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Original Article

Etiology, antibiotic susceptibility and prognostic factors of pediatric community-acquired sepsis in Addis Ababa, Ethiopia

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Abstract

Introduction: There is a scarcity of data on pediatric community-acquired sepsis (CAS) in Ethiopia. We sought to determine the etiology, role of *Streptococcus pneumoniae*, antibiotic susceptibility pattern, and prognostic factors in children with CAS in Addis Ababa, Ethiopia.

Methodology: A prospective cross-sectional study of 101 children aged 0-15 years with suspected CAS was performed at two major hospitals in Addis Ababa, Ethiopia. Blood culture, antibiotic susceptibility testing, amplification of the autolysin (*lyt*A) gene and typing *S. pneumoniae* by sequencing and Quellung reaction were performed. Data were analyzed using descriptive statistics and logistic regression.

Results: The prevalence of culture-positive CAS was 18.81% (19/101). *S. pneumoniae* (21.1%) (Serotypes 19A (n = 2), 33C and 12F) and *Klebsiella pneumoniae* (21.1%) were the most common causes of CAS. Half of *K. pneumoniae* isolates were resistant to gentamicin and ceftriaxone. The most common antibiotics used for treatment were a combination of ampicillin with gentamicin (47.5%). The presence of lower respiratory tract infections (LRTIs) in the preceding 3 months was an independent predictor associated with culture-proven sepsis (adjusted odds ratio (AOR), 7.02; 95% confidence interval (CI), 1.42 - 34.64; P = 0.02). The case-fatality rate was 11.9% (12/101). Presence of underlying comorbidity (AOR, 6.8; 95% CI, 1.59-28.7; P = 0.009) was an independent predictor of mortality.

Conclusions: *S. pneumoniae* and *K. pneumoniae* were the major causes of CAS and there was a substantial level of antibiotic resistance. Presence of LRTIs in the preceding 3 months was a predictor of culture-proven CAS whereas underlying comorbidity was a predictor of mortality.

Key words: Antibiotic resistance; community-acquired sepsis; Ethiopia; *Klebsiella pneumoniae*; *Streptococcus pneumoniae*.

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Introduction

Sepsis remains a huge burden and a leading cause of childhood morbidity and mortality especially in developing countries. Although Africa is likely to account for a significant proportion of the global burden of sepsis, there are limited reports on the epidemiology, management and outcomes of sepsis in African countries [1].

Information about etiological agents involved in causing community-acquired (CA) bacteremia and their antibiotic resistance pattern will help to improve empiric antibiotic therapy and consequently patient outcomes [2]. In developing countries, *Staphylococcus aureus*, *Klebsiella* spp. and *Escherichia coli* account for 55% (95% CI, 39–70%) of culture positive sepsis among neonates, while in infants the most prevalent pathogens are *S. aureus*, *E. coli*, *Klebsiella* spp., *S. pneumoniae* and *Salmonella* spp., which account for 59% (95% CI, 26–92%) of culture-positive sepsis [3].

The distribution of etiological agents of sepsis in the pediatric population is changing considerably as a result of the introduction of conjugate vaccines such as *Haemophilus influenzae* type b (Hib) vaccine and pneumococcal conjugate vaccines (PCVs). It has therefore become important to understand the role of childhood vaccinations not only on the rate and

distributions of causative organisms but also on the risk factors and long-term outcomes of sepsis [4].

Case-fatality rates due to CAS range from 6% to 13% and factors such as age, severity of illness at onset of sepsis, presence of comorbidities, bacteremia and etiology have been associated with mortality [4–7].

PCV10 was introduced in Ethiopia in October 2011 as a three dose primary series (3p+0) without any booster dose [8]. A study by Muhe and colleagues on CAS in children younger than three months of age in Addis Ababa Ethiopia performed two decades before the introduction of PCV10 in the country identified *S. pneumoniae* as the predominant cause of sepsis [9]. There is however a scarcity of data on the etiology of sepsis and the role of *S. pneumoniae* as a cause of sepsis after the introduction of PCV10 in the country.

Most of the previous studies on childhood sepsis in Ethiopia, have been on neonatal sepsis and do not distinguish clearly between CA and hospital-acquired (HA) sepsis [10–12]. Besides, *S. pneumoniae* infections including sepsis are often quite rare in neonates [13].

The aim of this study was therefore to determine the etiology, role of *S. pneumoniae* as a cause of sepsis, antibiotic susceptibility pattern of isolates and prognostic factors in children with CAS at two major hospitals in Addis Ababa, Ethiopia, five years after introduction of PCV10 in Ethiopia.

