Puerperal sepsis: Bacterial profile, Antimicrobial susceptibility patterns and Associated factors at Asella Referral and Teaching Hospital, Central Ethiopia: Cross sectional study

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Abstract

Background: Sepsis is a major cause of maternal death worldwide and caused by a variety of bacterial pathogens. In many developing countries including Ethiopia, the treatment of puerperal sepsis is based on empirical/syndromic that may promote antimicrobial resistance. Therefore the aim of this study was to investigate bacterial pathogens, their antimicrobial susceptibility patterns, and associated factors among women with suspected puerperal sepsis.

Methods: A cross-sectional study was conducted among puerperal sepsis suspected women attending Asella Referral and Teaching Hospital, from September 2020 to August 2021. A total of 174 study participants were enrolled. Sociodemographic and obstetric data of the participants were collected using a pretested structured questionnaire and checklist respectively. About 20 ml blood sample was collected from all study participants into BacT/ALERT® 3D blood culture bottles and incubated into BacT/ALERT® 3D automated blood culture system. Endocervical swab was also collected into Aime's transport media. Bacterial isolation and identification was done using standard bacteriological methods. Disc diffusion method was used to determine the antimicrobial susceptibility profiles of bacterial isolates. Data were entered into EpiData version 4.6 and transferred to SPSS version 25.0 for analysis.

Results: The overall positivity rate of bacterial isolates among puerperal sepsis suspected women was 48.9%. Out of these 87.1% of the isolates were Gram negative bacteria. The most common isolates were *E. coli* (54.1%) followed by *Klebsiella* spp. (23.5%) and *S. aureus* (10.6%). *E. coli* showed a higher resistance rate to Piperacillin (87%). *Klebsiella* spp. showed a higher resistance rate to Aztreonam (65%) and Ceftriaxone (65%). *S. aureus* showed a higher resistance rate to Trimethoprim-sulfamethoxazole (66.6%). In this study, 81.2% of the isolates were multi-drug resistant bacterial pathogens. Multivariate regression analysis showed no statistically significant association between sociodemographic, obstetrics factors, and having bacteria.

Conclusion: In this study the overall positivity rate in this study was 48.9%. *E. coli*, *Klebsiella* species, and *S. aureus* were the most common isolated bacteria. High numbers of multidrug-resistant bacterial isolates were identified. Our finding emphasizes the need for strengthening microbiology services for better management of patients.

Background

Sepsis is a major cause of maternal death worldwide. Puerperal sepsis is 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].

Study finding indicated that the most important predisposing factor for puerperal sepsis is delivery by cesarean section, while other factors also have a great contribution including home delivery related to unhygienic conditions using dirty materials, low socioeconomic condition, anemia, parity, prolonged rupture of amniotic membrane (PROM), frequent per-vaginal examinations, prolonged labor, and postpartum hemorrhage have a great contribution[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 are group A *streptococcus* (*Streptococcus pyogenes*), group B *streptococcus (Streptococcus agalactiae)*, *Staphylococcus aureus*, Methicillin-Resistant *Staphylococcus 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 with the support of evidence from patients' microbiology results. However, in many developing countries including Ethiopia, the treatment of puerperal sepsis is empirical/syndromic [5], mainly due to inadequate microbiological diagnostic services. This empirical treatment with different classes of antibiotic leads to a poorer patient outcomes and emergence of antimicrobial resistance(AMR) against various antibiotics that leads to the development of multidrug-resistant organisms (MDROs) such as methicillin resistance *S. aureus* (MRSA) and extended-spectrum β-lactamase (ESBL) producing Gram negative bacteria that challenges the puerperal sepsis treatment [6].

Though puerperal sepsis is a serious health issues in Ethiopia, there is inadequate information on prevalence, bacterial etiology agent and antimicrobial resistance 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.

Materials And Methods

Study Design, Period And Setting

A cross-sectional study was conducted from September 2020 to August 2021 at ARTH. ARTH is a public teaching and referral hospital found in the Arsi zone of Oromia regional state, central Ethiopia. The hospital has 347 beds of which 58 (16.7%) beds are found in Gynecology and Obstetrics departments/wards. According to the annual data of 2020 hospital report about 9,240 women were attended maternity ward/outpatients of ATRH for delivery and abortion/miscarriage cases.

