Emerging trends in the antimicrobial resistance among uropathogens

T. S. Shailaja[1], A. Mohan Kumar[2,*], Rajan J. Payyappilly[3]

[1]Professor & HOD, [2]Assistant Professor, [3]Professor & HOD, Dept. of Microbiology, [1,2]P. K. Das Institute of Medical Sciences, Vaniyamkulam, Palakkad, Kerala, India, [3]Academy of Medical Sciences, Pariyaram, Kannur Kerala, India

***Corresponding Author:**
Email: dkdrmogi@gmail.com

Abstract

Introduction: Antimicrobial resistance (AMR) is a global menace of recent times which does not spare the uropathogenic bacteria also. We aim to study the changes in bacteriological profile and antibiotic resistance pattern among the bacterial isolates of Urinary tract infections (UTI) in a multi-centric study from two different tertiary care hospitals located in rural parts of Kerala in two different time periods.

Materials and Methods: First study was conducted from Jul 2008 – Jun 2009 and the second from May 2016 - Apr 2017.

Results: Total numbers of urine samples processed in the first and second study are 3262 and 3772, of which 1096 and 1265 respectively showed significant bacteriuria. The predominant isolate is E.coli in both, with a percentage of 50.8 and 48.9 respectively followed by Klebsiella sp. and Enterococcus sp. There is a marked increase in the resistance rate of E.coli to cefotaxime, piperacillin tazobactam and imipenem. In case of first line drugs, there is a minimal fall in resistance rate except for ciprofloxacin. Similarly for aminoglycosides there is no significant increase in resistance.

Conclusion: The high prevalence of resistance to empirical drugs has long been appreciated in this area which precludes their use. Resistance to higher antibiotics also is on the rise. Very high rate of AMR is exhibited by the bacterial agents of UTI even in rural areas which necessitates routine culture and sensitivity mandatory in all cases. In order to stop this growing menace, judicious use of antibiotics has to be implemented.

Keywords: Antimicrobial resistance, Multicentric, Rural Kerala, Trends, Uropathogens.

Introduction

Antimicrobial resistance (AMR) is one of the major threats in clinical practice due to the injudicious and widespread use of antimicrobial drugs in general population. Similar to other clinical conditions, the bacterial agents of Urinary tract infections (UTIs) also are exhibiting high rates of drug resistance as reported in various Indian and international studies. In a study by Daniel F. Sahm et al from US in 2000, the resistance rates against empirical drugs were 39% to ampicillin, 15.6% to cephalothin, 3.7% to ciprofloxacin, 1% to nitrofurantoin and 18.6% to co-trimoxazole.[1] Whereas in another study conducted from Pune, India during 2015 -16 period, resistance of Gram negative bacteria to ampicillin, norfloxacin, co-trimoxazole, and nitrofurantoin were 98%, 94%, 79% and 14.7% respectively.[2] From this it is evident that antibiotic resistance is markedly increasing over the years, which needs to be studied periodically. Though there are a few international studies which describe the changing pattern of AMR among uropathogens, with the best of our knowledge only limited studies have been carried out in India.

In the present multi-centric study, we are trying to find out the emerging trends in resistance pattern among the bacterial agents of UTI from two different super specialty hospitals which are located in rural areas of Kerala.

Materials and Methods

First study is a retrospective study conducted in Academy of Medical Sciences, Pariyaram which is located in North Kerala from Jul 2008 – Jun 2009 and the second is a prospective one from PK Das Institute of Medical Sciences, Ottapalam which is in central Kerala from May 2016 - Apr 2017 and both the centers have got a rural location. In both the studies, we analyzed all urine culture samples and significant bacterial isolates from department of microbiology over a period of one year. A total of (3262 & 3772) 7034 samples from clinically suspected cases of UTI, both hospitalized and out patients, irrespective of their age and sex were included in the study. Most of them are clean voided mid stream samples and the rest, catheter samples and supra pubic aspirates. All samples are processed within 2 hours of collection. Microscopy and culture on Mac Conkey agar and 5% sheep blood agar are done. Significance of the isolates was assessed on the basis of colony count ($>10^5$ cfu/ml) and the clinical features. Colony counts $<10^5$ also are considered significant on an individual basis by correlating the microscopic findings, type of specimen and the clinical history.

