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ORIGINAL ARTICLE

SEPTICEMIA, BACTERIAL ISOLATES AND DRUG SUSCEPTIBILITY AMONG WOMEN ATTENDING DELIVERY AT DILCHORA HOSPITAL, DIRE DAWA, EASTERN ETHIOPIA

Daniel Demissie, MSc[1,2], Berhanu Seyoum, PhD[2,3], Melake Demena, MSc[2], Biruk Yeshitila, MSc[3*]

ABSTRACT

Background: *Puerperal sepsis is the second most frequent cause of maternal morbidity and mortality in resource limited settings and often occurs within the first 42 days after childbirth.*
Objective: *The aim of the study was to assess the prevalence of septicemia, its bacterial isolates, drug susceptibility patterns and associated factors among sepsis suspected women attending delivery at a referral hospital in Ethiopia.*
Method: *A cross sectional study was conducted with a sample size of 441 women in the age group 15-49 years at Dil-chora hospital, Dire Dawa, Eastern Ethiopia from May 1 to July 30, 2016. Socio-demographic and clinical data were collected using structured interview questionnaires. Blood was collected aseptically and inoculated into a broth medium and cultured aerobically for 48 hours. Antimicrobial susceptibility pattern of isolated bacteria was determined by Kirby Bauer disc diffusion method. Data were analyzed using SPSS version 16. Binary logistic regression was used to test for association. Significant variables were further adjusted using multivariate analysis.*
Result*: The prevalence of septicemia was 12.9% of suspected cases and coagulase negative staphylococcus was found to be the most frequent isolate (28.1%) followed by E. coli (22.8%), Pseudomonas aeruginosa (10.5%) and Proteus spp (3.5%). Multiple vaginal examinations and multiple pregnancies were associated with the occurrence of sepsis.*
Conclusion: *The prevalence of septicemia was 12.9%. Coagulase negative staphylococci and E. coli were the predominant bacteria isolated. Most of bacterial isolates were resistant against commonly used antibiotics such as ampicillin, amoxicillin and tetracycline.*
Key words: *Septicemia, Puerperal sepsis, maternal septicemia, Antimicrobial resistance.*

INTRODUCTION

The World Health Organization ranks maternal sepsis as the sixth leading cause of disease burden for women aged 15-44 years, after depression, HIV/ AIDS, tuberculosis, abortion and schizophrenia. As many as 5.2 million new cases of maternal sepsis are thought to occur annually and an estimated 62,000 maternal deaths will result from the condition (1). Added to the burden of loss of women's lives caused by sepsis are the long-term consequences of infertility and the association of maternal sepsis with over one million infection related neonatal deaths every year (2,3).

World Health Organization Technical Working Group on the prevention and management of puerperal infections put forward the definition of puerperal sepsis as infection of the genital tract occurring at any time between the rupture of membranes or labor, and the 42nd day postpartum in which two or more of the following are present: pelvic pain, fever, oral temperature 38.5°C or higher on any occasion, abnormal vaginal discharge, presence of pus, abnormal smell/foul odor of discharge (4).

The timely and appropriate use of antibiotics is currently the only way to treat bacteremia. However, the treatment most often begins before a definite causative agent is identified (5). Risk factors for puerperal sepsis surround three central components: community-related factors, delivery conditions and maternal co morbid conditions (6).

Among the Millennium Development Goals, achieving the goal for maternal health posed the greatest challenge in Sub- Saharan Africa, to which Ethiopia contributed considerably (6). It has been reported that Ethiopia is one of the six countries that contribute about 50% of the maternal deaths; the others being India, Nigeria, Pakistan, Afghanistan and the Democratic Republic of Congo (7). Ethiopia

[1]Ehiopian Public Health Institute, Addis Ababa, Ethiopia.
[2]Haramaya University, College of Health and Medical Sciences, Harar, Ethiopia.
[3]Armauer Hansen Research Institute, Addis Ababa, Ethiopia.
*Corresponding Author E-mail: biruk_23@yahoo.com or biruky01@gmail.com

Progress on reducing maternal mortality has stalled since 2005 when the country managed to reduce maternal mortality rate (MMR) to 676 per 100,000 births in 2010/11 from 871 in 2000/01 (8).
Identification of bacterial pathogens and associated risk factors of septicemia helps health facilities or health bureaus to take effective risk-reduction measures to reduce maternal morbidity and mortality due to septicemia in the area.

