FREQUENCY OF EXTENDED SPECTRUM β-LACTAMASES (ESBLS) AND AMP C β-LACTAMASE PRODUCING *ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE* ISOLATED FROM NEONATAL SEPSIS IN A TERTIARY CARE HOSPITAL, LAHORE

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ABSTRACT

Background and Objectives: ESBLs and Amp C producers are resistant to variety of antimicrobials which has made treatment strategies complicated. The main objective of study is to determine the frequency of ESBLs and Amp C beta lactamase producing Escherichia coli and Klebsiella pneumonia isolated from neonatal sepsis in a tertiary care hospital Lahore.

Methods: It is a descriptive study carried out on blood samples from clinically suspected septicemic neonates. 95 positive blood cultures with clinical signs and symptoms of sepsis were included in this study in PGMI, Lahore. The blood cultures were taken before the start of antimicrobial therapy. Blood culture reports were assessed for identification by standard methods. Escherichia coli and Klebsiella pneumoniae were identified by using API 20E kit. Antimicrobial susceptibility testing was carried out by Modified Kirby Bauer disk diffusion method on Mueller Hinton agar using CLSI protocols. ESBLs screening and confirmation was done according to CLSI recommendations. Boronic acid disk test was used for detection of Amp C producers.

Results: Out of the 450 blood samples, 95 (21.1%) were culture positive. Among 95 positive blood cultures, Gram negative organisms were recovered from 56 (58.9%). Among Gram negative organisms 13 (23.2%) Esherichia coli and 10 (17.8%) Klebsiella pneumoniae were isolated. 46.1% and 60% isolates of Esherichia coli and Klebsiella pneumoniae were ESBLs producers. Pure Amp C production was seen in 30.7% and 20% of E.coli and K.pneumoniae respectively. However, co-production was observed in 23% E.coli and 20% K.pneumonia isolates. ESBLs producing E.coli and K.pneumoniae showed 100% resistance to amoxicillin-clavulanic acid, ceftazidime, ceftriaxone, cefotaxime and aztreonam. Similarly pure Amp C producers showed 100% resistance to cefoxitin, ceftazidime, ceftriaxone, cefotaxime and aztreonam respectively.

Conclusion: It is very important to differentiate between ESBLs and Amp C producers. Boronic acid compound is a practical and most efficient method to detect Amp C beta lactamase enzymes.

Key Words: Neonatal sepsis, ESBLs, Amp C, boronic acid compounds.

INTRODUCTION

Neonatal sepsis is associated with increased mortality and morbidity.[1] Poor health practices, hospital acquired infections, cheaper antibiotics all gather to increase the incurable rate of neonatal sepsis.[2] Different studies have been carried out on neonatal sepsis which showed tremendous rise of resistance to the first line antibiotics. The greatest challenge today is the emerging disaster of neonatal sepsis in combination with antimicrobial resistance to commonly used antibiotics.[3]

B-lactamase enzymes are commonly categorized in two schemes, the Ambler molecular classification scheme and Bush-Jacoby-Medeiros functional classification scheme. The Ambler molecular strategy separates these enzymes into four main classes, namely class A-D according to protein homology. The Bush-Medeiros-Jacoby classification system groups are classified on the basis of their functional characteristics.[4,5] ESBLs are beta-lactamases belonging to group 2be in the Bush-Jacoby-Medeiros system and to class A in the Ambler system. They are plasmid encoded and hydrolysecephalosporins and monobactams. They do not hydrolyze cephamycins or carbapenems and are inhibited by clavulanic acid.[6]

Amp C β-lactamases are the enzymes belonging to group 1 of Bush functional classification and class C in the Ambler system. They impart resistance to peni-

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cillins, cephalosporins and monobactams except 4th generation cephalosporins and are not inhibited by β-lactamase inhibitor.[7] They can be differentiated from ESBLs in their hydrolysis of cephamycins along with other cephalosporins except fourth generation and no inhibition by clavulanic acid.[8]