Methodology

Study design and setting

We carried out a prospective cross-sectional study from September 1, 2016 to August 30, 2017. The study was carried out in pediatric emergency departments of two large hospitals in Addis Ababa, Ethiopia; Tikur Anbessa Specialized Hospital (TASH) and Yekatit 12 Hospital.

Participant selection and inclusion criteria

Among children between the ages of 0 to 15 years presenting to the two pediatric emergency departments (PEDs) during the study period, the ones that were included in the study were, those presenting with suspected CAS and who had not taken antibiotics within two weeks prior to presenting to the hospital. CAS was defined as a case of suspected sepsis in children with no hospital or health care admissions in the two weeks prior to the current admission [14] and identified from samples taken within 48 h of admission [15]. Suspected sepsis was categorized based on the clinical decision of the attending physician and was defined as meeting the systemic inflammatory response syndrome (SIRS) criteria [16].

Data collection and outcome measurement

Trained research nurses approached parents/guardians of 101 children, suspected with CAS during the study period. After obtaining informed consent, a structured questionnaire was used to obtain sociodemographic data and other relevant clinical data. List of antibiotics that were used for management of patients was extracted from medical records. Final outcome (discharge or death) was registered and length of stay in the hospital was recorded in days. The microbiological outcomes assessed were: culture positive CAS, pneumococcal CAS and antibiotic susceptibility pattern among isolates.

Laboratory procedures

Sample collection

At enrollment, venous blood (1 mL for < 1 month-olds and 2-5 mL for > 1 month-olds) was drawn aseptically and transferred into 20 ml Brain Heart Infusion (BHI) broth (Oxoid*, Cheshire,* England) bottles and mixed gently by inverting the bottle. In addition, 1-2 mL of blood, collected using Ethylenediaminetetraacetic acid (EDTA) vials was available for 69 children and was frozen at -80 °C for PCR.

Culture and identification

The inoculated BHI broth was cultured aerobically. After 24 hrs of incubation, Gram stain was done followed by subculture on Blood agar, Chocolate agar and MacConkey agar (Oxoid, Cheshire*,* UK). Culture bottles that did not show growth were further incubated for 7 days and subcultured before being reported negative. Initial identification of bacteria was made by Gram stain, hemolytic activity on sheep blood agar plates, optochin sensitivity, bile solubility, coagulase test, colony morphology on MacConkey agar and growth on Mannitol salt agar at the Armauer Hansen Research Institute (AHRI), Addis Ababa, Ethiopia. Further identification was then performed on all isolates by Matrix-assisted laser desorption/ionization time of flight mass spectrometry at Ghent University, Ghent, Belgium. Coagulase-negative staphylococci (CoNS) and *Micrococcus* spp were considered as contaminants when identified in the blood cultures.

Antibiotic susceptibility testing

Antibiotic susceptibility testing on a selected panel of antibiotics that are used locally was done using the Kirby-Bauer disk diffusion method [17]. For pneumococcal isolates, penicillin resistance was

initially measured using oxacillin discs and for isolates with zones ≤ 19 mm, minimum inhibitory concentrations (MICs) were determined using E-Test strips (bioMerieux, Marcy-l'Etoile, France). Test results of both disc diffusion and MICs were interpreted according to the Clinical and Laboratory Standard Institute (CLSI) criteria [18]. American Type Culture Collection (ATCC) strains: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Streptococcus pneumoniae* ATCC 49619 were used for quality control.

DNA extraction and amplification of *lyt*A genes

DNA extraction was performed on available whole blood samples obtained from 69 children. Initial pretreatment of the samples was performed as described previously [19]. DNA extraction was then carried out with the DNeasy® 96 Blood and Tissue Kit (Qiagen, Venlo, The Netherlands), following manufacturer's instructions. Amplification of a 101-bp fragment of the *lyt*A gene was carried out as previously described [20].

