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AMPC B-LACTAMASE PRODUCING BACTERIA INDUCED NEONATAL SEPSIS AND ITS ASSOCIATION TO ANTIBIOTIC MANAGEMENT AND EXCESSIVE USE OF INVASIVE PROCEDURES

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Abstract:

***Objective**: We aimed to determine the antimicrobial profile and occurrence of the AmpC β-lactamase which produces the bacteria.*

***Methods:** Our research was carried out on 1914 blood samples of the all suspected cases of neonatal septicemia at Allied Hospital, Faisalabad in the timeframe of October 2016 to July 2017. Gram staining was used for the identification of the isolates and API 20E & NE tests were also processed. Screening of the negative gram isolates was carried out for bacteria producing AmpC β-lactamase against cefotaxime, ceftazidime and cefoxitin resistance, it was confirmed through base inhibition method.*

***Results:** Gram-positive and negative bacteria cases were identified respectively as 54 gram-negative (8.49%) and 582 gram-positive (91.5%). In the negatively gram isolates, 141 AmpC producers (22%) were hundred percent resistant against cefoxitin, co-amoxiclav, cefotaxime, ceftazidime, cefixime, cefuroxime, cefpodoxime, ceftriaxone, amikacin, gentamicin and aztreonam. Low level of resistance was found against sulbactam cefoperazone, cefepime, ciprofloxacin, piperacillin-tazobactam & meropenem with respective proportions of 24.8%, 30.4%, 20.5%, 10.6% & 2.1%. Every isolate had sensitivity against imipenem. Because of various interventions patients were treated with AmpC β-lactamases where intravenous (I/V) line was observed nasogastric tube, Ambu bag, endotracheal tube, ventilator & surgery having respective proportions of 37.6%, 8.5%, 3.5%, 2.1% & 0.7%.*

***Conclusion:** Excessive utilization of the third generation cephalosporins and invasive procedures is to be marginalized and inhibited in order to avoid AmpC β-lactamases emergence in the children (neonates).*

Keywords: *Antimicrobial Resistance, AmpC β-lactamase, Multidrug-Resistant, Neonatal Sepsis and Bacteria.*

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INTRODUCTION

β-lactamases are produced through numerous bacteria and it is a bacterial enzyme providing resistance against β-lactam antibiotics including carbapenems, penicillin, monobactams and cephalosporins. AmpC β-lactamases hydrolyze with a broader spectrum of cephalosporins which includes cefotaxime, cefoxitin, ceftriaxone and ceftazidime [1]. Presence of these enzymes can be found in gram-negative bacteria including Escherichia coli, Salmonella species, Klebsiella species, Enterobacter species, Shigella, Pseudomonas aeruginosa, Citrobacter species, Providencia, Serratia marcescens, Morganella morganii and Proteus mirabilis [2].

The standard method for the detection is lacking to detect AmpC β-lactamases; whereas, numerous strategies are used for the screening of AmpC. AmpC detection is made through the three-dimensional test with the help of indicator drugs such as ceftazidime, cefoxitin or cefotaxime [3]. Inhibitor-Based Method is also used to detect AmpC β-lactamases which uses boronic acid as AmpC enzyme inhibitor [4].

Klebsiella pneumonia, Escherichia coli, Acinetobacter baumannii, Citrobacter diversus, Pseudomonas aeruginosa, Enterobacter cloacae, Staphylococcus aureus and Streptococcus pyogenes were mostly involved in the bacteria that caused neonatal sepsis [5]. Higher resistance levels were developed because of the 3rd generation of the cephalosporins. The sulbactam, carbapenems and amikacin were also used for the management of neonatal sepsis that was caused because of the AmpC produced strains; whereas, in case of organism mutations they can also cause resistance to carbapenem [1]. For the inhibition of the AmpC β-lactamase, we can also utilize Boronic acid [4]. We can also avoid multidrug resistance through limited use of the 3rd generation of cephalosporins for neonatal sepsis [6].

Unjustified use of the 3rd generation cephalosporins, invasive procedures and prolonged stay in the hospital were a major factor of risk. We aimed to determine the antimicrobial profile and occurrence of the AmpC β-lactamase which produces the bacteria.