The first plasmid encoded ESBLs was discovered in isolates of *Klebsiella ozaenae* in Germany in 1983.[6] The Clinical and Laboratory Standards Institute suggests screening of *Escherichia coli*, *Klebsiella pneumoniae, Klebsiella oxytoca* and *Proteus mirabilis* for potential ESBLs producers. Phenotypic tests have been developed for the recognition of ESBLs carrying strains.[16] There are no CLSI approved protocols to identify Amp C β lactamase enzymes.[7] Boronic acid compounds are considered as reversible inhibitors of Amp C β lactamase enzymes.[9] Many studies have confirmed the use of boronic acid as a simple, highly sensitive, specific and practical method to detect Amp C β lactamases among Gram negative bacteria.[10-12] There are many reports in which *Escherichia coli* and *Klebsiella pneumoniae* have carried both ESBLs and Amp C enzymes.[10,13]

ESBLs and Amp C producers are resistant to a variety of antimicrobials, their progression is a critical global health concern which has made treatment strategies complicated for the increased number of hospitalized patients. The aim of this study is the early detection of ESBLs and Amp C production in *Escherichia coli* and *Klebsiella pneumoniae* in neonates in our set up and to formulate antibiotic policy according to our sensitivity patterns. Organisms producing Amp C β lactamase give positive ESBLs screening test but fail to confirm giving misleading results. So it is mandatory to detect these separately and to distinguish between both types of enzymes.

MATERIALS AND METHODS

The present study was conducted in Microbiology Laboratory of Pathology Department, Post Graduate Medical Institute, Lahore to determine frequency of ESBLs and Amp C beta lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from neonatal sepsis in a tertiary care hospital Lahore. It is a descriptive study, carried out in 6 months duration (1st July'14- 31st Dec'14) and 450 samples of blood of clinically diagnosed patients of neonatal septicemia were collected from Pediatric Department, Lahore General Hospital.

All blood culture bottles were brought to Microbiology Laboratory of Post Graduate Medical Institute (PGMI) and were placed in an incubator at 37°C overnight. The blood samples were processed according to standard guidelines. First subculture from broth bottles was done on Blood, MacConkey and Chocolate agar plates on day 2. At the same time smears were prepared for Gram staining. The sub culture plates were incubated at 37°C for 24 hours and observed next day for any visible growth. If no growth occurred then second and third subculture were done at day 4 and 7 or when signs of positivity appeared.

Preliminary identification was based on Gram staining, catalase test, oxidase test and motility by hanging drop method. *Escherichia coli* and *Klebsiella pneumoniae* were identified by using API 20E kit. Antimicrobial susceptibility testing was carried out by Modified Kirby Bauer disk diffusion method on Mueller Hinton agar using CLSI protocols.[14,15]

ESBL Screening

After the confirmation of *Escherichia coli* and *Klebsiella pneumoniae* by API 20E, screening was done by Kirby Bauer disk diffusion method on Mueller Hinton agar. The antibiotic susceptibility disks Aztreonam (30µg), Cefotaxime (30µg), Ceftazidime (30µg) and Ceftriaxone (30µg) were placed on Mueller Hinton agar. The plates were incubated for 24 hours at 37°C. Zone diameters were measured and interpreted according to CLSI guidelines. The isolates with reduced sensitivity against two or more than two cephalosporins was interpreted as suspected ESBLs producer.[16]