PCRSeqTyping and serotyping

DNA extraction from *S. pneumoniae* isolates was carried out by alkaline lysis as described previously [21]. Amplification and sequencing of the 1061 bp of the *cps*B-gene (encoding for phosphotyrosine phosphatase) were done using PCRSeqTyping as previously described [22]. Twenty µl of the amplicons were sent for sequencing to GATC Biotech (Constance, Germany) and sequencing was performed using the Sanger sequencing technique. *cps*B sequences were used to interrogate GenBank database (http://www.ncbi.nlm.nih.gov/blast). Serotype was assigned based on a BLAST bit score of > 99% sequence identity of the query *cps*B nucleotide sequence with the reference sequences from GenBank. Because correct serotype could not be assigned by PCRSeqTyping for two of the isolates, serotyping was performed on all isolates by the Quellung reaction [23] using pool and group antisera, obtained from the Statens Serum Institut (Copenhagen, Denmark).

Statistical analysis

Data were initially entered in ReDCap (Vanderbilt, USA), exported into Excel and analyzed using PASW Statistics 20 software (SPSS Inc., Chicago, Ill. USA). Age was stratified into four groups: < 28 days, 28 days-1 year, 2-5 years and ≥ 6 years. Continuous variables were reported as median and interquartile range (IQR). Descriptive statistics was used to analyze sociodemographic, clinical and laboratory characteristics. To identify factors associated with culture-proven CAS and prognostic factors of CAS, bivariate analysis using binary logistic regression was performed. Variables which were significant at $P < 0.1$ were then used in multivariable model. Variables in the multivariable analysis were considered as significant when $P < 0.05$. Results from the binary logistic regression analyses were reported as CORs and AORs with 95% CIs.

Table 1. Sociodemographic characteristics of 101 children suspected with CAS, aged 0-15 years at two hospitals in Addis Ababa, Ethiopia.

Variables	Category	All children (n = 101)	Culture positive (n = 19)	Culture negative (n = 82)	P-value*
		No. (%)	No. (%)	No. (%)	
Gender	Male	58 (57.4)	12 (20.7)	46 (79.3)	0.58
	Female	43 (42.6)	7 (16.3)	36 (83.7)	--
Age	< 28 days	19 (18.8)	2 (10.5)	17 (89.5)	0.45
	28 days-1 year	66 (65.3)	13 (19.7)	53 (80.3)	0.79
	2-5 years	12 (11.9)	3 (25)	9 (75)	0.34
	≥ 6 years	4 (4)	1 (25)	3 (75)	--
Premature birth	Yes	15 (14.9)	3 (20)	12 (80)	0.89
	No	86 (85.1)	16 (18.6)	70 (81.4)	--
Birth weight	< 2.5 kg	16 (15.8)	1 (6.2)	15 (93.8)	0.19
	≥ 2.5 kg	85 (84.2)	18 (16.2)	67 (78.8)	--
Day care/ pre-school attendance	Yes	13 (12.9)	4 (30.8)	9 (69.2)	0.25
	No	88 (87.1)	15 (17)	73 (83)	--
Child has siblings aged < 5 years	Yes	33 (32.7)	9 (28.1)	23 (71.9)	0.26
	No	68 (67.3)	10 (14.7)	58 (85.3)	--
Breast feeding child	Yes	73 (72.3)	12 (16.4)	61 (83.6)	0.33
	No	28 (27.7)	7 (25)	21 (75)	--

* P-value of bivariate analysis.

Table 2. Clinical and laboratory characteristics of 101 children suspected with CAS, aged 0-15 years at two hospitals in Addis Ababa, Ethiopia.