METHODS:

Our research was carried out on 1914 blood samples of the all suspected cases of neonatal septicemia at Allied Hospital, Faisalabad in the timeframe of October 2016 to July 2017. Gram staining was used for the identification of the isolates and API 20E & NE tests were also processed. Screening of the negative gram isolates was carried out for bacteria producing AmpC β-lactamase against cefotaxime, ceftazidime and cefoxitin resistance, it was confirmed through base inhibition method. Blood samples were incubated at a temperature of 37°C. MacConkey agar plates were used for culturing after incubation of the blood samples. Identification of the bacteria was made through colony morphology, oxidase test, lactose fermentation, Gram's staining, API 20E & 20NE and biochemical tests [7]. Disc diffusion method was used for the screening of the isolates [8]. Isolates were resistant against cefotaxime, ceftazidime and cefoxitin which positive after screening for AmpC β-lactamases. Further confirmation was also made by using boronic acid through inhibition disc method. For this method, we used cefotaxime-clavulanate and ceftazidime-clavulanate by applying them on the agar plate. Detection of isolates was made as a producer of AmpC in case of a keyhole formation (synergism) in between boronic acid and (cephalosporin + clavulanate) [9].

Every bacterial stain was tested for sensitivity on 90 mm Muller Hinton agar plates. Strains were controlled through Klebsiella pneumoniae (ATCC-700603) and E. coli (ATCC-25922). Various antibiotics were used to test isolates which included aztreonam (30 µg), amikacin (30 µg), cefotaxime (30 µg), cefixime (5 µg), cefpodoxime (30 µg), amoxicillin-clavulanate (20/10 µg), cefoxitin (30 µg), cefepime (30 µg), meropenem (10 µg), imipenem(10 µg), ceftriaxone (30 µg), ceftazidime (30 µg), sulbactam cefoperazone (75 / 30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), piperacillin-tazobactam(100 / 10 µg) and cefuroxime (30 µg). With the application of the mentioned antibiotics overnight incubation was made at 37°C. CLSI guidelines were used for the measurement of zonal diameter after incubation process [8]. We also reviewed the record of the patients. Various other investigations were also documented which included a nasogastric tube, intravenous line, endotracheal tube, Ambu bag, surgery and ventilator.

RESULTS:

Gram-positive and negative bacteria cases were identified respectively as 54 gram-negative (8.49%) and 582 gram-positive (91.5%). In the negatively gram isolates, 141 AmpC producers (22%) were hundred percent resistant against cefoxitin, co-amoxiclav, cefotaxime, ceftazidime, cefixime, cefuroxime, cefpodoxime, ceftriaxone, amikacin, gentamicin and aztreonam. Low level of resistance was found against sulbactam cefoperazone, cefepime, ciprofloxacin, piperacillin-tazobactam & meropenem with respective proportions of 24.8%, 30.4%, 20.5%,

10.6% & 2.1%. Every isolate had sensitivity against imipenem. Because of various interventions patients were treated with AmpC β-lactamases where intravenous (I/V) line was observed nasogastric tube, ambu bag, endotracheal tube, ventilator & surgery having respective proportions of 37.6%, 8.5%, 3.5%, 2.1% & 0.7% as reflected in Table – I.

Table – I: Distribution of AmpC β-lactamase producing bacteria isolated in neonatal sepsis (141).

Bacteria	Number	Percentage
Enterobacter cloacae	80	56.7
Enterobacter sakazakii	20	14.2
Escherichia coli	14	9.9
Citrobacter freundii	8	5.7
Klebsiella pneumoniae	8	5.7
Klebsiella oxytoca	4	2.8
Serratia marcescens	3	2.1
Acinetobacter baumannii	2	1.4
Pseudomonas aeruginosa	1	0.7
Aeromonas hydrophila	1	0.7

