Original Research Article

Clinico-bacteriological profile of uropathogens with special reference to colistin susceptibility among Multi drug resistant isolates from a tertiary care hospital in South India

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Abstract

Background: Identification of microorganisms causing urinary tract infections (UTI) and their antimicrobial susceptibilities for different antimicrobial agents is important for providing appropriate treatment to the patients with UTI. Thus, this study was aimed to study the microbiological profile of UTI cases, their antibiotic susceptibility profile and assessment of biofilm formation in bacteria isolated from catheterised patients. **Methods:** Mid-stream urine samples were collected and were subjected to microscopy and culture. All positive cultures were identified by MALDI-TOF and their antimicrobial susceptibility was determined using VITEK 2. The minimum inhibitory concentration (MIC) of colistin was determined by MIKROLA TEST® MIC colistin kit. Biofilm formation of organisms isolated from catheter associated UTI was performed by tissue culture plate method. **Results:** The commonest pathogen associated with UTI in this study was found to be *Escherichia coli* (68%), followed by *Klebsiella pneumoniae* (17%), *Pseudomonas aeruginosa*(5%), *Enterococcus faecalis* (3%), *Acinetobacter baumannii* (3%), *Staphylococcus aureus* (2%), *Enterobacter cloacae* (1%) and *Proteus mirabilis* (1%). Majority of *Escherichia coli* (47/67, 70.15%) and *Klebsiella pneumoniae*(5/17, 29.41%) isolates were found to be ESBL producers. Antimicrobial susceptibility testing showed that 59 % (59/100) isolates were multi drug resistant, 47 (79.66%) among them were *E. coli* followed by 11(18.64%) *Klebsiella pneumonia* and 1 (1.69%) *Pseudomonas aeruginosa*.One (5%) *Klebsiella pneumonia* isolate was found resistant to colistin. All 12 isolates from catheterized patients were found to be biofilm producers. **Conclusion:** Present study highlights increased resistance of uropathogens towards cefuroxime and norfloxacin in south coastal Karnataka.

Keywords: Uropathogens, Colistin, Minimum inhibitory concentration, Multi drug resistance, Biofilm.

Introduction

High burden, acute care hospitals have identified urinary tract infections (UTI) as the most common type of healthcare associated infection (HAI), resulting in more than 30% of all reported infections.[1] Almost all healthcare-associated UTIs (HA-UTI) are commonly attributed to iatrogenic interventions. Prolong unnecessary use of catheters and long stay in hospital are also the important reasons for catheter associated urinary tract infection (CAUTI).[2] Bacteria possess rapidly evolving strategies which help them to adapt to various changing environments. These causative agents of UTI and antibiotic resistance pattern of different microorganisms causing UTI have changed over past few years.

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Correct identification of microorganisms causing these infections and detection of their antimicrobial susceptibilities for different antimicrobial agents is very important for providing appropriate treatment to the patients with UTI.[3] These days, UTI is one of the commonest infectious diseases encountered in clinical practice, and increasing antimicrobial resistance is a matter of concern for treating such patients. Bacteria adhere themselves to surface aggregate in a hydrated polymeric matrix of their own synthesis to produce biofilms.[4] Biofilm forming bacteria are more resistant to antimicrobial agents leading to treatment failures. Hence, detection of biofilm production by uropathogens is important.

Widespread use of antimicrobial agents without testing the susceptibility of clinical isolates results in development of multidrug resistance and most of the time colistin becomes the last option to manage major infections caused by multi drug resistant (MDR) Gram negative bacteria.[5] A plenty of research is carried out on testing the sensitivity of antimicrobial agents on organisms isolated from UTI

cases, but the literature available on Multi drug resistant uropathogens and testing their susceptibilities to colistin/polymyxin compounds by broth micro dilution method is comparatively less.[6] In our centre, automated systems like MALDI-TOF and VITEK 2 were used for identification and checking antimicrobial susceptibility of different clinical isolates, but colistin could not be reported by VITEK 2, so this study was planned to test MDR isolates by MICROLATEST® MIC COLISTIN kit which works on broth micro dilution method to evaluate the colistin susceptibility and an assessment of biofilm formation in microorganisms causing UTI in catheterised patients. In addition to that, it was also planned to investigate the microbiological profile of pathogens isolated from UTI case along with the antibiotic susceptibility profile.

Materials and Methods

Study Settings and Design

A prospective observational study was conducted at a tertiary care hospital in south coastal Karnataka for a period of 6 months after obtaining approval from institutional ethics committee. All adult patients (>18 years) admitted to the hospital with symptoms of UTI were included in the study after obtaining a written consent. Patients unwilling to give consent were excluded from the study. A detailed history of the patient's demographic details, chief complaints on admission, presence of a urinary catheter, risk factors, any surgery, and previous history of UTI was taken in a systematically designed proforma.

