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RESEARCH ARTICLE

Bacterial Profile, Antimicrobial Susceptibility Patterns, and Associated Factors of Puerperal Sepsis in Asella, Central Ethiopia: A Cross-sectional Study

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Abstract:
Sepsis, caused by various bacterial pathogens, is a significant contributor to maternal mortality worldwide. In many developing countries, including Ethiopia, empirical or syndromic treatment is commonly employed for puerperal sepsis, which may promote antimicrobial resistance (AMR). We conducted a cross-sectional study to investigate bacterial pathogens, their antimicrobial susceptibility patterns, and associated factors among women with suspected puerperal sepsis attending Asella Referral and Teaching Hospital from September 2020 to August 2021. A total of 174 participants were enrolled, and the sociodemographic and obstetric data were collected using a pretested structured questionnaire and checklist, respectively. Blood samples (approximately 20 ml) were collected from all study participants and incubated in BacT/ALERT® 3D automated blood culture system. In addition, endocervical swabs were collected in Amies transport media. Bacterial isolation and identification were performed following standard bacteriological methods. Antimicrobial susceptibility profiles of bacterial isolates were determined using the disc diffusion method. Data were entered into EpiData version 4.6 and analyzed using SPSS version 25.0. The overall positivity rate of bacterial isolates among puerperal sepsis-suspected women was 48.9%. Among these, 87.1% of the isolates were Gram-negative bacteria. The most common isolates were *Escherichia coli* (54.1%), followed by *Klebsiella* spp. (23.5%) and *Staphylococci aureus* (10.6%). High resistance rates were observed in *E. coli* to piperacillin (87%), in *Klebsiella* spp. to aztreonam (65%) and ceftriaxone (65%), and in *S. aureus* to trimethoprim-sulfamethoxazole (66.6%). Multidrug-resistant bacterial pathogens accounted for 81.2% of the isolates in this study. Multivariate regression analysis did not reveal any statistically significant association between the presence of bacteria and the sociodemographic and obstetrics factors. Our findings emphasize the urgency of strengthening microbiology services to optimize patient management and combat AMR in puerperal sepsis.

Keywords: Puerperal sepsis, Antimicrobial resistance, Associated factors, Asella

1. Introduction

Sepsis is a major cause of maternal death worldwide. Puerperal sepsis is an infection of the genital tract occurring at any time between the rupture of membranes or labor and the 42nd days of postpartum. The risk of a woman in a developing country dying from a maternal-related cause during her lifetime is about 33 times higher compared to a woman living in a developed country [1,2].

The study findings indicate that the most significant predisposing factor for puerperal sepsis is delivery by cesarean section. At the same time, other factors also contribute to puerperal sepsis significantly, including home delivery under unhygienic conditions using dirty materials, low socioeconomic conditions, anemia, high parity, prolonged rupture of amniotic membrane, frequent per-vaginal examinations, prolonged labor, and postpartum hemorrhage [3].

A wide variety of bacteria (Gram-negative and Gram-positive) are responsible for causing puerperal sepsis. The major bacterial pathogens causing sepsis in the puerperium include Group A *Streptococcus* (*Streptococcus pyogenes*), Group B *Streptococcus* (*Streptococcus agalactiae)*, *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), *Escherichia coli*, *Klebsiella* species, *Pseudomonas aeruginosa, Proteus* species, *Citrobacter* species, *Clostridium septicum*, and *Morganella morganii* [4].

In developed countries, puerperal sepsis is treated based on the evidence from patients' microbiology results. However, in many developing countries, including Ethiopia, the treatment of puerperal sepsis is empirical or syndromic [5], mainly due to of inadequacy of microbiological diagnostic services. This empirical treatment, which involves the use of different classes of antibiotics, can lead to poor patient outcomes and the emergence of antimicrobial resistance (AMR) against various antibiotics. This, in turn, contributes to the development of multidrug-resistant organisms, such as MRSA and extended-spectrum β-lactamase (ESBL)-producing Gram-negative bacteria that challenge the puerperal sepsis treatment [6].

