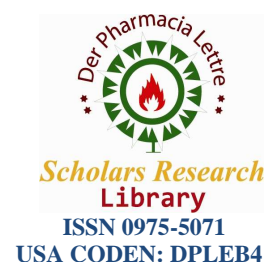




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Bacterial profile and drug susceptibility pattern of neonatal sepsis in Gondar University Hospital, Gondar northwest Ethiopia

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ABSTRACT

We aimed to determine the bacterial profile and describe the drug susceptibility pattern of neonatal sepsis in patients seen at Gondar University Hospital neonatal unit, Gondar, Northwest Ethiopia. A cross sectional study was conducted involving patients with clinical signs of sepsis who were admitted to Gondar University Hospital neonatal unit between July 2011 and June 2012. Venous blood sample was collected aseptically and inoculated in to Trypton soya blood culture medium for 2-14 days aerobically, checking for growth of bacteria every 3 days. Bacterial isolates were identified using standard procedures and antibacterial susceptibility test was done following agar disc diffusion method. A total of 181 neonates (99 male and 82 female) were included in the study. Out of this, 58 showed positive blood culture for bacteria with infection rate of 32.1%. The most common bacterial isolate was *Staphylococcus aureus* (29.3%) followed by *Klebsiella ozaenae* (17.2%), *Escherichia coli* (10.3%), non lactose fermenter gram negatives (10.3%), *K. pneumoniae* (8.6%) and coagulase negative staphylococci (6.8%). *S. aureus* showed high level of drug resistance for tetracycline (16, 94.1%), ampicillin (14, 82.4%), amoxicillin (14, 82.4%), chloramphenicol (4, 82.4%), trimetoprim sulphamethoxazole (14, 82.4%) and ceftriaxone (13, 76.4%). Among the total isolates of *S. aureus*, 41.2% of them were found to be methicillin resistant (MRSA). *K. ozaenae* showed extremely high level of resistance against amoxicillin (10, 100%), chloramphenicol (10, 100%), ampicillin (9, 90%) and tetracycline (7, 70%). Multiple drug resistance was observed in 85.7% of gram positive and 92.1% of gram negative isolates. There is exceedingly high rate of resistance of bacterial isolates to different antibiotics commonly prescribed. Bacterial profile suggesting the best choice of antibiotics are important for physicians when treatment of the septic neonates has to be initiated before the result of the blood culture is known.

Keywords: Neonate, Sepsis, bacterial profile, drug susceptibility

INTRODUCTION

Neonatal sepsis is a frequent cause of morbidity and mortality. It is a clinical syndrome characterized by systemic signs of infection and bacteremia in the first month of life [1]. The disease is the single most important cause of neonatal death in the community, accounting for over half of the deaths. If diagnosed early and treated aggressively with antibiotics and good supportive care, it is possible to save most cases of neonatal sepsis [2]. The disease remains an important cause of morbidity and mortality in Ethiopia [3]. Hospital based incidence of neonatal sepsis is high and this high incidence is related to the prevalence of predisposing factors and lack of basic amenities for optimal hygiene [4, 5, 6].

The spectrum of organisms that cause neonatal sepsis changes over time and varies from region to region. Epidemiological data from developing countries shows differences in the incidence, risk factors, pattern and antimicrobial sensitivities of pathogens and mortality from that of developed countries [7, 8].

Management of newborn with sepsis consists essentially of appropriate antibiotic therapy and supportive care. The choice of antibiotic therapy is best guided by the knowledge of etiologic agent. This, however, is usually not immediately possible. Thus, it is customary to initiate treatment with an empirical choice of antibiotic(s) that is informed by the epidemiology of causative agents and sensitivity patterns in a given locality [9].

This study was, therefore, conducted to determine the bacterial profile and antibiotic sensitivity pattern of bacterial isolates from blood cultures of neonates with septicemia admitted to Gondar University Hospital neonatal unit.

MATERIALS AND METHODS

Study area and population

This is a cross sectional study conducted among neonates admitted at Gondar University Hospital, neonatal unit between July 2011 and July 2012. Gondar University Hospital is located in Gondar town, 727 km north from the capital city of Ethiopia, Addis Ababa. It is one of the oldest health institutions in Ethiopia rendering primary and referral health services to over five million people in Northwest Ethiopia.

Neonates (0-28 days) with clinical sign and symptoms of sepsis at the time of admission or who developed sepsis during their hospital stay were included in this study. Neonates with congenital malformation or dysmorphic features and those who started antibiotic therapy were excluded. Neonatal sepsis was categorized according to its time of onset as early-onset sepsis (0-7 days) and late-onset sepsis (8-28 days).