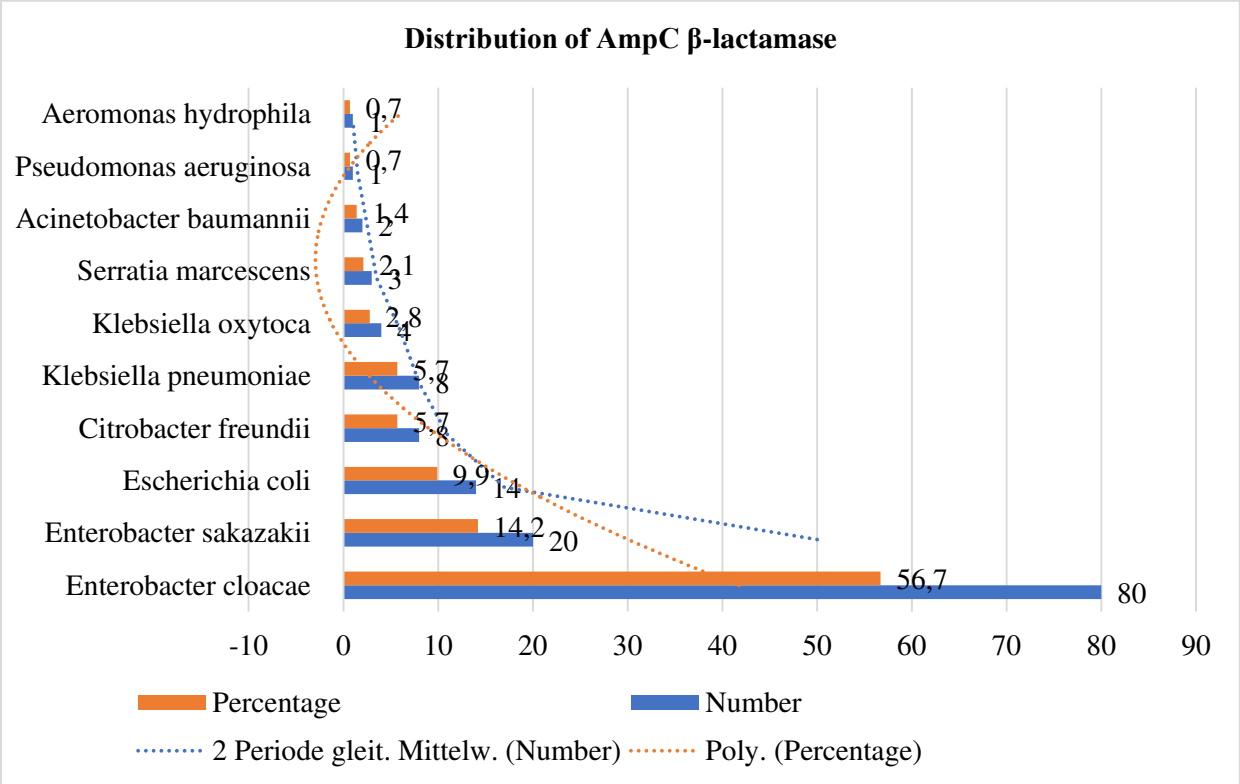
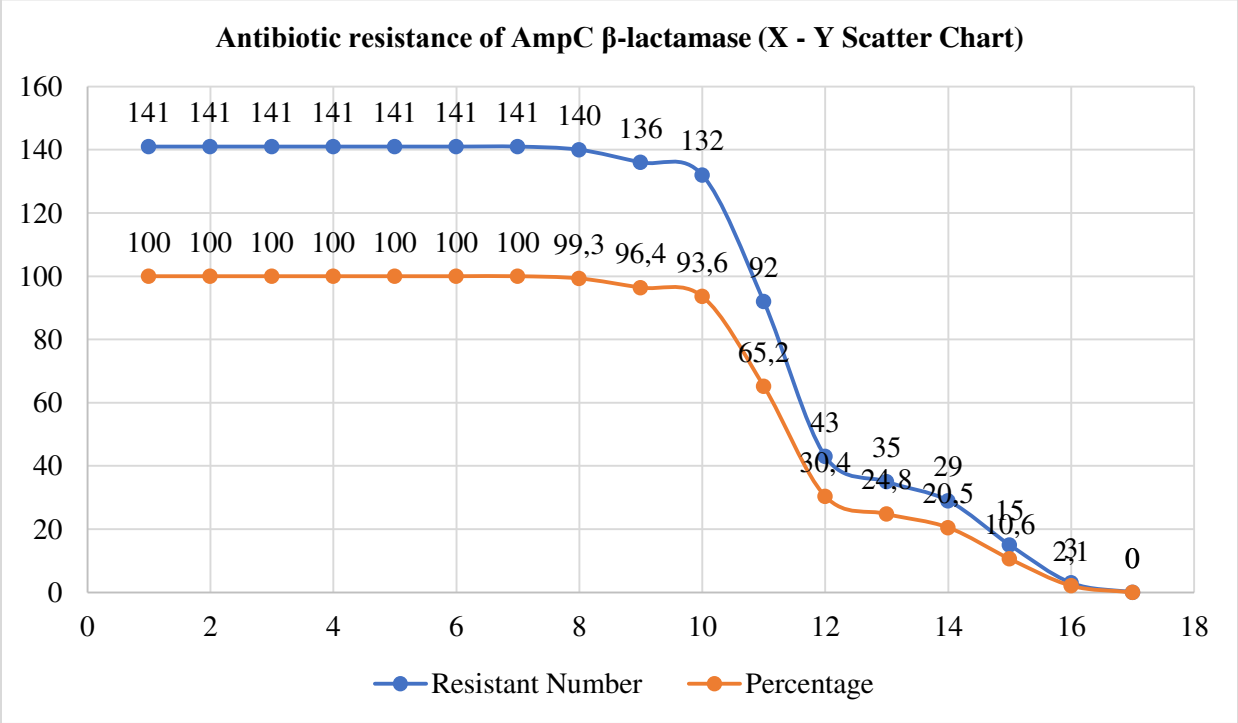


