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ORIGINAL ARTICLE

Frequency and Antibiotic Resistance Profile of *Escherichia coli* in Neonatal Sepsis

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ABSTRACT

Background: In developing countries, the most common cause of neonatal mortality is neonatal sepsis. Currently, the most significant and common hospital acquired pathogen is *Escherichia coli*, associated with many problems such as septicemia, pneumonia and meningitis in the neonates. Due to emerging antibiotic resistance in microorganisms, the current antibiotics which are extensively used are insufficient to resolve the issues.

Objectives: The aim of the study was to find out the frequency and antibiogram assay of *Escherichia coli* in neonatal sepsis.

Methodology: The research study was cross sectional study directed by the Department of Neonatology, Bahawal Victoria Hospital, Bahawalpur. The study duration for the current study was from September 2019 to January 2021. Blood samples were collected from all the suspected neonates for isolation and identification of *E.coli*, and standard microbiological protocols were used for the identification. Further a modified Kirby Bauer method of disk diffusion was used for antibiotic sensitivity testing.

Results: During the defined study duration, total 150 blood cultures were found positive for neonatal sepsis. Prevalence of *E.coli* in these 150 positive blood cultures was 70% (n = 105). The most effective antibiotics observed in our study were gatifloxacin, imipenam and amikacin, while the least effective antibiotics were vancomycin, ampicillin, gentamycin and linezolid. Gatifloxacin shows 100% sensitivity against all isolates of *E.coli*, while all *E.coli* isolates show 100% resistance to vancomycin.

Conclusion: Our study concluded that *E.coli* is a major cause for neonatal sepsis in neonates admitted at Bahawal Victoria Hospital, Bahawalpur. The resistance pattern was alarmingly increased as observed in the currently available antibiotics. Therefore, surveillance of this emerging resistance is needed in these antibiotics. Furthermore, in order to limit the resistant strains of the pathogens, there is a need of effective infections control program.

Keywords
Antibiotics, Escherichia coli, Frequency, Neonatal Sepsis, Susceptibility Pattern, Sepsis.

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INTRODUCTION

Neonatal sepsis denotes to an infection of the bloodstream in newborn infants of around <28 days old. It is a disseminated disease with positive blood culture, and considered more common in underdeveloped countries than developed countries[1]. Annually, about 30% to 50% of the death of neonates occur due to neonatal sepsis, hence making it renowned as the most common cause of death in neonates[2]. Neonatal sepsis has been classified into EOS (Early Onset Sepsis) and LOS (Late Onset Sepsis). EOS takes place within first 7 days of life while LOS take place

after first 7 days of life[3]. The associated risk factors for EOS include labor and delivery, while the risk factors for LOS include care providers or hospital staff. Even with the advancement in the health care system, the significant cause of neonatal mortality and morbidity remains neonatal sepsis, instigated by gram-negative bacilli[4]. Recently in Nepal, sepsis caused by gram-negative bacteria have been identified[5]. Out of total sepsis cases in neonates, about 18% to 78% of neonatal sepsis has been reported to be caused by gram-negative bacteria[6, 7].

The most common bacteria in under-developed countries, for EOS are *Escherichia coli*, *Klebsiella* species and *Staphylococcus aureus,* while *S. aureus*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes* are common bacteria that cause LOS[8]. However, the bacteria causing sepsis in neonates in underdeveloped countries are different as compared to developed countries. In well-developed states, the leading bacteria that cause EOS are *Group B streptococcus* and *E. coli,* while coagulase-negative staphylococci trailed by *Group B streptococcus* and *S. aureus* are responsible to cause LOS. Additionally, in developing countries, the bacteria that cause EOS and LOS sepsis are alike, particularly in the same hospital setup[9], with reported bacteria including *Salmonella* Spp*, Klebsiella* Spp, *E. coli, Pseudomonas aeruginosa*, coagulase negative staphylococci, *S. aureus, S. pyogenes* and *S. pneumonia*[10, 11].

The reported incidence of neonatal sepsis are 1-8 cases/thousand live births. Among these, meningitis have been stated to occur in 1/6 neonatal sepsis patients[12]. Increased morbidity, mortality, and prolong hospital stay have been observed in neonates with neonatal sepsis than neonates having no sepsis[13]. Due to infections, approximately 1.6 million neonates die every year in developing countries[14]. In Pakistan, approximately 7% of the global neonatal deaths occur[15], out of which 33% deaths occur due to sepsis infection[16]. Currently, amongst these, the most significant and common hospital acquired pathogen is *Escherichia coli,* associated with many problems such as septicemia, pneumonia, and meningitis in the neonates.

