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# Neonatal sepsis in Sana'a city, Yemen: a predominance of *Burkholderia cepacia*

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## Abstract

**Background:** Neonatal sepsis is a global concern with increasing morbidity and mortality. The burden of neonatal sepsis is highest in developing countries, especially in those lacking proper surveillance systems. The causative pathogens and their drug-resistance levels vary between countries with emergence of multidrug resistance organisms. Thus, accurate records on the recent trends of organisms causing neonatal sepsis will provide vital information for appropriate intervention. We aimed to investigate neonatal sepsis, identify its associated factors and causative pathogens and to assess the antibiotic susceptibility patterns in Sana'a city, Yemen.

**Methods:** A cross-sectional study was conducted on neonates admitted to intensive care units of six hospitals in Sana'a city, Yemen, in the period from January 15, to March 30, 2020. Natal and prenatal medical data were collected using well-structured questionnaire. Neonates were subjected to sepsis work-up including blood culture, complete blood count and C-reactive protein. Organisms were identified by Gram staining and analyzed by the VITEK II system for bacterial bio-typing and antibiotic susceptibility testing.

**Findings:** Of the 199-neonates with suspected neonatal sepsis, 154 (77.38%) had culture-proven sepsis. Early-onset neonatal sepsis (EOS) was higher (50.25%; 100/199) than late-onset neonatal sepsis (LOS) (27.13%; 54/199). Multivariable analysis identified vaginal delivery as an independent risk factor for neonatal sepsis  $p=0.005$ . Majority of isolated bacteria (74.39%) were gram-negative with *Burkholderia cepacia* (39%) and *Klebsiella oxytoca* (13%) being the most common pathogens of EOS and LOS. The most common gram-positive pathogens were *Staphylococcus haemolyticus* (9.1%) and *Staphylococcus epidermidis* (7.1%). *B. cepacia* showed multidrug resistance except for cefepime. All *Klebsiella* species isolates (100%) and most *Pantoea* species (93%) were ESBL and carbapenemase positive. All *Escherichia coli* and *Acinetobacter baumannii* isolates were ESBL positive. A significant number of gram-positive bacteria showed resistance to vancomycin.

**Conclusion:** The study findings show a high proportion of neonatal sepsis among neonates admitted to hospitals in Sana'a city with antibiotic-resistant *B. cepacia* being the single most common pathogen causing EOS and LOS. Findings also emphasize the emerging threat of multidrug-resistant bacteria in neonatal units and will help develop evidence-based management of neonatal sepsis in Yemen.

**Keywords:** Sepsis, Bacteria, Antibiotic resistance, VITEK II, Blood culture, Gram-negative, *Burkholderia cepacia*

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## Background

Neonatal sepsis is a leading cause of neonatal morbidity and mortality worldwide. The global burden of neonatal sepsis and other neonatal infections was estimated



at 22 million disability-adjusted life-years (95% UI: 18.9–28.0) [1]. In developing countries, neonatal sepsis is responsible for more than 50% of neonatal deaths. It includes EOS, which occurs within 72 h after birth and LOS [2]. The two types of infections have different clinical manifestations, epidemiology, and pathogens patterns [3–6].

Pathogens causing neonatal sepsis are either acquired from the maternal flora or postnatally from the hospital or community [7]. Bacteria are the most common pathogens, and they vary in the distribution between countries, regions and according to the disease onset EOS versus LOS [7]. The commonly involved bacteria in developing countries include *Klebsiella* species, *Staphylococcus aureus*, *E. coli*, and Group B *Streptococcus* [8, 9]. Recently, *B. cepacia*, a bacterial pathogen that has intrinsic drug resistance and ability to live inside drug products [10, 11], has emerged as a significant pathogen causing neonatal sepsis [12–16].

Yemen is suffering of an ongoing war that started in 2015. The war has destroyed the health system, rendering health facilities either not functioning or partially functioning [17]. There is a paucity of information about neonatal sepsis including its types, causative pathogens patterns and antibiotic susceptibility. Therefore, this study aimed to investigate neonatal sepsis, identify its associated factors and causative pathogens, and to assess the antibiotic susceptibility patterns in Sana'a city, Yemen. Findings from this study will fill gaps of knowledge about neonatal sepsis in Yemen, helping public health authorities to develop an effective control strategy to combat the disease, and guide Yemeni physicians to implement an evidence-based approach for the clinical management of neonatal sepsis.

## Methods

All methods were carried out in accordance with the relevant guidelines and regulations.