Inclusion And Exclusion Criteria

Puerperal sepsis suspected women who fulfilled the WHO criteria for puerperal sepsis [5] and gave written consent were our study participants and women with a sign of infection before delivery or abortion/miscarriage and women who were not willing to give both blood culture and endocervical swab samples due to different reason were excluded from this study.

Sample Size Determination

The sample size was determined using a single population proportion formula as follows: n = Z2p (1-p)/ d2; 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] d = the margin of error, taken as 5%. Accordingly, the sample size were 174 puerperal sepsis suspected women who fulfilled the WHO criteria for puerperal sepsis.

Data Collection

Socio-demographic data of study participants were collected by face to face interview using a structured questionnaire by interviewing the study participants. Obstetric and clinical data of the study participants were collected using standard checklist. Data was collected by midwifery nurses in consultation with gynecologist.

Sample Collection And Processing

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

Culturing And Identification Of Bacterial Isolates

Both aerobic and anaerobic blood culture bottles were incubated into 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 seven days with the machine were subcultured on a blood agar plate (Oxoid Ltd Basingstoke, Hampshire, UK), a chocolate agar plate (incubated at 5% CO2 atmosphere in an anaerobic incubator) and a MacConkey agar plate (Oxoid Ltd Basingstoke, Hampshire, UK) and examined for growth after 24–48 hours of incubation. All blood culture bottles negative by the machine after the 7th day were discarded and the results were recorded as “no growth”.

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

Preliminary identification of those sub-cultured bacterial isolates was done based on cultural characteristics such as colonial morphology, hemolysis pattern and Gram’s reaction. Further identification of Gram negative bacteria was carried out by performing

common biochemical tests like motility, indole, glucose and lactose fermentation, citrate utilization, urease, gas and H_2S production, oxidase tests. For Gram positive bacteria biochemical tests like catalase, coagulase and CAMP tests, were done [9].

Antimicrobial Susceptibility Testing (Ast)

AST was performed on Mueller-Hinton (Oxoid, UK) using disc diffusion technique for the following antibiotic discs. For Gram positive: - Piperacillin (30µg), Cefoxitin (30µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Trimethoprim-sulphamethaxazole (25µg), Amikacin (30µg), Gentamicin (10µg), Erythromycin (15 µg) and Clindamycin (2µg). For Gram negatives, Piperacillin (30µg), Cefoxitin (30µg), Ceftazidime (10µg), Ceftriaxone (30µg), Meropenem (10µg), Aztreonam (30µg), Ciprofloxacin (5µg), Amikacin (30µg), Gentamicin (10µg), Nitrofurantoin (100µg), Trimethoprim-sulphamethaxazole (25µg). Antibiotic breakpoints were defined according to European Committee on Antimicrobial Susceptibility Testing (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 quality of antibiotic disk.

Quality Control

The sterility of culture media was ensured by incubating 5% of each batch of the prepared media at 37^0c for 24 hours. Culture media, staining and biochemical test reagents and antibiotic disk Performance were 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 through the all assays [10].

Data analysis

Data were entered into Epi-Data version 4.6 and transferred to SPSS version 25.0 for analysis. Basic descriptive measures were determined to summarize and present findings. Logistic regression was performed and all variables with a p-value < 0.25 in bivariate regression analysis were further analyzed in multivariate regression analysis. Odds ratio and 95% confidence interval were computed and statistical significance was declared at p-value less than 0.0

Ethical Consideration

All methods were carried out in accordance with in accordance with the declaration of Helsinki. Ethical clearance was obtained from Jimma University, Health Institute Ethics Review Committee (Ref No; IRB000153/2020) and from Institutional Ethical Review board of Arsi University (Ref No; A/U/H/S/C/120/13084/2012). Informed consent was obtained from each participants. Participant's information was kept confidential, and positive results were delivered to the concerned physicians for management of the patients.

Result

Socio-demographic and obstetric characteristics

A total of 174 puerperal sepsis suspected women were enrolled in this study. The median age of the participants was 25 years (IQR: 21-30). The majority of study participants (51.1%) were between 25-34 years old, rural residence (63.2%), married (93.7%) and females (55.7%) (Table 1).

