Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 5, Issue, 08, pp.2256-2259, August, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

PREVALENCE AND ANTIMICROBIAL RESISTANCE PATTERN WITH SPECIAL REFERENCE TO EXTENDED SPECTRUM β-LACTAMASE PRODUCING KLEBSIELLA ISOLATES IN A TERTIARY CARE HOSPITAL

***Ambica, R. and Sahana, P.**

Department of Microbiology, Bangalore Medical College and Research Institute, Bangalore – 560002, Karnataka, India

ARTICLE INFO

Article History:
Received 12th May, 2013
Received in revised form
10th June, 2013
Accepted 30th July, 2013
Published online 23rd August, 2013

Key words:
ESBL,
Klebsiella spp.,
Prevalence.

ABSTRACT

Extended spectrum β- lactamases (ESBLs) are a group of enzymes which confer resistance to cephalosporins, monobactams and related oxyimino β-lactams. ESBLs are encoded by transferable conjugative plasmids which may also carry resistant determinants to other antimicrobials like aminoglycosides, limiting treatment options. ESBL's are more prevalent in *Klebsiella spp.* than any other members of family enterobacteriaceae. The present study was carried out to determine the antimicrobial susceptibility pattern and to detect ESBL production in *Klebsiella spp.* from clinical isolates in a tertiary care hospital. 100 isolates of *Klebsiella spp.* derived from various clinical specimens processed as per the standard guidelines were considered for the present study. Antimicrobial susceptibility testing by Kirby-Bauer technique was done according to Clinical Laboratory Standards Institute (CLSI) 2011 guidelines. 41 *Klebsiella* isolates which were considered presumptively positive for ESBL production by Kirby-Bauer method were later confirmed by the phenotypic double disc diffusion test using cefotaxime & cefotaxime/clavulanic acid discs. An increase of >/= 5 mm in the latter disc was considered as confirmatory. A majority of these isolates were also resistant to aminoglycosides. All isolates were found to be susceptible to carbapenems. The study revealed a high prevalence of ESBL producing *Klebsiella spp.* in our hospital. Enhanced infection control, coupled with antibiotic stewardship programs backed by simple and effective detection methods are required to limit the spread of ESBL-producers.

Copyright © 2013 Ambica, R. and Sahana, P. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Extended spectrum β-lactamase (ESBL) producing *Klebsiella* species were first reported in 1983 from Germany and since then a steady increase in resistance against Cephalosporins has been seen.[3] Antimicrobial resistance has increased dramatically in both nosocomial and community settings. β-lactam antibiotics are the most widely used group of antimicrobial agents. The most common mechanism of resistance among gram-negative pathogens to β-lactam involves the synthesis of β-lactamases, especially ESBL. ESBL's belong to group 2 β-lactamases, subgroup 2be ('e' for extended spectrum of activity), which are capable of inactivating third-generation Cephalosporins as well as Monobactams. The emergence of ESBL producing organisms is a serious concern worldwide. ESBLs arise because of mutation in TEM-1, TEM-2 or SHV-1 genes commonly found in members of famiy enterobacteriaceae. ESBLs not belonging to these groups have also been described. ESBL's are more prevalent in *Klebsiella spp.* than any other members of family enterobacteriaceae.[1,2,3] The present study was undertaken in the department of microbiology, BMCRI, Bangalore to isolate and identify *Klebsiella spp.* from clinical samples, analyze the anti-microbial resistance pattern of the clinical isolates and to detect ESBL production in *Klebsiella spp.*

MATERIALS AND METHODS

This descriptive study is conducted in the Department of Microbiology attached to Victoria Hospital, Minto Institute of Ophthalmology and Vani Vilas Hospitals of Bangalore Medical College & Research Institute, Bangalore, India over a period of 3 months from May to July 2011. *Processing of samples*: A total of 100 isolates will be obtained from various clinical samples like blood, urine, sputum, pus and conjunctival swabs. All samples were inoculated on MacConkey's media and Chocolate agar media, incubated at 37°C for 24 hours and colonies are processed. In case of blood samples, blood was incubated at 37°C for 24 hours in Brain Heart Infusion Broth and sub-cultures done on alternative days for a period of 1 week. *Klebsiella* isolates that were obtained as a pure and predominant growth from the clinical specimens were considered for the present study.