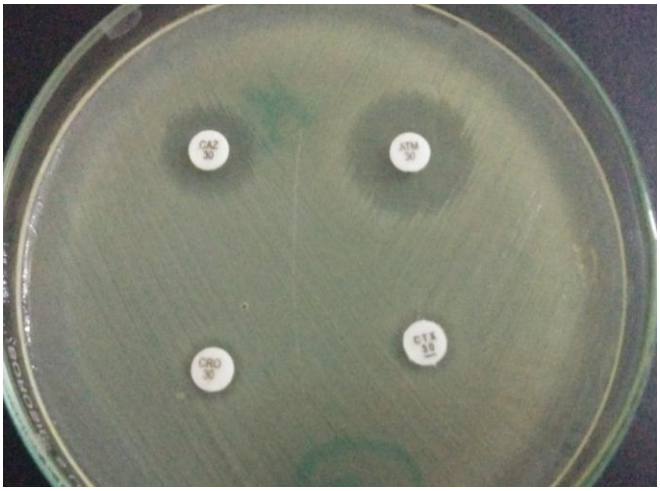

Fig. 1: *ESBLs Screening.*

ESBLs Phenotypic Confirmation

Phenotypic confirmatory test of provisional ESBLs producers was carried out by combined disk method by using antimicrobial disks of Ceftazidime (30µg) and Cefotaxime (30µg) alone and in combination with Clavulanic acid (30/10µg) on Mueller Hinton agar plates. A disk of Ceftazidime containing 30µg of antibiotic and Ceftazidime + Clavulanic acid disc containing 30+10µg of antibiotic were put on Mueller Hinton agar plate at a distance of 30mm from each other. Similarly a disk of cefotaxime containing 30µg of antibiotic and ceftazidime + clavulanic acid disc containing 30+10µg of antibiotic were placed. The plates were incubated for

24 hours at 37°C. Zone diameters were measured and interpreted according to CLSI guidelines. Isolates showing increase in the zone of inhibition of ≥ 5mm for ceftazidime or cefotaxime in combination with Clavulanic acid versus its zone alone were labeled as confirmed ESBLs producer.[16]

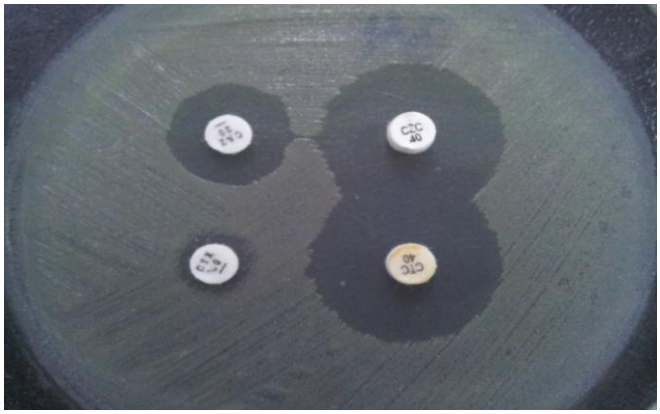

Fig. 2: *Phenotypic confirmatory test of ESBLs producing strain. Isolates showing zone diameter around the disc containing ceftazidime or cefotaxime in combination with Clavulanic acid ≥ 5mm than its zone diameter tested alone.*

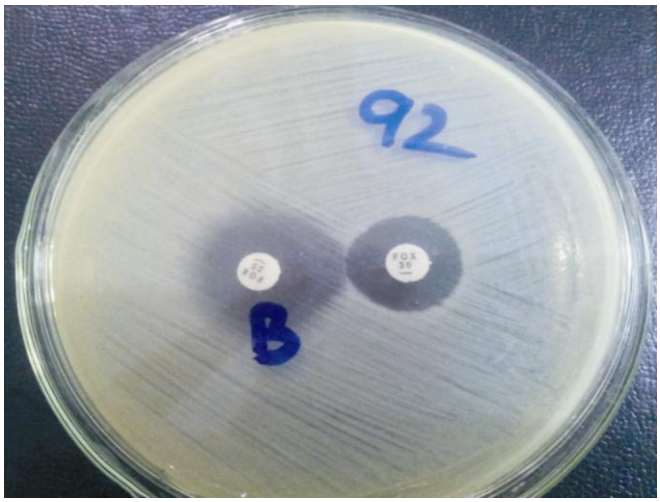

Fig. 3: *Inhibitor based method for Amp C production. Isolates showing zone diameter around the disc containing cefoxitin+boronic acid ≥ 5mm than zone diameter around the disk containing cefoxitin alone.*