Proportion of bacterial isolates

The overall culture positivity rate (either from blood or endocervical swab) was 48.9%. Of this 89.4 bacterial growth were from endocervical swabs, whereas 9(10.6%) bacterial species growth were from blood culture. Majorly 74 (87.1%) of the growth were Gram negative bacteria. The most frequently isolated bacteria were E. coli 46 (54.1%) followed by Klebsiella species 20 (23.5%) and S. aureus 9 (10.6%), respectively (Figure 1)

Antimicrobial susceptibility test profile of Gram negative bacteria

As shown in Table 2, among the total 74 Gram-negative bacterial isolates, a high resistance rate was found to piperacillin (73%), trimethoprim-sulfamethoxazole (60.8%), and aztreonam (60.8%). A lower resistance rate was observed to Amikacin (12.2%) and Meropenem (20.3%). E. coli showed high level of resistance to Piperacillin (87%), and all Acinetobacter spps were resistance to five of the total 11 tested drugs. From 74 Gram negative isolates 64 (86.5%) were MDR (resistant to at least two or more different classes of tested antibiotics).

AST profile of Gram positive bacteria

As summarized on table 3, a high rate of resistance was observed to Trimethoprim-sulfamethoxazole (63.6%) and Erythromycin (54.5%). A lower rate of resistance was observed for Ceftriaxone (9%), Cefoxitin (9%), and Piperacillin (9%). S. aureus (which is the dominant isolate) showed high level of resistance to Trimethoprim-sulfamethoxazole (66.7%) and Erythromycin (66.7%). From 11 Gram positive isolates 5(45.5%) were MDR (Table 3).

Discussion

In Ethiopia, many measures have been taken to address maternal and newborn health problems, but mortality and morbidity rates are still high. This is mainly due to the fact that the causes of maternal mortality and morbidity have not been clearly identified [11].

In the current study the proportion of bacterial isolates from blood culture was 10.6% which is comparable with the study conducted in Dire Dawa, Ethiopia 12.9% [4] and Tanzania 11.2% [12]. However, it is higher than the study findings from Zimbabwe 2% [13] and USA 3.2% [14] and much lower than reported from Bahir Dar, Ethiopia 33.7% [8], India 68.65%[15] and Sudan 72.9% [16].

The proportion of bacterial isolates from an endocervical swab was 89.4%, which is in line with the study done in Nigeria with a proportion of 82.7% [17] and Tanzania 90.5% [18]. However it was higher than the studies from Tanzania 43.6%, [12] India 52.6% [19], and Zimbabwe 68.2% [13]. Higher proportion was reported from Nigeria 99.2% [20]. Proportion of differences of our study compared with other studies might be due to differences in infection prevention practice among different health institution, management of laboring mothers by clinicians, and availability of microbiology laboratory facility for identification of etiologic agents and determining their antimicrobial susceptibility profile.

The most frequently isolated Gram negative bacteria were *E. coli* (54.1%) and *Klebsiella* species (23.5%). This is similar with the study report from Tanzania and Zimbabwe [12] where *E. coli* and *Klebsiella* species were the majority bacterial isolates from puerperal septic patients. However, our findings is different from other study findings where group A streptococcus, group B streptococcus, *Bacteroides species, Pseudomonas aeruginosa, Proteus, and Enterococcus species* were the dominant isolates [14, 15, 21, 22].

The dominancy of *E. coli* and *Klebsiella* species could be that these bacteria *are* normal flora of the gastrointestinal tract and which are a significant cause of hospital-acquired infections (urinary tract infection, pneumonia, and septicemia) especially among immune-compromised individuals [23].

The most frequently isolated gram positive bacteria was *S. aureus* (10.6%) which is comparable with other studies where *S. aureus* isolates were the common pathogenic bacteria isolated from puerperal sepsis patients [17, 24, 25]. *S. aureus* is frequently found on the skin that can easily cause contamination during vaginal delivery or delivery with caesarian section [26].

