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**COLLEGE OF HEALTH SCIENCES**  
**DEPARTMENT OF MEDICAL LABORATORY SCIENCES**



**Magnitude of Pediatric Sepsis and Antimicrobial Resistance at Adama Medical College Hospital, Adama, Ethiopia.**

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This is to certify that the thesis prepared by Megfira Redwan, entitled "**Magnitude of Pediatric Sepsis and Antimicrobial Resistance at Adama Medical College Hospital, Adama, Ethiopia**", and submitted in partial fulfillment of the requirements for a Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards concerning originality and quality.

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**Stop sepsis. Save lives.**

# I Table of Contents

<b>I</b>	<b>Table of Contents .....</b>	<b>iii</b>
	<b>List of Tables.....</b>	<b>vi</b>
	<b>List of Figures.....</b>	<b>vii</b>
	<b>Abbreviations/Acronyms .....</b>	<b>viii</b>
	<b>Abstract.....</b>	<b>ix</b>
<b>1</b>	<b>Introduction .....</b>	<b>1</b>
1.1	Background .....	1
1.2	Statement of the problem .....	3
1.3	Significance of the study .....	4
<b>2</b>	<b>Literature Review.....</b>	<b>5</b>
<b>3</b>	<b>Objectives.....</b>	<b>11</b>
3.1	General Objectives .....	11
3.2	Specific Objectives.....	11
<b>4</b>	<b>Materials and Methods .....</b>	<b>12</b>
4.1	Study Area.....	12
4.2	Study design and study period .....	12
4.3	Population.....	12
4.3.1	Source Population .....	12
4.3.2	Study population .....	12
4.4	Inclusion and exclusion criteria.....	13
4.4.1	Inclusion criteria.....	13
4.4.2	Exclusion criteria.....	13
4.5	Study Variables .....	13
4.5.1	Dependent variables .....	13

4.5.2	Independent variables.....	13
4.6	Sample size determination and Sampling technique.....	13
4.6.1	Sample size determination .....	13
4.6.2	Sampling technique .....	14
4.7	Data collection and laboratory processes .....	14
4.7.1	Blood specimen collection and transportation .....	14
4.7.2	Extended-spectrum beta-lactamase .....	15
4.7.3	Carbapenemase production test.....	16
4.7.4	MRSA test.....	16
4.8	Data Quality Assurance.....	17
4.8.1	Pre-analytical phase.....	17
4.8.2	Analytical phase .....	17
4.8.3	Post-analytical phase .....	17
4.9	Data Processing and Analyses.....	18
4.10	Operational definitions.....	18
4.11	Ethical consideration .....	19
<b>5</b>	<b>Results .....</b>	<b>20</b>
5.1	Prevalence, Age, and Sex Distribution.....	20
5.2	Frequency and distribution of bacterial isolates.....	20
5.3	Antibiotic resistance pattern of bacterial isolates.....	21
5.4	Magnitude of Methicillin Resistant <i>Staphylococcus aureus</i> (MRSA) .....	24
5.5	Magnitude of ESBL Gram-negative bacteria isolates.....	24
5.6	Magnitude of carbapenem resistant gram-negative isolates .....	24
<b>6</b>	<b>Discussion.....</b>	<b>26</b>
<b>7</b>	<b>Limitations .....</b>	<b>29</b>

<b>8</b>	<b>Conclusion.....</b>	<b>30</b>
<b>9</b>	<b>Recommendation.....</b>	<b>31</b>
<b>10</b>	<b>References .....</b>	<b>32</b>
<b>11</b>	<b>Appendix .....</b>	<b>40</b>
11.1	Annex 1: English version of participant information sheet for guardians .....	40
11.2	Annex 2: Amharic version of participant information sheet for guardians.....	43
11.3	Annex 3: Procedure for specimen collection, processing and interpretation.....	46
<b>12</b>	<b>Declaration .....</b>	<b>64</b>

## List of Tables

Table 1: Prevalence, Age, and Sex Distribution .....	20
Table 2: Antibiotic resistance patterns of Gram-negative bacteria ( <i>Enterobacteriaceae</i> , <i>Acinetobacter</i> , and <i>Pseudomonas</i> species) isolated from children with suspected sepsis. .....	22
Table 3: Multidrug resistance frequency of <i>Enterobacteriaceae</i> , <i>Acinetobacter</i> , <i>Pseudomonas</i> and other species .....	23
Table 4: Prevalence of ESBL-producing and carbapenemase-resistant gram negative and MRSA among bacterial Isolates.....	25

## List of Figures

Figure 1: Frequency and distribution of bacteria isolated from children investigated for pediatric sepsis .....	21
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## Abbreviations/Acronyms

AHMC	Adama Hospital Medical College	ICU	Intensive Care Units
AMR	Antimicrobial resistance		
APHRC	Adama Public Health Research Center	IPSCC	International Pediatric Sepsis Consensus Conference
AST	Antimicrobial Susceptibility Testing	LMIC	Low- and Middle-Income Countries
CDC	Center for Disease Control	MCIM	modified carbapenem inactivation method
CDT	Combination disk test	MDR	Multidrug Resistance
CLSI	Clinical laboratory standards institute	MHA	Mueller-Hilton Agar
CONS	Coagulase negative staphylococci	MRSA	Methicillin resistant staphylococcus aureus
CRGN	Carbapenem-resistant gram- negative bacterial pathogens	NICU	Neonate intensive care unit
CRO	Carbapenemase Resistant organisms	PEPFAR	President's Emergency Plan for AIDS Relief
ESBL	Extended spectrum beta-lactamase	SPSS	Statistical Package for Social Sciences
GNB	Gram-negative bacteria	TSB	Tryptic soy broth
HC	Health center	ZOI	Zone of inhibition

## Abstract

**Background:** Pediatric sepsis and antimicrobial resistance are a leading cause of severe illness and death among children, especially in developing countries.

**Objective:** The aim of this study was to determine the magnitude of pediatric sepsis and antimicrobial resistance at Adama Medical College Hospital, Ethiopia.

**Methods:** A cross-sectional study was conducted at Adama Hospital Medical College from March 2024 to January 2025. Blood was collected for culture in aseptic techniques and Tryptic Soya Broth was used for inoculation. Following incubation, subculturing was performed on blood agar, chocolate agar and MacConkey agar plates. For samples that showed bacterial growth, identification was carried out based on colony morphology, Gram staining, and standard biochemical tests. Once the bacterial species were identified, antibiotic susceptibility testing was performed using the disk diffusion methods. Additional tests were conducted to detect extended-spectrum beta-lactamase (ESBL) production, carbapenemase resistance, and methicillin-resistant *Staphylococcus aureus* (MRSA). Statistical analysis was performed using SPSS version 26.

**Result:** Among 400 blood cultures done, 144 (36%) showed bacterial growth. The bacteria that were most dominant in this study were *Staphylococcus aureus* 36 (25%), *Acinetobacter baumannii* 25 (17.4%), *Enterobacter cloacae* 13 (9%), *Klebsiella pneumoniae* 8 (5.6%), and *Serratia marcescens* 8 (5.6%). *Enterobacteriaceae* most often showed resistance to ampicillin (94.1%), cefotaxime (89.2%), cefepime (86.2%), and ceftazidime (84.6%). MDR frequency of *Enterobacteriaceae* was 87%, and Gram-positive was 33.3%. MRSA was found to be 88.9%, ESBL production was detected in 87.5% of *Citrobacter koseri* (n=8), 85.7% in *Providencia stuartii* (n=7), and 83.3% in *Enterobacter cloacae* (n=12), and carbapenemase resistance was detected in 100% of *Pantoea agglomerans* (n=2) and 50% of *Acinetobacter baumannii* (n=6).

**Conclusion:** The prevalence of pediatric sepsis and the level of multidrug resistance (MDR) among the children studied were notably high. However, certain antibiotics from the carbapenem class, along with amikacin, demonstrated effective antimicrobial activity. The rates of methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum beta-lactamase (ESBL)-producing organisms were also significantly higher than expected.

**Keywords:** Pediatric, sepsis, ESBL, MRSA, carbapenemase, AMR, Ethiopia

# 1 Introduction

## 1.1 Background

Sepsis is the development of bacteria and their toxins in the blood, resulting in septicemia, a systemic sickness, which can make the host's defenses against infection inadequate, leading to sepsis, an organ failure with a high risk of death (1,2). It is a significant international child health problem with increasing occurrences and a high mortality rate due to its inflammatory response to an infection (3). This study focuses on children; a child is any person under the age of 18 (4).

A systemic infection that occurs in children and is a significant cause of death and sickness to newborns and children is called pediatric sepsis (5). It is estimated that pediatric sepsis claims the lives of 2.9 million children less than the age of five each year on a global scale and developing countries in particular have higher incidence and higher mortality (6). According to some research, up to 20% of children get sepsis, and 1% pass away from sepsis-related reasons (7). In low- and middle-income countries, including Ethiopia, it is suggested that there are around 19 million instances of sepsis and 5 million deaths from sepsis annually (8). The most common pathogens seen are various types of *Acinetobacter*, *Enterobacter*, *Klebsiella*, and *Pseudomonas*, as well as *S. aureus* and *E. coli*, which constitute significant causes of sepsis, indicating differences across geographic areas (9).

The problem of pediatric sepsis is made more difficult by the increasing threat of antibiotic resistance (AMR), which, according to the WHO, arises when bacteria show insensitivity to antimicrobial medications. AMR signifies a phenomenon that occurs naturally and transpires gradually within pathogens due to genetic transformations (10). The global distribution of resistance to various antimicrobial agents, including cephalosporins, fluoroquinolones, penicillins, aminoglycosides, monobactams, macrolides, and carbapenems, is a matter of great concern (9). Improper administration of antibiotics for sepsis due to antimicrobial resistance and prolonged hospitalization has been linked to increased mortality rates in children, with the impact being more severe in newborns (11). In the last two decades, multi-drug resistance has reached a pandemic level in various bacterial pathogens (12). Geographical and regional location, healthcare infrastructure, and current practices all influence this resistance pattern (13). An important factor in the poor prognosis of pediatric sepsis is the increasing frequency

of resistance of bacteria to antibiotics, such as *Acinetobacter baumannii*, *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, which are especially worrisome (14).

One important mechanism among the several types of AMR is the development of extended-spectrum beta-lactamase (ESBL) in gram-negative bacteria, which is the ability of certain bacteria to produce unique enzymes known as ESBLs that degrade and eliminate specific antibiotics, thereby rendering the bacterium resistant. It has a significant impact on critical sepsis in infants living in poor nations (15). Because of the rise and quick global dissemination of antibiotic resistance, gram-negative bacteria (GNB) are particularly alarming, especially those that produce carbapenemase, which are bacteria that produce the enzymes known as carbapenemase, which break down and render ineffective carbapenem antibiotics, including ertapenem, imipenem, and meropenem.

This poses a serious threat to global health because they make infections difficult to treat by producing enzymes that break down antibiotics; the absence of novel antibiotics exacerbates the issue, and due to the extensive use of such antimicrobials, novel resistance mechanisms have emerged, leading to a large increase in the incidence of carbapenem-resistant gram-negative bacterial infections (CRGNs) over the past few decades (16–18)

The threat is further increased by the growing detection of MRSA, which makes treatment options even more challenging. Any strain of *Staphylococcus aureus* that has become resistant to methicillin and its derivatives is referred to as MRSA. It poses a major risk to public health (19,20). The prevalence of *S. aureus* bacteremia in pediatric patients varies by area, ranging from 3.7 to 15 occurrences per 100,000 persons, with the highest rates occurring in infants less than one year old (15,21)

This research was initiated in response to the growing concern of pediatric sepsis, with the aim of investigating bacterial origins and resistance patterns to provide better treatment techniques and clinical outcomes in children.

## 1.2 Statement of the problem

Research reveals that there were 48.9 million diagnoses of sepsis, resulting in 11 million losses of life, which accounted for 20% of global deaths. It is estimated by WHO that in 2020, 41% of sepsis cases were children under five, and the death rate among intensive care patients with septicemia was 42% (8,17). Pediatric sepsis accounts for around 30% of infant fatalities in underdeveloped nations (22). With proper interventions targeted at this crucial stage of life might spare roughly 3 million lives (23).

Despite medical progress, pediatric sepsis still has a terrible prognosis, and many underdeveloped settings lack access to contemporary laboratory techniques like blood culture, which is the best test for diagnosing sepsis. Therefore, it forces us to start effective antibiotics as soon as possible for patients with blood infections, even before the test results are available, which could lead to AMR (23). According to a systematic review done in 2022, in Ethiopia, Uganda, Kenya, and Sudan, the prevalence of pediatric sepsis was 38.31%, 24.4%, 18.28%, and 39.26 percent, respectively (24). Like many developing countries, Ethiopia faces this major problem, accounting for 33% of all newborn deaths, according to a study. Moreover, multi-drug-resistant bacteria complicate the management of sepsis, with antimicrobial resistance linked to 30% of deaths (25) .

The magnitude of pediatric sepsis diseases distribution differs by location and time that needs a regular investigation of certain location. This emphasizes the necessity of additional thorough and focused research like this one. There are published research findings on pediatric sepsis in different areas of Ethiopia, including Oromia region. However, when compared to the size of the population, the existing studies are limited, and most papers tend to focus on neonates not older children. To address these gaps, further research is needed to understand the mechanisms underlying pediatric sepsis and AMR, and to develop effective strategies for their prevention and management. Hence, this study aimed to determine the magnitude of pediatric sepsis and antimicrobial resistance at Adama Medical College Hospital, Adama, Ethiopia.

### **1.3 Significance of the study**

This research is important because it has the possibility to enhance the management and outcomes of children with bacterial sepsis. By determining the prevalence of pediatric sepsis and the frequency of identified pathogens, this study will guide evidence-based therapy and help in the development of antibiotic administration programs in the study site. The analysis of MDR patterns will help choose the right antibiotics and lower the chance of medication not succeeding. This will also help healthcare providers manage children with sepsis, reducing morbidity and mortality. Additionally, this research can be utilized to develop strategies to prevent the development and dissemination of resistance to antibiotics, which is a global concern.

Overall, the results of this investigation will have implications for clinical practice, public health policies, and research, and this research has the potential to improve pediatric sepsis management and address the challenges of antimicrobial resistance in Ethiopia. It will also fill gaps in literature, paving the way for other researchers to explore this topic further.

## 2 Literature Review

### Prevalence of Pediatric Sepsis

An estimated three million children die from sepsis each year, with significant mortality rates seen in those who need to be admitted to an intensive care unit. The WHO and the Global Sepsis Alliance recognize the need for better early sepsis detection and stress the importance of this effort (26). It is one of the leading causes of illness and death worldwide (17,27). To improve clinical outcomes, adequate and targeted antimicrobial therapy requires early and accurate identification of the causative bacteria diagnosis of the bacteria (19,28).