Table – II shows the resistance of 141 bacteria producing AmpC against ceftazidime, co-amoxiclav, cefuroxime, cefotaxime, ceftriaxone, cefixime and cefpodoxime. Various rates of resistance have been analyzed in the given tabular data.

Table – II: Antibiotic resistance of AmpC β-lactamase producing bacteria

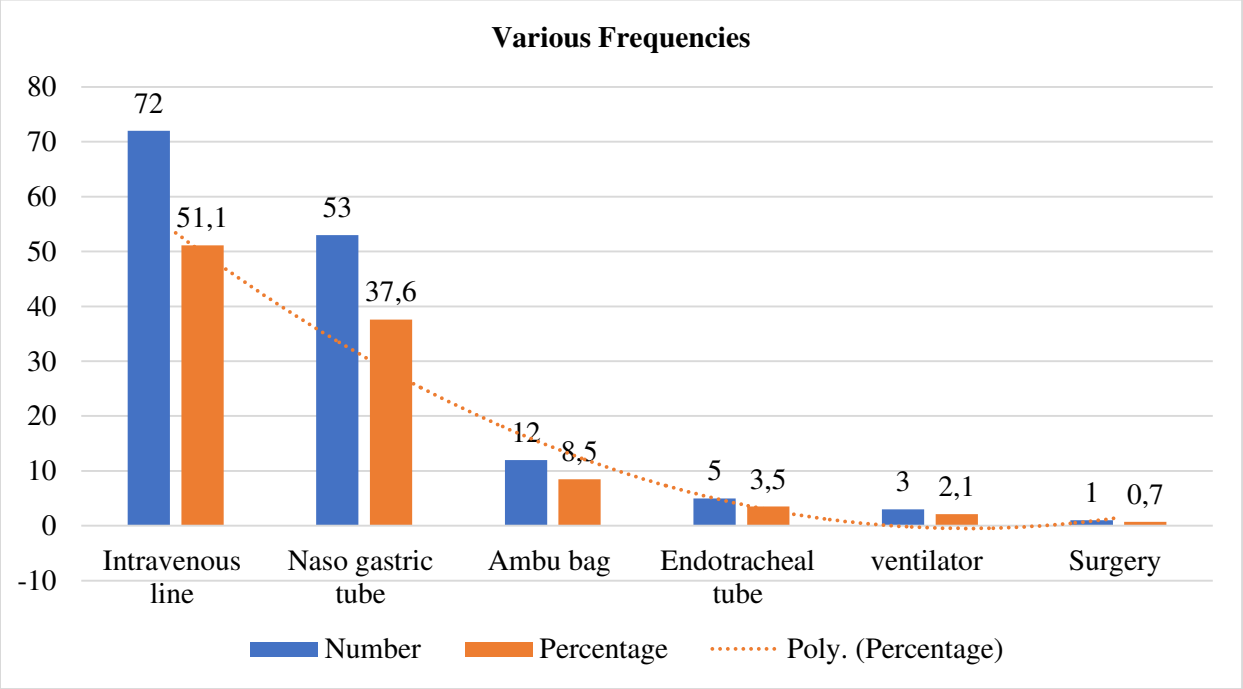
Antibiotics	Resistant Number	Percentage
Co-amoxiclav	141	100
Ceftazidime	141	100
Ceftriaxone	141	100
Cefotaxime	141	100
Cefuroxime	141	100
Cefixime	141	100
Cefpodoxime	141	100
Cefoxitin	140	99.3
Gentamicin	136	96.4
Amikacin	132	93.6
Aztreonam	92	65.2
Cefepime	43	30.4
Sulbactam+cefoperazone	35	24.8
Ciprofloxacin	29	20.5
Piperacillin+tazobactam	15	10.6
Meropenem	3	2.1
Imipenem	0	0