***Antibiotic susceptibility testing*:** was done by Kirby-Bauer disc diffusion technique according to CLSI guidelines. A lawn culture of the test organism was made on Muller Hilton Agar (MHA) medium after adjusting the inoculum to 0.5 McFarland units. Discs were placed and incubated at 37°C overnight, and the reading was recorded.[6] The antibiotic susceptibility pattern of the isolates to a panel of antibiotics including amikacin, ciprofloxacin, gentamicin, imipenen, piperacillin and piperacillin-tazobactum were recorded.

***Detection of ESBL isolates*:** *Klebsiella* isolates reported as cephalosporin resistant were screened for ESBL detection using the disc diffusion method as per CLSI guidelines. ESBL screening was performed by Kirby Bauer disc diffusion technique using ceftazidime (10 μg), cefpodoxime (30 μg), cefotaxime (30μg), cefoperazone (30 μg) and aztreonam (30 μg) disks. Resistance to any of these drugs was presumptively considered as positive for ESBL. Confirmation of ESBL phenotype was performed by the double disk diffusion method using antibiotic discs containing a combination of cephalosporins plus

Corresponding author: Dr. Ambica, R.
Department of Microbiology, Bangalore Medical College & Research Institute, Bangalore – 560002. Karnataka. India

clavulanic acid in conjunction with the corresponding cephalosporin disc alone. The antibiotic disks used were: cefotaxime (CTX 30 µg) and cefotaxime plus clavulanic acid (CTX/CA 30/10 µg). *Klebsiella pneumoniae* ATCC 700603 (ESBL positive) is used as quality control strain. The tests are interpreted according to CLSI guidelines: >/= 5 mm increase in the zone of inhibition from CTX/CLA containing disk versus the corresponding CTX disk is considered positive for ESBL.

RESULTS

In the present study 100 isolates of *Klebsiella spp.* were obtained from various clinical samples like sputum, pus, blood and urine and were screened for susceptibility to various antibiotics. Of the 100 clinical isolates of *Klebsiella spp.* screened according to CLSI guidelines, 41 isolates which were detected to be ESBL producers by disc diffusion method were confirmed using the double disc diffusion technique. ESBL producing *Klebsiella spp.* were predominantly noted in the age group of 20-40 years, the maximum distribution in the age-group 30-40 years-26% (11/41 were positive), followed by the age group 20-30 years-19% (8 positive samples were obtained). Also, the distribution of positive isolates were found to be slightly more in males- 56% (23/41 among males), compared to females-44% (18/41 samples positive). (Male: Female: 1.28:1). The sample-wise distribution of the ESBL producing *Klebsiella* isolates was determined. The maximum number of ESBL producers were obtained from pus samples (60%), followed by sputum samples (24%), urine samples (9%) and blood samples (4%), as depicted in Figure 1.

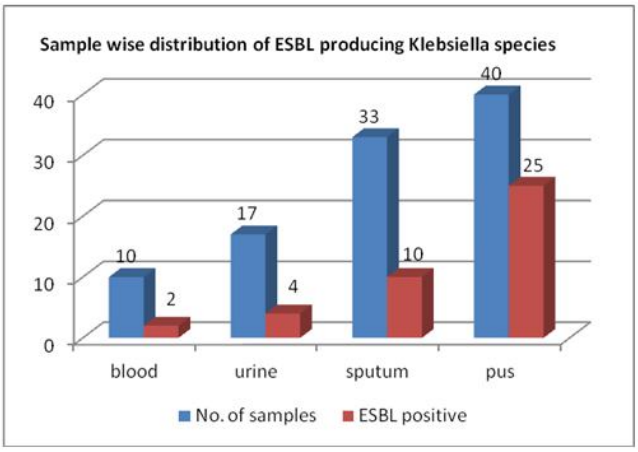

Figure 1. Sample wise distribution of isolates of *Klebsiella spp* and ESBL positivity pattern

