Volume : 4 | Issue : 1 | January 2015 • ISSN No 2277 - 8179

Research Paper

Frequency of Extended Spectrum Beta Lactamase producing Gram negative bacteria isolated from blood cultures at children hospital in Baghdad

Biology

KEYWORDS : Septicemia, Gram negative bacteria ,Extended-spectrum β-lactamase.

Nadheema Hammood Hussein	Department of Biology, College of Science, Al-Mustansiryah University./Baghdad/Iraq.
Khetam Habeeb Rasool	Department of Biology, College of Science, Al-Mustansiryah University./Baghdad/Iraq.
Jumaah Dakel Hussein	Fattima-AL-Zahra Hospital for Pediatric and Obstetric /Ministry of Health /Baghdad/Iraq.

ABSTRACT

Aims: The prevalence of extended spectrum beta-lactamases (ESBLs) producing strains and their resistance to beta lactam antibiotics has had a daily increase. Because of the importance of these enzymes in Gram negative bacteria. This study was carried out to investigate it's frequency in Gram negative bacteria isolated from blood cultures at a children hospital in Baghdad. Subjects and Methods: Blood samples (1-2) ml were collected from children suspected with septicemia prior to initiation of antimicrobial therapy, cultured, and the bacterial isolates were identified at species level .The antibiotic susceptibility test was done by using Vitek-2 system . Detection of ESBL producing strains was done by Vitek-2 system and screening test .Results: Frequency of gram negative bacteria during one year of study (1 January 2013 to 1 January 2014) were 169 (13.56%) out of 1246 blood specimens and its frequency was higher in male than female patients, 107(63.3%) vs. 62 (36. most effective antibiotic on these isolates was Imipenem followed by Amikacin. From 169 Gram negative bacteria 113(66.86%) and 103(60.94%) isolates were positive ESBL producing bacteria by Vitek-2 system and screening test, respectively and the commonest ESBL producing gram negative isolates were K. pneumonia (82.05%) followed by E. coli (75%) and Acinetobacter spp. (72.72%).

Introduction

Infections of blood stream are life-threatening and require rapid identification and antibiotic susceptibility testing of the causative agent in order to facilitate specific antimicrobial therapy (1). Blood stream infection (BSI) is a serious problem that needs immediate attention and treatment. It is a cause of high mortality especially if caused by multidrug resistant bacteria (2,3). Extended-spectrum cephalosporins, such as those of third-generation (e.g. ceftriaxone, cefotaxime, ceftazidime) and fourth-generation (e.g. cefepime), are frequently used antibiotics for the treatment of severe infections, because of their ample spectrum, strong bactericidal activity, and low toxicity. However, during the past three decades, an increasing number of extended-spectrum cephalosporins resistant Gram-negative pathogens have been reported worldwide (4). Nowadays Extended Spectrum Beta Lactamase **(**ESBL) producing microbes are emerging threat. The main mechanism for this ESBL resistance by production of enzyme called extended spectrum β-lactamases (ESBLs). The β lactamase enzymes produced by the organisms break down the structural beta-lactam ring of β-lactam antibiotics. Many genera of gram negative bacteria possess a naturally occurring, chromosomally mediated β-lactamase and also some are

Plasmid mediated β- lactamases (5,6). The majority of ESBL producing strains are *Enterobacteriacea*e members such as *Klebsiella pneumoniae, Klebsiella oxytoca* and *Escherichia coli* (7). According to the Infectious Diseases Society of America (IDSA), ESBL-producing *Klebsiella* and *E. coli* belong to the six most important multidrug resistant (MDR) pathogens for which new therapies are urgently needed (8). ESBL

Producing microorganisms are very dynamic and constitutes an increasing problem due to their hydrolyzing activity against extended spectrum third generation cephalosporins and the monobactam (aztreonam) which often employed in the treatment of hospital acquired infections (9). This study aims to detect the frequency of ESBL producing Gram negative bacteria isolated from children blood cultures at a children hospital in Baghdad.