Amp C Phenotypic Confirmation

The principle of this test is based on the use of Boronic acid compounds.Disks containing boronic acid were prepared as follows: 120 mg of phenylboronic acid (benzeneboronic acid) was dissolved in 3ml of dimethyl sulfoxide. 3ml of sterile distilled water was added in the solution. 20µl of stock solution was dispensed onto disks containing cefoxitin. Disks were allowed to dry for 30 min and utilized instantly. A disk containing 30µg of cefoxitin and a disk containing 30µg of cefoxitin and 400µg of boronic acid were placed on the Mueller Hinton agar plate. Inoculated plates were incubated for 24 hours at 37°C. An organism demonstrating a zone diameter around the disk containing cefoxitin + boronic acid ≥ 5mm than the zone diameter around the disk containing cefoxitin alone was considered an Amp C producer.[10,13]

Statistical analysis was done by SPSS 20. Fisher's exact test was performed to determine statistically significant differences among the ESBLs, Amp C, and Co-producing *Esherichia coli* and *Klebsiella pneumoniae* isolates.

RESULTS

Duration of study was 6 months (1st July'14- 31st Dec' 14) and 450 samples of blood of clinically diagnosed patients of neonatal septicemia were collected and processed in the Microbiology laboratory of Postgraduate Medical Institute Lahore. Out of the 450 blood samples, 95 (21.1%) were culture positive.Among 95 positive blood cultures, Gram negative organisms were recovered from 56 (58.9%), mostly belonging to Enterobacteriaceae family.

Frequency of *Escherichia coli* and *Klebsiella pneumoniae* isolated from neonatal sepsis is given in table 1. Among Gram negative organisms, 23.2% (13) of *Escherichia coli*and 17.8% (10) of *Klebsiella pneumoniae* were isolated.

Table 1: *Frequency of Escherichia coli and Klebsiella pneumoniae in suspected cases of neonatal septicemia.*

S/No	*Organism Isolated*	*Number (n = 56)*	*Percentage %*
1.	*Escherichia coli*	13	23.2%
2.	*Klebsiella pneumonia*	10	17.8%

Frequency of resistance among ESBLs, Amp C and co-producing *Escherichia coli* recovered from the suspected cases of neonatal septicemia are given in table 2. In our study, ESBLs producing *Escherichia coli* showed 100% resistance to amoxycillin-clavulanic acid, ceftazidime, ceftriaxone, cefotaxime and aztreonam. Pure Amp C producing *E.coli* showed 100% resistance to cefoxitin, ceftazidime, ceftriaxone, cefotaxime and aztreonam. Co-producers showed 100% resistance to augmentin, ceftazidime, cefotaxime, ceftriaxone and aztreonam. Not a single isolate was resistant to imipenem.

Frequency of resistance in ESBLs, Amp C and co-producing *Klebsiella pneumoniae* recovered from the suspected cases of neonatal septicemia are given in table 3. ESBLs producing *Klebsiella pneumoniae* showed 100% resistance to amoxycillin-clavulanic acid,

ceftazidime, ceftriaxone, cefotaxime and aztreonam. Pure Amp C producing *K.pneumoniae* showed 100% resistance to cefoxitin, ceftazidime, ceftriaxone, cefotaxime and aztreonam. Co-producers showed 100% resistance to augmentin, ceftazidime, cefotaxime, cefoxitin and aztreonam. Not a single isolate was resistant to imipenem.