Identification of the isolates is done by standard bacteriological methods.[3] Antibiotic susceptibility testing of the isolates is performed by Kirby-Bauer disc diffusion method on Mueller Hinton Agar as per CLSI guidelines[4] using Hi Media, India antibiotic discs. Antibiotic discs used are ampicillin (10µg), norfloxacin (10µg), ciprofloxacin (5µg), nitrofurantoin (300µg), co-

trimoxazole (1.25/23.75µg), cephalothin (30µg), cefuroxime (30µg), cefotaxime (30µg), ceftazidime (30µg), gentamicin (10µg), piperacillin (100µg), amikacin (30µg), amp-sulbactam (10/10µg), piperacillin-tazobactam (100/10µg), imipenem (10µg), meropenem (10µg), high level gentamicin (120µg), tetracycline (30µg), cefoxitin (30µg), vancomycin (30µg), linezolid (30µg).

Using CLSI phenotypic confirmatory test, ESBL production is checked with cefotaxime & cefotaxime clavulanate disc among the members of Enterobacteriaceae. Quality control is performed by standard strains, *E.coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923.

Analysis of the isolates based on hospital status of the patients is done in both studies because marked variation in the distribution of bacterial agents and their resistance pattern is observed between admitted and out patients.

Results

During the one year study period from Jul 2008 – Jun 2009, 3262 samples were subjected to culture and microscopy of which 1096 (33.6%) gave significant bacterial growth. Among the 3772 urine samples processed from May 2016 - Apr 2017, significant bacterial growth is obtained from 1265 (33.5%) samples.

E.coli stands as the most common isolate in both 2008 and 2016 studies. Not much difference is observed among the rate of isolation of other bacterial pathogens also in both the studies (Fig. 1)

In both the studies more number of isolations are from admitted patients and predominance of Gram positive cocci, *Pseudomonas* and other NFGNB is also noted among them compared to outpatients (OP). Distribution of members of Enterobacteriaceae is also similar in both time periods with predominance in OP (Table 1.).

In the 2016-17 study, 64.7% of Enterobacteriaceae are ESBL producers and 15.75% are carbapenem resistant. Of the Staphylococcal isolates, 79.5% is methicillin resistant. Vancomycin resistance is 0.65% in *Enterococci* and 0% among *Staphylococci*. Whereas 66.3% of staphylococcal isolates from the first study is found to be methicillin resistant and no case of vancomycin resistance is observed among *Staphylococci* and *Enterococci*. 42.75% of *E.coli* and *Klebsiella* isolates from 2008- 09 study are ESBL producers. (Table 2.)

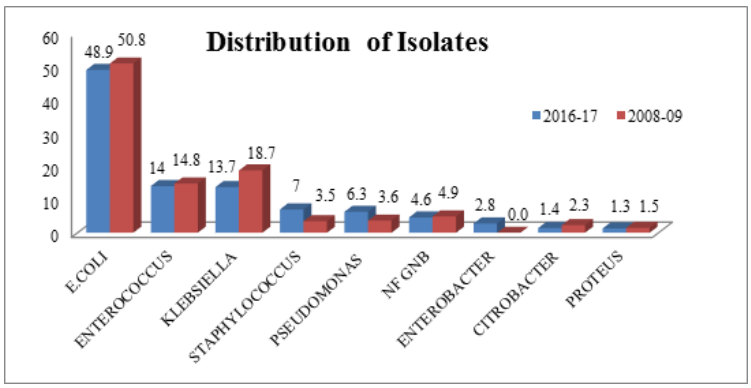

Fig. 1: Distribution of bacterial agents

Table 1: Distribution of bacterial agents according to the hospital status of patients

Organism	2008-09		2016-17	
	OP	IP	OP	IP
E.coli	52	49.8	57.7	43.5
Klebsiella sp	19.3	18.2	16.5	11.9
Proteus sp	2.7	0.5	1.4	1.2
Citrobacter sp	1.9	2.6	2.5	0.8
Enterobacter sp	0	0	3.5	2.4
Pseudomonas sp	2	4.9	3.9	7.8
Other NFGNB	4.2	5.5	3.1	5.5
Enterococcus sp	13.9	15.4	7.4	18.1
Staphylococcus sp	4	4	3.9	8.8
Total	43.8	56.1	38	61.6