The objective of this study was to determine the prevalence of septicemia, its bacterial isolates, drug susceptibility pattern and associated factors among suspected women attending delivery in a referral hospital in Ethiopia.

MATERIALS AND METHODS

A cross sectional study was conducted at Dil-Chora Hospital from May to July 2016. Total sample size for the study was 441 considering a single population proportion formula with 50% prevalence of septicemia and a 15% non-response rate. A convenient sampling technique was used. Women suspected for septicaemia were consecutively enrolled until the desired sample size was attained. Primary data on respondent's socio-demographic and obstetric clinical variables were gathered using a structured questionnaire.

The questionnaire was prepared by reviewing the literature (9, 10, 11). From each study participant, 10 ml of venous blood was collected following standard procedures and inoculated into 50 ml of tryptone soya broth (12, 13). The blood culture bottle was then incubated at 37^0C aerobically and examined macroscopically. Culture that had turbid appearance or lysis of erythrocytes was picked up and sub-cultured (12). Three to five colonies of bacteria from pure culture were picked with an inoculating loop and transferred into a tube containing 5ml nutrient broth and incubated again at 37^oC for 3-5 hours with turbidity of the suspension adjusted to a density of 0.5 McFarland standard (14) and antibiotic susceptibility test was performed with Kirby-Bauer disk diffusion method (15).

Data was entered and cleaned using Epi-Data version 3 and analysis was done using SPSS version 16 statistical software program. Logistic regression was used to determine the relative association of independent variables to the outcome variable with Odds ratio at 95% confident interval.

A p value <0.05 was considered statistically significant. The study was approved by Haramaya University, College of Health and Medical Sciences, Health Research Ethics Review Committee.

RESULTS

Socio-demographic characteristics

A total of 441 septicemia suspected women were enrolled in this study.

The majority of them 262(59.4%) were in the age range of 25-34 years with a mean age of 26.55. Among them, 433(98.2%) were married and 128 (31.5%) had an educational level of grade 1-8 while 33(7.5%) had higher education (>12 grade) and 344 (78%) study participants were urban dwellers. The mode of delivery for 71% of the subjects was vaginal and the rest were cesarean sections. Among the study participants, 181(41%) had visited a health institution for antenatal care (ANC) during their pregnancy, but only 28 (6.3%) had completed the full course of ANC follow up.

Prevalence of septicemia and proportion of bacterial isolates

The prevalence of septicemia in this study was (12.9%). The majority of the isolates, (57.9%) were Gram-negative organisms. *Coagulase negative staphylococcus (CONS)* was found to be the most frequent isolate (28%) followed by *E. coli* (22.8%), *Salmonella* (14%), *Klebsiella spp* (10.5%), and *Pseudomonas aeruginosa* (7%), while *Proteus spp.* were found in relatively small numbers (3.5%) (Table 1).

Table 1: Frequency of bacterial pathogens isolated from blood culture among suspected women attending delivery at Dil-chora hospital Dire Dawa, Eastern Ethiopia from May to July 2016.

Bacterial Isolates	Frequency	Percent (%)
CONS	16	28.1
E. coli	13	22.8
S. aureus	8	14
Salmonella Spp	8	14
P. aeruginosa	6	10.5
Klebsiella spp	4	7
Proteus species	2	3.5
Total	57	100

Antimicrobial susceptibility pattern for Gram negative bacteria

The overall rate of sensitivity of Gram-negative bacterial isolates was in the range of 3.1% to amoxicillin to 88% to ciprofloxacin. Generally, the isolates showed high frequency of sensitivity to ciprofloxacin (88%) and to ceftriaxone (81.8%) followed by gentamicin (67%). Lower frequency of resistance (< 30%) was observed to ciprofloxacin (6%), ceftriaxone (12.1%), gentamicin (24%), chloramphenicol (18%) and nalidixic acid (30%) [Table 3]. Among, the predominantly isolated Gram-negative bacteria, *E. coli* was found to be highly sensitive to ciprofloxacin (92.3%), ceftriaxone (92.3%) and gentamicin (84%) whereas high frequency of resistance was observed to ampicillin (100%), amoxicillin (92.3%), and tetracycline (84.6 %).