E. coli, the most common Gram-negative isolates, showed higher sensitivity to Amikacin (89.1%), Gentamycin (80.4%), and Cefoxitin (78.3%), and least sensitivity to Piperacillin (13%). This is comparable with a study conducted in the USA where 90.5% of *E. coli* were sensitive to gentamicin [14]. High penicillin resistance could be due to negative selective pressure exerted by the overuse of this antibiotic. It is known that *E. coli* has resistant genes for beta-lactam agents including piperacillin [27]. *Klebsiella* species which is the second common isolate showed relatively high (65%) resistance to Ceftriaxone. This finding agreed with a study done in Bahir Dar, Ethiopia where 57.1% of *Klebsiella* species were resistant to ceftriaxone [8].

All (100%) *S. aureus* isolates were sensitive to Ceftriaxone and Cefoxitin, which is similar with study findings reported from Nigeria where *S. aureus* were 100% sensitive to ceftriaxone, ceftazidime, ciprofloxacin, and ofloxacin [20]. 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%) [28], while higher value was reported from Zimbabwe, 10.9% [13]. High rate of MDR was observed among *Klebsiella* species (90%) *and E.*

coli (87%). This might be that ESBL producing Enterobacteriaceae has intrinsic resistance mechanisms, most importantly, they have chromosomal and plasmid-encoded beta-lactam hydrolyzing enzymes [29].

In the current study, multivariate regression analysis did not show a statistically significant association between socio-demographic and obstetrics factors with having bacterial pathogen. This could probably be due to the small sample size and a cross-sectional study design. However, women with ages greater than 34 years 12 (70.6%) demonstrated a higher proportion of culture-positive bacterial infection. This is inconsistent with another study findings where the majority of women admitted with puerperal sepsis were above thirty years of age 65.11% [30]. This could probably be due to decreasing in immunity as age increases and having multiple deliveries.

Women having education above secondary school showed lower positivity rate (27.3%) which is in line to another study from Bahir Dar, Ethiopia [8]. This could be as the educational status of women increases their health-seeking behavior and their standard of living increases.

This study has limitations. The sample size was too small to perform further data analysis on the risk factors of puerperal sepsis at the study site. The species of the isolates were not confirmed by VITEK or MALDI-TOF, and the AST results were also not confirmed by VITEK or molecular techniques.

In conclusion nearly 50% of mothers suspected of having puerperal sepsis had bacterial growth in blood or endocervical smear. In this study the percentage of bacterial isolates from blood cultures was 10.6%, which is comparable to the growth rate in adult sepsis at the same hospital of previous study. The main causes of puerperal sepsis at the study site were *E. coli* and *Klebsiella* spp. among Gram-negative bacteria and *S. aureus* among Gram-positive isolates. Therefore, our study warrants the need for a microbiology laboratory with high compliance during this resistance era.

Abbreviations

PROM: prolonged rupture amniotic membrane; MRSA: Methicillin-Resistant *Staphylococcus aureus*; MDROs: multidrug-resistant organisms; ARTH: Asella Referral and Teaching Hospital; ESBL: extended-spectrum β-lactamase

Declarations

Author's contributions

AA, BG, GM, and AK were responsible for the formulation and designing of the research topic, acquisition of data and data analysis, interpretation of results and drafting of the manuscript. GM, AK, and AF were contributed in the designing the study, supervised the data collection process and data analysis, writing as well as the review of the drafted manuscript. AK and GM were critically reviewed and approved the manuscript. All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the analysis. All authors read and approved the final manuscript.

Competing interests

We verify that we all the authors have agreed to share the outcome of this manuscript equally and there is no conflict of interest among us.

Availability of data and materials

All the data pertinent to this study are presented in the manuscript. Raw data can be presented by principal investigator upon reasonable request.

Consent for publication

Not applicable.

Ethics and consent to participate

All methods were carried out in accordance with in accordance with the declaration of Helsinki.

The study was approved by 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 sought from each study participant prior to inclusion in the study and confidentiality was assured.

