Original Research Article

Five years trend of bacteriological profile and antibiogram of urinary tract infections at a rural medical college hospital in North Kerala, India: 2012-16

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ABSTRACT

Background and Objective: Urinary tract infections (UTI) are amongst the most common bacterial infections in developing countries. The etiology of UTI and the antibiotic resistance of uropathogenes vary in regions and change through times. Regular surveillance of the changing trends in its bacteriological profile and antibiotic sensitivity pattern is therefore mandatory. This study aims to find out the changing trends in the prevalence and antibiotic susceptibility patterns of urinary isolates of over five consecutive years.

Materials and Methods: A retrospective, record based study was conducted on all culture and sensitivity (C/S) reports of urine samples obtained in the microbiology laboratory in a tertiary care centre, Central Kerala (January 2012- December 2016). The C/S reports which were positive for significant growth were analyzed to find out its prevalence and antibiotic susceptibility patterns. Descriptive statistics was used for data analysis and the results were expressed in percentages.

Result: Of the 14105, urine specimens received, 27.93% were positive. Highest rate of UTI is seen in female patients (67.58%) and geriatric age group 47.58%. *E.coli* and Klebsiella are the two most common isolates from all five years of study period and constituted ~ 72% of total. The year wise analysis of antibiotic resistance showed fluctuating pattern. The resistance rate to drugs like Piperacillin –tazobactam and carbepenem showed increasing drug resistance. *E.coli* was found to be more sensitive to Amikacin and nitrofurantoin.

Conclusion: Drug resistant strains are markedly high in our area. Antibiotic resistance does not show a consistent trend over years and vary from region to region. Therefore each institution should have an antibiotic policy based on the local antibiogram which is to be renewed regularly.

1. Introduction

Urinary tract infections (UTIs) constitute a major burden of bacterial infections world over.[1] It accounts for approximately 150 million cases annually.[2] Apart from being the most common cause of nosocomial infection among hospitalized patients, it is also the second most common cause of hospital visit.[2] UTI are a significant cause of morbidity in elderly people, and in females of all ages.[3] Of the various uropathogens, the Gram-negative bacteria such as *Escherichia coli, Klebsiella* species, *Pseudomonas aeruginosa*, and *Acinetobacter* species cause most of the UTIs, and Gram-positive bacteria such as *Enterococcus* species and Staphylococcus species also contribute to causing UTIs.[4] *E.coli* is the most common cause of both community acquired and hospital acquired cases.[4] Inappropriate and empirical usage of wide spectrum antibiotics, immunosuppression and prolonged hospitalization are some of the major factors that elevate the chances of infection.[4] Treatment becomes even more

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challenging in the presence of risk factors such as old age, comorbidity, and immunocompromised state.[5] The antibiotic susceptibility pattern of uropathogens varies with types of organism, different environments and geographical locations. Therefore periodic evaluation of changing trends in the etiology and resistance pattern is necessary to update this information. The present study is carried out with the aim of finding out rate of UTI and distribution of urinary isolates in different age and sex groups and their antibiotic susceptibility pattern in a tertiary care centre in North Kerala, India.

2. Materials and Methods

A retrospective study was conducted in the Department of Clinical Microbiology, of 570-bedded multi-speciality medical college Hospital in North Kerala after clearance from the Institutional Ethics Committee (No. IEC/MES/26/2017). This study was conducted with an objective to determine the etiological bacterial pathogens of the Urinary tract infection and the antibiotic sensitivity pattern of pathogens isolated.

All the positive urine culture samples of outpatients and inpatients above the age of 18 years, who were suspected of urinary tract infection from January 2012 to December 2016, were included in the study. Samples included in the study were midstream clean catch urine, urine from a patient with an indwelling catheter and suprapubic/ cysto-scopic aspiration. All samples collected were immediately transported to the Diagnostic Microbiology Department and processed within 2 hour. All urine samples were inoculated onto cysteine lactose electrolyte deficient (CLED) medium (Himedia, Mumbai, India) using a calibrated loop (volume-0.005 ml) and were incubated for 18-24 h at 37°C. Samples showing significant growth, bacteria growing $>10^5$ colony-forming units (CFU/mL) with single morphotype or up to 2 types, were considered significant and processed further for identification and susceptibility testing. Gram-positive organisms were processed, if isolated as pure growth even when the colony counts were $<10^4$CFU/mL. Susceptibility testing was done by Kirby-Bauer disk diffusion method and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[6] The quality control of the disc was tested by *Escherichia coli* ATCC 25922 and Staphylococcus aureus ATCC 25923.