Table 2: *Frequency of resistance among ESBLs, Amp C and co-producing Escherichia coli to different antibiotics (n = 13).*

Antimicrobials	*Pure ESBLs Producers (n = 6) %*	*Pure Amp C Producers (n = 4) %*	*Co-production of ESBLs and Amp C (n = 3) %*
Amoxycillin-clavulanic acid(AMC)	100	75	100
Piperacillin-tazobactam (TZP)	33.3	50	33.3
Cefoxitin (FOX)	66.6	100	66.6
Ceftazidime (CAZ)	100	100	100
Ceftriaxone (CRO)	100	100	100
Cefotaxime (CTX)	100	100	100
Cefipime (FEP)	s83.3	50	66.6
Imipenem (IPM)	0	0	0
Aztreonam (AZT)	100	100	100
Gentamicin (CN)	83.3	50	66.6
Ciprofloxacin (CIP)	50	75	66.6
Trimethoprim-sulfamethoxazole (SXT)	50	50	33.3

Table 3: *Frequency of resistance among ESBLs, Amp C and co-producing Klebsiella pneumoniae to different antibiotics (n = 10).*

Antimicrobials	*Pure ESBLs Producers (n=6) %*	*Pure Amp C producers (n=2) %*	*Co-production of ESBLs and Amp C (n=3) %*
Amoxycillin-clavulanic acid(AMC)	100	50	100
Piperacillin-tazobactam (TZP)	16.6	50	50
Cefoxitin (FOX)	33.3	100	100
Ceftazidime (CAZ)	100	100	100
Ceftriaxone (CRO)	100	100	100
Cefotaxime (CTX)	100	100	100
Cefipime (FEP)	83.3	50	50
Imipenem (IPM)	0	0	0
Aztreonam (AZT)	100	100	100
Gentamicin (CN)	33.3	50	50
Ciprofloxacin (CIP)	83.3	0	50
Trimethoprim-sulfamethoxazole (SXT)	50	50	50

Table 4 shows the frequency of ESBLs producers in *Escherichia coli* and *Klebsiella pneumoniae*. Out of 13 *Escherichia coli* isolates in the present study, 6 (46.1%) isolates were ESBLs producers. Whereas among 10 *Klebsiella pneumoniae* 6 (60%) were ESBLs producers.

The distribution of pure Amp C producers in *Escherichia coli* and *Klebsiella pneumonia* is shown in table 5. Out of 13 *Escherichia coli,* 4 (30.7%) isolates were pure Amp C producers. However among 10 *Klebsiella pneumoniae* 2 (20%) were pure Amp C producers.

Table 6 shows the co-production of ESBLs and Amp C in *Escherichia coli* and *Klebsiella pneumoniae*. Out of 13 *Escherichia coli,* 4 (23%) isolates were combined ESBLs and Amp C producers. Whereas among 10 *Klebsiella pneumoniae* 2 (20%) showed co-production of ESBLs and Amp C.

Table 4: *Frequency of ESBLs producers in Escherichia coli and Klebsiella pneumoniae isolates.*

Organisms	*Number of Isolates*	*Pure Amp C Producers*	
		No.	*%*
Escherichia coli	13	4	30.7*
Klebsiella Pneumonia	10	2	20

*Statistically there was no significant difference (p value >0.05)

Table 5: *Frequency of pure Amp C producers in Escherichia coli and Klebsiella pneumoniae isolates.*

Organisms	*Number of Isolates*	*Pure Amp C Producers*	
		No.	*%*
Escherichia coli	13	6	46.1
Klebsiella Pneumonia	10	6	60*

*Statistically there was no significant difference (p value □0.05)

Table 6: *Frequency of co-producers of ESBLs and Amp C in Escherichia coli and Klebsiella pneumoniae isolates.*

Organisms	*Number of Isolates*	*Co-producers of ESBLs and Amp C*	
		No.	*%*
Escherichia coli	13	3	23*
Klebsiella Pneumonia	10	2	20

*Statistically there is no significant difference (p value □0.05)

