai A., Shakya J., Khanal H. (2019) Journal of Medical Biomedical and Applied Sciences, 7(12), 312–319.
[21] Ingti B., Dhar D., Chakravarty A., Bhattacharjee A. (2016) International Journal of Infectious Diseases, 455(1), 41054.
[22] Alqasim A., Jaffal A.A., Alyousef A.A. (2018) International Journal of Microbiology, 3026851.