Table 2: Antibiotic resistance pattern of isolates

Organism	Study	AMP	CEP	CIP	COT	NIT	AK	GEN	CAZ	A/S	PIT	IPM
E.coli	2008	96	89	64	61	15	7	49	47	44	3	2
	2016	91.1	83.7	66.4	58.8	17.8	7.8	35.1	70.3	75	25.5	7.9
Klebsiella sp	2008	97	77	46	61	55	17	55	43	48	11	2
	2016	99.4	82.1	59.5	64.2	75.1	26	49.1	67.1	83.2	45.1	17.3
Pseudomonas sp	2008	ND	ND	57	ND	ND	44	54	50	ND	21	5
	2016	80	78.8	52.5	100	77.5	40	47.5	52.5	80	33.8	25
Other NFGNB	2008	ND	ND	71	60	66	30	46	4	39	4	54
	2016	100	100	60.3	46.1	93.1	63.8	62.1	74.1	55.2	48.3	50
Enterococcus sp	2008	66	ND	65	58	31	ND	ND	ND	51	18	24
	2016	35	ND	78	80.2	18.1	59.3	55.9	ND	52	35	ND
Staphylococcus sp	2008	83	72	64	58	14	37.5	ND	ND	35	ND	ND
	2016	96.6	81.8	73.9	44.3	0	71.6	55.7	79.5	83	81.8	ND

Discussion

Due to the injudicious use of common antibiotics in the therapy of UTI, especially fluoroquinolones, uropathogens are increasingly becoming multi drug resistant (MDR) making the empiric treatment of all forms of UTI highly difficult.

When we analyze the distribution of pathogens in both the studies, no significant change is noted over the years. *E.coli* comes to 50% of total isolates in both 2008 and 2016 studies and is the most frequent one. Both studies show more number of isolates from hospitalized patients which represent more of complicated UTI cases than OP.

Complicated UTI is defined as an infection associated with a structural or functional abnormality of the genitourinary tract or the presence of an underlying disease. Here the spectrum of bacteria that can cause infections includes more of non-fermenters (*Pseudomonas sp*.) and Gram- positive bacteria. Furthermore, bacteria causing these types of infection are more likely to be resistant to antibiotics. In our studies also we could demonstrate more number of similar bacterial isolates among admitted patients.

E.coli is the predominant isolate in both OP and inpatients (IP) in 2008 & 2016 studies followed by *Klebsiella* and *Enterococci* with a percentage of 14. In contrary to Europe and US, rate of isolation of *Klebsiella* is more in other Asian and African countries which ranges from 13.8 to 25.5 %.[5-9]

In the study conducted during 2008-09 period itself, bacterial agents of UTI exhibited very high level of resistance against the first line drugs like ampicillin (85.5%), co-trimoxazole (60%), norfloxacin (62%), ciprofloxacin (61%) and cephalothin (66%). The high prevalence of resistance to empirical drugs has long been appreciated in this area and precludes their use in empirical therapy. In the study conducted in 2016-17, resistance of empirical drugs except ciprofloxacin has reduced a little which could be explained by the reduced use of these agents subsequent to the high level of resistance prevailing in the area. A study from Canada also reported a relatively steady resistance rate of 17.7% and 19.1% for co-trimoxazole among *E.coli* during 1998 to 2009.[10] Similarly in a study from Guadeloupe, France, also resistance rates for amoxicillin, co-trimoxazole and amikacin have been stable during 2003 -2014. On the contrary, they reported a significant increase in the resistance to ciprofloxacin.[11]

In a study on community acquired UTI in Europe during 2007, *E.coli* resistance to mecillinam, cefadroxil, nitrofurantoin, gentamicin and third-generation cephalosporins was <2% and resistance levels for amoxicillin/ clavulanic acid was 2.0-8.9%, ciprofloxacin 0.5-7.6%, ampicillin 21.2-34.0%, and trimethoprim/sulfamethoxazole 14.4-18.2%.[12] From the above data one can appreciate the wide disparity in the resistance patterns between India and Europe.

As per infectious disease society of America (IDSA), fluoroquinolones for 7–10 days is recommended as first-line initial therapy for uncomplicated pyelonephritis in areas where the resistance rate of E.coli is <10%.[13] The mean resistance of ciprofloxacin for *E.coli* in the present study is 65% which precludes the use of fluoroquinolones in the empirical therapy of UTI in Kerala.

