Egyptian Journal of Medical Microbiology Volume 25 / No. 1 / January 2016 37-44

ORIGINAL ARTICLE

Study of Bacteria Causing Septicemia in Neonatal Intensive Care Unit

[1]Hussien El-Shiekh, [2]MahaGaafar, [1]Mohamed Yosri , [2]Dina Hassan, [1]Hassan Said.*

[1]Botany and Microbiology Department, Faculty of Science, El-Azhar University, Egypt
Regional Center of Mycology and Biotechnology El-Azhar University, Egypt
[2]Clinical and Chemical Pathology Department, Faculty of Medicine, Cairo University, Egypt

Key words:

Neonatal septicemia, bacterial agents, antibiotic susceptibility, ESBL-producing organism

ABSTRACT

Background: *Septicemia continues to be a major cause of neonatalmorbidity and mortality worldwide. Blood culture is the gold standard for diagnosis of septicemia.* ***Objectives:*** *To determine the common causative bacterial agents of neonatal sepsis and their antimicrobial susceptibility in NICU.* ***Methodology:*** *Five hundred and fifty one suspected cases of septicemia who were admitted to the Neonatal Intensive Care Unit (NICU) of Abu El Rich El Mounira pediatric Hospital from 1st January 2013 to 31ST December2013were included. Blood cultures were withdrawn for all cases. Organisms were isolated and identified by conventional biochemical reactions and sensitivity was tested by Kirby Bauer disc diffusion technique. Also screening for Extended Spectrum B lactamase (ESBL) producing bacteria was done.* ***Results:*** *Blood Culture was positive in 87(15.7%) of cases. There was a predominance of Gram negative organisms (58.6%) over Gram positive organisms (36.8%) and Fungi (4.6%). Gram negative bacteria showed high susceptibility to impenem and merpenem and reduced susceptibility to ampicillin and ampicillin-sulbactam, while Gram positive bacteria were sensitive to vancomycin and rifampicin and had reduced susceptibility to ampicillin. ESBL Production was found in 37.3%of Gram negative strains. Thirty one isolated pathogens were found to be hospital acquired, of which 64.5% were Gram Negative while 32.3% were Gram positive represented 32.3% and fungi 3.2%were fungi.* ***Conclusions:*** *Neonatal sepsis in our NICU is mainly caused by Gram negative organisms, which are developing resistance to commonly used antibiotics. This emphasizes the need to implement infection control policies at the hospital level for effective management of spread ESBL producing organisms and Multidrug resistance.*

INTRODUCTION

Globally, sepsis is still one of the major causes of morbidity and mortality in neonates, in spite of recent advances in health care units[1]. More than 40% of under-five deaths globally occur in the neonatal period, resulting in 3.1 million newborn deaths each year [2].The majority of these deaths usually occur in low-income countries and almost 1 million of these deaths are attributed to infectious causes including neonatal sepsismeningitis, and pneumonia[3]. On the otherhand, the survivors of neonatal sepsis are vulnerable to short and long-term neurodevelopmental morbidity[4].

**Corresponding author:*
Dina Mohammed Hassan MD.
Lecturer of Clinical and Chemical Pathology,
Faculty of Medicine-Cairo University, Cairo, Egypt.
Email: dinamhassan@kasralainy.edu.eg, Tel: 01006911788

Neonatal sepsis is defined as a clinical syndrome in an infant 28 days of life or younger, manifested by systemic signs of infection and isolation of a bacterial pathogen from the bloodstream[5]. Diagnosis and management of sepsisare a great challenge facing neonatologists in NICUs. Clinical diagnosis of presentation is difficult due to nonspecific signs and symptoms. In addition, laboratory diagnosis is time consuming. This matter necessitates the initiation of empirical antibiotic therapy till the suspected sepsis is ruled out. At the same time, increased multidrug resistant organisms make the treatment options fewer and the effective treatment is delayed[6].

Neonatal sepsis is caused by Gram-positive and Gram negative bacteria and *Candida*[7]. The diversity of organisms causing sepsis varies from region to another and changes overtime even in the same place[8].

This is attributed to the changing pattern of antibiotic use and changes in lifestyle. Many factors

contribute to the susceptibility of the neonate to sepsis, which can influence the incidence of neonatal sepsis. Incidence also varies from nursery to nursery depending on conditions predisposing infants to infection [7].

In neonatal intensive care units (NICUs), hospital acquired infections represent a significant problem and their rates appear to be increasing, especially because of the longer survival of very low birth weight infants, the growing complexity of invasive treatments and more children are hospitalized for longer periods[9].

