ISSN 2394–806X (Print), ISSN 2454-5139 (Electronic)
IJHRMLP, Vol: 04 No: 01 January, 2018
Printed in India

ORIGINAL PAPER

Prevalence and antibiogram of uropathogens in a tertiary care hospital in Manipur, India

Devi Bishwabati Yumlembam[1], Laifangbam Supriya[2], Singh Rajkumar Manojkumar[3], Huidrom Smeeta[4], Singh Huidrom Lokhendro[5]

***Received** on September 15, 2017; **editorial approval** October 20, 2017*

ABSTRACT

***Introduction**: Due to high variability of resistance patterns which vary even over short periods of time, periodic evaluation of such activity is essential. **Aim**: To document the prevalence, antibiotic susceptibility and resistance patterns of uropathogens in the area to ensure appropriate therapy. **Methods**: This is a cross sectional study on 5108 urine samples from June 2014 to May 2015. Antibiotic susceptibility testing was done by Kirby- Bauer disc diffusion method and compared. Gram positive cocci and Gram negative bacilli detected were further subjected to detection of Methicillin resistant Staphyloccocussureus (MRSA) by Cefoxitin disc diffusion tests and detection of Extended Spectrum Beta lactamases (ESBL) by ESBL screening test and confirmed by combined disc diffusion test(CDT). **Results**: Out of 5108 samples subjected to bacterial culture, 2940(57.56%) showed positive growth out of which Escherichia coli, 1300(44.2%) was the most common organism isolated followed by Staphylococcus aureus 914(31%), Klebsiella pneumonia 362(12.3%), Enterococcus 172(5.85%), Acinetobacter 80(2.72%), Pseudomonas 72(2.44%), Proteus 40(0.81%), and Staphylococcus saprophyticus 16(0.54%). On further evaluation, 1026(56.06%) isolates of Gram negative bacilli(GNB) family showed presence of ESBL and 722(78.99%) isolates of Staphylococcus aureus were positive for (MRSA)tests. **Conclusion**: Continual surveillance is required to detect changes in prevalence rates of different uropathogens. Increased prevalence of Acinetobacter was detected in our study. Monitoring of MRSA, ESBL production and antimicrobial susceptibility testing (AST) is necessary to avoid treatment failure and development of further resistance in patients with UTI.*

***Keywords**: Urinary tract infection, Acinetobacter*

INTRODUCTION

Despite the widespread availability of antibiotics, urinary tract infections (UTIs) represent one of the most common diseases encountered in medical practice today, with an estimated 150 million UTIs per annum worldwidewith a lifetime risk greater than 50% in females.[1, 2] More than 95% of UTIs are caused by a single species out of which Escherichia coli was the most frequently associated bacteria in both the community and hospital acquired cases.[3] Other Gram negative bacteria isolated includes Klebsiella, Proteus, Pseudomonas and Enterobacter. Gram positive bacteria account for 5 to 10% of UTIs and include Staphylococci, Streptococci and Enterococci.[4]

Pathogenic organisms show highly variable patterns of resistance over short durations depending on different regions and sites of isolation of the organisms.[5] The present study was carried out to generate and update this information.

METHODS

5108 consecutive urine samples were studied from both outpatients and inpatients of all age groups irrespective of sex, religion and socio-economic status with symptoms suggestive of UTI. It was a prospective study done for a 1 year period from June 2015-May 2016 with approval from the Institutional Ethics Committee.

Address for correspondence:
[1]Post graduate Trainee
Mobile: +919436899218
Email: dr.bishwayumlem@gmail.com
[2]Associate Professor (**Corresponding Author**)
Email: slaifangbam@gmail.com
[3]Associate Professor,[4]Associate Professor,[5] Professor and Head
Department of Microbiology,Jawaharlal Nehru Institute of Medical Sciences (JNIMS), Imphal East-05

Samples were collected and processed immediately.[6] Samples with significant pyuria were subjected to culture by semi-quantitative method.[7] Significant bacteriuria were determined and organisms were identified by their colony morphology, Gram stain and biochemical reactions adopting standard methodology.[8] Antibiotic susceptibility testing (AST) was done as per CLSI recommendations.[9]

All isolates of S. aureus were tested for presence of Methicillin Resistant S.aureus (MRSA) strain by cefoxitin disc diffusion method. A standard strain of Methicillin sensitive Staphylococcus aureus (MSSA) ATCC 29213 was used as control strain.[10]

