Pattern of Blood Stream Infections and their antibiotic susceptibility profile in a Neonatal intensive care unit of a tertiary care hospital; a current perspective

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Abstract

Objective: To determine the pattern of blood stream infections and their antibiotic susceptibility profile with infectivity predictors in a neonatal setting.

Methods: The descriptive cross-sectional study was conducted at the Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from December 1, 2016,to April 30, 2018, and comprised blood culture samples received in Bactec/BactAlert paediatric bottles from neonates aged 0-30 days admitted in the neonatal intensive care unit. The samples were processed as per the standard guidelines. Antibiotic susceptibility was checked as per guidelines of the Clinical and Laboratory Institute. VITEK 2 system was used for rapid identification and minimum inhibitory concentrations of the drugs. SPSS 24 was used for data analysis.

Results: Out of 640 samples, 172(27%) were culture-positive. Among them, 98(57%) were gram-negative rods, 50(29%) gram-positive cocci and 24(14%) were fungi. Of the 172 pathogens identified, *Klebsiella pneumoniae* was 39(22.7%) followed by Candida species 24(14%) and methicillin-resistant Coagulase-negative *staphylococci* 20(11.6%). Of *Klebsiella pneumoniae* isolates, 26(58%) were extended spectrum β-lactamase producers. Among *Acinetobacterbaumanii*, 11(58%) were extensively drug resistant and Carbapenem-resistant strains were 20(91%). Also, 4(8%) isolates of *Enterococcus faecium* were vancomycin-resistant.

Conclusion: Majority of the isolates causing blood stream infections in neonatal intensive care unit were multidrug resistant, posing a therapeutic challenge for the neonatologists.

Keywords: Blood stream infections, Extended spectrum β-lactamase, Multidrug resistant, Neonatal intensive care unit, Carbapenem resistant organism. (JPMA 69: 1668; 2019). **doi: 10.5455/JPMA.298528.**

Introduction

Neonatal blood stream infections (BSIs) are one of the major causes of high mortality in neonatal intensive care units (NICUs), accounting for around 1.6 million deaths each year in developing countries. Early onset sepsis (EOS) manifests within 72h of life, or later due to breach in infection control practices or interventions, and manifests as late onset sepsis (LOS) after 72h of birth.[1] Neonates have an immature immune system, making them more vulnerable to invasive infections which they may acquire during delivery from the maternal birth canal or surroundings. Neonatal infections are difficult to diagnose, as the presentation is often with non-specific signs and symptoms. The risk of sepsis is even higher with preterm and low birth weight (LBW) infants.[2] There has been emergence of multi-drug resistance (MDR), which is resistance to at least one drug in three or more classes, carbapenem-resistant organisms (CRO),which is resistance to either one or any of 2 carbapenems, and extensive drug resistance (XDR)which is non-susceptibility to ≥1 agents in all but ≤2 categories.[3]

Neonatologists must start treatment with empirical antibiotics in suspected cases in order to curtail the mortality and morbidity associated with neonatal sepsis. The choice of empirical treatment must take into account the prevalent pathogens and their current antibiotic resistance patterns.[4] The antibiotic policy must be tailored according to the local trends since the organisms responsible for neonatal sepsis vary in different geographical regions. Therefore, there is a need for

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constant surveillance of the prevailing pathogens and their pattern of resistance in NICU.

The current study was planned to determine the pattern of BSIs and their antibiotic susceptibility patterns, and to have a look at the risk factors for septicaemia for the formulation of local treatment guidelines.

Material and Methods

The descriptive cross-sectional study was conducted at the Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from December 1, 2016, to April 30, 2018, and comprised blood culture samples received from the NICU of Combined Military Hospital, Rawalpindi, from neonates aged 0-30 days. Permission was taken from the institutional ethics review committee, and informed consent was taken from all the parents/attendants of the neonates. Neonatal risk factors like LBW (≤2500 g) and prematurity (≤37weeks) were analysed. Neutrophilia ≥12 × 10^9/l and neutropenia <4 × 10^9/l, serial risein C-reactive protein (CRP) levels ≥10mg/l and thrombocytopenia ≤100,000/µl were also noted.

A laboratory-confirmed blood stream infection (LCBSI) was defined as isolation of positive peripheral-blood culture with signs of sepsis. Incoagulase-negative *staphylococci* (CoNS) and Candida (C.), evidence of indwelling catheters were checked. Otherwise, CoNS isolated from a single blood culture was considered contaminantion the absence of clinical signs of sepsis and time of positivity of culture.