The life of the neonate with neonatal sepsis can be saved by early diagnosis, early treatment with antibiotics and proper supportive care. The bacteria responsible for neonatal sepsis have developed antimicrobial resistance and therefore cannot be easily treated with the commonly used antibiotics[12]. Hence, for the selection of appropriate antibiotics, it is essential to know the causative agent for neonatal sepsis which can be determined by the antimicrobial susceptibility testing. Additionally, geographical variations have also been reported depending upon the bacteria causing sepsis and the antibiotics used for their treatment at that specified geographical location. Hence, continuous surveillance is needed to observe the variation in the epidemiology of microorganisms, sensitivity of antibiotics, and antibiotics used to determine the emerging resistance[13]. Since, limited data is available about the neonatal sepsis, causative organisms for neonatal sepsis, and their antimicrobial profile. Therefore, our study was directed to describe the frequency and antibiotic susceptibility pattern of *E. coli* for better management of neonatal sepsis.

MATERIALS AND METHODS

This research study was cross sectional study, directed by the Department of Neonatology, Bahawal Victoria Hospital, Bahawalpur. The duration of this study was from September 2019 to January 2021. The study was approved by the Hospital Committee for Research and Ethics for neonatal sepsis sample collection. A consent form was also signed from the guardians of all included neonates. Only the cases having positive blood culture were included in our study while negative blood culture cases and premature neonates were excluded from our study. Blood samples (5ml) were taken from all suspected neonates and sent to the diagnostic laboratory of the hospital for further investigation. The reports of blood culture were divided into positive and negative reports and only positive reports of blood culture were included in this study. Standard blood culture bottles were used for inoculation in the same laboratory and all the samples were incubated for 5 days. *E.coli* was isolated from all positive blood culture samples by standard microbiological procedures. For identification of the bacteria, Gram staining and biochemical tests were done. After confirmation of *E. coli,* all isolates were processed for antibiogram assay. Modified Kirby Bauer method of disk diffusion was used for antibiogram assay. Mueller-Hinton agar was used for disc diffusion method and different antibiotic discs were used in antimicrobial sensitivity testing that includes Ciprofloxacin (5µg),

Amikacin (30µg), Ampicillin (10µg), Netilmycin (30µg), Cefotaxime (30µg), Ceftazidime (30µg), Imipenem (10µg), Piperacillin (100µg), Gatifloxacin (5µg), Gentamicin (10µg), Tobramycin (10µg), Linezolid (30µg) and Vancomycin (30µg). The inhibitory zones were measured according to guidelines of Clinical and Laboratory Standards Institute (CLSI)[17]. Data was entered and analyzed by using SPSS version 23. Mean and standard deviation were documented for continuous variables while for categorical data, frequency, and percentages were reported. All the data were presented in figures and tables.

RESULTS

During this one-year study, 150 sepsis positive blood cultures were found. In 60% (n=90) cases Late Onset Sepsis (LOS) was observed, while in 40% (n=60) Early Onset Sepsis (EOS) was observed in our study (Table **1**). Out of 150 positive blood cultures, 53.33% (n=80) were baby boys, while 46.66% (n=70) were baby girls (Fig**. 1**). The prevalence of *E.coli* in these 150 positive blood cultures was 70% (n=105) (Fig. **2**).

On the basis of antibiotic susceptibility testing, ampicillin & cefotaxime, currently used as empirical therapy at Bahawal Victoria Hospital, Bahawalpur were observed resistant in 80% and 65.71% respectively, to most of the *E.coli* isolates in our study. The most effective antibiotics observed in our study were gatifloxacin, imipenem, and amikacin and these were effective against *E.coli* as 100%, 80% and 80%, respectively. While, the antibiotics to which *E.coli* shows more resistance were vancomycin, gentamycin and linezolid and resistance observed in these antibiotics was 100%, 80% and 80%, respectively. In other antibiotics like piperacillin, ciprofloxacin, netilmycin, and tobramycin the resistance observed was 40%, 60%, 65.71% and 31.42%, respectively (Table **2**).

Table 1. Distribution of Patients on the Basis of Types of Sepsis.

Type of Sepsis	Number of Patients (n)	Percentage (%)
Early Onset Sepsis	60	40%
Late Onset Sepsis	90	60%

