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Prevalence of Extended Spectrum Beta-Lactamase Producing Klebsiella Pneumoniae Isolated From Urinary Tract Infected Patients

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ABSTRACT

Background: Klebsiella pneumoniae, one of the bacterial agents associated with urinary tract infection has been often implicated as a major extended spectrum beta-lactamase (ESBL) producer in last few decades. This study was designed to assess the prevalence of ESBL producing Klebsiella pneumoniae in urinary isolates at a tertiary care hospital in Kathmandu, Nepal, from July to December 2014.

Methods: One thousand nine hundred eighty six mid-stream urine specimens were collected aseptically from the clinically suspected patients of urinary tract infections attending Capital Hospital and Research Center, Kathmandu. The samples were processed following standard guidelines as recommended by American Society for Microbiology (ASM) and the isolates including Klebsiella spp. were identified using the specific biochemical and sugar fermentation tests recommended by ASM. Antibiotic sensitivity testing was done by modified Kirby-Bauer disk diffusion method and interpreted following Clinical and Laboratory Standards Institute (CLSI) guidelines. Klebsiella pneumoniae isolates showing resistance upon initial screening with ceftriaxone (30 µg) disc were then confirmed for ESBL production by phenotypic confirmatory disc diffusion test (PCDDT) using ceftazidime (30 µg) and ceftazidime + clavulanic acid (30 µg + 10µg) and cefotaxime (30 µg) and cefotaxime + clavulanic acid (30 µg +10µg) disc as per CLSI guidelines.

Results: Out of a total 1986 specimens investigated, Escherichia coli was isolated in 309 (83.9%) and Klebsiella pneumoniae in 38 (10.3%) cases. Initial screening with ceftriaxone disc revealed 18 isolates of Klebsiella pneumoniae to be resistant. Further testing by PCDDT method confirmed 7 (18.4%) Klebsiella pneumoniae isolates to be ESBL producers.

Conclusions: Compared to some earlier studies done in Nepal, higher prevalence of ESBL-producing Klebsiella pneumoniae was observed warranting a national surveillance for routine monitoring of ESBL producing Klebsiella pneumoniae isolates.

Keywords: Extended spectrum beta-lactamase (ESBL); klebsiella pneumonia; Nepal; prevalence; urinary tract infection (UTI).

INTRODUCTION

Longer exposure of bacterial strains to the most commonly used antibiotics, Beta-lactams, for treatment of infection has caused induction of an enzyme extended-spectrum beta-lactamases (ESBLs) in bacteria, showing extended activities against newly developed Beta-Lactams.[1,2] Recent global surveys have shown, a significant increase in the ESBL rate.[3-10] Klebsiella pneumoniae, an important pathogen causing urinary tract infections (UTIs), pneumonia, and intra-abdominal infections in both community and hospital settings has been frequently found to be ESBL producers.[11] Infections caused by ESBL-producing pathogens are problematic when co-existed with resistance to other antimicrobial class, limiting the available therapeutic options,[12] with delay in the detection and reporting causing increased

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morbidity, mortality and healthcare costs.[13]

However, we lack enough evidence on the current epidemiology and clinical pattern of K. pneumoniae in Nepal.[14,15] Hence, this study aimed to determine the prevalence of ESBL-producing K. pneumoniae in urine samples of patients visiting a tertiary care hospital of Kathmandu, Nepal.

METHODS

This prospective study was conducted in the department of Pathology and Microbiology Laboratory of Capital Hospital and Research Center, Kathmandu. A total of 1986 clean catch mid-stream urine (MSU) samples collected in a wide mouthed sterile container were processed in between July to December 2014 from the patients attending to the hospital.

All samples were inoculated on MacConkey's agar and Blood agar, incubated at 37˚C for overnight, and colonies from significant bacterial growth (≥ 105 colony forming unit / ml) plate were processed. The organisms were identified by standard techniques. K. pneumoniae isolates obtained as a pure and predominant growth from the clinical specimens were only considered for this study.

