

## RESEARCH ARTICLE

# Treatment outcomes, antibiotic use and its resistance pattern among neonatal sepsis patients attending Bahawal Victoria Hospital, Pakistan

Muhammad Atif<sup>1\*</sup>, Rabia Zia<sup>1</sup>, Iram Malik<sup>1</sup>, Nafees Ahmad<sup>2</sup>, Sajjad Sarwar<sup>3</sup>

**1** Department of Pharmacy Practice, Faculty of Pharmacy, The Islamia University of Bahawalpur, Bahawalpur, Punjab, Pakistan, **2** Department of Pharmacy Practice, Faculty of Pharmacy and Health Sciences, University of Balochistan, Quetta, Balochistan, Pakistan, **3** Department of Pulmonology, Bahawal Victoria Hospital, Bahawalpur, Punjab, Pakistan

\* [pharmacist\\_atif@yahoo.com](mailto:pharmacist_atif@yahoo.com)



## Abstract

### Background

Sepsis is one of the major causes of neonatal mortality in Pakistan. This study aimed to investigate the treatment outcomes, antibiotic use and its resistance pattern among neonatal sepsis patients attending a tertiary care hospital in Pakistan. We also aimed to identify the factors affecting mortality in neonatal sepsis patients.

### Methods

A descriptive, cross-sectional study was conducted in the pediatric wards of the Bahawal Victoria Hospital, Bahawalpur, Pakistan. All eligible neonatal sepsis patients who were registered at the study site from January 1, 2019 to June 30, 2019 were included in the study. The data collection form included information on patient's characteristics, antibiotic use and its sensitivity pattern, laboratory and microbiological data, and final treatment outcomes. Treatment outcomes included, discharged (with treatment success), leave against medical advice (LAMA), discharged on request (DOR) and death. Multivariable binary logistic regression analysis was used to find the independent factors associated with death. A p-value of less than 0.05 was considered statistically significant.

### Results

Among the total 586 patients, 398 (67.9%) were male, 328 (56%) were preterm, 415 (70.8%) were diagnosed with early onset sepsis, 299 (51%) were born with low birth weight. Most of the patients (n = 484, 82.6%) were treated with amikacin+cefotaxime at the start of treatment. Culture was positive in 52 (8.9%) patients and the most commonly identified bacteria included, *Klebsiella* species (n = 19, 36.5%) followed by *E. coli* (n = 15, 28.5%) and *Staphylococcus aureus* (n = 8, 15.4%). The identified bacterial isolates showed high level of resistance against the antibiotics initiated at the start of the treatment, while resistance

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against piperacillin+tazobactam, imipenem, vancomycin and linezolid was very low. Just under half of the patients (n = 280, 47.8%) successfully completed the treatment (i.e., discharged with treatment success), while 123 (21%) patients died during treatment. In multi-variable binary logistic regression, the factors which still remained significantly associated with neonatal death included, preterm delivery (AOR 9.59; 95% CI 4.41, 20.84), sub-optimal birth weight (AOR 5.13; 95% CI 2.19, 12.04), early onset sepsis (AOR 2.99; 95% CI 1.39, 6.41) and length of hospital stay (AOR 0.76; 95% CI 0.67, 0.88).

## Conclusion

The mortality rate associated with sepsis was high in our study cohort. The bacterial isolates showed high level of resistance against the antibiotics started as the empiric therapy. Rational use of antibiotics can decrease the adverse outcomes in neonatal sepsis patients.

## Introduction

Neonatal sepsis is defined as a systemic infection which occurs before 28 days of newborn's life [1]. It is manifested by hemodynamic, post-inflammatory and immunosuppressive changes that can lead to substantial mortality and morbidity [2]. The clinical signs and symptoms of neonatal sepsis include, hypothermia or fever, respiratory problems such as apnea and cyanosis, difficulty in feeding, abdominal distension, diarrhea, vomiting, oliguria, lethargy and irritability [2]. On the basis of onset of symptoms, neonatal sepsis could be either early-onset sepsis (EOS) or late-onset sepsis (LOS). The EOS manifests itself within first 72 hours and is vertically transmitted [3]. Whereas, the LOS manifests itself after 72 hours of child birth and is mainly acquired horizontally from the environment [4].

Neonatal sepsis is the leading cause of mortality worldwide, but compared with high income countries, its prevalence and mortality rates are high in low and middle-income countries (LMICs) probably due to poor hygiene and suboptimal practices for infection control [5]. The neonatal mortality rate (per 1000 live births) in Pakistan has declined from 55 in 2013 to 42 in 2018 [6, 7], however, it is much higher compared with the developed countries like the United States (US), the United Kingdom (UK) and Canada where it is less than 5 per 1000 live births. The neonatal mortality rate in Pakistan is even much higher than its neighboring countries, for example, India (23 per 1000 live births), Iran (9 per 1000 live births) and China (4 per 1000 live births) [8]. Compared with the global scenario, Pakistan appears to be lagging behind in achieving the target set (12 or fewer neonatal deaths by 2030) by the Every Newborn Action Plan (ENAP) [9]. Among the known causes of neonatal mortality in Pakistan, sepsis accounted for 17.2% of the total deaths (2015 data) [10].