Materials and Methods

Period of Study

This study was conducted during a period of one year from 1 January 2013 until 1 January 2014**.**

Collection of blood specimens

Blood samples were collected from inpatients suspected with blood stream infections prior to initiation of antimicrobial therapy. For each blood culture, 5 mL from infants and children (≥0.5 mL for infants <1 month of age, ≥1 mL for children between 1 month and 36 months of age, and ≥4 mL for children ≥36 months of age) (10).

Isolation and Identification of bacterial isolates

Blood specimens were cultured and microorganisms isolated from all specimens according to standard microbiology methods (11). then microorganisms were identified at species level by using Vitek-2 system (Bio-Merieux)**,** using ID-GNB cards according to the manufacturer's instructions.

Antibiotic Susceptibility Test

Antibiotic susceptibility test towards cephalosporins and other groups of antibiotics including; Ampicillin, Amoxicillin/Clavulanic acid, Ampicillin/ Sulbactam, Piperacillin/Tazobbactam, Cefazolin, Ceftriaxone, Ceftazidime, Cefepime, Ciprofloxacin, Gentamicin, Imipenem, Meropenem, Nitrofurantoin, Levofloxacin, Tobramycin and Trimethoprim/Sulfamethoxazole was done by Vitek-2 system (Bio-Merieux , France) using AST cards according to the manufacturer's instructions.

Phenotypic Detection of ESBL producing strains

1- Detected by Vitek-2 system

Strains were reported as ESBL-positive or ESBL-negative with the Vitek- 2 automated system using the AST-N69 cards, in accordance with the manufacturer's instructions.

2- Screening test

The organism was swabbed on to a Mueller-Hinton agar plate. Antibiotic discs ceftazidime (30 µg) (Al-Razi/ Iraq) and cefotaxime (30 µg) (Al-Razi/ Iraq) were placed on the surface of the agar and incubated. If a zone diameter of ≤ 22 mm for ceftazidime and ≤ 27 mm for cefotaxime was recorded, the strain was considered as 'suspicious' for ESBL, producer (12,13). *Escherichia coli* ATCC 25922 were used as negative control.

Statistical analysis

The Chi-square (χ^2) test was employed for comparison among groups. P value ≤ 0.05 was considered statistically significant.

Volume : 4 | Issue : 1 | January 2015 • ISSN No 2277 - 8179

Results

Study patients

Through a period of one year, blood specimens were collected from 1246 patients suspected with blood stream infections. During this study we noticed that the majority of patients were within the age grouping less than three months and male patients were higher than female patients, 752 (60.35%) vs. 494 (39.65%) out of 1246 children patients.

Blood culture

Out of 1246 blood specimens, the frequency of gram negative bacteria isolated from blood cultures that indicates bacteremia in the studied children patients were 169 (13.56%) cases (figure-1).

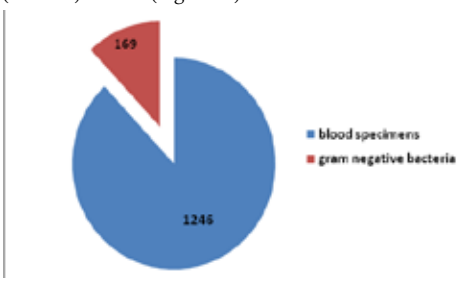

Figure-1: Numbers of gram negative bacteria

According to age and gender, frequency of positive gram negative bacteria in blood culture was higher in male than female patients, 107(63.3%) vs. 62 (36.7%). The majority of patients with positive gram negative bacteria blood culture were within the age group less than three months, 97 (57.4%) patients (*P-value* < 0.05).