Routine disc diffusion susceptibility testing was performed by Kirby- Bauer's disc diffusion method as described by CLSI.[16] Various antimicrobial discs used were: amikacin (30 µg), gentamicin (10 µg), norfloxacin (10 µg), nitrofurantoin (300 µg), nalidixic acid (30 µg), co-trimoxazole (1.25/23.75 µg), ceftriaxone (30 µg), ceftazidime (30 µg), imipenem (10 µg).

According to CLSI guidelines, strains showing zone of inhibition of ≤ 25 mm for ceftriaxone were selected for conformational test for ESBL.[17]

ESBL production was confirmed among potential ESBL-producing isolates by phenotypic tests. Microbial sensitivity tests were done on the Mueller-Hinton agar plates with disc diffusion method according to the Kirby-Bauer method.[18] 3rd-generation cephalosporins, ceftazidime (30 µg) disc and ceftazidime + clavulinic acid (30 µg + 10 µg) disc and Cefotaxime (30 µg) and Cefotaxime + clavulanic acid (30 µg +10µg) disc were placed with 25 mm apart. An increase of ≥ 5 mm in zone of inhibition for ceftazidime + clavulinic acid compared to ceftazidime or Cefotaxime + clavulanic acid compared to Cefotaxime was confirmed as ESBL producers.

The antimicrobial agent discs were obtained from HI-Media Laboratories Pvt. Ltd., Mumbai, India. K. pneumoniae ATCC 700603 (ESBL positive) strain was used as control throughout the study.

RESULTS

Out of 1986 mid-stream urine samples were subjected for culture, 368 (18.53%) samples showed significant bacteriuria, E. coli was the most common organism isolated from UTI cases (83.96%), followed by K. pneumoniae (10.33%). Other less common isolates were Proteus spp. (1.90%), Staphylococcus aureus (1.63%) and Pseudomonas spp. (1.09%) (Table 1).

Table 1. Distribution of uropathogens among the growth positive UTI cases.

Name of the organisms	Number (percentage)	Percentage
E. coli	309	83.96
Klebsiella pneumoniae	38	10.33
Proteus spp.	7	1.90
Staphylococcus aureus	6	1.63
Pseudomonas spp.	4	1.09
Enterobacter spp.	3	0.82
Acinetobacter spp.	1	0.27
Total	**368**	**100**

Initial screening of these isolates for ESBL production showed 18/ 38 (47.37%) of Klebsiella pneumoniae strains to be ceftriaxone resistant. Phenotypic confirmatory disc diffusion test (PCDDT) revealed 7/38 (18.42%) of Klebsiella pneumoniae isolates to be ESBL positive.

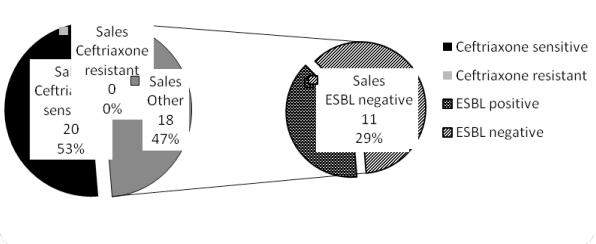

Figure 1. ESBL pattern of Klebsiella pneumonia.

ESBL positive K. pneumoniae were isolated from a wide range of age group, i.e., 16-67 year. Out of the 18.42% (7/38) confirmed ESBL positive K. pneumoniae isolates, 71.42% (5/7) were seen in female population of wide age distrubution. Remaining 28.58% (2/7) ESBL positive K. pneumoniae isolates were seen among the age group of 46 and above male population studied (Table 2).

Table 3. Comparing anthropometric and biochemical measurements between <10 % or ≥ 10 % risk of CVD predictions.