Sample collection and examination

After getting written consent from the guardians, 3ml of blood was collected from neonates aseptically and inoculated into Trypton soya broth and incubated at 37°C for 2-14 days aerobically, checking for turbidity or growth every 3 days. Sub-cultures were made on blood agar, chocolate agar and McConkey agar after 24 hours. Bacterial growths obtained were identified by the standard bacteriological techniques [10]. Susceptibilities to common antibiotics were determined by Kirby-Bauer disc-diffusion method [10, 11]. The susceptibility of the bacterial isolates were tested against amoxicillin (30mg), ampicillin (10 mg), ceftriaxone (30 mg), chloramphenicol (30 mg), norfloxacin (30 mg), penicillin (30 mg), ciprofloxacin (5 mg), tetracycline(30 mg), methicillin (5mg), trimethoprim-sulfamethoxazole (5mg), and vancomycin (30 mg). The zones of inhibition were measured and compared with National Committee for Clinical Laboratory Standards (NCCLS) guidelines [12, 13]. *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) susceptible to all antimicrobial agents were used as a control strains. Isolates showing an intermediate level of susceptibility were classified as resistant and multiple drug resistance was defined as resistance to two or more drugs.

Data analysis

Blood culture results were collected and analyzed for the frequency of different bacterial isolates and their antibiotic susceptibility pattern. Data were entered and analyzed using SPSS version 16 software. Frequencies of different variables were calculated in percentages. In all cases, p value of less than 0.05 was taken to indicate level of statistical significance.

Ethical issues

Written informed consent was obtained from mothers/caretakers of neonates after explaining the purpose and objective of the study. The results were communicated to the treating physician for immediate and appropriate treatment. Ethical clearance was obtained from the Institutional Review Board of University of Gondar.

RESULTS

A total of 181 clinically suspected neonatal sepsis cases were included in this study. Ninety nine (54.7%) of them were males and 82 (45.3%) were females with a male to female ratio of 1.2:1. The mean age of the population was 4.15 days with standard deviation of 5.4 days. The mean duration of hospital stay was 5.6 days with a standard deviation of 4 days.

Table 1: Antibiotic susceptibility pattern of bacteria isolated from neonates with sepsis, Gondar University Hospital, 2011-2012

		<i>Co Neg Staph</i>	<i>S aureus</i>	<i>Entero Bacter</i>	<i>Kleb Siella Spp</i>	<i>Klebsiella Ozonae</i>	<i>Group B streptococci</i>	<i>Lact ferm gr-ve</i>	<i>Non lact ferm gr -ve</i>	<i>Klebsiella Pneum Onae</i>	<i>Serra tia</i>	<i>Shige Lla</i>	<i>Salmo nella</i>	<i>E coli</i>	<i>Citro bacter</i>	TOTAL
		N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	
AMP	S	1 25%	3 17.6%	0	0	1 10%	ND	ND	0	0	0	0	0	0	0	5 8.6%
AMX	S	3 75%	3 17.6%	0	0	0	ND	ND	0	0	0	1 50%	ND	ND	ND	7 12.1%
CRO	S	2 50%	4 23.5%	0	0	0	ND	1 100%	4 66.7%	4 80%	0	1 50%	1 50%	4 66.7%	ND	21 36.2%
CAF	S	1 23%	3 17.6%	0	ND	4 40%	ND	1 100%	3 50%		0	1 50%	1 50%	3 50%	0	17 29.3%
CIP	S	3 75%	8 47.1%	ND	1 100%	5 50%	ND	ND	4 66.7%	5 100%	0	1 50%	1 50%	5 83.3%	0	33 58.9%
VANC	S	4 100%	17 100%	1 100%	1 100%	ND	ND	ND	ND	ND	0	ND	ND	ND	ND	22 37.9%
NOR	S	3 75%	11 64.7%	1 100%	1 100%	4 40%	ND	0	4 66.7%	3 60%	ND	2 100%	1 50%	5 83.3%	0	34 56.3%
PEN	S	2 50%	10 58.8%	1 100%	0	ND	ND	ND	1 16.7%	ND	ND	ND	1 16.7%	ND	ND	15 25.9%
SXT	S	1 25%	3 17.6%	0	0	4 40%	ND	ND	1 16.7%	ND	ND	ND	ND	ND	ND	9 15.5%
TE	S	1 25%	1 5.88%	0	0	3 30%	ND	ND	3 50%	0	ND	2 100%	0	3 50%		13 22.4%
MET	S	2 50%	10 58.8%	1 100%	ND	ND	ND	0	ND	ND	ND	ND	ND	ND		15 25.9%
Total isolates		4 6.8%	17 29.3%	1 1.72%	1 1.72%	10 17.2%	1 1.72%	1 1.72%	6 10.34%	5 8.62%	1 1.72%	2 3.44%	2 3.44%	6 10.34%	1 1.72%	58 100%