All the blood cultures were taken in Bactec (Becton Dickinson, USA) and BacT/ALERT 3D (BioMérieux microbial identification system, USA) bottles and incubated in their respective automated systems. Sub-cultures were performed on 5% sheep blood agar, and MacConkey agar after the bottles flagged positive. The growth obtained after overnight incubation at 35±2^O C in ambient air, it was further identified by standard routine biochemical tests and automated system (VITEK 2, BioMerieux, France). *Candida* species were identified using Candida chrome agar (BioMerieux, France) and biochemical test by API 20C Aux (BioMerieux, France).

Antibiotic susceptibility testing was done by both modified Kirby-Bauer disk diffusion method on Mueller Hinton agar (Oxoid, UK) and VITEK 2 system. The antibiotic Discs (Oxoid, UK) of different concentrations were applied, as per the Clinical and Laboratory Standard Institute guidelines 2016-17.[5] *Escherichia (E.) coli* ATCC 25922, *Pseudomonas(P.)aeruginosa* ATCC27853 and *Staphylococcus (S.) aureus* ATCC 25923 were used as control strains. Screening for Methicillin-resistant *Staphylococcus aureus* (MRSA), mecA-mediated oxacillin resistance was detected by using cefoxitin (30 µg) disc. Resistance to ceftazidime (30 µg) disk was used as a screening method for the detection of extended spectrum beta-lactamase(ESBL),confirmed by double-disk synergy test.[5]

Data obtained was analysed using SPSS 24. Demographic data was assessed using descriptive statistics. Mean and standard deviation (SD) were calculated for numerical variables, like age. Categorical variables were expressed using frequencies and percentages. P<0.05 was considered statistically significant.

Results

Of the640 samples, 209(32%) were positive. Of them, 37(17.7%) isolates were excluded for being CoNS from a single blood culture. As such the sample stood at 172(27%). The mean age at admission of the subjects was 6.67±2 days (range: 0-28days), and102(60%) were boys. Overall, 136(79%) subjects were LBW, 134(78%)were preterm, 100 (58%) were LOS and 72 (42%)were EOS. (Table 1).

Among these neonates,98(57%) developed bacteraemia /sepsis associated with gram-negative rods (GNR), and of them, 65(66%) isolates were Enterobacteriaceae, 33(34%) were non-Enterobacteriaceae. Also, 50(29%) cases were of gram-positive septicaemia (GPS) and 24(14%) C. species (spp.) (Table 2).

Table-1: Demographic and Laboratory Findings of the neonates.

Demographic Findings and neonatal risk factors	Laboratory Findings	Positive Blood Culture with details of microbes vs onset of sepsis
Gender Male= 102(60%) Female = 70 (40%) Preterm: 78% (p-value ≤0.01) Male=77 (58%) Female= 57(42%) Low birth weight (≤ 2500 g): 79% (p-value≤0.01) Male= 81(59%) Female= 55(41%) Age of onset of sepsis(0-28 days) 0-3 days(EOS)= 72(42.19%) 4-28 days (LOS) = 100(58%)	Increased serial CRP= 137(80%) Leukocytosis= 132(77%) Leukopenia = 40(23%) Thrombocytopenia= 100(58%)	Total= 172 GNR = 98(57%) In EOS = 42(24%) In LOS = 56 (32.5%) GPC = 50(29%) In EOS = 19 (11%) In LOS = 31(18%) Candida spp = 24(14%) In EOS = 12(7%) In LOS = 12 (7%)

CRP: C-reactive protein, GPC: Gram-positive cocci, GNR: Gram-negative rods, EOS: Early onset sepsis, LOS: Late onset sepsis

Table-2: Frequency of various organisms isolated from blood culture in EOS and LOS (n=172).

Isolates	EOS 42% (0-3 days)	LOS 58%(4-29 days)
Klebsiella pneumonia (39)	12 (16.4%)	27(27.3%)
Acinetobacterbaumannii(22)	12 (16.4%)	10 (10%)
MRCoNS(20)	7(9.6%)	13 (13.1%)
Serratiamarcescens(18)	8(11%)	10 (10.1%)
Staphylococcus aureus(2),*MRSA*(10)	2(2.7%)	10 (10.1%)
Burkholderiacepacia(9)	3(4%)	6 (6%)
Enterococcus faecium(9)	7(9.5%)	2 (2%)
Enterococcus fecalis(7)	1(1.4%)	6 (6%)
Escherichia coli (4)	4(5.5%)	-
Stenotrophomonas maltophilia (2)	-	2 (2%)
Citrobacterfreundii(2)	1(1.4%)	1(1%)
Enterobacter cloacae(2)	2(2.74%)	-
Streptococcus pyogenes(1)	1(1.4%)	-
Steptococcusanginosus(1)	1 (1.4%)	-
Candida parapsilosis(9)	3(4%)	6 (6%)
Candida albicans(6)	5(6.8%)	1(1%)
Candida glabrata(5)	4(5.%)	1(1%)
Candida tropicalis (2)	-	2(2%)
Candida krusei(2)	-	2 (2%)
Total (172)	73 (42%)	99(58%)