DISCUSSION

The main focus of study was to determine the frequency of ESBLs and Amp C beta-lactamases in *Escherichia coli* and *Klebsiella pneumoniae* from suspected cases of neonatal septicemia in a tertiary care hospital, Lahore. ESBLs producing *E.coli* and *K.pneumoniae* are the foremost cause of neonatal infections in neonatal intensive care units around the globe.[17]

Different studies had been carried out by various researchers who also reported predominance of Gram negative organisms recovered from cultures of blood in suspected cases of neonatal sepsis.[18] Frequency of *Escherichia coli* and *Klebsiella pneumoniae* isolated from neonatal sepsis is given in table 1. Among Gram negative organisms, 23.2% (13) of *Escherichia coli* and 17.8% (10) of *Klebsiella pneumoniae* were isolated. Similar results were also observed in other studies in which the most common organism was *Klebsiella pneumoniae* (21.4%).[19,20]

In present study, patterns of antimicrobial susceptibilities were studied in *Escherichia coli* and *Klebsiella pneumoniae* isolated from suspected cases of neonatal septicemia. Susceptibility testing was carried by modified Kirby Bauer disc diffusion method. Frequencies of resistance among ESBLs, Amp C and co-producing *E.coli* and *K.pneumoniae* recovered from the suspected cases of neonatal septicemia are given in tables2 and 3.

The observations are also in accordance with other researchers. A study was conducted in Lahore which revealed that Amp C producing *E.coli* were 100% resistant to ceftazidime, ceftriaxone, cefotaxime and cefoxitin.[21] 70-100% resistance was observed among augmentin, cefotaxime and ceftazidime in Amp C producing *Escherichia coli* and *Klebsiella pneumoniae*.[10] Another study showed a multi-drug resistant pattern in ESBLs producing *E.coli* and *K.pneumoniae*. Maximum resistance (100%) was recorded among cefotaxime and ceftazidime.[22]

In present study 100% susceptibility was seen among imipenem. Our results correspond to the findings of many other studies which showed 100% susceptibility rate to imipenem in ESBLs and Amp C producers in *Esherichia coli* and *Klebsiella pneumoniae*.[10]

Table 4 shows the frequency of ESBLs producers in *Escherichia coli* and *Klebsiella pneumoniae* isolates. An observation was made in India and they showed 48% and 44% ESBLs production in *Escherichia coli* and *Klebsiella pneumoniae* respectively.[23] Similar trends were also reported in Lahore in which ESBLs production was seen in 72% *Esherichia coli* and 65.8%

Klebsiella pneumoniae.[22]

Contrary to our results, a study was carried out in India in 2012 who detected a very high frequency of ESBLs production in almost 80% of *Escherichia coli* and *Klebsiella pneumoniae* isolates.[24]Similar trends were also noticed in Zambia in which 100% *K.pneumoniae* isolates were ESBLs producers.[25] Whereas some reported a very low frequency of ESBLs production (31.5%).[26]

The distribution of pure Amp C producers in *Escherichia coli* and *Klebsiella pneumoniae* in our study is shown in table 5.Boronic acid derivatives considered as reversible inhibitors of Amp C β lactamase enzymes.[9] A similar observation was made in Turkey who reported Amp C beta lactamase production in 39.5% *E.coli* and *K.pneumoniae* isolates.[10] Another study was conducted in Oman who gave 40.8% and 35.9% frequency of Amp C beta lactamase producing *E.coli* and *K.pneumoniae*.[11] Amp C enzyme was detected in 40% of clinical isolates of *E.coli* by using boronic acid method.[12]

Nigeria reported a high frequency i.e 56.2% and 43.7% Amp C β-lactamase production in *E.coli* and *Klebsiella* spp respectively.[28] On the other hand a very low frequency of Amp C production was also reported by other researchers.[8]