Numerous invitational procedures were observed as 72 intravenous line (I/V) 51.1%, 53 nasogastric tubes 37.6%, 5 endotracheal tubes 3.5%, 12 Ambu bag 8.5%, 3 ventilators 2.1% and 1 Surgery 0.7% as shown in Table – III.

Table – III: Interventional Frequency among AmpC β-lactamase harbouring neonates

Interventions	Number	Percentage
Intravenous line	72	51.1
Nasogastric tube	53	37.6
Ambu bag	12	8.5
Endotracheal tube	5	3.5
ventilator	3	2.1
Surgery	1	0.7



DISCUSSION:

Mortality and morbidity are very much associated with the neonatal sepsis in Pakistani neonates. Gram-positive and negative bacteria cases were identified respectively as 54 gram-negative (8.49%) and 582 gram-positive (91.5%), these outcomes can be compared with other research studies where gram-negative was more than gram positive with the proportion of 92.8% to 7.2% respectively [10]. Another research reported gram negative and positive respectively 80.4% and 20.6% in neonatal sepsis [8]. Different outcomes have been reported by Muhammad et al. as they reported higher gram-negative bacteria frequency than gram positive bacteria with respective proportions of 54.6% to 45.4% [11]. Microbiologists face real challenge AmpC β-lactamases identification. Rate of bacteria production through AmpC β-lactamase was observed as 22.0% in this particular research; whereas, other studies reported as 26.8%, 19.61%, 20.7% and 35.5% [12 – 15].

Most prevalent AmpC producing isolates were Enterobacter sakazakii and Enterobacter cloacae. The emergence of the resistance is mostly linked with the Enterobacter species which is against cephalosporins than associated bacteria in case if treated with a broader spectrum of cephalosporins [1]. Gram-negative had a repeated nosocomial pathogen of Enterobacter species which caused 11% cases of pneumonia [16]. Every isolate produced by AmpC were resistant to ceftazidime, co-amoxiclav, cefuroxime, cefotaxime, ceftriaxone, cefixime and cefpodoxime. A research observed antimicrobial resistance establishment of AmpC producing pattern of Gram-negative bacteria to cefoxitin, gentamicin, amikacin, aztreonam, cefepime and sulbactam-cefoperazone having respective proportions of 99.3%, 96.4%, 93.6%, 65.2%, 30.4% & 24.8% [15]. The resistance of the AmpC β-lactamases was reported against aztreonam and cefoxitin [11, 14]. We did not observe a higher level of resistance of the

AmpC producers to sulbactam-cefoperazone and cefepime which is comparable with the outcomes of other same research studies [15, 17].

According to the outcomes of this particular research, ciprofloxacin resistance was 20.5% and numerous isolates had a sensitivity to various antibiotic as observed in another research such as sensitivity to ciprofloxacin was 30% [20]. Resistance to piperacillin-tazobactam was reported in our research as 10.6% that can be compared with another research which reported the same as 78% [18]. Resistance to meropenem was reported as 4.9% and no isolate was resistant against imipenem. It reflects the sensitivity of majority of isolates to carbapenems. Antibiotic susceptibility was studied by Beatrice and Herman about AmpC β-lactamase producing a pattern on carbapenems and Enterobacteriaceae as an effective antimicrobial medicine to treat AmpC producing Enterobacteria induced infection [19, 20].