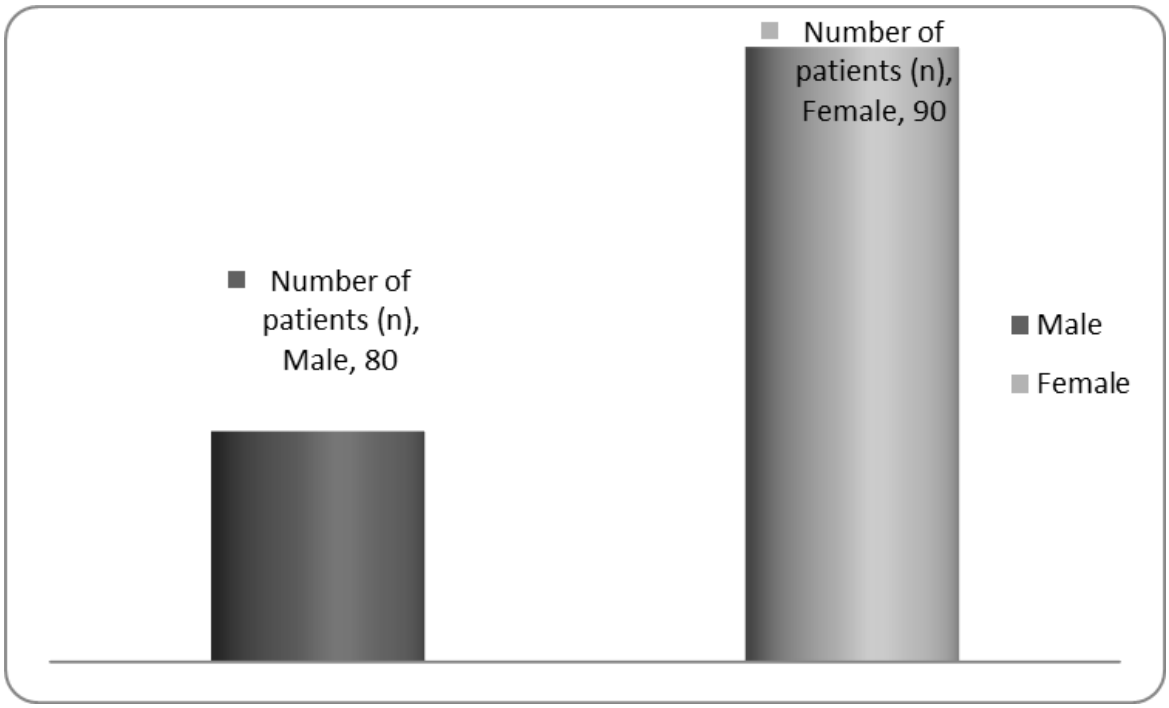

Figure 1. Gender-wise distribution of neonates.

Table 2. Antibiotic Susceptibility Pattern of *E.coli* in Neonatal Sepsis.

S. No.	Antibiotics Used	Concentrations	Antibiotics Susceptibility	*E.coli* Isolates
1	Ampicillin	10µg	S	21 (20)
			R	84 (80)
2	Cefotaxime	30µg	S	36 (34.28)
			R	69 (65.71)
3	Ceftazidime	30µg	S	42 (40)
			R	63 (60)
4	Piperacillin	100µg	S	63 (60)
			R	42 (40)
5	Ciprofloxacin	5µg	S	42 (40)
			R	63 (60)
6	Gatifloxacin	5µg	S	105 (100)
			R	00 (00)
7	Netilmycin	30µg	S	36 (34.28)
			R	69 (65.71)
8	Tobramycin	10µg	S	72 (68.57)
			R	33 (31.42)
9	Imipenam	10µg	S	94 (89.52)
			R	11 (10.47)
10	Linezolid	30µg	S	21(20)
			R	84 (80)
11	Vancomycin	30µg	S	00(00)
			R	105 (100)
12	Gentamicin	10µg	S	21 (20)
			R	84 (80)
13	Amikacin	30µg	S	94 (89.52)
			R	11 (10.47)

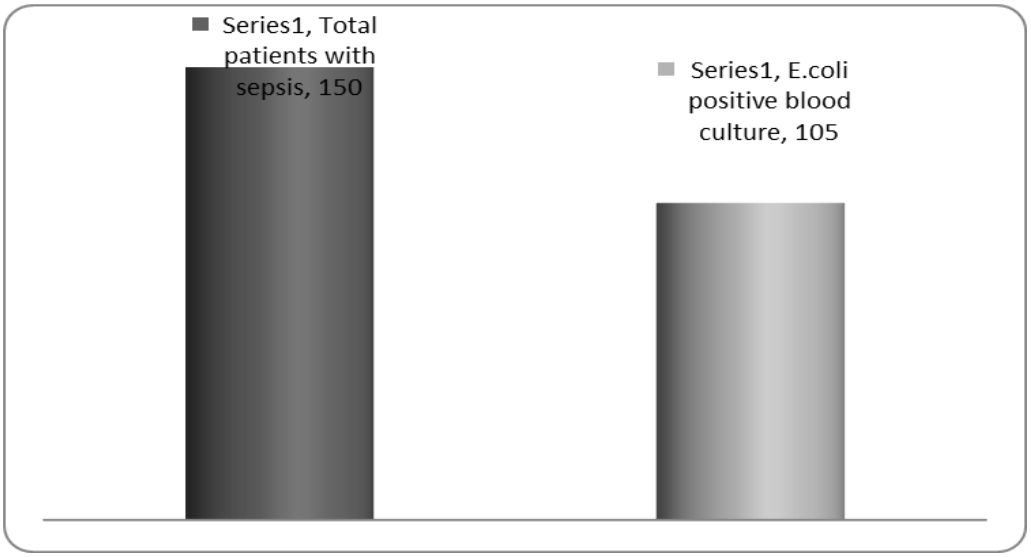

Figure 2. Frequency of isolated *E.coli* in neonatal sepsis.