All Gram negative bacilli were further subjected to detection of Extended spectrum Beta-lactamases (ESBL) by CLSI recommended ESBL screening test.[9] Potential ESBL isolates were then put up for Combined disk test (CDT) for confirmation.[11]

RESULTS

Of the 5108 consecutive urine samples, 3356 were found to contain significant bacteriuria and were included in the study of which 2940 showed positive growth, 384 showed insignificant growth and 32 were declared contaminated due to growth of more than two organisms seen after overnight incubation at 37°C. Out of 2940 positive growth samples, 971 (33.02%) belonged to IPD (In Patient Department) and 1969(66.97%) to OPD (Out Patient Department). Among the IPD patients, maximum urine samples were received from Gynaecology and Obstetrics Department followed by Surgery and Medicine along with their Allied Departments respectively.

Out of a total of 2940 urine samples showing positive growth, 2323(79.01%) samples were from females, thus showing a female predominance, of which the highest isolation rate was found in the (21-30) years age group with 775 samples showing positive growth, thus revealing the increased vulnerability of the reproductive age group to UTIs. Amongst the males, maximum samples with positive growth were seen in patients in the age group of 61-70 years old. The male: female ratio is 1:3.8.

Table 1 shows the distribution of bacterial isolates with Escherichia coli, 1300(44.2%) being the most common organism isolated irrespective of gender or age group followed by Staphylococcus aureus 914(31%), Klebsiella pneumonia 362(12.3%), Enterococcus 172(5.85%), Acinetobacter 80(2.72%), Pseudomonas 72 (2.44%), Proteus 40(0.81%), and Staphylococcus saprophyticus 16(0.54%).

Table 1 Gender wise distribution of bacterial isolates

Organisms	Male	Female	Total (%)
Esherichia coli	339	961	1300 (44.2%)
Staphylococcus aureus	82	832	914 (31%)
Klebsiellapneumonia	68	294	362 (12.3%)
Enterococcus	44	128	172 (5.85%)
Acinetobacter	24	16	80 (2.72%)
Pseudomonas	44	68	72 (2.44%)
Proteus	12	12	24 (0.81%)
Staphylococcus saprophyticus	4	12	16 (0.54%)
TOTAL	617	2323	2940 (100%)

Table 2 Drugs sensitivity pattern of Gram Negative Bacilli

Antibiotic	Escherichia coli,n(%)	Klebsiellapneumoniae, n (%)	Proteus,n(%)	Acinetobacter, n(%)	Pseudomonas aeruginosa,n(%)
Ceftazidime(30µg)	483(37.15%)	148(40.88%)	20(83.33%)	60(75%)	42(58.33%)
Cefotaxime(30µg)	459(35.30%)	145(40.05%)	19(79.16%)	54(67.5%)	41(56.94%)
Ceftriaxone(30µg)	442(34%)	143(39.50%)	19(79.16%)	52(65%)	41(56.94%)
Cefpodoxime(10µg)	445(34.23%)	147(40.60%)	18(75%)	52(65%)	39(54.16%)
Aztreonam(30µg)	518(39.84%)	153(42.26%)	20(83.33%)	53(66.25%)	42(58.33%)
Ceftazidime + Clavulanate(30/10µg)	794(61.07%)	255(70.44%)	21(87.5%)	54(67.5%)	44(61.11%)
Imipenem(10µg)	1300(100%)	304(83.97%)	22(91.66%)	64(80%)	66(91.66%)
Nitrofurantoin(300µg)	1139(87.61%)	293(80.93%)	11(45.83%)	ND*	ND*
Norfloxacin(10µg)	640(49.23%)	181(50%)	18(75%)	ND*	39(54.16%)
Gentamicin(10µg)	1042(80.15%)	362(100%)	11(45.83%)	31(38.75%)	29(40.27%)
Ciprofloxacin(10µg)	ND*	ND*	ND*	35(43.75%)	53(73.61%)
Ampicillin(10µg)	ND*	ND*	ND*	31(38.75%)	ND*
Piperacillin + Tazobactum(30/10µg)	1189(91.46%)	341(94.19%)	24(100%)	69(95.83%)	64(88.88%)
Amikacin(30µg)	1105(85%)	328(90.60%)	24(100%)	36(45%)	65(90.27%)

**Not Done*

On the other hand, Gram positive bacteria included in the study showed maximum sensitivity withVancomycin and Linezolid for Staphylococcus aureus, Linezolid for Enterococcus and Nitrofurantoin, Linezolid and Vancomycin for Staphylococcus saprophyticus.

