The Professional Medical Journal
www.theprofesional.com

DOI: 10.17957/TPMJ/15.3022

ORIGINAL PROF-3022

E-COLI AND KLEBSIELLA PNEUMONIAE;
FREQUENCY AND PATTERN OF EXTENDED SPECTRUM BETALACTAMASE (ESBL): A STUDY AT LADY READING HOSPITAL (LRH) PESHAWAR

Fazli Bari[1], Muhammad Akram[2], Hamidullah Shah[3], Muhammad Imtiaz Shafiq[4]

1. Department of Microbiology, Lady Reading Hospital, Peshawar (KPK), Pakistan.
2. Department of Biochemistry SFINHS, Shaikh Zayed Postgraduate Medical Institute, Lahore, Pakistan.
3. Department of Pathology, Lady Reading Hospital, Peshawar (KPK), Pakistan.
4. Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore, Pakistan.

Correspondence Address:
Muhammad Akram
Associate Professor Biochemistry
SFINHS/SKBZMDC, Shaikh Zayed Postgraduate Medical Institute, Lahore.
aakramskzmdc@yahoo.com.

Article received on:
13/07/2015
Accepted for publication:
00/00/2015
Received after proof reading:
12/10/2015

ABSTRACT… Objectives: To evaluate extended spectrum betalactamase (ESBL) in E.coli and Klebsiella pneumoniae in bacterial cultures and its frequency at LRH. **Study Design:** Cross sectional analytical study. **Setting:** Lady Reading Hospital, Peshawar. **Period:** June 2013 to December 2013. **Methodology:** Total of 1037 bacterial isolates including 614 E.coli and 423 of Klebsiellapneumoniae were evaluated. All cases were subjected to double disc diffusion method for ESBL detection using amoxacilln-clavulanic acid and a third generation cephalosporin as all ESBLs are hydrolysed by clavulanic acid. The data were analysed using SPSS-16. **Results:** Out of 1037 cases five ninety two (55%) were males and four fourty five (45%) were females. Of these, E. Coli were 614 (59.2%) and K. Pneumoniae were four twenty three (40.8%). Of these 1037 isolates, four hundred & ninety five(47.7%) tested positive for ESBL enzyme. Frequency of ESBL positivity in E.coli isolates was 264 (43%) and in Klebsiellapneumoniae isolates was 231 (54.6%).Frequency of ESBL in pus was 34.3%(152/395),in urine, it was 31.8%(141/368), in blood it was 28.6%(127/233) and in sputum it was 5.1% (23/41). Unit wise frequency of ESBL was surgical & allied 24.6% (109/283), medical and allied 21.4%(95/241), paediatrics 18.5% (82/203), obstetrics & gynaecology 23.2%(103/178) and outpatients 12.1 %(54/132). No significant correlation between ESBL positivity, gender, unit or specimen was found. **Conclusion:** ESBL positive isolates of E.coli and K.pneumoniaeshould be properly detected in routine laboratory workflow to avoid unnecessary use of otherwise effective antibiotics.These results indicate that such organisms are highly prevalent in our Hospital and need immediate infection control measures to reduce their further spread.

Key words: E.coli, K.pneumoniae, Extended Spectrum Beta Lactamase (ESBL), Hospital Acquired Infections (HAI).

Article Citation: Bari F, Akram M, Shah H, Shafiq MI. Escherichiacoli and klebsiella pneumonia; frequency and pattern of extended spectrum betalactamase (ESBL): a study at Lady Reading Hospital (LRH) Peshawar. Professional Med J 2015;22(10):1362-1366. **DOI:** 10.17957/TPMJ/15.3022