Although puerperal sepsis is a serious health issue in Ethiopia, there is scarce information on the prevalence, bacterial etiology agents, and AMR profile of the isolates that cause puerperal sepsis [7,8]. Therefore, the aim of this study was to investigate bacterial pathogens, their antimicrobial susceptibility patterns, and associated factors in women with puerperal sepsis at Asella Referral and Teaching Hospital (ARTH), Central Ethiopia.

2. Materials and methods

2.1. Study design, period, and setting

A cross-sectional study was conducted from September 2020 to August 2021 at ARTH, a public teaching and referral hospital located in the Arsi zone of Oromia regional state, Central Ethiopia. The hospital has a total of 347 beds, with 58 (16.7%) beds dedicated to the Gynecology and Obstetrics Department. According to the hospital's annual report for 2020, approximately 9,240 women sought care at the maternity ward/outpatient clinic of ATRH for delivery and abortion/miscarriage cases.

2.2. Inclusion and exclusion criteria

Puerperal sepsis-suspected women who fulfilled the World Health Organization (WHO) criteria for puerperal sepsis [5] and provided written consent were included as study participants. Women with signs of infection before delivery or abortion/miscarriage and those unwilling to provide both blood culture and endocervical swab samples for various reasons were excluded from this study.

2.3. Sample size determination

The sample size was determined using a single population proportion formula [9] as follows:

$$n = Z2p(1-p)/d^2 \quad \text{(I)}$$

Where n = The number of postpartum or aborted/miscarriage women to be involved in this study; Z = Standard normal distribution value at 95% CI, which is 1.96; p = The prevalence of puerperal sepsis determined at 12.9% [4]; and d = The margin of error, taken as 5%. Accordingly, the sample size was 174 puerperal sepsis-suspected women who fulfilled the WHO criteria for puerperal sepsis.

2.4. Data collection

Sociodemographic data of study participants were collected through face-to-face interviews using a structured questionnaire. Obstetric and clinical data of the study participants were collected using a standard checklist by midwifery nurses in consultation with a gynecologist.

2.5. Sample collection and processing

Two bottles of blood samples (approximately 10 ml for each vial) were collected from all study participants using a sterile vacutainer needle aseptically after proper disinfection. The blood samples were placed into separate blood culture bottles (BacT/ALERT® 3D aerobic and anaerobic vials). Endocervical swab samples were also collected from all study participants following standard protocol, and they were put in Amies transport medium (Copan Italia Spa, Italy) for transportation to the Laboratory of Hirsch Institute of Tropical Medicine (HITM), Asella, Ethiopia, immediately. At the laboratory, bacterial isolation, identification, and antimicrobial susceptibility test (AST) were performed.

2.6. Culturing and identification of bacterial isolates

Both aerobic and anaerobic blood culture bottles were incubated in the BacT/ALERT® 3D automated blood culture system, following the standard instructions of the manufacturer. Considering the Gram staining result, all positive blood cultures showing growth within 7 days in the machine were sub-cultured on a blood agar plate (Oxoid Ltd Basingstoke, Hampshire, UK), a chocolate agar plate (incubated at 5% CO_2 atmosphere in an anaerobic incubator) and a MacConkey agar plate (Oxoid Ltd Basingstoke, Hampshire, UK). These sub-cultures were then examined for growth after 24 – 48 h of incubation. Blood culture bottles that showed negative in the machine after the 7th day were discarded, and the results were recorded as "no growth."

Endocervical swab samples were cultured on a blood agar plate (Oxoid Ltd Basingstoke, Hampshire, UK), chocolate agar plate (incubated at 5% CO_2 atmosphere in an anaerobic incubator), and MacConkey agar plate (Oxoid Ltd Basingstoke, Hampshire, UK). The samples were examined for growth after 24 – 48 h of incubation.