Among the first line drugs, nitrofurantoin alone maintains a reasonably low resistance rate, 15 and17.5% among *E.coli* in 2008 and 2016 respectively and in other Indian studies, resistance rates among *E.coli* range from 5% to 24.4%.[14-17] According to other Indian authors, resistance rate is 6–17% for *Enterococcus* sp.[14,17] whereas in our studies it is 31 & 18.1% respectively. The major strength of nitrofurantoin is its action at multiple sites which includes inhibition of bacterial carbohydrates, DNA, RNA, and total protein synthesis. It is active against most common uropathogens including *E.coli, Citrobacter* spp., *Staphylococcus sp.* and *Enterococcus* sp. whereas, *Enterobacter* sp. and *Klebsiella* spp. are only moderately inhibited and *Proteus* group, *Serratia* spp., *Pseudomonas* spp., and *Acinetobacter* spp. are

mostly resistant.[18] IDSA recommend the use of nitrofurantoin for uncomplicated UTIs due to the high urinary concentration achieved. For complicated UTI where plasma and tissue concentration is also important, nitrofurantoin cannot be recommended because of inadequate tissue concentration.

While there was a significantly increasing trend for amikacin resistance in *Klebsiella*, the mean resistance in *E.coli* in 2016 is 7.8% in contrast to 7% in 2008 which correlates with the European study where the mean aminoglycoside resistance in *E.coli* showed no significant change between 2011 and 2014, in contrast to *Klebsiella.*[13]

Unlike the first line drugs, a marked increase in resistance to third generation cephalosporins, beta lactam- beta lactamase inhibitor combinations and carbapenems is noted in our study which correlates well with a report on current European trends of resistance in Gram-negative uropathogens where mean resistance for third generation cephalosporin increased significantly between 2011 and 2014.[13] In the second study, among the members of Enterobacteriaceae isolated, 64.7% are ESBL producers and 15.75% were carbapenem resistant whereas in 2008 it was only 42.7% and 2 % respectively. A recent study from southern part of Kerala also shows a high rate of ESBL production of 38.2%.[19] The prevalence of Carbapenem resistant Enterobacteriaceae in a Mumbai hospital was found to be 12.26% in 2013.[20] In contrary, an annual Canadian national surveillance study (CANWARD) which tested 2,943 urinary pathogens from January 2007 to December 2009, ESBL production was only 1.5% among Enterobacteriace.[10] A significantly increasing trend for carpabenem-resistant *Klebsiella* was observed by Hala et al from Europe.[13]

Among the Staphylococcal isolates, 66.3 and 79.5% are MRSA from 2008 and 2016 studies respectively. A marked fall in ampicillin resistance is observed from 66% in 2008 to 35% in 2016 among enterococcal isolates which can be explained by the reduced use of ampicillin in clinical practice due to the prevailing high resistance. Vancomycin resistance is not exhibited by any of our staphylococcal isolates and a minimal rise in VRE is observed in the study.

The isolates that are tested in a laboratory based study may be mostly from previous antibiotic treatment failed or from patients with other underlying risk factors. Hence this study, like any other laboratory based study, may bias towards an over reporting of resistance in patients with UTI and necessitate more patient based studies in this area.

Conclusion

Over a period of 8years, not much change in the distribution of bacterial agents of UTI is observed in this area. The emergence of antibiotic resistant bacteria generally has been correlated with the rise and fall of antibiotic use in clinical practice. From the present study, we could realize that high level of resistance against first line drugs used in the treatment of UTI has been prevailing in Kerala for several years and the resistance against the higher agents like third generation cephalosporins, beta lactam – beta lactamase inhibitor combinations and carbapenems are also increasing in an alarming rate even in rural settings. The possible explanation for such a high level of resistance is the uncontrolled antibiotic prescribing practices of our region. Antibiotics are a precious commodity, and we should do what we can to preserve the activity of these, to use in clinical practice.