A very low sensitivity rate was observed for amoxicillin (0%) and tetracycline (15.4%). Among the Salmonella species isolated in 8 blood samples,7/8 (87.5%) were sensitive to ceftriaxone and ciprofloxacin (each) and 6/8 (75%) to nalidixic acid whereas 6/8(75%) of them were resistant to ampicillin, 5/8 (62.5%) to amoxicillin, 4/8(50%) to tetracycline and 2/8(37.5%) to SXT (Table 2). All 4 isolates of *Pseudomonas aeruginosa* were resistant to ampicillin, amoxicillin, tetracycline and gentamicin but 3 of 4 isolates (75%) were susceptible to ciprofloxacin (75%). (Table 2).

Antimicrobial susceptibility pattern of Gram positive bacteria

The susceptibility of Gram-positive bacteria (n=24) isolated from blood culture against common antimicrobial agents ranged from 8% to 92% of isolates (**Table 3**).

Generally, Gram-positive isolates showed high frequency of susceptibility to gentamicin (91.7%), erythromycin (87.5%), ceftriaxone (75%), chloramphenicol (75%) and ciprofloxacin (71%). The proportion of resistance of Gram-positive isolates was (83%) for ampicillin, 79 % for amoxicillin, 54.1% for tetracycline and 33.3% for chloramphenicol while lower frequency of resistance (< 30%) was observed against erythromycin, ceftriaxone and ciprofloxacin.

Possible Associated Factors of Septicemia

According to the finding of this study, septicemia was associated with number of pregnancy, multiple vaginal examination and ANC follow up. In the bivariable analysis a statistically significant association was observed between rural residence and the occurrence of septicemia. After the Odds of infection adjustment with other associated risk factors in multivariable analysis however, this association was no more evident (p=0.60, AOR: 1.852, 95% CI: 0.975, 3.516) (Table 4).

The frequency of septicemia among participants who had multiple pregnancy (> five) was found to be high. Mothers who were pregnant more than five times were found to have statistically significant association with the occurrence of septicemia. The Odds of infection was also significant after adjustment with other associated risk factors (p=0.035, AOR: 0.797, 95% CI: 0.645, 0.984) (Table 4).

Table 2.: Antimicrobial susceptibility pattern of Gram-negative bacterial isolates from blood culture among sepsis suspected women attending delivery at Dil-chora Referral Hospitals, Dire Dawa, Eastern Ethiopia from May to July 2016.

Bacterial isolates no and %	S I R	Antimicrobial agents / No. of bacterial isolates (%) n=33								
		AMP	CRO	C	CIP	GEN	NA	AMO	TTC	SXT
E. coli 13(39.4%)	S	0(0)	12 (92.3)	10 (76.9	12 (92.3)	11 (84)	10 (77)	0(0)	2(15.4)	11(77)
	I	0(0)	1(7.7)	1(7.7)	1(7.7)	1(7.7)	1(7.7)	1(7.7)	0(0)	0(0)
	R	13(100)	0(0)	2 (15.4)	0(0)	1(7.7)	2 (15.3)	12 (92.3)	11 (84.6)	2(15.4)
Salmo-nella.Spp 8(24.2%)	S	1(12.5)	7(87.5)	6(75)	7(87.5)	5 (62.5)	6(75)	1(16.6)	2(25)	5(62.5)
	I	1(12.5)	1(12.5)	1 (12.5)	1(12.5)	1 (12.5)	1(12.5	2(25)	2(25)	1(12.5)
	R	6(75)	0(0)	1 (12.5)	0 (0)	2(25)	1(12.5	5(62.5)	4(50)	2(37.5)
Klebsiella 6(18.2%)	S	0(0)	6(100)	5(83)	5(83.3)	4 (66.7)	2(33.3	0(0)	2(33.3)	2(33.3)
	I	0(0)	0(0)	1 (16.6)	0(0)	1 (16.7)	0(0)	1(16.7)	0(0)	0(0)
	R	6(100)	0(0)	0(0)	1(16.7)	1 (16.6)	4 (66.7)	5(83.3)	4(66.7)	4(66.7)
P.aeruginosa 4(12.2%)	S	0(0)	0(0)	1(25)	3(75)	0(0)	1(25)	0(0)	0(0)	0(0)
	I	0(0)	0(0)	0(0)	0(0)	0(0)	1(25)	0(0)	0(0)	0(0)
	R	4(100)	4(100)	3(75)	1(25)	4 (100)	2(50)	4(100)	4(100)	4(100)
Proteus Spp 2(6.1%)	S	0(0)	2(100)	1(50)	2(100)	2 (100)	1(50)	0(0)	1(50)	1(50)
	I	0(0)	0(0)	1(50)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	R	2(100)	0(0)	0(0)	0(0)	0(0)	1(50)	2(100)	1(50)	1(50)
Total 33(100)	S	1(3)	27 (81.8)	23 (69.7	29(88)	22 (67)	20 (61)	1(3.1)	7(21.2)	19 (57.6)
	I	1(3)	2(6.1)	4 (12.1)	2(6)	3(9)	3(9)	4(12.1)	2(6.1)	1(3)
	R	31(94)	4(12.1)	6 (18.2)	2(6)	8(24)	10 (30)	28 (84.8)	24 (72.7)	13 (39.4)