INTRODUCTION

Members of the family Enterobacteriaceaeare the main cause of hospital acquired infections in the world. Among them E.coli and K.pneumoniae which are normal gastrointestinal flora, are the most prevalent pathogens in this family.[1] Not only these organisms are ubiquitous in hospital environment, there is also increased burden of antibiotic pressure. Patients are overcrowded in hospital units and are being looked after by busy physicians and surgeons thus further contributing to the increased incidence of hospital acquired infections.[2,3] We believe that resistance to cephalosporins is the main cause of failure in the treatment of hospital acquired infections due to E. Coli and K. pneumoniae. Because of wide spread use of this group of antibiotics, Entetrobacteriacea have evolved various mechanisms of resistance, the most important of them being Extended Spectrum Beta Lactamases (ESBL).[2] ESBLs have as much as 200 variants including TEM (Temoria) and SHV (Sulphydryl).[4] These enzymes are called extended spectrum because they hydrolyse a wide variety of beta-lactam antibiotics including penicillins, cephalosporins and monobactam.[3] These enzymes are mostly plasmid mediated in E.coli and K.pneumoniae[4]. The hallmark of these enzymes is their inhibition by beta lactamase inhibitors like clavulanic acid, sulbactam and tazobactam.[5] Hospital acquired infection rates are

different in various parts of the world and depend upon many factors like severity of the disease, immune status of the patient and antibiotic administration in hospitals.[5,6] Correct detection and timely surveillance of ESBL producing Enterobacteriaceae in the hospital can greatly influence emperical therapy of high risk patients with severe hospital acquired infection.[5,7,12] The main aim of our study was to determine the frequency of ESBL producing E.coli and K.pneumoniae to reduce the antibiotic therapy failure and, therefore to minimise hospital stay, mortality, morbidity and economy burden.[8,9]

Methodology

In this cross-sectional analytical study, a total of 1037 isolates, including 614 E. Coli and 423 of K.pneumonie from different departments of Lady Reading Hospital, Peshawar were included during June 2013 to December 2013.

Antibiotic susceptibility profile of the isolates was determined by Kirby Bauer disc diffusion method. A turbidity suspention of bacterial isolate in normal saline equivalent to 0.5M Mcfarl and standard was prepared under strict aseptic conditions. A sterilized swab was immersed in the suspention and then squeezed with the inner side of the vial to express excess of fluid. A full plate of Mueller Hinton agar(150mm)was innoculated with the swab, rotating the plate at 60° three times to ensure a uniform bacterial lawn. Antibiotic containing discs (oxoid) of cefpodoxime 10mcg,cefotaxime 30mcg, ceftazidime 30mcg and aztreonam 30mcg were applied with a forcep.[14] Use of four discs increases the sensitivity of ESBL detection.[14] Plates were incubated at 35±2°C in ambient air for 16-18 hours as per CLSI recommendations. Zones of clear inhibition, if any, were recorded with a rular in mm. All isolates with zones of inhibition as depicted in table were suspected of potential ESBL producer and therefore, were subjected to Double Disc Synergy Test.

Antibiotic	Disc content and zone break point
Cefpodoxime	10mcg:≤17mm
Ceftazidime	30mcg:≤17mm
Cefotaxime	30mcg:≤22mm
Aztreonam	30mcg:≤17mm

Table-I. Screening test for extended spectrum betalactamases

Double Disc Synergy Test

In this test, amoxacillin-clavulanic acid 20/10mcg disc is placed in the centre of the Muellar Hinton plate already innoculated with the test organism. Discs of cefpodoxime, ceftazidime, cefotaxime and aztreonam are placed around it with a centre to centre distance of about 30 mm. Clear zones of inhibition and distortion were evaluated after 18 hour incubation. A zone of extention of the edge

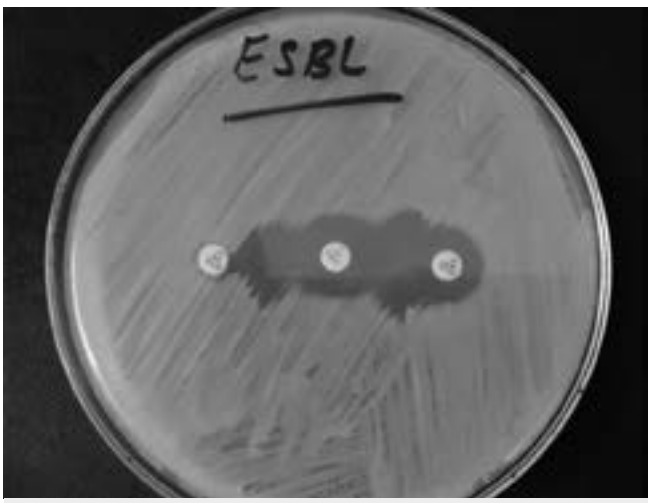

Diagram showing enhancement of ceftazidime and cefotaxime discs towards coamoxiclav disc