The type of pathogens causing neonatal sepsis differ among countries, however, literature from developing countries suggest that although gram-negative organisms predominate, but some of the gram-positive bacteria such as *Staphylococcus aureus* and *coagulase negative staphylococcus* (CoNS) may also cause neonatal sepsis [11–14]. The World Health Organization (WHO) has recommended to use injectable gentamicin and ampicillin as a first-line therapy for the hospitalized neonatal sepsis patient, and according to a recent review, there are no evidences which suggest to change this therapy [15]. However, due to emergence of antimicrobial resistance and sepsis associated complications, use of many other antibiotics (either alone or in combination) such as cephalosporins, imipenem, vancomycin, piperacillin/tazobactam

(PT), amikacin, metronidazole and clindamycin has been recommended in many current international guidelines [15].

Antibiotic resistance is a growing problem worldwide and poses a threat to survival rates from serious infections [16], including neonatal sepsis. Globally, about 214,000 neonates die each year owing to sepsis caused by bacteria resistant to first-line antibiotics [17]. Though there is no availability of data on this aspect from Pakistan, but seriousness of the issue can be gauged by prevailing inappropriate use of antibiotics among pediatrics, availability of inappropriate antibiotic pack sizes for pediatrics and poor adherence to treatment guidelines [18, 19]. Most importantly, there is a variation in the pathogen profile and their susceptibility pattern against antimicrobials among various countries, and it changes over time [14]. According to an estimate, a total of 25,692 neonates in Pakistan succumb to resistant sepsis infections each year [17]. In this regard, it is important to know the bacterial isolates and their resistance pattern against commonly used antibiotics in neonatal sepsis not only at a country level but also at all levels of healthcare [3]. Based on the fact that neonatal mortality rate is very high in Pakistan [20] and sepsis is one of the major causes of neonatal mortality [6, 10], such evidence based data will help the clinicians to initiate an empiric therapy which can decrease the probability of adverse outcomes among the patients. Despite the gravity of matter, only a few studies were conducted in Pakistan among neonatal sepsis patients [11, 21, 22], however, antibiotic use pattern and final treatment outcomes have not been discussed in detail. Moreover, patient's attributes associated with the final treatment outcome are still under explored in Pakistan. Therefore, the aim of this study was to investigate the treatment outcomes, antibiotic use and its resistance pattern among neonatal sepsis patients in Pakistan. We also identified the independent factors associated neonatal mortality in our study cohort.

## Methodology

### Study setting

This study was conducted in the pediatric wards of the Bahawal Victoria Hospital (BVH), Bahawalpur, Punjab, Pakistan. The BVH has a bed capacity of 1600, and it serves large population living in the southern part of the province of Punjab [23]. The hospital has more than 20 departments including, medicine, surgery, pediatrics, radiology, pulmonology, obstetrics and gynecology, pathology, dermatology, cardiology, neurology, nephrology, physiotherapy, endocrinology, and allied medicine. The BVH has two pediatric wards named as Pediatric Ward 1 and Pediatric Ward 2 with the respective bed capacity of 94 and 82, respectively.

At the study setting, neonatal sepsis is diagnosed on the basis of clinical signs and symptoms (i.e., temperature instability, feeding problems, convulsions, lethargy and respiratory distress) [13], laboratory findings such as C-Reactive Protein (CRP), complete blood count (CBC) (clinical sepsis) and/or blood/urine cultures [12, 24]. Given the extremely high burden on microbiological laboratory at the BVH, all presumptive cases of neonatal sepsis are not necessarily diagnosed through positive cultures. At the time of admission in the pediatrics wards, neonatal sepsis patients (diagnosed clinically) are put on empiric parenteral antibiotics. However, the doctors recommend to do cultures in patients who do not show clinical improvement. On an average, cultures are ordered after two days of initiating empiric therapy.

### Study design and study population

This was a descriptive, cross-sectional study. All neonates admitted in the Pediatric Ward 1 and Pediatric Ward 2 from January 1, 2019 to June 30, 2019, and diagnosed with EOS and LOS were included in the study. Neonates with extremely low birth weight (i.e., <1000 g) and those suffering from pneumonia, meningitis and congenital abnormalities were excluded from

the study [13, 25, 26]. Similarly, those with incomplete medical records were also excluded from the study. Using a prevalence of 37.5% (reported in previous study from the same study setting [27]), a target sample size of 358 was calculated using simple population formula.

### Microbiological investigations

The BVH follows standard microbiological techniques. The skin is disinfected using standard methods before collecting the venous blood samples. Afterwards, 1 to 3 ml blood sample is drawn from the neonate under aseptic condition and transferred to the BACTEC PedsPlus™ (Becton Dickinson, Ireland) culture vial. The vial is then incubated at 37 °C for up to seven days. The culture vial is examined on daily basis for bacterial growth and turbidity [13]. The pure bacterial isolates are obtained using subculture technique and later the organisms are identified using methods described by Yadav et al. [28]. Antibiotic susceptibility testing is performed using disc diffusion method (Kirby-bauer method) as per Clinical and Laboratory Standards Institute (CLSI) guidelines [29].

### Outcomes variables

Treatment outcomes, antibiotic usage, and antibiotic resistance and sensitivity pattern were taken as the outcome variables.

Treatment outcomes were categorized into discharge (with treatment success), leave against medical advice (LAMA), discharge on request (DOR) and death.

- *Discharge*: Neonatal sepsis patient who was discharged from the ward after successfully completing the treatment.
- *Leave against medical advice*: Neonatal sepsis patient who left the ward without completing the treatment due to assorted reasons, such as financial issue or not satisfied with the treatment or hospital environment.
- *Discharge on request*: Neonatal sepsis patient who left the ward on request but without completing the treatment.
- *Death*: Patient died while on sepsis treatment.