DISCUSSION

In spite of significant progress in hygienic conditions, new antimicrobial agent introduction and new advanced techniques for early diagnosis and treatment of neonatal sepsis is still considered as a major cause of neonatal mortality and morbidity[13]. Sepsis of neonate has high prevalence around the globe and is a leading health issue in neonates. According to previous studies, neonatal sepsis incidence were 1-10/thousand normal live births, while the incidence of neonatal sepsis in premature neonates is 1/250 premature live births[3].

The best technique for sepsis diagnosis is blood culture[9]. During one year study, total 150 positive sepsis samples from blood culture were found. In 60% (n = 90) cases, Late Onset Sepsis (LOS) was observed, while in 40% (n = 60) Early Onset Sepsis (EOS) was observed in our study. These results are in contrast with the studies reported in other underdeveloped countries like Iran, where the LOS:EOS is 22.5%:77.5%, and Bangladesh with the ratio as 29.3%:70%[18, 19]. Our findings are in accordance with the previous reports from Saudi Arabia, where the ratio of EOS:LOS was 39%:61%, Pakistan 42%:58% and in Libya 31%:69% was observed[20-22].

Moreover, the prevalence of *E.coli* in these 150 positive blood cultures was 70% (n = 105). This finding is consistent with the early reports from underdeveloped countries where they reported that the major sepsis causing bacteria is gram-negative rods. Our results are in accordance with the earlier research which reported 77.1% prevalence of *E.coli* [23]. Similarly, in our study, out of 150 positive blood cultures, 53.33% (n = 80) were baby boys, while 46.66% (n = 70) were baby girls. This finding is consistent with the earlier study done in Dow University, Karachi where 1:0.9 male to female ratio was reported[24].

Antibiotic susceptibility testing revealed that ampicillin and cefotaxime, which are presently utilized as empirical treatment at Bahawal Victoria Hospital in Bahawalpur, were resistant to 80% and 65.71% of the *E.coli* isolates in our study, respectively. Gatifloxacin, imipenem, and amikacin were the most effective antibiotics in our study, with 100%, 80%, and 80% efficacy against *E.coli,* respectively. Vancomycin, gentamycin, and linezolid were the antibiotics to which *E.coli* showed the most resistance, with resistance rates of 100%, 80%, and 80%, respectively. Resistance to other antibiotics such as piperacillin, ciprofloxacin, netilmycin, and tobramycin was found to be 40%, 60%, 65.71 %, and 31.42 %, respectively. Earlier study which was done by Jhoshi *et al.,* in neonates reported high resistance in gram negative bacteria to the majority of penicillin's and cephalosporin's, which might be due to beta-lactamase production[25]. In this study, all the *E.coli* isolates show sensitivity to imipenem. This high sensitivity of *E. coli* to imipenem might be due to lack of selective pressure to rare prescription of this antibiotic. These findings are in accordance with the study done by Marzban *et al.* that high sensitivity of all gram-negative bacteria to imipenem (95%), with resistance to imipenem shown by only one *E.coli* strain[26]. A previous study done by Anwer *et al.* reported the effectiveness of aminoglycosides against gram negative bacteria and they suggested the reservation of aminoglycosides only for severe infections[27].

CONCLUSION

Our study concluded that *E. coli* is a major causative agent for neonatal sepsis in neonates admitted at Bahawal Victoria Hospital, Bahawalpur. The resistance pattern was alarmingly increased which was observed in the currently available antibiotics. Therefore, surveillance of this emerging resistance is needed in currently used antibiotics. Furthermore, in order to limit the resistant strains of the pathogens, there is a need of effective infections control program. Our study proposed amikacin in the empirical antibiotic therapy, to decrease the morbidity & mortality from neonatal sepsis. To control this antimicrobial resistance, there should be judicial use of antibiotics with perfect dosage and duration.

ETHICAL APPROVAL

The study was approved by the Hospital Committee of Bahawal Victoria Hospital, Bahawalpur, for Research and Ethics of neonatal sepsis sample collection. A consent form was also signed from the guardians of all included neonates.

CONFLICTS OF INTEREST

None.

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None.

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None.

LIST OF ABBREVIATIONS

CLSI	Clinical and Laboratory Standards Institute
EOS	Early Onset Sepsis
LOS	Late Onset Sepsis

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