FREQUENCY AND DETECTION OF EXTENDED SPECTRUM BETALACTAMASE IN ESCHERICHIA COLI AND KLEBSIELLA PNEUMONIAE: A STUDY AT LADY READING HOSPITAL PESHAWAR

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ABSTRACT

Objectives: To detect extended spectrum beta lactamase in E. coli and Klebsiella pneumonia in bacterial cultures and its frequency at Lady Reading Hospital, Peshawar.

Methodology: This cross sectional analytical study was conducted at LRH between June 2013 to December 2013, a total of 1037 bacterial isolates including 614 E. coli and 423 of Klebsiella pneumoniae 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: Patients' mean age was 40 years. Out of 1037 cases,592 (55%) were males and 445 (45%) were females. Of these, E. Coli were 614 (59.2%) and K. Pneumoniae were 423 (40.8%).Frequency of ESBL positivity in E. coli isolates was 264 (43%) and in Klebsiella pneumonia isolates was 231 (54.6%). Frequency of ESBL in pus was 34.3%(152/395),in urineit 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 &gynaecology23.2% (103/178) and outpatients 12.1 %(54/132). No significant correlation between ESBL positivity and age, gender, unit or specimen was found.

Conclusion: ESBL positive isolates of E. coli and K. pneumoniae account for a very high percentage of hospital-acquired infections. These results should be considered while prescribing penicillins and cephalosporins for treating gram-negative acquired infections.

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

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INTRODUCTION

Entetro-bacteriacae, also known as Coliforms, are the main cause of hospital acquired infections in the world. Among them E. coli and K. pneumoniae are the most prevalent members 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]. 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 Entetro-bacteriacae have evolved various mechanisms of resistance, the most important of them being Extended Spectrum Beta Lactamases (ESBL)[2,3]. Extended Spectrum Beta Lactamases have as much as 200 variants including TEM (Temoria) and SHV (Sulphydryl)(4). 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 antibiotics administration in hospitals[5,6]. Correct detection and timely surveillance of ESBL producing Entetrobac peiriacaein a hospital can greatly influence empirical therapy of high risk patients with se-

vere 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 minimize 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 method. A suspension of bacterial isolates in normal saline equivalent to 0.5M Mcfarland standard was prepared under strict aseptic conditions. A sterlised swab was immersedin the suspension and then squeezed with the inner side of the vial. A full plate of Mueller Hinton agar(150mm) was inoculated with the swab, rotating the plate at 60^0 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. 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.

Antibiotic Disc content and zone break point were used as:

Cefpodoxime 10mcg:≤17mm

Ceftazidime 30mcg:≤17mm

Cefotaxime 30mcg:≤22mm

Aztreonam 30mcg:≤17mm

Double Disc Synergy Test

All isolates with zones of inhibition were suspected of potential ESBL producer and therefore, were subjected to 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 extension of the edge of any of the cephalosporin or aztreonam discs towards amoxicillin-clavulanateindicates the isolate is an ESBL producer. In case of no such zone, the isolate is not ESBL producing.

All data were analyzed with software SPSS, version 16. p values of less than 0.05 were considered significant.

RESULTS

Mean age of the patients was 40 years. Five hundred and ninety two were male (57%) and four hundred and forty five (43%) were female. Out of 443 ESBL positive cases, 222 were males and 221 were females. E. Coli accounted for six hundred and fourteen (59.2%) and K. Pneumoniae for four hundred and twenty three (40.8%) samples. The details are given in table 1 and figure 1.

Frequency of ESBLs in E. coli was 44.6% and in K. pneumoniae, it was 39.9%. fig.1

Specimen and unit wise distribution is given in table 2 and 3 respectively.

DISCUSSION

Members of the family Entero-bacteriacae 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].

Our study at lady Reading Hospital indicate that ESBL producing E. Coli and K. Pneumonie are endemic 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 surgical sites 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 becomecolonized with these pathogens leading to serious UTI and septicemia. 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 in vitro sensitivity[11, 12].

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

Table 1: Demographic details

Age	Frequency	Sex		Specimen			
		Male	Female	Pus	Urine	blood	Sputum
0-09	97	42	55	23	24	15	6
10-19	141	91	50	69	27	24	8
20-29	283	134	152	135	96	55	07
30-39	298	183	115	43	65	63	10
40-49	131	86	27	86	103	53	06
>50	105	56	46	39	53	23	04
Total	1037	592	445	395	368	233	41

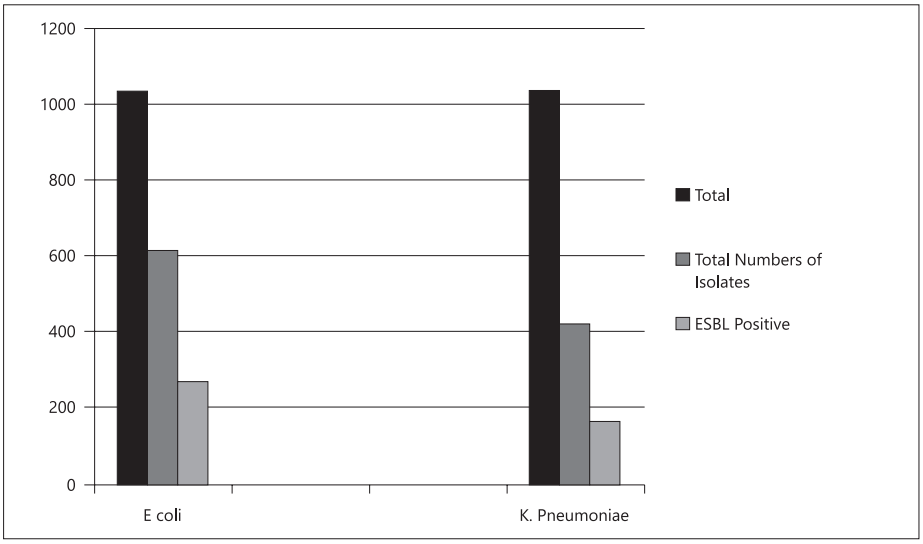
Table 2: Specimen wise distribution of cases

Specimen	Total	ESBL Positive	ESBL Negative
Pus	395	152 (14.7%)	243 (23.5%)
Urine	368	141 (13.6%)	227 (21.9%)
Blood	233	127 (12.2%)	106 (10.2%)
Sputum	41	23 (2.2%)	18 (1.7%)
	1037 (100%)	443 (42.7)	594 (57.3)

Table 3: Unit wise distribution of cases

Unit	Total	ESBL Positive	ESBL Negative
Surgical and allied	283	109 (10.5%)	174 (16.8%)
Medical and Allied	241	95 (9.2%)	146 (14.1%)
Peadiatrics	203	82 (7.9%)	121 (11.7%)
Obstetrics&Gnynaecology	178	103 (9.9%)	75 (7.2%)
Outpatients	132	54 (5.2%)	78 (7.5%)
	1037 (100%)	443 (42.7)	594 (57.3)

Figure 1: ESBL positivity in E. Coli & K. pneumoniae



profile is quite low and patients are being treated on best guess concept[11]. 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 klebsiellae pneumoniae 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.

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CONTRIBUTORS

FB conceived the idea, planned the study, and drafted the manuscript. HS helped acquisition of data and did statistical analysis. RWdrafted and critically revised the manuscript. All authors contributed significantly to the submitted manuscript.