It was shown in table -1 that from 169 gram negative bacteria, *Escherichia coli* strains were the most frequently isolated gram negative bacteria in blood specimens from patients followed by *Klebsiella pneumoniae* and then by *Acinetobacter spp* ., *Pseudomonas spp.*, *Enterobacter spp.*, *Serratia spp.*, *Citrobacter spp* and *Salmonella spp.*, respectively.

Table-1: Frequency of gram negative bacteria isolated from blood specimens.

Gram negative bacteria	Number of strains (%)
Escherichia coli	48 (28.40)
Klebsiella pneumoniae	39 (23.08)
Acinetobacter spp.	33 (19.53)
Pseudomonas spp.	20 (11.83)
Enterobacter spp.	19 (11.24)
Serratia spp.	5(2.96)
Citrobacter spp	3 (1.78)
Salmonella spp.	2 (1.18)
Total	169 (100)

Antimicrobial sensitivity test results of Gram negative bacteria from blood specimens to various antimicrobial drugs are shown on table -2. It was shown that the most effective antibiotic on these isolates was Imipenem followed by Amikacin. Gram negative bacteria in this study were mostly resistant to Ampicillin, and Tobramycin. Also *Klebsiella pneumoniae* isolates followed by *E. coli* isolates were mostly resistant to cephalosporins than other gram negative bacteria under study.

Phenotypic Detection of ESBL

Phenotypic detection of ESBL was done by two methods includingVitek-2 system and screening test (figure-3).The frequency of ESBL producing gram negative bacteria was shown in table -3, according to table-3 we found from 169 gram negative bacteria 113(66.86%) and 103(60.94%) isolates were positive ESBL producing bacteria by Vitek-2 system and screening test, respectively. The commonest ESBL producing gram negative isolates were *K. pneumonia* (82.05%) followed by *E. coli* (75%), *Acinetobacter spp.* (72.72%), *Pseudomonas spp.* (55%), *Enterobacter spp* .(47.37%) and *Serratia spp.* (20%), respectively by Vitek-2 system as shown in table-3, and there was no ESBL producer isolate of *Citrobacter spp* and *Salmonella spp.*

Figure-2: Screening test for ESBL producers

Table-2: Antimicrobial sensitivity patterns of gram-negative bacteria isolated from blood cultures of children .

Antimicrobial drug	Escherichia coli n=48		Klebsiella pneumonia n=39		Acinetobacter spp. n=33		Pseudomonas spp. n=20		Enterobacter spp. n=19		Serratia spp. n=5		Citrobacter spp. n=3		Salmonella spp. n=2	
	S* %	R %	S %	R %	S %	R %	S %	R %	S %	R %	S %	R %	S %	R %	S %	R %
Amikacin	100	-	94	6	80	20	80	20	80	20	80	20	90	10	80	20
Amoxicillin-Clavulanic acid	40	60	35	65	17	83	-	100	-	100	60	40	30	70	10	90
Ampicillin	-	100	-	100	-	100	-	100	-	100	-	100	-	100	-	100
Aztreonam	-	100	-	100	-	100	-	100	20	80	50	50	80	20	80	20
Cefepime	30	70	14	86	30	70	20	80	70	30	20	80	75	25	30	70

Ceftazidime	25	75	12	88	30	70	45	55	60	40	80	20	100	-	100	-
Ceftriaxone	25	75	14	86	30	70	50	50	55	45	80	20	80	20	100	-
Gentamicin	50	50	44	56	40	60	60	40	70	30	65	35	45	55	80	20
Imipenem	100	-	100	-	100	-	100	-	100	-	100	-	100	-	100	-
Tobramycin	-	100	-	100	-	100	-	100	-	100	-	100	-	100	-	100
Trimethoprim-Sulphamethoxazole	42	58	30	70	6	94	-	100	10	90	15	85	40	60	20	80

Table-3: Frequency of ESBL producing gram-negative bacteria isolated from blood cultures of children.