Of the 100 isolates of *Klebsiella* species, 100% susceptibility was found to carbapenems (imipenem). Among the non β-lactam antibiotics, piperacillin-tazobactam (PT) was found to be the most effective with a susceptibility of 86%, followed by piperacillin (82%), fluoroquinolones (53%). They were found to be least susceptible to aminoglycosides (36%). The susceptibly pattern to different antibiotics in shown in Figure 2.

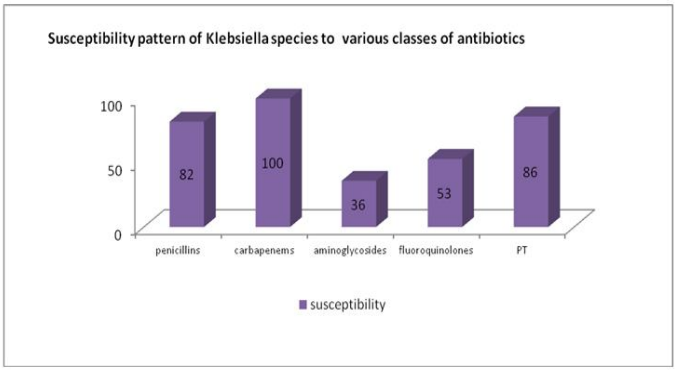

Figure 2. Antibiogram of *Klebsiella spp* encountered in the present study

The multidrug resistance was found to be significantly higher among ESBL producers compared to non-ESBL producing strains. Like in non-ESBL producing strains, in ESBL producers too, the highest resistance was shown to aminoglycosides, but significantly more than non-ESBL producers-87% (38/41 ESBL positive strains showed resistance to aminoglycosides). This was followed by resistance to fluoroquinolones-51% (21/41 ESBL positive strains showed resistance to fluoroquinolones), penicillins-19% (8/41 strains showed resistance to penicillins), piperacillin + tazobactum (PT) - 17% (7/41 ESBL positive strains showed resistance). ESBL producing *Klebsiella spp.* were also found to be 100% susceptible to carbapenems (imipenem), as in non-ESBL producing strains. The comparative study of antibiotic resistance among ESBL and non-ESBL producers is shown in Figure 3.

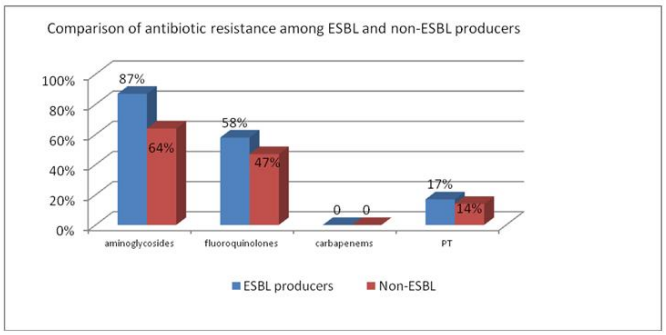

Figure 3. Resistance pattern of ESBL and non-ESBL producing isolates of *Klebsiella spp*