Variables	Category	All children (n = 101)	Culture positive (n = 19)	Culture negative (n = 82)	P-value*
		No. (%)	No. (%)	No. (%)	
URTI in the last 3 months	Yes	9 (8.9)	2 (22.2)	7 (77.8)	0.78
	No	92 (91.1)	17 (18.5)	75 (81.5)	--
LRTI in the last 3 months	Yes	7 (6.9)	4 (57.1)	3 (4.9)	**0.02**
	No	94 (93.1)	15 (16.7)	79 (84)	--
Hospital admission in the last 3 months	Yes	17 (16.8)	3 (17.6)	14 (72.4)	0.89
	No	84 (83.2)	16 (19)	68 (81)	--
Signs and symptoms	Fever (> 37.5 °C)	92 (91.1)	17 (18.5)	75 (81.5)	0.78
	Hypothermia	9 (8.9)	2 (22.2)	7 (77.8)	0.78
	Tachypnea	67 (67.3)	11 (16.4)	56 (83.6)	0.39
	Tachycardia	66 (65.3)	13 (19.7)	53 (80.3)	0.76
	Respiratory distress	55 (54.5)	8 (14.5)	47 (85.5)	0.23
	Apnea	10 (9.9)	1 (10)	9 (90)	0.46
	Vomiting	71 (70.1)	14 (19.7)	57 (80.3)	0.72
	Irritability	75 (74.3)	15 (20)	60 (80)	0.61
	Hypo-perfusion	15 (14.9)	14 (16.3)	72 (83.7)	0.13
	Hypotension	7 (6.9)	1 (14.3)	16 (85.7)	0.75
Underlying comorbidity	Yes	33 (32.7)	4 (12.1)	29 (87.9)	0.24
	Respiratory	11(10.9)	0 (0)	11 (100)	--
	Gastrointestinal	10 (9.9)	1 (10)	9 (90)	--
	Cardiovascular	7 (6.9)	1 (14.3)	6 (85.7)	--
	Hematologic	3 (3)	0 (0)	3 (100)	--
	Renal	2 (2)	2 (100)	0 (0)	--
	No	68 (67.3)	15 (22.1)	53 (77.9)	--
Primary infection focus identified	Yes	65 (64.4)	9 (13.8)	56 (86.2)	**0.09**
	Respiratory	45 (44.6)	6 (13.3)	39 (86.7)	--
	Abdominal	16 (15.8)	0 (0)	16 (100)	--
	Skin and soft tissue	2 (2.0)	1 (50)	1 (50)	--
	Urinary tract	2 (2.0)	2 (100)	0 (0)	--
	No	36 (35.6)	10 (27.8)	26 (72.2)	--
PCV vaccination status[a]	Vaccinated	70 (69.3)	13 (18.6)	57 (81.4)	0.93
	1 dose	33 (32.7)	6 (18.2)	27 (81.8)	--
	2 doses	12 (11.9)	2 (16.7)	10 (83.3)	--
	Fully vaccinated	25 (24.8)	5 (20)	20 (80)	--
	Unvaccinated	31 (30.7)	6 (19.4)	25 (80.6)	--
Nutritional status[b]	Well-nourished	61 (60.4)	14 (23)	47 (77)	0.28
	Moderately malnourished	10 (9.9)	1 (10)	9 (90)	--
	Severely malnourished	30 (29.7)	4 (13.3)	26 (86.7)	--
Laboratory results					
White blood cells (cells/mL)	< 5000	6 (5.9)	1 (16.7)	6 (83.3)	0.74
	5000-15, 500	39 (38.7)	10 (25.6)	29 (74.4)	0.76
	15, 500-17, 500	10 (9.9)	2 (20)	8 (80)	0.78
	>17, 500	46 (45.5)	6 (13)	40 (87)	--
Immature neutrophils (%)	> 10%	73 (72.3)	16 (21.9)	57 (78.1)	0.21
	≤ 10%	28 (27.7)	3 (10.7)	25 (89.3)	--
Platelets (per mL)	< 150,000	20 (19.8)	4 (20)	16 (80)	0.88
	≥ 150,000	81(80.2)	15 (18.5)	66 (81.5)	--

CAS: community-acquired sepsis; LRTI: lower respiratory tract infection; PCV: pneumococcal conjugate vaccine; URTI: upper respiratory tract infection; PICU: pediatric intensive care unit; [a] Vaccinated with at least one dose of PCV10; [b] WHO child growth standard of weight for age z score (WAZ) was used to determine nutritional status. Accordingly, well-nourished: z-score ≥ -2.0 <, moderately malnourished: -3.0 < z-score < -2.0; severely malnourished: z-score < -3.0; *P-value of bivariate analysis.

Ethical approval

The study procedures were in accordance with the Helsinki Declaration. The study was approved by the AHRI/All Africa Leprosy Rehabilitation and Training Hospital (ALERT) Ethics Review Committee, Addis Ababa University Institutional Review Board, Yekatit 12 Medical College Ethics Committee and the National Research Ethics Review Committee (No. 310/194/2017). The parents and/or guardians of all participants gave written informed consent.