Studies show that approximately 75,000 pediatric patients in the United States and 360,000 in China are treated annually for severe sepsis, with an incidence rate of 1.8 per 1,000. Alarming findings have also been reported in parts of Europe, Asia, and South Africa (3,29). While an investigation done in Myanmar encompassed 42% of pediatrics bacterial sepsis (21). Findings in Nigeria revealed that among children with sepsis, the incidence of positive blood cultures was 25.2%, most frequent bacterium found in patients was *S. aureus* (30). This explains the gravity of the situation with pediatric sepsis and how much it needs attention, so papers like this are essential.

As stated earlier, over 19 million instances of sepsis and 5 million deaths are thought to occur annually in low- and middle-income nations, including Ethiopia (8). Various Studies done in Northwest of Ethiopia, specifically in Gondar, at different times reported that the proportion of newborns admitted with pediatric sepsis were 11.1%, 32.1%, and as high as 46.6% (31–33). Separate investigation conducted in Gondar at different times showed 20.7%, and 64.8% positive bacterial isolates for pediatric sepsis, with a 65% prevalence of multidrug resistance (MDR) identified (34,35).

Furthermore, a study done in the northeast parts of Ethiopia at Comprehensive Specialized Hospitals in Woldia and Dessie showed a prevalence of 79.4% (36). Which is a much higher prevalence rate than that found in Gonder. When we look at the south of Ethiopia. A study done in Wolaita Sodo Town showed a significant decrease in the prevalence of children with sepsis, 33.8% (37). In other parts of southern Ethiopia, such as Arbaminch General Hospital, 78.3% of children admitted had sepsis, while the remaining 21.7% were admitted for other illnesses (38). which was a much lower rate (39).

In the western part of Ethiopia, there was a study done in the Wollega zone. Their result showed sepsis was present in 78.7% of their cases, more than three-fourths (40). A somewhat similar result was also found in southern parts of the Oromia region in Shashemene, where the prevalence of sepsis was 77.9% (41). Results found in the western part of Ethiopia are more consistent than in the west and the south. Meanwhile, studies found in Addis Ababa, the capital city of Ethiopia, showed a somewhat consistent result of bacterial pediatric sepsis from different hospitals with a prevalence of 17.1%, 18.81%, and 21% for positive bacterial isolates of culture (25,42,43). Various parts of the country have given various results, so filling the gap is needed more than ever and finding out how it has escalated over the years.

### **Prevalence of different bacterial species in pediatric sepsis**

Turning now into the pathogens that are most or least prevalent, a bacteria that is typically regarded as the primary cause of sepsis in developed countries is Group B *Streptococcus*. However, in sub-Saharan Africa, *Klebsiella* species, *Enterobacter* species, *Escherichia coli*, and *Pseudomonas* species were found to be the major causes of blood culture-positive isolates (44). A review done regarding LMICs, including African and South Asian countries, found that *Escherichia coli*, *Serratia marcescens*, and *Klebsiella pneumoniae* gram-negative bacteria were the most prevalent pathogens causing sepsis in these countries. For example, *K. pneumoniae* was predominantly found in Ethiopia, while *Serratia marcescens* was more prevalent in Nigeria, India, and South Africa (45). *Escherichia coli*, *Klebsiella* spp., and *Staphylococcus aureus* accounted for 55% of positive bacterial cultures for sepsis among children in developing countries, while *E. coli*, *Klebsiella* spp., *S. aureus*, and *S. pneumoniae* were the most dominant pathogens in infants, which accounted for 59% of positive bacterial cultures (43). Infants with *Pseudomonas aeruginosa*, *Serratia marcescens*, and *E. coli* infections had the highest mortality rates (46).

Correspondingly, in countries like the United Kingdom and the United States, the leading organisms causing sepsis are Group B *Streptococcus* (GBS) (about 43%), *Staphylococcus aureus*, and *Streptococci*, while among gram-negative isolates, *Escherichia coli* (25.29%) was mainly found (19,47). In contrast, a study done in India showed a prevalence of 7.6% of sepsis, with 48.21% of it being *Klebsiella pneumoniae*, while CONS (8.92%) and *Enterococci* (16.67%) were recovered amongst the gram-positive organisms (7). There was also a study done in western Mexico; their main isolated bacteria were *Klebsiella pneumoniae* and Gram-

negative bacilli (20). A study done in China showed both gram-positive and gram-negative bacteria prevalence, such as CONS and *Staphylococcus aureus*. While *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Salmonella* were observed among the Gram-negative bacteria (48).

On the other hand, in studies in Africa, such as in Libya, their most dominant bacteria found were Gram-positive bacteria (53.33%), which included *Staphylococcus aureus* (20%) and *Staphylococcus epidermidis* (15%) (49). While also a study done in Ethiopia, the principal MDR strains were *S. aureus*, accounting for 61.2%, and *K. pneumoniae*, constituting 74%. (33).

### **Antimicrobial Resistance**

One of the biggest risks to world health today is the acceleration of antimicrobial resistance (AMR) in bacterial diseases. A recent study estimated that AMR resulted in 1.27 million attributable deaths worldwide in 2020 (50). When bacteria continue to multiply in the presence of antimicrobial medications, resistance develops (51). Antibiotic resistance poses a serious concern to the world today and is growing over time in all nations, resulting in significant economic costs. (52). The third most prominent global factor contributing to mortality is attributed to the emergence of resistance to antibiotics (53).

The growing resistance of bacteria to commonly used antibiotics is putting children's lives at risk, especially in hospital settings. In western Mexico, researchers found that the majority of hospital-acquired bacteria were no longer responding to essential antibiotics: nearly all were resistant to ampicillin, and high levels of resistance were also observed in gentamicin, amikacin, and cefotaxime at 98.5%, 84.4%, 65.6%, and 83.3%, respectively (20). The situation is even more alarming in some regions. A study from Iraq revealed that the standard antibiotics recommended by the World Health Organization for treating sepsis in children were entirely ineffective; some bacterial strains were resistant to every drug tested (54). In Dar es Salaam, sepsis continues to take lives, with a reported mortality rate of 9.4%. Although resistance to certain drugs, including amikacin and ciprofloxacin, was still relatively low (under 40%), nearly all bacterial strains were resistant to ampicillin, and a large portion resisted ceftriaxone as well. *Pseudomonas aeruginosa* strains showed a worrying pattern: while they remained treatable with amikacin, they were completely resistant to ampicillin, gentamicin, and ceftriaxone (55). These patterns show just how unpredictable and region-specific antimicrobial resistance can be, and how urgently new strategies are needed to protect pediatric patients

The next area of concern is the challenge of controlling antimicrobial resistance in Ethiopia, which is further complicated by the vast development of excessive antimicrobial prescribing, abuse, and self-purchase without proper identification of the proper pathogen (8,56). This can be seen in findings from a study done at the University of Gondar Hospital, Northwest Ethiopia: 76% of the bacterial isolates had a multidrug resistance (MDR) antimicrobial susceptibility pattern. The result demonstrated that the best antibiotics for Gram-positive bacterial isolates were ciprofloxacin, clindamycin, chloramphenicol, and gentamicin, whereas the best antibiotics for Gram-negative pathogens were chloramphenicol, meropenem, and amikacin. Forty-five percent of *Staphylococcus aureus* were found to be methicillin resistant (57).

Overall findings suggest that accurate microbiological diagnosis and antibiotic susceptibility testing (AST) are crucial in the management of pediatric sepsis (10,58). Timely and accurate microbiological diagnosis and AST can help reduce morbidity, mortality, and the overuse of antibiotics in sepsis (19). AMR being one of the ever-growing health crises is a sign more than ever research needs to be done.

### **ESBL Producing Gram-negative Bacteria**

Among the most alarming resistance pathways observed in isolates is ESBL production, particularly among Gram-negative bacteria. Infections brought on by ESBL-producing bacteria have led to huge consequences, like lower rates of antimicrobial and medical reaction and prolonged durations in hospitals (59). The WHO and CDC have taken the initiative to formulate and release a comprehensive worldwide strategy focused on combating AMR and classifying MRSA- and ESBL-producing microorganisms, along with other MDR bacteria, as powerful threats (60,61). Beta-lactam medications, including aminoglycosides (such as gentamicin), carbapenems, cephalosporins, extended-spectrum penicillins, fluoroquinolones (such as ciprofloxacin), and monobactams, are widely prescribed antibiotics for treating infections caused by gram-negative bacteria (62).

ESBL-producing bacteria are becoming a major threat in the treatment of pediatric sepsis, especially in low-resource settings. In Tanzania, 76% of cases involved Gram-negative bacteria, 77% of which were ESBL producers. *Klebsiella pneumoniae* was the leading cause of these infections and related deaths (63). Similarly, in Ethiopia, various members of the *Enterobacteriaceae* family were isolated from children with sepsis, including *Enterobacter* spp., *Proteus* spp., *Citrobacter* spp., *Morganella* spp., *Providencia* spp., *Salmonella* spp., and

*Serratia* spp. Among them, *Klebsiella* spp. and *E. coli* were the most common ESBL-producing Gram-negative isolates (9). A recent meta-analysis of several dispersed and sparse studies conducted in Ethiopia found an overall prevalence of 18% (61). These findings highlight the increasing burden of drug-resistant infections in children and the challenges they pose to effective treatment.

### **Carbapenem-resistant Gram-negative Bacteria**

The other, more severe type of AMR is the incidence of carbapenem-resistant gram-negative bacterial infections (CRGNs), which has increased dramatically over the past few decades because of the widespread use of such drugs, which has led to the adoption of novel resistance mechanisms (64). Resistance to carbapenem frequently coexists with resistance to a variety of other antibiotics, such as beta-lactams and other medications like quinolones and aminoglycosides. Infections with these extremely resistant bacteria can cause severe illness and a 40–50% chance of mortality, according to numerous studies (65,66).

Globally, carbapenem-resistant gram-negative (CRGN) bacteria represent a significant risk to public health because of their high rates of morbidity and mortality (67). The best therapeutic therapy for CRGN infections is still unknown due to resistance to carbapenems and the most widely used medicines (68). Consequently, the WHO has assigned CRGN as a disease of crucial importance, and the CDC has identified it among the three greatest issues related to antibiotic resistance (69,70).

The burden of carbapenem-resistant Gram-negative bacteria (CRGNs) in pediatric sepsis is increasingly evident across different regions. Findings in Turkey and Italy showed that the presence of CRGNs in their research was 32% and 78.6% (66,71). A systematic review done on Africa showed a 30.34% overall incidence of carbapenem resistance. While 35.57% and 34.35%, respectively, of gram-negative bacteria were resistant to imipenem and meropenem. (72). While a study done in Ethiopia showed a higher pooled prevalence of carbapenem resistance, 47.4% and 69%, respectively (73,74). These numbers are deeply concerning and point to the urgent need for more in-depth studies to understand the drivers of resistance and guide more effective treatment strategies.

### **Methicillin resistant *staphylococcus aureus* (MRSA)**

Further escalating the threat, MRSA organisms are increasingly detected, complicating treatment options even more severely. Even when properly treated with antibiotics, *Staphylococcus aureus* bacteremia (SAB) can result in significant illness and death (75). In pediatric cases, the prevalence of *S. aureus* bacteremia varies by area, ranging from 3.7 to 15 cases per 100,000 children. Infants less than one year old have very high rates (76,77).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant public health concern, known for causing difficult-to-treat infections with higher mortality rates compared to methicillin-sensitive strains (78,79). Findings from a meta-analysis done globally show the incidence of MRSA in the pediatric population is 5% (80). While in a study done in South Korea, MRSA prevalence was observed in pediatric septicemia to be 44.8% (78). This result is somewhat consistent with findings conducted in the northwest of Ethiopia; the prevalence of MRSA among the pediatric cases was 45.8% (58). As seen, the result of MRSA seems to be higher than in the rest of the global world; hence, emphasis needs to be given to this area of antimicrobial resistance. The findings found in many literatures shows a significant fluctuation in many geographic area; specifically in Ethiopia this calls for more studies and a representative sample size analysis.

## **3 Objectives**

### **3.1 General Objectives**

To determine the magnitude of pediatric sepsis and antimicrobial resistance at Adama Hospital Medical College Hospital, Adama, Ethiopia.

### **3.2 Specific Objectives**

- To describe bacterial etiologies of pediatric sepsis.
- To assess the multidrug resistance level of bacteria isolated from children suspected of sepsis.
- To determine the prevalence of extended-spectrum beta-lactamase-producing gram-negative bacteria among children suspected of sepsis.
- To determine the level of carbapenem-resistant gram-negative bacteria among children suspected of sepsis.
- To assess the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) among children suspected of sepsis.

## **4 Materials and Methods**

### **4.1 Study Area**

The study was carried out in Adama, Oromia, Ethiopia, at Adama Hospital Medical College (AHMC). Located in Adama town, approximately 100 kilometers from Addis Ababa, Ethiopia, it is one of the first medical hospitals in Oromia. The hospital was elevated to medical college in 2011 due to its location, patient volume, and staffing level. The hospital serves more than six million people from five regions: Oromia, Amhara, Afar, Somalia, and Dire-Dawa. It can accommodate an average of 1000 patients every day with its 232 beds, six medical case teams (OPDs), and numerous specialty clinics (80). The pediatric and NICU ward sepsis suspected cases recorded in this hospital an average are 5 patients per day.

The laboratory process was performed at Adama Public Health Research and Referral Laboratory Center, which was first established in 2004 and then expanded by PEPFAR and officially opened on 31 January 2011. It provides services for 9 zones, 14 town administrations, 58 hospitals, 704 HCs, and 2 neighboring regions (Amhara & Afar) as a referral laboratory. This research was done in the clinical bacteriology department, which is nationally accredited (81).

### **4.2 Study design and study period**

A cross-sectional investigation was carried out at a hospital from March 2024 to January 2025.

### **4.3 Population**

#### **4.3.1 Source Population**

All pediatric patients who sought medical services at AHMC throughout the research period.

#### **4.3.2 Study population**

Pediatric patients were suspected of developing bacterial sepsis during the study period and fulfilled the inclusion criteria.

## **4.4 Inclusion and exclusion criteria**

### **4.4.1 Inclusion criteria**

- All pediatric groups, including neonates up to 15-year-old children.

### **4.4.2 Exclusion criteria**

- Patients receiving antibiotics at the time of sample collection.
- Patients whose guardians/parents didn't consent to take part in the research.

## **4.5 Study Variables**

### **4.5.1 Dependent variables**

The following dependent variables are defined:

- Magnitude of pediatric sepsis.
- Magnitude of multidrug-resistant bacteria among children suspected of sepsis.
- Prevalence of ESBL-producing gram-negative bacteria
- Prevalence of carbapenem-resistant gram-negative bacteria
- Prevalence of methicillin-resistant *Staphylococcus aureus* among isolated gram-positive bacteria

### **4.5.2 Independent variables**

The following independent variables are defined: Age, sex, and underlying cause of sepsis.

## **4.6 Sample size determination and Sampling technique**

### **4.6.1 Sample size determination**

For this investigation, the necessary sample size was determined with a pediatric sepsis prevalence of 63.87% that was found in Jimma, Ethiopia (82).

The following formula was used to determine the sample size:

$$n = Z^2_{(\alpha/2)} P (1 - P) / d^2$$

Where: where the estimated proportion is denoted by P-.