Age group	ESBL positive K. pneumonia		
	Male	Female	Total
< 15	0	0	0
16 - 30	0	2	2
31 - 45	0	1	1
46 - 60	1	1	2
> 60	1	1	2
Total	**2**	**5**	**7**

With regard to the antibiotic susceptibility pattern of ESBL positive K. pneumoniae isolates, Imipenem (100%) was found to be the most effective antimicrobial drug followed by Nitrofurantoin (71.43%) whereas, Nalidixic acid (28.57%) was the least effective antibiotic (Figure 2).

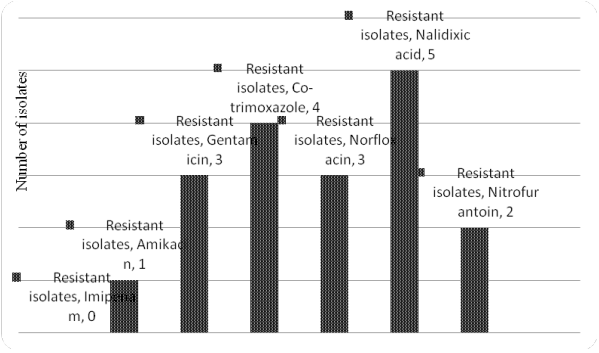

Figure 2: Antibiotic resistivity pattern of ESBL positive Klebsiella pneumoniae

DISCUSSION

Urinary Tract Infections (UTIs) are the second most common bacterial infectious diseases in humans acquired both in the community and hospital settings. ESBLs have emerged gradually during the last decades in species of Enterobacteriacae and their prevalence reach alarming rates.[19] Infection caused by such pathogens often limits therapeutic options and cause treatment failures.[20,21] The present study was carried out to detect ESBL producing K. pneumoniaeand determine the antimicrobial susceptibility pattern of the isolates among the patients visiting Capital Hospital and Research Center Kathmandu, Nepal.

In this study, initial screening for ESBL production showed 47.37% K. pneumoniae strains to be ceftriaxone resistant. Confirmation test (PCDDT) revealed that ESBLs prevalence was 18.42%, which is in concordance with the 16.55% ESBL positive K. pneumoniae reported in a similar study conducted in Nepal[22] and in India.[23] The frequency of ESBL producer in studies done in Bangladesh, other study done in India was higher than our study.[24,25] It may be due to steadily increasing the incidence of ESBL producing strains among the clinical isolates, also the prevalence of ESBLs among clinical isolates varies from country to country and from institution to institution.[26]

The high occurrence of ESBLs in Klebsiella spp. is of great concern since infections caused by this bacterium are very common and resistance of the organism may be due to the presence of capsule that gives some level of protection to the cells, presence of multidrug resistance efflux pump, and greater efficiency to acquire and disseminate resistance plasmid.[27] This study revealed a higher occurrence of ESBL producing uropathogen in the adult age group of 16-45 years, which is in concordance to a study done in Pakistan.[28] This study attempted to access the antibiotic susceptibility patterns to the ESBL producing K. pneumoniae isolates. It is essential for rationalizing both prophylaxis and treatment regimens.[29] In this study, ESBL producing K. pneumoniae showed 100% sensitivity for Imipenem which is parallel to the findings of study done by Alipourfard and Nili (2010) and Chander and Shrestha (2013). Another drug of choice for ESBL producing K. pneumoniae is Nitrofurantoin, showed 71.43% effectivity. The result is in harmony with report from similar study conducted at Indore, India.30 Meanwhile, Cotrimoxazole and gentamicin (42.86%) showed intermediate susceptibility with Nalidixic acid (28.57%) proving to be least effective drug in our study. The finding is in line with studies done in India and Pakistan.[30,31]

Several studies have demonstrated that, a modifiable risk factor for the development of ESBL-producing organisms is the use of third-generation cephalosporins. Hence, use of Imipenem instead of third-generation cephalosporin significantly decreases the development of ESBL production in bacteria.[25,31] The ESBL producing organisms are increasing rapidly and becoming a major problem in the area of infectious disease which includes multi drug resistance, difficulty in detection and treatment and increase in mortality of patients. Of all available anti-microbial agents, carbapenem are the most sensitive and reliable treatment options for infections caused by ESBL producing isolates. However, overuse of carbapenem may lead to resistance of other gram-negative organisms. Therefore, restricting the use of third-generation cephalosporins, along with implementation of infection control measures, are the most effective means of controlling and decreasing the spread of ESBL producing isolates.