The demographic details of the patients, the pathogen isolated and the antibiotic susceptibility pattern were collected. All samples were stratified by year, gender, ward and age wise (18–30, 31–45, 46–60 and >60 years). The year-wise cumulative antibacterial resistance rate was calculated for Gram-positive and Gram-negative organisms separately and analysed for change in the rate. The collected data were entered into Microsoft Excel and analysed.
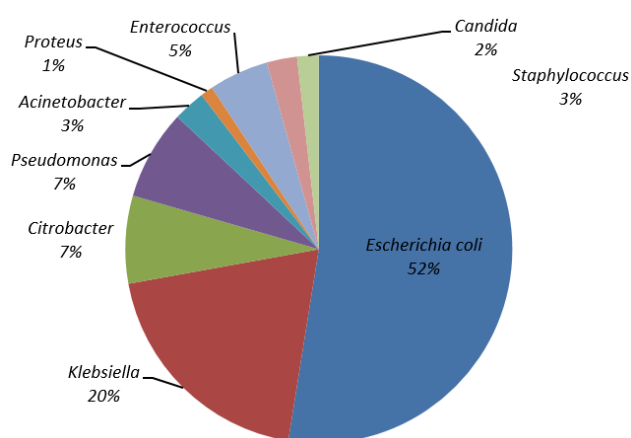

Fig. 1: Distribution of Uropathogens

3. Result

During the study period from January 2012 to December 2016, a total of 14,105 urine samples were received in our lab. Of these, 3939 (27.93%) were positive. Among the positive cultures, 32.42% were from male patients and 67.58% from female patients. In the ward wise distribution 48.84%, 41.08% and 10.08% the positive samples were from outpatients, Inpatients and high risk areas respectively. The geriatric patients above 61 years constituted 47.78% of the positive samples. The details of positive samples with regard to sex, ward and age group is given in detail in Table 1.

Of 3939 pathogens isolated, the gram negative bacteria constituted for 3572 (90.68%), *Enterobactericiae spp.* constituted 3171 (80.50%). Among them *Escherichia coli, Klebsiella sp, Citrobacter sp*, and *Proteus sp* accounted for 52.53%, 19.62%,7.31% and 1.04% respectively. Non fermenter Gram negative bacilli were 401(10.18%), *Pseudomonas spp* (295, 7.49%) and *Acinetobacter spp* (106, 2.69%). The gram positive bacteria constituted for 296 (7.51%), *Enterococcus sp* (196, 4.98%) and Staphylococcus sp accounted for 100 (2.54%). Fungus Candida sp. constituted 71 (1.80%). It is given in detail inTable 2.

Among all the isolated uropathogens in female patients the prevalence rate of *Escherichia coli* was 72.21%, followed by *Klebsiella sp*. 65.20%, *Pseudomonas sp.* 58.64%, *Proteus sp*. 58.54%, *Citrobacter sp*. 58.33% where as in male patients *Acinetobacter sp*. was isolated in 47.1% of cases followed by *staphylococcus sp.* 42%, *Citrobacter sp.* 41.67% given in Table 3.

The antimicrobial resistance spectrum assessment revealed that in Enterobactericiae isolates, there was a steady rise in resistance for 3rd generationCephalosporins (3GC) till the year 2014 followed by an increase in sensitivity in 2015 and 2016. Gentamycin resistance steadily declined from 2012 to 2016. Amikacin resistance was highest in 2014 except for proteus sp. which showed

Table 1: Demographic details of the Urine Sample received during the study period (2012-16)

		2012	2013	2014	2015	2016	Total Number	Percentage
Total sample		3207	3329	3432	3615	3729	14105	
Positive		638	779	653	832	1037	3939	
		19.89	23.40	19.03	23.02	27.81	27.93	
Gender								
	Male	210.0	226.0	195	286	360	1277	32.42
	Female	428	553	458	546	677	2662	67.58
Age								
	18-30	89	122	121	111	144	587	14.90
	31-45	98	110	85	115	126	534	13.56
	46-60	133	183	144	207	269	936	23.76
	61-75	238	255	222	274	372	1361	34.55
	>75	80	109	81	125	126	521	13.23
Ward								
	OP	237	363	328	393	603	1924	48.84
	IP	326	326	268	362	336	1618	41.08
	HRA	75	90	57	77	98	397	10.08

Table 2: Year-wise distribution of the Pathogens Isolated from the urine during the study period.(in %)