Since a 95% confidence range was utilized,  $Z(\alpha/2)$  will be 1.96 d, where d is the margin of error, which in this case is 0.05.

The level of error is represented by  $\alpha$ -, which is  $n = 1.962 \times 0.6387 (1 - 0.6387)/0.05^2 = 354.12$  rounded to 354

for a 10% non-respondent rate, this will be 393.

#### **4.6.2 Sampling technique**

Until the necessary sample size was reached, study participants who satisfied the inclusion criteria were included using a convenience sampling procedure.

### **4.7 Data collection and laboratory processes**

#### **Data collection**

Skilled nurses who were working for the NICU and pediatric ward were recruited and trained on blood sample collection for blood culture. Additional details such as age, sex, and ID were collected.

#### **4.7.1 Blood specimen collection and transportation**

After properly locating a suitable vein and disinfecting the vein puncture site using iodine tincture and 70% isopropyl alcohol, the blood sample was drawn from each patient. Depending on the age, 1 to 10 ml of blood was collected, for example. For neonates (0-28 days), 1- 2 ml was collected, while for adolescents (13+ years), 10 ml of blood sample was collected and transferred into 2 bottles of blood culture bottle containing tryptose soy broth (TSB). Each vial of blood culture was taken to the microbiology lab as soon as it was collected.

#### **Blood culture**

Each blood culture bottle was incubated aerobically for up to 7 days at 37°C or until a sign of growth was detected. Every 24 hours, macroscopically, each one was examined for signs of bacterial growth, such as gas generation, turbidity, hemolysis, or the development of distinct colonies. Whatever the bacterial development stage, each blood culture was subcultured onto

sheep blood agar and MacConkey agar after 24 hours, 48 hours, and 72 hours and finally on the 7th day on chocolate agar.

### **Isolation and identification**

After isolating the pure colony that grew on the subculture media. The etiologic agent is determined by the colony's morphological features, Gram stain, and the outcomes of biochemical tests. Coagulation, catalase, and mannitol salt agar were used to identify gram-positive bacteria, while citrate utilization, triple sugar iron, urea, oxidase, Lysine Iron Agar, DNase, and motility tests were used to identify gram-negative bacteria (83). More in-depth detail according to the CLSI guidelines is in the annex.

While the growth of the microorganism in blood culture is diagnostic in the patient, the failure to produce it does not exclude the diagnosis. The volume of blood drawn, the time of collection, and the quantity of samples taken are all variables that could affect the pathogen recovery from the blood. Blood culture sensitivity may be reduced in neonates with low or sporadic bacteremia and maternal antibiotic exposure (83).

### **Antimicrobial susceptibility testing**

Following the guidelines set forth by the Clinical and Laboratory Standards Institute (CLSI 2023), antimicrobial susceptibility testing was conducted for isolated organisms on Kirby-Bauer's disk diffusion on MHA. For isolated bacteria, proper antibiotic discs for antimicrobial susceptibility tests were utilized. For gram positives, cefoxitin, oxacillin, and penicillin were used. For gram negatives, drugs from the cephalosporin, penicillin, aminoglycoside, carbapenem family, etc. According to the CLSI guidelines, it was used for the appropriate bacteria (84).

#### **4.7.2 Extended-spectrum beta-lactamase**

##### **Screening for potential ESBL-producing isolation**

As suggested by CLSI guidelines, isolates that exhibited an inhibitory zone size of  $<22$  mm and/or  $\leq 27$  mm with ceftazidime (30  $\mu\text{g}$ ) and with cefotaxime (30  $\mu\text{g}$ ), respectively, were chosen for confirmation of ESBL production using CDT. These isolates were regarded as possible ESBL-producers (screening ESBL positive) (84).

##### **Confirmation of ESBLs with the combination disc test**

Before inoculating the bacteria into a Mueller-Hinton agar plate, a 0.5 McFarland suspension of the isolate is prepared. Then the antibiotic disks (with and without clavulanic acid) are placed 25 mm apart (center to center). The plate was then incubated at  $35 \pm 2^\circ\text{C}$  for 16–18 hours. Interpretation: Positive for ESBL if there was a zone diameter increase or equal to 5 mm for either ceftazidime + clavulanic acid vs ceftazidime alone, or cefotaxime + clavulanic acid vs cefotaxime alone (84).

### **4.7.3 Carbapenemase production test**

#### **Screening for carbapenemase**

Carbapenemase enzyme screening was done by using meropenem (10  $\mu\text{g}$ ) and imipenem (10  $\mu\text{g}$ ) and looking for a zone of inhibition. If the zone of inhibition for imipenem is  $<19$  mm and that for meropenem is  $<16$  mm, it is indicative of carbapenemase production. Further confirmation was done by using a modified carbapenem inactivation method (MCIM) technique (84).

#### **Phenotypic confirmatory test for carbapenemase**

Phenotypic confirmatory testing for carbapenem-resistant isolates to produce carbapenemase was carried out using the modified carbapenem inactivation technique (MCIM). A 10-g meropenem disc was put in the tube along with a 1  $\mu\text{l}$  loop of the tested isolate (but 10  $\mu\text{l}$  for *Pseudomonas*) that had been emulsified in 2 ml of trypticase soy broth (TSB) for 4 hours and 15 minutes of incubation. It was then placed on a Mueller-Hinton agar (MHA) plate that had been inoculated with *E. coli* ATCC 25922. The plate was then incubated at  $35^\circ\text{C}$  for 18 to 24 hours, during which time a zone of inhibition measuring 6 to 15 mm and the presence of pinpoint colonies within a zone measuring 16 to 18 mm were deemed positive to produce carbapenemase (84).

### **4.7.4 MRSA test**

A cefoxitin disk was used to detect methicillin-resistant *S. aureus* (MRSA). After inoculating a known *S. aureus* into an MHA agar plate, a cefoxitin disk was placed on it and incubated for 24 hours at  $35^\circ\text{C}$ . The bacterial growth surrounding the antibiotic disc was measured for the zone of inhibition (ZOI) diameter ( $< 21$  mm). After measuring the clear zones (inhibition

zones), the results were converted into sensitive (S) and resistant (R) categories using the Clinical and Laboratory Standard Institute's (CLSI) interpretation table (84).

## **4.8 Data Quality Assurance**

Data quality was guaranteed using standardized data collecting tools, appropriate training before data collection, and close monitoring by the lead investigator during the data collection process. In the context of laboratory analysis, the pre-analytical, analytical, and post-analytical phases of quality assurance found in the microbiological laboratory standard operating procedures were taken into consideration. Additionally, the laboratory analysis process involved the participation of skilled and knowledgeable laboratory specialists.

### **4.8.1 Pre-analytical phase**

Following a verbal and written consent request, nurses filled out all the necessary paperwork, labeled the bottle with the patient's ID number, and used a syringe to aseptically draw blood from the participants. Specimens were delivered to the microbiology lab in five to ten minutes after being collected.

### **4.8.2 Analytical phase**

Every method, piece of equipment, and material was well managed and monitored. The culture media's quality was checked for sterility and the capacity to cultivate the control bacterial strains. Standard reference strains of the American Type Culture Collection *E. coli* (ATCC-25922), *Klebsiella pneumoniae* ATCC 700603, *P. aeruginosa* (ATCC-27853), and *S. aureus* (ATCC-25923) were used as control bacteria strains for both media and antibiotic discs, and the inoculum density of the bacterial suspension was standardized for the susceptibility test using a densitometer or nephelometer that is calibrated to check the equivalent of a 0.5 McFarland standard. The APHRC microbiology laboratory's standard operating procedures (SOPs) were closely adhered to, and the senior microbiologist verified the results (85).

### **4.8.3 Post-analytical phase**

The patient's identification number and the outcomes were recorded properly. Before the results were provided to the caregiver and the clinician, the department head reviewed and checked the reports to ensure that there were no mistakes in the test results. When there were significant

public health implications, appropriate action was taken. The isolates would then be disposed according to the guidelines by first Segregating and categorizing immediately after use: Infectious/biological waste (e.g., culture plates, pipette tips, swabs), Sharps (e.g., needles, blades), Non-infectious waste (e.g., paper towels, packaging) and Chemical waste (e.g., stains, reagents) and followed by autoclaving all infectious waste (e.g., culture media, bacterial cultures) at 121°C for 15–30 minutes to render it non-infectious before disposal. Finally, incineration or burial was used for infectious/autoclaved wastes in many labs.

## **4.9 Data Processing and Analyses**

The Statistical Package for Social Sciences (SPSS) version 26 program was used to evaluate all the data once it had been encoded into Microsoft Excel. Frequencies and percentages were examples of descriptive statistics that were employed. Lastly, the findings are displayed using tables, figures, and words.

## **4.10 Operational definitions**

**Sepsis:** Participants who showed sign and symptoms such as fever ( $\geq 38^{\circ}\text{C}$ ) or hypothermia ( $< 36^{\circ}\text{C}$ ), especially in neonates, fast breathing (tachypnea), vomiting or diarrhea etc according to the WHO guidelines.

**Pediatrics:** A practice that focuses on the health and treatment of newborns, children, and adolescents up until they turn 15.

**Multi-Drug Resistance:** It is the ability of a bacterium to withstand three or more classes of antibiotics.

**ESBL production:** The ability of certain bacteria to produce enzymes known as extended-spectrum beta-lactamases that degrade and eliminate third generation cephalosporins thereby rendering the bacterium resistant.

**MRSA isolate:** It is a strain of *Staphylococcus aureus* bacteria that makes infections difficult to cure because it is resistant to methicillin and other beta-lactam medicines.

**Carbapenemase-resistant organisms (CRO):** It refers to bacteria that produce the enzymes known as carbapenemase, which break down and render ineffective carbapenem antibiotics, including imipenem, and meropenem.

#### **4.11 Ethical consideration**

The Department of Research and Ethical Review Committee of Medical Laboratory Science, College of Health Science, Addis Ababa University, DRERC/748/24/MLS as well as the institutional review board of the AHMC research directorate, provided their ethical clearance. The suspected children's parents or guardians were notified and asked for their permission. The patient's clinician was informed of every confirmed case of pediatric sepsis. Every piece of information included in the study was kept private.

## 5 Results

### 5.1 Prevalence, Age, and Sex Distribution

In this study, a total of 400 sepsis suspected children (newborn to 15 years of age) were included, of which 59.5 % (n=238/400) were male. Among all 400 blood cultures performed, 47% (n=188/400) yielded growth, where 36% (n=144/400) were pathogenic bacteria while 11% (n=44/400) were possible contaminants. Newborns have the highest incidence of bacterial sepsis at 54.5% (n=72/132), followed by infants at 34.5% (n=49/142). (Table 1).

**Table 1: Prevalence, Age, and Sex Distribution**

Demographic Data		Culture Result			
		Bacterial Growth n(%)	No bacterial Growth n(%)	Possible Contaminants n(%)	Total n(%)
<b>Sex</b>	Male	81 (34%)	134 (56.3%)	23 (9.7%)	238 (59.5%)
	Female	63 (38.9%)	78 (48.1%)	21 (13%)	162 (40.5%)
	Total	144 (36%)	212 (53%)	44 (11%)	400 (100%)
<b>Age group</b>	Newborn (<28 days)	72 (54.5%)	47 (35.6%)	13 (9.8%)	132 (33%)
	Infants ( $\leq 12$ month)	49 (34.5%)	70 (49.3%)	23 (16.2%)	142 (35.5%)
	Toddler (1-3 year)	5 (25%)	14 (70%)	1 (5%)	20 (5%)
	Early childhood (3--5 years)	10 (28.6%)	22 (62.9%)	3 (8.6%)	35 (8.75%)
	Middle childhood (6-11 years)	5 (11.6%)	35 (81.4%)	3 (7%)	43 (10.75%)
	Adolescence $\geq 12$ years)	3 (10.7%)	24 (85.7%)	1 (3.6%)	28 (7%)
Total		144 (36%)	212 (53%)	44 (11%)	400 (100%)

### 5.2 Frequency and distribution of bacterial isolates

Out of 400 blood cultures, a total of 144 pathogenic bacteria were identified. Gram-negative isolates accounted for 75% (n=108/144), while gram-positive isolates accounted for 25% (n=36/144). From the isolated gram-negative bacteria, the *Acinetobacter baumannii* complex was the most frequent at 17.4% (n=25/108), followed by *Enterobacter cloacae* at 9% (n=13/108). *Citrobacter koseri* (*Citrobacter diversus*) 5.6% (n=8/108), *Klebsiella pneumoniae* 5.6% (n=8/108), *Providencia stuartii* 5.6% (n=8/108), and *Serratia marcescens* 5.6% (n=8/108). From the identified gram-positive bacteria, *Staphylococcus aureus* was the most prevalent, accounting for 25% (n=36/144) of all pathogenic bacteria isolates. Other less frequent and rare species detected were *Moraxella catarrhalis*, *Shigella species*, *Salmonella species*, *Burkholderia cepacia* Complex, *Pasteurella multocida*, and *Stenotrophomonas*

*maltophilia*, which were 6.9% (n=10/144) in total. Figure 1 demonstrates the prevalence of bacterial isolates from hospitalized pediatric sepsis patients.