Results

Characteristics of the study participants

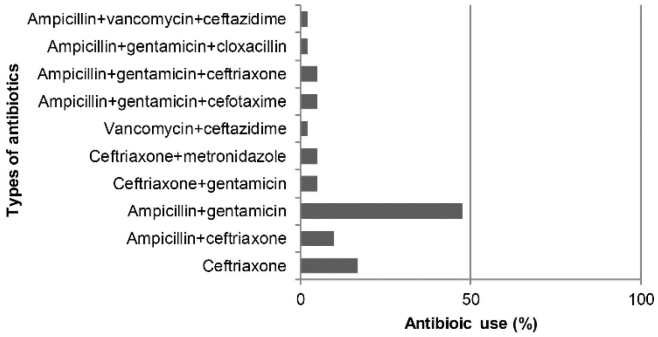
Among 377 children with suspected sepsis admitted to TASH and Yekatit 12 hospitals during the study period, 101 (26.8%) met the study inclusion criteria and were enrolled. Fifty eight (57.4%) of the children were boys and the median age of the enrolled children was 2 months IQR (1 - 5.5) (Table 1). Most children (65.3%) were aged between 28 days and 1 year, 19 (18.8%) were neonates, 12 (11.9%) between 2-5 years and 4 (4%) were 6 years or older.

The most common sign was fever (> 37.5 °C), and was seen in 91.1% of the children, followed by irritability (74.3%) and vomiting (70.1%) (Table 2). A total of 32.7% of the children had comorbidities, with gastrointestinal comorbidities (8.9%) being the most frequent. The focus of infection was determined in 64.4% of the cases and respiratory tract (44.6%) was the most common. Based on the data obtained from vaccination cards or words of parents/guardians, 69.3% of the children have received at least one dose of PCV10. Malnourishment (moderate or severe) was seen in 39.6% of the children.

Etiology of pediatric community-acquired sepsis

A pathogen was identified in 19 (18.8%) and a contaminant in 15 (14.9%) of the blood cultures. *S. pneumoniae,* 21.1% (4/19) and *Klebsiella pneumoniae,* 21.1% (4/19) were the two most common causes of CAS. Other important pathogenic isolates were: *Streptococcus oralis*, 10.5% (2/19)*; Streptococcus pyogenes,* 10.5% (2/19); *Citrobacter koseri,* 5.3% (1/19)*; E. coli,* 5.3% (1/19); *H. influenzae,* 5.3% (1/19); *Moraxella catarrhalis,* 5.3% (1/19); *S. aureus,* 5.3% (1/19); *Streptococcus anginosus*, 5.3% (1/19) and *Streptococcus mitis* 5.3% (1/19). Non-pathogenic bacteria which were considered contaminants were: CoNS (n = 12) and *Kocuria* spp (n = 3).

Figure 1. Types of antibiotics and their frequency of usage for the management of CAS among 101 children aged 0-15 years at two hospitals in Addis Ababa, Ethiopia.



Factors associated with culture-proven sepsis

Seven (6.9%) of the children had LRTIs within three months prior to admission. Among them 4 (57.1%) had culture-proven sepsis. In multivariable analysis, presence of LRTIs within three months prior to admission was an independent predictor associated with culture-proven sepsis (AOR, 7.02; 95% CI, 1.42-34.64; P = 0.02) (Table 3).

Use of antibiotics

The most common antibiotics used for the management of children with CAS were a combination of ampicillin with gentamicin, 47.5% (48/101) followed by ceftriaxone, 16.7% (17/101) (Figure 1). Combinations of ampicillin and gentamicin and /or along with additional antibiotics were used in 59.4% (60/101) of the cases. Ceftriaxone alone or along with other antibiotics were used in 41.6% (42/101) of the cases. Combinations of ampicillin/gentamicin or ceftriaxone alone or along with other antibiotics were used in 96% (97/101) of the cases overall.

Table 3. Factors associated with culture-proven CAS, among children aged 0-15 years at two hospitals in Addis Ababa, Ethiopia.

Variable	Category	Bivariate analysis		Multivariable analysis	
		OR (95% CI)	P-value	AOR (95% CI)	P-value**
Primary infection focus identified	Yes	0.99(0.152-1.151)	0.09	1.00 (0.556-1.803)	0.99
	No	Ref			
LRTIs in the last 3 months	Yes	7.02 (0.029-0.702)	0.02	7.02 (1.424-34.64)	**0.02**
	No	Ref			

**P-value of multivariable analysis.

Antibiotic susceptibility

All of the four *S. pneumoniae* isolates were susceptible to penicillin while two were resistant to trimethoprim/sulfamethoxazole and chloramphenicol, while one was resistant to erythromycin and none to tetracycline (Table 4). Half of the *K. pneumoniae isolates* were resistant to gentamicin and ceftriaxone.

