Increasing antibiotic resistance amongst Uropathogenic E. coli isolated from inpatients with symptomatic UTI - a three year study

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Abstract

Introduction: *Escherichia coli* (E.coli) is one of the most common uropathogen worldwide. In the past few years, uropathogenic E.coli has become increasingly resistant to many drugs. **Materials and Methods:** This prospective observational study was conducted from January 2014 to December 2016. Semiquantitative urine culture was done on blood agar and MacConkey agar using standard technique. Cultures showing a significant growth of 10^5 CFU/ml or more were further processed. Identification and analysis of antibiotic susceptibility patterns was done by VITEK®2 (bioMerieux, USA). **Results:** A total of 674 E. coli samples were included in the final analysis. The isolates showed highest overall sensitivity to imipenem (75.82%) and nitrofurantoin (72.25%) whereas highest resistance was observed in case of amoxicillin-clavulinic acid (84.12%) and cephalosporins (71.96-83.23%), followed by ciprofloxacin (72.70%), amikacin (64.39%) and TMP-SMX (64.24%). Over three years duration, a trend of increasing resistance was observed in the case of cephalosporins, ciprofloxacin and trimethoprim-sulfamethoxazole (TMP-SMX). Percentage of MDR E.coli also increased over three years. **Conclusion:** There is an increase in antibiotic resistance amongst uropathogenic *Escherichia coli* over the past three years. Routine surveillance of resistance patterns should continue in the hospitals and antibiotic policy should be constantly updated. Judicious use of antibiotics is the need of the hour.

Keywords: Uropathogenic E. coli, Increasing resistance, MDR, urinary tract infections

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Introduction

Uropathogenic *Escherichia coli* (UPEC) is the most common etiological agent responsible for uncomplicated urinary tract infections (UTI) [1]. It causes urinary tract infections in community as well as hospital settings. It is estimated that nearly 40% of women and 12% of men will experience a symptomatic UTI in their lifetime and about 25% of these women will experience a recurrence within 6 to 12 months [2]. UTI in the hospital setting is a very common phenomenon owing to long hospital stays, catheterisation, procedures performed and drug pressure. A lot of monetary and personnel resource is spent each year in treating the infection. In the past few years, uropathogenic E.coli have become increasingly

resistant to many drugs [3,4] . These include oral drugs like TMP-SMX, nitrofurantoin, ciprofloxacin, oral cephalosporins as well as injectables like cephalosporins, aminoglycosides and carbapenems. Hospital acquired strains are known to be more resistant than community acquired ones [5,6].

It is important to understand the changing trends of antibiotic susceptibility of isolates, in order to better modify the empirical therapy used.

The main objective of this study was to determine antibiotic susceptibility profile of E.coli causing UTI in the hospital setting along with monitoring the trend of antibiotic susceptibility over three years. This study presents a three year comparison of the same from a tertiary care hospital in western India.

Material and Methods

Study design: This prospective observational study was conducted at our institute from January 2014 to December 2016.

Setting: The institute where study was conducted is a tertiary care centre and caters to patients on both outpatient and inpatient basis. The hospital is 1200 bedded.

Participants: Urine samples from patients admitted to the hospital and having symptomatic urinary tract infection were collected and processed.

Inclusion criteria: Inpatients of both sexes and more than 18 years of age having symptomatic urinary tract infection were included in the study.

Exclusion criteria: Patients less than 18 years of age, outpatients, patients on immunosuppressive drugs, HIV positive and patients unwilling to participate in the study were excluded from the study

Data source: Prospective data collection was done from the culture and antibiotic susceptibility results obtained in the microbiology laboratory at our centre.

Study size: A total of 2376 Urine samples were received in microbiology laboratory for urine culture in the three year study duration.

Culture: Semi quantitative urine culture was done on Blood agar and MacConkey agar using standard technique. Cultures showing a significant growth of 10^5 CFU/ml or more were further processed. Cultures with mixed growth on the culture media were excluded.

Identification and Antibiotic Susceptibility Testing: Identification and analysis of Antibiotic susceptibility patterns was done by VITEK®2 (bioMerieux, USA), as per the manufacturer's instructions.