Preliminary identification of those sub-cultured bacterial isolates was based on cultural characteristics, such as colonial morphology, hemolysis pattern, and Gram reaction. Further identification of Gram-negative bacteria was carried out using common biochemical tests, including indole, glucose and lactose fermentation, citrate utilization, urease, gas and H_2S production, and oxidase tests. Motility was determined by observing bacterial movement under a microscope. For Gram-positive bacteria, biochemical tests, such as catalase, coagulase and CAMP tests, were conducted [10].

2.7. AST

AST was performed on Mueller–Hinton agar (Oxoid, UK) using the disc diffusion technique with the following antibiotic discs: For Gram-positive bacteria, the disc contains piperacillin (30 μg), cefoxitin (30 μg), ceftriaxone (30 μg), ciprofloxacin (5 μg), trimethoprim-sulfamethaxazole (25 μg), amikacin (30 μg), gentamicin (10 μg), erythromycin (15 μg), and clindamycin (2 μg); for Gram-negative bacteria, the disc contains piperacillin (30 μg), cefoxitin (30 μg), ceftazidime (10 μg), ceftriaxone (30 μg), meropenem (10 μg), aztreonam (30 μg), ciprofloxacin (5 μg), amikacin (30 μg), gentamicin (10 μg), nitrofurantoin (100 μg), and trimethoprim-sulfamethaxazole (25 μg). Antibiotic breakpoints were interpreted according to the European Committee on AST (EUCAST) guidelines, version 9.0 [10]. *E. coli* (ATCC-25922), *S. aureus* (ATCC-25923), and *P. aeruginosa* (ATCC 27853) were included as reference strains to assure the quality of antibiotic discs.

2.8. Quality control

The sterility of culture media was ensured by incubating 5% of each batch of the prepared media at 37°C for 24 h. The quality of the culture media, staining and biochemical test reagents, and antibiotic disc performance was assured by including international standard control strains, such as *E. coli* (ATCC 25922) for Gram-negative bacteria, *S. aureus* (ATCC 25923) for Gram-positive bacteria, throughout all assays [11].

2.9. Data analysis

Data were entered into Epi-Data version 4.6 and transferred to Statistical Package for the Social Sciences (SPSS) version 25.0 for analysis. Basic descriptive measures were used to summarize and present findings. Logistic regression was performed, and all variables with $P < 0.25$ in the bivariate regression analysis were further analyzed in multivariate regression analysis. The odds ratio and 95% confidence interval were computed, and statistical significance was declared at $P < 0.05$.

2.10. Ethical consideration

All methods were carried out in accordance with the Declaration of Helsinki. Ethical clearance was obtained from the Health Institute Ethics Review Committee of Jimma University

(Ref. No.: IRB000153/2020) and from the Institutional Ethical Review Board of Arsi University (Ref. No.: A/U/H/S/C/120/13084/2012). Informed consent was obtained from each participant. Participant information was kept confidential, and positive results were delivered to the concerned physicians for patient management.

3. Results

3.1. Sociodemographic and obstetric characteristics

A total of 174 puerperal sepsis-suspected women were enrolled in this study. The participants' median age was 25 years (interquartile range: 21 – 30). The majority of study participants (51.1%) were between 25 and 34 years old, resided in rural areas (63.2%), were married (93.7%), and had no history of genital mutilation (55.7%) (**Table 1**).

3.2. Proportion of bacterial isolates

The overall culture positivity rate (from either blood or endocervical swab) was 48.9%. Among these, 89.4% (76) of bacterial growth was from endocervical swabs, while 10.6% (9) of bacterial growth was from blood culture. The majority of the growth (74 [87.1%]) comprised Gram-negative bacteria. The most frequently isolated bacteria were *E. coli*, with 46 (54.1%) occurrences, followed by *Klebsiella* spp. with 20 (23.5%) occurrences and *S. aureus* with 9 (10.6%) occurrences (Figure 1).