Pneumococcal serotypes and lytA PCR

Using PCRSeqTyping, only two of the isolates were typed to serotype level (19A (n=2)) while the other two were only typed to subtype level (44, 12B/F, 32A/F and 35A/B/C/F, 33A/C/F). All isolates were then serotyped using the Quellung reaction. The ones initially identified to subtype level by PCRSeqTyping were identified as serotypes 12F and 33C and there was 100% concordance between PCRSeqTyping and Quellung for the remaining two isolates. All the four pneumococcal isolates were non-PCV10 types. Three of the four children had been vaccinated with PCV10; one of the three has been fully vaccinated while the other two have received two doses. Whole blood was available for only two of the four cases from which pneumococci was isolated and only one was *lyt*A-PCR positive.

Factors associated with mortality

The overall case-fatality rate was 11.9% (12/101). The length of stay for the children who died was 5 (IQR: 3-14) days. Seven of the children who died (*i.e*. 58.8% of mortality cases), were aged between 28 days and 1 year, which is also the age group of most of the children in the study (65.3%). However, in bivariate analysis, compared to children aged > 6 years, children within this age group were less likely to die (COR, 0.1; 95% CI, 0.01-0.98; and P = 0.048) (Table 5). Children with body temperature of 37.5 °C - 38.5 °C were also less likely to die (COR, 0.2; 95% CI, 0.05-0.82; P = 0.03) compared to those with > 38.5 °C. In a multivariable analysis, children with comorbidities were 6.8 times more likely to die than children without comorbidities (AOR, 6.8; 95% CI, 1.59-28.7; P = 0.009). A pathogenic bacterium was isolated from only one of the children who died and it was a multi-drug resistant *K. pneumoniae*. There was no association between culture positivity and mortality (P = 0.34).

Discussion

Results from our study indicate that the prevalence of culture-proven sepsis was 18.8%. This finding is lower than the results obtained in other studies in Gondar (32.1%) and Addis Ababa (27.9% and 44.7%), Ethiopia [10,11,24]. The lower prevalence of culture-proven sepsis in this study is probably due to our focus on CA cases and the wide age range of the study population. Most previous studies on pediatric sepsis in Ethiopia focus on neonatal sepsis with nosocomial origin.

S. pneumoniae (21.1%) and *K. pneumoniae* (21.1%) were the two most common causes of sepsis. The prevalence of pneumococcal sepsis was 3.96% (4/101). In a previous study in Addis Ababa, Ethiopia performed long before the introduction of PCV10; *S. pneumoniae* was identified as the most common cause of sepsis in children younger than 3 months of age while *K. pneumoniae* was uncommon [9]. A recent report from Switzerland has indicated that after widespread vaccination with PCV7 and a transition to PCV13 in

Table 4. Antibiotic susceptibility pattern of bacterial isolates among children suspected with CAS, aged 0-15 years, number of non-susceptible isolates.

Bacterial species	Total N	Antibiotics									
		PEN	AMX	AMC	CRO	ERY	TE	SXT	GEN	C	CIP
Gram positive											
Streptococcus pneumoniae	4	0	-	-	-	1	0	2	-	2	-
Streptococcus pyogenes	2	1	1	1	0	1	1	1	-	0	0
Streptococcus oralis	2	-	-	-	0	0	1	-	-	0	1
Staphylococcus aureus	1	1	1	0	0	0	0	0	0	0	0
Streptococcus anginosus	1	-	-	-	0	0	0	-	-	0	0
Streptococcus mitis	1	-	-	-	0	1	0	-	-	0	0
Gram negative											
Klebsiella pneumoniae	4	-	4	1	2	3	1	2	2	2	0
Citrobacter koseri	1	-	1	0	1	1	1	1	0	0	0
Escherichia coli	1	-	1	1	1	0	1	1	0	0	0
Haemophilus influenzae	1	-	1	1	1	0	0	0	-	0	0
Moraxella catarrhalis	1	-	-	0	-	0	0	0	-	-	-

-: not tested; PEN: Penicillin; AMX: Amoxicillin; AMC: Amoxicillin-clavulanate; CRO: Ceftriaxone; ERY: Erythromycin; TE: Tetracycline; SXT: Trimethoprim/sulfamethoxazole; GEN: Gentamicin; C: Chloramphenicol; CIP: Ciprofloxacin; [a] Number of non-susceptible isolates.