Culture results with isolation of E. coli were included in the final analysis. Repeated isolates of E. coli from the same patient were excluded.

Multi Drug Resistant *Escherichia coli*: Isolates resistant to three or more classes of antibiotics were considered as multi drug resistant (MDR).

Statistical methods: Statistical analysis of the test results was done using SPSS (version 20.0.0).

Categorical variables were compared using chi square test. A p value of <0.05 was considered to be statistically significant.

Ethical considerations: The study was approved by the institutional ethics committee.

Results

Baseline characteristics: A total of 2376 samples sent for urine culture were processed in the study period. 1287 (54.16%) showed significant growth(10^5 CFU/ml) and 57 samples showed mixed growth.

Escherichia coli was isolated from 674/1287 (52.36%) samples and was the most common uropathogen isolated.

These isolates were included in the final analysis.

The number of E. coli isolates obtained were 280, 237 and 157 in the year 2014, 2015 and 2016 respectively.

Antibiotic susceptibility patterns: The antibiotic susceptibility of E. coli to the following drugs was included in the final analysis: amoxycillin- clavulinic acid, ciprofloxacin, cefepime, ceftriaxone, cefuroxime, nitrofurantoin, trimethoprim- sulfamethoxazole (TMP-SMX), amikacin, gentamicin and imipenem.

The overall antibiotic susceptibility patterns of the Uropathogenic E.coli isolated in three years are presented in Table 1.

The isolates showed highest overall sensitivity to imipenem (75.82%) and nitrofurantoin (72.25%) whereas highest resistance was observed in case of amoxycillin- clavulinic acid (84.12%) and cephalosporins (71.96-83.23%), followed by ciprofloxacin (72.70%), amikacin (64.39%) and TMP-SMX (64.24%) in the three year duration.

Table-1: Overall antibiotic susceptibility of Uropathogenic *Escherichia coli* isolated from 2014 - 2016 (n=674).

S.no	Antibiotic tested	Sensitive (Percentage)	Resistant (Percentage)	Total
1	Amoxycillin- clavulinic acid	107 (15.88)	567 (84.12)	674
2	Ciprofloxacin	184 (27.30)	490 (72.70)	674
3	Cefepime	189 (28.04)	485 (71.96)	674
4	Ceftriaxone	185 (27.45)	489 (72.55)	674
5	Cefuroxime	113 (16.77)	561 (83.23)	674
6	Nitrofurantoin	487 (72.25)	187 (27.75)	674
7	TMP-SMX	241 (35.73)	433 (64.24)	674
8	Amikacin	434 (64.39)	240 (35.61)	674
9	Gentamicin	384 (56.97)	290 (43.03)	674
10	Imipenem	511 (75.82)	163 (24.18)	674

Comparative analysis: It was observed in the comparative analysis of the three year data that there was no significant difference in susceptibility of E. coli to amoxycillin- clavulinic acid, nitrofurantoin, and imipenem. However there are interesting trends to be observed in case of ciprofloxacin, cephalosporins, TMP-SMX and aminoglycosides [Table 2].

The Susceptibility to ciprofloxacin decreased over three years with 42.5%, 21.52% and 8.91% isolates being reported as sensitive in 2014, 2015 and 2016 respectively. The decrease in antibiotic susceptibility was statistically significant in 2014 vs 2015 (p value<0.001) and 2015 vs 2016 (p value<0.001).

The susceptibility to cephalosporins tested also decreased over three years. The decrease of susceptibility to cefepime was significant in 2014-2015 [45% vs 23.20%(p value<0.001)] and 2015-2016 [23.20% vs 5.10%(p value<0.001)]. The susceptibility to ceftriaxone also significantly decreased in 2014-2015[43.93% vs 21.94% (p value <0.001)] and 2015-2016[21.94% vs 6.37%(p value <0.001)]. Similarly the decline in sensitivity to cefuroxime was found to be significant in 2014-2015 [28.93% vs 9.70% (p value<0.001)] and 2015-2016 [9.70% vs 5.73%(p value<0.001)].

Sensitivity to TMP-SMX was lesser in 2015 and 2016 as compared to 2014. The decrease was significant in 2014 vs 2015[42.85% vs 28.27% (p value<0.001)] but insignificant in 2015 vs 2016. However if data from 2014 was compared to 2015 and 2016 combined, the decrease in susceptibility was statistically significant[42.85% vs 30.71%(p value<0.001)].