Table 6 shows the co-production of ESBLs and Amp C in *Escherichia coli* and *Klebsiella pneumoniae*. Detection of both ESBLs and Amp C enzymes was seen in 39.1% and 36.3% of *E.coli* and *Klebsiella* spp.[8] Another study was conducted in Turkey, which showed 38% co-production of ESBLs and Amp C among *E.coli* and *Klebsiella pneumoniae*.[10] Similar results were also showed in a study which reported 43.7% and 56.2% combined ESBLs and Amp C production in *E.coli* and *Klebsiella* spp.[27]

It is ***concluded*** that organisms producing Amp C β lactamase give positive ESBLs screening but fail to confirm, giving misleading results. So it is mandatory to detect these separately and differentiate between Amp C and ESBLs producers. ESBLs testing should be routinely carried out in microbiology laboratories in all bacterial isolates showing resistance to third generation cephalosporins. Boronic acid compound is a practical and most efficient method to detect plasmid mediated Amp C β lactamase enzymes in *Esherichia coli* and *Klebsiella pneumoniae*. It also differentiates ESBLs enzymes from Amp C enzymes. It seems to be very efficient in categorizing these varieties of resistant strains.

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Authors' Contribution

SQ: Data collection, literature search, materials & methods, result writing, contributed to discussion writing. IJ: Conceived the idea, contributed in literature search, materials & methods, result and discussion writing. SM: Contributed in literature search, writing results based on analysis. MSA: Analysis of data, reviewed the article and gave expert opinion. All authors contributed equally in this study.

Conflict of Interest

The authors have no financial relationship with any organization. The authors have no conflict of interest in the subject matter or materials discussed in this manuscript.

REFERENCES

1. Tsai MH, Chu SM, Lee CW, Hsu JF, Huang HR, Chiang MC et al. Recurrent late-onset sepsis in the neonatal intensive care unit: incidence, clinical characteristics and risk factors. Clin Microbiol Infect. 2014; 20 (11): 0928-35.
2. Laxminarayan R, Matsoso P, Pant S, Brower C, Barter D, Klugman K et al. Access to effective antimicrobials: a worldwide challenge. The Lancet. 2016; 387 (10014): 168-175.
3. Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE et al. Global, regional and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systemic analysis. The Lancet. 2015; 385 (9966): 430-440.
4. Ambler RP, Coulson AF, Frere JM, Ghuysen JM, Joris B, Forsman M et al. A standard numbering scheme for the class A beta-lactamases. Biochem J. 1991; 276: 269–70.
5. Bush K, Jacoby GA. Updated functional classification of β-lactamases. Antimicrob Agents Chemother. 2010; 54 (3): 969-976.
6. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: A clinical update. Clin Microbiol Rev. 2005; 18 (4): 657–86.
7. Jacoby GA. Amp C β lactamases. Clin Microbiol Rev. 2009; 22 (1): 161-182.
8. Rudresh SM, Nagarathnamma T. Two simple modifications of modified three-dimensional extract test for detection of Amp C β lactamase among the members of family Enterobacteriaceae. Chron Young Sci. 2011; 2 (1): 42-46.
9. Winkler ML, Rodkey EA, Taracila MA, Drawz SM, Bethel CR, Papp-Wallace KM., et al. Design and exploration of novel boronic acid inhibitors reveals important interactions with a clavulanic acid-resistant sulfhydral-variable (SHV) β-lactamase. J Med Chem. 2013; 56 (3): 1084-1097.
10. Yilmaz NO, Agus N, Bozcal E, Oner O, Uzel A. Detection of plasmid mediated AmpC β lactamase in Escherichia coli and Klebsiella pneumonia. Indian J Med Microbiol. 2013; 31 (1): 53-9.
11. Barua T, Shariff M, Thukral SS. Detection and Charac-