3.3. AST profile of Gram-negative bacteria

As shown in **Figure 2**, among the total 74 Gram-negative bacterial isolates, high resistance rates to piperacillin (73%), trimethoprim-sulfamethoxazole (60.8%), and aztreonam (60.8%) were observed, while low resistance rates to amikacin (12.2%) and meropenem (20.3%) were observed. *E. coli* showed a high level of resistance to piperacillin (87%), and all *Acinetobacter* spp. were resistant to five of the total 11 tested drugs. Of the 74 Gram-negative isolates, 64 (86.5%) were multidrug-resistant (multidrug-resistant [MDR], resistant to at least two or more different classes of tested antibiotics).

3.4. AST profile of Gram-positive bacteria

As summarized in **Table 2**, high resistance rates to trimethoprim-sulfamethoxazole (63.6%) and erythromycin (54.5%) were observed. On the other hand, low resistance rates to ceftriaxone (9%), cefoxitin (9%), and piperacillin (9%) were observed. *S. aureus* (which is the dominant isolate) showed a high level of resistance to trimethoprim-sulfamethoxazole (66.7%) and erythromycin (66.7%). Of the 11 Gram-positive isolates, 5 (45.5%) were MDR (**Table 2**).

Table 1. Sociodemographic characteristics of puerperal sepsis-suspected women at Asella Referral and Teaching Hospital, Central Ethiopia, from September 2020 to August 2021

Variable	Categories	Frequency (n [%])	Bacterial isolates	
			Yes (n [%])	No (n [%])
Age group (years)	18–24	68 (39.1)	30 (44.1)	38 (55.9)
	25–34	89 (51.1)	39 (43.8)	50 (56.2)
	35–42	17 (9.8)	12 (70.6)	5 (29.4)
Residence	Rural	110 (63.2)	51 (46.4)	59 (53.6)
	Urban	64 (36.8)	30 (46.9)	34 (53.1)
Education	No education	40 (23.0)	21 (52.5)	19 (47.5)
	Primary	67 (38.5)	35 (52.2)	32 (47.8)
	Secondary	56 (32.2)	22 (39.3)	34 (60.7)
	More than secondary	11 (6.3)	3 (27.3)	8 (72.7)
Occupation	Housewife	50 (28.7)	16 (32.0)	34 (68.0)
	Farmer	104 (59.8)	54 (51.9)	50 (48.1)
	Employee	20 (11.5)	11 (55.0)	9 (45.0)
History of genital mutilation	Yes	77 (44.3)	40 (51.9)	37 (48.1)
	No	97 (55.7)	41 (42.3)	56 (57.7)

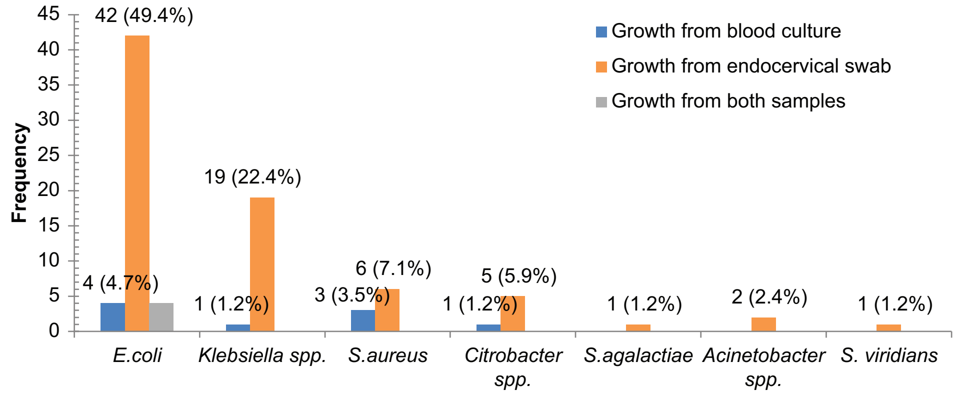

Figure 1. Frequency of bacterial isolates from blood culture and endocervical swab samples of puerperal sepsis suspected women.