Collection of Urine Samples

For non-catheterised patients, early morning mid-stream urine samples were collected. For catheterised patients, sample was collected from catheter port following standard protocols. Samples were taken in a 50ml sterile universal container with tight screw cap. Urine containers were properly labelled, indicating – patient's name, age and other important details along with adequately filled requisition forms.

Microscopy: Ten microliters of the sample was placed on to a clean glass slide. It was air dried, heat fixed and Gram stain was performed for the presence of bacteria and pus cells.

Isolation, Identification and Antimicrobial susceptibility testing

A 4mm diameter nichrome wire loop, dispensing 0.01ml of sample, was used for semi-quantitative streaking on 5% sheep blood agar and MacConkey agar. All plates were then incubated at 37ºC in bacteriological incubator overnight. The bacterial colonies were identified and quantified (n colony = n x 100) as colony forming units (cfu) per ml of urine. Counts equal to or in excess of 100,000 cfu /ml were taken as significant bacterial load. The isolates were further processed for identification and antimicrobial susceptibility testing (AST) by using MALDI-TOF (Matrix- Assisted Laser Desorption/ Ionization- Time of Flight), (Biomerieux- Diagnostics) and VITEK-2 (Biomerieux- Diagnostics) systems respectively. Subsequently, the organism was sub cultured on MacConkey agar and incubated at 37ºC for 18-24 hours. Pure colonies were then stored in Soybean-Casein Digest Medium (Tryptone soya broth, Himedia) containing glycerol (used for bacterial culture) at -70°C till further use.

Detection of minimum inhibitory concentration of colistin for MDR isolates

The minimum inhibitory concentration (MIC) for MDR isolates was detected by using MIKROLATEST® MIC colistin kit following the manufacturer's guidelines. *E.coli* ATCC 25922 was used as control strain. Briefly 0.5 McFarland bacterial suspensions of isolates were prepared in sterile physiological saline by removing few colonies from 18-24 hours pure culture on blood agar. Sixty microliters of bacterial suspension were inoculated into a tube with 13 ml of cation-adjusted Muller Hinton broth II and the contents in the tubes were mixed and 100µl of suspension was inoculated into well of the antibiotic coated microtiter plate. Inoculated microtiter plates were inserted into polyethene bag (to prevent evaporation during incubation time) provided in the kit and were incubated at 37°C overnight. Evaluation of the results was done by reading the plates against a grey background or against natural/ artificial dispersed light visually. Results were interpreted by visual reading of plate.

Detection of Biofilm production by the organisms isolated from catheter associated UTIs

Ten millilitres of Brain Heart Infusion (BHI) broth with additional 2% sucrose was inoculated with loopful of bacterial colonies from 5% sheep blood agar plates and incubated for 18-24 hr at 37°C. Turbidity of the broth was matched to 0.5 McFarland standard and the broth was diluted to 1:100 with fresh medium. Each well of flat bottom 96 well tissue culture plate (Tarson Products Pvt. Ltd) was added with 0.2 mL of the diluted culture. Broth without bacterial culture was added to control well as sterility control. The plates were incubated at 37°C for 24 hrs. To remove free-floating bacteria, the constituents of the well were smoothly removed. Additionally, the washing step was performed by adding 0.2mL of phosphate buffer saline for 4 times. 150µl of 100% methanol was added to each well for 30 minutes as a fixative to fix biofilms formed by adherent microorganisms. After fixation, 150µl of 0.1% crystal violet was added to stain the wells for 30 min. Removal of excess dye was performed by washing the wells with deionized water. The above procedure was performed in triplicates. OD values (optical densities) of adherent microorganisms were read at a wavelength of 570 nm and mean was calculated. [7-9] OD values were classified as stated by Panda et al [7] and Christensen et al.[9]

Statistical analysis

Recorded data was analysed in SPSS v 16. Associations between 2 categorical variables were explored by cross-tabulation. Age of the patient, gender, organisms causing UTI were recorded. Antibiotic susceptibility patterns, presenting clinical features and predisposing risk factors associated with UTI were included in the model.

Results

Over a span of six months of study duration, 100 urine samples were culture positive. The mean ± SD age of the patients enrolled for present study was found to be 55.49 ± 16.12 years, with male-female ratio of 1:0.92. Complete clinical and demographic details are given in Table 1.