The findings of the study have important implications for community-based research and programs to improve maternal and neonatal health and survival not only in Egypt, but also in other similar developing world settings.

METHODLOGY

1. **Study design:** A cross sectional study was conducted in our study including 551suspected cases of septicemia admitted to Neonatal Intensive Care Unit (NICU) of the of Abu Rich El Monira pediatric Hospitalin Egypt from the 1st of January , 2013 to 31st of December, 2013.
2. **Isolation of bacteria:** A blood sample of 0.5 to 1.0 ml was withdrawn aseptically after the venipuncture site was wiped with 70% alcohol followed by two cycles of 2 min of disinfection with povidone-iodine, then inoculated into BACTEC pediatric blood culture bottles (BD Diagnostic Systems).Bottles were transported to the laboratory as soon as possible and immediately incubated in the instrumented Blood Culture Systems which is BD BACTEC™ 9050. Blood cultures were processed using the standard technique described by Cruickshank et al[10] and were cultured on specific culture media to isolate the causative organisms. Identification of the recovered bacteria was done by colony characteristics, Gram staining and biochemical tests. For Gram positive organisms identification was done by Catalase and Coagulase test. For Gram negative organisms, identification was done by biochemical reactions as Simon's Citrate test, MIO (Motility, Indole, Ornthin), TSI (Triple Sugar Iron) and Urease Tests. Regarding Candida isolates confirmation was done by subculture on Sabouraud dextrose agar media and germ tube was done for differentiation between candida albicans and non albicans[11].
3. **Confirmation of Gram Negative isolates by API strips:** The identification of the 51Gram negative bacterial isolates was performed using API strips inoculated and incubated as described by the manufacturer (bio MerieuxVitek System, France). The strips were API 10S, API 20 NE and API 20E. Examination of the strips was conducted after 18-23 h, and the results from the 24 h analysis were used. The results were read and analyzed using analytical profile index.
4. **Antimicrobial Susceptibility testing:** Antimicrobial susceptibility testing was performed for all blood culture isolates by Kirby–Bauer disc diffusion method as recommended in the Clinical Laboratory Standards Institute (CLSI 2012) guidelines[12].
5. **Phenotypic confirmatory test for ESBL production by Gram Negative isolates:**
   a. Double disk synergy test (DDST) was performed using disks of 30 µg each of cefpodoxime (CPD), ceftazidime (CAZ), cefotaxime (CTX), ceftriaxone (CRO), aztreonam (ATM) (Oxoid Co. England) along with AMC (amoxicillin 20µg and clavulenic acid 10 µg).The disks were placed at the distance 20 mm from each other (centre to centre) and incubated at 37ºC overnight. A clearly visible extension of the edge of the inhibition zone of any disk towards the amoxicillin clavulenic disk was interpreted as positive for Clavulenic acid(CLA)synergy [13].
   b. Cephalosporin/clavulanate combination discs: A 0.5 MacCfurland suspension of the isolate is inoculated into a 10-mm Müller Hinton agar plate using the antimicrobial discs ceftazidime (30 µg), ceftazidime /clavulenic acid (30/10 µg). After incubation, the zone of inhibition around each of the discs is measured. An increase of > 5 mm in zone diameter for either antimicrobial agent tested in combination with clavulenic acid versus its zone when tested alone indicates positive for Extended Spectrum Beta Lactamase (ESBL) production [14].
6. **Statistical Analysis:** Summary of measures was reported as mean and standard deviation (SD) for quantitative variables and percentages for categorical variables. The differences indistribution were evaluated using the chi-square test for categorical variables. Value≤ 0.05 was considered statistically significant. All the statistical analyses were performed using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

RESULTS

During the period of study 551 blood culture bottles were received in the lab from which 87(15.7%) were positive. Forty six (52.8%) of the isolates were from male patients and 41(47.2%) were from female patients resulting in an overall male to female ratio of 1.3: 1. However, no significant difference was detected regarding sex (P> 0.05).

Distributions of pathogens isolated from blood culture of infants involved in the current study are presented in table 1. There was a predominance of Gram negative organisms 51(58.6%) over Gram positive organisms 32(36.8%) and fungi 4(4.6%). *Klebsiella pneumoniae* was the most frequently isolated organism 27 (31.0%), followed by *Coagulase Negative Staphylococcus* (CoNS) 19 (21.8%), *Staphylococcus aureus* 11(12.6%), *E.coli* 8 (9.2%). *Pseudomonas aeruginosa* 8(9.2%)**,** other less frequently isolated organisms were *Acinetobacter spp.* 4 (4.6%), *Candida spp.* 4 (4.5%)**,** *Enterobacter aerginosa*3 (3.4%), *Streptococcus Pneumonia*1 (1.5%), *Streptococcus Viridans*1 (1.5%)**,** *Salmonella typhi*1 (1.5%)

Table 1: Distribution of isolated pathogens causing septicemia.