### Study design and subjects

This was a cross-sectional study conducted on neonates admitted to neonatal units in six major hospitals in Sana'a city; Al-Kuwait, C-Plas, Al-Gumhory, Al-Sabeen, AL-Thawra, and Family hospitals, in the period from January 15 to March 30, 2020. Neonates who were admitted for at least 72 h with suspected sepsis during the study period were included. Neonates with congenital anomalies and hemolytic jaundice were excluded from the study.

### Diagnosis of neonatal sepsis

Neonatal sepsis was suspected based on the presence of clinical signs or risk factors according to the international recommendations [18] and confirmed as neonatal sepsis

if blood culture was positive [18, 19]. Neonatal sepsis was then classified into EOS and LOS [19].

### Questionnaire

Bio and socio-demographic data were collected using a standard questionnaire by face-to-face interviews with the neonate's mothers. The health condition of the mothers and clinical manifestations of the neonates were collected by physicians using standard tools. Written informed consent was obtained from guardians of all neonates after explaining the study objectives. All procedures described in this study were approved by the Research and Ethics Committee of the Faculty of Medicine and Health Sciences, University of Science and Technology, Sana'a (Approval No. EAU/UST122).

### Laboratory investigations

Laboratory investigations were performed according to standard microbiological techniques [20]. Under aseptic conditions, trained nurses collected blood samples that were used for laboratory investigations of complete blood counts, C-reactive proteins and blood culture. For culture, at least 1 ml of blood was inoculated into BacT/Alert PF plus culture bottle (BIOMERIEUX, France, LOT 4053532) and incubated until the BacT/Alert instrument (BACTEC 9050, Becton Dickinson) signals it either as positive or negative. All positive samples were sub-cultured on chocolate agar, blood agar, and MacConkey agar and incubated at 37 °C for 24–48 h. Gram-staining was conducted to differentiate between gram-positive and gram-negative bacteria. A sufficient number of colonies of pure culture was used to suspend the microorganism in 3.0 ml of sterile saline test tube. Pure bacterial suspension was added to bacterial specific identification and sensitivity testing kit device and analyzed by the VITEK II system for bacterial bio-typing and antibiotic susceptibility patterns as instructed in the product information manuals (BIOMERIEUX). VITEK® GN ID identification card (lot 2410933203) was used to characterize gram-negative bacteria and VITEK® GP ID identification card (lot 2420938203) was used to characterize gram-positive bacteria. Different cards were used to perform antibiotic susceptibility tests; AST-GN 87 (lot; 6770912203), AST-GN 72 (lot; 5921083103) and AST GN75 (lot; 5951129403) for gram-negative bacteria and AST-GP67 (lot; 1321137103) for gram-positive bacteria. All procedures were conducted for routine diagnostic and therapeutic reasons.

### Statistical analysis

Data were analyzed using IBM SPSS Statistics for Mac, version 23.0 (IBM Corp., Armonk, NY, USA). Categorical variables were presented in frequencies. The association

between independent and dependent variables was tested using Pearson's Chi-squared with reporting odds ratio (OR) and 95% confidence interval (CI). Fisher's exact test was used when applicable. Multivariable analysis using the binary logistic regression model was conducted, including all variables, and the adjusted OR with its corresponding 95%CI was reported. A  $p$ -value of  $<0.05$  was considered significant.

### Ethical consideration

The study protocol was approved by the Research and Ethics Committee of the Faculty of Medicine and Health Sciences, University of Science and Technology, Sana'a, Yemen (Approval No. EAU/UST122).

## Results

### Characteristics of the patients

A total of 199 neonates admitted to referral hospitals in Sana'a city, Yemen, were enrolled in this study. Nearly half of neonates (84, 42.2%) were preterm, majority of them (70, 83.3%) were moderates to late preterm. More than half of neonates (109, 54.8%) were low birth weight. A total of 113 neonates (56.8%) were born by vaginal delivery. The age of 133 neonates (66.8%) was less than 72 h on admission. Tachypnea, lethargy and poor feeding were the most common clinical manifestations observed among neonates (Table 1).

