Original Article

Frequency and sensitivity pattern of Extended Spectrum Beta Lactamase producing isolates in a tertiary care hospital laboratory of Pakistan

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Abstract

Objective: To determine frequency, distribution and sensitivity pattern of Extended-Spectrum ß Lactamase (EBSL) producing organism at a tertiary care hospital in Pakistan.

Methods: All members of enterobacteriacae isolated between April and August 2002 were studied. Isolates were speciated according to standard biochemical tests. Susceptibility testing was performed by Kirby-Bauer method. ESBL was detected using double disc method using cefotaxime versus cefotaxime plus clavulanate according to NCCLS. Statistical analysis was performed by SPSS version 10. Test of significance were calculated using chi-square test.

Results: During the study period, 1137/2840 (40%) of the isolates tested were found to be ESBL producing. ESBL positivity was detected in 50% Enterobacter sp., 41% E.coli and 36% K.pneumoniae. ESBL production was noted in 52% of nosocomial isolates tested (415/799). ESBL was more frequent in patients at the extremes of ages (under 5 years and more than 60 years). Cross-resistance to non-beta lactam antibiotics (flouoroquinolones, aminoglycosides and co-trimoxazole) was also more frequent in ESBL producing organisms ($p<0.05$).

Conclusion: A high frequency of ESBL positivity amongst our isolates is documented which is alarming in low-income settings where expensive second line agents are unavailable. Our data supports urgent need for regular screening and surveillance for these organisms (JPMA 55:436;2005).

Introduction

Extended-spectrum ß-lactamase (ESBL) producing organisms are a major problem in the area of infectious disease[1] conferring resistance to all ß-lactam antibiotics except cephamycins and carbapenems.[2] In addition, ESBL-producing organisms frequently show cross-resistance to many other classes of antibiotics; including aminoglycosides and fluoroquinolones[3] thus treatment of these infections is often a therapeutic challenge.

The frequency of ESBL-producing organisms differs significantly in accordance with geographic location.[4-6] The ESBL positivity rate amongst K.pneumoniae is reported at 45% in Latin America and 7% in the United States. In New York, on the other hand, surveillance of 15 hospitals in Brooklyn report, 34% ESBL positivity was found in K. pneumoniae.[5] Similarly, although frequency of ESBL producing E.coli in Europe, North, Latin America and Western Pacific is reported at 1-8%[4], its prevalence in the Asia Pacific region and South Africa is reported at more than 20%.[7] Mathur et al from India recently reported 68% ESBL positivity rate in their enterobacteriacae isolates[8] while Shah et al and Zaman et al have reported a frequency of 48% and 35% respectively from Pakistan (Table).[9,10]

Detection of ESBL is a major challenge for the clinical microbiology laboratory.[11] Its presence in bacterial cells does not always produce phenotypic resistance resulting in some ESBL isolates appearing susceptible to third-generation cephalosporin in vitro. However treatment of these isolates with third-generation cephalosporins is frequently ineffective.[12,13]

Table. Reported ESBL positivity rates from Asia.

Year	Country	% (n) (ESBL)	Reference
2002	Pakistan	48 (400)	9
2002	China, Japan, Taiwan, Singapore, Philippine	>20 (2193)	7
2002	India	68 (678)	8
2000	Hong Kong	11-13 (1174)	25
1999	Pakistan	35 (200)	10

A proficiency testing project for clinical laboratories participating in the National Nosocomial Infections Surveillance System indicated that as many as 58% of laboratories failed to detect and report ESBL isolates correctly.[14] In some studies, 37% of ESBL producing organisms were misreported[15] whereas in another study only 7 out of 38 laboratories correctly identified and reported these organisms.[16] These data suggest that improvement in the ability of clinical laboratories to detect ESBLs is needed. National Committee for Clinical Laboratory Standards (NCCLS) and British Society for Antimicrobial and Chemotherapy (BSAC) have published standardized criteria for screening, confirmatory testing and reporting of these organisms.[17,18] All ESBL producing organisms

should be reported as being resistant to all penicillins, cephalosporins and aztreonam.