Type of organism	N(%)
Gram Negative Bacteria	51(58.6)
Klebsiella pneumoniae	27(31.0)
E.coli	8(9.2)
Enterobacter aerginosa	3(3.4)
Salmonella typhi	1(1.5)
Pseudomonas aeruginosa	8(9.2)
Acinetobacter spp.	4(4.5)
Gram Positive Bacteria	32(35.6)
Staphylococcus aureus.	11(12.6)
Coagulase Negative staphylococcus	19(21.8)
Streptococcus Pneumonia	1(1.5)
Streptococcus Viridans	1(1.5)
Fungi	4(4.5)
Candida spp.	4(4.5)
Total	87(100.0)

N: Number

The resistance of Gram-negative organisms to the most relevant antibiotics is described in table 2.

Klebsiella pneumonia which is the most common isolated Gram negative isolate showed 96.4%reduced susceptibility to ampicillin, 82.2% to ampicillin+sulbactam, 71.5% to amoxcillin-clavulenic, third generation cephalosporins (ceftriaxone 89.3%, cefatizidime85.7%) fourth generation cephalosporins (cefepime 82.2%). They showed also reduced susceptibility 44.4% tolevofloxacin, 44.4% to cefoxtin, 37.0% to ciprofloxacillin,37.0%to amikacin, They were highly susceptible to impenemby 74.0%,to merpenemby67.8%,and sulphameth-trimethby 67.8%.

E.coli showed 87.5% reduced susceptibility to ampicillin, 87.5% to ampicillin+sulbactam, 62.5% to amoxcillin-clavulanic, 62.5% to cefoxtin, third generation cephalosporins (ceftriaxone75.0%, cefatizidime87.5%) fourth generation cephalosporins (cefepime 62.5%). They showed also reduced susceptibility 50.0% to Levofloxacin, 62.5% to ciprofloxacillin, 62.5% to amikacin. They were susceptible to impenem by87.5%, merpenem by75.0%and sulphameth-trimethby 75.0%.

The resistance of Gram-positive organisms to the tested antibiotics is presented in table 3.

Coagulase negative Staphylococci which is the most common isolated Gram positive bacteria showed 84.2% reduced susceptibility to ampicillin ,78.1% to erythromycin, They showed also reduced susceptibility 63.2% to sulphameth-Trimeth, 57.8% to doxycyclin, 52.6% to clindamycin,42.1% to chloramphenicol,42.1% to gentamycin, 36.8% to Amoxcillin-clavulenic,36.8% to azithromycin, and 31.6% to cefoxtin. They were all susceptible to vancomycin 100%, and 68.4% to rifampicin.

Staphylococcus aureus showed 100% reduced susceptibility toampicillin, 81.8% to amoxcillin-clavulenic, 72.8% to sulphameth-trimeth, 72.8% to azithromycin, and 72.8%to chloramphenicol. They showed also reduced susceptibility to erythromycin 54.5%, doxycyclin 54.5%, clindamycin 45.5%,cefoxitin 36.4**%**, They were all susceptible to vancomycin 100%, and 63.6% were susceptible to rifampicin**.**

Table 2: Reduced susceptibility pattern of the isolated Gram Negative organisms to different antibiotics

Antibiotic	*Klebsiella pneumoniae*		*E.coli*		*Pseudomonas aeruginosa*		*Acinetobacter spp.*		*Enterobacter spp.*		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Ampicillin	26	96.4	7	87.5	7	87.5	4	100.0	3	100	47	92.2
Amoxcillin-clavulenic	19	71.5	5	62.5	5	62.5	4	100.0	1	33.3	34	66.6
Ampicillin+Sulbactam	22	82.2	7	87.5	7	87.5	4	100.0	2	66.6	42	82.3
Impenem	7	25.9	1	12.5	2	25.0	1	25.0	0	0.00	11	21.6
Cefoxtin	15	55.6	5	62.5	7	87.5	4	100.0	2	66.6	33	64.7
Ceftriaxone	24	89.3	6	75.0	8	100.0	4	100.0	3	100.0	45	88.2
Cefatizidime	23	85.7	7	87.5	8	100.0	4	100.0	2	66.6	44	86.2
Cefepime	22	82.2	5	62.5	7	87.5	4	100.0	2	66.6	41	80.4
Amikacin	17	63.0	3	37.5	6	75.0	3	75.0	1	33.3	30	58.8
Sulphameth-Trimeth	8	32.2	2	25.0	6	75.0	3	75.0	1	33.3	20	39.2
Merpenem	8	32.2	2	25.0	4	50.0	1	25.0	0	0.00	15	29.5
Ciprofloxacillin	17	63.0	3	37.5	3	37.5	2	50.0	0	0.00	25	49.0
Levofloxacin	15	55.6	4	50.0	2	25.0	3	75.0	0	0.00	24	47.1

% was correlated to the total number of each bacterial species.