		2012	2013	2014	2015	2016	Total	%
Gram negative bacteria							3572	90.68
Enterobacteriaeceae							3171	80.50
	Escherichia coli	366	414	355	420	514	2069	52.53
	Klebsiella sp	114	164	125	173	197	773	19.62
	Citrobacter sp	65	51	56	48	68	288	7.31
	Proteus sp	4	11	5	9	12	41	1.04
Non fermentor GNB							401	10.18
	Pseudomonas sp	41	63	49	57	85	295	7.49
	Acinetobacter sp	14	16	9	20	47	106	2.69
							401	10.18
Gram positive bacteria							296	7.51
	Enterococcus sp	20	50	27	54	45	196	4.98
	Staphylococcus sp	14	10	9	30	37	100	2.54
Fungus								
	Candida sp	-	-	18	21	32	71	1.80
		638	779	653	832	1037	3939	100.00

Table 3: Gender wise prevalence of uropathogens

	Male	Female	
Escherichia coli	575 (27.79%)	1494 (72.21%)	2069
Klebsiella Sp	269 (34.80%)	504 (65.20%)	773
Citrobacter sp	120 (41.67%)	168 (58.33%)	288
Proteus sp	17 (41.46%)	24(58.54%)	41
Pseudomonas sp	122 (41.36%)	173 (58.64%)	295
Acinetobacter sp	50 (47.17%)	56 (52.83%)	106
Enterococcus sp	56 (28.57%)	140 (71.43%)	196
Staphylococcus sp	42 (42.00%)	58 (58.00%)	100

steady rise in resistance pattern till 2016. The beta lactam-beta lactamase inhibitor (PT) resistance has increased in Proteus sp., Klebsiella sp., Citrobacter sp., steadily. There was no resistance observed for meropenem in the first two years (2012, 2013), in all the isolates of enterobactericiae and non fermenter gram negative bacilli but later years showed emergence of resistance which gradually increased till 2016 (Tables 4 and 5).

The gram positive cocci showed increase in resistance to ampicillin and amoxyclav, but resistance pattern of ciprofloxacin was highest in 2012 and it gradually decreased by 2016. During the study period, we have not encountered any resistance to Vancomycin, Linezolid and Teicoplanin. We have observed increase in resistance to Amikacin over 5 years for *Staphylococcus sp.*

We observed that the *Acinetobacter spp* has emerged as a Multi –drug resistant pathogen, with increasing resistance to 3GC, Amikacin, Piperacillin Tazobactum and Meropenem to 97.87%, 23.40%, 38.30% and 80.85% respectively. Similar, trend was observed in *Pseudomonas spp.*, *E.coli* and *Klebsiella sp* with increased resistance to these drugs (Tables 4 and 5).

4. Discussion

Continuous survey of antimicrobial resistance (AMR) plays an important role in the empiric treatment of UTI. Although AMR is rising all over the world, there is a significant difference in the resistance pattern across different geographical areas and also susceptibility of uropathogens varies with time.[2,7] It is therefore important to keep a continuous record of the rates of AMR in the clinically important pathogens at various regions across the world.

The present study reports a prevalence rate of 27.9 % among the patients suspected of having UTIs. Similar prevalence rates have been reported by Indian authors 22.78%[8] and 32%.[9] However a study from Cameroon in 2016,[10] the positivity rate was 59.8%.

The study revealed that females (67.58%) were more susceptible to UTI than males (32.42%), which is similar to other studies.[9,11] Females are more prone to develop UTI, due to the characteristic anatomy of the urethra and the effect of normal physiological changes that affect the urinary tract like short urethra, its proximity to the anus, and dilation of the urethra and stasis of urine during pregnancy.[9] In this study maximum numbers of isolates were from aged people i.e. above 61 years (47.8%). This result is in comprehension with other studies,[3,12] but unlike other studies where high prevalence was found in age group of 26 to 44 years.[13] Predisposing factors for elderly persons for UTI include chronic diseases, functional abnormalities and certain medicines, and in women low estrogen levels which make urethra more susceptible to UTI. Infections due to Gram-negative organisms are commonly encountered in the clinical practice.