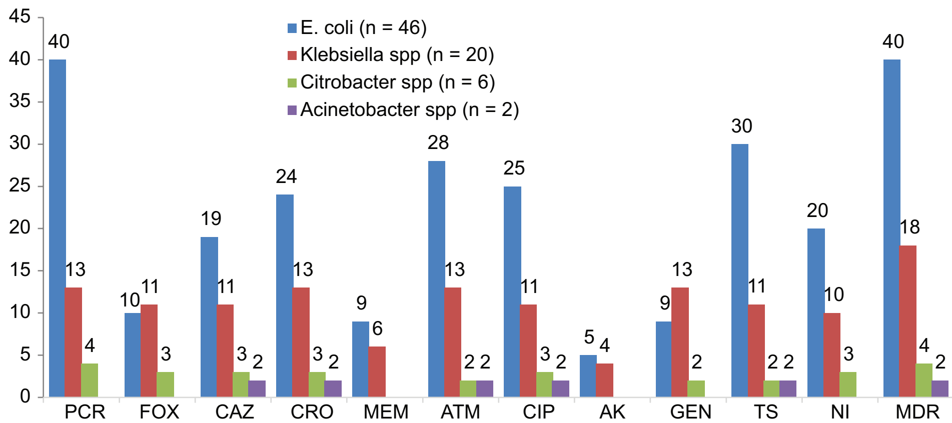

Figure 2. Resistance patterns of Gram-negative bacterial isolates from puerperal sepsis women. Abbreviations: AK: Amikacin; ATM: Aztreonam; CAZ: Ceftazidime; CIP: Ciprofloxacin; CRO: Ceftriaxone; FOX: Cefoxitin; GEN: Gentamicin; MDR: Multidrug-resistant; MEM: Meropenem; NI: Nitrofurantoin; PCR: Piperacillin; TS: Trimethoprim-sulfamethoxazole.

Table 2. Antimicrobial susceptibility patterns of Gram-positive bacterial isolates from puerperal sepsis-suspected women at Asella Referral and Teaching Hospital, Central Ethiopia, from September 2020 to August 2021

Bacterial isolates (n)	Resistance (n [%])									MDR
	AST	PCR	FOX	CRO	CIP	AK	GEN	TS	E	CD	
S. aureus (9)	S	8 (88.9)	9 (100)	9 (100)	6 (66.7)	7 (77.8)	7 (77.8)	3 (33.3)	3 (33.3)	7 (77.8)	3 (33.3)
	R	1 (11.1)	-	-	3 (33.3)	2 (22.2)	2 (22.2)	6 (66.6)	6 (66.7)	2 (22.2)	
S. agalactiae (1)	S	1 (100)	1 (100)	1 (100)	-	-	-	-	1 (100)	1 (100)	1 (100)
	R	-	-	-	1 (100)	1 (100)	1 (100)	1 (100)	-	-	
S. viridians (1)	S	1 (100)	-	-	-	-	-	1 (100)	1 (100)	-	1 (100)
	R	-	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	-	-	1 (100)	
Total (11)	S	10 (91.0)	10 (91.0)	10 (91.0)	6 (54.5)	7 (63.6)	7 (63.6)	4 (36.4)	5 (45.5)	8 (72.7)	5 (45.5)
	R	1 (9.0)	1 (9.0)	1 (9.0)	5 (45.5)	4 (36.4)	4 (36.4)	7 (63.6)	6 (54.5)	3 (27.3)	

Abbreviations: AK: Amikacin; AST: Antimicrobial susceptibility test; CD: Clindamycin; CIP: Ciprofloxacin; CRO: Ceftriaxone; E: Erythromycin; FOX: Cefoxitin; GEN: Gentamicin; MDR: Multidrug-resistant; PCR: Piperacillin; R: Resistant; S: Susceptible; TS: Trimethoprim-sulfamethoxazole; *S. aureus: Staphylococcus aureus; S. agalactiae: Streptococcus agalactiae; S. viridians: Streptococcus viridians.*

4. Discussion

In Ethiopia, numerous measures have been taken to address maternal and newborn health problems; however, mortality and morbidity rates remain high. This persistent challenge is attributed to the ongoing difficulty in clearly identifying the underlying causes of maternal mortality and morbidity [12].