Table 1: Clinical and Demographic data of study population, N=100

Parameters	N (%)
Age (years)	
18-30	9 (9)
31-45	20 (20)
46-60	27(27)
>60	44
Gender	
Male	48 (48)
Female	52 (52)

Co-morbidities	
Diabetes mellitus (Type 2)	46 (46)
Hypertension	36 (36)
Urological intervention	8 (8)
History of renal calculi	2 (2)
History of recurrent UTI	1 (1)
Presenting sign and symptoms	
Dysuria	63 (63)
Pain/Swelling of testis, epididymis or prostatitis	13 (13)
Fever (>38℃)	30 (30)
Chills and rigors	04 (04)
Suprapubic tenderness	04 (04)
Gross hematuria	03 (03)
Urinary incontinence	03 (03)
Urgency	79 (79)
Frequency	69 (69)
Abdominal pain	52 (52)
Recent onset of confusion	01 (01)
Purulent discharge around the catheter	02 (02)
Tenderness at the site of catheterization	08 (08)

The most common predisposing factor among patients with UTI was found to be diabetes (46%) followed by hypertension (36%), urinary intervention (8%), renal calculi (2%) and history of recurrent UTI (1%). Frequency of different uropathogens isolated in this study along with their MDR status is given in Table 2.

Table 2: Frequency of isolated organisms and their MDR status

Serial Number	Organism Isolated	Number of Isolates (%)	MDR strains (%)
1	Escherichia coli	67 (67)	47 (70)
2	Klebsiellapneumoniae	17 (17)	11 (65)
3	Enterococcus faecalis	03 (03)	00 (00)
4	Pseudomonas aeruginosa	06 (06)	01 (16)
5	Proteus mirabilis	01 (01)	00 (00)
6	Staphylococcus aureus	02 (02)	00 (00)
7	Acinetobacterbaumannii	03 (03)	00 (00)
8	Enterobacter cloacae	01 (01)	00 (00)
	Total	100	59

Common pathogens associated with UTI in the present study were found to be *Escherichia coli* (68%), *Klebsiellapneumoniae* (17%), *Pseudomonas aeruginosa*(5%), *Enterococcus faecalis* (3%), *Acinetobacter baumannii* (3%), *Staphylococcus aureus* (2%), *Enterobacter cloacae* (1%) and *Proteus mirabilis* (1%). Majority of *Escherichia coli* (47/67, 70.15%) and *Klebsiella pneumoniae*(5/17, 29.41%) isolates were found to be ESBL producers. Antimicrobial susceptibility testing showed that 59 % (59/100) isolates were multi drug resistant, 47 (79.66%) among them was *E. coli* followed by 11(18.64%) *Klebsiella pneumoniae* and 1(1.69%) *Pseudomonas aeruginosa.*A detailed description of drug sensitivity patterns of most commonly isolated uro-pathogens is given in Table 3.

Table 3: Sensitivity pattern of most commonly isolated Uropathogens

Sr. No.	Name of Antibiotic	Most common uropathogens					
		Escherichia coli (N= 67)			*Klebsiella pneumoniae* (N=17)		
		S (%)	I	R (%)	S	I	R
1	Amikacin	62 (93%)	02	03 (4%)	11 (65%)	00	06 (35%)
2	Amoxi-Clavulanic	25 (37%)	04	38 (57%)	06 (35%)	02	09 (53%)
3	Ampicillin	08 (12%)	01	58 (87%)	00 (0%)	00	17 (100%)
4	Ceftriaxone	18 (27%)	02	47 (70%)	07 (41%)	00	10 (59%)
5	Cefuroxime	13 (19%)	03	51 (76%)	06 (35%)	00	11 (65%)
6	Norfloxacin	21 (31%)	01	45 (67%)	06 (35%)	00	11 (65%)
7	Cotrimoxazole	45 (67%)	00	22 (33%)	09 (53%)	00	08 (47%)
8	Gentamycin	52 (78%)	00	15 (22%)	10 (59%)	01	06 (35%)
9	Cefoperazone-Sulbactum	50 (75%)	03	14 (21%)	11 (65%)	00	06 (35%)
10	Cefepime	35 (52%)	00	32 (48%)	11 (65%)	00	06 (35%)
11	Imipenem/Meropenem	59 (88%)	03	05 (7%)	11 (65%)	02	04 (24%)
12	Piperacillin-Tazobactum	41 (61%)	05	21 (31%)	08 (47%)	00	09 (53%)

Out of total study population (n=100), 14 (14%) patients developed urosepsis. Among these 14 patients, *E.coli* was isolated from 11 cases (79%) followed by *K.pneumoniae* and *P.aeruginosa* from two and one cases respectively. Out of these 14 isolates, 10 were multi drug resistant. Fifty two (52%) isolates were ESBL producers, out of these 52 cases, *E.coli* accounting for 47 (90%) ESBL producers followed by 5 (10%) strains of *K.pneumoniae.*

Biolfilm production was seen in the micro-organisms isolated from catheterised patients. In catheterized patients (n=12), all strains were biofilm producers. Biofilm production was detected by tissue culture plate method (TCP). OD (optical densities) values of stained adherent microorganisms were detected using ELISA – auto reader at a wavelength of 570 nm and were classified as stated by Christensen et al, shown in table number 4.