The most common combination of resistance among ESBL producers was shown against aminoglycosides and fluoroquinolones. 56% of the ESBL producers showing resistance against aminoglycosides also showed resistance to fluoroquinolones. (23/41 ESBL positive organisms showed resistance to both groups of antibiotics. It was also shown that by adding a monobactam (tazobactam) to the penicillin (piperacillin), the resistance was reduced from 19% to 17%. Among the third generation cephalosporins and monobactams used in the antibiotic susceptibility testing, resistance to any one of them was considered ESBL positive, according to CLSI 2011 guidelines.[6] 41/100 isolates, were tested to be ESBL positive. Among the ESBL producers, the maximum resistance was shown to ceftazidime-73% (30/41 isolates resistant), followed by aztreonam-62.5% (25 isolates resistant), ceftriaxone-36% (15 isolates resistant), cefoperazone-17% (7/41 organisms were found to be resistant). All the isolates were found to be susceptible to cefpodoxime. The most common combination of resistance was found against ceftazidime (third generation cephalosporin) and aztreonam (monobactam)- 43% of ESBL producers were found to be resistant to both these groups (18/41 resistant),followed by resistance to ceftazidime and ceftriaxone- 21% of the isolates were found to be resistant to both these groups (9/41 isolates resistant). ESBL production was confirmed by phenotypic disc diffusion test when the zone of inhibition increased >/= 5 mm in the presence of clavulanic acid. Cefotaxime (CTX 30 µg) and cefotaxime plus clavulanic acid (CTX/CA 30/10 µg) antibiotic disks were used. All the 41 samples were confirmed to be ESBL producers by phenotypic double disc diffusion technique.

DISCUSSION

Bacterial infections are a major focus of concern for infection control programs in hospitals. Such infections may occur as an outbreak or may become established as a regular occurrence. Before the advent of molecular biologic techniques to assess the genetic relationships between nosocomially acquired organisms, typing methods that assessed phenotypic differences between organisms were widely used. Phenotypic methods like biotyping and assessment of antimicrobial susceptibly test pattern may potentially be used to type *Klebsiella* isolates harbouring ESBL's. Recently, an inhibition enzyme-linked

immune-sorbent assay method has been developed which overcomes this technical problem. Phage typing, bacteriocin typing, analytical iso-electric focusing and multi-locus enzyme electrophoresis are the other methods used to discriminate ESBL-producing strains.[4,5] The plasmid mediated resistance against cephalosporins can spread among related and unrelated Gram negative bacteria. Since ESBL-positive isolates show false susceptibility to extended spectrum Cephalosporins in standard disk diffusion tests, it is difficult to reliably detect ESBL production by the routine techniques. Specific methods recommended by CLSI 2011 have to be adopted. Laboratories using disc diffusion methods for antibiotic susceptibility testing can screen for ESBL production by noting specific zone diameters which indicate a high level of suspicion for ESBL production. Cefpodoxime, ceftazidime, aztreonam, cefotaxime or ceftriaxone are used. However, the use of more than one of these agents for screening improves the sensitivity of detection. If any of the zone diameters indicate suspicion for ESBL production, phenotypic confirmatory tests should be used to ascertain this diagnosis.[6,7]

The CLSI advocates use of cefotaxime (30µg) or ceftazidime (30µg) disks with or without clavulanate(10µg) for phenotypic confirmation of the presence of ESBL's in *Klebsiella spp.* It is recommended that the disk tests be performed with confluent growth in Mueller-Hinton agar. A difference of ≥5 mm between zone diameters of either of the cephalosporin discs and their respective cephalosporin/ clavulanate disc is taken to be the phenotypic confirmation of ESBL production.[8] ESBLs are encoded by transferable conjugative plasmids which also quite often code resistant determinants to other antibiotics (for example, aminoglycosides).Therefore, antibiotic options in the treatment of ESBL-producing organisms are extremely limited.[(9)] Carbapenems are the treatment of choice for serious infections due to ESBL-producing organisms, yet carbapenem-resistant isolates, achieved by the combination of porin loss and β-lactamase production have recently been reported. ESBL-producing organisms may appear susceptible to some extended-spectrum cephalosporins. However, treatment with such antibiotics has been associated with high failure rates.[10] ESBLs compromise the efficacy of β–lactam antibiotics, with the exception of the cephamycins and carbapenems by hydrolysis of the β–lactam ring. Antimicrobial therapy is frequently limited for the treatment of ESBL producers because they are often multi-drug resistant (MDR).[11]