In the current study, the proportion of bacterial isolates from blood culture was 10.6%, which is comparable with the findings of a study conducted in Dire Dawa, Ethiopia (12.9%) [4], and Tanzania (11.2%) [13]. However, it is higher than the reported rates from Zimbabwe (2%) [14] and the USA (3.2%) [15] and much lower than those reported from Bahir Dar, Ethiopia (33.7%) [8], India (68.65%) [16], and Sudan (72.9%) [17].

In this study, the proportion of bacterial isolates from endocervical swabs was 89.4%, which is consistent with the findings of studies conducted in Nigeria, with a proportion of 82.7% [18], and Tanzania, with 90.5% [19]. In contrast, another study from Tanzania reported a lower proportion (43.6%), while proportions reported in [13] India (52.6%) [20] and Zimbabwe (68.2%) [15] were also lower. A higher proportion was reported in Nigeria (99.2%) [21]. The differences in proportions between our study and others might be attributed to variations in infection prevention practices among different health institutions, the management of laboring mothers by clinicians, and the availability of microbiology laboratory facilities for identifying etiologic agents and determining their antimicrobial susceptibility profiles.

The higher proportion of bacterial isolates from an endocervical swab compared to a blood sample could be due to puerperal sepsis-causing bacteria, such as *S. aureus* and *E. coli*, which may be secondary invaders or vaginal contaminants introduced during sample collection. This is supported by a study conducted in Benin, Southern Nigeria, which found these microorganisms to be the most commonly isolated from the genital tracts of asymptomatic pregnant women [22].

The most frequently isolated Gram-negative bacteria were *E. coli* (54.1%) and *Klebsiella* spp. (23.5%). This finding is consistent with a study reported from Tanzania and Zimbabwe [12], where *E. coli* and *Klebsiella* spp. were also the predominant bacterial isolates from puerperal septic patients. However, our findings differ from those of other studies, which reported Group A *streptococcus*, Group B *streptococcus, Bacteroides* spp.*, P. aeruginosa, Proteus* spp.*, and Enterococcus* spp. as the dominant isolates [14,15,23,24].

The dominancy of *E. coli* and *Klebsiella* spp. could be attributed to their presence as normal flora of the gastrointestinal tract. In addition, they are significant etiological agents of hospital-acquired infections, such as urinary tract infections, pneumonia, and septicemia, particularly among immune-compromised individuals [25,26].

The most frequently isolated Gram-positive bacteria was *S. aureus* (10.6%) which is consistent with other studies reporting *S. aureus* isolates as a common pathogenic bacteria isolated from puerperal sepsis patients [18,27]. *S. aureus* is frequently found on the skin and can easily cause contamination during both vaginal and cesarean deliveries [28].

E. coli, the most common Gram-negative isolates, showed high sensitivity to amikacin (89.1%), gentamycin (80.4%), and cefoxitin (78.3%), but low sensitivity to piperacillin (13%). These findings are consistent with a study conducted in the USA, where 90.5% of *E. coli* isolates were sensitive to gentamicin [15]. The high penicillin resistance observed could be attributed to negative selective pressure exerted by the overuse of this antibiotic. *E. coli* is known to have resistant genes for beta-lactam agents, including piperacillin [29]. *Klebsiella* spp., the second most common isolate, showed relatively high (65%) resistance to ceftriaxone, which is in agreement with a study conducted in Bahir Dar, Ethiopia, where 57.1% of *Klebsiella* spp. were resistant to ceftriaxone [8].