Escherichia coli (52.53 %)was the most common Gram-negative bacillus, followed by Klebsiella species(20%) and Pseudomonas species (7.5%) which is consistent with many other studies.[14,15] Our most common isolate *E. coli* was 52.53% the rate is less compared to western studies where it was 85%[16] but similar to other Indian studies.[14] Enterobacteriace have several factors responsible for their attachment to the uroepithelium. These bacteria colonize the urogenital mucosa with adhesin, pili, fimbriae and P1-blood group phenotype receptor making them the most commonly isolated organisms.[17]

Among gram positive isolates *Enterococcus sp.* is the most common isolated organism (5%), followed by Staphylococcus aureus (2.54%), which is consistent with other study.[11] However, studies from other parts of the country have shown different isolation rates, probably due to variation in geographical location or population.[4] While the study from north India done reported a higher prevalence of gram positive isolates (21.79%).[18] Non-fermenters are ubiquitous in the environment, able to survive in the hospital environment and can spread among hospitalized patients. They are emerging nosocomial pathogens especially in seriously ill patients and are responsible for causing a variety of infections. We have seen an upward trend in its isolation from 2012 to 2016

Antimicrobial resistance all over the world is on rise and isolates from our study were resistant to multiple drugs. Also resistance pattern varies with time which makes it difficult to start a desired treatment.[4] Most of the isolates were resistant to multiple antibiotics at our setting. *E.coli*

Table 4: Year-wise Overall Resistance pattern of uropathogens (in %)

Organism/antibiotics	2012	2013	2014	2015	2016	Average
			Resistance (%)			
Escherichia coli (2069)	366	414	355	420	514	
Ampicillin	97.27	95.17	93.52	93.57	89.49	93.80
Amoxyclav	76.78	73.91	90.99	96.90	95.14	86.74
3GC	75.14	76.57	81.41	78.57	75.49	77.43
Cotrimoxazole	61.75	68.84	61.97	62.14	57.98	62.53
Gentamicin	61.20	47.83	34.37	33.81	25.49	40.55
Amikacin	13.66	11.11	16.06	11.67	15.37	13.57
Ciprofloxacin	83.88	75.85	72.11	70.95	64.20	73.4
Piperacillin Tazobactum	0.00	9.90	56.34	62.14	64.9	38.65
Meropenem	0.00	0.00	22.25	43.8	46	22.41
Nitrofurantoin	30.33	23.43	22.25	20.00	41.83	27.56
Klebsiella sp (773)	114	164	125	173	197	
	79.82	76.83	91.20	94.80	100.00	88.53
3GC	74.56	70.12	80.80	72.83	73.10	74.28
Cotrimoxazole	71.05	59.76	61.60	55.49	54.31	60.44
Gentamicin	65.79	53.66	38.40	39.31	38.58	47.14
Amikacin	20.18	21.95	24.00	22.54	25.89	22.91
Ciprofloxacin	79.82	64.63	65.60	52.02	58.88	64.19
Piperacillin Tazobactum	0.00	23.78	67.20	43.93	39.5	34.88
Meropenem	0.00	0.00	29.60	22.54	23.35	15.09
Citrobacter sp (289)	65	51	57	48	68	
Ampicillin	96.92	94.12	100.00	95.83	95.59	96.49
Amoxyclav	70.77	70.59	91.07	97.92	98.53	85.77
3GC	66.15	66.67	91.07	89.58	80.88	78.87
Cotrimoxazole	66.15	62.75	57.14	60.42	55.88	60.46
Gentamicin	63.08	47.06	44.64	37.50	42.65	46.98
Amikacin	27.69	23.53	32.14	25.00	20.59	25.79
Ciprofloxacin	78.46	58.82	69.64	60.42	60.29	65.52
Piperacillin Tazobactum	0.00	13.73	67.86	43.75	77.94	40.65
Meropenem	0.00	0.00	7.14	10.41	50	13.51
Nitrofurantoin	52.31	52.94	57.14	60.42	54.41	55.44
Proteus sp (38)	4	11	5	9	9	
Ampicillin	75.00	100.00	100.00	77.78	66.67	83.89
Amoxyclav	25.00	81.82	100.00	44.44	100.00	70.25
3GC	75.00	36.36	80.00	44.44	33.33	53.82
Cotrimoxazole	50.00	45.45	80.00	77.78	44.44	59.53
Gentamicin	75.00	36.36	80.00	22.22	22.22	47.16
Amikacin	0.00	9.09	0.00	11.11	22.22	8.4
Ciprofloxacin	75.00	45.45	60.00	11.11	33.33	44.97
Piperacillin Tazobactum	0.00	0.00	60.00	11.11	22.22	18.66
Meropenem	0.00	0.00	0.00	0.00	11.11	2.22

Table 5: Year-wise Overall Resistance pattern of uropathogens (in %)