### Culture-confirmed neonatal sepsis and its associated factors

Majority of neonates 77.38% (154/199) had positive blood culture. Of them, 50.25% (100/199) had EOS. Neonates born by vaginal delivery were at three-times higher risk of neonatal sepsis (OR=3.08, 95% CI 1.54, 6.16;  $p=0.002$ ) than those whose deliveries were through Cesarean section. Although most neonates born at home acquired neonatal sepsis (OR=2.8; 95% CI 0.81, 9.87;  $p=0.09$ ), the significance of association was in the borderline (Table 2). Multivariable analysis using binary logistic regression model identified vaginal delivery as an independent risk factor for neonatal sepsis (adjusted OR = 3.0; 95% CI 1.40, 6.33,  $p=0.005$ ).

### Clinical manifestations and hematological factors associated with culture-confirmed sepsis

Culture confirmed neonatal sepsis was significantly associated with hyperthermia ( $p=0.045$ ) and convulsion ( $p=0.01$ ). Poor feeding, jaundice, irritability, and lethargy were not significantly associated with neonatal sepsis. Elevated C-reactive protein was significantly associated with neonatal sepsis ( $p=0.001$ ). One-third (32%) of neonates with proven sepsis had normal white cell

**Table 1** Characteristics of neonates with suspected sepsis admitted to referral hospitals in Sana'a city, Yemen, in the period from January 15 to March 30, 2020 (N = 199)

Variable	n (%)
Gender	
Female	77 (38.7)
Male	122 (61.3)
Gestational age (weeks)	
$\geq 37$	115 (57.8)
$< 37$	84 (42.4)
Mode of delivery	
Cesarean section	86 (43.2)
Vaginal delivery	113 (56.8)
Place of delivery	
Hospital	170 (85.4)
Home	29 (14.6)
Premature rupture of membrane	
No	139 (69.8)
Yes > 18 h	60 (30.2)
Birth weight (g)	
$\geq 2500$	90 (45.2)
$< 2500$	109 (54.8)
Age of neonate (h)	
$> 72$	66 (33.2)
$\leq 72$	133 (66.8)
Clinical manifestations	
Poor feeding	120 (60.3)
Convulsion	17 (8.5)
Irritability	35 (17.5)
Jaundice	58 (29.1)
Tachypnea	135 (67.8)
Apnea	11 (5.5)
Lethargy	129 (64.8)
Poor sucking	102 (51.2)
Hypothermia	9 (4.5)
Hyperthermia	36 (18.0)

counts and two-thirds (59%) had normal platelets counts (Table 3).

### Pathogens causing neonatal sepsis

Table 4 represents the distribution of pathogens causing neonatal sepsis. Out of the 154 neonates with culture-confirmed sepsis, 152 were infected by bacteria, while two were infected by *Candida albicans*. Of the 152 neonates with proven bacterial culture, nine had mixed bacterial growth. We isolated 161 bacterial pathogens, 119 (74%) were gram-negative while 42 (26%) were gram-positive. *B. cepacia* (37%) was the most common organism causing neonatal sepsis with higher prevalence

**Table 2** Factors associated with culture-confirmed neonatal sepsis among septic neonates admitted to referral hospitals in Sana'a city, Yemen, in the period from January 15 to March 30, 2020 (N = 199)

Variable	N	Culture-confirmed neonatal sepsis		
		n (%)	OR (95% CI)	p value
Gender				
Female	77	60 (77.9)	Reference	
Male	122	94 (77)	0.95 (0.48, 1.88)	1.0
Gestational age (weeks)				
≥ 37	115	89 (77.4)	Reference	
< 37	84	65 (77.4)	0.99 (0.51, 1.95)	1.0
Mode of delivery				
Cesarean section	86	57 (66.3)	Reference	
Vaginal delivery	113	97 (85.8)	3.08 (1.54, 6.16)	0.002*
Place of delivery				
Hospital	170	128(75.3)	Reference	
Home	29	26 (89.9)	2.80 (0.81, 9.87)	0.09
Premature rupture of membrane				
No	139	111(79.9)	Reference	
Yes > 18 h	60	43 (71.7)	0.63 (0.31, 1.28)	0.26
Birth weight (g)				
≥ 2500	90	68 (75.6)	Reference	
< 2500	109	86 (78.9)	1.21 (0.62, 2.35)	0.61

N, number of suspected neonates; n, number of culture-confirmed septic neonates; OR, odds ratio; CI, confidence interval

\*Was confirmed as independent risk factor using binary logistic regression model (adjusted OR = 3.0; 95% CI 1.40, 6.33, p = 0.005)

among EOS (38%) than LOS (35%), followed by *K. oxytoca* (11.6%), which was higher among LOS (14.8%) than EOS (10.0%). *Pantoea agglomerans* caused neonatal sepsis in nine neonates (5.8%). The main gram-positive pathogens were *S. haemolyticus* (9.1%), *S. epidermidis* (7.1%) and *Staphylococcus hominis* (5.1%). Coagulase-negative Staphylococci (CONS) was the most common gram-positive organisms. *S. haemolyticus* emerged as the most frequently CONS.