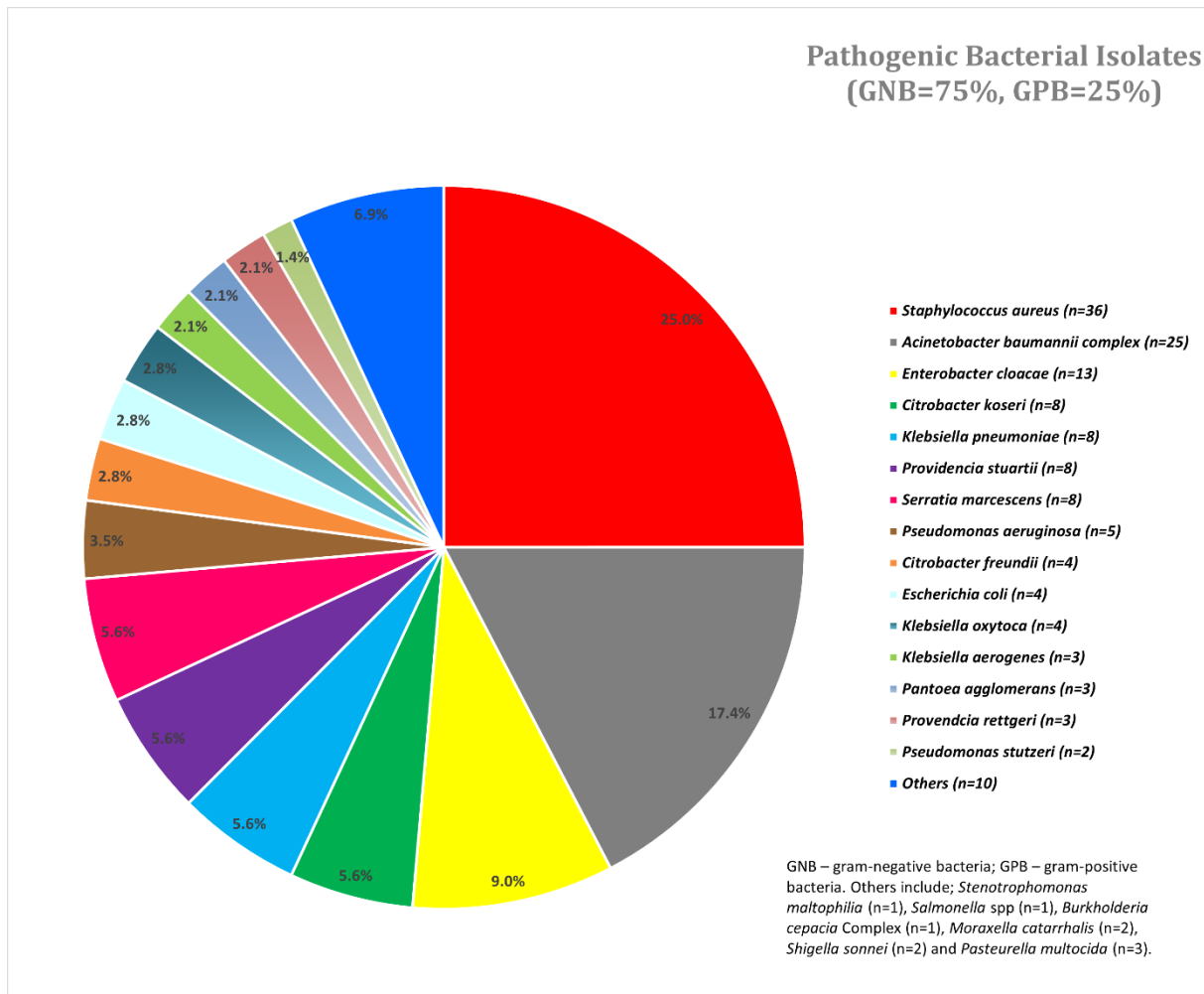


Figure 1: Frequency and distribution of bacteria isolated from children investigated for pediatric sepsis

### 5.3 Antibiotic resistance pattern of bacterial isolates

Among *Enterobacteriaceae*, resistance to ampicillin (94.1%), cefotaxime (89.2%), cefepime (86.2%), ceftazidime (84.6%), amoxicillin-clavulanate (84.6%), and cefuroxime (75.4%) was high, while amikacin (17%), imipenem (15%), chloramphenicol (23.5%), and meropenem (26.2%) showed low resistance. The antibiotic intermediate patterns and resistance to *Enterobacteriaceae*. *Serratia marcescens* (n=8) demonstrated the maximum resistance (100%) to ampicillin, amoxicillin-clavulanate, cefotaxime, ceftazidime, cefepime, and cefuroxime, while resistance to meropenem was 50% for *Serratia marcescens*. (Table 2).

**Table 2: Antibiotic resistance patterns of Gram-negative bacteria (*Enterobacteriaceae*, *Acinetobacter*, and *Pseudomonas* species) isolated from children with suspected sepsis.**

Gram negative bacteria isolates	AMK R n(%)	AMP R n(%)	AMC R n(%)	AZM R n(%)	FEP R n(%)	CAZ R n(%)	CTX R n(%)	CXM R n(%)
<i>Enterobacteriaceae</i>								
<i>C. freundii</i> (n=4)	1(25)	4(100)	4(100)	-	3(75)	3(75)	3(75)	4(100)
<i>C. koseri</i> (n=8)	2(25)	8(100)	6(75)	-	8(100)	7(87.5)	8(100)	7(87.5)
<i>E. cloacae</i> (n=13)	1(8)	13(100)	13(100)	-	12(92)	11(85)	12(92)	11(85)
<i>E. coli</i> (n=4)	1(25)	3(75)	2(50)	-	3(75)	3(75)	2(50)	3(75)
<i>K. aerogenes</i> (n=3)	2(67)	3(100)	3(100)	-	3(100)	3(100)	3(100)	3(100)
<i>K. oxytoca</i> (n=4)	0	4(100)	2(50)	-	3(75)	3(75)	3(75)	1(25)
<i>K.pneumoniae</i> (n=8)	0	8(100)	6(75)	-	7(87.5)	7(87.5)	7(87.5)	4(50)
<i>P. agglomerans</i> (n=3)	0	2(100)	2(100)	-	1(50)	2(100)	2(100)	2(100)
<i>P. rettgeri</i> (n=3)	0	3(100)	2(67)	-	2(67)	3(100)	3(100)	2(67)
<i>P. stuartii</i> (n=8)	1(12.5)	6(75)	7(87.5)	-	1(87.5)	6(75)	7(87.5)	4(50)
<i>Salmonella spp.</i> (n=1)	-	0	-	0	-	-	-	-
<i>S. marcescens</i> (n=8)	3(37.5)	8(100)	8(100)	-	8(100)	8(100)	8(100)	8(100)
<i>S. sonnei</i> (n=2)	-	1(100)	-	0	-	-	-	-
<b>Total <i>Enterobacteriaceae</i> (n=69)</b>	<b>11(17)</b>	<b>64 (94.1)</b>	<b>55 (84.6)</b>	<b>0</b>	<b>56 (86.2)</b>	<b>55 (84.6)</b>	<b>58 (89.2)</b>	<b>49 (75.4)</b>
<i>Pseudomonadaceae</i> species								
<i>P. aeruginosa</i> (n=5)	0	-	-	-	4(80)	2(60)	-	-
<i>P. stutzeri</i> (n=2)	1(50)	-	-	-	1(50)	1(50)	-	-
<b>Total <i>Pseudomonadaceae</i> (n=7)</b>	<b>1(14.3)</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>5(71.4)</b>	<b>3(57.1)</b>	<b>-</b>	<b>-</b>
<i>Acinetobacter</i> species								
<i>A. baumannii</i> (n=25)	0	-	-	-	17(68)	10(40)	14(56)	-
Gram negative bacteria isolates	CIP R n(%)	C R n(%)	CRO R n(%)	GEM R n(%)	IMI R n(%)	MEM R n(%)	TZP R n(%)	SXT R n(%)
<i>Enterobacteriaceae</i>								
<i>C. freundii</i> (n=4)	2(50)	2(50)	-	3(75)	0	1(25)	1(25)	2(50)
<i>C. koseri</i> (n=8)	2(50)	1(12.5)	-	2(50)	1(12.5)	2(25)	2(25)	3(37.6)
<i>E. cloacae</i> (n=13)	10(76.9)	3(23.1)	-	7(53.8)	2(15.4)	3(23.1)	10(76.7)	7(53.8)
<i>E. coli</i> (n=4)	0	1(25)	-	1(25)	0	1(25)	1(25)	2(50)
<i>K. aerogenes</i> (n=3)	3(100)	0	-	3(100)	0	0	2(66.7)	3(100)
<i>K. oxytoca</i> (n=4)	2(50)	1(25)	-	2(50)	1(25)	1(25)	3(75)	2(50)
<i>K. pneumoniae</i> (n=8)	5(62.5)	2(25)	-	5(62.5)	1(14.3)	1(14.3)	4(50)	6(87.5)
<i>Pseudomonadaceae</i> species								
<i>P. agglomerans</i> (n=3)	0	1(50)	-	1(50)	1(50)	2(100)	1(50)	1(50)
<i>P. rettgeri</i> (n=3)	2(66.7)	0	-	(66.7)	0	1(33.3)	2(66.7)	1(33.3)
<i>P. stuartii</i> (n=8)	0	0	-	6(75)	1(12.5)	1(12.5)	3(37.5)	0
<i>S. spp.</i> (n=1)	1(100)	0	1(100)	-	-	-	-	1(100)
<i>S.marcescens</i> (n=8)	3(37.5)	4(50)	-	4(50)	3(37.5)	4(50)	6(75)	5(62.5)
<i>S.sonnei</i> (n=2)	0	1(50)	1(50)	-	-	-	-	0
<b>Total <i>Enterobacteriaceae</i> (n=69)</b>	<b>31 (45.6)</b>	<b>16(23.5)</b>	<b>2(66.7)</b>	<b>37 (56.9)</b>	<b>10(15)</b>	<b>17 (26.2)</b>	<b>35 (53.8)</b>	<b>34(50)</b>
<i>Pseudomonadaceae</i> species								
<i>P. aeruginosa</i> (n=5)	0	-	-	0	1(20)	2(40)	1(20)	-
<i>P. stutzeri</i> (n=2)	1(50)	-	-	0	0	1(50)	1(50)	-
<b>Total <i>Pseudomonadaceae</i> (n=7)</b>	<b>1(14.3)</b>	<b>-</b>	<b>-</b>	<b>0</b>	<b>2(14.3)</b>	<b>3(42.9)</b>	<b>2(28.6)</b>	<b>-</b>
<i>Acinetobacter</i> species								
<i>A. baumannii</i> (n=25)	5(20)	-	-	4(16)	1(4)	6(24)	2(8)	-
Others gram-negative	AMP R n(%)	AMC R n(%)	CRO R n(%)	CXM R n(%)	CAZ R n(%)	CIP R n(%)	C R n(%)	MEM R n(%)
<b>Total (n=7)</b>	<b>3(100)</b>	<b>3(60)</b>	<b>2(66.7)</b>	<b>2(66.7)</b>	<b>1(100)</b>	<b>0</b>	<b>1(33.3)</b>	<b>0</b>
Others gram-negative	AZM R n(%)	LEV R9 n(%)	TE R n(%)	P R n(%)	ERY R n(%)	SXT R n(%)	CTX R n(%)	
<b>Total (n=7)</b>	<b>3(60)</b>	<b>2(50)</b>	<b>2(40)</b>	<b>2(66.7)</b>	<b>3(42.9)</b>	<b>1(33.3)</b>		
Gram positive bacteria	FOX R n(%)	GEN R n(%)	OXA R n(%)	P R n(%)				
<i>S. aureus</i>	32 (88.9)	12 (33.3)	31 (86.1)	35 (97.2)				

Legend: I-intermediate, R-resistance, AMC (amoxicillin/clavulanate), AMP (ampicillin), CRO-ceftriaxone, CAZ-ceftazidime, CXM-cefuroxime, FEP-cefepime, CTX-cefotaxime, AZM-azithromycin, Ciprofloxacin (CIP), ERY-erythromycin, C-chloramphenicol, P-penicillin, SXT-trimethoprim-sulfamethoxazole, TZP-piperacillin/tazobactam, TE-tetracycline, IMI-imipenem, LEV-levofloxacin, MEM-meropenem, and GEN-gentamicin. *Moraxella catarrhalis* (n=2), *Burkholderia cepacian complex* (n=1), *Stenotrophomonas maltophilia* (n=1), and *Pasterulla maltocida* (n=3) are other gram-negative bacteria.

While in the non-fermenter group, *A. baumannii* demonstrated the maximum resistance to cefepime (68%), cefotaxime (56%), and ceftazidime (40%). On the other hand, *Pseudomonadaceae* showed a high resistance to antibiotics like cefepime (71.4%), ceftazidime (57.1%) and meropenem (42.9%). The only pathogenic gram-positive bacteria found was *S. aureus*, it showed a resistance of 97.2%, 88.9%, 86.1%, and 33.3% to penicillin, ceftazidime, oxacillin, and gentamicin, respectively.

### Multidrug resistance level

There was an overall MDR frequency of 87% (n=60/69) for *Enterobacteriaceae*. Across all studied drug classes, only 1.4% of *Enterobacteriaceae* exhibited 0% resistance (R-0). R-7 (1.4%) was the highest antibiotic class to show resistance. (Table 3)

**Table 3: Multidrug resistance frequency of *Enterobacteriaceae*, *Acinetobacter*, *Pseudomonas* and other species**

Bacteria family	Resistance frequency (%)	R-0 n(%)	R-1 n(%)	R-2 n(%)	R-3 n(%)	R-4 n(%)	R-5 n(%)	R-6 n(%)	R-7 n(%)	R-8 n(%)	R-9 n(%)	R-10 n(%)	MDR n(%)
<i>C. freundii</i> (n=4)		0	0	12(5)	0	0	2(50)	1(25)	0	0	0	0	3(75)
<i>C. koseri</i> (n=8)		0	0	1(12.5)	2(25)	4(50)	0	1(12.5)	0	0	0	0	7(87.5)
<i>E. cloacae</i> (n=13)		0	0	1(7.7)	2(15.4)	4(30.8)	4(30.8)	2(15.4)	0	0	0	0	12(92.3)
<i>K. aerogenes</i> (n=3)		0	0	0	1(33.3)	0	2(66.7)	0	0	0	0	0	3(100)
<i>K. oxytoca</i> (n=4)		0	1(25)	0	1(25)	0	2(50)	0	0	0	0	0	3(75)
<i>P. agglomerans</i> (n=3)		1(33.3)	0	0	0	1(33.3)	1(33.3)	0	0	0	0	0	2(66.7)
<i>Enterobacteriaceae</i>													
<i>P. stuartii</i> (n=8)		0	1(12.5)	1(12.5)	6(75)	0	0	0	0	0	0	0	6(75)
<i>Salmonella species</i> (n=1)		0	0	0	1(100)	0	0	0	0	0	0	0	1(100)
<i>S. marcescens</i> (n=8)		0	0	0	2(25)	2(25)	1(12.5)	3(37.5)	0	0	0	0	8(100)
<i>S. sonnei</i> (n=2)		0	0	2(100)	0	0	0	0	0	0	0	0	0
<i>P. rettgeri</i> (n=3)		0	0	0	1(33.3)	1(33.3)	1(33.3)	0	0	0	0	0	3(100)
<i>E. coli</i> (n=4)		0	0	0	2(75)	1(25)	0	0	0	0	0	0	4(100)
<i>K. pneumoniae</i> (n=8)		0	0	0	2(25)	2(25)	3(37.5)	0	1(12.5)	0	0	0	8(100)
<b>Total (n=69)</b>					21(30.4)	15(21.7)	16(23.2)	7(10.1)	1(1.4)	0	0	0	60(87)
<i>P. stutzeri</i> (n=2)		1(50)	0	0	0	0	1(50)	0	0	0	0	0	1(50)
<i>P. aeruginosa</i> (n=5)		1(20)	1(20)	3(60)	0	0	0	0	0	0	0	0	0
<b>Total (n=7)</b>		2(28.6)	1(14.3)	3(42.9)	0.0	0.0	1(14.3)	0	0	0	0	0	1(14.3)
<i>Acinetobacter</i>													
<i>A. baumannii</i> (n=25)		3(12)	12(48)	5(20)	3(12)	2(8)	0	0	0	0	0	0	5(20)
<b>Others gram-negative</b>													
<b>Total (n=7)</b>		0	1(16.7)	2(33.2)	1(16.7)	1(16.7)	1(16.7)	0	0	0	0	0	3(50)
<b>Gram positive</b>													
<i>S. aureus</i> (n=36)		1(2.78)	3(8.3)	20(55.6)	12(33.3)	0	0	0	0	0	0	0	12(33.3)

Legend: others include *Pasteurella multocida* (n=3), *Burkholderia cepacia complex* (n=1), *Moraxella catarrhalis* (n=2) and *Stenotrophomonas maltophilia* (n=1)

None of the bacterial isolates found showed >R-8 resistance level. The highest level of MDR observed in the *Enterobacteriaceae* family is in *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella aerogenes* (*Enterobacter aerogenes*), *Providencia rettgeri*, and *Serratia marcescens*, all of which are 100%. *A. baumannii* overall MDR frequency was 20% (n=5/25). However, the

*Pseudomonas* species had an MDR incidence of 14.3%. In *Pseudomonas stutzeri*, an overall MDR frequency of 50% was found.