	Resistance (%)					
Organism/antibiotics	*2012*	*2013*	*2014*	*2015*	*2016*	*Average*
Pseudomonas sp (295)	41	63	49	57	85	
3GC	87.80	36.5	89.80	98.25	95.29	81.52
Cotrimoxazole	82.93	73.02	89.80	98.25	85.88	85.97
Gentamicin	56.10	60.32	44.90	38.60	37.65	47.51
Amikacin	14.63	36.51	22.45	26.32	34.12	26.80
Ciprofloxacin	75.61	61.90	61.22	45.61	48.24	58.51
PiperacillinTazobactum	0.00	12.70	63.27	70.17	84.71	46.17
Meropenem	0.00	0.00	17	35.08	44.71	19.35
Acinetobacter sp (106)	14	16	9	20	47	
Ampicillin	100.00	100.00	100.00	100.00	100.00	100
3GC	85.71	81.25	100.00	95.00	97.87	91.96
Cotrimoxazole	78.57	81.25	66.67	35.00	25.53	57.40
Gentamicin	85.71	68.75	44.44	20.00	31.91	50.16
Amikacin	42.86	37.50	44.44	15.00	23.40	32.64
Ciprofloxacin	78.57	75	77.78	75.00	72.34	75.73
PiperacillinTazobactum	0.00	25.00	55.56	75.00	80.85	47.28
Meropenem	0.00	0.00	11.11	50.00	38.30	19.88
Nitrofurantoin	71.43	62.50	66.67	75.00	55.32	66.18
Enterococcus sp (196)	20	50	27	54	45	
Ampicillin	35.00	56.00	48.15	42.59	53.33	47.01
Amoxyclav	30.00	44.00	100.00	98.15	51.11	64.65
Gentamicin	55.00	66.00	48.15	42.59	31.11	48.57
Ciprofloxacin	75.00	72.00	88.89	66.67	42.22	68.95
Staphylococcus sp (100)	14	10	9	30	37	
Ampicillin	35.71	60.00	88.89	96.67	89.19	74.09
Amoxyclav	28.50	20.00	66.67	83.33	83.78	56.45
3GC	7.14	20.00	33.33	83.33	97.30	48.22
Cotrimoxazole	21.42	30.00	44.44	33.33	32.43	32.32
Gentamicin	14.27	20.00	22.22	30.00	21.62	21.62
Amikacin	7.14	10.00	11.11	16.67	16.22	12.22
Ciprofloxacin	0.00	20.00	44.44	43.33	40.54	29.66

identified in this study were highly resistant to ampicillin < 93% and amoxyclav <85%). Studies that were conducted in India by Kothari A et al.[19] showed that, the isolates of *E.coli* showed high resistance towards Ampicillin, amoxycilav which is in agreement with our study. Enterobactericiae family also offered around 75% resistance towards 3 GC and fluroquinolones while comparitvely less resistance to amikacin and gentamycin 13% and 40%. Resistance to fluroquinolones showed a downward trend in last five years. Similar study from central Kerala also documented a decreasing trend over years.[20] In the present study, Amikacin and nitrofurantoin was found to be more sensitive among *E.coli*. This is similar to other study.[12] There was no resistance to carbepenem in the first two years which slowly increased and in 2016 it was 46%. Multiple mechanisms like production of beta-lactamases, blocking the entry of these antibiotics, or by efflux pumps which actively pump out these antibiotics, have led to rise in the resistance against carbapenems.[21] Carbapenems are often used as the last line of defense against resistant Gram-negative infections and resistance to it could result in higher cost, increased morbidity, mortality and prolonged hospitalization.

The rate of resistance to carbapenem among Gram-negative bacilli seen in our institution could possibly be due to the fact that this is a reference center, and that many of the patients had prior contact with other healthcare institutions and history of antibiotic use.

The limitations of the study were that it was a single centric, retrospective laboratory based and limited to the cases for which cultures were requested from the clinic. Information on antibiotics administered prior to culture or data on subsequent treatment and its outcome in this study population would have added meaningful data to allow a better understanding of the prevalent practice in diagnosis and treatment of UTI.

5. Conclusion

This retrospective study provided information on the common uropathogens and their drug resistance pattern. In this study, high prevalence of UTI was found in female gender and in geriatric patients. *E. coli* (53%) was the most common pathogen causing UTI. The uropathogens showed high levels of resistance to multiple urinary antimicrobial agents leading to limited option of treatment. Empirical Antimicrobials like ampicillin and amoxicillin have developed resistance to such a level that, prescribing them would definitely lead to treatment failure. In order to prevent development of resistance, antibiotic susceptibility patterns must be continuously and periodically evaluated to select the appropriate regimen to treat UTI and to avoid complications. Institutional Antibiotic policy can be tailored to achieve superior therapeutic outcome.

6. Source of Funding

None.

7. Conflict of Interest

None.

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