**Antibiotic susceptibility**

Most isolated pathogens showed antimicrobial resistance (AMR) to the commonly used antibiotics (ampicillin, gentamicin, amikacin), cephalosporins and carbapenems (Tables 5 and 6). *B. cepacia*, the most common pathogen caused neonatal sepsis in this study, was highly resistant to ampicillin/sulbactam, gentamicin, tobramycin, tetracycline, amoxicillin/clavulanic acid, cefalotin, cefazolin, cefuroxime, ceftoxitin, cefpodoxime, imipenem, and amikacin. However, it was 100% susceptible to cefepime. *K. oxytoca* was susceptible to gentamicin, ciprofloxacin, levofloxacin, tetracycline, nitrofurantoin, and trimethoprim/sulfamethoxazole. *Klebsiella* species (100%) and *Pantoea*

**Table 3** Association of clinical manifestations and hematological factors with culture-confirmed sepsis among septic neonates admitted to referral hospitals in Sana'a city, Yemen, in the period from January 15 to March 30, 2020 (N = 199)

Variable	N	Culture-confirmed neonatal sepsis		
		n (%)	Chi-square value	p value
Clinical manifestations				
Poor feeding				
No	79	58 (73.4)	1.18	0.277
Yes	120	96 (80.0)		
Convulsion <sup>a</sup>				
No	182	137(75.3)		0.015
Yes	17	17 (100)		
Irritability <sup>a</sup>				
No	164	124(75.6)		0.266
Yes	35	30 (85.7)		
Jaundice				
No	141	107(75.9)	0.62	0.430
Yes	58	47 (81.0)		
Lethargy				
No	70	52 (74.3)	0.59	0.411
Yes	129	102(79.1)		
Respiratory rate <sup>a</sup>				
Normal	53	40 (75.5)		0.96
Tachypnea	135	105 (77.8)		
Apnea	11	9 (81.8)		
Temperature <sup>a</sup>				
Normal	154	115 (74.7)		0.045
Hyperthermia	36	33 (91.7)		
Hypothermia	9	6 (66.7)		
Hematological factors				
C-reactive protein				
Non-reactive < 6 ml/dl	74	48 (64.9)	11.3	0.001
Reactive	113	97 (85.8)		
WBC <sup>a</sup>				
Normal	65	49 (75.4)		0.891
High	126	99 (78.6)		
Low	6	5 (83.3)		
Platelets <sup>a</sup>				
Normal	124	91 (73.4)		0.158
High	5	5 (100)		
Low	68	57 (83.8)		

N, number of suspected neonates; n, number of culture-confirmed septic neonates; normal WBC 4000–10,000/mm<sup>3</sup>; normal platelets 150,000–450,000; normal respiratory rate, 30–50permint

<sup>a</sup> Fisher's exact test was used

species (93%) were carbapenemase positive and ESBL positive. All isolates of *E. coli* and *A. baumannii* were ESBL

**Table 4** Organisms causing neonatal sepsis among neonates admitted to referral hospitals in Sana'a city, Yemen, in the period from January 15 to March 30, 2020 (N = 154)