### Data collection

A data collection form was developed after literature review [13, 30, 31]. The data collection form consisted of data on patient's gender, residence, gestational age at birth, birth weight (very low birth weight, VLBW, < 1500 g; low birth weight, LBW, < 2500 g; normal birth weight, NBW, ≥ 2500 g), mode of child birth, diagnosis (EOS/LOS), treatment strategy, laboratory parameters, blood and urine culture, bacterial isolates, antimicrobial sensitivity and resistance pattern, and treatment outcomes [32]. The data were obtained from the medical records of the neonates and microbiology laboratory culture registers. Prior to the start of data collection, data collectors were guided and trained by the supervisor (MA) on how to collect data from the records.

### Data analysis

Statistical Package for Social Sciences (IBM Corp. released 2012. IBM SPSS statistics for windows, version 20.0, Armonk, NY: IBM Corp.) was used to analyze the data. The findings of the study were presented as counts (n) and proportions (%) for the categorical variables, and mean and standard deviation (SD) for the continuous variables. Chi-square test was used to

test the statistically significant difference among the categorical variables. Due to high death rate among the study population, logistic regression analysis was used to identify the independent factors associated with it. The variables which were statistically significant in simple logistic regression analysis were entered into multivariable logistic regression analysis to identify the final predictors. A p-value of less than 0.05 was considered statistically significant.

### Ethical considerations

Pharmacy Research Ethics Committee (PREC) at the Islamia University of Bahawalpur (Reference: 74/S-2019-/PREC) approved the design and conduct of the study. At the study setting, the medical record of a patient is anonymized in a way that it only contains name of the patient and the hospital registration number without any further information. Therefore, the identity of the patients could not be disclosed to the data collectors or the principal investigator. Nevertheless, the PREC waived the requirement for informed consent.

### Results

During the study period, a total of 722 neonatal sepsis patients were enrolled at the study site. Out of these, 136 (18.83%) were excluded from the study because either their medical records were incomplete/missing or they were extremely low birth weight neonates. As a result, a total of 586 patients were included in the final analysis. Most of the patients were male ( $n = 398$ , 67.9%), while more than 75% of the patients were residents of rural areas ( $n = 454$ , 77.5%). Out of the total 586 patients, 328 (56%) were preterm, while 299 (51%) were born with low birth weight. A total of 415 (70.8%) neonates were diagnosed with EOS. C-section was relatively dominant mode of child delivery ( $n = 326$ , 55.6%). Only 52 (8.9%) neonates were culture positive, while a large number of population ( $n = 414$ , 70.6%) were culture negative (Table 1).

### Antibiotics initially started among the neonatal sepsis patients

At the start of treatment, most of the patients ( $n = 484$ , 82.6%) were treated with amikacin+cefotaxime, while 102 (17.4%) patients were given amikacin+ceftriaxone as a start treatment.

### Bacterial isolates identified among the neonatal sepsis patients

A total of 52 specimens were culture positive. Among them, *Klebsiella* species were dominant ( $n = 19$ , 36.5%) followed by *E. coli* ( $n = 15$ , 28.8%). *Staphylococcus aureus* was identified in eight (15.4%) specimens (Table 2).

### Antibiotic sensitivity and resistance pattern against bacterial isolates

Out of the total 52 positive cultures, cefotaxime was tested in 35 isolates (34 gram-negative, one gram-positive). Out of these, 27 (77.1%) gram-negative and one (2.9%) gram positive isolates were resistant to this antibiotic. Similarly, ceftriaxone was tested in 39 isolates and it showed almost similar resistance pattern to cefotaxime. Amikacin was tested in 41 isolate (40 gram-negative and one gram-positive) and it was resistant in 15 (36.6%) gram negative and one (0.2%) gram positive isolates. Imipenem, piperacillin+tazobactam, vancomycin and linezolid showed better sensitivity against the bacterial isolated (Table 3). For details on number of resistant drugs against each of the identified bacteria, please refer to S1 File.

### Percentage resistance of bacterial isolates against antibiotics

Among the most common gram negative bacterial isolates (i.e., *Klebsiella* species and *E. coli*) identified in this study, highest level of resistance was seen against all tested cephalosporins,

**Table 1. Sociodemographic and clinical characteristics of the patients (N = 586).**

Variable	n (%)
<b>Gender</b>	
Male	398 (67.9)
Female	188 (32.1)
<b>Gestational age at birth</b>	
Preterm (< 37 weeks of gestation)	328 (56)
Term	258 (44)
<b>Birth weight</b>	
Very low birth weight	131 (22.4)
Low birth weight	299 (51)
Normal birth weight	156 (26)
<b>Diagnosis</b>	
Early onset sepsis	415 (70.8)
Late onset sepsis	171 (29.2)
<b>Mode of child birth</b>	
Spontaneous vaginal delivery	260 (44.4)
Cesarean section	326 (55.6)
<b>Residence</b>	
Rural	454 (77.5)
Urban	132 (22.5)
<b>Length of hospital stay</b>	
≤6 days	262 (44.7)
≥6 days	324 (55.3)
<b>Culture status</b>	
Culture positive	52 (8.9)
Culture negative	414 (70.6)
Specimen not sent for culture	120 (20.5)
<b>Specimen type for culture</b>	
Blood	338 (57.7)
Urine	128 (21.8)

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including those started as an empiric therapy (i.e., cefotaxime, ceftriaxone). 11 (66.1%) *Klebsiella* isolates were resistant to amikacin, but only one (7.7%; out of 12) *E. coli* isolates were resistant to amikacin. Most common gram negative isolates showed low level of resistance against piperacillin+tazobactam and imipenem (Table 4).