ND: Not done AMP: ampicilin AMX: amoxicilin CRO: ceftriaxone CAF: chloramphenicol CIP: ciprofloxacin VANC: vancomycin
NOR: norfloxacin PEN: penicillin SXT: trimetoprim sulphametoxazole TE: tetracycline MET: methicillin

Out of the total of 181 samples studied, 58 showed positive blood culture for bacteria with infection rate of 32.1%. The most common bacterial isolate was *S. aureus* (29.3%) followed by *K. ozaenae* (17.2%), *E. coli* (10.3%), non lactose fermenter gram negatives (10.3%), *K. pneumoniae* (8.6%) and coagulase negative staphylococci (6.8%). *S. aureus* showed high level of drug resistance against tetracycline (16, 94.1%), ampicillin (14, 82.4%), amoxicillin (14, 82.4%), chloramphenicol (4, 82.4%), trimetoprim sulphametoxazole (14, 82.4%) and ceftriaxone (13, 76.4%). Among the total isolates of *S. aureus*, 41.2% of them were found to be methicillin resistant (MRSA). *K. ozaenae* showed extremely high level of resistance against amoxicillin (10, 100%), chloramphenicol (10, 100%), ampicillin (9, 90%) and tetracycline (7, 70%). Multiple drug resistance (resistance to two or more drugs) was observed in 85.7% of gram positives and 92.1% of gram negative isolates (Table 1).

Table 2: Distribution of bacterial isolated from neonates based on their age at admission

Organisms isolated	Age at times of admission			Total isolates
	0 -2days	3 – 7 days	8 – 28 days	
<i>Staphylococcus aureus</i>	5 (31.3%)	7 (30.4%)	5 (26.3%)	17 (29.3%)
Coagulase negative staphylococcus	0(0)	2(8.7%)	2(10.52%)	4(6.8%)
<i>Enterobacter spp</i>	1(6.3%)	0(0)	0(0)	1(1.72%)
<i>Klebsiella spp</i>	0(0)	1(4.3%)	0(0)	1(1.72%)
<i>Klebsiella ozaenae</i>	4(25%)	3(13%)	3(15.8%)	10(17.2%)
Alpha hemolytic streptococci	0(0)	0(0)	1(5.3%)	1(1.72%)
Lactose fermenter gram negative bacteria	0(0)	1(4.3%)	0(0)	1(1.72%)
Non lactose fermenter gram negative bacteria	2(12.5%)	2(8.7%)	2(10.5%)	6(10.34%)
<i>Klebsiella pneumonia</i>	3(18.3)	1(4.3%)	1(5.3%)	5(8.62%)
<i>Serratia spp</i>	0(0)	1(4.3%)	0(0)	1(1.72%)
<i>Shigella spp</i>	0(0)	0(0)	2(10.52%)	2(3.44%)
<i>Salmonella spp</i>	0(0)	2(8.7%)	0(0)	2(3.44%)
<i>Escherichia coli</i>	1(6.3%)	3(14.1%)	2(10.52%)	6(10.34%)
<i>Citrobacter spp</i>	0(0)	0(0)	1(5.3%)	1(1.72%)
TOTAL	16(27.6%)	23(39.7%)	19(32.8%)	58(100%)

Among the culture proven sepsis cases 16 (27.6%) of the neonates were presented within the first 48 hours of life, 23 (39.7%) between 3-7 days of life and 19 (32.8%) after the first week of life. There were 39 episodes of early onset neonatal sepsis (0-7days) and 19 episodes of late onset neonatal sepsis (7-28days) in this study (Table 2).

The predominant organisms during the first 48 hours of life were gram negatives, accounting for 11 (68.7%) of the 16 isolates. Between 3 and 7 days of life, gram negative and gram positive isolates accounted for 14 (60.9%) and 9 (39.1%) of the 23 isolated organisms, respectively. Between 8 and 28 days of life, gram positive and gram negative organisms accounted for 7 (36.8%) and 12 (63.2%) of the 19 organisms isolated, respectively. The single predominant gram positive organism in all age group was *S. aureus*. Among gram negatives, *K. ozaenae* was the predominant causative agent in age groups of less than 48 hrs and greater than 7 days. In those between 3 and 7 days of age, both *K. ozaenae* and *E. coli* were found to be the predominant organisms (Table 2).