Isolated organism	Proportion of isolated pathogen n (%)		
	Early onset <sup>a</sup> sepsis (N = 100)	Late onset sepsis (N = 54)	Total (N = 154)
Single infection			
<i>Burkholderia cepacia</i>	38 (38.0)	19 (35.2)	57 (37.0)
<i>Klebsiella oxytoca</i>	10 (10.0)	8 (14.8)	18 (11.6)
<i>Pantoea agglomerans</i>	7 (7.0)	2 (3.7)	9 (5.8)
<i>Pseudomonas aeruginosa</i>	4 (4.0)	2 (3.7)	6 (3.8)
<i>Klebsiella pneumoniae</i>	2 (2.0)	2 (3.7)	4 (2.5)
<i>Pantoea dispersa</i>	3 (3.0)	1 (1.8)	4 (2.5)
<i>Acinetobacter baumannii</i>	2 (2.0)	0 (0)	2 (1.3)
<i>Acinetobacter lwoffii</i>	2 (2.0)	1 (1.8)	3 (1.9)
<i>Enterobacter cloacae complex</i>	0 (0)	1 (1.8)	1 (0.64)
<i>Escherichia coli</i>	1 (1.0)	1 (1.8)	2 (1.3)
<i>Achromobacter denitrificans</i>	0 (0)	1 (1.8)	1 (0.64)
<i>Sphingomonas paucimobilis</i>	1 (1.0)	0 (0)	1 (0.64)
<i>Staphylococcus haemolyticus</i>	8 (8.0)	4 (7.4)	12 (7.8)
<i>Staphylococcus epidermidis</i>	6 (6.0)	5 (9.2)	11 (7.1)
<i>Staphylococcus hominis</i>	4 (4.0)	2 (3.7)	6 (3.8)
<i>Staphylococcus aureus</i>	2 (2.0)	1 (1.8)	3 (1.9)
<i>Staphylococcus saprophyticus</i>	2 (2.0)	0 (0)	2 (1.3)
<i>Enterococcus faecalis</i>	0 (0)	1 (1.8)	1 (0.64)
<i>Candida albicans</i>	2 (2.0)	0 (0)	2 (1.3)
Double infection			
<i>B. cepacia</i> and <i>S. hominis</i>	2&2(2.0)	0 (0)	2 (1.3)
<i>B. cepacia</i> and <i>S. haemolyticus</i>	0 (0)	1&1 (1.8)	1 (0.64)
<i>K. oxytoca</i> and <i>Serratia marcescens</i>	1&1(1.0)	0 (0)	1 (0.64)
<i>P. agglomerans</i> and <i>K. oxytoca</i>	0 (0.0)	1&1 (1.8)	1 (0.64)
<i>K. pneumoniae</i> and <i>E. cloacae complex</i>	1&1 (1.0)	0 (0.0)	1 (0.64)
<i>A. baumannii</i> and <i>S. saprophyticus</i>	1&1 (1.0)	0 (0.0)	1 (0.64)
<i>E. cloacae complex</i> and <i>E. faecalis</i>	0 (0)	1&1 (1.8)	1 (0.64)
<i>S. haemolyticus</i> and <i>Streptococcus. agalactiae</i>	1&1 (1.0)	0 (0.0)	1 (0.64)
Total number of isolated organisms	106	57	163

N, number of patients

<sup>a</sup> Early onset sepsis  $\leq$  72 h and Late onset sepsis > 72 h

positive (Table 5). A significant number of gram-positive isolates were resistant to vancomycin. Most gram-positive bacteria were ceftazidime screening positive and sensitive to moxifloxacin, linezolid and rifampicin. *S. haemolyticus* isolates were resistant to ampicillin and gentamicin (Table 6). *Staphylococcus* species showed resistance to fluoroquinolones (ciprofloxacin).

## Discussion

In this multicentric cross-sectional study we report a high proportion of culture-confirmed neonatal sepsis, accounting for two-thirds (77.38%) of admitted neonates in the referral hospitals in Sana'a city in Yemen. EOS was more common than LOS and *B. cepacia* emerged as the

predominant causative organism of both EOS and LOS. Majority of the isolated pathogens were resistant to commonly used antibiotics.

Culture confirmed sepsis was high in our study. However, the proportion of culture-confirmed neonatal sepsis varied between studies from developing countries; 62.8% in Pakistan [21], 57% in Yemen [22], 45.9% in Egypt [23], 44.7% in Ethiopia [24], 24% in Tanzania [25] and 12.6% in Nepal [26]. The differences in the proportion of neonatal sepsis between countries may be due to several factors including sample size and the different used techniques.

In this study, EOS was about two times higher than LOS, which is consistent with other reports from developing countries [22, 23, 26, 27]. However, it is

**Table 5** Antibiotic resistance levels of gam-negative bacteria isolated from septic neonates admitted to referral hospitals in Sana'a city, Yemen, in the period from January 15 to March 30, 2020