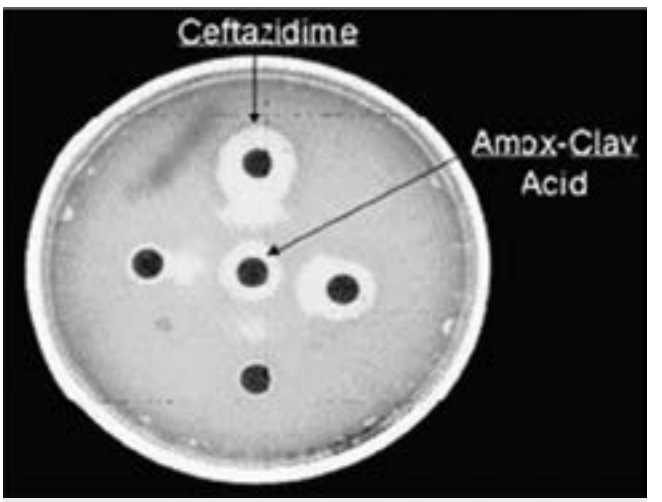

Diagram showing inhibition zone extention of various 3rd generation cephalosporin towards coamoxiclav

of any of the cephalosporin or aztreonam discs towards amoxicillin-cavulanic acid indicates the isolate is an ESBL producer as depicted in the diagrams.[14] In case of no such zone, the isolate is not ESBL producing.

All data were analyzed with SPSS16 software. p values less than 0.05 were considered significant.

RESULTS

Five hundred and ninety two were male (57%) and four hundred and forty five (43%) were female. E. Coli accounted for six hundred and fourteen (59.2%) and K. Pneumoniae for four hundred and twenty three (40.8%).

Frequency of ESBLs in E. coli was 44.6%(264/443) and in K.pneumoniae, it was 39.9%(231/443). Fig-1.

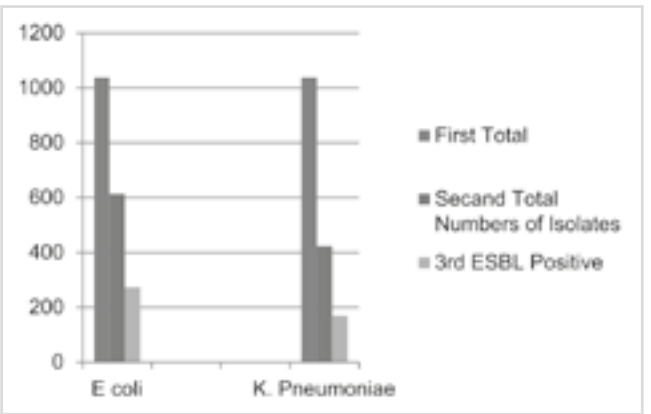

Figure-1. Showing ESBL positivity in E. Coli & K. pneumoniae

Specimen received were pus in three hundred and ninety five cases(38.1%), urine in three hundred and sixty eight cases(35.5%), blood in two hundred and thirty three cases (22.5%) and sputum in forty one cases (03.9%).

Specimen	Total No.	ESBL Positive	ESBL Negative
Pus	395	152(34.3%)	243
Urine	368	141(31.8%)	227
Blood	233	127(28.6%)	106
Sputum	41	23(5.1%)	18

Table-II. Showing ESBL frequency in various specimen

Unit-wise distribution was surgical and allied two hundred and eighty three (27.3%), medical and allied two hundred and forty one (23.2%) paediatrics two hundred and three (19.5%), obstetrics and gynaecology one hundred and seventy eight (17.2%) and outpatients one hundred and thirty two (12.7%). Table-III.