Table 4: Biofilm producing uropathogens in catheterized patients

Name of organism	Number of isolates(n=12)	Biofilm formation	
		Moderate	Strong
Escherichia coli	4	04	00
Acinetobacterbaumannii	2	01	01
Klebsiellapneumoniae	2	01	01
Enterococcus faecalis	1	01	00
Pseudomonas aeruginosa	3	01	02

In present study, 8 isolates were moderate biofilm producers and 4 isolates were high biofilm producers as shown in figure 1.

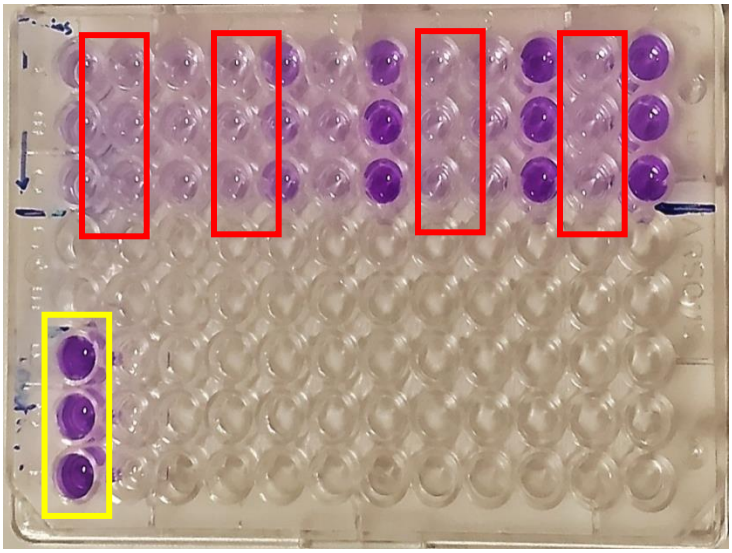

Fig. 1:Screening of biofilm producers by TCP method: moderate and high (Red box) slime producers along with known positive control (Yellow box) differentiated with crystal violet staining in 96 well tissue culture

Among strong biofilm producers, two isolates were *P.aeruginosa* followed by one isolate of *K. pneumoniae* and *Acinetobacter baumannii* each.Colistin drug sensitivity was performed for MDR isolates (n=20) by minimum inhibitory concentration by broth microdilution method. *E.coli* ATCC 25922 was used as control strain to determine the MIC. Among 20 MDR isolates, 14 were MDR *E.coli* and 6 were MDR *K.pneumoniae*. Nineteen out of 20 strains were sensitive to colistin showing MIC range 0.25-0.5 mg/l. One strain of *K.pneumoniae* was found to be resistant to colistin with MIC 4mg/l as shown in Fig 2.

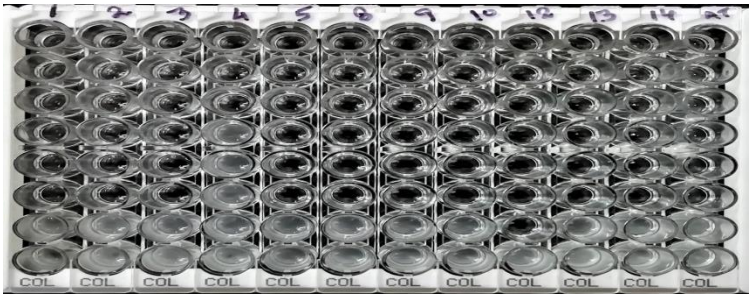

Fig 2: Micro broth dilution test. Arrow showing MIC value of resistant strain. (MIC=4mg/l)