Table 3: Antimicrobial susceptibility pattern of Gram-positive bacteria isolated from blood culture among suspected women at Dil-chora Referral Hospitals, Dire Dawa, Eastern Ethiopia from May to July 2016.

Bacterial isolates no and %	S I R	Antimicrobial agents / No. of bacterial isolates (%)								
		AMP	CRO	C	CIP	GEN	NA	AMO	TTC	SXT
CONS 16(66.7)	**S**	2(12.5	14 (87.5)	13(81)	12(75)	15 (93.8)	15 (93.8)	2(12.5)	10(63	2(12.5)
	I	1(6.5)	1(6.25)	0(0)	1(6.3)	0(0)	1(6.2)	1(6.2)	1(6.3)	2(12.5)
	R	13(81)	1(6.25)	3(19)	3(18.6)	1(6.2)	0(0)	13(81)	5(31.3	12(75)
S .aureus 8(33.3)	**S**	0(0)	4(50)	5(62.5	5(62.5)	7(87.5)	6(75)	1 (12.5)	3(37.5)	1(12.5)
	I	1(12.5	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(12.5)	0(0)
	R	7(87.5	4(50)	3 (37.5)	3(37.5)	1(12.5)	2(25)	7(87.5)	4(50)	7(87.5)
Total 24(100)	**S**	2(8.4)	18(75)	18(75	17(71)	22 (91.7)	21 (87.5)	3(16.6	13 (58.3)	3(33)
	I	2(8.3)	1(4.2)	0(0)	1(0)	0(0)	1(4.2)	1(4.3)	2(12.5)	3(12.5)
	R	20(83)	5(20.8)	6 (33.3)	6(25)	2(8.3)	2(8)	20 (79.1)	7(29.2)	13 (54.1

Table 4: Factors associated with septicemia among sepsis suspected women attending delivery at Dil-Chora Referral Hospitals, Dire Dawa, Eastern Ethiopia from May to July 2016.