The most frequent co-resistances found in ESBL producing organisms are amino-glycocides, fluroquinolones, tetracyclines, chloramphenicol and sulphamethoxazole-trimethoprim.[12] ESBLs are more prevalent in *Klebsiella spp* than any other enterobacterial species. The prevalence of ESBL producing *Klebsiella spp* is reported to be varying from 6 to 87% in India. The prevalence of ESBL producing *Klebsiella* in the present study was noted to be 41%, which is comparable to the findings of Shukla I et al from Uttar Pradesh.[13,9] The age distribution of cases harbouring isolates of ESBL producing *Klebsiella* noted in the present study, which was highest among the 20 – 40 years group is comparable to the study conducted in Saudi Aramco Dhahran Health Centre. Male predominance of cases (Male: Female:: 1.28:1) yielding ESBL producing *Klebsiella spp* noted in the study is comparable to the observations of the Canadian Ward Surveillance Study. [2,11] Majority of ESBL producers were detected from pus samples in the present study (60%), followed by sputum, urine and blood, which correlates with the findings of Shiji MP et al from Mangalore, Karnataka, wherein 70% of the ESBL producing strains were isolated from pus and the rest from urine samples.[12] The role of other drugs in the treatment of *Klebsiella* infections is very limited as multi-drug resistance among ESBL producers is on the rise. Imipenem, was found to be the most effective antimicrobial in vitro with all the ESBL and non-ESBL producing strains exhibiting 100% susceptibility against the carbapenem, which compares favourably with the findings of Jain A et al in a study conducted in Lucknow, India, wherein of the 100 isolates of *Klebsiella spp* tested, all the isolates were found to be sensitive to imipenem and meropenem, except one.[13]

In the present study, the *Klebsiella* isolates were found to be 86% sensitive to piperacillin-tazobactum, which can be compared to the results of the study conducted in Mangalore (98% susceptibility). Fluoroquinolones were once considered as an alternative therapy for treatment of ESBL producing *Klebsiella* infections. However in the present study, 47% of *Klebsiella* isolates exhibited resistance to Fluoroquinolones. This can be explained by resistance mechanisms associated with alterations in target enzymes (DNA gyrase/ topoisomerase 4) or due to impaired access to the target enzymes due to changes in porin expression. The *Klebsiella* isolates were found to be least susceptible to aminoglycosides (64 % resistance). These findings can be compared to the results of the Canadian study, which reported 57.1 % and 71.4% resistance against fluoroquinolones and Aminoglycosides respectively.[12,1,11] The multidrug resistance was found to be significantly higher among ESBL producers compared to non-ESBL producing strains as mentioned in Table 1. These observations are comparable to the findings of Jain A et al.[13] Among the ESBL producers, the maximum resistance was shown to ceftazidime (73%), followed by aztreonam (62.5%), ceftriaxone (36%) and cefoperazone (17%). These findings compare favourably with the results of a study from Kurnool, Andhra Pradesh.[14]

Table 1. Comparison of antimicrobial resistance among ESBL and non-ESBL producing strains of Klebsiella

Antimicrobial	Non ESBL producers (n=59)	ESBL producers (n=41)
Aminoglycosides	29 (49.15%)	38 (92.68%)
Fluoroquinolones	20 (33.89%)	21 (51.21%)
Piperacillin	11 (18.64%)	8 (19.51%)
Piperallin-Tazobactum	10 (16.94%)	7 (17.03%)

Conclusions

A high prevalence of ESBL producing *Klebsiella spp.* was noted in the present study in our tertiary care hospital. Judicious use of antimicrobials, coupled with effective infection control measures and detection methods for multidrug resistant organisms are the need of the hour. The authors emphasize the importance of creating awareness among health care personnel about the present scenario of multidrug resistance and lack of treatment options for countering the infections caused by these organisms.

