Original Article

Pathology

Detection of Beta-Lactamase and Extended Spectrum Beta-Lactamase from Bacteria Causing Neonatal Sepsis

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ABSTRACT

Objectives: The aim of the present study was to detect Beta-Lactamase and ESBL (Extended Spectrum of Beta-Lactamase) by bacteria causing neonatal sepsis

Study Design: Observational Study.

Place and Duration of Study: The study was conducted at the department of Microbiology, Basic Medical Science Institute of JPMC during the period of August 2009- July 2010. The blood samples were taken from babies admitted at National Institute of Child Health.

Materials and Methods: The study included 200 babies between the ages of 1 to 30 days who were presumed to have sepsis on clinical grounds. Neonates who had already been given antibiotics prior to admission and those who had congenital anomaly were excluded. Two hundred (200) blood samples were processed for blood culture. In the laboratory, each specimen were inoculated on differential and selective (Blood agar, MacConkey agar and Chocolate agar) media accordingly. For beta-lactamase production, we performed Chromogenic Cephalosporin method and for ESBL (Extended Spectrum of Beta-Lactamase) we performed double disc diffusion method.

Results: Two strains of *staphylococcus* and single isolated strain of *Haemophilus influenzae* yielded positive beta-lactamase production. Two strains of each *Enterobacter cloacae* and *Klebsiella pneumoniae* and one strain of *Escherichia coli* were positive for ESBL production.

Conclusion: According to our study, prevalence of beta-lactamase and ESBL in the total number of bacteria was low in NICH Karachi. Therefore, beta-lactam antibiotics remain the drug of choice in infections. ESBL detection must be routinely performed in clinical laboratories, as false reporting would result in treatment failure.

Key Words: Beta-Lactamase, ESBL (Extended Spectrum of Beta-Lactamase), DDDT (Double Disc Diffusion Test).

INTRODUCTION

The beta-lactamases are the group of enzymes capable of hydrolyzing the beta-lactam bond of both penicillin and some cephalosporins, thereby causing these antibiotics to become inactive[1].The most significant mechanism of resistance to the β-lactam antimicrobial agents is the production of β-lactamase enzymes[2]. Beta-lactamases enzymes destroy the beta-lactam ring by two major mechanisms of action. Firstly, most common beta-lactamases have a serine-based mechanism of action. Secondly, a less commonly encountered group of beta-lactamases is the metallo beta-lactamases a class of beta-lactamases that require bivalent metal ions, usually zinc for their activity.[1, 3].Beta lactamases are the major defense mechanisms of gram-negative bacteria against the beta-lactam antibiotics[4, 5].

Extended-spectrum beta-lactamase (ESBLs) are plasmid mediated enzymes[6]. These new enzymes are naming extended-spectrum beta-lactamases (ESBLs) to reflect the fact that they are derivatives of older enzymes but have the capability to hydrolyze a broader spectrum of beta-lactam drugs. Extended-spectrum β-lactamase (ESBL)–producing gram-negative bacteria are emerging pathogens and resistance to ß-lactam antimicrobial agents, especially extended-spectrum cephalosporins and other antimicrobial agents is on the rise worldwide [7,8] in all age groups[9]. ESBL-producing strains of the *Enterobacteriaceae* have been reported to cause outbreaks of infections[10, 11], leading to serious antibiotic management concerns[12]. Clinicians, microbiologists, infection control practitioners, and hospital epidemiologists are concerned about ESBL-producing bacteria because of the increasing incidence of such infections, the limitations of effective antimicrobial drug therapy, and adverse patient outcomes. ESBLs producing Enterobacter has been increasingly reported recently [13].

Chromogenic cephalosporin, nitrocefin has been found effective and specific in detecting all known B-lactamases including the Staphylococcal penicillinases and Haemophilus influenza[14, 15]. Oxoid Beta-lactamase (nitrocefin) touch sticks (Code no: BR0066A) are available[16]. Several clinical tests have been developed for the detection of B-lactamases. These tests provide rapid information predictive of the development of resistance[15].

Risk factors that have been associated with the acquisition of ESBL-producing organisms are usage of central venous or arterial catheters, emergency intra-

abdominal surgery, and lung or gastrointestinal tract pathologies[17].

There have been an increasing number of reports of community acquired methicillin-resistant Staphylococcus aureus. Staphylococcus aureus is an important human pathogen causing both community and hospital-associated infections. Penicillin and related β-lactams have dramatically reduced the morbidity and mortality of S. aureus infections, but steadily rising resistance threatens to erode their utility. Either of two mechanisms mediate staphylococcal resistance to β-lactam antibiotics i.e. production of beta lactamase and production of an altered target penicillin binding protein[18].

MATERIALS AND METHODS

This study included 200 babies between the ages of 1 to 30 days who were presumed to have sepsis on clinical grounds. Neonates who had already been given antibiotics prior to admission and those who had congenital anomaly were excluded. Two hundred (200) blood samples were processed for blood culture. In the laboratory, each specimen was inoculated on differential and selective (Blood agar, MacConkey agar and Chocolate agar) media accordingly. For beta-lactamase production, we performed Chromogenic Cephalosporin method and for ESBL (Extended Spectrum of Beta-Lactamase) we performed double disc diffusion method.