**Table 2. Type of bacteria identified in the culture positive patients.**

Bacterial isolates	Specimen type		Total n (%)
	Blood n	Urine n	
<b>Gram-negative</b>			
<i>Klebsiella species</i>	15	4	19 (36.5)
<i>E.coli</i>	4	11	15 (28.8)
<i>Citrobacter species</i>	5	-	5 (9.6)
<i>Pseudomonas aeruginosa</i>	3	-	3 (5.8)
<i>Enterobacter species</i>	1	-	1 (1.9)
<i>Serratia species</i>	1	-	1 (1.9)
<b>Gram-positive</b>			
<i>Staphylococcus aureus</i>	7	1	8 (15.4)
<b>Total</b>			<b>52 (100)</b>

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**Table 3. Overall status of antibiotic resistance and sensitivity against bacterial isolates.**

Antibiotic tested	Gram negative		Gram positive	
	R (%)	S (%)	R (%)	S (%)
Amoxicillin + Clavulanic acid (n = 41)	26 (63.4)	12 (29.3)	2 (4.9)	1 (2.4)
Piperacillin+ Tazobactam (n = 46)	1 (1.2)	38 (82.6)	0 (0)	7 (15.2)
Imipenem (n = 38)	0 (0)	37 (97.8)	0 (0)	1 (2.6)
Cefoperazone + sulbactam (n = 41)	7 (17)	34 (82.9)	0 (0)	0 (0)
Cefepime (n = 36)	21 (58)	15 (41.7)	0 (0)	0 (0)
Cefixime (n = 27)	24 (88.9)	3 (11.1)	0 (0)	0 (0)
Ceftazidime (n = 29)	18 (62)	10 (34.5)	0 (0)	1 (3.4)
Cefotaxime (n = 35)	27 (77.1)	7 (20)	0 (0)	1 (2.9)
Ceftriaxone (n = 39)	30 (76.9)	8 (20.5)	0 (0)	1 (2.5)
Ciprofloxacin (n = 40)	19 (47.5)	20 (50)	0 (0)	1 (2.5)
Levofloxacin (n = 41)	12 (30.8)	28 (71.8)	0 (0)	1 (2.5)
Amikacin (n = 41)	15 (36.6)	25 (61)	0 (0)	1 (0.2)
Vancomycin (n = 9)	0 (0)	2 (22.2)	0 (0)	7 (77.8)
Linezolid (n = 8)	0 (0)	1(12.5)	0 (0)	7 (87.5)
Clarithromycin (n = 8)	1 (1.2)	0 (0)	1(1.2)	6 (75)
Oxacillin (n = 4)	1 (25)	0 (0)	0 (0)	3 (75)
Metronidazole (n = 6)	1 (16.7)	0 (0)	4 (66.7)	1 (16.7)
Meropenem (n = 1)	0 (0)	0 (0)	0 (0)	1 (100)

Note: Calculations were based on number of samples with positive bacterial growth and in which specific antibiotic was tested for sensitivity and resistance against antibiotic

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**Table 4. Percentage resistance of bacterial isolates against antibiotics.**

Antibiotic	<i>Klebsiella species</i>		<i>E.coli</i>		<i>Citrobacter species</i>		<i>Pseudomonas aeruginosa</i>		<i>Enterobacter species</i>		<i>Serratia species</i>		<i>Staphylococcus aureus</i>	
	R/(R+S)	R%	R/(R+S)	R%	R/(R+S)	R%	R/(R+S)	R%	R/(R+S)	R%	R/(R+S)	R%	R/(R+S)	R%
Amoxicillin + Clavulanic Acid	12/ (12+3)	80	10 / (10+5)	66.7	3 / (3+1)	75	0 / (0+2)	0	1 / (1+0)	100	0 / (0+1)	0	2 / (2+1)	66.7
Piperacillin+ Tazobactam	1 / (1+15)	6.25	0 / (0+13)	0	0 / (0+5)	0	0 / (0+3)	0	0 / (0+1)	0	0 / (0+1)	0	0 / (0+7)	0
Imipenem	0 / (0+15)	0	0 / (0+13)	0	0 / (0+4)	0	0 / (0+3)	0	0 / (0+1)	0	0 / (0+1)	0	0 / (0+1)	0
Cefoperazone + Sulbactam	5 / (5+13)	27.8	1 / (1+12)	7.7	1 / (1+4)	20	0 / (0+3)	0	0 / (0+1)	0	0 / (0+1)	0	0 / (0+0)	-
Cefepime	9 / (9+5)	64.3	11 / (11+3)	78.6	1 / (1+3)	25	0 / (0+2)	0	0 / (0+1)	0	0 / (0+1)	0	0 / (0+0)	-
Cefixime	9 / (9+0)	100	10 / (10+1)	90.9	2 / (2+0)	100	2 / (2+1)	66.7	1 / (1+0)	100	0 / (0+1)	0	0 / (0+0)	-
Ceftazidime	8 / (8+3)	72.7	9 / (9+2)	81.8	1 / (1+1)	50	0 / (0+2)	0	0 / (0+1)	0	0 / (0+1)	0	0 / (0+1)	0
Cefotaxime	15 / (15+2)	88.2	12/ (12+1)	92.3	0 / (0+3)	0	0 / (0+1)	0	0 / (0+0)	-	0 / (0+0)	-	0 / (0+1)	0
Ceftriaxone	16 / (16+2)	88.9	12 / (12+2)	85.7	2 / (2+3)	40	0 / (0+0)	-	0 / (0+0)	-	0 / (0+1)	0	0 / (0+1)	0
Ciprofloxacin	8 / (8+10)	44.4	10 / (10+3)	76.9	0 / (0+4)	0	1 / (1+2)	33.3	0 / (0+1)	0	0 / (0+0)	-	0 / (0+1)	0
Levofloxacin	5 / (5+14)	26.3	6 / (6+6)	50	0 / (0+4)	0	1 / (1+2)	33.3	0 / (0+1)	0	0 / (0+1)	0	0 / (0+1)	0
Amikacin	11 / (11+7)	66.1	1 / (1+12)	7.7	2 / (2+2)	50	1 / (1+2)	33.3	0 / (0+1)	0	0 / (0+1)	0	0 / (0+1)	0
Vancomycin	0 / (0+0)	-	0 / (0+2)	-	0 / (0+0)	-	0 / (0+0)	-	0 / (0+0)	-	0 / (0+0)	-	0 / (0+7)	0
Linezolid	0 / (0+0)	-	0 / (0+1)	-	0 / (0+0)	-	0 / (0+0)	-	0 / (0+0)	-	0 / (0+0)	-	0 / (0+7)	0
Clarithromycin	1 / (1+0)	100	0 / (0+0)	-	0 / (0+0)	-	0 / (0+0)	-	0 / (0+0)	-	0 / (0+0)	-	1 / (1+6)	14.3
Oxacillin	0 / (0+0)	-	1 / (1+0)	100	0 / (0+0)	-	0 / (0+0)	-	0 / (0+0)	-	0 / (0+0)	-	0 / (0+3)	0
Metronidazole	0 / (0+0)	-	1 / (1+0)	100	0 / (0+0)	-	0 / (0+0)	-	0 / (0+0)	-	0 / (0+0)	-	4 / (4+1)	80
Meropenem	0 / (0+0)	-	0 / (0+0)	-	0 / (0+0)	-	0 / (0+0)	-	0 / (0+0)	-	0 / (0+0)	-	0 / (0+1)	0

