Original Article

Quantification of Extended Spectrum β-Lactamase Producing Isolates among Gram Negative Bacteria in Hospitalized Patients with Blood Stream Infections

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ABSTRACT

Objective: To quantify ESBL producing microorganisms among hospitalized patients with blood stream infections,
Study Design: Descriptive study
Place and Duration of Study: This study was conducted at the Department of Pathology, Frontier Medical College, Abbottabad from Jan 2016 to Dec 2016.
Materials and Methods: Bacterial isolates were recognized based on colony morphology and biochemical characteristics. Modified Kirby-Bauer method using Mueller Hinton agar was used to check for antibiotic sensitivity. ESBL producers were identified using double disc synergy test.
Results: Overall 152 samples yielded a growth of gram negative bacteria. Out of these 152 positive samples, 80% comprised of E. coli while 12% were K. pneumoniae, 05% were P. aeruginosa and 03% were P. mirabilus. The prevalence of ESBL producing bacteria was low, 1.97%. Most of them were observed in male patients as compared to female patients. Likewise, their incidence was similar among different age groups but there was no ESBL producing organism observed in patients who were less than 20 years of age. As per the site of involvement, ESBL producing bacteria were most commonly seen in specimens received from ICU followed by medical ward.
Conclusion: Infections caused by ESBL producing microorganisms are not uncommon in clinical practice. Detection of such bacteria is pivotal in both community and hospital acquired infections as rapid identification and characterization of such resistant bacteria will aid in minimizing the spread of these infections as well as in selecting appropriate antimicrobial therapy.
Key Words: ESBL, gram negative

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INTRODUCTION

β–lactam antibiotics are frequently used in the management of infectious diseases and therefore, emerging and increasing resistance to these drugs is common. Persistent exposure to β–lactam antibiotics has led to mutations and continued production of β–lactamases which has resulted in bacterial resistance to even newer groups of β–lactam antibiotics.[1,2] These enzymes have been labeled as extended spectrum β–lactamase (ESBL).

Infections with these ESBL producing microorganisms pose a new and significant risk in hospitalized patients globally. They lead to both hospital and community acquired infections and are associated with higher mortality.[3-5] The geographical distribution of such clinical isolates varies greatly depending on geography and institution. The prevalence of ESBL producing Enterobacteriaceae varies between 0 – 25% among different institutes in United States but the national average is 3%.[6] Similarly, ceftazidime resistance in K. pneumoniae isolates varies from 5% in non-intensive care unit to 10% in intensive care unit (ICU).[6] In Japan, there is a very low incidence of ESBL producing isolates among K. pneumoniae and E. coli where only 0.3% of K. pneumoniae and < 0.1% of E. coli isolates were ESBL positive.[7]

It is remarkable that certain specific ESBLs are solely present in certain geographical areas or regions. For example, infection with TEM-10 is quite new in Europe while it is responsible for many disparate outbreaks in United States for many years.[8-11] On the other hand, TEM-3 is found frequently in France but it is undetected in United States.[12] TEM refers to Temoniera who was a patient from Greece from whom this strain was first isolated.[13]

Indiscriminate and injudicious use of antibiotics especially broad spectrum cephalosporins is a major

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cause of emergence of ESBL producing microorganisms.[14] Duration of inpatient and specifically ICU stay, catheterization, mechanical ventilation and intubation, severity of disease and prior antibiotic use are few of the risk factors which are associated with the emergence of ESBLs.[6]

MATERIALS AND METHODS

This descriptive study was performed Department of Pathology, Frontier Medical & Dental College, Abbottabad from Jan 2016 to Dec 2016. Samples, received in the Pathology department for culture and sensitivity from hospitalized patients, were included. Bacterial isolates were recognized based on colony morphology and biochemical characteristics. Modified Kirby-Bauer method using Mueller Hinton agar was used to check for antibiotic sensitivity of the gram negative bacteria using a set of antibiotics comprising of imipenem, aztreonam, levofloxacin, amoxicillin with clavulanic acid, ceftazidime and cefoperazone with sulbactam. ESBL producers were identified using double disc synergy test as described by Jarlier et al.[15] Test organisms were swabbed on the surface of Mueller Hinton agar plate. A disc of amoxicillin and clavulanic acid (20µg and 10µg) was positioned in the center while cephalosporin (ceftriaxone, cefotaxime, and that of ceftazidime, 30µg each) and aztreonam (30µg) discs were placed around the central disc at a distance of 30mm. Extension of zone of inhibition around cephalosporin/aztreonam discs towards central disc were labeled as ESBL producers. E. coli ATCC 25922 was used as a negative while a recognized ESBL producer were used as positive control.[5]

RESULTS

Overall 152 samples yielded a growth of gram negative bacteria. Out of these 152 positive samples, 80% comprised of E. coli while 12% were K. pneumoniae, 05% were P. aeruginosa and 03% were P. mirabilus. The prevalence of ESBL producing bacteria was low, 1.97%. Most of them were observed in male patients as compared to female patients, Table 1.