The difference in antibiotic susceptibility to amikacin between 2014 and 2015 as well as 2015 and 2016 was statistically insignificant. Susceptibility to gentamicin was significantly lesser in 2015 as compared to 2014(p value<0.001) where as the difference between 2015 and 2016 was insignificant. However the difference was significant when the results from 2014 were compared to 2015 and 2016 combined[64.64% vs 51.5%(p value<0.001)].

MDR E.coli: Isolates resistant to three or more classes of antibiotics were considered as multi drug resistant (MDR). The percentage of MDR E. coli isolated from urine samples in 2014, 2015 and 2016 were 53.92%, 64.13% and 79.61% respectively. The most common phenotype of MDR E. coli isolated was resistant to either of the cephalosporins, ciprofloxacin and TMP-SMX. The rates of resistance to two of the most commonly used drugs for UTI - ciprofloxacin and TMP-SMX, were highest in 2016 at 91.09% and 65.60% respectively and 98/157(62.42%) isolates were concomitantly resistant to both. Concurrent resistance to ciprofloxacin, TMP-SMX and nitrofurantoin was observed in 28/157(17.83%) isolates.

Table 2: A three year comparison of the antibiotic susceptibility patterns of Uropathogenic *Escherichia coli*.

S.No	Antibiotic tested	2014 (n=280)		2015 (n=237)		2016 (n=157)	
		S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
1	Amoxycillin-clavulinic acid	41 (14.64)	239 (85.36)	29 (12.23)	208 (87.77)	37 (23.36)	120 (76.44)
2	Ciprofloxacin	119 (42.50)	161 (57.5)	51 (21.52)	186 (78.48)	14 (8.91)	143 (91.09)
3	Cefepime	126 (45%)	154 (55%)	55 (23.20)	182 (76.80)	8 (5.10)	149 (94.90)
4	Ceftriaxone	123 (43.93)	157 (56.07)	52 (21.94)	185 (78.06)	10 (6.37)	147 (93.63)
5	Cefuroxime	81 (28.93)	199 (71.07)	23 (9.70)	214 (90.30)	9 (5.73)	148 (94.27)
6	Nitrofurantoin	202 (72.14)	78 (27.86)	167 (70.46)	70 (29.54)	118 (75.15)	39 (24.85)
7	TMP-SMX	120 (42.85)	160 (57.15)	67 (28.27)	170 (71.73)	54 (34.40)	103 (65.60)
8	Amikacin	183 (65.36)	97 (34.64)	147 (62.02)	90 (37.98)	104 (66.24)	53 (33.75)
9	Gentamicin	181 (64.64)	99 (35.36)	121 (51.05)	116 (48.95)	82 (52.23)	75 (47.77)
10	Imipenem	209 (74.64)	71 (25.36)	185 (78.06)	52 (21.94)	117 (74.5)	40 (25.5)

Discussion

Antibiotic resistance amongst uropathogenic E. coli is increasing every year as found in this study. The highest overall resistance was seen in case of cephalosporins, ciprofloxacin and amoxycillin-clavulinic acid.

Resistance to flouroquinolones in uropathogenic E. coli is a global phenomenon now and varied resistance rates to ciprofloxacin have been reported from different geographical regions. In a study conducted on outpatients with community acquired UTI in Spain, the authors reported 14% resistance to ciprofloxacin [7]

whereas Timothy et al.[8] from Nigeria reported 31% resistance to ciprofloxacin amongst uropathogenic E.coli isolated from inpatients. Karaca Y et al. in 2005 [9], reported that ciprofloxacin resistance rates in E.coli varied from 5.2% to 27.6% in the ten year study period. Our study found the overall resistance to ciprofloxacin to be 72.70% in the three year study period. This is similar to the resistance rates reported by Indian studies in recent times [6,10]. Our study showed that the resistance to ciprofloxacin in E.coli increased significantly each year from 2014 to 2016. The high

resistance rates and significant increase in resistance to ciprofloxacin found in this study may be attributed to two factors: high usage of ciprofloxacin for treating various clinical conditions and use of ciprofloxacin as empirical therapy for treating UTI while awaiting culture reports. In a surveillance study from The Netherlands, a similar yearly increase in resistance to flouroquinolones in E.coli was reported in 2000 with the projection of an increase in resistance further as flouroquinolones remain the most commonly prescribed antibiotics [11].