Out of the 52 specimen which showed bacterial growth, Klebsiella = 19, E.coli = 15, pseudomonas = 3, Enterobacter = 5, Citrobacter = 5, Serratia = 1, Staphylococcus aureus = 8; R = number of resistant isolates; R% = percentage of resistant isolates; S = number of sensitive isolate

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**Table 5. Modification in the antibiotic treatment based on physician's judgment, and culture and sensitivity results.**

Modified treatment	Modified after c/s n (%)	Modified after negative culture n (%)	c/s not done but treatment modified n (%)	Total n (%)
Amikacin+ceftriaxone	3 (4.2)	60 (84.5)	8 (11.3)	71 (40.6)
Vancomycin+ceftriaxone	6 (20.7)	22 (75.9)	1 (3.4)	29 (16.6)
Vancomycin+ meropenem	6 (66.7)	3 (33.3)	0 (0)	9 (5.1)
Cefoperazone+sulbactam+Ampicillin	0 (0)	25 (86.2)	4 (13.8)	29 (16.6)
Cefoperazone+sulbactam+B.penicillin	0 (0)	8 (100)	0 (0)	8 (4.6)
Imipenem+amikacin	5 (100)	0 (0)	0 (0)	5 (2.8)
Imipenem+cefotaxime	2 (100)	0 (0)	0 (0)	5 (1.1)
Imipenem+ceftriaxone	5 (100)	0 (0)	0 (0)	2 (2.8)
Cefoperazone+sulbactam+Amikacin	0 (0)	15 (88.2)	2 (11.8)	17 (9.7)
<b>Total</b>	<b>27 (15.4)</b>	<b>133 (76)</b>	<b>15 (8.6)</b>	<b>175 (100)</b>

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### Modification in antibiotic treatment among the neonatal sepsis patients

Among the total 586 patients, antibiotic treatment was modified in 175 (29.9%) patients either based on physician's judgment and/or culture and sensitivity results. Out of 175 patients in whom therapy was modified, amikacin+cefotaxime was prescribed as a start treatment in 168 patients (96%), while remaining seven (4%) patients were on amikacin+ceftriaxone. With regard to modifications, amikacin+ceftriaxone was started as a modified treatment in 71 (40.6%) cases, while cefoperazone+sulbactam+ampicillin and vancomycin+ceftriaxone combinations were started as modified treatment in 29 (16.6%) cases each. Interestingly, only 27 (15.4%) treatment modifications were based on culture and sensitivity results, while the remaining 148 (84.6%) modifications were made on physician's judgment ([Table 5](#)).

### Treatment outcomes among the neonatal sepsis patients

With regard to treatment outcomes among the total 586 patients, 280 (47.8%) were discharged (with treatment success), 122 (20.8%) LAMA, 61 (10.4%) were DOR and 123 (21%) died during treatment ([Table 6](#)). A detailed description of treatment outcomes with regard to patients' characteristics is provided in [S2 File](#).

### Factors associated with death among neonatal sepsis patients

In simple logistic regression analysis, the factors which were significantly associated with neonatal death included; preterm delivery (OR 16.87; 95% CI 8.05, 35.35); sub-optimal birth weight (OR 7.86; 95% CI 3.58, 17.28), EOS (OR 6.81; 95% CI 3.37, 13.80), cesarean delivery (OR 2.06; 95% CI 1.35, 3.14) and length of hospital stay (OR 0.79; 95% CI 0.70, 0.89). In multi-variable binary logistic regression, the factors which still remained significantly associated with neonatal death included; preterm delivery (AOR 9.59; 95% CI 4.41, 20.84); sub-optimal birth weight (AOR 5.13; 95% CI 2.19, 12.04), EOS (AOR 2.99; 95% CI 1.39, 6.41) and length of hospital stay (AOR 0.76; 95% CI 0.67, 0.88) ([Table 7](#)).