Pathogen	Ampicillin sulbactam		Ampicillin		Amo/CA		Piperacillin/tazobactam		Cefalotin		Cefazolin		Cefuroxime	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
<i>B. cepacia</i>	54	100	60	100	28	100	60	15	27	100	60	100	28	100
<i>K. oxytoca</i>	20	100	20	100	3	100	20	100	3	100	20	100	3	100
<i>P. agglomerans</i>	NA	NA	NA	NA	9	100	9	100	9	100	10	100	9	100
<i>P. aeruginosa</i>	6	100	6	100	4	100	6	0	4	100	6	100	4	75
<i>K. pneumoniae</i>	5	100	5	100	1	100	5	100	1	100	5	100	1	100
<i>P. dispersa</i>	NA	NA	NA	NA	NA	NA	4	100	NA	NA	4	75	NA	NA
<i>A. baumannii</i>	3	33	1	100	3	33	3	33	1	100	3	100	1	100
<i>A. lwoffii</i>	3	100	3	100	2	100	3	33	3	100	3	100	2	100
<i>E. cloacae complex</i>	NA	NA	NA	NA	1	100	3	33	1	100	3	100	1	100
<i>E. coli</i>	2	50	2	100	NA	NA	2	100	NA	NA	2	100	NA	NA
<i>A. denitrificans</i>	1	0	1	100	1	0	1	0	1	100	1	100	1	100
<i>S. marcescens</i>	NA	NA	NA	NA	NA	NA	1	100	NA	NA	1	100	NA	NA
<i>S. paucimobilis</i>	1	100	1	100	NA	NA	1	100	NA	NA	1	100	NA	NA
Pathogen	Cefuroxime axetil		Cefoxitin		Cefpodoxime		Ceftazidime		Ceftriaxone		Cefepime			
	N	%	N	%	N	%	N	%	N	%	N	%		
<i>B. cepacia</i>	28	100	59	100	28	100	60	3.3	60	10	60	0		
<i>K. oxytoca</i>	3	100	20	100	3	100	20	100	20	100	20	100		
<i>P. agglomerans</i>	9	100	10	100	9	100	10	100	10	100	10	100		
<i>P. aeruginosa</i>	4	100	6	100	4	75	6	0	6	66	6	0		
<i>K. pneumoniae</i>	1	100	5	80	1	100	5	100	5	100	5	100		
<i>P. dispersa</i>	NA	NA	4	75	NA	NA	4	75	4	75	4	75		
<i>A. baumannii</i>	1	100	3	100	1	100	3	100	3	100	3	33		
<i>A. lwoffii</i>	2	100	3	100	2	100	3	33	3	0	3	0		
<i>E. cloacae complex</i>	1	100	3	100	1	100	3	33	3	33	3	33		
<i>E. coli</i>	NA	NA	2	50	NA	NA	2	100	2	100	2	100		
<i>A. denitrificans</i>	1	100	1	100	1	100	1	0	1	100	1	0		
<i>S. marcescens</i>	NA	NA	1	0	NA	NA	1	100	1	100	1	100		
<i>S. paucimobilis</i>	NA	NA	1	100	NA	NA	1	0	1	100	1	0		
Pathogen	Gentamicin		Tobramycin		Ciprofloxacin		Levofloxacin		Tetracycline		FT		SXT	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
<i>B. cepacia</i>	59	100	60	100	60	98	60	80	28	100	60	100	60	5
<i>K. oxytoca</i>	20	0	20	100	20	0	20	0	3	0	20	0	20	0
<i>P. agglomerans</i>	10	100	10	100	10	0	10	0	9	0	10	100	10	0
<i>P. aeruginosa</i>	6	66	6	66	6	17	6	33	4	100	6	100	6	66
<i>K. pneumoniae</i>	5	20	5	100	5	0	5	0	1	0	5	20	5	40
<i>P. dispersa</i>	4	100	4	100	4	25	4	0	NA	NA	4	75	3	33
<i>A. baumannii</i>	3	66	3	66	3	33	3	33	1	0	3	100	3	100
<i>A. lwoffii</i>	3	100	3	100	3	0	3	66	2	100	3	100	3	66
<i>E. cloacae complex</i>	3	33	3	33	3	33	3	0	1	0	3	33	3	33
<i>E. coli</i>	2	0	2	50	2	50	2	50	NA	NA	2	0	2	0
<i>A. denitrificans</i>	1	100	1	100	1	0	1	0	1	0	1	100	1	0
<i>S. marcescens</i>	1	100	1	100	1	0	1	0	NA	NA	1	100	1	0
<i>S. paucimobilis</i>	1	100	1	100	1	0	1	100	NA	NA	1	100	1	0