Table 3: Reduced susceptibility pattern of the isolated Gram positive organisms to different antibiotics.

Antibiotic	*Coagulase Negative staphylococcus*		*Staphylococcus aureus*		*Streptococcus sp.*		Total	
	No.	%	No.	%	No.	%	No.	%
Ampicillin	11	100.0	16	84.2	1	50.0	28	87.5
Vancomycin	0	0.00	0	0.00	0	0.00	0	0.00
Amoxcillin-clavulenic	9	81.8	12	63.1	0	0.00	21	65.6
Rifampicin	4	36.4	6	31.6	0	0.00	10	31.2
Cefoxtin	7	63.6	13	68.4	-	-	20	62.5
Gentamycin	6	54.5	11	57.9	2	100.0	19	59.3
Clindamycin	6	54.5	9	47.4	1	50.0	16	50.0
Erythromycin	5	45.5	15	78.9	2	100.0	22	68.7
Sulphameth-Trimeth	8	72.8	7	36.8	-	-	15	46.8
Doxycyclin	5	45.5	8	42.2	-	-	13	40.6
Chloramphenicol	8	72.8	11	57.9	2	100.0	21	65.6
Azithromycin	8	72.8	12	63.2	-	-	22	68.7

% was correlated to the total number of each bacterial species

Hospital acquired pathogens:

Thirty one isolated pathogens were found to be hospital acquired, of which Gram Negative Bacteria were found to be the predominant isolates representing 64.5%, while Gram positive bacteria represented 32.3% and fungi represented 3.2% of isolates.

In the present study total of 19(37.3%) Gram negative strains were positive for ESBL Production, ESBL was detected in 9 (47.4 %) isolates of *Klebsiella pneumonia*,6 (31.6 %) isolates of *E. coli.,* 3 (15.8 %) isolates of *Pseudomonas aeruginosa*, 1 (5.3 %) isolates of *Enterobacter spp.* and in none of the isolates of *Acinetobacter spp.* and *Salmonella typhi.* Sixteen (84.2%) of ESBL positive isolates by DDST were positive by combined disc method, also 3 of ESBL negative isolates by DDST were positive by combined disc method and 32 of ESBL negative isolates by DDST were negative by combined disc method. DDST is in agreement with combined disc method for detection of ESBL positive cases this result showed in table 4 and 5**.**

Table 4: Relation between combined disc and double disc synergy test in detection of ESBL.

		Double disc synergy (DDST)		Total	Kappa
		+ve	-ve		
Combined disc	+ve	16	3	19	0.8
		84.2%	15.8%	100.0%	
	-ve	0	19	19	
		0.0%	100.0%	100.0%	

Table 5: Sensitivity and specificity of double disc synergy test compared tocombined disc.

Item	TP	FN	TN	FP	Sens.	Spec.	Accuracy
DDST	14	2	16	0	87.5%	100.0%	87.5%

DISCUSSION

Neonatal sepsis is the commonest cause of neonatal morbidity and mortality worldwide. In the World Health Organization2000–2003 report, neonatal sepsis and pneumonia were responsible for about 1.6 million deaths each year, mainly inresource-poor countries[15]. In this study, documented neonatal sepsis with positive culture was 15.7 %. This is low compared to about 20% yield reported by BaltimoreandGladstone[16] and higher than previous studies done by Begum et al.[12] where positive cases were 13.0%[17]. Much higher rates of45.9% was also described by Eman et al.[18]. These varying proportions may be due to the difference in methodology used and the areas of study, as regional variation are known to occur.

The organisms causing neonatal septicemia differ from area to area and also change with respect to time, even in the same area, which may be due to different livingconditions[19]. Our study showed that the Gram negative bacteria constituted the major group of isolates (58.6%) of neonatal septicemia cases compared to(36.8 %) Gram Positive bacteria and (4.6%) Candida. This finding is similar to that of another study which showed that Gram negative bacteria were responsible in most cases of neonatal sepsis[20]. Other authors in other studies reported Gram positive bacteria as the commonest cause of neonatal sepsis[21, 22].