Beeta-Lactamase Production Test: For beta-lactamse production Chromogenic Cephalosporin method[14] was performed. In this study, we used nitrocefin discs[15], which were commercially available. These are filter paper discs impregnated with nitrocefin. Discs were moistened and were placed in a Petri dish to prevent it from drying. Then colonies of test organisms were applied on discs.

Interpretation of Beta-Lactamse Production: In positive case of beta-lactamase production, the disc, which contained colonies of Staphylococcus aureus, turned pink red colored within 60 min. The disc, which contained colonies of Haemophilus influenza turned pink colored upto 10 minutes. In colonies of weak positive cases, there was light pink color but in negative cases, there were no change in colour[19].

Extended Spectrum Beta-Lactamase (ESBL) Production Test: Extended spectrum beta-lactamase (ESBL) production was carried out by double disc diffusion test (DDDT) method of[20]. According to double disc diffusion method, susceptibility disc containing Augmentin (Amoxicillin 10 ug + Clavulanic acid 20 ug) was placed in the center of plate containing Muller Hinton Agar. Cefotaxime, Ceftazidime, Ceftriaxone and Azotreonam (30 ug) each were placed at a distance of 30mm (center to center) of Augmentin. Enhancement of inhibition zone of towards Augmentin disc indicating synergy of Clavulanic acid with any one of the test antibiotics, was taken as presumptive evidence of ESBL production[21,22] .The quality control strain used for DDDT were E.coli ATCC 25922[2, 22.]

Discs Used For Double Disc Diffusion Test

DISCS	Concentrations	Oxoid Disc Codes
Augmentin	20+10ug (C.A+Amp)	
Ceftazidime	30ug	CAZ30
Cefotaxime	30ug	CTX30
Ceftriaxone	30ug	CRO30
Azactum	30ug	ATM30

Interpretation of Extended spectrum beta-lactamase (ESBL) production: An organism was interpreted as an ESBL producer if there is an increase or extension of >5mm in the inhibition zone in the area between the Augmentin (amoxicillin-clavulanic acid) disc and any one of the four cephalosporin discs[20].

RESULTS

Out of 200 cases 96 had positive blood cultures of which 73 (76.04%) were gram negative and 23 (23.96%) were gram positive bacterial isolates. Out of 96 cases 3 were detected as beta lactamase producers and 5 were ESBL producer

Table No.1: Beta-lactamase positive isolates of bacteria with percentage

S.No	Bacteria	Beta-lactamase positive isolates	%age
1	Staphylococcus aureus n=10	2	20%
2	Haemophilus influenzae n=1	1	100%

Table No.2: ESBL positive isolates of bacteria isolates with percentage

S.No	Bacteria isolated	ESBL positive isolates	%age
1	Enterobacter cloaca n=23	2	8.69
2	Klebsiella pneumoniae n=16	2	12.50
3	Escherichia coli n=17	1	5.88

DISCUSSION

In our region, most centres do not routinely do testing for ESBL production. Which results in the dissemination of ESBL-producing strains? The spread of ESBLproducing bacteria observed within and between hospitals remains undetected for long periods. The consequence can be serious outbreaks, particularly in the intensive-care units. Detection of ESBL is a

major challenge for the clinical microbiology laboratory as its detection has major impact on therapy. Moreover, presence of an ESBL also has significant infection control implications[23].

In our study none of the isolates of Pseudomonas aeruginosa, Citrobacter freundii, Neisseris meningitididis were positive for ESBL production by the method we used; whether they were actually non-producers, or whether some of them did produce β-lactamases but not inhibited by clavulanate It is supported by study done by[21]. It needs to be further investigated. The commonest ESBL producers have been E.coli and K. pneumoniae. According to a large number of studies[24, 25] and in our study, both these bacteria were ESBL producer.

In this study out of ten isolates of Staphylococcus aureus, eight isolates (resistant to penicillin or cephalosporins) were tested for beta-lactamase production. Among which only two were beta-lactamase positive i.e only 20% strains were beta-lactamase-producing. It is in contrast to study performed by[26] according to them 83.2% were beta-lactamase-producing strains .Single isolated Haemophilus influenzae which were resistant to penicillins and cephalosporins was also positive for beta-lactamase production test . It correlates with findings obtained by[27]. In recent study from Saudi Arabia it was found that there were Klebsiella pneumonia (11.3%), Enterobacter spp (10.14%) and E-coli (9.6%), were positive for ESBL production test[28] , also in our study Enterobacter cloacae (8.69%) Klebsiella pneumoniae (12.5%) and E-coli (5.88%) were positive for ESBL production. In the present study, prevalence of ESBL was low, supported by other study[29].

CONCLUSION

According to our study, prevalence of beta-lactamase and ESBL in the total number of bacteria was low in NICH Karachi. The low prevalence was due to selection of the neonates who were not taking antibiotics. Therefore, beta-lactam antibiotics remain the drug of choice in infections. ESBL detection must be routinely performed in clinical laboratories, as false reporting would result in treatment failure.

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