Table 3 Antibiotic sensitivity of Gram Positive Bacteria

Antibiotics	S. aureus	Enterococcus	S. saprophyticus
Norfloxacin(10µg)	65(7.11%)	112(65.11%)	10 (62.5%)
Nitrofurantoin(300µg)	716(78.33%)	155(90.11%)	16(100%)
Ampicillin(10µg)	114(12.47%)	14(8.13%)	8(50%)
Cotrimoxazole(25µg)	365(39.93%)	ND*	7(43.75%)
Cefoxitin(30µg)	173(18.92%)	ND*	ND*
Clindamycin(2µg)	498(54.48%)	ND*	11(68.75%)
Vancomycin(30µg)	914(100%)	164(95.34%)	16(100%)
Gentamycin(10µg)	724(79.21%)	ND*	13(81.25%)
Gentamycin(120µg)	ND*	130(75.58%)	ND*
Linezolid(30µg)	914(100%)	170(98.83%)	16(100%)
Fosfomycin(50µg)	712(77.89%)	130(75.58%)	13(81.25%)
Ciprofloxacin(5µg)	372(40.70%)	24(13.95%)	7(43.75%)

**Not Done*

Out of 914 staphylococcus aureus isolated, 722(78.99%) showed resistance to cefoxitinand were, therefore, positive for the detection of methicillin resistant staphylococcus aureus (MRSA) strain.

All enterobacterioceae isolates were subjected to ESBL screening and confirmatory test by combined disc test (CDT). Out of a total of 1830 Gram negative bacilli, 1407 isolates were positive in the ESBL screening test and 1026(56.06%) isolates were confirmed positive by combined disc test.

Table 4 Results for ESBL screening and confirmatory test

Organism	No of organism isolated (n)	ESBL results	
		Screening,n(%)	CDT,n(%)
Escherichia coli	1300	913	768(59.07%)
Klebsiellapneumonia	362	247	206(56.90%)
Proteus	24	10	3(12.5%)
Acinetobacter	72	32	15(20.83%)
Pseudomonas	80	46	34(42.5%)
TOTAL	1830	1407	1026(56.06%)

DISCUSSION

UTI is emerging as an important bacterial infection both in the community as well as amongst hospital acquired infections.[12] It is documented to be more common in females; our study is rightly in agreement with this generalization. This can be attributed to the fact that females become more susceptible to UTIs after the age of 6 months due to their shorter urethra thus providing easy access of bacteria to the bladder.[13]

This study was done to generate data on the etiologic agents causing UTI and their AST patterns in relation to different factors such as age and gender in the region. By using this database, we have made an attempt to define the population that is most amenable to empirical therapy. However, it is to be noted that the safety and efficacy of such empirical therapy depends upon periodic assessment of antimicrobial resistance profiles.

A comparison of the results of our study with the resistance rates previously published in this region showed a broadly similar picture but with a few exceptions.[14]

Escherichia coli (44.2%) was the most common organismidentified in our study as observed in other studies from other parts of India and also from different countries across the world such as Israel, Iran, Kuwait, Nigeria, Britain and USA.[15]

Due to their widespread indiscriminate use, easy availability, and over the counter sale, drug resistanceamong uropathogens has increased over the past few decades which are heading us toward the use of higher spectrum antibiotics. Hence, the magnitude of this problem should be assessed properly in an accurate way.[16]

In the present study, maximum isolates of Escherichia coliwere sensitive to Imipenem. Among the Enter obacteriaceae, majority of the isolates have shown high resistance to cephalosporin which was once a commonly used drug for UTI but, this broad spectrum molecule has almost entirely lost its efficacy due to lack of rational use.[17]Amongst all antimicrobials used, nitrofurantoin was found to be a reasonably efficacious agent against almost all uropathogens in our study and similar results were also reported from other studies.[18]

Amongst the gram positive organism, the most common organism isolated was Staphylococcus aureus which showed maximum sensitivity to vancomycin and linezolid. This is in concordance with other studies.[19]