**Table 6. Treatment outcomes among the patients.**

Outcome	n (%)
Discharged (with treatment success)	280 (47.8)
Leave against medical advice	122 (20.8)
Discharge on request	61 (10.4)
Death	123 (21)

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**Table 7. Factors associated with death among neonatal sepsis patients: Multivariable binary logistic regression analysis.**

Independent variable	B	S.E	p-value	AOR (95% CI)
Preterm delivery	2.26	.396	<0.0005	9.59 (4.41, 20.84)
Sub-optimal birth weight	1.63	.435	<0.0005	5.13 (2.19, 12.04)
Early onset sepsis	1.09	.389	.005	2.99 (1.39, 6.41)
Cesarean delivery	0.10	.252	.688	1.10 (0.68, 1.81)
Length of hospital stay*	-0.271	.070	<0.0005	0.76 (0.67, 0.88)

Note: Sub-optimal birth weight includes both very low birth weight and low birth weight

\*entered as continuous variable; Hosmer and Lemeshow test (8.300),  $p = 0.405$ ; Nagelkerke R Square (0.357); Model summary = Chi square (152.79),  $df (5)$ ,  $p < 0.0005$

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Fig 1 presents the differences in the important study parameters with regard to diagnosis (i.e., EOS and LOS).

## Discussion

The lack of recognition of neonatal sepsis as a major public health concern is primarily attributable to paucity of detailed data, especially in developing nations [33]. To our knowledge, this is the first study that simultaneously assessed treatment outcomes, antibiotic use and its resistance pattern among neonatal sepsis patients in a tertiary care hospital of Pakistan. In most of the patients, diagnosis of sepsis was made on clinical findings rather than microbiological. We found that just over 47% ( $n = 280$ ) of the patients were discharged from hospital after clinical improvements (i.e., discharged with treatment success). However, more than 30% ( $n = 183$ ) of the patients left the pediatric wards either against the medical advice or on request. More than 20% ( $n = 123$ ) of the neonatal sepsis patients died during the treatment which is alarming. *Klebsiella* species and *E. Coli* were the most commonly identified gram negative bacterial isolates and these showed high level of resistance against parenteral antibiotics started at the time of treatment. We also noted that most of the modifications in the treatment were based on physician's judgement, but not on microbiological reports. This study also identified the independent factors associated with death among the neonatal sepsis patients.

In the current study, most of the neonatal patients (67.9%) were male and this is in agreement with the previous study conducted in Pakistan [34]. Greater susceptibility of male to suffer from infections might be due presence of single x (x) chromosome. The x chromosome has several genes that regulate the production of immunoglobulins. Females have double x (xx) chromosomes which lead to more production of immunoglobulins making them less vulnerable to infections [35]. Our study findings also showed that a greater percentage (77.5%) of the patients were from rural areas. Similarly, most of the neonatal sepsis patients were those who had less than optimal (VLBW and LBW) birth weight, and were suffering from EOS. These attributes are related to patient's mother in a way that mothers living in rural areas of Pakistan might have poor socioeconomic status [20], unhealthy living environment [36], lack of health-care facilities leading to unhygienic obstetric practices (intrapartum transmission from mothers) and poor nursery practices thus predisposing the newborns at the risk of infections [37]. Earlier studies have shown that preterm babies and those with less than optimal birth weight have underdeveloped immune system, which makes newborns susceptible to infections [13, 38, 39].

The results of our study revealed that diagnosis of the presumptive sepsis patients were made on the basis of physician's judgment and laboratory investigations (CBC and CRP). For 120 patients, the physicians did not send the sample for bacteriological profile, and for the remaining 466 patients in whom bacteriology was requested, only 52 (11.7%; out of 446) were