In developing countries, many laboratories do not routinely detect ESBL production, a practice which is likely to result in misreporting and hence treatment failures. On the other hand, in areas with low ESBL levels it may not be cost effective to test for ESBL on a routine basis.[19] It is therefore essential that ESBL positivity rates are monitored and that decision regarding appropriate laboratory practices made in light of local/regional ESBL data. Moreover correct reporting would limit inappropriate antimicrobial usage and hence decrease emergence and extension of antimicrobial resistance worldwide. In this paper, ESBL positivity rates among isolates from Karachi, Pakistan are discussed along with implications with regard to laboratory practices in developing countries. Therefore frequency, distribution and sensitivity pattern of EBSL producing organism in samples submitted to a tertiary care referral hospital laboratory was determined.

Material and Methods

This descriptive study was performed at a 550 bed tertiary care hospital located in Karachi, Pakistan. Clinical microbiology laboratory of the hospital receives samples from in and outpatients presenting to the tertiary care centre as well as from referrals other hospitals, clinics and general practitioners across the city.

All enterobacteriacae isolated between April to October 2002 (2840 isolates) were studied for ESBL production. These included 1248 isolates from patients presenting to our hospital (including inpatients, and patients from emergency room, consulting clinics as well as from our community health centre). While 1590 isolates were from referrals outside (i.e. from other hospitals, clinics and general practitioners across the city).

Enterobacteriaceae growing in clinical specimens were identified using routine biochemical tests.[20] Kirby Bauer was performed in accordance with NCCLS guidelines[17] using Mueller Hinton agar (Oxoid). ESBL detection method used was double disc method using cefotaxime (30 µg) in comparison to cefotaxime plus clavulanate (30+10 µg) (Oxoid) according to NCCLS criteria.[17]

SPSS version 10 was used to enter and analyze data. Descriptive analysis was carried out and test of significance was calculated using chi-square test.

Results

During the study period 2840 isolates of enterobacteriaceae were identified. Of these, 2016 (71%) were E.coli, 429 (15%) K.pneumonae and 256 (9%) Enterobacter sp. Overall 1137/2840 (40%) were ESBL producing. The sources of ESBL positive isolates included urine (n=784), blood (n=119), sterile body fluids (CSF, pleural and peritoneal fluids) (n= 86), respiratory specimens (n= 62) and central lines (n=20). Frequency of ESBL positivity was highest amongst Enterobacter species 50% (n=256) followed by E.coli 41% (n=2016), Klebsilla species 36% (n=429), Morganella species 27% (n=111), Proteus species 20% (n=51) with Citrobacter sp. showing the lowest positivity rate 14% (n=22).

Out of 2840 enterobacteriaceae isolates, 1248 isolates from patients presenting to the tertiary care centre itself were further analyzed in terms of location and ESBL positivity. Unfortunately, the remaining isolates from outside referrals could not be analyzed due to incomplete clinical information. While ESBL positivity was more than 30% in all study areas, highest positivity rate 52% (n=799) was significantly noted amongst inpatient isolates. The positivity rate was also high in isolates from the emergency room 45% (n=269) and consulting clinics 39% (n=140). Whereas ESBL isolation rate was significantly lower, 30% (n= 40) in community health centre patients as compared to inpatients. ($p>0.01$)

Age wise break up of ESBL positivity rate is shown in Figure 1. Mean age of patients with ESBL producing organisms was 47 (Range: 1-95) versus 43 (Range 1-100) for non ESBL producers ($p<0.01$). Furthermore ESBL production is significantly more in patients less than 5 years and more than 60 years of age ($p<0.01$) (Figure 1).

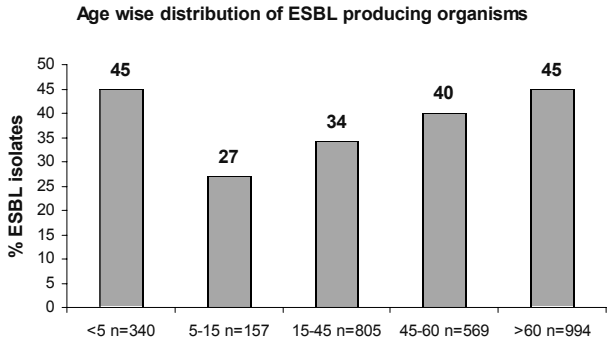

Figure 1. Age wise distribution of ESBL producing organisms exhibiting increase frequency of ESBL isolates at extremes of ages. % ESBL isolates represents ESBL positive isolates divided by total number of isolates obtained from a particular age group.