EOS; Early onset sepsis, LOS; Late onset sepsis, MRCoNS: Methicillin-resistant Coagulase-negativestaphylococci, MRSA: Methicillin-resistant Staphylococcus aureus.

Table-3a: Antibiotic Susceptibility Pattern of Gram negative Isolates causing Sepsis (n=98).

Antibiotics	K.p (39)	A.b (22)	Serratia spp (18)	B.cepacia(9) S.maltophila (2)	E.coli (4)	E.cloacae(2) C.freundii (2)	% Resist
Ampicillin	NT	NT	NT	NT	4	NT	100%
Co-amoxiclav	39	NT	NT	NT	4	NT	100%
Ceftrixaone	38	22	16	NT	4	4	96.5%
Cefipime	26	22	11	NT	4	4	77%
Ceftazidime	26	22	11	2	4	4	70.4%
Meropenem	19	20	3	0	2	0	45%
Gentamycin	27	14	12	NT	3	2	69%
Amikacin	16	14	1	NT	0	0	47%
Cotrimoxazole	26	9	4	2	0	0	42%
Ciprofloxacin	20	19	11	5	2	0	58%
Levofloxacin	20	19	11	3	2	0	56%
Polymyxin	0	0	NT	NT	0	0	0
Pip/Tzp	26	19	11	NT	3	4	72.4%

Pip/ Tzp; Piperacillin/Tazobactum, NT: not tested, K.p: klebisella pneumonia, A: Acinetobacter, E.coli: Escherichia coli, E: Enterobacter, C: citrobacter, S: Stenotrophomonas.

Antibiotic susceptibility pattern of the isolates was noted in detail (Table 3a/b).In total, 50 gram-positive cocci (GPC),32(64%) Staphylococci and 10(31%)S.aureus were found methicillin-resistant. Among Enterococci,4(8%) were vancomycin (VRE) resistant. Besides, 24(14%) cases of BSI were caused by C. spp. Amphotericin B showed no resistance.

Discussion

Early institution of effective antimicrobial therapy in suspected neonatal sepsis is imperative for a better outcome. There is considerable geographical variation in patterns of the bacterial infections and antibiotic susceptibility profiles. In the present study the culture positivity rate was 27%, which is higher than reported in an Iranian study.[6]

Reserving broad-spectrum therapy for high-risk infants and quickly de-escalating once culture results are available is one strategy for improving neonatal outcomes. Though blood culture is time-consuming it is still the gold standard for diagnosis of sepsis. It helps in prognosis, guiding and monitoring response to therapy and for epidemiological purposes.[7]

Neonatal risk factors such as birth asphyxia, LBW and prematurity showed association with culture-proven neonatal septicaemia ($p<0.05$), which has been established by other studies as well.[8,9] In thisstudy, GNRs were the leading cause of BSIs in both EOS and LOS, which is similar to the results of other regional studies.[10,11] In contrast, CoNS in LOS, Group B *Streptococcus and E. coli* in EOS were the commonest causative agents in developed countries, as reported by various studies.[12,13]

In LOS, *K. Pneumoniae* and MR-CoNS while in EOS, *A.baumannii* followed by Serratia marcescens appeared as predominant isolates in the present as well as in an Egyptian study.[14]

Among gram-positive isolates, 40% MR-CoNS, 24% MRSA, 14% *E. fecalis* and 18% *E.faecium* have been reported in the present study, which is comparable with other studies.[15,16]

In our study, 8% *enterococci* were found to be vancomycin-resistant (VRE).This percentage is lower than that reported in various regional studies.[10,16,17] The gene that confers in VRE is transmissible to *staphylococci* and may lead to rise in vancomycin-resistant *Staphylococcus aureus* (VRSA) strains as well.