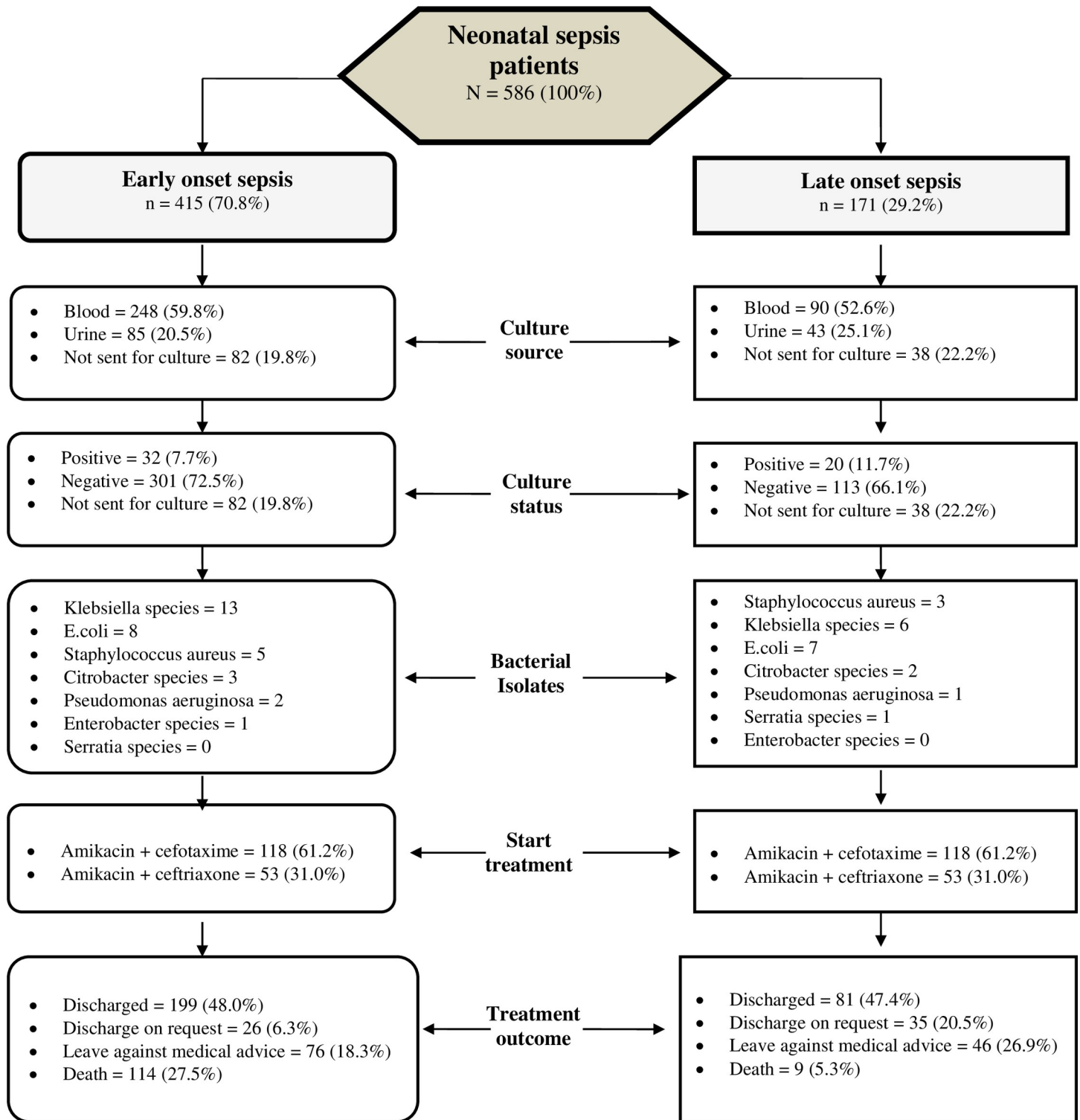


Fig 1. Differences in important study parameters between neonates with early and late onset sepsis.

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culture positive. Studies from other developing countries also reported low culture positivity rates among neonatal sepsis patients, for example, Bangladesh (9%), India (36%) and Egypt (43%) [12, 40, 41]. Low sensitivity of cultures might be due to wrong sampling collection and processing procedures, improper sample transportation, use of antibiotics prior to sample collection, slow growing bacteria, and viral, fungal or parasitic infections [12].

*Klebsiella species* and *E. coli* were the most common gram negative and *Staphylococcus aureus* was the only gram positive bacterial isolate identified in this study. This finding is comparable to what was observed in a previous Pakistani study [11]. Studies from other developing countries like Nepal, Egypt, Ghana reported that the most common bacterial isolates among neonatal sepsis patients were *Klebsiella species*, *Pseudomonas aeruginosa*, *E. coli*, *Enterobacter species*, CoNS and *Staphylococcus aureus* [12–14, 28]. In order to treat sepsis, the physicians in our study prescribed amikacin+cefotaxime combination in most of the patients (82.6%) followed by amikacin+ceftriaxone combination (17.4%). Alarming, in this study, most of the commonly identified bacterial isolates showed high level of resistance against cephalosporins. Similarly, over 60% of *Klebsiella* isolates showed resistance against amikacin. A systematic review showed that *Klebsiella species* were highly resistant to cephalosporins (84%) in Asian countries [42]. Two other systematic reviews and meta-analysis also concluded that most common bacterial isolates responsible for causing neonatal sepsis in LMICs were resistant to or had reduced susceptibility to the WHO recommended ampicillin and gentamicin combination therapy and to third generation cephalosporins [15, 43].

It is important to note that despite being aware of the most common bacterial isolates and their resistance against the standard antibiotic therapy at the BVH, the physicians in the current study neither tailored the therapy according to need of the patient nor according to the WHO recommendations. In the current scenario, the WHO recommended ampicillin+gentamicin (first-line) and third generation cephalosporins (second-line) may not be the viable options because the most common bacterial isolates in the present study were resistant to penicillins and cephalosporins (Tables 3 and 4). However, piperacillin+tazobactam, imipenem, vancomycin and linezolid showed better sensitivity against these bacterial isolates. Studies from other developing countries like India, Nepal, Egypt showed that these aforementioned antibiotics showed promising results in their healthcare settings in terms of antibacterial activity against most common bacterial isolates involved in neonatal sepsis [44–47]. Selection of empiric antibacterial therapy according to antibiotic resistance pattern in locally prevalent bacterial isolates is consistent with the recommendations of most of the neonatal sepsis treatment guidelines [15]. A study from Israel provided the evidence that the piperacillin+tazobactam combination plus amikacin were safe to use in neonatal sepsis patients and effectively/successfully eradicated (>90%) gram-negative and gram-positive organisms [48]. Similarly, a study from the US also advocated the superiority of piperacillin+tazobactam over ampicillin+gentamicin in terms of effectiveness and safety profile among neonatal sepsis patients [49]. Many guidelines, for example, Surviving Sepsis Campaign, British National Formulary (children), BMJ Clinical Evidence and American Academy of Pediatrics) have suggested the judicious use (benefits outweigh harmful effects) of piperacillin+tazobactam, imipenem, vancomycin and linezolid [15]. Based on antibacterial spectrum of these antibiotics, piperacillin+tazobactam or imipenem seems to be the better options for gram-negative and gram-positive bacteria [50] and vancomycin or linezolid for gram-positive bacteria [51]. However, considering the probability of adverse consequences arising from the long-term use of broad spectrum antibiotic [13], mandatory culture and susceptibility investigations are crucial in order to timely switch to narrow-spectrum antibiotics.