Acknowledgements

The authors express gratitude to the faculty of the department of microbiology, Victoria Hospital, BMCRI, Bangalore for their valuable help in processing of clinical specimens for the present study. The project was done under ICMR-STS grant.

REFERENCES

1. Daniella Fabiola dos Santos, Fabiana Cristina Pimenta. Extended-spectrum β-lactamases producing *Klebsiella pneumoniae* isolated in 2 hospitals in Goiania/Brazil. *Braz J Microbiol* 2008; 39(4).
2. Khanfar. ESBL producers in the hospital and community settings. *J Infect Dev Ctries* 2009; 3(4): 295-299.
3. Emery C.L., Weymouth L.A. Detection and clinical significance of extended spectrum β-lactamases in a tertiary-care medical centre. *J.Clin.Microbiol* 1997; 35(8):2061-7.
4. Hansen, D.S., F. Mestre, S.Alberti, S.Hernandez-Alles, D. Alvarez, A.Domenech-Sanchez, J. Gill, S.Merino, J.M. Tomas, and V.J.Benedi.1999. Klebsiella pneumonia lipopolysacchride O typing: revision of prototype strains and O – group distribution among clinical isolates from different sources and countries. *J.Clin.Microbiol*.37:56-62.
5. Bauernfeind, A., E.Rosenthal, E.Eberlein, M. Holley, and S.Schweighart.1993.Spread of Klebsiella pneumonia producing SHV-5 beta-lactamase among hospitalized patients.*Infection* 21:18-22.

6. Clinical and Laboratory Standards Institute (CLSI). Performance standards of anti-microbial disc susceptibility tests; Nineteenth informational supplement. Wayne PA USA CLSI; 2011;40-42
7. Agarwal P, Ghosh AN. Prevalence of extended spectrum β-lactams among Escherichia coli and Klebsiella pneumonia isolates in a tertiary care hospital. *Indian J Pathol Microbiol* 2008; 51: 139-142.
8. David L.Paterson and Robert A.Bonomo. Extended-Spectrum β-lactamases: a Clinical Update.Clin Microbiol Rev.2005 October; 18(4): 657-686.
9. Shukla I, Tiwari R, Agarwal M. Prevalence of extended spectrum β-lactamase producing klebsiella pneumoniae in a tertiary care hospital. *Indian J Med Microbiol* 2004; 22: 87-91.
10. Paterson dl, Hujer KM, Hujer AM et al. (2003). Extended spectrum β-lactamases in Klebsiella pneumoniae bloodstream isolates from seven countries: dominance and widespread prevalence of SHV- and CTX-M-type β-lactamases. *Antimicrob Agents Chemother* 47(11); 3554-3560.
11. PJ Bandry, M. McCracken. Prevalence and characterization of extended spectrum β-lactamases producing enterobactereciae isolated in Canadian hospitals: Results from CANWARD 2007 *Can J Infect Dis Med Microbiol* 2009; 20(suppl A):43A-48A.
12. Shiji.MP, Yashavant. R. Detection of extended spectrum β-lactamases production and multidrug resistance in clinical isolates of E.coli and K.pneumoniae in Mangalore. *Journal of Clinical and Diagnostic Research* 2010 June; 4: 2442-2445.
13. Jain. A, Mondal.R. Prevalence and antimicrobial resistance pattern of extended spectrum β-lactamase producing Klebsiella spp isolated from cases of neonatal septicemia. *Indian J Med Res* 125, January 2007, pp89-94.
14. Sarojamma Vemula, Ramakrishna Vadde. Prevalence of ESBL-producing Klebsiella pneumonia isolates in a tertiary care hospital. *ISRN Microbiology*, 2011:5402-5407.

****