Isolates		Escherichia coli n=48	Klebsiella pneumonia n=39	Acinetobacter spp. n=33	Pseudomonas spp. n=20	Enterobacter spp. n=19	Serratia spp. n=5	Citrobacter spp. n=3	Salmonella spp. n=2	Total Number
Number of positive ESBL producing isolates	Vitek-2 system	N=36 (75%)	N=32 (82.05%)	N=24 (72.72%)	N=11 (55%)	N=9 (47.37%)	N=1 (20%)	N=0 (0%)	N=0 (0%)	N=113 (66.86%)
	Screening test	N=34 (70.83%)	N=31 (79.48%)	N=22 (66.66%)	N=9 (45%)	N=6 (31.58%)	N=1 (20%)	N=0 (0%)	N=0 (0%)	N=103 (60.94%)

Discussion

The results of our study showed that the majority of patients were within the age of ≤ three months and the percentage of infection were higher in male(39.65%) than female(60.35%) which is in harmony with the study of Vijayakanthi,et al (2013).*Escherichia coli* strains were the most frequently isolated gram negative bacteria in blood specimens from patients followed by *Klebsiella pneumoniae* and then by *Acinetobacter spp* ., *Pseudomonas spp.*, *Enterobacter spp.*, *Serratia spp.*, *Citrobacter spp.* and *Salmonella spp.*, respectively. Amongst the important ESBL producing gram negative bacilli *Klebsiella pneumonia, Escherichia coli, Proteus mirabilis, Enterobacter spp., Citrobacter spp., Acinetobacter spp.* and Pseudomonas aeruginosa were reported (Shukla ,et al. 2004;Malhotra,et al.2008).

Due to the importance of drug susceptibility tests for the clinical management of patients infected

by ESBL bacteria we studied the antimicrobial profile of these isolates and we found that the most effective antibiotic on these isolates was Imipenem (100%), followed by Amikacins similar results have been reported by other researchers as well (Luzzaro et al.2006, El-Bialy and Abu-Zaid *2009*;Hafeez,et al. 2009).Gram negative bacteria in this study were mostly resistant to Ampicillin, and Tobramycin. Also *Klebsiella pneumonia* isolates followed by *E. coli* isolates were mostly resistant to cephalosporins than other gram negative bacteria under study. In our country, testing for ESBL production is not routinely done by all hospitals. This may allow ESBL-producing strains within or between hospitals to remain undetected for long periods leading to serious outbreaks particularly in intensive care units, resulting in the failure of therapy dependent on second- and third-generation cephalosporins (Yagi et al.2000; Yan et al.2000)

Production of ESBL can varies according, the geographical distribution and bacterial species(Manchanda et al.2005,Baharulla,et al.2013) ,our study showed that the commonest ESBL producing gram negative isolates were *K. pneumonia* (82.05%) followed by *E. coli* (75%), *Acinetobacter spp.* (72.72%), *Pseudomonas spp.* (55%), *Enterobacter spp* .(47.37%) and *Serratia spp.* (20%),this percentage was higher than the study of Shehabia et al., (2000) who reported an incidence of ESBL production in *Klebsiella pneumonia* and *Escherichia coli* isolates in the ICU of Jordan University Hospital, to be 70% and 38%, respectively. The proportion of ESBL-producing isolates in Japan was 4.3% in *E. coli and 3.1% in K. pneumoniae in 2006* (Yamaguchi et al.2007) . Batchoun, et al(2009) reported that ESBL production *Escherichia coli* isolates, found in 21 out of a total of 195 isolates recovered (10.8%)and in 60/84 (72.4%) of the *Klebsiella pneumoniae* isolates. ESBL was detected in 86·6% of Klebsiella spp., 73·4% of Enterobacter spp. and 63·6% of Escherichia coli strains (Jain et al.2003). It was also observed that 74·4–80·9% of these ESBL producers were resistant to cefotaxime and 47·6–59·5% were resistant to Ceftazidime in routine susceptibility testing; these results reflect the misuse or overuse of beta lactam drugs. We designed our study to investigate the prevalence of ESBL-producing Gram-negative rods in septicemic Iraqi neonates.