With regard to treatment outcomes, nearly 50% of our patients were discharged from the wards after successful completion of treatment. In contrast, an Ethiopian study showed that

more than 80% of the patients were discharged after successfully completing the treatment [30]. Importantly, 23% of neonatal sepsis patients left the BVH against the medical advice. Previous studies from Pakistan and India also stated that 25% of neonatal patients left the pediatric wards against the medical advice [52, 53]. Some of the reasons which may provoke the patients to LAMA include, financial problems, not satisfied with the medical care, preferences for other hospitals and communication gap between the health care providers and parents. Alarming, 21% of the neonatal sepsis patients enrolled in this study died during the treatment, which demand better treatment compliance through regular monitoring of CRP levels, review of culture and frequent consideration of other physiological parameters to timely start interventions [54, 55]. Nevertheless, the mortality rate reported in our study is almost similar to the mortality rate reported in Egypt (22%) and Iran (19%) [12, 56]. Similarly, data retrieved from multiple studies conducted in various developing countries presented a wide range of neonatal mortality rate related to infection (8–80%) [57]. In developing nations, including our setting [27, 58, 59], high neonatal mortality rates are widely known to be attributed to poor quality management of seriously ill neonates due to absence of neonatal specialists and neonatal intensive care units, delays in newborn resuscitation, unavailability of warming devices, and non-utilization of antenatal care.

Careful selection of antibiotics in neonatal sepsis patients can greatly reduce the risk of death [60]. For example, Table 5 shows that the physicians modified antibiotic treatment in 175 (29.9%) patients either based on their clinical judgement and/or culture and sensitivity results. Further analysis of the data revealed that none of the patients died in whom imipenem (in combination) was started as a modified treatment. Moreover, one patient died among those in whom vancomycin (in combination) was started as a modified treatment. Similarly, cefoperazone+sulbactam (in combination with other antibiotics) also showed promising results (data not shown in results, for details please refer to S3 and S4 Files). These findings clearly signify the importance of microbiological data in tailoring the antibiotic regimen for neonatal sepsis patients and ultimately curbing sepsis related mortality at our study setting. Beside the appropriate selection of antibiotics, there are some other factors which may predict mortality among neonatal sepsis patients. For example, in our study, preterm neonates (9.59 times), those having suboptimal birth weight (5.13 times) and suffering from EOS (2.99 times) had higher chances of dying compared to term neonates, those having optimal birth weight and had LOS, respectively. As discussed earlier, neonates with these particularities have weakened immune system thus making them more prone to death due to sepsis [4, 61]. This study also demonstrated that neonatal sepsis patients with longer hospital stay had lesser chances of death than those with shorter hospital stay. One possible reason for this finding could be that the severely ill patients died earlier than those who had mild to moderate infection.

Our study has a few limitations. First, this study was conducted at only one tertiary care hospital of the Punjab province of Pakistan. Therefore, the findings should be generalized with care for whole of Pakistan as type of pathogens and antibiotic use pattern may vary across the country. Second, due to retrospective nature of the study, we were unable to capture some of the important variables (not available in patient files) (for example, maternal variables, prior use of antibiotics by the neonates, etc.) which could have affected the treatment outcomes. Third, small sample and low culture positivity rates are few other limitations of the study. Hence, multicenter studies with larger sample size are required to validate our findings. Moreover, future studies should also report treatment outcomes, and antibiotic use and susceptibility pattern among neonatal sepsis patients with meningitis, pneumonia etc.

## Conclusion

Just under half of the total neonatal sepsis patients successfully completed the treatment (i.e., discharged with treatment success), whereby the mortality rate was high in the study cohort. *Klebsiella species*, *E. coli* and *Staphylococcus aureus* were the most common bacterial isolates identified in the study. Most of the bacterial isolates showed high level of resistance against empiric treatment. Preterm, less than optimal birth weight and EOS babies were more likely to die from sepsis. Given the antibacterial spectrum of the tested antibiotics, the combination of piperacillin+tazobactam with amikacin could be recommended as an empiric therapy for neonatal sepsis patients at our study setting. Imipenem in combination with other antibiotics may also be considered as a treatment option.

## Supporting information

**S1 File. Number of resistant drugs with regard to identified bacteria.**  
(DOCX)

**S2 File. Treatment outcomes with regard to the patient characteristics.**  
(DOCX)

**S3 File. Percentage of death with regard to modified treatment.**  
(DOCX)

**S4 File. Number of deaths with regard to culture status.**  
(DOCX)

**S1 Data.**  
(SAV)

## Author Contributions

**Conceptualization:** Muhammad Atif, Rabia Zia, Iram Malik.

**Data curation:** Rabia Zia.

**Formal analysis:** Muhammad Atif, Rabia Zia, Iram Malik, Nafees Ahmad, Sajjad Sarwar.

**Methodology:** Muhammad Atif, Rabia Zia, Iram Malik, Nafees Ahmad, Sajjad Sarwar.

**Supervision:** Muhammad Atif.

**Validation:** Muhammad Atif, Rabia Zia, Iram Malik, Nafees Ahmad, Sajjad Sarwar.

**Writing – original draft:** Muhammad Atif, Rabia Zia, Sajjad Sarwar.

**Writing – review & editing:** Muhammad Atif, Iram Malik, Nafees Ahmad.

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