**Table 5** (continued)

Pathogen	Imipenem		Ertapenem		Meropenem		Amikacin		ESBL		Carbapenemase	
	N	%	N	%	N	%	N	%	N	%	N	%
<i>B. cepacia</i>	7	100	NA	NA	53	13	53	100	–	–	NA	NA
<i>K. oxytoca</i>	1	100	20	100	20	100	20	65	20	100	20	100
<i>P. agglomerans</i>	7	100	8	100	10	100	10	100	10	100	10	100
<i>P. aeruginosa</i>	2	100	NA	NA	6	0	6	66	–	–	–	–
<i>K. pneumoniae</i>	NA	NA	5	80	5	80	5	40	5	100	5	80
<i>P. dispersa</i>	NA	NA	4	50	4	50	4	25	4	75	4	50
<i>A. baumannii</i>	1	0	NA	NA	NA	NA	NA	NA	3	100	NA	NA
<i>A. lwoffii</i>	NA	NA	NA	NA	1	100	3	100	–	–	–	–
<i>E. cloacae</i> complex	NA	NA	3	33	3	33	3	33	3	33	1	Pos
<i>E. coli</i>	NA	NA	2	0	2	0	2	0	2	100	–	–
<i>A. denitrificans</i>	1	0	NA	NA	1	0	1	100	1	Pos	–	–
<i>S. marcescens</i>	NA	NA	1	0	1	0	1	0	1	Pos	–	–
<i>S. paucimobilis</i>	NA	NA	NA	NA	1	100	1	100	–	–	Pos	Pos

N: the number of isolated bacteria tested for antibiotic sensitivity; %: the percentage of resistant bacteria; NA: not analyzed; pos: positive; Amo/CA: amoxicillin/clavulanic acid; FT: nitrofurantoin; SXT: trimethoprim/sulfamethoxazole; ESBLPos: positive, ESBL-positive, resistance for all penicillins, cephalosporins and aztreonam

**Table 6** Antibiotic resistance levels of gam-positive bacteria isolated from septic neonates admitted to referral hospitals in Sana'a city, Yemen, in the period from January 15 to March 30, 2020

Pathogen	Cefoxitin screen, positivity		Benzylpenicillin		Ampicillin		Oxacillin		Gentamicin high level		Streptomycin high level		Gentamicin		Ciprofloxacin		Levofloxacin		Moxifloxacin		Inducible clindamycin resistance	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
<i>S. haemolyticus</i>	10	100	NA	NA	4	100	NA	NA	NA	NA	NA	NA	13	85	10	90	10	90	10	50	10	20
<i>S. epidermidis</i>	11	63	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	11	9.0	11	9	11	9	11	0	11	27.3
<i>S. hominis</i>	8	87.5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	8	37.5	8	25	8	25	8	0	8	50
<i>S. aureus</i>	3	66.6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	3	0	3	66.6	3	66	3	0	3	33.3
<i>E. faecalis</i>	NA	NA	NA	NA	NA	NA	NA	NA	2	0	2	0	NA	NA	2	0	2	0	NA	NA	NA	NA
<i>S. saprophyticus</i>	3	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	3	0	3	0	3	0	3	0	3	66.6
<i>S. agalactiae</i>	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1	S	NA	NA	NA	NA	NA

  

Pathogen	Erythromycin		Clindamycin		Quinupristin/Linezolid dalfopristin		vancomycin		Tetracycline		Tigecycline		Nitrofurantoin		Rifampicin		Trimethoprim/sulfamethoxazole			
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%		
<i>S. haemolyticus</i>	14	92.8	14	28.5	10	0	14	0	14	28.5	10	10	10	0	10	0	10	14	43	
<i>S. epidermidis</i>	11	36.3	11	36.3	11	9	11	9	11	36.3	11	45.4	11	0	11	0	11	9	11	54.5
<i>S. hominis</i>	8	75	8	50	8	0	8	0	8	25	8	37.5	8	0	8	12.5	8	12.5	8	25
<i>S. aureus</i>	3	66	3	66.6	3	0	3	0	3	33.6	3	33.3	2	0	3	0	3	0	3	0
<i>E. faecalis</i>	2	100	NA	NA	2	100	2	0	2	50	2	50	2	0	2	0	NA	NA	NA	NA
<i>S. saprophyticus</i>	3	100	3	66.6	3	0	3	0	3	66.6	3	0	3	0	3	33.3	3	0	3	0
<i>S. agalactiae</i>	NA	NA	1	0	1	0	1	0	1	0	1	100	1	100	NA	NA	NA	NA	NA	NA

N: the number of isolated bacteria tested for antibiotic sensitivity, %: the percentage of resistant bacteria, NA: not analyzed

inconsistent with reports from developed countries where LOS is the predominant type of neonatal sepsis [28, 29]. The predominance of EOS in developing countries can be attributed to low quality health services and poor hygiene. This is mainly due to illiteracy, ignorance, cultural beliefs, and prejudices [19]. Infection may be of

the maternal genital tract or from delivery rooms or neonatal units [19, 30].