Conclusion

Routine detection test of ESBL in all culture-positive samples growing gram-negative isolates and proper managements are recommended in order to control spread of these organisms as the infections by ESBL-producing organisms are a significant problem in neonate's intensive care units in our country.

Volume : 4 | Issue : 1 | January 2015 • ISSN No 2277 - 8179

REFERENCE

1. Berit, E. C.; Maria, P. S.; Jorg, G.; Salima, M.; Martin, K.; Oleg, K. (2006). Identification and characterization of bacterial pathogens causing bloodstream infections by DNA microarray. J Clin Microbiol; 44(7): 2389-2397. | 2. Murty, D.S.; Gyaneshwari, M. (2007). Blood cultures in pediatric patients: A study of clinical impact. Indian J Med Microbiol 2007; 25: 220-224. | 3. Diekema, D.J.; Beekmann, S.E. (2003). Come of nosocomial and community-onset bloodstream infection. J Clin Microbiol; 41(8): 3655 -3660. | | 4. Reinert RR, Low DE, Rossi F, Zhang X, Wattal C, Dowzicky MJ. | Antimicrobial susceptibility among organisms from the Asia/ | Pacific Rim, Europe and Latin and North America collected as | part of TEST and the in vitro activity of tigecycline. J Antimicrobial | Chemotherapy. 2007;60(5):1018-29. | 5. Aruna, S. Ramya, S and Balagurunathan, R. (2013). Prevalence of ESBL pathogens in Salem Hospitals and its control. Adv. Appl. Sci. Res., 2013, 4(1):277-284. | 6. Paterson DL, Hujer KM, Hujer AM, Yeiser B, Bonomo MD, Rice LB, Antimicrobial Agents Chemother, 2003, 47, 3554-60. | 7. Ami Y.V., Dogra J.D., Kulkarni M.H., Bhalekar P.N. Extended | spectrum beta lactamase producing Escherichia coli and Klebsiella | pneumoniae in diabetic foot infection. Journal of Pathological Microbiology. 2008; 51(3): 370-372. | 8. Talbot GH, Bradley J, Edwards JE Jr, Gilbert D, Scheld M, Bartlett JG. Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. Clin Infect | Dis. 2006;42(5):657-68. | 9. Dechen C. T., Das S., Adhiakari L., Pal R., Singh T.S.K. Extended spectrum beta lactamases detection in gram negative bacilli of nosocomial origin. Journal of Global Infectious Diseases. 2009; 1(2):22-28. | 10. Connell, T. G.; Rele, M.; Cowley, D. (2007). How reliable is a negative blood culture result? Volume of blood submitted for culture in routine practice in a children's hospital. Pediatrics; 119: 891-894. | 11. Murray, P. R.; Baron, E.J.; Jorgensen, J.H. (2003). editors: Manual of Clinical Microbiology, ed 8, Washington DC, ASM Press. | 12. National Committee of Clinical Laboratory Standards, 2000. Performance standards for antimicrobial disk Susceptibility test, 7th edition, Approved Standards, NCCLS Document: Wayne PA., pp: 20. | 13-Poovendran, P., Vidhya, N. and Murugan, S. (2013). Antimicrobial Susceptibility Pattern of ESBL and Non-ESBL Producing Uropathogenic Escherichia coli (UPEC) and Their Correlation with Biofilm Formation. Intl. J. Microbiol. Res., 4 (1): 56-63 | | | 14-Vijayakanthi N., Bahl D., Kaur N., Maria A., and DubeyN .K.