Journal of Clinical Research Letters, ISSN: 0976–7061 & E-ISSN: 0976–707X
Vol. 1, Issue 2, 2010, PP-01-03

Detection of extended spectrum beta lactamases in blood culture isolates

Rajesh K.R.*, Mathavi S., Indra Priyadarsini R.
Department of Microbiology, Vinayaka Mission's Kirupananda Variyar Medical College, Salem, 636308,Tamilnadu, India, biocare.rajesh@gmail.com

Abstract- Background and Objectives: ESBLs have evolved as an important mechanism of resistance in Gram-negative bacteria. Our study is designed to detect ESBL-producing Gram negative Bacilli from blood culture isolates. Materials and Methods: Totally 548 blood samples, collected over a period of 6months were tested. The blood samples were directly added to brain heart infusion (BHI) broth and incubated at 370C. Subculture was made into MacConkey and Blood agar plates. The susceptibility of the pure culture isolates to Third Generation Cephalosporins (3 GC)- Ceftriaxone, Ceftazidime, Cefotaxime and to the other antibiotics were tested by Disc Diffusion Method. Gram Negative Bacilli isolates with resistance or with reduced susceptibility to Ceftriaxone, Ceftazidime and Cefotaxime were selected for the study. Phenotypic confirmation of ESBLs is done by Double Disc Synergy Test. Results: A total of 548 blood samples were collected, out of which there was growth of Gram Negative Bacilli in 63 samples in which 27 isolates were found to be ESBL producers (42.85 %).Conclusion: The emergence and spread of organisms producing Extended-spectrum Beta lactamases can be prevented by repeated surveillance and prudent use of antibiotics.
Key words: ESBL, Blood culture, Gram negative bacilli

Introduction

Extended-spectrum Beta lactamases (ESBLs) are plasmid-mediated enzymes that confer resistance to the Third Generation cephalosporins(3GC) such as cefotaxime, ceftriaxone and ceftazidime. ESBLs have evolved as an important mechanism of resistance in Gram-negative bacteria. ESBL-producing organisms also contain resistance determinants to other important antibiotic groups, such as Aminoglycosides and Fluoroquinolones, limiting the therapeutic options [1]. Delay in managing the infections with ESBL producers is associated with increased mortality and prolonged hospital stay [2,3]. Knowledge of ESBL-producing organisms from blood culture could therefore be advantageous in aiding appropriate antibiotic choice at the earliest opportunity and improving outcomes. Our study is conducted to detect ESBL-producing Gram negative Bacilli from blood culture isolates.

Materials and Methods

A total of 548 Non-repetitive blood samples, recovered over a period of 6months (Apr. 2010 to Sep 2010) were processed at a tertiary care hospital. Immediately after collection, 5-7 ml blood was directly added to brain heart infusion (BHI) broth and incubated at 370 C. Subculture were made into MacConkey and Blood agar plates. The subculture was repeated for up to seven days until the final result was negative. The isolates obtained were identified by conventional identification methods. The identified isolates were subjected to Antimicrobial susceptibility Testing by Disc Diffusion Method as per CLSI guidelines.

Antimicrobial Susceptibilit Test

The susceptibility of the isolates to Third Generation Cephalosporins (3 GC) Ceftriaxone, Ceftazidime, Cefotaxime and to the other antibiotics such as Amikacin, Ampicillin, Ciprofloxacin, Cefipime, Gentamicin, Cotrimoxazole and Imipenem was determined by Disc Diffusion Method [4]. The results were interpreted as per CLSI guidelines [5]. *Escherichia coli* ATCC 25922 strain was used for quality control. Gram Negative Bacilli isolates with resistance or with reduced susceptibility to Ceftriaxone (‹ 21 mm), Ceftazidime (‹ 18 mm) and Cefotaxime (‹ 20 mm) were selected for the study.

ESBL detection by Double Disc Synergy Test (DDST)

In the DDST, synergy was determined between a disc of Augmentin (20 mcg of Amoxicillin & 10 mcg of Clavulanic Acid) and 30 mcg disc of each of the 3 GC antibiotics which were placed at a distance of 30 mm apart on a lawn culture of the resistant isolate under test on MHA. (HiMedia, India) [6]. Test organism was considered to produce ESBL if the zone size around the test antibiotic increased towards the Augmentin disc. This increase occurs because the Clavulanic acid present in the Augmentin disc inactivates the ESBL produced by the test organisms [7].

Results

Fig.1 shows that most of the isolates from blood samples in this study were *Pseudomonas*

Copyright © 2010, Bioinfo Publications

aeruginosa (42.85%), *Escherichia coli* (25.39%) standing second followed by Acinetobacter species (17.46%). A total of 548 blood samples were collected, out of which there was growth of Gram Negative Bacilli in 63 samples. These 63 isolates were subjected to Antimicrobial Susceptibility Testing and DDST for phenotypic confirmation of ESBL in which 27 isolates were found to be ESBL producers (42.85 %). The distribution of the ESBL producing isolates are described in Table 1.

Discussion

Blood Stream Infections are life threatening and have very poor prognosis. Blood Stream Infections (BSIs) are considered to be the leading cause of morbidity and mortality among the health care infections [8]. They are always associated with sepsis and septic shock which requires admission in critical care units [9]. Invasive procedures, prolonged hospital stay, decreased immunity, poor hand washing techniques are some of the factors which are predisposing for BSI's [10]. Bacteria generally implicated in BSIs commonly originate from Respiratory, Genitourinary and Intra-abdominal sites [11].