Table No.1. Distribution of ESBL producing bacteria as per gender

Gender	Characteristics	Number	%age
Male	Total Isolates	97	63.82%
	ESBL Producing Isolates	02	1.31%
Female	Total Isolates	55	36.18%
	ESBL Producing Isolates	01	0.66%

Likewise, their incidence was similar among different age groups but there was no ESBL producing organism observed in patients who were less than 20 years of age, Table 2. As per the site of involvement, ESBL producing bacteria were most commonly seen in specimens received from ICU followed by medical ward, Table 3.

Table No.2: Incidence of ESBL producing bacteria among different age groups

Age, (in years)	Total Isolates	ESBL Producing Isolates
0 – 20	39, 25.66%	00, 00%
21 – 40	59, 38.82%	01, 0.65%
41 – 60	34, 22.37%	01, 0.66%
> 61	20, 13.15%	01, 0.66%
Total	152, 100%	03, 1.97%

Table No.3: Stratification of ESBL producing bacteria according to the site of isolation

Hospital Site	Total Isolates	ESBL Producing Isolates
ICU	68, 44.74%	02, 1.31%
Medical Ward	50, 32.89%	01, 0.66%
Surgical Ward	29, 19.08%	00, 00%
Gynecology Ward	05, 3.29%	00, 00%
Total	152, 100%	03, 1.97%

Both E. coli and K. pneumoniae were sensitive to imipenem and levofloxacin while E. coli showed moderate resistance to amoxicillin plus clavulanic acid and ceftazidime, Figure 1. P. aerugenosa was sensitive to most of the antibiotics. P. mirabilus was sensitive to levofloxacin and cefoperazone plus sulbactam while it showed moderate resistance to imipenem, aztreonam and amoxicillin plus clavulanic acid.

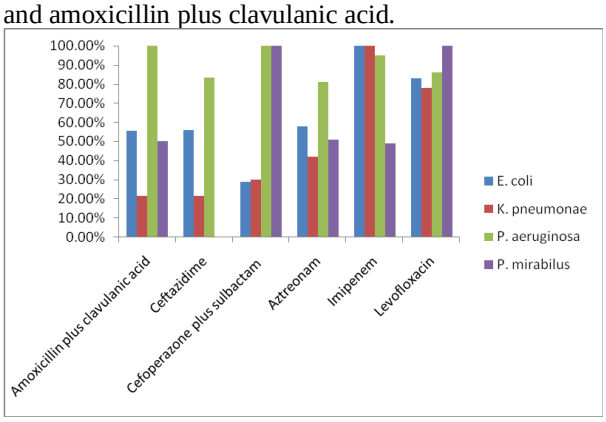

Figure No.1: Antibiotic sensitivity of gram negative bacteria

DISCUSSION

Resistance of infectious organisms to conventional antimicrobial drugs has become a global issue with serious consequences on the outcome of infectious diseases. Misuse or overuse of antibiotics has played a major role in acquiring this resistance to antibiotics.[2] There is a very high risk of treatment failure in patients who have acquired infections, which were caused by ESBL positive organisms, when given extended

spectrum β–lactam antibiotics. Thus, all microorganisms which were established for ESBL production should be reported as resistant to all extended spectrum β–lactam antibiotics irrespective of their antibiotic susceptibility test outcomes.[16]

The prevalence of ESBL producing bacteria was low (1.97%) in our study. Similarly, Gray et al have also reported a very low (0.7%) rate of ESBL positive bacteria in their study which was conducted in Malawi.[17] Likewise, only 3.8% of blood isolates in patients from Dakar, Senegal, were ESBL producing microorganisms.[18] Correspondingly, the rate of ESBL was 6% in Saudi Arabia according to Khanfar et al whereas 15.4% of these were detected among hospitalized patients while 4.5% were detected in out-patients.[19] But, on the other hand, the prevalence of ESBL positive bacteria was found to be 10-25% in Southern Europe while it was reported to be 25.8% amongst E. coli and K. pneumoniae isolates in Eastern Europe.[20, 21] The variable rate of ESBL producing microorganisms, being lower in low income counties and higher in higher income countries, could be due to the fact that poor quality or higher cost of antibiotic drugs in developing countries while their overuse in developed counties could lead to this discrepancy in results.[21]

The prevalence of E. coli was higher (80%) in our study. Khanfar et al also reported that majority (83%) of their isolates comprised of E. coli. [19] Likewise, most of the isolates were E. coli in a study conducted by Al-Zarouni et al in UAE.[22]

Knowledge of regional resistance patterns of pathogens, timely initiation of appropriate empirical antimicrobial therapy and strict enforcement of infection control measures could help not only to contain this emerging problem of antimicrobial resistance among microorganisms but also provide a cost effective way of reducing economic burden related to such infections, through prevention, on already over-burdened health system especially in developing countries.

CONCLUSION

Infections caused by ESBL producing microorganisms are not uncommon in clinical practice. Detection of such bacteria is pivotal in both community and hospital acquired infections as rapid identification and characterization of such resistant bacteria will aid in minimizing the spread of these infections as well as in selecting appropriate antimicrobial therapy.

Author's Contribution:

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Conflict of Interest: The study has no conflict of interest to declare by any author.

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