#### **5.4 Magnitude of Methicillin Resistant *Staphylococcus aureus* (MRSA)**

Among the bacteria that were most common was *Staphylococcus aureus*, 36 (25%), found in this study. All *Staphylococcus aureus* were screened for MRSA using a cefoxitin screening test. Disk diffusion was used to confirm MRSA, with cefoxitin disk diffusion being the preferred phenotypic test for routine detection. Among 36 *Staphylococcus aureus* isolates, 32 (88.9%) were resistant to the MRSA phenotype. (Table 4)

#### **5.5 Magnitude of ESBL Gram-negative bacteria isolates**

It was observed that 90 out of 108 Gram-negative isolates were suspected of ESBL production, where 80% (72/90) were resistant to ceftazidime and cefotaxime. A confirmatory test was performed by combination disks. 70.8% (n=51/72) of the gram negatives were positive for ESBL production. The highest ESBL production was observed from *Citrobacter koseri* (*Citrobacter diversus*) at 87.5% (n=7/8), *Providencia stuartii* at 85.7% (n=6/7), *Enterobacter cloacae* at 83.3% (n=10/12), and *Klebsiella pneumoniae* at 80% (n=4/5). Less frequent positive isolates for ESBL production were *Acinetobacter baumannii* complex 53.8% (n=7/13) and *Serratia marcescens* 50% (n=4/8). While the few other different gram-negative species were also positive for ESBL, 68.4% (n=13/19). (Table 4)

#### **5.6 Magnitude of carbapenem resistant gram-negative isolates**

First, carbapenem resistance screening was done according to CLSI guidelines. Out of 95 GNB isolates that were screened, 25 (26.3%) were suspected of carbapenemase production. Screening for carbapenem resistance was done on 25 isolates; 10 (40%) were confirmed for carbapenemase production. Carbapenemase production was most frequently seen among *Pantoea agglomerans*, 100% (n=2/2); *Acinetobacter baumannii* complex was 50% (n=3/6); and *Citrobacter koseri* (*Citrobacter diversus*), 50% (n=1/2). While the least frequent gram negatives were *Enterobacter cloacae*, 33.3% (n=1/3), and *Serratia marcescens*, 25% (n=1/4). Few other gram-negative species were 12.5% (n=2/8). (Table 4)

**Table 4: Prevalence of ESBL-producing and carbapenemase-resistant gram negative and MRSA among bacterial Isolates**

<b>Gram-negative Bacteria</b>	<b>ESBL producing n(%)</b>	<b>Carbapenemase producing n(%)</b>
<i>Acinetobacter baumannii</i> (n=13)	7(53.8)	3/6(50)
<i>Citrobacter freundii</i> (n=3)	2(66.6)	-
<i>Citrobacter koseri</i> (n=8)	7(87.5)	1/2(50)
<i>Enterobacter cloacae</i> (n=12)	10 (83.3)	1/3 (33.3)
<i>Escherichia coli</i> (n=2)	2(100)	-
<i>Klebsiella aerogenes</i> (n=2)	2(100)	-
<i>Klebsiella oxytoca</i> (n=3)	2(66.6)	-
<i>Klebsiella pneumoniae</i> (n=5)	4(80)	-
<i>Pantoea agglomerans</i> (n=2)	2(100)	2/2(100)
<i>Providencia stuartii</i> (n=7)	6(85.7)	-
<i>Providencia rettgeri</i> (n=3)	2(66.6)	-
<i>Pseudomonas aeruginosa</i> (n=2)	0	-
<i>Pseudomonas stutzeri</i> (n=1)	0	-
<i>salmonella spp.</i> (n=1)	1(100)	-
<i>Serratia marcescens</i> (n=8)	4(50)	1/4(25)
Total (n=72)	51 (70.8)	10(40)
<b>Gram-positive Bacteria</b>	<b>Isolate (n)</b>	<b>MRSA (%)</b>
<i>Staphylococcus aureus</i> (n=36)	32	88.9

## 6 Discussion

The total amount of pathogenic bacteria found in pediatric sepsis in the current study was 36% (n=144/400), which is greater than studies done previously in Addis Ababa (9,25,86), Gondar (43), Northwest Ethiopia (8) and Southern Ethiopia (39) and with the findings in another developing world such as Libya (49) and Nigeria (87). Several factors could contribute to the higher sepsis magnitude in the current study. First, in our study, a significant proportion of sepsis patients presented with advanced stages that potentially resulted in a higher bacterial load at the time of sampling that yielded growth. Additionally, geographical and epidemiological differences, including a higher burden of infectious diseases, may also have contributed to the elevated culture yield. In this study, newborns were the most affected with bacterial sepsis than other pediatrics group like adolescents (>11 years.) This observation was in concordance with a study done in southern parts of Ethiopia (39), Nigeria (87), Bangladesh (88), and China (48). The plausible reason for this is that newborns have a weak immune system compared to older children, who have better immunity to fight pathogenic bacteria.

In this study's findings, Gram-negative bacteria 108 (75%) were more dominant overall in both and species diversity compared to Gram-positive ones 36 (25%). This was in agreement with previous studies done in Shashemene (41) and Northeast Ethiopia (36) and India (53) but contrary to studies done in other parts of Africa (72), Libya (49) and Southern part of Ethiopia (39) While *Staphylococcus aureus* was the most frequently isolated single species, this finding is in contrary with other studies done in Gondar, Mekelle, other parts of Ethiopia and Bangladesh (42,83,88–91). The majority of Gram-Negative bacteria are *Acinetobacter baumannii* (17.4%), *Enterobacter cloacae* (9%), *Klebsiella pneumoniae*, *Citrobacter koseri* (*Citrobacter diversus*), *Serratia marcescens* are all (5.6%) These findings are in accordance with those done in Libya (49) and Addis Ababa (9). These findings of gram-negative bacteria being isolated more frequently than gram-positive organisms among pediatric sepsis cases may reflect the changing epidemiology of sepsis globally, where gram-negative pathogens are increasingly recognized as major causative agents, particularly in developing nations like Ethiopia. The high burden of hospital-acquired infections could have contributed to this finding. Furthermore, the ability of Gram-negative bacteria to persist in hospital environments and resist disinfection may have facilitated their transmission.

Ampicillin, amoxicillin-clavulanate, cefotaxime, cefuroxime, ceftazidime, and cefepime showed the most frequent resistance to *Enterobacteriaceae*, which also showed an MDR level of 87%, while amikacin, meropenem, imipenem, ciprofloxacin, and chloramphenicol showed low resistance to *Enterobacteriaceae*. Higher levels of antibiotic resistance among *Enterobacteriaceae* were consistent with previous researches in Gondar (34), Wolaita Sodo (37) Addis Ababa, Ethiopia (11,25). *Enterobacter cloacae*, the most frequent isolate from *Enterobacteriaceae*, which showed the highest resistance to ampicillin, amoxicillin/clavulanate, cefotaxime, ceftazidime, cefuroxime, and cefepime. While from non-fermenter bacteria, *Acinetobacter baumannii* complex, the most common isolate, had the greatest level of resistance (>55%) to cefotaxime and cefepime, also with a total frequency of MDR of 20%. These results were comparable to other studies done in Pakistan (92), Gondar (57) and Woldia and Dessie (36). Antimicrobial resistance (AMR) prevalence in this study is higher, maybe due to the fact that for pediatric issues that were not infectious in nature, medicines were misused as an empirical treatment. Additionally, many children with culture-confirmed bacteremia were born in a medical facility, where hospital-acquired infections account for most sepsis cases. MRSA found in this study was 88.9%; this result was higher than other studies in Wolaita Sodo (37) and Addis Ababa (34), China (93) and Germany (50). But it is still less than those found in Congo (94) and Egypt (95). This situation raises serious concern and suggests a very high resistance gene pool, perhaps due to gross misuse and inappropriate usage of the antibiotic drugs.

Third-generation cephalosporins in this study were also used in abundance and resistance were most shown in cefotaxime, ceftazidime, and cefuroxime. This is in alignment with studies done in Pakistan (92) and Tanzania (63) but less than those in Bangladesh (88). ESBL was found to be 70.8 % in this current study, which is higher than most findings in Africa (72), Turkey (66) and southern part of Ethiopia (39). But less than a study done in Tanzania (96), Jimma (82) and Iran (97). one of the reasons that third generation cephalosporins resistance and ESBL production was high could be due to their widespread availability. Many poor nations (LMIC) treat severe sepsis with third-generation cephalosporins as the first line of treatment at district, regional, and tertiary healthcare facilities, even though the WHO recommends them as a second-line antibiotic (63). This resistance is made worse by the frequent use of these medications most times without culture-based diagnosis. This result emphasizes how critical it

is to improve diagnostic procedures and implement more focused, evidence-based antibiotic regulations.

In this study, 25 bacterial isolates were resistant to meropenem or imipenem; only 40% (n=10) of the isolates produced carbapenemase enzymes. This result is somewhat greater than a study done in Italy (71), Turkey (66), and Africa (98) in contrast, it is less than studies done in India (99) and Argentina (100). The possible reason in our findings may reflect that some bacteria are becoming resistant through other means due to the poor antibiotic practices in the area (giving stronger antibiotics too fast or without proper diagnosis and justification). This calls for wise use of strong antibiotics or administering them proper testing and diagnosis.

## **7 Limitations**

This study has certain limitations even though it offers insightful information about the bacterial makeup and patterns of antibiotic resistance in pediatric sepsis. First off, because only one location was used, the study's findings might not be as generalizable to other hospitals or areas. We also depended on culture-based techniques, which may have underestimated the actual burden of infection by omitting organisms that are picky or slow-growing. Finally, clinical outcome data that may have been used to evaluate the wider effects of resistant infections on patient outcomes, such as mortality or treatment success rates, were not recorded.

## 8 Conclusion

A high magnitude of pediatric sepsis was determined at AHMC. Notably, infants and newborns were the most affected, with pediatric sepsis showing their vulnerability to infections. Pediatric sepsis was found to be mostly caused by gram-negative bacteria, while the most frequently found was gram-positive *Staphylococcus aureus*. *Acinetobacter baumannii* and *Enterobacter cloacae* were primary sepsis-causing agents.

Antibiotic susceptibility patterns revealed that third- and fourth-generation cephalosporins were used the most, but they were the least effective, unlike amikacin and the carbapenem family, which were the most effective. Consequently, a high level of MDR, MRSA, and ESBL was also observed. This shows that we further need to bring attention to the growing problem of antimicrobial resistance in pediatric care.

All things considered, these results emphasize the need to reconsider our antibiotic selections and give early detection and prevention top priority, particularly in high-risk populations like newborns and infants. Stronger antimicrobial management and ongoing monitoring are obviously required to better safeguard this susceptible group.

## 9 Recommendation

To tackle the issues highlighted in this study, neonatal and infant healthcare systems should be reinforced by implementing early sepsis screening, continuous monitoring, and educating frontline health workers. Hospitals facing high burdens should form specialized neonatal sepsis teams and incorporate risk assessment instruments in pediatric treatment. Reinforcement of infection prevention and control (IPC) practices is essential, especially in neonatal and pediatric units, emphasizing stringent hygiene, equipment sterilization, and regular antimicrobial monitoring. Given the significant resistance to third-generation cephalosporins, empirical treatment protocols must be updated according to the latest local antibiograms, supplemented by routine audits to encourage appropriate antibiotic utilization. Carbapenems are effective but should be reserved for severe infections or those confirmed to be resistant, and should only be used after culture and sensitivity testing.

Quick diagnostic testing is crucial for the early identification of ESBL-producing organisms, backed by continuous monitoring to inform prompt treatment choices. Current resistance trends should be reflected in updated national pediatric treatment guidelines, while hospitals implement regular bacteriological monitoring. Finally, ongoing research and consistent training for healthcare professionals on antimicrobial resistance and sepsis management are essential for enhancing outcomes and decreasing mortality

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# 11 Appendix

## 11.1 Annex 1: English version of participant information sheet for guardians

### I. Participant information sheet

Department of Medical Laboratory Science, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

**Title of the Research Project: Magnitude of pediatric bacterial sepsis and antimicrobial resistance at Adama Medical College Hospital, Adama, Ethiopia.**

First of all, I would like to thank you in advance for your cooperation and consent in participation in this study. Please read or listen when it is read for you about the general information of the study. If you have any questions regarding the study please ask freely.

#### **Background information**

**Background:** Pediatric sepsis causes high morbidity and mortality worldwide especially in developing country. Therefore, the knowledge of the causative agents of neonatal sepsis and antimicrobial susceptibility pattern will be helpful in the control and selection of empiric antimicrobial therapy.

**Aim of the study:** The purpose of this study is to determine the magnitude, antimicrobial resistance and outcome of pediatric sepsis among patients at AHMC, Adama, Ethiopia.

**Benefits for participants:** Study participants will not have any financial incentives or other inducements from participating on this study. However, based on the diagnosis result you will be treated accordingly. Most importantly, the result of the study will be beneficial to design effective prevention and control measure for pediatric sepsis. Hence, you are indirectly benefiting other patients and the society in this respect.

**Risks and complications:** There are no anticipated risks to your participation. As routine laboratory procedure blood sample will be taken once from your peripheral vein. During sample collection you may feel some discomfort but this does not produce serious pain.

**Confidentiality:** There is no sensitive issue that you will be asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential. Participants will not be prohibited to stop or withdraw at any time from the study. Only interested participants can retrieve their own lab result using their code number. The information collected about you will be coded using numbers. No personal information will be disclosed to third party or will not appear in any report from this study.

**Assurance of Principal Investigator:** I put my signature below to confirm you that I take over the responsibility for the scientific ethical and technical conduct of the research project and for provision of progress reports for all stakeholders of the research project.

Megfira Redwan (PI)

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

**Note:** If you have any questions about this study, you should feel free to ask now or anytime throughout the study by contacting

**PI Address:** Megfira Redwan. Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia

**E-mail:** [maghfira25readwan98@gmail.com](mailto:maghfira25readwan98@gmail.com)

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## II. Consent form

I have been informed about the study which plans to determine the magnitude and antimicrobial resistance of pediatric sepsis among patients at Adama, Ethiopia. The objective and the application of the study were briefly explained to me. Moreover, I have been well informed of my right to refuse information, decline to cooperate and drop out of the study if I want and none of my actions will have any bearing at all on my child 's overall health care. It is therefore with full understanding of the situation that I agreed to give the informed assent voluntarily to the researcher to give my child 's blood for the mentioned study. I agreed that the specimen would be tested for sepsis. I have had the opportunity to ask questions about the project and received clarification to my satisfaction in a language I understand. I was also informed that results for the analysis of blood will be given to the Doctor who follow my child and that I may ask the information if I want.