In the present study, maximum numbers of non-fermenters were reported from Acinetobacter species followed by Pseudomonas species which is in contrast with other studies showing Pseudomonas as the leading non-fermenting bacteria. Most of

the Acinetobacter cases were isolated in the months of June and July correlating with the fact that Acinetobacter shows increased prevalence during hot and humid climates. Further evaluation was done to determine the factors associated with this recent increase in Acinetobacter speciesin our institute. It was noted that, at the time of infection, 46 patients were from various ICUs, 23 patients were from medicine wards and 11 patients were from surgery wards. The high incidence seen in the ICUs leads us to suspect the possibility that a minor epidemic might have occurred during these months. Out of a total of 23 patients from medicine wards, 16 patients were HIV positive and were on ART treatment and 3 patients were diabetic. Of the 11 patients from surgery wards, 5 patients had urinary catheters and 1 patient was on mechanical ventilation after a prior surgery. All of these factors hintat an increased prevalence of Acinetobacter infection in an immuno compromised host. Another important contributing factor is irregular infection control practices at present in the institute as ours is primarily a new medical college and Hospital Infection Control Committee (HICC) though established is not fully functional.[20]

Conventional antimicrobials are usually ineffective against Pseudomonas infections. In our study, it showed maximum sensitivity to Imipenem. In the recent years, carbapenems are being used widely for Pseudomonad infections; we recommend that its use should be restricted to special circumstancesin order to preserve its long term efficacy.[21]

The overall MRSA prevalence in our study was 78.99% which is comparable to a study done in the area.[24] In our study, MRSA detection was done by Cefoxitin Disk test as cefoxitin disk diffusion zones are much easier to read compared to oxacillindisk which gives hazy zones frequently and can be commonly misinterpreted. Also, oxacillinshould be read using transmitted light, unlike cefoxitin, to ensure correct interpretation.[25]

ESBL production, Amp C production, reflux mechanism and porin deficiency are the different mechanisms of drug resistance in GNB among which ESBL production is the most common. In our study, 56.06% of gram negative bacilli were ESBL producers, which is in contrast with studies of Rekha *et al*. (30%) but is comparable to Anup Saha et al.[21, 22]

We appreciate some shortcomings of our work as it lacks clinical information. This study was based on laboratory data only so we failed to provide information on categorization of UTI patients into symptomatic or asymptomatic and complicated or uncomplicated.

CONCLUSION

Although the etiologic pathogens of UTI remain same over the years, their prevalence and resistance rates through different mechanisms such as MRSA and ESBL production are ever changing as in the case of Acinetobacter in our study. Therefore continuous monitoring is critical to generate local population specific data to choose appropriate empiric pharmacotherapy for UTI.

Acknowledgement: Authors are profoundly thankful to Microbiology Department, JNIMS and its skilful technical staffs.

Conflict of interest: None.

Ethical clearance: Obtained from JNIMS Ethical Committee.

Source of funding: None.

Authors' contribution: (i) We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. It does not infringe any copyright or violate any other right of any third parties, (ii) Dr. Yumlembam Bishwabati Devi- Performed the laboratory tests and drafted the manuscript (iii) Dr. Supriya Laifangbam- Conceived the idea and contributed for intellectual content and scientific revision of the manuscript, (iv) Dr. Rajkumar Manojkumar Singh- Scientific revision of the manuscript, (v) Dr. Smeeta Huidrom- Scientific revision of the manuscript, (vi) Dr. Lokhendro Singh- Scientific revision of the manuscript.

The article has not been published (whole or in part) else where and is not being considered for publication elsewhere in any form, except as provided here in. All authors have contributed sufficiently in the article to take public responsibility for it and all authors have reviewed the final version of the above manuscript and approve it for publication.

REFERENCES

1. Stamm WE, Norrby SR. Urinary tract infections: disease panorama and challenges. J Infect Dis 2001;183: S1-S4.
2. Daoud Z, Sokhn ES, Mastri K, Cheaito K, Haidar-Ahmad N, Matar GM, et al. Escherichia coli isolated from urinary tract infections of lebanese patients between 2005 and 2012: Epidemiology and Profiles of Resistance. *Front Med (Lausanne)* 2015;2(66*)*.
3. Shaaban MT, Ghozlan HA, El Maghraby MM. Susceptibility of bacteria infecting urinary tract to some antibiotics and essential oils. *Journal of Applied Pharmaceutical Science* 2012;02(04):90-98.
4. Gupta V, Yadav A, Joshi R M. Antibiotic resistance pattern in uropathogens. *Indian J Med Microbiol* 2002;20;96-8.
5. Colle JG, Dugoid JP, Fraser AG. Laboratory strategy in the diagnosis of infective syndrome. In: Colle JG, Dugoid JP, Fraser AG, Editors. Mackie and Mc-Cartney Practical Medical Microbiology 14thed. London:Churchill Livingstone; 1996.p.53-94.
6. Hanna-Wakim RH, Ghanem ST, El Helou MW, Khafaja SA, Shaker RA, Hassan SA, et al. Epidemiology and characteristics of urinary tract infections in children and adolescents. Front Cell Infect Microbiol 2015;5:45.
7. Koneman EW, Allen SD, Janda WM, Schreckenberger PC. The Enterobacteriaceae. In: Koneman's color atlas and textbook of diagnostic microbiology. 6thed. Philadelphia PA: Lippincott Williams and Wilkins; 2006.p. 53-94.
8. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J ClinPathol* 1966;45:493-6.
9. Clinical Laboratory Standards Institute. Performance Standard for Antimicrobial Susceptibility Testing. NCCLS