Characteristics	Septicemia Yes	No	COR	95% CI	p-value	AOR	95%CI	P-value
Age: 15-24	1(13.3%)	111(86.7%)	1	1		1	1	
25-34	29(12.1%)	233(88.9%)	0.813	0.429-1.541	0.523	0.633	0.043-9.276	0.593
35-44	1(20.4%)	39(79.6%)	1.674	0.707-3.965	0.241	0.668	0.047-9.596	0.739
≥45	1(50%)	1(50%)	6.529	0.390 -109.37	0.192	0.317	0.020-5.051	0.767
Residence: Rural	19(19.6%)	78(80.4%)	1.962	1.072-3.589	0.029*	1.852	0.975-3.516	0.60
Urban	38(11.1%)	306(88.9%)	1			1		
Edu. Status: Illiterate	13(15.5%)	71(84.5%)	1.025	0.334-3.144	0.666	1.266	0.217-7.404	0.793
Read and Write	9(13.9%)	56(86.2%)	0.900	0.276-2.940	0.965	0.722	0.142-3.669	0.694
Primary (1-8)	13(9.4%)	126(90.6%)	0.578	0.190-1.753	0.333	0.742	0.158-3.475	0.705
High school (9-12)	17(14.2%)	103(85.8%)	0.924	0.314-2.725	0.886	0.610	0.138-2.693	0.514
Higher education(>12)	5(15.2%)	28(84.8%)	1	1		1	1	
Marital status: Single	1(16.2%)	5(83.3%)	1	1		1	1	
Widowed	0(0%)	2(100%)	0.327	0.028-3.742	0.368	3.989	0.680-6.406	0.081
Married	56(12.9%)	377(87.1%)	0.182	0.008-4.263	0.290	2.533	0.24-40.333	0.179
Mo. income(birr)<500	12(14.6%)	70(85.4%)	0.932	0.414-2.099	0.865	0.871	0.282-2.687	0.810
501-1000	11(15.1%)	62(84.9%)	0.965	0.419-2.221	0.933	0.876	0.307-2.498	0.805
1001-1500	3(4%)	72(96%)	0.227	0.063-0.808	0.022*	3.693	0.895-	0.071
1501-2000	15(13.9%)	93(86.1%)	0.877	0.409-1.880	0.736	1.336	15.242	0.542
>2000	16(15.5%)	87(84.5%)	1	1			0.527-3.387	1
Vag.Exam1 time	1(1.7%)	29(7.5%)	1	1		1		
Two time	7(12.3%)	123(28.9%)	1.650	0.195-13.944	0.645	0.526	0.654-3.559	0.328
Three Time	19(33.3%)	111(25.8%)	4.964	0.638-38.637	0.126	0.496	0.699-3.201	0.299
Four Time	13(54.4%)	62(16.1%)	6.081	0.759-48.731	0.089	1.337	1.035-	0.926
Five time	17(22.8%)	59(15.4%)	8.356	1.059-65.901	0.044*	0.644	28.991	0.042*
							0.503-0.824	
Delivery mode Vaginal	43(13.7%)	270(86.3%)	1	1		1		
CS	14(10.9%)	114(89.1%)	1.297	0.683-2.463	0.427	2.087	0.883-4.934	0.094
ANC Visit No	3(13.6%)	19(86.4%)	1	1		1		
One visit	7(7.6%)	85(92.4%)	0.579	0.127-2.638	0.480	1.625	0.542-4.883	0.609
Two visits	15(12.7)	103(87.3%)	0.302	0.092-0.990	0.048*	1.819	0.959-3.449	0.067
Three visits	26(14.4)	155(85.6%)	0.534	0.186-1.530	0.243	1.060	0.645-6.585	0.331
>Four visits	6(24.4%)	22(78.6%)	0.615	0.228-1.661	0.338	1.542	0.294-8.094	0.091
Hospital Stay<Two days	2(6.7%)	28(93.3%)	1	1		1	1	1
Three days	5(4.6%)	104(95.4%)	0.673	0.124-3.655	0.647	12.648	1.36-16.73	0.074
Four days	9(7.2%)	116(92.8%)	1.086	0.222-5.309	0.919	7.501	2.00-8.062	0.721
Five days	20(23.3%)	66(76.7%)	4.242	0.929-19.383	0.062	3.174	1.79-4.911	0.069
>= six days	21(23.1%)	70(76.9%)	4.200	0.923-19.111	0.063	0.854	0.332-2.19	0.073
No of pregnancy First	11(10%)	99(90%)	1	1		1		
Second pregnancy	16(10.8%)	132(89.2%)	1.091	0.485-2.454	0.833	0.608	0.005-6.328	0.609
Third pregnancy	6(9%)	61(91%)	0.886	0.311-2.516	0.819	0.932	0.203-6.581	0.221
Fourth pregnancy	9(16.4%)	46(83.6%)	1.761	0.682-4.543	0.242	0.814	0.645-4.300	0.387
>=fifth pregnancy	15(24.6%)	46(75.4%)	2.935	1.251-6.887	0.013*	1.797	0.645-0.984	0.035*

DISCUSSION

The result of this study demonstrated that prevalence of bacterial isolates causing septicemia was 12.9 % among blood cultures of septicemia suspected mothers. This finding was relatively comparable with a Lahore (Pakistan) study with 16.6% (17), but much lower than studies done in Sudan, 72.9 % (18) and India, 68.65% (19). The possible explanation for this variation might be due to differences in methodology, sample size and epidemiological difference of the etiological agent. Sepsis is extremely variable due to geographical conditions prevailing in a particular community, with regards to hygiene during performing delivery. The rate of reproductive tract infections, including those transmitted sexually, may also create variation in prevalence (20).