Candida spp. were isolated only in 4 cases (4.6%), similar findings were found in other studies done by Kavitha et al in year 2014where candida isolates represented (5.05%)[23] and Eman*et al*[18] it represented (2.86%)[18] but this was lower than several other reports showing frequency of isolation of13.6-19.6% of cases[24].

In this study, the most frequent isolate was *Klebsiella pneumonia*e 27(31.0%) This was in accordance with other studies[25, 26]. The pattern ofisolated organisms in our study slightly differs from the findings inIran[27], where *Pseudomonas aeruginosa* was the most common causeof neonatal sepsis followed by *Klebsiella spp.* and *E. coli.* In a similar study from Bangladesh, Nepal and Pakistan, *E. coli* was the leading cause of neonatal sepsis followed by *Klebsiella spp.* [28].

In our study, *Coagulase Negative Staphylococcus* was the second most common isolated organism, similar results where CoNS had been reported as the most common organism from blood culture isolates were found [29,30]. In the present study 21.8% of isolates were CoNS. The clinical significance of CoNS when isolated from blood cultures should be always evaluated. Some studies have reported that up to 85% of CoNS representing contamination rather than true bacteremia[31]. However, CoNS have become an important nosocomial pathogenpartly because of the increasing use of medical devices such as long term indwelling catheters, vascular grafts, and prosthetic heart valves and joints[32]. On the other hand Mustafa and Ahmed in year 2014 reported in their study *Staphylococcus aureus*as the most common causative agent of neonatal bacteremia [25].

The problem of antimicrobial resistance is highlighted by the World Health Organization (WHO) and combating antimicrobial resistance has been selected as the theme for World Health Day[33]. In this study, *Klebsiella pneumonia* isolates were found to be highly resistant to routinely used antibiotics, followed by *Pseudomonas aeruginosa* and *Escherichia coli.* All Gram negative isolates were having considerable sensitivity to amikacin, levofloxacin and ciprofloxacin but were highly susceptible to impenem (74.4%) and meropenem (70.5%) and highly resistant to ampicillin, amoxicillin-clavulanic acid, cefotaxime, ceftriaxone, ceftazidime. cefepime. Aurangzeb et al. reported considerable resistance of Gram negative bacteria to commonly used antibiotics such as ampicillin, amoxicillin, ceftazidime, cefotaxime andcomparatively low resistance to gentamicin, impenem and ciprofloxacin[34].

In our study, only two isolates of *Pseudomonas aeruginosa* were recovered from blood cultures exhibiting resistance to all antibiotics tested. This result is in accordance with another study done in Egypt[18].

Among the Gram-Positive isolates, all isolates were highly resistant to ampicillin, amoxicillin-clavulanic acid, erythromycin, azithromycin and chloramphenicol but all isolates were highly susceptible to vancomycin (100%) and rifampicin (67.7%).

In our study, all CoNS isolates showed high resistance to ampicillin and cefoxitin, erythromycin, azithromycin, and gentamicin. These results are in agreement with a previous study.[18]

Interestingly, all staphylococcal isolates were sensitive to Vancomycin as previously found in other reports [35] but it's overprescription may result in the development of vancomycin-resistant strains such as enterococci.

According to our finding, best sensitivity among Gram negative isolates was observed with impenem followed by meropenem while among Gram-positive isolates was with vancomycin and is followed by rifampicin. The high resistance rates found in this study may be associated with the frequent use of antimicrobial drugs for both prophylactic and therapeutic treatment of hospitalized newborns[36]. In view of the above facts the strategy of antibiotic usage in neonates should be reviewed periodically even in the same hospital.

In our study a NICU hospital acquired infection rate of 35.6% was reported. A wide variations in the reported incidence of hospital acquired infection rates in literature according to the NICUs , as in the United States, hospital acquired infection rates vary from 6 to greater than 40%[37], and in one study the incidence has been reported to be as high as 70% [38]. This discrepancy in hospital acquired infection rates could be not only attributed to the infection control policies and the Surveillance system in every place, but also due to differences in defining, identifying and reporting hospital acquired infection as there are differences in reporting it, particularly the time of onset.

The present study showed that 37.3% of Gram negative strains isolated from blood specimens were ESBL producers. This high prevalence is related to the less controlled use of antibiotics in Egypt, where many drugs are still available over the counter. Few studies have investigated the prevalence of ESBL in Egyptian hospitals. Abdallah et al in year2015detected 48.9% ESBL-producing *Enterobacteriaceae* among strains isolated from patients in the intensive care unit at El-Ahrar General Hospital, Zagazig, Egypt[39]. While Bouchillon et al conducted the PEARLS study in 2001–2002, and found that 38.5% of *Enterbacteriaceae* isolates did produce an ESBLresistance[40]. A lower ESBL prevalence rate (16%) was found among 120isolates collected between May 2007 and August 2008 at the Theodor Bilharz Research Institute, Cairo, Egypt[41].