terization of Amp C B-Lactamases in Indian Clinical Isolates of *Escherichia coli, Klebsiella pneumoniae* and *Klebsiellaoxytoca*. Univer J Microbiol Res. 2013; 1 (2): 15-21.
12. Handa D, Pandey A, Asthana AK, Rawat A, Handa S, Thakuria B.Evaluation of phenotypic tests for the detection of Amp C beta-lactamase in clinical isolates of *Escherichia coli*. Indian J Pathol Microbiol. 2015; 56 (2): 135-138.
13. Kaur DC, Puri J, Kulkarni SS, Jayawant A. Prevalence of Amp C β lactamases in clinical isolates of E.coli from a tertiary care rural hospital. Int J Pharm Sci. 2015; 7 (6): 165-168.
14. Cheesbrough M. District Laboratory Practice in Tropical Countries. 2nd Ed., New Delhi: Cambridge University Press; 2006 Mar 2.
15. Koneman EW, Alien SD, Janda WM, Schreckenberger PC, Winn WC. Color Atlas and Textbook of Diagnostic Microbiology. 6th Ed., New York: Lippincott Williams and Wilkins; 2006.
16. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twenty third informational supplement update. CLSI document M100-S22 U. Clinical and Laboratory Standards Institute, Wayne, PA; 2013.
17. Cantey JB, Sreeramoju P, Jaleel M, Trevino S, Gander R, Hynan LS, et al. Prompt control of an outbreak caused by extended-spectrum beta lactamase producing *Klebsiella pneumoniae* in a neonatal intensive care unit. J Pediatr. 2013; 163 (3): 672-9.
18. Liaquat I, Khan NS, Naveed A. Bacteriological spectrum of Neonatal Sepsis. J Rwp Med Coll. 2015; 19 (1): 29-32.
19. Goheer L, Khattak ZS. Early onset neonatal sepsis (Risk factors & clinic-bacteriological profile). Pak Pediatr J. 2014; 38 (4): 205-10.
20. Raha BK, Baki MA, Begum T, Nahar N, Jahan N, Begum M. Clinical, Bacteriological Profile & Outcome of Neonatal Sepsis in a Tertiary Care Hospital. Med Today. 2014; 26 (1): 18-21.
21. Jameel NA, Ejaz H, Zafar A, Amin H. Multi drug resistant Amp C β-lactamase producing *Escherichia coli* isolated from a paediatric hospital. Pak J Med Sci. 2014; 30 (1): 181-184.
22. Majda Q, Najma A, Summyia B. Evaluation of extended spectrum beta-lactamase mediated resistance in *Escherichia coli* and *Klebsiella* in urinary tract infection at a tertiary care hospital. Biomedica. 2013; 29: 78–81.
23. Rupinder B, Geeta W, Shikha J. Prevalence of extended spectrum β-lactamases in multidrug resistant strains of Gram negative Bacilli. J Acad Indus Res. 2013; 1 (9): 558–560.
24. Nasa P, Juneja D, Singh O, Dang R, Singh A. An observational study on bloodstream extended-spectrum beta lactamase infection in critical care unit: incidence, risk factors and its impact on outcome. Eur J Intern Med. 2012; 23 (2): 192-195.
25. Mumbula EM, Kwenda G, Samutela MT, Kalonda A, Mwansa JCL, Mwenya D, et al. Extended spectrum β lactamase producing *Klebsiella pneumonia*e from the neonatal intensive care unit at the university teaching hospital in Lusaka, Zambia. J Med Sci Tech. 2015; 4 (2): 85-91.
26. Thakur S, Pokhrel N, Sharma M. Prevalence of multidrug resistant enterobacteriaceae and extended spectrum β-lactamase producing *Escherichia coli* in urinary tract infection. Res J Phrm Bbiolg Chem Sci. 2013; 4 (2): 1615-1624.
27. Akujobi CO, Odu NN, Okorondu, SI. Detection of Amp C beta lactamases in clinical isolates of *Escherichia coli* and *Klebsiella*. Afr J Cln Exper Microbiol. 2012; 13 (1): 51-55.