Unit	Total No.	ESBL Positive	ESBL Negative
Surgical and allied	283	109(24.6%)	174
Medical and Allied	241	95(21.4%)	146
Peadiatrics	203	82(18.5%)	121
Obstetrics & Gynaecology	178	103(23.2%)	75
Outpatients	132	54(12.1%)	78

Table-III. Indicating ESBL frequency in different units

Master table

Age	Freq-uency	Sex		Specimen				Unit				
		Male	Female	Pus	Urine	blood	Sputum	Surgical & Allied	Medical & Allied	Peadiatrics	Obs & Gynae	Out-patients.
0-09	97	42	55	23	24	15	6	25	20	25	24	26
10-19	141	91	50	69	27	24	8	34	30	29	28	22
20-29	283	134	152	135	96	55	07	65	40	39	30	24
30-39	298	183	115	43	65	63	10	73	60	40	33	20
40-49	131	86	27	86	103	53	06	58	70	50	36	22
>50	105	56	46	39	53	23	04	28	21	20	27	18
Total	**1037**	**592**	**445**	**395**	**368**	**233**	**41**	**283**	**241**	**203**	**178**	**132**

ESBL Total	Male	Female	Pus	Urine	Blood	Sputum	Surgical & Allied	Medical & Allied	Peadiatrics	Obs & Gynae.	Outpatients
443	222	221	152 (34.3%)	141 (31.8%)	127 (28.6%)	23 (5.1%)	109 (24.6%)	95 (21.4%)	82 (18.5%)	103 (23.2%)	54 (12.1%)

Among specimen, the frequency of ESBLs in pus was 34.3%, in urine, it was29.5%, in blood it was 24.1% and in sputum, it was11.9%.

Unit wise distribution of ESBLs was 24.6% for surgical and allied, 21.4% for medical and allied, 18.5% for paeditrics, 23.2% for obstetrics and gynaecology and 12.1% for outpatients.

There was no significant correlation between ESBL producing isolates and age, gender, unit and specimen.

DISCUSION

Members of the family Enterobacteriecae are main causes of hospital acquired infections. Both E. Coli and K.pneumonie are presently resistant to several groups of antimicrobial agents including penicillins and cephalosporins.[13] This is due to several risk factors often acting concurrently and making therapeutic options difficult.[14,15] Amongst these risk factors, ESBL production is far more important & necessitates prompt surveillance in hospital environment.

Our study at lady Reading Hospital indicate that ESBL producing E. Coli and K. Pneumoniae are more frequent and are reported from each unit. Amongst specimen, pus was having the highest yield of ESBLs followed by urine, blood and sputum. Different types of wounds especially post-surgical site infections are highly exposed to contaminations with these isolates[16]. Lack of contact precautions and failure to follow infection control measures contribute to infection with these bacteria. Implants, i/v lines and follys' catheters can readily become colonised with these pathogens leading to serious UTI and septecemia. These risk factors have been mentioned in other studies from Pakistan.[1,17]There are several phenotypic methods for the detection of ESBL in E. Coli and K.Pnumoniae. The Double Disc Diffusion method is simple and can be carried out in routine laboratory workflow[9]

We suggest that any E. Coli and K. Pneumoniae found resistant to any one of the third generation cephalosporin should be suspected of being a potential ESBL producer and should be subjected to the double disc synergy test. The use of more than one discs increases the sensitivity of ESBL detection.[10] If the isolate is confirmed ESBL positive, it should be reported resistant to all penicillins, cephalosporins (except for cephamycines) and monobactam irrespective of their in vitro sensitivity.[5]

The increased frequency of ESBLs is closely linked with the injudicious use of different groups of antibiotics especially cephalosporins. These antibiotics are freely available over the counter and are used indiscriminately. This factor is especially important at our hospital where the trend of bacterial culture and sensitivity profile is quite low and patients are being treated on best guess concept. Same situation is developing in community acquired infection where ESBLs frequency is 12.1%.Again widespread use of cephalosporins may be the most important driving force.[18]

CONCLUSION

All isolates of E.coli and klebsiellaepneumoniae should be properly detected for ESBL confirmation and reported accordingly. Antibiotics like penicillins, cephalosporins and monobactam must be prescribed with care following correct susceptibility reports. Drastic measures need to be followed to reduce the burden of this menace in hospital environment.

REFERENCES

1. Kausar J, Afia Z, Rumina H. **Frequency and sensitivity pattern of extended spectrum beta lactamase producing isolates in a tertiary care hospital laboratory of Pakistan.** J Pak Med Assoc 2005;55:436-9.

2. Hafeez R, Aslam M, Mir F, Tahir M, Javaid I, Ajmal AN. **Frequency of extended spectrum beta lactamase producing gram negative bacilli among clinical isolates.** Biomedica 2009;25:112-5.