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Tables

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

Variable	*Frequency*	*Percentage*
Age group (years)		
18-24	68	39.1
25-34	89	51.1
35-42	17	9.8
Residence		
Rural	110	63.2
Urban	64	36.8
Marital status		
Single	1	0.6
Married	163	93.7
Divorced	8	4.6
Widowed	2	1.1
Maternal Education		
No education	40	23.0
Primary	67	38.5
Secondary	56	32.2
More than secondary	11	6.3
Maternal occupation		
Housewife	50	28.7
Farmer	104	59.8
Employee	20	11.5
History of Female		
Genital Mutilation	77	44.3
Yes	97	55.7
No		

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

Isolates	Antibiotics No (%)											
		PCR	FOX	CAZ	CRO	MEM	ATM	CIP	AK	GEN	TS	NI	MDR
E. coli (n=46)	S	6 (13.0)	36 (78.3)	27 (58.7)	22 (47.8)	37 (80.4)	18 (39.1)	21 (45.7)	41 (89.1)	37 (80.4)	16 (34.8)	26 (56.5)	40 (87.0)
	R	40 (87.0)	10 (21.7)	19 (41.3)	24 (52.2)	9 (19.6)	28 (60.9)	25 (54.3)	5 (10.9)	9 (19.6)	30 (65.2)	20 (43.5)	
Klebsiella spp.(n=20)	S	7 (35.0)	9 (45.0)	9 (45.0)	7 (35.0)	14 (70.0)	7 (35.0)	9 (45.0)	16 (80.0)	7 (35.0)	9 (45.0)	10 (50.0)	18 (90.0)
	R	13 (65.0)	11 (55.0)	11 (55.0)	13 (65.0)	6 (30.0)	13 (65.0)	11 (55.0)	4 (20.0)	13 (65.0)	11 (55.0)	10 (50.0)	
Citrobacter spp. (n=6)	S	2 (33.3)	3 (50.0)	3 (50.0)	3 (50.0)	6 (100)	4 (66.7)	3 (50.0)	6 (100)	4 (66.7)	4 (66.7)	3 (50.0)	4 (66.7)
	R	4 (66.7)	3 (50.0)	3 (50.0)	3 (50.0)	-	2 (33.3)	3 (50.0)	-	2 (33.3)	2 (33.3)	3 (50.0)	
Acinetobacter spp. (n=2)	S	2 (100)	2 (100)	-	-	2 (100)	-	-	2 (100)	2 (100)	-	2 (100)	2 (100)
	R	-	-	2 (100)	2 (100)	-	2 (100)	2 (100)	-	-	2 (100)	-	
Total (n=74)	S	20 (27.0)	50 (67.6)	39 (52.7)	32 (43.2)	59 (79.7)	29 (39.2)	33 (44.6)	65 (87.8)	50 (67.6)	29 (39.2)	41 (55.4)	64 (86.5)
	R	54 (73.0)	24 (32.4)	35 (47.3)	42 (56.8)	15 (20.3)	45 (60.8)	41 (55.4)	9 (12.2)	24 (32.4)	45 (60.8)	33 (44.6)	

Key: PCR: Piperacillin, FOX: Cefoxitin, CAZ: Ceftazidime, CRO: Ceftriaxone, MEM: Meropenem, ATM: Aztreonam, CIP: Ciprofloxacin, NI: Nitrofurantoin, TS: Trimethoprim-sulfamethoxazole, AK: Amikacin, GEN: Gentamicin, S: Susceptible, R: Resistant, MDR: Multi Drug Resistant.

Table 3: 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	Resistance No (%)										MDR
	AST	PCR	FOX	CRO	CIP	AK	GEN	TS	E	CD	
S. aureus (n=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)	
Strep. agalactiae (n=1)	S	1 (100)	1 (100)	1 (100)	-	-	-	-	1 (100)	1 (100)	1 (100)
	R	-	-	-	1 (100)	1 (100)	1 (100)	1 (100)	-	-	
Strep. Viridians (n=1)	S	1 (100)	-	-	-	-	-	1 (100)	1 (100)	-	1 (100)
	R	-	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	-	-	1 (100)	
Total (n=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)	

Key: PCR: Piperacillin, FOX: Cefoxitin, CRO: Ceftriaxone, CIP: Ciprofloxacin, NI: Nitrofurantoin, TS: Trimethoprim-sulfamethoxazole, AK: Amikacin, GEN: Gentamicin: E: Erythromycin, CD: Clindamycin, S: Susceptible, R: Resistant