(2013) Frequency and Characteristics of Infections Caused by Extended-Spectrum Beta-Lactamase-Producing Organisms in Neonates: A Prospective Cohort Study . BioMed Research . | | 15-Shukla I., Tiwari R., Agrawal M. Prevalence of extended spectrum-lactamase producing Klebsiella pneumonia in a tertiary care hospital. Indian J Med Microbiol .2004; 22: 87-91. | | 16- Malhotra VL, Khandpur N, Dass A, Mehta G. Prevalence of extended spectrum beta lactamases producing clinical isolates from patients of urinary tract infection in a tertiary care hospital in Delhi. J Commun Dis 2008; 40 (4): 269-72. | | 17-Luzzaro F, Mezzatesta M, Mugnaioli C, Perilli M, Stefani S, Amicosante G, Rossolini GM, Toniolo1 A. (2006).Trends in Production of Extended-Spectrum ß-Lactamases among Enterobacteria of Medical Interest: Report of the Second Italian Nationwide Survey. J of Clin Microbiol. 44 (5): 1659-1664. | | 18-El-Bialy A.A., and Abu-Zeid A. Prevalence and Risk Factors of Extended-Spectrum β-Lactamase Producing Kelbsiella Pneumoniae in Neonatal Intensive Care Unit Egyptian Journal of Medical Microbiology, January 2009 , 18(1)141-149. | | 19-Hafeez R., Aslam M., Mir .F, Tahir M. .Javaid I. ,Aasma A.and Ajmal A. Frequency of extended spectrum beta lactamase producing gram negative bacilli among clinical isolates. Biomedica 2009 .(25): 112 – 115 . | | 20-Yagi T., Kruokawa H., N. Shibata, K. Shibayama, and Y. Arakawa, A preliminary survey of extended-spectrum β- lactamases (ESBLs) in clinical isolates of Klebsiella pneumonia and Escherichia coli in Japan. FEMS Microbiology Letters, vol.184, pp. 53–56, 2000. | | 21-Yan J.J., S. M.Wu, S. H. Tsai, J. J.Wu, and I. J. Su.Prevalence of SHV-12 among clinical isolates of Klebsiella pneumonia producing extended-spectrumβ-lactamases and identification of a novel AmpC enzyme (CMY-8) in southern Taiwan .Antimicrobial Agents and Chemotherapy, vol. 44, pp. 1438–1442, 2000. | 22- Manchanda V, Sing NP, Goyal R, Kumar A, Thukral S.S. Phenotypic characteristics of clinical isolates of Klebsiella pneumoniae and evaluation of available phenotypic techniques for detection of extended spectrum β-lactamases. Indian J Med Res 2005; 122: 330-37. | | 23Baharulla;Syedulla;Shakirulla;Azizulla;Iqbal H.;Wahab,A;Rehman,A.(2013) Prevalence and antimicrobial susceptibility pattern of ESBL producing Gram negative causing nosocomial infection.Int.J.Res.Pharm.Sci.4(2):171-176. | | | 24- Shehabia A.A., A. Mahafzah, I. Baadran, F. A. Qadar, and N. Dajani. High incidence of Klebsiella pneumoniae clinical isolates to extended-spectrum β-lactam drugs in intensive care Units. Diagnostic Microbiology and Infectious Disease, vol. 36, no. 1, pp. 53–56, 2000. | | 25- Yamaguchi K, Ishii Y, Iwata M, Yoshida H, Satoh T, et al. (2007) Nationwide surveillance of parenteral antibiotics containing meropenem activities against clinically isolated strains in 2006. Jpn J Antibiot 60: 344-377. | | 26- Batchoun R.G., Samer F. Swedan, and AbdullahM. Shurman(2009) Extended Spectrum β-Lactamases among Gram-Negative Bacterial Isolates from Clinical Specimens in Three Major Hospitals in --Microbiology.1-8. | | 27-Jain A, Roy I., Gupta M.K., Kumar M.and S. K. Agarwal S.K.Prevalence of extended-spectrum –lactamase producing Gram-negative bacteria in septicemic neonates in a tertiary care hospital. Journal of Medical Microbiology (2003), 52, 421–425. |