CONCLUSION

Imipenem was the only antibiotic found to be the most effective against ESBL producing K. pneumoniae, a rational use of carbapenem should be sought through proper regulations in clinical settings so as to prevent any possible selection of carbapenem resistant strains in future. Compared to other studies conducted in Nepal, a higher prevalence of ESBL-producing K. pneumoniae was observed during the study, warranting a national surveillance for routine monitoring of ESBL producing K. pneumoniae isolates. It is recommended that such study be done to have routine monitoring of ESBL producing pathogens in clinical isolates and all healthcare professionals should work together with pharmacist and laboratory personnel to overcome this problem.

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REFERENCES

1. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. Clinical Microbiology Reviews. 2005;18(4):657-86.

2. Pitout JD, Laupland KB. Extended-spectrum β-lactamase-producing Enterobacteriaceae: an emerging public health concern. The Lancet Infectious Diseases. 2008;8(4):159-66.

3. Gupta V. An update on newer β-lactamases. Indian Journal of Medical Research. 2007;126(5):417-27.

4. Livermore DM, Canton R, Gniadkowski M, Nordmann P, Rossolini GM, Arlet G, Ayala J, Coque TM, Kern-Zdanowicz I, Luzzaro F, Poirel L, Woodford N. CTX-M: changing the face of ESBLs in Europe. Antimicrob. Chemother. 2007;59(2):165-74.

5. St¨urenburg E, Mack D. Extended-spectrum β- lactamases: implications for the clinical microbiology laboratory, therapy, and infection control. Journal of Infection. 2003;47(4):273-95.

6. Perez F, Endimiani A, Hujer KM, Bonomo RA. The continuing challenge of ESBLs. Current Opinion in Pharmacology. 2007;7(5):459-69.

7. Romero ED, Padilla TP, Hernández AH, Grande RP, Vázquez MF, García IG, García-Rodríguez JA, Muñoz Bellido JL.Prevalence of clinical isolates of Escherichia coli and Klebsiella spp. producing multiple extended-spectrum β-lactamases. 2007; 59(4):433-7.

8. Kuo KC, Shen YH, Hwang KP. Clinical implications and risk factors of extended-spectrum β-lactamase producing Klebsiella pneumoniae infection in children: a casecontrol retrospective study in a medical center in southern Taiwan. Journal of Microbiology, Immunology and Infection. 2007;40(3):248-54.

9. Hoşoğlu S, Gündes S, Kolayli F, Karadenizli A, Demirdağ K, Günaydin M, Altindis M, Caylan R, Ucmak H. Extended-spectrum β-lactamases in ceftazidime-resistant Escherichia coli and Klebsiella pneumoniae isolates in Turkish hospitals.Indian Journal of Medical Microbiology. 2007;25(4):346–50.

10. Messai Y, Iabadene H, Benhassine T, Alouache S, Tazir M, Gautier V, Arlet G, Bakour R. Prevalence and characterization of extended-spectrum β-lactamases in Klebsiella pneumoniae in Algiers hospitals (Algeria). Pathologie Biologie. 2008; 56(5):319–25.

11. Podschun R, Ullmann U.Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin. Microbiol. Rev. 1998;11:589–603.

12. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. Clin Microbiol Rev. 2005;18:657–86.

13. Kollef MH.The importance of appropriate initial antibiotic therapy for hospital-acquired infections. Am J Med. 2003;115:582-4.