AmpC β-lactamase producing bacteria was managed in numerous interventions such as nasogastric (NG) tube, an intravenous line (IV), an endotracheal tube (ETT), Ambu bag, surgery and ventilator support. Higher frequency was reported in 72 IV cases (51.1%). Authors also reported IV as risk factors linked with the nosocomial bacteremia and ambu bags as a cause of pathogenic transmission [21, 22]. Microbial colonization is also associated with the utilization of ETT which is a significant reason for the ventilator linked pneumonia [23]. Infection association was probed by an author which reported infection rate associated to a ventilator as (0.44 / 1000 days on a ventilator); whereas, central-line as (4.6 / 1000 days on a catheter) [24]. Pathogens can also be associated with various surgical procedures and contaminated apparatus. Bloodstream infections are also associated with sepsis and invasive procedures [25].

CONCLUSION:

AmpC β-lactamase producing bacteria induced neonatal sepsis causes mortality and treatment failure. Various epidemiological research studies are required to study the infection source during hospitalization. Excessive use of the invasive procedure is to be limited to reduce complications and unjustified broader spectrum cephalosporins are also to be restricted. Antibiotic utilization policy needs to be reviewed for the reduction of AmpC producing bacteria emergence. Excessive utilization of the third generation cephalosporins and invasive procedures is to be marginalized and inhibited in order to avoid AmpC β-lactamases emergence in the children (neonates).

REFERENCES:

1. Rojo D, Pinedo A, Clavijo E, García-Rodriguez A, García V. Analysis of risk factors associated with nosocomial bacteremia's. J Hosp Infect. 1999;42(2):135-341. doi: 10.1053/jhin.1998.0543.
2. Mirza IA, Hussain A, Abbasi SA, Malik N, Satti L, Farwa U. Ambu bag as a source of Acinetobacter baumannii outbreak in an intensive care unit. J Coll Physicians Surg Pak. 2011;21(3):176-178. doi: 03.2011/JCPSP.176178.
3. Baudry T, Ader F. Non-invasive mechanical ventilation to prevent ICU-acquired infection. Infect Disord Drug Targets.2011;11(4):384-348. doi: 10.2174/187152611796504782.
4. Navoa-Ng JA, Berba R, Galapia YA, Rosenthal VD, Villanueva VD, Tolentino MC, et al. Device-associated infections rates in adult, pediatric, and neonatal intensive care units of hospitals in the Philippines: International Nosocomial Infection Control Consortium (INICC) findings. Am J Infect Control. 2011;39(7):548-554. doi: 10.1016/j.ajic.2010.10.018.
5. Horrath, R, Collignon P. Controlling intravascular catheter infections. Aust. Prescr 2003. doi: 10.18773/austprescr.2003.029.
6. Kaistha N, Mehta M, Singla N, Garg R, Chander J. Neonatal septicemia isolates and resistance patterns in a tertiary care hospital of North India. J. Infect. Dev. Ctries. 2009;4(1): 55-57.doi: 10.3855/jidc.625.
7. Cheesbrough M. District laboratory practice in tropical countries (2) Cambridge University press, United Kingdom2000:124-143.
8. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility tests20th ed. approved standard, CLSI document M100-S20, Vol.30. 2010. Wayne, PA: CLSI.
9. Jacoby GA, Walsh KE, Walker VJ. Identification of extended spectrum, AmpC, and carbapenem-hydrolyzing beta lactamases in Escherichia coli and Klebsiella pneumoniae by disk tests. J Clin Microbiol. 2006;44(6):1971-1976. doi:10.1128/JCM.00062-06.
10. Aletyeb SMH, Khosravi AD, Dehdashtian M, Kompani F, Mortazavi SM, Aramesh MR. Identification of bacterial agents and antimicrobial susceptibility of neonatal sepsis: A 54-month study in a tertiary hospital. Afr J Microbiol.2011;5(5):528-531. doi: 10.5897/AJMR10.224.
11. Muhammad Z, Ahmed A, Hayat U, Wazir MS,