2011, incidence of pneumococcal sepsis still remained substantial and was responsible for 25% of the CAS episodes in 2015 [25]. All four *S. pneumoniae* isolates from this study were non-PCV10 serotypes with two of them being serotype 19A. In countries that have introduced PCVs, there is an increase in diseases due to non–vaccine serotypes [26]. In Brazil, after introduction of PCV10, the proportion of serotype 19A among invasive pneumococcal disease strains increased from 2.8% in 2005–2009 (pre-PCV period) to 16.4% in 2016-2017 (6 to 7 years post-PCV) [27]. Lack of comprehensive pre-vaccine serotype data in Ethiopia and the limited sample size and isolates in the current study means that we could not make comparisons. Our findings however warrant further large scale studies in Ethiopia to assess the role *S. pneumoniae* as a cause of sepsis in the post-PCV period.

Although they did not clearly distinguish between CA and HA cases, various studies on neonatal sepsis in Africa [28–31] have identified *Klebsiella* spp. as the most common cause. Kabwe and colleagues who analyzed 13 studies on neonatal sepsis in Africa, have reported that *Klebsiella* spp. account for 32% (323/1009, range 0–59%) of the isolates identified [32].

Blood culture contamination is a source of frustration for clinicians and microbiologists and leads to unnecessary and or prolonged course of antibiotics and repeated testing for the patients [33]. CoNS are the most common skin commensals that are isolated from blood cultures but are also important causes of sepsis specially in the presence of indwelling catheters and in very low birth weight infants [34]. CoNS have been previously reported in many studies from Africa, including Ethiopia, as important causes of neonatal sepsis [12,28,32]. However, isolation of CoNS from blood is considered pathogenic if it is obtained from at least two different sites and because this might be difficult in infants, a combination isolation of CoNS from blood stream and clinical symptoms is often the best approach to determine their pathogenicity [35,36]. However, in many of the studies that report CoNS as pathogens, it is not clear whether these criteria have been met. In this study, 12 CoNS were isolated and were considered to be contaminants because we did not find enough evidence to classify them as pathogens.

We performed *lyt*A PCR for detection of *S. pneumoniae* from whole blood samples that were available from 69 patients. Enhanced diagnosis of pneumococcal bacteremia by using *lyt*A PCR which resulted in up to 10.7% increased yields compared to blood culture has been previously reported [19]. In our study *lyt*A PCR did not increase sensitivity. Whole blood was available for two of the samples from which *S. pneumoniae* were isolated and only one was positive for *lyt*A PCR. Possible reasons for low sensitivity could be the small number of samples and limitations in transport and storage of samples.

Presence of LRTIs in the preceding three months was an independent predictor of culture-proven CAS in this study. Studies indicate that in children with sepsis, the most common site of infection is respiratory [37]. The most common focus of infection (44.6%) in this study was also respiratory. Recurrent pneumonia, defined as at least two pneumonia episodes in a year or three in a lifetime, occurs in 7.7%–9% of all children with CAP [38]. It is therefore possible that in this study recurrent LRTIs might have led to sepsis. Further studies which describe recurrent LRTIs among children in Ethiopia should therefore be piloted.

The Ethiopian standard treatment guideline recommends ampicillin and gentamicin as a first line and ceftriaxone as a second line antibiotic therapy for pediatric sepsis [39]. Among 96% of the cases, either the first line antibiotics alone or with additional antibiotics; or the second line antibiotics alone or with other antibiotics were used for management of the

Table 5. Risk factors associated with mortality from CAS among children aged 0-15 years at two hospitals in Addis Ababa, Ethiopia.

Variables[a]	Category	Final outcome		Odds of mortality			
		Discharged (n = 89) (%)	Died (n = 12) (%)	Bivariate COR (95% CI)	*P*[b]	Multivariable AOR (95% CI)	*P*[b]
Age	< 28 days	19.1	16.7	0.1 (0.01-1.36)	0.09	0.33(0.02-4.92)	0.42
	28 days - 1 year	66.3	58.3	0.1 (0.01-0.98)	**0.048**	0.48(0.04-5.31)	0.55
	2 - 5 years	12.4	8.3	0.1 (0.005-1.56)	0.09	0.21(0.01-4.49)	0.32
	≥ 6 years	2.2	16.7				
Body temperature (Axillary)	< 36 °C	10.1	8.3	0.4 (0.04-3.55)	0.40	0.5(0.05-5.2)	0.54
	> 37.5-38.5 °C	58.4	25	0.2 (0.05-0.82)	**0.03**	0.2(0.05-0.99)	0.08
	> 38.5 °C	31.5	66.7				
With comorbidity	-	72.7	27.3	8.13 (2.03-32.55)	**0.003**	6.8 (1.59-28.7)	**0.009**