I \_\_\_\_\_ hereby give my assent for giving of the requested information and my child 's blood for this study.

Participant code: \_\_\_\_\_ Signature: \_\_\_\_\_ Date: \_\_\_\_\_

## 11.2 Annex 2: Amharic version of participant information sheet for guardians

አርዕስት፡- በአዳማ ከተማ በአዳማ ሆስፒታል ሜዲካል ኮሌጅ የልጆች እና ህፃናት የደም ውስጥ በሽታ አምጫ ተህዋስያን ስርጭት፤ ለተለያዩ ጾረ ባክቴሪያ ያላቸው አይበገሬነት ናየበሽታውን አጋላጭ ሁኔታዎችን ስለማጥናት

አጠቃላይ መረጃ፡- በጥናቱ በመሳተፍዎ ከልብ እያመሰገንን ከመወሰንዎ በፊት ይህን ቅፅ በትክክል አንብቡ ወይም ሲነበብልዎ በትክክል ያዳምጡ

እንዲሁም ግልጽ ያልሆነ ልዎትን ነገር በሙሉ በነፃነት ይጠይቁ ስለጥናቱ መረጃ፡- የልጆች እና ህፃናት የደም ውስጥ ተህዋስያን በሽታ በአለም አቀፍ ደረጃ በተለይም በታዳጊ ሀገራት ከፍተኛ የሆነ ህመም ና ሞት ያስከትላል። በተደረጉ ጥናቶች የተለያዩ አጋላጭ ሁኔታዎች እንዳሉላ ማወቅ ተችሏል። ስለዚህ የበሽታውን አምጫ ተህዋስያን ስርጭትና ለጸረተህዋስያን ያላውን ግትርነት ማወቅ በሽታውን ለመቆጣጠርና ለመከላከል እንዲሁም ተገቢውን መድሃኒት ለማዘዝ ይረዳል።

የጥናቱ አላማ፡- በአዳማ ከተማ በአዳማ ሆስፒታል ሜዲካል ኮሌጅ የልጆች እና ህፃናት የደም ውስጥ በሽታ አምጫ ተህዋስያን (ባክቴሪያ) ስርጭት ፤ ለተለያዩ ጾረባክቴሪያ ያላቸው ግትርነትና የበሽታው አጋላጭ ሁኔታዎችን ማጥናት ናማወቅ ነው።

ጥናቱ ለተሳታፊዎች ያለው ጥቅም፡- በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለም ነገር ግን በምርመራው ውጤት መሰረት የመታከም እድል ይኖረታል በተጨማሪም የጥናቱ ውጤት የደም ውስጥ ህመም ለመቆጣጠርና ለመከላከል ስለሚጠቅም በተዘዋዋሪ መንገድ ሌላ ህመምተኛ ንዲሁም ህብረተሰቡን የመጥቀም እድል ያገኛሉ።

በጥናቱ ተሳታፊዎች ላይ ያለው ጉዳትና ተዛማጅ ችግር፡- በዚህ ጥናት በመሳተፍ ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም ለዚህ ጥናት የሚያገለግል የደም ናሙና የሚወሰድ ሲሆን ከመጠነኛ የህመም ስሜት በስተቀር በጤናዎ ላይ ምንም ጉዳት አይደርስም።

የመረጃ ሚስጥራዊ አጠባበቅ፡- መረጃ በሚሰጡበት ወቅትም ሆነ ከዛ በኋላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘው በስም ሳይሆን በመለያ ቁጥር ይሆናል በጥናቱ ላይ

እያሉ በፈለጉት ጊዜ የማቆም ወይም የማቋረጥ መብት አልዎት የላብራቶሪ ውጤትዎን ማወቅ ከፈለጉ የመለያ ቁጥሮን በመጠቀም በሚሰጥዎ የቀጠሮ ጊዜ መውሰድ ይችላሉ።

ጥናቱን የሚያካሄደው ሰው ማረጋገጫ፡- ለዚህ ጥናት ሃላፊነቱን ለመውሰድ፣ ማናቻውንም ጥናቱ የሚመለከቱ ጉዳይ ክትትል ለማድረግና ለሚመለከተው አካል መግለጫ ለመስጠት በፊርማዬ አረጋግጣለሁ።

ፊርማ \_\_\_\_\_

ቀን \_\_\_\_\_

ማንኛውንም ጥያቄ መጠየቅ ለሚሹ የሚቀጥለውን አድራሻዬን መጠቀም ይችላሉ።

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ተንቀሳቃሽ ስልክ 0922770477

II. የፈቃደኝነት ማረጋገጫ ቅጽ (ለቤተሰብ/አሳዳጊ)

በአዳማ ከተማ በአዳማ ሆስፒታል ሜዲካል ኮሌጅ የልጆች አና ህፃናት የደም ውስጥ በሽታ አምጫ ተህዋስዎን ስርጭት ለተለያዩ ጸረባክቴሪያ ያላቸው አይበገሬነትና የበሽታው አጋላጭ ሁኔታዎችን ለማጥናት በሚል ርእስ ላይ በሚደረገው ጥናት ላይ ለመሳተፍ ሲሆን የጥናቱ አላማና ጥቅም በሚገባ ተገልጿል። በመጠይቁ ላይ የሚሞላው የኔ ሙሉ መረጃም በሚስጥር እንደሚያዝ ተነግሮኛል። በተጨማሪም በጥናቱ ውስጥ ልጄን አለማሳተፍ መብቴ እንደሆነና በማንኛውም ጊዜ ከጥናቱ በራሴ ውሳኔ መውጣት እንደምችልና በዚህም ምክንያት ምንም አይነት መጉላላት በልጄ ላይ እንደማይደርስ በሚገባ ተረድቻለሁ ስለሆነም ሁኔታውን በሚገባ በማጤን በፍቃደኝነት ልጄን በምርምሩ ላይ ለማሳተፍ ለተመራማሪው ፍቃደኝነቴን ሰጥቻለሁ። በተጨማሪም ልጄ የሚሰጠው የደም ናሙና ለተጠቀሰው ጥናት ብቻ እንደሚውል ተነግሮኝ ተስማምቻለሁ። ማንኛውም ያልገባኝን ነገር የመጠየቅ እድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለሁ። በተጨማሪም የሁሉም የላብራቶሪ ምርመራ ውጤቶች

በወቅቱ ክትትል ለሚያደርገው የጤና ባለሙያ እንደሚሰጥ እና ውጤቱን ማወቅ ከፈለኩ ማግኘት እንደምችል ተነግሮኛል።

እኔ ----- የተባልኩ ግለሰብ ይህን ሁሉ በመገንዘብ በምርምር ላይ ስለ ልጄ መረጃና የደም ናሙና እንዲወሰድ ተስማምቻለሁ።

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## 11.3 Annex 3: Procedure for specimen collection, processing and interpretation

### I. Laboratory procedure for collection and culturing of Blood

1. Using a pressure cuff, locate a suitable vein in the arm. Deflate the cuff while disinfecting the vein puncture site.
2. Wearing gloves, thoroughly disinfect the vein puncture site as follows:
  - Using 10% tincture of iodine and a circular action, swab the area beginning at the point.
  - Using 70% ethanol, cleanse an area of about 50 mm in diameter. Allow it to air-dry.
3. Lift back the tape or remove the protective cover from the top of the culture bottle(s). Wipe the top of the bottle using an ethanol-ether swab.
4. Using a sterile syringe and needle, withdraw about 1-2 ml of blood per culture for neonates and upto 5ml for pediatrics.
5. Insert the needle through the rubber liner of the bottle cap and
6. Dispense accordingly into the trypto soya broth culture medium bottle.
7. Using a fresh ethanol-ether swab, wipe the top of each culture bottle and replace the tape or protective cover(s). Without delay, mix the blood with the broth.  
**Important:** The blood must not be allowed to clot in the culture media because any bacteria will become trapped in the clot.
8. Clearly label each bottle with the name and number of the patient, and the date and time of collection.
9. As soon as possible, incubate the inoculated media. Protect the cultures from direct sunlight until they are incubated.

### II. Laboratory procedure for Gram staining technique

1. Labeling the slides clearly with the date and patient's name and number.
2. Making of smears by spread evenly covering an area about 15-20mm diameter on a slide.
3. Drying of smears after making smears, the slide should be left in a safe place to air-dry, protected from flies and dust.

4. Fix the dried smear by using heat or alcohol (methanol).
5. Cover the fixed smear with crystal violet stain for 30-60 seconds.
6. Rapidly wash off the stain with clean water. If the tap water is not clean, use filtered water or clean boiled rainwater.
7. Tip off all the water and cover the smear with Lugol's iodine for 30-60 seconds.
8. Wash off the iodine with clean water.
9. Decolorize rapidly (few seconds) with 3% acetone alcohol. Wash immediately with clean water.
10. Cover the smear with neutral red or safranin stain for 2 minutes.
11. Wash off the stain with clean water
12. Wipe the back of the slide clean and place in a draining rack for the smear to air-dry.
13. Examine the smear microscopically, first with the 40 X objective to check the staining and to see the distribution of materials and then with the oil-immersion objective to look for bacteria and cells.

**Result:**

- Gram positive bacteria -----dark purple
- Gram-negative bacteria -----pale to dark red

### **III.Laboratory procedure for Biochemical testing**

#### **1. Biochemical tests for gram positive bacteria:**

Gram-positive cocci will be identified based on their gram reaction, catalase, coagulase and mannitol salt agar tests results.

#### **2. Catalase test**

Catalase test to differentiate staphylococci which produces the enzyme catalase from streptococci which are non- catalase producing.

**Principle:** Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer.

## **Procedure**

1. Pour 2-3 ml of 3% hydrogen peroxide to a test tube
2. Using a sterile wooden stick take the test organism & immerse into the H<sub>2</sub> O<sub>2</sub> solution.56
3. Look for immediate bubbling

## **Interpretation**

- Active bubbling . . . . . Positive catalase test
- No bubbles . . . . . Negative catalase test

## **Control**

- Positive catalase control: *Staphylococcus* species
- Negative catalase control: *Streptococcus* species

## **3. Coagulase test**

This test is used to identify *S. aureus* which produces the enzyme coagulase

## **Principle**

Coagulase causes plasma to clot by converting fibrinogen to fibrin.

## **Procedure**

1. Place a drop of physiological saline on two separate slides
2. Emulsify the test organism in each of the drop to make thick suspension
3. Add one drop of plasma to one of the suspensions and mix gently.
4. Look for clumping of the organism within 10 seconds

## **Interpretation**

- Clumping within 10 secs . . . . . *S. aureus*
- No clumping within 10 secs . . . . . No bound coagulase

## **Controls**

- Positive coagulase control: *Staphylococcus aureus*
- Negative coagulase control: *Escherichia coli*.

#### **4. Mannitol salt agar test**

##### ***Principle***

Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, and Beef Extract provide the nitrogen, vitamins, and carbon in Mannitol Salt Agar. D-Mannitol is the carbohydrate source. In high concentrations, Sodium Chloride inhibits most bacteria other than staphylococci. Phenol Red is the pH indicator. Agar is the solidifying agent. Bacteria that grow in the presence of a high salt concentration and ferment mannitol produce acid products, turning the Phenol Red pH indicator from red to yellow. Typical pathogenic *staphylococci* ferment mannitol and form yellow colonies with yellow zones. Typical non-pathogenic staphylococci do not ferment mannitol and form red colonies.

##### ***Procedure***

Inoculate specimen on medium as a primary isolation or inoculate isolated colonies onto medium for differentiation. Incubate at 37°C for 24 hours. Look for colony morphology.

##### ***Result***

- Positive: yellow colony and may have a yellow halo around the colony.
- Negative: no growth of bacteria (*E. coli*) or growth with colorless or pink colony (CONs)

#### **5. Biochemical test for gram negative bacteria**

Identification of gram-negative bacteria will be based on their test result with a series of biochemical tests.

#### **6. Indole test**

##### ***Principle***

The test organism is cultured in a medium which contains tryptophan. Indole production is detected by Kovac's or Ehrlich's reagent which contains 4 (p)-dimethyl amino benzaldehyde. This reacts with the indole to produce a red colored compound. Kovac's reagent is recommended in preference to Ehrlich's reagent for the detection of indole from enterobacteria.

##### ***Method***

1. Inoculate the test organism in a bijou bottle containing 3 ml of sterile tryptone water.
2. Incubate at 35–37 O C for up to 48 hr.
3. Test for indole by adding 0.5 ml of Kovac’s reagent. Shake gently. Examine for a red color in the surface layer within 10 minutes.

***Interpretation***

- Red surface layer . . . . . Positive indole test
- No red surface layer . . . . . Negative indole test

**7. Urease test (Christensen’s (modified) urea broth):**

***Principle***

The test organism is cultured in a medium which contains urea and the indicator phenol red. When the strain is urease producing, the enzyme will break down the urea (by hydrolysis) to give ammonia and carbon dioxide. With the release of ammonia, the medium becomes alkaline as shown by a change in color of the indicator to pink red.

***Procedure***

1. Inoculate heavily the test organism in a bijou bottle containing 3 ml sterile Christensen’s modified urea broth.
2. Incubate at 35–37 O C for 3–12 h (preferably in water bath for a quicker result).
3. Look for a pink color in the medium.

***Interpretation***

- Pink color . . . . . Positive urease test
- No pink color . . . . . Negative urease test

**8. Triple Sugar Iron (TSI) Agar Slant**

***Principle***

TSI agar tests are used to determine whether gram negative bacilli utilize glucose and lactose or sucrose fermentative and produce hydrogen sulfide. It contains 1% lactose, 1% sucrose and 0.1% glucose and peptone. Phenol red and ferrous sulphate serves as an indicator for acidification of medium and H<sub>2</sub>S production.

### ***Procedure***

1. Using a sterile inoculating needle, stab the butt of the TSI agar slant twice then streak back and forth along the surface of the agar with the organism.
2. Incubate at 37 O C for 18 to 24 h.
3. Look for color change and gas production.

### ***Interpretation***

- If acid slant–acid butt (yellow–yellow): glucose and sucrose and/or lactose fermented.
- If alkaline slant–acid butt (red–yellow): glucose fermented only.
- If alkaline slant–alkaline butt (red–red): glucose not fermented.
- The presence of black precipitate (butt) indicates hydrogen sulfide production, and Presence of splits or cracks with air bubbles indicates gas production.

## **9. Manitol test**

### ***Principle***

The test organism is cultured on a medium which contains mannitol. The microbe can ferment the carbohydrate (sugar) mannitol as a carbon source. If mannitol fermented to produce acid end product, the ph. indicator phenol red changes to yellow.