In the present study we observed that 47.4% *Klebsiella pneumoniae* and31.6% *E. coli* isolates were ESBL producers. This is in agreement with a study conducted at Menoufia University, where ESβL producing Gram Negative Bacilli (GNB) were mostly *Klebsiella spp*. (54.3%) followed by *E. coli* (19.6%)[42]. In 2003,Jain *et* al detected ESBL in 86.6% of *Klebsiella pneumoniae* and 63.6% of *Escherichia coli*[43]. Also study done by Gandhi et al year 2013, ESBL production was seen in 52.9% of *Escherichia coli* and 50% of *Klebsiella pneumonia* isolates[44].

In the present study, the Double disc synergy test (DDST) was able to detect 16out of the 19 ESBL-producers detected by the combined disc method and thus showing a substantial agreement with combined disc (Kappa=0.8) and a sensitivity of 84.2%. This finding is in agreement with Daef et al in year2009 who reported that 21 out of 23 (91.3%) potentially ESBL-producing *Enterobacteriaceae* were positive by DDST[45]. The Combined disc test was previously compared with DDST and it was found to be an inexpensive alternative for the DDST, for the detection of ESBL producers. The DDST may miss few ESBL cases because of the problem of optimal disc spacing and the correct storage of the clavulenic acid containing discs. Assuming that a laboratory is currently testing the sensitivity for ceftazidime by using the disc diffusion test and it required only one disc to be added to the sensitivity plate by combined disc test and would screen all Gram negative bacteria in the diagnostic laboratory for ESBL production. This method is technically simple and inexpensive[46].

The Clinical and Laboratory Standards Institute (CLSI) therefore, also recommended the use of Combined disc test for the phenotypic confirmation of the ESBL producers among *E. coli* and *K. pneumoniae* [12].

CONCLUSION

Antibiotic resistance is today a global problem. Neonatal septicemia is a life-threatening emergency, and rapid treatment with antibiotics is essential for a favorable outcome. In our study *Klebsiella pneumoniae* and *CONS* together with *Staph aureus* are the leading causative agents of neonatal sepsis in our unit. They were resistant to commonly used antibiotics, thus according to antibiotic sensitivity results, appropriate initial empirical antibiotic therapy for neonatal sepsis should be replaced. Every unit should carefully follow the bacterial spectrum and resistance patterns of microorganism responsible for neonatal infections to design a specific empirical antibiotic regimen .Appropriate infection control policies and procedure are essential to prevent spread of ESBL and multidrug resistant organisms in our NICU.