The results show a high rate of resistance to TMP-SMX (64.2%) which is higher than those reported from other geographical regions [7], similar to the rate reported by Niranjan V et al[12] in a study conducted on inpatients in India, and lesser as compared to some published studies from India [6,10]. In our study, higher resistance rates to TMP-SMX were observed in 2015 and 2016 combined as compared to a 2014. In a 10 year study from turkey, resistance rates to TMP-SMX varied from 38.5% to 69.3%[9]. Such variations of resistance rates amongst hospitals and varying resistance rates with time may be attributable to different/changing practices involving use of TMP-SMX for therapy.

Resistance to cephalosporins ranged from 71.96% to 83.23% in the present study which is similar to recent reports from Indian Hospitals [6,12]. World wide, cephalosporin resistance in uropathogenic E.coli has been reported to be in the range of 15.7% to 83% depending upon the geographical region and inpatient or outpatient setting [8,13,14,15]. Resistance to cephalosporins significantly increased over three years of study period. This may be attributable to excess use of cephalosporins in the community and hospital settings. In our hospital set up cephalosporins are very commonly used as first line empirical therapy for treating many clinical conditions. Findings of the present study highlight the hazardous effects of the same.

The percentage of MDR E. coli found by this study is comparable to that of recently published studies [12,14]. The number of MDR E.coli has steadily increased over the three year duration. Indiscriminate usage of antibiotics in the outpatient and inpatient care system is possibly the reason behind this. Many published studies have clearly established that prior antibiotic usage is a risk factor for infection with MDR E.coli [16,17,18,19,20].

According to Infectious diseases society of America and European society of Microbiology and infectious diseases, flouroquinolones, TMP-SMX, nitrofurantoin and amoxycillin-clavulinic acid are the recommended first line drugs for uncomplicated UTI [21].

However the recommendations for the first line treatment should be decided according to the local susceptibility patterns of a region/ country. Recently published NCDC guidelines for antibiotic use in infectious diseases recommend nitrofurantoin/ TMP-SMX/ ciprofloxacin as first line antibiotics for treating uncomplicated and Piperacillin- Tazobactam/ amikacin for complicated UTI[22]. Beta lactam antibiotics are not the choice for empirical therapy possibly owing to their very high resistance rates as reflected in our study results with only 16% strains of E.coli being susceptible to amoxycillin -clavulinic acid.

Nitrofurantoin susceptibility is found to be nearly 72% in the present study and it did not vary over the three years significantly. However the declining susceptibility of ciprofloxacin is a major concern as it still remains as a first line drug. Ciprofloxacin has been recently named as one of the "alert drugs" by NCDC which means that it is among the drugs most frequently prescribed irrationally and should be used only when clearcut microbiological indications exist. Switching over to susceptible/narrow spectrum antibiotics after obtaining culture results while treating UTI is also strongly recommended by NCDC [22]. These measures might be able to reduce or stop the progression of the existing fluoroquinolone resistance.

Strengths of the study: This study presents a comparative analysis of susceptibility of *Escherichia coli* over three years duration. Therefore not only we are able to observe the trend of increasing antibiotic resistance but also the data will be helpful in fine tuning the antibiotic policy for empirical therapy in patients with UTI. According to the findings of this study, flouroquinolones should be used as sparingly as possible.

Limitations: The data on actual antibiotic usage could not be collected. A correlation of antibiotic usage and increasing resistance patterns would have provided us with a better insight into the resistance pattern.

Future directions: Routine surveillance of antibiotic susceptibility patterns of uropathogenic *Escherichia*

coli, especially to ciprofloxacin, should continue. Indiscriminate usage of antibiotics should be strictly controlled.

Conclusion

There is an increase in antibiotic resistance amongst uropathogenic *Escherichia coli* over the past three years. Judicious use of antibiotics is the need of the hour.

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