3. Rafique S, Mehmood A, Qayyum M, qazilbash AA. **Prevalence patterns of community-based and nosocomial urinary tract infection caused by Escherichia coli.** Pak J BiolSci 2002; 5:494-6.

4. Shah AA, Hasan F, Ahmed S, Hameed A. **Prevalence of**

extended-spectrum beta-lactamases in Nosocomial and outpatients (ambulatory). Pak J Med Sci 2003; 19:187-91.

5. Clinical and Laboratory Standards Institute (CLSI). **Performance standards for antimicrobial susceptibility testing:** twentieth informational supplement, M100-S20. Vol. 30. Wayne, PA: CLSI,2010:1-153.

6. Gupta V, Yadav A, Joshi RM. **Antibiotic resistance pattern inuropathogens.** Indian J Med Microbiol 2002; 20:96-8.

7. Zaman G, Karamat KA, Abbasi SA, Rafi S, Ikram A. **Prevalenceof extended spectrum beta lactamase (ESBL) producing Enterobacteriaceae in Nosocomial isolates.** Pak Armed Forces Med J1999; 49:91-6.

8. Nathisuwan S, Burgess S, Lewis JS 2nd. **Extended-Spectrum Lactamases: Epidemiology, Detection, and Treatment.** Pharmacotherapy 2001;21:920-8.

9. Winokur P, Jones RN, Pfaller MA (2000). **Characterization of strains from Europe, North and Latin America, and Western Pacific that express an extended-spectrum ß-lactamase phenotype: report from the SENTRY antimicrobial surveillance program (1997-1999).** Presented at the 40th interscience conference on antimicrobial agents and chemotherapy. Toronto, Canada, September 17-20, 2000.

10. Hirakata Y, Matsuda J, Miyazaki Y, Kamihira S, Kawakami S, Miyazawa Y, et al. **Regional variation in the prevalence of extended-spectrum beta-lactamase-producing clinical isolates in the Asia-Pacific region (SENTRY 1998-2002).** DiagnMicrobiol Infect Dis 2005;52(4):323-9.

11. Chaudhary U, Aggarwal R. **Extended spectrum -lactamases (ESBL) - An emerging threat to clinical therapeutics.** Indian J Med Microbiol 2004;22(2):75-80.

12. Angel DM, Ramon HJ, Martinez-Martinez L, Rodriguez-Bano J, Pascual A. **Extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiellapneumoniae in Spanish hospitals; 2nd multicenter study (GEIH-BLEE project, 2006).** EnfermInfeccMicrobiolClin 2009;27(9):503-10.

13. Giamarellos-Bourboulis EJ, Papadimitriou E,Galanakis N, Antonopoulou A, Tsaganos T, Kanellakopoulou K, et al. **Multidrug resistance to antimicrobials as a predominant factor influencing patient survival.** Int J Antimicrob Agents 2006;27(6):476-81.

14. Aminzadeh Z, Sadat KM, Sha'bani M. **Bacteriuria by extended-spectrum Beta-lactamase-producing Escherichiacoli and Klebsiellapneumoniae: isolates in a governmental hospital in South of Tehran, Iran.** Iran J Kidney Dis2008;2(4):197-200.

15. Akyar I. **Antibiotic resistance rates of extended spectrum beta-lactamase producing Escherichia coli and Klebsiella spp. strains isolated from urinary tract infections in a private hospital.** MikrobiyolBul 2008;42(4):713-5.

16. Rupp ME, Fey PD. **Extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae: considerations for diagnosis, prevention and drug treatment.** Drugs 2003;63(4):353-65.

17. El-Khizzi NA, Bakheshwain SM. **Prevelance of extended-spectrum beta-lactamases among Enterobacteriaceae isolated from blood culture in a tertiary hospital.** Saudi Med J 2006;27(1);37-40.

18. Pavia AT, Byington CL. **Extended-spectrum beta-lactamase-producing pathogens in a children's hospital: a 5-year experience.** Am J Infect Control 2009;37(6):435-41.

AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Dr. Fazli Bari	Focal to conduct research & hypothesis designing & article arrangement	
2	Muhammad Akram	Contribute to statiscal data analysis	
3	Hamidullah Shah	Contribution in reults interpretation	
4	Muhammad Imtiaz Shafiq	Manuscript design/ finalization	