Document 2013;23 Suppl:M100-S23.

10. Jain A, Agarwal A,Verma RK. Cefoxitin disc diffusion test for detection of methicillin-resistant staphylococci. *Journal of Medical Microbiology* 2008;57;957-61.
11. Singh RM, Singh HL. Comparative evaluation of six phenotypic methods for detecting extended-spectrum beta-lactamase-producing enterobacteriaceae. J Infect Dev Ctries 2014;8(04):408-15.
12. Shaikh S, Fatima J, Shakil S, Rizvi SMD, Kamal MA. Antibiotic resistance and extended-spectrum beta-lactamases: types, epidemiology and treatment. Saudi J Biol Sci 2015;22:90–101.
13. Deshpande KD, Pichare AP, Suryawanshi NM, Davane MS. Antibiogram of gram negative uropathogens in hospitalized patients. Int J Recent Trends SciTechnol 2011;1(2):56–60.
14. Urvashi C, Chitra Y, Lokhendro H. Bacterial uropathogens in urinary tract infection and antibiotic susceptibility pattern of patients attending jnims hospital, Imphal. *J Evolution Med Dent Sci* 2013;2(50):9769-74.
15. Benachinmardi K, Padmavathy M, Malini J, Navaneet BV. Microbiological profile and antibiogram of uropathogens in paediatric age group. *Int J of Health Allied Sci* 2015;4(1):61-4.
16. Madhavi VN, Subbulu P. Prevalence and antibacterial susceptibility testing pattern of bacterial pathogens causing urinary tract infections in community. *J Evolution Med Dent Sci* 2016;5(30):1528-31.
17. Haque R, Akter ML, Salam MA. Prevalence and susceptibility of uropathogens: a recent report from a teaching hospital in bangladesh. *BMC Res Notes* 2015;8:416.
18. Kothari A, Sagar V. Antibiotic resistance in pathogens causing community-acquired urinary tract infections in India: a multicenter study. J Infect Dev Ctries 2008;2:354–8.
19. Gade ND, Qazi MS. Fluoroquinolone therapy in *staphylococcus aureus* infections: where do we stand? J Lab Phys 2013;5:109–12.
20. Gales AC, Jones RN, Forward KR, Inares JL, Sader HS, Verhoef J. Emerging importance of multidrug-resistant acinetobacter species and stenotrophomonasmaltophilia as pathogens in seriously ill patients: geographic patterns, epidemiological features, and trends in the sentry antimicrobial surveillance program (1997-1999). *Clinical Infectious Diseases* 2001;32(Suppl 2);104-13.
21. Rekha B, Pai R, Scaria B, Shetty A, Pinto H. Microbiological profile of the uropathogens isolated from pediatric patients from a tertiary care centre. *Karnataka Paediatr J* 2010;24:42-5.
22. Saha A, Majumdar T, Dasgupta A, Saumandal P. Prevalence of extended spectrum beta-lactamases (ESBLs) among uropathogens at a tertiary care hospital in Tripura. *The health Agenda* 2015;3(2):55-62.
23. Taneja N, Rao P, Arora J, Dogra A. Occurrence of ESBL and Amp-C beta-lactamases and susceptibility to newer antimicrobial agents in complicated UTI. *Indian J Med Res* 2008;127:85-8.
24. Joshi S, Ray P, Manchanda V, Bajaj J, Chitnis DS, Gautam V. et al. Methicillin resistant staphylococcus aureus (MRSA) in India: prevalence & susceptibility pattern. *Indian J Med Res* 2013;137(2);363-69.
25. Broekema NM, Van Tam T, Monson TA, Marshall SA, Warshauer DM. Comparison of cefoxitin and oxacillin disk diffusion methods for detection of *mec*a-mediated resistance in *staphylococcus aureus* in a large-scale study. *Journal of Clinical Microbiology* 2009;47(1):217–9.