In this study, the majority of the isolates (57.9 %) were Gram-negative organisms. This is contrary to a study done in Gondar, Ethiopia (9) where the majority of isolates were Gram-positive bacteria, 69%. A study from Oman also reported 57.8% of isolates to be Gram-positive (21). However, in the study from Lahore, Pakistan, 60% of the isolates were Gram-negative bacilli (17). A recent study from India in 2016 reported a 67.4% proportion of Gram negative bacilli comparable to our current study (19). This variation might be related to the difference in the practice of using medical devices with which some Gram-positive bacteria are known to be directly associated or due to epidemiological difference of the etiological agents involved (22).

Increased antimicrobial resistance to the commonly used antibiotics leaves clinicians with very few choices of drugs for the treatment of septicemia (23). In this study, susceptibility pattern of Gram-negative bacteria showed that most of the isolates were sensitive to ciprofloxacin (88%), ceftriaxone (85%), chloramphenicol (66%), gentamicin (66%) and nalidixic acid (61%) which is similar to studies reported from Gondar, Ethiopia (9), Sudan (18), Nigeria (24) and Oman (21). This finding could be explained at least in part by the practice of the tendency to reserve third generation cephalosporin in the region limiting access without prescription (25) and to the fact that the drugs are quite expensive and, therefore, not likely to be purchased as frequently as cheaper alternatives.

For the most predominant Gram-negative isolate *E. coli,* higher susceptibility results were observed to ceftriaxone 92%, ciprofloxacin 92%, gentamicin 84% and chloramphenicol, 77%.

In the current study, resistance of *E. coli* was 100% to ampicillin and 92% each to amoxicillin and tetracycline.

This finding is comparable with a Gondar study (70%-85%) (90 and similar resistance frequency has been documented in Nigeria, (85 – 95%) (24), Oman (75-100%) (21) and India (70-100%)(26). This might signal an indiscriminate and continuous use of subtherapeutic doses of commonly available antimicrobials, frequent inappropriate prescription of these drugs by physicians and a growing tendency of self-prescription and misuse. This could further complicate the management of septic patients and increase morbidity and mortality (24).

In the present study, mothers who delivered babies more than five times were observed to have suffered more frequently from sepsis and this was statistically significant (p=0.017, COR: 2.812, 95% CI: 1.1.201, 6.587), (p=0.035, AOR: 0.797, 95% CI: 0.645, 0.984). In a similar study from Pakistan, 78.3 % of affected mothers were grand multiparous having a parity of five and above (27).

This study also showed that frequency of vaginal examination (>4) was a factor that was significantly associated with occurrence of sepsis (p=0.044, COR: 8.536, 95% CI: 1.059, 65.901), (p=0.001, AOR: 0.646, 95% CI: 0.505, 0.526). A similar study in Egypt reported that frequent vaginal examinations (OR = 5.1) were significantly related to the occurrence of puerperal sepsis (29). Studies done in India (20), Sudan (18) and Pakistan (28) showed that women who had high frequency of vaginal examination were more likely to have septicemia than those who had a lower frequency of vaginal examination. Multiple vaginal examinations allow infections to ascend from the vagina directly and lead to sepsis. Multiple vaginal examinations make mothers vulnerable to infection (20). Improving infection control measures during delivery, limiting the frequency of vaginal examinations, and avoiding all unhygienic practices related to delivery are strongly recommended (29).

Conclusion

The overall prevalence of septicemia was 12.9% (n=57/441). The results of this study showed the etiologic agents of septicemia were mainly Gram-negative bacteria, and that the predominantly isolated bacteria were *Coagulase negative staphylococci* and *E. coli.* Most of the bacterial isolates are resistant against commonly used broad spectrum antibiotics such as ampicillin, amoxicillin and tetracycline.

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