DISCUSSION

Bacterial isolates from neonatal sepsis show variation from place to place which indicate that there is a need for continuous monitoring of causative organisms and their drug susceptibility pattern at a local level. This study, which is the first of its kind in Northwest Ethiopia, showed a very high prevalence of bacterial causes of neonatal sepsis and alarmingly high level of antibiotic resistance in the isolates.

Even if this study did not include the possible predisposing risk factors it is suspected that the high incidence of *S. aureus* and *K. ozaenae* in this study can be associated with the poor hygienic condition that is seen around this area. In this study 58 samples were culture positive with a blood culture positivity rate of 32.1%. Gram negative sepsis was encountered in 62.18% of culture proven cases. A relatively higher blood culture positivity rate in septicemic neonates (44.7%) had been reported by Shitaye *et al* [15] in Addis Ababa. A low blood culture isolation rate in this study might be due to several reasons, one of which being administration of antibiotics before blood collection. Shitaye *et al* also identified *Klebsiella spp.* (39.2%) as a predominant organism. This result is in contrast to this study finding which showed *Staphylococcus aureus* (29.3%) as the leading causative agent.

There were 39 (67.3%) episodes of early onset neonatal sepsis (<7days) while there were 19 (32.8%) episodes of late onset neonatal sepsis (8-28days) in this study. Other studies by Mathur *et al* [16] in India and Mokoulo *et al* [17] in Nigeria also reported a higher percentage of early onset neonatal sepsis. About 64.1% of early onset and 63.2% of late onset neonatal sepsis infections were due to gram negative organisms with *Klebsiella ozaenae* accounting for majority of the cases. The distribution of organisms in this study was different from the study done in

Nigeria [17], which demonstrated that gram positives accounted for majority of the cases in late onset neonatal sepsis with 38.8% being *S. aureus*.

It is known that Gram negative organisms are dominant flora in pregnant females increasing the probability of these organisms gaining access to nurseries and causing infection [18]. *Staphylococcus aureus* was the most frequent gram positive isolate in this study while Maryam [19] and Roy [20] have reported *Staphylococcus epidermidis* as the most common gram positive organism.

Drug resistance of microorganisms causing neonatal sepsis is a rapidly emerging, potentially a disastrous problem. The situation is worse in developing countries because of lack of legislation, over the counter sale of antibiotics, poor sanitary conditions, lack of surveillance of the standards of maternity homes and the practices of traditional birth attendants who deliver almost 80% of all births [24, 29]. The antimicrobial susceptibility pattern differs in different studies done in Ethiopia [14] as well as elsewhere studies [25-27]. In this study, we observed that methicillin resistance was observed in 41.2% of the *S. aureus* species and 50% of coagulase negative *Staphylococcus* species. Similarly high rates of methicillin resistance against *S. aureus* (28%) were observed in a study conducted in Tanzania [28]. In this study *S. aureus* showed a sensitivity of 100% to vancomycin, 64.1% for norfloxacin and 58.8% to penicillin. Most of the gram negative organisms were sensitive to norfloxacin, ciprofloxacin and ceftriaxone. Multiple drug resistance (resistance to two or more drugs) was observed in 85.7% of gram positives and 92.1% of gram negative isolates. This result is almost comparable with other studies done in Ethiopia [14, 15] and other countries [29, 30, 31, 32, 33, 34].

It is important to maintain surveillance for nosocomially acquired organisms and factors that predispose to neonatal sepsis. A better understanding of this issue would facilitate the necessary behavioral changes in the care of newborn and rational antibiotic therapy in management of neonatal sepsis. More studies are also needed from this area that addresses maternal risk factors for neonatal sepsis in order to provide a better care of the newborn at risk for sepsis. In summary, the predominant bacterial isolates causing neonatal sepsis are gram negative bacilli particularly *Klebsiella ozaene*, non lactose fermenting gram negatives and *E.coli*. *Staphylococcus aureus* is the most common of the total (both gram positive and gram negative) isolated organisms. There is exceedingly high rate of resistance of microorganisms to different antibiotics commonly prescribed.

Bacterial profile suggesting the best choice of antibiotics are important for physicians when treatment of the septic neonates has to be initiated before the result of the blood culture is known, as rational use of appropriate antibiotics can markedly decrease the neonatal morbidity and mortality due to sepsis.

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