REFERENCES

1. Wu JH, Chen CY, Tsao PN, Hsieh WS, and Chou HC.Neonatal sepsis: a 6-year analysis in a neonatal care unit in Taiwan. Pediatrics and Neonatology.2009; 50(3): 88–95
2. WHO .World Health Organization. Antimicrobial resistance 2011. Fact sheet number 194
3. Black RE, Cousens S, Johnson HL et al. Global, regional,and national causes of child mortality in 2008: a systematic analysis.2010; The Lancet 375(9730): 1969–1987
4. Stoll BJ, Hansen N, Fanaroff A et al. Changes inPathogens causing early-onset sepsis in very-low-birth-weight Infants. The New England Journal of Medicine.2002; 347(4): 240–247
5. 5.Edwards MS and Baker CJ. Sepsis in the newborn.In Krugman's Infetious Diseases of Children.2004; 11th ed.: 545-61
6. Patel SJ and Saiman L. Antibiotic resistance in neonatal intensive care unit pathogens: mechanisms, clinical impact, and prevention including antibiotic stewardship. Clinics inPerinatology.2010; 37(3): 547–563
7. 7.Jumah DS and Hassan MK. Predictor of mortality outcome in neonatal sepsis.Medical Journal of Basrah University.2007; 25: 11–18
8. Shrestha S, Adhikari N, Rai BK and ShreepailiA.Antibiotic resistance pattern of bacterial isolates in neonatal care unit. Journal of theNepal MedicalAssociation.2010; 50(4):277– 281
9. José M,CernadasC, Silvia Fernandez Jonusaset al. Clinical outcome of neonates with nosocomial suspected sepsis treated with cefazolin or vancomycin. Non-inferiority, randomized, controlled trial Arch Argent Pediatr.2014; 112(4):308-314 – 308
10. Cruickshank K, Duguio JP and Marnion BP.Test for sensitivity to antimicrobial agents, In Medical Microbiology, Edinburgh: Churchill Livingstone.1980; 12(2), 196
11. Krieg NR.Bergey's Manual of systematic Bacteriology. Vol. The Williams and Wilikins Company, Library of Congress, Baltimore, USA.1984
12. Clinical and Laboratory Standard Institute .Performance standards for antimicrobial susceptibility testing; twentieth informational supplement. CLSI document M100-S22. Clinical and Laboratory Standards Institute, Wayne, PA.2012
13. Jarlier V, Nicolas M-H, Fournier G and Philippon A. Extended board-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in Enterbacteriaceae: hospital Prevalence and susceptibility patterns. Rev Infect Dis.1988; 10:86-878
14. Sanders M, Fille M, BauernfeindA, StemplingerI and FeyP. Extended spectrum beta–Lactamase (ESBL)-producing Enterbacteriaceae Considerations for diagnosis, prevention and drug treatment. Drugs.1996; 63:353-365
15. Posfay-Barbe KM, Zerr DM, and Pittet D, (2008)."Infection controlin paediatrics," The Lancet Infectious Diseases.2008;8(1):19–31
16. Baltimore RS, Huie SM, Meek JI, Schuchat A, O'Brein KL. Early-onset neonatal sepsis inthe era of group B Streptococcal prevention. Pediatrics.2001; 108:1094-98
17. Begum M, Hassan ZS, HaqueM, JahanN, Chowdhury K, RobA. W, (2013). Study of Bacteriological pathogen causing neonatalsepsis at NICU in Ad-din medical college hospital.Northern International Medical College Journal.2013;9(2): 352–365
18. Eman M. Shehab El-Din R, MohamedM. El-Sokkary A, Mohamed RBand Hassan R.Epidemiology of Neonatal Sepsis and Implicated Pathogens: A Study from Egypt: BioMed Research International.Volume 2015, Article ID 509484, 11 pages
19. Shrestha P, Das BK, Bhatta NK, Jha DK, Das B, Setia A, Tiwari A.Clinical and bacteriological profile of blood culture positive sepsis in newborns. J. Nepal. Pediatr. Soc.2007;27(2):64-67
20. Muhammad Z, Ahmed A, Hayat U, Wazir MS, Rafiyatullah, Wagas H. Neonatal sepsisCausative bacteria and their resistance to antibiotics. J Ayub Med Coll Abbottabad .2010;22(4):33-6
21. Li Z, Xiao Z, Zhong Q, Zhang Y, and Xu F.116 cases ofneonatal early-onset or late-onset sepsis: a single center retrospectiveanalysis on pathogenic bacteria species distributionand antimicrobial susceptibility," International Journal of Clinicaland Experimental Medicine.2013;6(8): 693–699
22. Ballot DE, Nana T, Sriruttan C and Cooper PA. "Bacterialbloodstream infections in neonates in a developing country,"ISRN Pediatrics, vol. 2012,Article ID 508512, 6 pages
23. Kavitha H,Anuradha K, Venkatesha D.Comparison of Susceptibility of Various Candida Species Isolated From Neonatal Septicaemia to Voriconazole and Fluconazole: IOSR Journal of Pharmacy and Biological Sciences.2014; e-ISSN: 2278-3008, p-ISSN:2319-7676. 9(2): 78-81
24. Agarwal J, Bansal S, Mailk GK, Jain A. Trends in neonatal septicemia, Emergence of non-albican Candida, Indian Pediatr.2004; 41, 712–5
25. MustafaM and Ahmed SL. Bacteriological profile and antibiotic susceptibility patterns in neonatal septicemia in view of emerging drug resistance: J Me d A l li e d S c i.2014; 4 (1): 02-08
26. MondolSK, Nag DR., Bandyopadhyay R, Chakraborty D and SinhaSK. Neonatal sepsis: Role of a battery ofimmunohematological tests in early