Discussion

Bacterial UTI is one of the main cause for seeking medical attention. Early accurate identification of pathogens and careful selection of antibiotic agents is important to manage patients with UTIs effectively. [10]Females have been documented to have a higher risk and prevalence of UTIs, as compared to males in various previous studies.[11,12] Though there was no significant difference in prevalence of UTI among female and male in present study, it was observed that majority of patient among men were above 45 years and incidences of UTI tends to increase with age as they are more likely to develop prostate problems. Flow of urine can be slowed down by enlarged prostate gland, thus increasing the risk of infection.[13] The most common clinical presentation observed in present study was increased urgency (79%) followed by increased frequency (69 %), dysuria (63%) and abdominal pain (52%) which was in lines of previous findings.[14, 15] In a study, Gould *et al*. [2] reported a prevalence of 10%–15% for developing bacterial infection in catheterised patients, 12 patients in present study also developed catheter associated infection. In present study, the most common predisposing factor among patients was found to be diabetes (46%) followed by hypertension (36%). Elevated blood sugar levels have negative effect on the immune system and decreased immunity in

diabetic patients is a significant risk factor for acquiring UTI. Eshwarappa*et al* [16] reported diabetes as the commonest factor associated with complicated UTI and Nicolle *et al* [17] reported higher risk of developing UTI in diabetic patients. George *et al* [18] also reported that diabetics have higher prevalence of UTI (44.4%) than non-diabetics (29.4%). In present study, of the 67 (67%) isolates of *E. coli*, 86% of strains were ampicillin resistant. Such increased level of resistance has been reported from different studies all over India. This result is in congruence to another study done in south India by Somashekara*et al.*[19]A study done by Gupta *et al* [20] in North India reported 76% ampicillin resistance which is correlating with the results from the present study. Other studies done by Manjunath*et al* [21] and Murugan*et al* [22] reported 90% and 96% resistance to ampicillin respectively. The resistance of *Escherichia coli* to ceftriaxone was 70% in the present study which is comparable to the study done by Murugan*et al* (76%) [22]. Resistance to norfloxacin in this study was 66% which is comparatively lower than the other studies from South India.[20-22] Among K. *Pneumoniae isolates*, resistance against cefuroxime was 64% which is comparatively higher than other studies done by Mohammed *et al* (46% in Libya) [23],Bitew *et al.* (44% in Ethiopia) [24] and Manjunath*et al* (43% in India) [21]. Resistance in *K. Pneumonia* against Norfloxacin was also 64% which is much higher than other studies done by Somashekhara *et al* (38%) [19], Manjunath*et al* (34%) [21] and Murugan*et al* (23%) [22]in south India. The possible reason for this high resistance can be due to higher usage of this drug in UTI cases in our setting.

Biofilm detection was performed only in catheterized group of patients as almost 80% of UTIs involve catheter-associated urinary tract infections (CAUTIs). In catheterized patients (n=12), all strains were biofilm producers. Among this *E coli* (n=4) was a predominant biofilm producer which is correlating with the study done by Maharjan*et al* [25] and Ponnusamy*et al* [26].Among these biofilm producing strains (n=12), 4 strain (33%) were strong and 8 (67%) were moderate biofilm producers, which is unlike other studies done by Hassan et al [27] and Pragyan*et al*[28]. According to Hassan *et al* 22.7% were high and 41% strains were moderate biofilm producers by TCP method whereas study done by Pragyan*et al* showed 11% strains were strong biofilm producers and 34.7% were moderate biofilm producers.Twenty MDR isolates were selected with the control strain (*E. coli* ATCC 25922) to determine the Colistin MIC values. Among 20 MDR isolates, 14 were MDR *E. coli* and 6 were MDR *K. pneumoniae*. Nineteen out of 20 strains were sensitive to colistin showing MIC range 0.25-0.5 mg/l. One isolate of *K. pneumoniae*was found to be resistant to colistin with MIC = 4 mg/l but was shown sensitive by VITEK-2 automated system. Studies done by Tan *et al* [29] and Ng *et al* [30] concluded that colistin susceptibility testing by VITEK -2 is unreliable. Like their findings Chew *et al* [31] also reported discrepancies in resistance pattern against colistin by VITEK-2 and BMD method. This highlights the importance of using gold standard methods while assessing susceptibility to colistin/polymyxin compounds as most of the time colistin becomes the last option available to treat severe infections caused by MDR organisms. However, one of the major limitations of the present study was its limited sample size.

Conclusion

The commonest pathogens associated with UTI in present study were found to be *Escherichia coli* (68%), followed by *Klebsiella pneumoniae* (17%). Increased resistance to cefuroxime and norfloxacin among uropathogens in present study highlights the need of surveillance of antimicrobial susceptibility pattern for establishing an appropriate infection control program and antibiotic policy. Further we recommend strict monitoring of unnecessary usage of fluoroquinolones and cephalosporins in this region.

Data availability

All datasets generated or analyzed during this study are included in the manuscript.

Ethics Statement

Ethical clearance was taken from institutional ethics committee with IEC number 46/2019 and informed consent was taken from all the patients before enrolling them in study.

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