### ***Procedure***

1. Inoculums from pure colony inoculated in a test tube of mannitol broth.
2. Incubate at 35-37 O C for 24 hr
3. Look for yellow color in the medium

### ***Interpretation***

- Yellow color.....Positive mannitol test
- Red color .....Negative mannitol test

## **10. Citrate utilization test using Simmon’s citrate agar**

### ***Principle***

The medium contains citrate as the sole source of carbon and inorganic ammonium salt as the sole source of nitrogen. Bacteria that can grow on this medium produce an enzyme, citrate-

permease, capable of converting citrate to pyruvate. Pyruvate can then enter the organism's metabolic cycle for the production of energy. Growth is indicative of utilization of citrate, an intermediate metabolite in the Krebs cycle.

### ***Procedure***

1. Streak the slant back and forth with light inoculums picked from the center of a well isolated colony.
2. Incubate aerobically overnight at 35–37 O C for up to 4-7days.
3. Observe a color change from green to blue.

### ***Interpretation***

- Blue. . . . . Positive citrate test
- Green. . . . . Negative citrate test

### ***Controls***

A positive citrate test reaction is obtained with *Klebsiella pneumonia* and a negative reaction with *Escherichia coli*.

## **11. Motility Test (using motility agars)**

### ***Principle***

Motility agar will be prepared and inoculated with a straight inoculating needle making a single stab about 1-2cm down into the medium. The motility will be examined after 35°C for 24hour. Motility will be indicated by the presence of diffuse growth (appearing as coloring of the medium) away from the line of inoculation. But if the bacteria are non-motile, the growth of the bacteria will be along the stab, diffusion will not occur.

## **12. Oxidase test**

### ***Principle***

A piece of filter paper is soaked with a few drops of oxidase reagent. A colony of the test organism is then smeared on the filter paper. Alternatively an oxidase reagent strip can be used. When the organism is oxidase-producing, the phenylenediamine in the reagent will be oxidized to a deep purple colour.

### ***Procedure***

1. Place a piece of filter paper in a clean petri dish and add 2 or 3 drops of freshly prepared oxidase reagent.
2. Using a piece of stick or glass rod (not an oxidized wire loop), remove a colony of the test organism and smear it on the filter paper.
3. Look for the development of a blue-purple colour within a few seconds.

### ***Interpretation***

- Blue-purple color . . . . . Positive oxidase test (within 10 seconds)
- No blue-purple color . . . . . Negative oxidase test (within 10 seconds)

### ***Controls***

- Positive oxidase control: *Pseudomonas aeruginosa*
- Negative oxidase control: *Escherichia coli*

## **13. Lysine Iron Agar**

### **Purpose**

This test is used to differentiate gram-negative bacilli based on decarboxylation or deamination of lysine and the formation of hydrogen sulfide (H<sub>2</sub>S).

### **Principle**

Lysine iron agar (LIA) contains lysine, peptones, and a small amount of glucose, ferric ammonium citrate, and sodium thiosulfate. The medium has an aerobic slant and an anaerobic butt. When glucose is fermented, the butt of the medium becomes acidic (yellow). If the organism produces lysine decarboxylase, cadaverine is formed. Cadaverine neutralizes the organic acids formed by glucose fermentation, and the butt of the medium reverts to the alkaline state (purple). If the decarboxylase is not produced, the butt remains acidic (yellow). If oxidative deamination of lysine occurs, a compound is formed that, in the presence of ferric ammonium citrate and a coenzyme, flavin mononucleotide, forms a burgundy color on the slant. If deamination does not occur, the LIA slant remains purple. Bromocresol purple, the pH indicator, is yellow at or below pH 5.2 and purple at or above pH 6.8.

Media: Enzymatic digest of gelatin (5 g), yeast extract (3 g), dextrose (1 g), l-lysine (10 g), ferric ammonium citrate (0.5 g), sodium thiosulfate (0.04 g), bromocresol purple (0.02 g), agar (13.5 g) per 1000 mL, pH 6.7.

### **Method**

1. With a straight inoculating needle, inoculate LIA by twice stabbing through the center of the medium to the bottom of the tube and then streaking the slant.
2. Cap the tube tightly and incubate at 35°C–37°C in ambient air for 18–24 h.

### **Expected Results**

Alkaline slant/alkaline butt (K/K)—lysine decarboxylation and no fermentation of glucose

Alkaline slant/acid butt (K/A)—glucose fermentation

*Note:* Patterns accompanied by a black precipitate of ferrous sulfide (FeS), which indicates production of H<sub>2</sub>S.

Red slant/acid butt (R/A)—lysine deamination and glucose fermentation.

### **Limitations**

*Proteus* spp. that produces hydrogen sulfide will not blacken the medium. Additional testing, such as triple sugar iron (TSI) agar, should be used as a follow-up identification method.

### **Quality Control**

Alkaline slant and butt: H<sub>2</sub>S positive: *Citrobacter freundii* (ATCC8090) Alkaline slant and butt: *Escherichia coli* (ATCC25922)

Alkaline slant and butt: H<sub>2</sub>S positive: *Salmonella enterica* subsp. *enterica* serovar Typhimurium (ATCC14028)

Red slant, acid butt: *Proteus mirabilis* (ATCC12453)

## **14. Deoxyribonucleic Acid Hydrolysis (DNase Test Agar)**

### **Purpose**

This test is used to differentiate organisms based on the production of deoxyribonuclease. It is used to distinguish *Serratia* spp. (positive) from *Enterobacter* spp., *Staphylococcus aureus* (positive) from other species, and *Moraxella catarrhalis* (positive) from *Neisseria* spp.

## **Principle**

The test is used to determine the ability of an organism to hydrolyze deoxyribonucleic acid (DNA). The medium is pale green because of the DNA–methyl green complex. If the organism growing on the medium hydrolyzes DNA, the green color fades and the colony is surrounded by a colorless zone.

Media: Pancreatic digest of casein (10 g), yeast extract (10 g), deoxyribonucleic acid (2 g), NaCl (5 g), agar (15 g), methyl green (0.5 g), pH 7.5.

## **Method**

1. Inoculate the DNase agar with the organism to be tested and streak for isolation.
2. Incubate aerobically at 35°C–37°C for 13–24 h.

## **Expected Results**

Positive: When DNA is hydrolyzed, methyl green is released and combines with highly polymerized DNA at a pH of 7.5, turning the medium colorless around the test organism.

Negative: If no degradation of DNA occurs, the medium remains green.

## **Limitations**

Agar must be inoculated with a suspension of a young broth culture (4 h old) or an 18–24-h overnight colony in 1–2 mL of saline.

## **Quality Control**

Positive: *Staphylococcus aureus* (ATCC25923)

Negative: *Escherichia coli* (ATCC25922)

*Note:* Several variations of this media are available that include agarose slants and toluene blue (positive result appears a deep pink) or precipitation of the DNA by flooding with hydrochloric acid resulting in a visible precipitation of the polymerized DNA in the absence of a dye.

## **15. Optochin (P disk) Susceptibility Test**

### **Purpose**

This test is used to determine the effect of optochin (ethylhydrocupreine hydrochloride) on an organism. Optochin lyses pneumococci (positive test), but alpha-streptococci are resistant (negative test).

### **Principle**

Optochin is an antibiotic that interferes with the ATPase and production of adenosine triphosphate (ATP) in microorganisms. The optochin-impregnated disk (TaxoP) is placed on a lawn of organism on a sheep blood agar plate, allowing the antibiotic to diffuse into the medium. The antibiotic inhibits the growth of a susceptible organism, creating a clearing, or zone of inhibition, around the disk. A zone of 14–16 mm is considered susceptible and presumptive identification for *Streptococcus pneumoniae*.

### **Method**

1. Using an inoculating loop, streak two or three suspect colonies of a pure culture onto half of a 5% sheep blood agar plate.
2. Using heated forceps, place an optochin disk in the upper third of the streaked area. Gently tap the disk to ensure adequate contact with the agar surface.
3. Incubate the plate for 18–24 h at 35°C in 5% CO<sub>2</sub>. *Note:* Cultures do not grow as well in ambient air, and larger zones of inhibition occur.
4. Measure the zone of inhibition in millimeters, including the diameter of the disk.

### **Expected Results**

Positive: Zone of inhibition at least 14 mm in diameter with 6-mm disk.

Negative: No zone of inhibition.

### **Limitations**

Equivocal: Any zone of inhibition less than 14 mm is questionable for pneumococci; the strain is identified as a pneumococcus with confirmation by a positive bile-solubility test.

### **Quality Control**

Positive: *Streptococcus pneumoniae* (ATCC6305)

Negative: *Streptococcus pyogenes* (ATCC12384)

## **16. Laboratory procedure for antimicrobial sensitivity testing**

### ***Procedure***

Emulsify colonies of similar appearance in small volume of nutrient broth. Match the turbidity of the suspension against the turbidity standard which has a similar appearance to an overnight broth culture.

1. With a sterile swab take sample from the suspension (squeeze the swab against the side of the test tube to remove the excess fluid).
2. Spread the inoculum evenly over the Muller-Hinton agar plate with the swab
3. Using a similar inoculation technique, inoculate an overnight broth culture of the Control organism evenly across the upper and lower third of the plate.
4. Using a sterile forceps or needle, place the antimicrobial disc on the inoculated plate
5. Incubate the plate aerobically at 35 O C for 18-24 hours.
6. Read the tests after checking that the bacterial growth of the test and control organism is neither too heavy nor too light.
7. Measure the radius of the inhibition zone. Interpret result based on the inhibition zone.
  - **Sensitivity (S):** Zone of radius is wider than, equal to, or not more than 3mm smaller than the control.
  - **Intermediate (I):** Zone radius is more than 3mm smaller than the control but not less than 3mm.
  - **Resistant (R):** No zone of inhibition or zone radius measure 2mm or less.

**Below are methodology's used in this study, adapted from Koneman's Color Atlas and Textbook of Diagnostic Microbiology (101) and Bailey & Scott's Diagnostic Microbiology (102).**

**TABLE 6-6** Key Identification Characteristics for the Most Common *Enterobacteriaceae*

	KIA	GAS	H <sub>2</sub> S	MR	VP	IND	CIT	PAD	URE	MOT	LYS	ARG	ORN	ONPG
<b>Tribe I: Escherichieae</b>														
<b>Genus: Escherichia</b>														
<i>E. coli</i>	A/A	+	-	+	-	+	-	-	-	+	+	-/+	+/-	+
<b>Genus: Shigella</b>														
Groups A, B, C	Alk/A	-	-	+	-	-/+	-	-	-	-	-	-	-	-
<i>S. sonnei</i>	Alk/A	-	-	+	-	-	-	-	-	-	-	-	+	+
<b>Tribe II: Edwardsielleae</b>														
<b>Genus: Edwardsiella</b>														
<i>E. tarda</i>	Alk/A	+	+	+	-	+	-	-	-	+	+	-	+	-
<b>Tribe III: Salmonelleae</b>														
<b>Genus: Salmonella</b>														
	Alk/A	+	+	+	-	-	+	-	-	+	+	+/-	+	-
<b>Tribe IV: Citrobactereae</b>														
<b>Genus: Citrobacter</b>														
<i>C. freundii</i>	A/A; Alk/A	+	+	+	-	-	+	-	+/-	+	-	+/-	-/+	+
<i>C. koseri</i>	Alk/A	+	-	+	-	+	+	-	+/-	+	-	+/-	+	+
<b>Tribe V: Klebsielleae</b>														
<b>Genus: Klebsiella</b>														
<i>K. pneumoniae</i>	A/A	++	-	-	+	-	+	-	+	-	+	-	-	+
<i>K. oxytoca</i>	A/A	++	-	-	+	+	+	-	+	-	+	-	-	+
<b>Genus: Enterobacter</b>														
<i>E. aerogenes</i>	A/A	++	-	-	+	-	+	-	-	+	+	-	+	+
<i>E. cloacae</i>	A/A	++	-	-	+	-	+	-	+/-	+	-	+	+	+
<b>Genus: Hafnia</b>														
<i>H. alvei</i>	Alk/A	+	-	-/+	+	-	-	-	-	+	+	-	+	+
<b>Genus: Pantoea</b>														
<i>P. agglomerans</i>	A/A; Alk/ A	-/+	-	-/+	+/-	-/+	+/-	-/+	-/+	+	-	-	-	+

235

<b>Genus: Serratia</b>														
<i>S. marcescens</i>	Alk/A	+	-	-/+	+	-	+	-	-	+	+	-	+	+
<b>Tribe VI: Proteeae</b>														
<b>Genus: Proteus</b>														
<i>P. vulgaris</i>	Alk/A	+/-	+	+	-	+	-/+	+	++	+	-	-	-	-
<i>P. mirabilis</i>	Alk/A	+	+	+	+/-	-	+/-	+	++	+	-	-	+	-
<b>Genus: Morganella</b>														
<i>M. morganii</i>	Alk/A	+	-	+	-	+	-	+	++	+	-	-	+	-
<b>Genus: Providencia</b>														
<i>P. rettgeri</i>	Alk/A	-	-	+	-	+	+	+	++	+	-	-	-	-
<i>P. stuartii</i>	Alk/A	-	-	+	-	+	+	+	+	-/+	+/-	-	-	-
<i>P. alcalifaciens</i>	Alk/A	+/-	-	+	-	+	+	+	+	-	+	-	-	-
<b>Tribe VII: Yersinieae</b>														
<b>Genus: Yersinia</b>														
<i>Y. enterocolitica</i>	Alk/A	-	-	+	-	+/-	-	-	+/-	<sup>b</sup>	-	-	+	+

<sup>a</sup>Swarming motility demonstrated on noninhibitory media.

<sup>b</sup>Nonmotile at 36°C, motile at 22°C.

KIA, Kligler's iron agar; H<sub>2</sub>S, hydrogen sulfide; MR, methyl red; VP, Voges-Proskauer; IND, indole; CIT, citrate; PAD, phenylalanine deaminase; URE, urease; MOT, motility; LYS, lysine; ARG, arginine; ORN, ornithine; ONPG, o-nitrophenyl-β-D-galactopyranoside; ++, strong positive reaction; +, 90% or more strains positive; -, 90% or more strains negative; +/-, 50% to 90% of strains positive; -/+, 50% to 90% of strains negative; shaded areas indicate key reactions; A/A, acid slant/acid butt; Alk/A, alkaline slant, acid butt.