Rafiyatullah, Waqas H. Neonatal sepsis: causative bacteria and their resistance to antibiotics. J Ayyub Med Coll Abbottabad.2010;22(4):33-36.
12. Mohamudha PR, Harish BR, Parija SC. AmpC beta lactamase among Gram negative clinical isolates from a tertiary hospital, South Asia. Braz. J. Microbiol. 2010; 41:596-602. doi: 10.1590/S1517-83822010000300009.
13. Wen-en L, Yuan J, Yin T, Hong-mei W. Emergence of AmpC enzymes and antibiotic resistance in Gram negative bacilli. J. Cent. South. Univ. 2006;31(1):134-137.
14. Manchanda V, Singh NP, Shamweel A, Eideh HK, Thukral SS. Molecular epidemiology of clinical isolates of AmpC producing Klebsiella pneumoniae. Indian J. Med. Mirobiol.2006;24(3):177-181.
15. Akujobi CO, Odu NN, Okorondu SI. Detection of AmpC beta-lactamases in clinical isolates of Escherichia coli and Klebsiella. Afr. J. Of Clin. Exper. Micrbiol. 2012;13(1): 51-55.doi: 10.4314/ajcem/v13i1.6.
16. Kaye KH, Fraimow, Abrutyes E. Pathogen resistant to antimicrobial agents: Epidemiology, molecular mechanisms and clinical management. Infect. Dis. Clin. North Am.2000;14(2).
17. Roh KH, Uh Y, Kim J, Kim H, Shin DH, Song W. First outbreak of multidrug resistant Klebsiella pneumoniae producing both SHV-12 type extended spectrum beta lactamase and DHA-1 type AmpC beta-lactamase ata Korean Hospital. Yonsei Med J. 2008;49(1): 53-57.doi: 10.3349/ymj.2008.49.1.53.
18. Luzzaro F, Brigante G, D'Andrea MM, Pini B, Giani T, Mantengoli E, et al. Spread of multidrug-resistant Proteus mirabilis isolates producing an AmpC-type beta lactamase: epidemiology and clinical management. Int J Anti microb Agents. 2009;33(4):328-333. doi: 10.1016/j.ijantimicag.2008.09.007.
19. Goossens, H, Grabein B. Prevalence and antimicrobial susceptibility data for extended spectrum beta-lactamases and AmpC producing Enterobacteriaceae from the MYSTIC program in Europe and United States (1997-2004). Diag Microbial Infect Dis. 2005;53(4):257-264. doi: 10.1016/j.diagmicrobio.2005.10.001.
20. Upadhyay S, Sen MR, Bhattacharjee A. Presence of different beta-lactamase classes among clinical isolates of Pseudomonas aeruginosa expressing Amp C beta-lactamase enzymes. J Infect Dev Ctries. 2009;4(4):239-242. doi:10.3855/jidc.497.
21. Jacoby GA. AmpC β-Lactamases. Clin Microbial Rev.2009;22(1):161-182. doi: 10.1128/CMR.00036-08.
22. Livermore DM. Beta-lactamases in laboratory and clinical resistance. Clin Microbiol Rev. 1995;8(4):557-584.
23. Poirel L, Naas T, Nicolas D, Collet L, Bellais S, Cavallo JD, et al. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-beta-lactamase and its plasmid- and integron-borne gene from a Pseudomonas aeruginosa clinical isolate in France. Anti microb. Agents Chemother. 2000;44(4): 891-897.doi: 10.1128/AAC.44.4.891-897.2000.
24. Beesley T, Gascoyne N, Knott-Hunziker V, Petursson S, Waley SG, Jaurin B, et al. The inhibition of class Cβ-lactamases by boronic acids. Biochem. J. 1983;209(1):229-233. doi: 10.1042/bj2090229
25. Mahmood A, Karamat KA, Butt T. Neonatal sepsis: high antibiotic resistance of the bacterial pathogens in a neonatal intensive care unit in Karachi. J Pak Med Assoc.2002;52(8):348-350.