[a] Among 29 variables in Table 1 and Table 2 used for bivariate analysis using binary logistic regression, 3 were significant at P < 0.1 and were further used in the multivariable analysis. [b] *P*-value of bivariate and multivariable logistic regression analyses, those in bold are with P < 0.05.

patients. Among *K. pneumoniae* isolates in this study, half were resistant to gentamicin and ceftriaxone. A recent review by Williams and colleagues revealed a high prevalence of *Klebsiella* spp non-susceptibility to gentamicin (median 49%, IQR 48–58%) and ceftriaxone (range 33–50%) in sub-Saharan Africa [40]. In developing countries, amikacin, which is effective against most MDR *Klebsiella* spp. and has comparable cost to gentamicin, has been recommended as an alternative to gentamicin as second-line treatment in combination with penicillin [3]. Our results indicate the need for continued surveillance of antibiotic resistance in Ethiopia and assessment of treatment options.

In the pediatric consensus definition of SIRS, abnormal core temperature is defined as a temperature of < 36 °C or > 38 °C measured by rectal, bladder, oral, or central catheter probe and a temperature of > 38.5 °C is used as it increases specificity [16] In our study, we used the local definition of fever (> 37.5 °C) measure via axillary route. We compared the final outcome of children with local definition of fever (> 37.5 °C) and fever as per the SIRS criteria (> 38.5 °C). In a univariate analysis, we were able to see that children with body temperature between 37.5 °C and 38.5 °C°C were less likely to die than children with body temperatures of > 38.5 °C. In a similar study performed in Dar es Salaam, Tanzania, George and colleagues found that children with a temperature of > 38.5 °C and > 2 SIRS criteria were seven times more likely to die (OR, 7.05; *P*, 0.01) [41].

There is evidence that the SIRS criterion for sepsis lacks specificity in identifying children with higher risk of mortality [42]. For adults, SIRS has now been abandoned as a requirement for sepsis and the Sepsis-3 definitions in which sepsis is defined as a dysregulated host response to infection resulting in organ dysfunction is now being applied [43]. This definition was however designed for adults and has not been validated for children. Recent efforts by the pediatric sepsis definition taskforce [44] are expected to come up with new definitions for neonates, children, and adolescents by incorporating lessons learned from application of the Sepsis-3 definitions in adults.

The case-fatality rate (CFR) in this study, (11.9%), was higher than findings from European childhood life-threatening infectious disease study report on CAS (6%, increasing to 10% in the presence of septic shock) [6]. In a recent population based cohort study in Switzerland, mortality was 18%, 12%, and 0% in early onset sepsis (EOS), hospital-acquired late onset sepsis (LOS), and community-acquired LOS [45]. This perhaps indicates the disparity in CFRs between developing and developed countries. This disparity was also highlighted in a recent meta-analyses of the global CFRs of pediatric severe sepsis and septic shock which indicated that CFRs were significantly higher in developing countries (31.7% [95% CI, 27.3%-36.4%]) compared to developed countries (19.3% [95% CI, 16.4%-22.7%]; P < .001) [46]. One of the noteworthy findings of this study is that children with underlying comorbidity were 6.8 times more likely to die than those without. Similarly, in a study on pediatric severe sepsis in the US, case-fatality rate was reported to be significantly higher in children with underlying comorbidity [4].

We acknowledge some limitations to our study. The sample size was small and more patients and data would have been useful for our analysis. Because the study hospitals are tertiary care facilities with shortage of beds, a selection bias might have occurred due to the possibility that children with comorbidities are preferentially admitted while those without comorbidities are referred to other health care centers. Since we did not perform serial blood cultures, the rate of culture positivity in the present study could be an underestimation. Limitations in proper transport and storage of whole blood might have also affected the results of *lyt*A PCR.

Conclusions

The study identified that *S. pneumoniae* and *K. pneumoniae* are the main etiological agents of CAS among children at the two pediatric emergency departments in Addis Ababa, Ethiopia. All the pneumococci isolates were non-PCV10 serotypes. There was a high rate of antimicrobial resistance to first and second line antibiotics used to treat sepsis, among gram negative isolates which indicates the need for enhanced surveillance of antimicrobial resistance and assessment of treatment options in Ethiopia. Presence of LRTIs in the preceding 3 months was a predictor of culture-proven CAS. Presence of comorbidity was a predictor of mortality. Further large scale studies on the etiology, antibiotic resistance and prognostic factors of CAS in Ethiopia are warranted.

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