This condition is even worsened if the Blood stream infections are caused by multidrug resistant organisms making the therapeutic interventions even more complicated. ESBL producing organisms are clinically proved and important cause for failure of cephalosporin therapy [12, 13]. ESBLs are carried by the genes present on plasmids which also carry genes for resistance to other antibiotics [14]. ESBL production coexisted with resistance to several other antibiotics like Quinolones, Aminoglycosides etc. in our study.

The incidence of ESBL producing gram negative bacilli (GNB) in blood isolates is 42.85% in this study. *Pseudomonas aeruginosa* (44.44 %) predominated amongst the ESBL producing blood isolates. *Acinetobacter species* (25.92 %) being the second commonest followed by E. coli (22.22 %). Our results indícate the increased prevalence of ESBL producing GNBs among blood culture isolates. This might be due to the factors like prolonged hospital stay, more invasive procedures, closeness to other patients etc. Moreover these patients have to be treated with heavy dose of antibiotics for a longer duration which in turn would lead to the emergence of multidrug resistant strains by selection pressure. Furthermore these patients have to be managed with empirical therapy till the culture and sensitivity report arrives, which always ends up in treatment failure due to the presence of ESBL producers.

Conclusion

High prevalence of ESBL producing strains in blood isolates (42.85 %) is found in our study. This might result in therapeutic failure of blood stream infections. Repeated surveillance, formulation of Antibiotic policy and proper prescription of antibiotics are the possible routes to prevent the rapid emergence and spread of ESBL producing organisms.

References

[1] Livermore D.M., Woodford N. (2004) *Guidance to diagnostic laboratories. http://www.hpa.org.uk/srmd/div_nsi_armrl/highlights.htm*

[2] Paterson D. L., Ko W. C., von Gottberg A. et al. (2001) *Journal of Clinical Microbiology*, 39, 2206–12.

[3] Brun-Buisson C., Legrand P., Philippon A. et al. (1987) *Lancet*, 2, 302–6.

[4] Bauer,A.W., Truck H. and Sherries J.C. (1990) *Am J Clin Pathol.,* 45, 493 – 496.

[5] Clinical and Laboratory Standards Institute (CLSI) (2010) *20th informational supplement:M100-S20U*, 30(15), 1-16.

[6] Vercauteren E., Descheemaeker P., Ieven M., Sanders C.G., Goossens H. (1997) *J Clin Microbiol.*, 35, 2191–2197.

[7] Subha S. Ananthan (2002) *IJMM*, 20(2), 92– 95.

[8] Deika D.J., Beekman S.E., Chapin K.C., Morel K.A., Munson E. and Doern G.V. (2003) *J. Clinical Microbiol.*, 41,3655-3660.

[9] Balk R. (2000) *Crit. Care. Clin.*, 16, 179–192.

[10] Weinstein M.P., Towns M.L., Quartey S.M., Mirrett S., Reimer L.G., Parmigiani G. and Reller L.B. (1997) *Clin. Infect. Dis.*, 24, 584 – 602.

[11] Jarvis W.R. (2002) *Infect. Control. Hosp. Epidemiol.*, 23, 236–238.

[12] Bush K. (2001) *Clin Infect Dis*, 32, 1085–9.

[13] Bradford P.A. (2001) *Clin Microbiol Rev.,* 14, 933–51.

[14] Paterson D.L. (2000) *Clin Microbiol Infect.*, 6, 460–3.

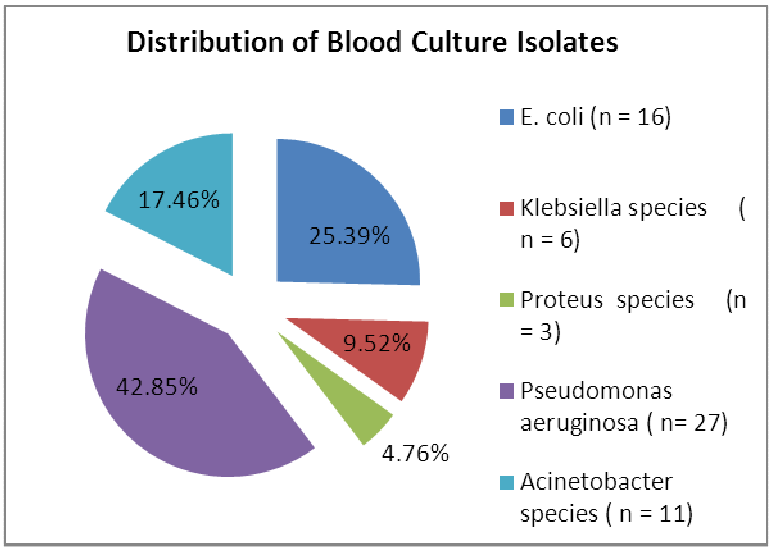

Fig. 1

Table 1 – Distribution of ESBL Positive Isolates

Organisms	**ESBL Positive (42.85 %) (n = 27)**
Escherichia coli	6 (22.22 %)
Klebsiella species	2 (7.40 %)
Proteus species	0 (0 %)
Pseudomonas aeruginosa	12 (44.44 %)
Acinetobacter species	7 (25.92 %)

Copyright © 2010, Bioinfo Publications