References

1. Daniel F. Sahm, Clyde Thornsberry, David C. Mayfield, Mark E. Jones, and James A. Karlowsky, Multidrug-Resistant Urinary Tract Isolates of *Escherichia coli:* Prevalence and Patient Demographics in the United States in 2000. Antimicrob Agents Chemother. (2001) 45(5): 1402–1406.
2. Dnyaneshwari Puroshottam Ghadage, Vrishali Avinash Muley, Jyotika Sharma, Arvind Vamanrao Bhore, Bacteriological Profile and Antibiogram of Urinary Tract Infections at a Tertiary Care Hospital. National Journal of Lab Med. (2016) 5(4): MO20-MO24.
3. Collee JG, Fraser AG, Marmion BP, Mackey SA, McCartney (2006) Practical Medical Microbiology. Tests for the identification of Bacteria, In: Collee JG, Miles RS, Watt B, editors. 14th ed. New Delhi, India: Elsevier; p.131-49.
4. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 26th ed. CLSI Supplement M100S. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
5. Lu PL, Liu YC, Toh HS, Lee YL, Liu YM, Ho CM, et al, Epidemiology and antimicrobial susceptibility profiles of Gram negative bacteria causing urinary tract infections in the Asia-Pacific region: 2009–2010 results from the Study for Monitoring Antimicrobial Resistance Trends (SMART). Int J Antimicrob Agents. (2012) 40:S37–43.
6. Hanley J, Branford I, Gugnani HC, Wilkinson C, Uhrin T, urinary bacterial pathogens and their antimicrobial susceptibility profile for the years 2005-2007 in St. Kitts. West Indies Med J. (2009) 58 (6) 571-574.
7. Kothari A, Sagar V. study, Antibiotic resistance in pathogens causing community acquired urinary tract infections in India: a multicenter. J Infect Dev Ctries. (2008) 2(5):354–8
8. Akoachere JF, Yvonne S, Akum NH, Seraphine EN, Etiologic profile and antimicrobial susceptibility of community-acquired urinary tract infection in two Cameroonian towns. BMC Res Notes (2012). 5:219.
9. Kalpana G, Thomas MH, Kurt GN, et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the infectious diseases society of America and the European society for microbiology and infectious diseases. *Clinical Infectious Diseases*, (2011) 52(5): e103–e120.
10. James AK, Philippe RS. L-W, Patricia JS, Melanie RD, Heather JA, Andrew W, et al. Antimicrobial Resistance in Urinary Tract Pathogens in Canada from 2007 to 2009: CANWARD Surveillance Study. Antimicrobial agents and chemotherapy. (2011) 55(7): 3169–75.
11. Stephanie G R, Joyce M, Celia D, Syndia S, Melanie F, Dorothee H, et al. Temporal trends and risks factors for

antimicrobial resistant Enterobacteriaceae urinary isolates from outpatients in Guadeloupe. BMC Microbiology. (2016) 16:121.
12. Kahlmeter G, Poulsen HO, Antimicrobial susceptibility of Escherichia coli from community-acquired urinary tract infections in Europe: the ECO·SENS study revisited. Int J Antimicrob Agents. (2012) 39(1):45-51
13. Hala K, Emma C, Tejal V, Trends in Antibiotic Resistance in Urologic Practice. European urology focus; (2016) 2(4): 363 –73.
14. Kaur N, Sharma S, Malhotra S, Madan P, Hans C, Urinary tract infection: Aetiology and antimicrobial resistance pattern in infants from a tertiary care hospital in northern India. J Clin Diagn Res, (2014) 8(10):DC01-03.
15. Niranjan V, Malini A, Antimicrobial resistance pattern in *Escherichia coli* causing urinary tract infection among inpatients. Indian J Med Res; (2014) 139(6):945-8.
16. Shaifali I, Gupta U, Mahmood SE, Ahmed J, Antibiotic susceptibility patterns of urinary pathogens in female outpatients. N Am J Med Sci, (2012) 4(4): 163-9.
17. Sahni RD, Balaji V, Varghese R, John J, Tansarli GS, Falagas ME, Evaluation of fosfomycin activity against uropathogens in a fosfomycin-naive population in South India: A prospective study. Future Microbiol. (2013) 8(5): 675-80.
18. Laishram S, Balaji V, Advantage and limitations of nitrofurantoin in multi-drug resistant Indian scenario. Ind J of Med Microbiol; (2015) 33(4): 477-481.
19. Anitha Madhavan, V Jayalakshmi, Occurrence of extended-spectrum beta-lactamase, AmpC and MBLase producers among multidrug-resistant Enterobacteriaceae causing urinary tract infection in a tertiary health-care teaching hospital. JACM**;** (2016) 18(2):80.
20. Pravin KN, Michelle SV, Prevalence of carbapenem resistant Enterobacteriaceae from a tertiary care hospital in Mumbai, India. Journal of Microbiology and Infectious Diseases; (2013) 3 (4): 207-210.

How to cite this article: Shailaja TS, Kumar AM, Payyappilly RJ. Emerging trends in the antimicrobial resistance among uropathogens. Indian J Microbiol Res 2018;5(1):47-51.