diagnosis,International Journal of Applied and Basic MedicalResearch.2012;2(1), 43-47
27. Movahedian AH, Moniri R, and Mosayebi Z. "Bacterial cultureof neonatal sepsis," Iranian Journal of Public Health.2006; 35(4): 84–89
28. Ahmed AS, Chowdhury MA, Hoque M, Darmstadt GL. Clinical and bacteriological profileof neonatal septicemia in a tertiary level pediatric hospital in Bangladesh. Indian Pediatr.2002; 39(11):1034-9
29. Dagnew M, Yismaw G, Gizachew M, Gadisa A, Abebe T, Tadesse et al.Bacterial profile and antimicrobialsusceptibility pattern in septicemia suspected patientsattending Gondar University Hospital, Northwest Ethiopia.Dagnew et al. BMC Research Notes. 2013;6:283
30. Meremikwu MM, Nwachukwu CE, Asuquo AE, Okebe JU, Utsalo SJ .Bacterial isolates from blood cultures of children with suspected septicaemia in Calabar, Nigeria. BMC Infectious Diseases. 2005;5:110
31. Towns ML, Quartey SM, Weinstein MB, Reimer LG, Reller LB. The clinical significance of positive blood cultures:a prospective, multicenter evaluation. In: Abstracts ofthe 93rd General Meeting of the American Society forMicrobiology, C-232. Washington, DC: American Society forMicrobiology.1993
32. Reimer LG, Wilson ML, Weinstein MP. Update on detection of bacteremia and fungemia. ClinMicrobiol Rev.1997; 10:444-65
33. 33.WHO. World Health Organization.Antimicrobial resistance 2011. Fact sheet number 194
34. Aurangzeb B and Hamdeed A. Neonatal sepsis in hospital born-babies: bacterial isolates and susceptibility patterns, J Coll Physicians Surg Pak.2003; 13(11): 629-632
35. Shah AJ, Mulla SA, and Revdiwala SB. Neonatal sepsis: high antibiotic resistance of the bacterial pathogens in a neonatal intensive care unit of a tertiary care hospital," Journalof Clinical Neonatology.2012; 1(2): 72–75
36. Loureiro MM, Moraes BA, Quadra MRet al. Study of multi-drug resistantmicroorganisms isolated from blood cultures of hospitalized newborns in Rio de Janeirocity, Brazil. Brazil J Microbiol.2002; 33(1):73-8
37. ZafarN, Wallace CM, Kieffer P, Schroeder P, Schootman M and Hamvas A. Improving survival of vulnerable infants increases neonatal intensive care unit nosocomial infection rate. Arch PediatrAdolesc Med.2001; 155: 1098-1104
38. Hwang JH, Choi CW and Chang YS.The efficacy of clinical strategies to reduce nosocomial sepsis in extremely low birth weight infants. J. Korean Med Sci.2005; 20: 177-181
39. Abdallah HM, Wintermans BB, Reuland EA, Koek A, al Naiemi N, Ammar AM et al. Extended-Spectrum β-Lactamase- and Carbapenemase-Producing Enterobacteriaceae Isolated from Egyptian Patients with Suspected Blood Stream Infection. PLoS ONE.2015; 10(5): e0128120
40. Bouchillon SK, Johnson BM, Hoban DJ, Johnson JL, Dowzicky MJ, Wu DH, et al. Determining incidence of extended spectrum beta-lactamase producing Enterobacteriaceae, vancomycin-resistant Enterococcus faecium and methicillin-resistant Staphylococcus aureus in 38 centres from 17 countries: the PEARLS study 2001–2002. Int J Antimicrob Agents. 2004; 24: 119–24
41. Fam N, Leflon-Guibout V, Fouad S, Aboul-Fadl L, Marcon E, Desouky D, et al.CTX-M-15-producing Escherichia coli clinical isolates in Cairo (Egypt), including isolates of clonal complex ST10 and clones ST131, ST73, and ST405 in both community and hospital settings. Microb Drug Resist. 2011; 17: 67–73
42. Eissa NA, Al-Ragehy NA, Koura BA et al ESβL producing Enterobacteriaceae and Pseudomonas aeruginosa causing nosocomial pneumonia in Menoufyia University. M.Sc.2007 Thesis (Microbiology), Faculty of Medicine, Cairo Unviersity, Egypt
43. Jain A, Roy I, Gupta MK, Kummar M, Agarwal SK. Prevalence of extended – spectrum beta – lactamase- producing gram – negative bacteria in septicamic neonates in a tertiary care hospital. J Med Microbiol.2003; May;52(Pt):421-5
44. Gandhi S, Ranjan KP, Ranjan N, Sapre N, Masani M. Incidence of neonatal sepsis in tertiary care hospital: an overview. Int J Med Sci Public Health.2013; 2(3):548–52
45. Daef AE, Aly AS, Seif El-Din AS, et al.Phenotypic and genotypic detection of extended spectrum beta-lactamase Klebsiella pneumoniae isolated from Intensive Care Units in Assuit University Hospital. Egyptian Journal of Medical Microbiology.2009; 18: 29-40
46. Shukla I, Tiwari R, Agarwal M. Prevalence of extended-spectrum β-lactamase producing Klebsiella pneumoniae in a tertiary care hospital. Indian J Med Microbiol.2004; 22(2):87-91.