Figures

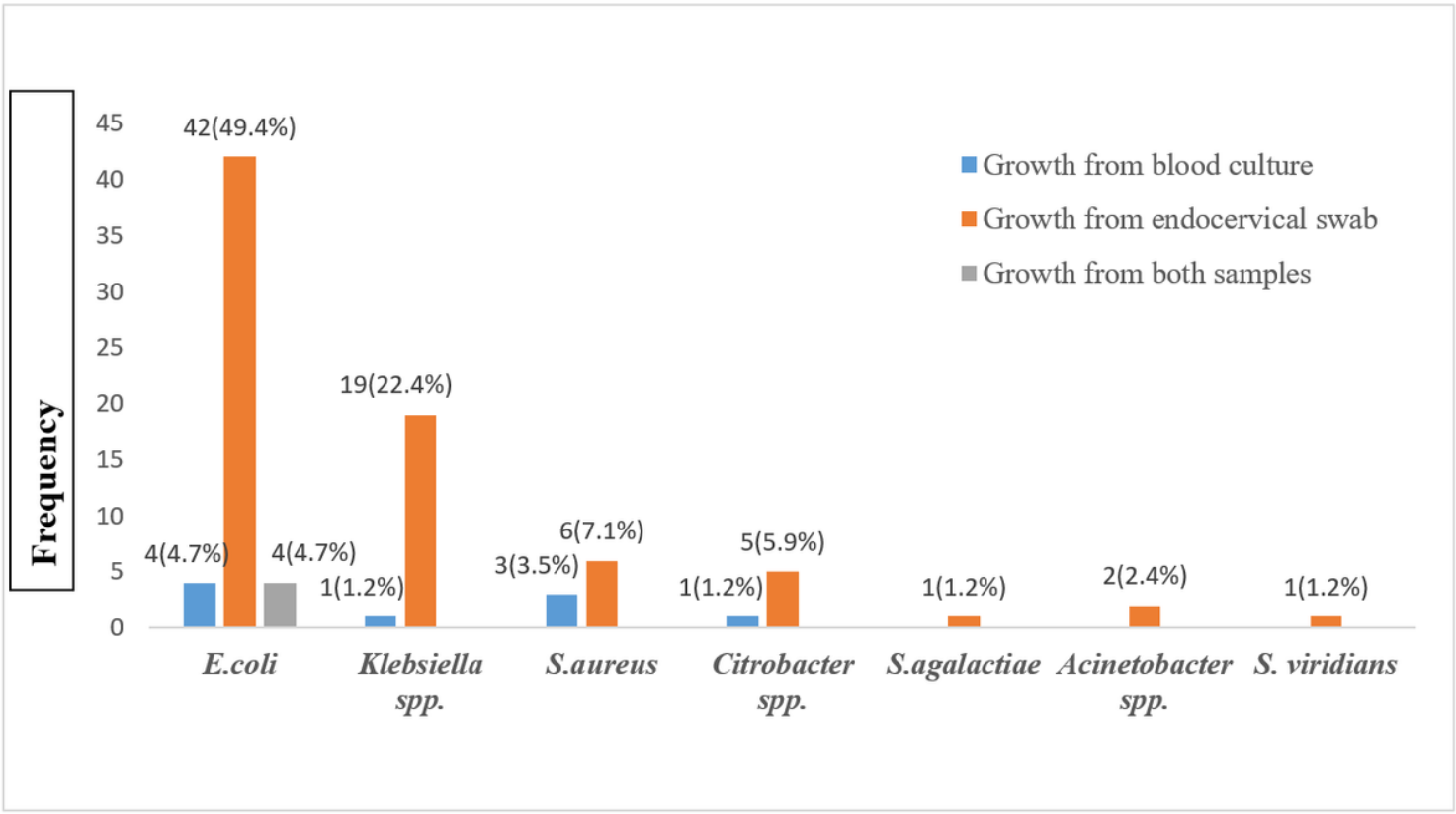

Figure 1

Frequency of bacterial isolates from blood culture and endocervical swab samples of puerperal sepsis suspected women at Asella Referral and Teaching Hospital, Central Ethiopia, from September 2020 to August 2021