14. Shrestha S, Amatya R, Dutta R. Prevalence of extended spectrum beta lactamase (ESBL) production in gram negative isolates from pyogenic infection in tertiary care hospital of Eastern Nepal. Nepal Med Coll J 2011;13(3):186-9.

15. Upadhyay AK, Parajuli P. Extended spectrum β lactamase producing multidrug-resistant Klebsiella species isolated at National Medical College and Teaching Hospital, Nepal. Asian J Pharm Clin Res 2013;6(4):161-4.

16. Clinical Laboratory Standard Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty first Informational Supplement (M100-S21). Wayne PA. Clinical and Laboratory Standards Institute (CLSI). 2011.

17. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method.

Am J Clin Pathol. 1966;45:493-6.

18. Zahar JR, Bille E, Schnell D, Lanternier F, Mechai F, Masse V, Nassif X, Lortholary O. Extension of β-lactamases producing bacteria is a worldwide concern. Med Sci 2009;25:939-44.

19. Rossolini GM, Mantengoli E, Docquier J, Musmanno RA, Coratza G. Epidemiology of infections caused by multiresistant gram-negatives: ESBLs, MBLs, panresistant strains. Microbiologica-Bologna, 2007;30(3):332.

20. Chander A, Shrestha CD. Prevalence of extended spectrum β lactamase producing Escherichia coli and Klebsiella pneumoniae urinary isolates in a tertiary care hospital in Kathmandu, Nepal. BMC Research notes. 2013;6:487.

21. Sarojamma V, Ramakrishna V. Prevalence of ESBL-Producing Klebsiella pneumoniae Isolates in Tertiary Care Hospital. ISRN Microbiology, 2011;Article ID 318348.

22. Alipourfard I, Nili NY. Antibiogram of Extended Spectrum β-lactamase (ESBL) producing Escherichia coli and Klebsiella pneumoniae isolated from Hospital samples. Bangladesh J Med Microbiol. 2010;04(01):32-6.

23. Chaudhary BL, Shrivastav S, Singh BN, Shukla S. Nosocomial infection due to Multidrug Resistant (MDR) Escherichia coli and Klebsiella pneumoniae in Intensive Care Unit. Int.J.Curr.Microbiol.App.Sci. 2014;3(8):630-5.

24. Bradford PA. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology and detection of this important threat. Clin Microbiol Rev 2001;14:933-51.

25. Paterson DL, Yu VL. Extended spectrum β-lactamases: a call for improved detection and control, Clin Infect Dis, 1999;29:1419-22.

26. Chaudhary U, Aggarwal R. Extended spectrum β-lactamases (ESBL) - An emerging threat to clinical therapeutics. Indian Journal of Medical Microbiology. 2004;22(2):75-80.

27. Yusha'u MM, Kumurya AS, Suleiman L. Prevalence of Extended spectrum β-lactamases among Enterobacteriaceae in Murtala Mohammed specialist hospital, Kano,N. Journal of Pure and Applied Sciences. 2010;3(1):169-72.

28. Ullah F, Malik SA, Ahmed J. Antimicrobial susceptibility pattern and ESBL prevalence in Klebsiella pneumoniae from urinary tract infections in the North West of Pakistan. African Journal of Microbiology. 2009;3(11):670-80.

29. Gupta RD, Sullivan R, French G, O'Brien T. Evidence-based prescriptions of antibiotics in urology: a 5-year review of microbiology. BJU Int. 2009;102:1634-7.

30. Pathak A, Marothi Y, Kekre V, Mahadik K, Macaden R, Lundborg CS. High prevalence of extended spectrum β-lactamase producing pathogens: results of a surveillance study in two hospitals in Ujjain, India. Infect Drug Resist 2012;5:65-73.

31. Abdullah FE, Mushtaq A, Irshad M, Rauf H, Afzal N, Rasheed A. Current efficacy of antibiotics against Klebsiella isolates from urine samples- a multi-centric experience in Karachi. Pak J Pharm Sci 2013;26(1):11–5.