Similar to previous studies neonates born by vaginal delivery were at a higher risk of neonatal sepsis compared with those delivered through Cesarean section [23, 27]. Further, culture confirmed sepsis was high (89.9%)

among home delivered neonates. Conversely, majority (75.3%) of the hospital delivered neonates had culture confirmed sepsis. These results emphasize on both the vertical and nosocomial transmission of the causative pathogens.

In this study, gram-negative bacteria were the most common cause of EOS and LOS, which is consistent with previous reports from developing countries [8, 9, 22, 31]. Unexpectedly, *B. cepacia* emerged as the most common cause of neonatal sepsis. It was found in all neonatal units. Moreover, it was resistant to aminoglycosides, fluoroquinolones and imipenem. *B. cepacia* has been reported to cause multiple hospital outbreaks and significant neonatal septicemia in different countries [12–16]. Also, it has been isolated from intravenous solutions, mouthwash, disinfectant and medical devices [31–33]. This organism is often overlooked and reported as *Pseudomonas* species [33, 34]. To our knowledge, this is the first report of *B. cepacia* from Yemen.

*Staphylococcus haemolyticus* was the most frequently isolated CONS in this study. Most isolated *S. haemolyticus* showed variable degrees of antibiotic resistance, which is the nature of *S. haemolyticus* to acquire antibiotic resistance due to unusual genome plasticity [35]. So, this organism has a great ability to survive in the hospital environment, especially on medical devices.

Our results of antimicrobial resistance profile are consistent with other findings. Though some of the previous reports were based on cultures from different kinds of medical specimens and performed by conventional methods [8, 24, 28, 36–39]. Most of the isolated gram-negative and gram-positive pathogens were resistant to commonly used antibiotics, penicillins, cephalosporins and carbapenems. Increased antimicrobial resistance in developing countries is due to multiple factors including poverty, self-medication, unregulated supply and drug smuggling, misguided practice and inappropriate prescriptions [40, 41]. Such factors may provoke changes in causative agents together with their change in antibiotic susceptibility patterns [40, 41]. Markedly, majority of neonates in this study received antibiotics before sampling. The presence of ESBL and carbapenemase-positive gram-negative bacteria is an alarming sign both locally and globally and increases the burden of neonatal sepsis in Yemen.

Proven sepsis was associated with elevated CRP. Elevated CRP indicates activation of the immune system and is commonly used as an indicator of bacterial sepsis [18, 19]. The variation in the total white cell counts and platelet counts is considered a hematological response to inflammation, but there was no association between the culture-positive and culture-negative groups regarding white cell or platelets counts. This may be explained by considering evolving neonatal immune system [18, 19].

## Conclusions

Culture-positive neonatal sepsis is high in Sana'a city with EOS representing two-thirds of the cases. *B. cepacia* followed by *K. oxytoca* and *S. haemolyticus* were the most common causes of both EOS and LOS. Majority of the isolated bacterial pathogens showed a high level of resistance to commonly used antibiotics. Vaginal delivery held high risk for developing neonatal sepsis. The study results emphasize the emergence of multidrug-resistant bacteria in the NICUs and might serve as a baseline for proper medical treatment of neonatal sepsis in Sana'a city.

## Abbreviations

CRP: C-reactive protein; ESBL: Extended-Spectrum  $\beta$ -lactamase; EOS: Early-onset sepsis; LOS: Late-onset sepsis.

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## Authors' contributions

AS and IA: study design, data acquisition and analysis, interpretation of data and manuscript preparation. AH: patient enrollment, data acquisition, analysis and interpretation of data. AIA and AbA: patient enrollment, acquisition, analysis and interpretation of patient data. WF and DS: study design, LC-MS experiments and analysis. OA, TA, FA: patient enrollment and acquisition of data. AbA: patient enrollment and data analysis. MM: data analysis, critically revised the manuscript. All authors read and approved the final manuscript.

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## Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

### Ethics approval and consent to participate

The study protocol was approved by the Research and Ethics Committee of the Faculty of Medicine and Health Sciences, University of Science and Technology, Sana'a, Yemen (Approval No. EAU/UST122). Written informed consent was obtained from guardian of all children after explaining the study objectives.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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