In the current study, there was a high rate of MDR and XDR microorganisms, a parallel trend also highlighted by a study.[18] Microorganisms like *A.baumannii*, S. marcescens, *Enterobactercloacae*, *C.freundiiare* intrinsically resistant to most β-lactam drugs.[5] Now, ESBL-producing *K.pneumoniae* and *E.coli* infections have become endemic inhospitals.[18,19] we observed ESBL production in 69% of *Enterobacteriaceae*

Table-3b: Antibiotic Susceptibility Pattern of Gram Positive and fungal Isolates causing Sepsis (n=74).

Antibiotic/ Antifungal	Gram positive(50)				Fungi (24)	% Resist	GPC/GNR (148) Overall % Resist
	MRCoNS(20)	*S.aures* (12) (MRSA:10)	*Entero Spp*(16)	*Strept Spp*(2)	[1]*C.albicans*(6) [1]*C.glabrata*(5) [1]*C. krusei*(2)* [1]*C.parapsilosis*(9) [1]*C.tropicalis* (2)**		
Ampicillin	20	12	13	0	NT	90%	91%
Co-amoxiclav	20	10	13	0	NT	86%	92%
Ceftrixaone	20	10	NT	0	NT	88%	94%
Cloxacillin	20	10	NT	NT	NT	94%	NT
Gentamycin	9	10	16	NT	NT	73%	69%
Clindamycin	6	2	NT	0	NT	23.5%	NT
Cotrimoxazole	11	8	NT	NT	NT	59%	46%
Ciprofloxacin	14	10	16	0	NT	80%	66%
Erythromycin	14	10	13 (NR)	0	NT	74%	NT
Levofloxacin	14	10	16	0	NT	80%	64%
Penicillin	20	12	16	0	NT	96%	NT
Teicoplanin	NT	NT	0	NT	NT	0	NT
Linezolid	0	0	0	0	NT	0	NT
Vancomycin	0	0	4	0	NT	8%	NT
1Fluconazole	NT	NT	NT	NT	10	40%	NT
Amphotericin B	NT	NT	NT	NT	0	0	NT

Strept: Streptococcus spp, Entero :Enterococcous(fecalis& faecium), S;Staphylococci, Antifungals: [1]Fluconazole: ** Intermediately susceptible, *Intrinsically resistant (NT), NT: not tested, NR: not reported

with significant rise in XDR *A.baumannii* strains as documented in a study.[19] Furthermore, it appeared as the second major cause of sepsis in the current study, but a study conducted in Bangladesh, Serratia held the same place.[20]

Increasing percentage of CRO (39%) in ICUs is another concern. Among these, *A. baumannii* (59%CRAb) had the highest proportion followed by *K.pneumoniae* (56% CRKp), a finding seen in studies from Greece and India as well.[21]

The current study revealed comparable susceptibility pattern as stated in literature.[9,22] Among them, 46% amikacin, 46%co-trimoxazole, 66% ciprofloxacin, 94% ceftriaxone, 71% piperacillin-tazobactam, 64% levofloxacin were found resistant in overall bacterial pathogens. Carbapenems, glycopeptides, polymyxins and linezolid were the best available options for gram-negative and gram-positive sepsis in our setup, but should be used cautiously to control developing resistance against them

C.spp. also emerged as one of the dominant pathogens accounting for 14% cases. There was a shift in trend from C. albicans to non-C. albicans C. spp.C. *parapsilosis* was found to be the most prevalent in our study, which is in contrast to another study.[23] It was also observed that gram-negative septicaemia and candidaemia showed progressive thrombocytopenia and raised serial CRP associated with difficulty in recovery and overstay in hospital compared to gram-positive organisms. Our findings showed agreement with a previous study.[24] It is recommended that while planning diagnostic and therapeutic options for neonatal sepsis, C. spp should also be kept in mind and efforts should be made for the isolation and identification as C. krusei and C. glabrata are usually resistant to conventional antifungal drug fluconazole. Moreover, amphotericin B, a drug of choice in neonatal sepsis, was found to be 100% susceptible.

The need of the hour is to implement strict infection control measures and rationalised use of broad-spectrum antibiotics, along with continuous surveillance to retard our journey towards the pre-antibiotic era.[25] A combination therapy will be useful to cover different organisms as well as to overcome growing resistance due to VRE and CRO. Thrombocytopenia and CRP are important tools of infectivity predictors primarily for monitoring the outcome.

Conclusion

Majority of the causative organisms were found to be highly resistant with very limited treatment options against septicaemia which is a dreadful clinical condition with potentially fatal outcome.

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