An analysis of cross resistance to other antibiotics amongst ESBL producing isolates showed that ESBL positive isolates had significantly higher resistance to other classes of antibiotics; aminoglycosides, quinolones

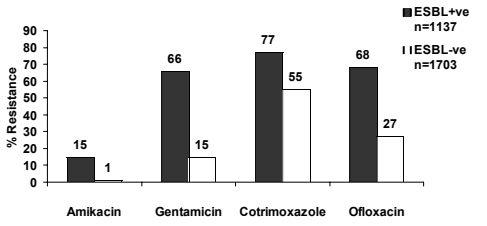

Figure 2. Comparison of sensitivity pattern of ESBL versus non ESBL to amikacin, gentamicin, cotrimoxazole and ofloxacin showing cross resistance to these antibiotics as well.

and cotrimoxazole ($p<0.01$) (Figure 2). No resistance was seen to carbapenems and only 23 (1%) isolates were resistant to piperacillin/tazobactam.

Discussion

ESBL producing organisms are among the fastest growing problems in the area of infectious diseases. Clinical microbiological laboratories can no longer rely on simple in vitro susceptibility data in the absence of the proper detection of ESBL. Two other studies from Pakistan[9,10] have also reported high frequencies of ESBL positivity rate. Shah et al from Pakistan reported that 48% of their ESBL positive isolates were from patients between 50-60 years of age.[9] Other studies report a mean ages of 83[21] and 58[22] years for ESBL as compared to 47 years in this study. The increasing prevalence in the lower age group is likely to be related to the overall increase in ESBL load in our setting, a hypothesis supported by other surveillance studies from Asia and Asian Pacific region reporting an alarming increase[7] in ESBL positivity.

This study observed highest positivity for ESBL production in Enterobacter sp. followed by E.coli. Zaman et al from Pakistan reported highest frequency of ESBL production in Klebsiella sp. followed by E.coli.[10] The SENTRY surveillance programme form Asia Pacific and South Africa reports that most common ESBL producer was Klebsiella sp.[23] Mathur et al[8] from India have also reported Klebsiella sp. as the top ESBL producing organism.

Antibiotic pressure is reported to result in a mutation in beta lactamase gene with production of ESBL.[3] Risk factors responsible for this high frequency in our setting have not been determined. However, the higher positivity rate in inpatient isolates as compared to those from patients presenting to the community health centre is likely to reflect greater antibiotic pressure amongst the inpatients. ESBL positivity was also high in patients from emergency room and consulting clinics which is likely to be a reflection of tertiary referrals and discharged inpatients being seen in these areas respectively. The importance of antibiotic pressure is further supported by the significantly higher ESBL positivity in isolates from patients at the extremes of ages where antimicrobial usage is likely to be higher.

One of the dilemmas of ESBL producing organism is that they are frequently resistant to antibiotics other than beta lactams as they contain plasmids with genes that encode resistance to aminoglycosides, quinolones and trimethoprim sulfmethoxazole.[24] Various studies[22,23] have documented that ESBL producing organisms showed reduced susceptibility to all antibiotics except amikacin and carbapenems. We note a similar phenomenon with increased resistance to all antibiotics in ESBL producing organisms in comparison with non-ESBLs. Thus treatment options in these infections are very limited.

In conclusion, we document a high prevalence of ESBL positivity amongst our isolates. Due to limited resources, several laboratories in developing countries do not routinely detect ESBL production. However, our data supports an urgent need for regular screening and surveillance for these organisms in this region. Increased ESBL positivity in isolates from patients at the extremes of ages most likely reflects high antimicrobial usage in this population. Moreover, cross-resistance in ESBL positive isolates to non-beta lactam agents severely limits therapeutic choices and is alarming particularly in low-income settings where expensive second line agents are unavailable.

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