All *S. aureus* isolates were sensitive to ceftriaxone and cefoxitin, which is similar to the study findings reported from Nigeria, where all *S. aureus* isolates were 100% sensitive to ceftriaxone, ceftazidime, ciprofloxacin, and ofloxacin [21]. The overall proportion of MDR in this study was 81.2%. Similar findings were reported from Bahir Dar Referral Hospital, Northwest Ethiopia (84%) [8] and Uganda (80%) [30], while a higher value was reported from Zimbabwe (10.9%) [14]. A high rate of MDR was observed among *Klebsiella* spp. (90%) *and E. coli* (87%). This might be due to ESBL-producing *Enterobacteriaceae*, which have intrinsic resistance mechanisms; most importantly, they have chromosomal and plasmid-encoded beta-lactam hydrolyzing enzymes [31].

In the current study, multivariate regression analysis did not show a statistically significant association between sociodemographic and obstetrics factors and the presence of a bacterial pathogen. This lack of significance may be attributed to the small sample size and the cross-sectional study design. However, among women aged >34 years, 12 (70.6%) demonstrated a higher proportion of culture-positive bacterial infection. This finding is inconsistent with another study where the majority of women admitted with puerperal sepsis were above 30 years of age, comprising 65.11% of cases [32]. The difference in proportions could probably be due to age-related changes in immunity and the effect of multiple deliveries on susceptibility to infections.

Women having education above secondary school showed a low positivity rate (3 in 81 [3.7%]) than those with lower than secondary education (38 in 81 [46.9%]) and no education (40 in 81 [49.4%]). This finding is consistent with another study conducted in Bahir Dar, Ethiopia [8]. The higher educational status of women could potentially increase health-seeking behavior and a higher standard of living, contributing to the lower rates of puerperal sepsis.

This study has certain limitations. First, the sample size was relatively small, which limited our ability to perform more detailed data analysis on the risk factors of puerperal sepsis at the study site. In addition, the species of the isolates were not confirmed by VITEK or MALDI-TOF, and the AST results were also not validated using VITEK or molecular techniques.

5. Conclusion

Bacterial growth was detected in the blood or endocervical smear of nearly 50% of mothers suspected of having puerperal sepsis. The percentage of bacterial isolates from blood cultures was 10.6%, which is consistent with the growth rate observed in the previous study on adult sepsis at the same hospital. The primary causative agents of puerperal sepsis at the study site were *E. coli* and *Klebsiella* spp. among Gram-negative isolates and *S. aureus* among Gram-positive isolates.

E. coli, the dominant isolated bacteria among Gram-negative isolates, showed high sensitivity against amikacin, gentamycin, and cefoxitin. In contrast, all isolates of *S. aureus* (the dominant Gram-positive isolate) were sensitive to ceftriaxone and cefoxitin.

It is important to note that the causative agents of puerperal sepsis and their antibiotic sensitivity patterns can vary over time and among different hospitals. Therefore, timely laboratory-based study results can assist health personnel in effectively managing women with puerperal sepsis.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

Conceptualization: Abduselam Abbiso Godana, Mulatu Gashaw, Getenet Beyene
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Writing review & editing: Getenet Beyene, Mulat Gashaw

Ethics approval and consent to participate

All methods were carried out in accordance with the Declaration of Helsinki. The study was approved by the Institutional Review Board (IRB) of Health Institute, Jimma University (Ref No: IRB000153/2020) and the Institutional Ethical Review Board of Arsi University (Ref No: A/U/H/S/C/120/13084/2012). Written informed consent was obtained from each study participant prior to inclusion in the study, and confidentiality was assured.

Consent for publication

The study participants were informed and they agreed that the data of the study can be published.

Availability of data

All the data pertinent to this study are presented in the manuscript. Raw data can be obtained from the corresponding author following a formal request.

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