**TABLE 19.7 TSI and LIA Reactions Used to Screen for Enteropathogenic Enterobacterales and *Aeromonas/Vibrio* spp.<sup>a,b</sup>**

TSI Reactions <sup>c</sup>	LIA Reactions <sup>c</sup>	Possible Identification
K/Ⓐ or K/A H <sub>2</sub> S+	K/K or K/NC H <sub>2</sub> S+	<i>Salmonella</i> serotypes <i>Edwardsiella</i> spp.
K/A H <sub>2</sub> S+	K/K or K/NC H <sub>2</sub> S+	<i>Salmonella</i> serotypes (rare)
K/Ⓐ	K/K or K/NC	<i>Salmonella</i> serotypes (rare)
K/A, H <sub>2</sub> S	K/K or K/NC H <sub>2</sub> S+	<i>Salmonella</i> serotype Typhi (rare)
K/Ⓐ	K/A H <sub>2</sub> S+	<i>Salmonella</i> serotype Paratyphi A (usually H <sub>2</sub> S-)
K/Ⓐ	K/A or A/A	<i>Escherichia</i> spp. <i>Salmonella</i> serotype Paratyphi A <i>Shigella flexneri</i> 6 (uncommon) <i>Aeromonas</i> spp. (oxidase positive)
K/A	K/K or K/NC	<i>Plesiomonas</i> sp. (oxidase positive) <i>Salmonella</i> serotype Typhi (rare) <i>Vibrio</i> spp. (oxidase positive)
K/A	K/A or A/A	<i>Escherichia</i> spp. <i>Shigella</i> groups A-D <i>Yersinia</i> spp.
A/Ⓐ H <sub>2</sub> S+	K/K or K/NC H <sub>2</sub> S+	<i>Salmonella</i> serotypes (rare)
A/A	K/A or A/A	<i>Escherichia</i> spp. <i>Yersinia</i> spp. <i>Aeromonas</i> spp. (oxidase positive) <i>Vibrio cholerae</i> (rare, oxidase positive)
A/A	K/K or K/NC	<i>Vibrio</i> spp. (oxidase positive)

A, Acid; Ⓐ, acid and gas production; H<sub>2</sub>S, hydrogen sulfide; K, alkaline; LIA, lysine iron agar; NC, no change; TSI, triple sugar iron agar.

<sup>a</sup>*Vibrio* spp. and *Aeromonas* spp. are included in this table because they grow on the same media as the Enterobacterales and may be enteric pathogens; identification of these organisms is discussed in Chapter 26.

<sup>b</sup>TSI and LIA reactions described in this table are only screening tests. The identity of possible enteric pathogens must be confirmed by specific biochemical and serologic testing.

<sup>c</sup>Details regarding the TSI and LIA procedures can be found in Chapter 12.

**TABLE 39.4 Biochemical and Physiologic Characteristics of *Moraxella catarrhalis* and Coccoid *Neisseria* spp.**

Organism	Growth On:			Rapid Fermentation Sugars								
	Modified Thayer-Martin <sup>a</sup>	Nutrient Agar at 35°C	Blood or Chocolate Agar at 25°C	Glucose	Maltose	Lactose	Sucrose	Fructose	Nitrate Reduction	Gas from Nitrate Reduction	0.1% Nitrite Reduction	Superoxol (30% H <sub>2</sub> O <sub>2</sub> )
<i>Moraxella catarrhalis</i> <sup>b</sup>	V	+	+	-	-	-	-	-	+	-	V	1 to 4+
<i>Neisseria cinerea</i> <sup>c</sup>	V	+	-	- <sup>d</sup>	-	-	-	-	-	-	+	Weak 2+
<i>Neisseria elongata</i> <sup>e</sup>	V	+	+	-	-	-	-	-	-	-	-	Neg
<i>Neisseria flavescens</i>	-	+	+	-	-	-	-	-	-	-	+ <sup>f</sup>	2+
<i>Neisseria gonorrhoeae</i> <sup>g</sup>	+	-	-	+	-	-	-	-	-	-	-	Strong 4+
<i>Neisseria lactamica</i>	+	V	V	+	+	+	-	-	-	-	+	1 to 3+
<i>Neisseria meningitidis</i>	+	-	-	+	+	-	-	-	-	-	V	1 to 4+
<i>Neisseria mucosa</i>	-	+	+	+ or (+)	+	-	+	+	+	+	+	Weak 2+
<i>Neisseria polysaccharea</i>	-	-	-	+	+	-	-	-	-	-	-	Weak 1+ to 3+
<i>Neisseria sicca</i> <sup>h</sup>	-	+	+	+ or (+)	+	-	+	+	-	-	+	2+
<i>Neisseria subflava</i> <sup>i</sup>	-	+	+	V	+	-	-	-	-	-	+	2+

<sup>a</sup>Growth defined as >10 colonies.  
<sup>b</sup>Butyrate and DNase positive.  
<sup>c</sup>*N. cinerea* may be differentiated from *N. flavescens* by a positive reaction with the amylase test.  
<sup>d</sup>Some strains of *N. cinerea* may appear glucose-positive in some rapid systems and be mistaken for *N. gonorrhoeae*. However, *N. cinerea* grows on nutrient agar at 35°C and reduces nitrate, unlike *N. gonorrhoeae*.  
<sup>e</sup>*N. elongata* subsp. *glycolytica* is positive for acid production from glucose.  
<sup>f</sup>*N. subflava* biovar *flava* is positive for acid production from fructose and *N. subflava* biovar *perflava* is positive for acid production from sucrose and fructose.  
<sup>g</sup>Only 2 of 10 strains were tested.  
<sup>h</sup>*Kingella denitrificans* may grow on modified Thayer-Martin agar and be mistaken for *N. gonorrhoeae* on microscopic examination. However, *K. denitrificans* can reduce nitrate and is catalase negative, unlike *N. gonorrhoeae*.  
<sup>i</sup>*Neisseria subflava* produces a yellow pigment on Loeffler agar; *N. sicca* does not.  
+, >90% of strains positive; (+), >90% of strains positive but reaction may be delayed (i.e., 2–7 days); -, >90% of strains negative; V, variable.  
Data compiled from Centers for Disease and Control; Carroll KC, Tenover MC, Landry ML, et al. *Manual of Clinical Microbiology*, 12th ed. Washington, DC: ASM; 2019; and Weyant RS, Moss CW, Weaver RE, et al, editors. *Identification of Unusual Pathogenic Gram-negative Aerobic and Facultatively Anaerobic Bacteria*, 2nd ed. Baltimore: Williams & Wilkins; 1996.

**TABLE 7-26 Identification of Oxidase-Positive, Nonmotile Coccobacilli<sup>a</sup>**

Organism	Characteristic Odor or Appearance	OF Dextrose	DNase	Urease	Phenylalanine Deaminase	Gelatin Hydrolysis	Nitrate Reduced	Growth on MacConkey	Nitrite Reduced	Catalase	Growth at 35°C
<i>Psychrobacter immobilis</i> (saccharolytic)	Odor of PEA agar (roses)	+	-	+	+	-	V (86)	V	NA	+	-
<i>Paracoccus yeei</i> (CDC EO-2)	D-shaped cells <sup>b</sup>	+	-	+	NA	-	+	V (82)	NA	V (82)	+
CDC EO-3	Yellow colonies (100)	+	-	+	NA	-	-	+	NA	+	+
CDC EO-4	Yellow colonies (83)	+	NA	+	NA	-	-	+	NA	+	+
CDC EF-4b		+	NA	-	NA	-	+	V (65)	V	+	+
<i>M. canis</i>		-	+	-	-	-	+	+	V	+	+
<i>M. catarrhalis</i>		-	+	-	V	-	+	-	+	+	+
<i>Psychrobacter phenylpyruvicus</i> <sup>c</sup>		-	-	+	+	-	V (89)	+	-	Weak	+
<i>D. urethralis</i>	Small coccoid cells	-	-	-	+	-	-	V (83)	+	+	+
<i>M. lacunata</i>		-	-	-	-	+	+	-	-	Weak	+
<i>M. nonliquefaciens</i>		-	-	-	-	-	+	V (17)	-	+	+
<i>M. osloensis</i>		-	-	-	-	-	V (26)	V (49)	-	Weak	+
<i>M. atlantae</i>	Spreading or pitting colonies	-	-	-	-	-	-	+	V (20)	Weak or -	+
<i>M. lincolnii</i>	Coccus-like to plump rods	-	-	-	NA	-	-	-	V	+	+

<sup>a</sup>Data obtained from references 251, 367, 493, 754, 1102, 1170, and 1172.  
<sup>b</sup>Gram's stain smears show coccoid to short thick rods that are frequently vacuolated. Cells have unstained centers but are peripherally stained and appear as O's.  
<sup>c</sup>*Brucella* species may be misidentified as *Psychrobacter phenylpyruvicus* in some commercial identification systems.  
+, 90% or more strains positive; -, 90% or more strains negative; V, 11%–89% of strains positive; NA, results not available; numbers in parentheses are percentage of strains giving positive reaction. Instructions for interpreting table using shaded boxes are provided under heading "Steps to Follow" on p.392.

**TABLE 7-31** Identification of Strongly Lactose-Positive Nonfermenters<sup>a</sup> (Oxidase +, Motility +,

Fluorescein -)

Organism	Arginine	Lysine	OF Mannitol	Urease	ONPG	Polymyxin B
<i>Burkholderia pseudomalle</i> <sup>b</sup>	+	-	+	V (43)	-	R
<i>Burkholderia cepacia</i> complex	-	+	+	V (45)	V (79)	R
<i>Rhizobium radiobacter</i>	-	-	+	+	+	S
<i>Burkholderia multivorans</i>	-	V (53)	+	+ (100)	+ (98)	R
<i>Ralstonia mannitolilytica</i> (Va-3)	-	-	+	+	-	R
<i>Ralstonia pickettii</i> (Va-1)	-	-	-	+	-	R
<i>Sphingomonas paucimobilis</i>	-	-	-	-	+	S (89)

<sup>a</sup>Data obtained from references 367, 438, and 1172.

<sup>b</sup>Also consider *P. aeruginosa*, *P. fluorescens*, and *P. putida* that are pyocyanin and pyoverdinin-negative.

+, 90% or more strains positive; -, 90% or more strains negative; V, 11%–89% of strains positive; numbers in parentheses are percentage of strains giving positive reaction; R, resistant; S, susceptible. Instructions for interpreting table using shaded boxes are provided under heading "Steps to Follow" on p.392

*Acinetobacter baumannii* and *Sphingobacterium* species are strongly lactose-positive, but are excluded from this table because they are nonmotile (see Tables 7-24 and 7-27).

**TABLE 21.3**

**Biochemical and Physiologic Characteristics *Burkholderia cepacia* Complex and Other Species**

Organisms	Growth at 42°C	Nitrate Reduction	Gas From Nitrate	Gelatin Liquefied	Arginine Dihydrolase	Lysine Decarboxylase	Urea Hydrolysis	Oxidizes Glucose	Oxidizes Lactose	Oxidizes Mannitol	Oxidizes Xylose
<i>Burkholderia cepacia</i> complex	v	v	-	v	-	+/v	v	+	+/v	V	+/v
<i>Burkholderia mallei</i>	-	+	-	-	+	-	v	+	V	-	V
<i>Burkholderia pseudomallei</i>	+	+	+	v	+	-	v	+	+	+	+

ND, No data; v, variable; +, >90% of strains are positive; -, >90% of strains are negative.

**TABLE 7-33** Identification of Lysine-Positive Nonfermenters

	Polymyxin B	DNase	OF Mannitol
<i>Burkholderia cepacia</i> complex <sup>a</sup>	R	-	+
<i>Stenotrophomonas maltophilia</i> <sup>b</sup>	S	+	-

<sup>a</sup>For further separation of the *B. cepacia* complex, see Table 7-9.

<sup>b</sup>Occasional strains of *S. maltophilia* may be oxidase positive.

+, 90% or more of strains are positive; -, 90% or more of strains are negative.

**TABLE 21.4 Biochemical and Physiologic Characteristics of *Pseudomonas* spp.**

Organisms	Growth at 42°C	Nitrate Reduction	Gas From Nitrate	Gelatin Liquefied	Arginine Dihydrolase	Lysine Decarboxylase	Urea Hydrolysis	Oxidizes Glucose	Oxidizes Lactose	Oxidizes Mannitol	Oxidizes Xylose
<i>Pseudomonas aeruginosa</i>	+	+	+	v	+	-	v	+	-	v	+
<i>Pseudomonas alcaligenes</i>	v	54%	-	-	12%	-	-	-	-	-	-
<i>Pseudomonas fluorescens</i>	-	-	-	+	+	-	v	+	v	v	+
<i>Pseudomonas mendocina</i>	+	+	+	-	+	-	v	+	-	-	+
<i>Pseudomonas monteilii</i>	-	-	-	-	+	-	v	+	-	-	-
<i>Pseudomonas mosselii</i>	-	-	-	+	+	-	ND	+	-	v	-
<i>Pseudomonas pseudoalcaligenes</i>	94%	+	-	-	78%	-	3%	9%	-	-	18%
<i>Pseudomonas putida</i>	-	-	-	-	+	-	v	+	v	v	+
<i>Pseudomonas stutzeri</i>	v	+	+	-	-/+*	-	v	+	-	+	+
<i>Pseudomonas veronii</i>	-	+	+	v	+	ND	v	+	ND	+	+

ND, No data; v, variable; %, percent of positive strains; +, >90% of strains are positive; -, >90% of strains are negative.  
 Updated from: Carroll KC, Pfaller MA, Landry ML, et al. *Manual of Clinical Microbiology*. 12th ed. Washington, DC: ASM; 2019.

**TABLE 7-34 Identification of Pigmented Nonfermenters<sup>a</sup> (Oxidase +, Motility +, Fluorescein -, 10% Lactose -, Acetamide -, Lysine -)**

Organism	Characteristic, Color, or Appearance	KIA/H <sub>2</sub> S	OF Fructose	Arginine	Gas from Nitrate	Indole	OF Mannitol	OF Xylose	Esculin Hydrolysis	Growth in 6.5% NaCl
<i>Roseomonas</i> sp. <sup>b</sup>	Pink, mucoid	-	+	-	-	-	V	V	-	-
<i>Methylbacterium</i> sp. <sup>b</sup>	Coral, dry	-	V (50)	-	-	-	-	V	-	-
<i>Shewanella alga</i>	Tan	+	-	-	NA	-	-	-	-	+
<i>Shewanella putrefaciens</i>	Tan	+	V	-	-	-	-	-	-	-
<i>Massilia timonae</i>	D/Nase Pos, Pale yellow	-	-	-	-	-	-	+	+	-
<i>Brevundimonas vesicularis</i>	Tan/orange Vancomycin susceptible	-	-	-	-	-	-	V (11)	+	-
<i>Pseudomonas mendocina</i> (Vb-2) <sup>c</sup>	Pale yellow	-	+	+	+	-	-	+	-	+
CDC Vb-3 <sup>c</sup>	Pale yellow	-	+	+	+	-	V (88)	+	-	+
<i>Pseudomonas stutzeri</i> (Vb-1)	Yellow, wrinkled	-	+	-	+	-	V (70)	+	-	+
<i>Balneatrix alpica</i>	Pale yellow	-	+	-	-	+	+	NA	-	NA
<i>Burkholderia cepacia</i> complex	Yellow	-	+	-	-	-	+	V	V (67)	-
<i>Sphingomonas paucimobilis</i>	Yellow	-	+	-	-	-	-	+	+	-
CDC group O-1 <sup>d</sup>	Yellow, urea -	-	+	-	-	-	-	-	+	-
CDC group O-2 <sup>d</sup>	Yellow, urea V (12)	-	+	V (22)	-	-	-	-	V (64)	-

<sup>a</sup>Data obtained from references 255, 367, 400, 560, 645, 906, and 1172.  
<sup>b</sup>*Methylbacterium* species appear as dark colonies under long-wave UV light due to absorption of UV light. *Roseomonas* species do not absorb UV light and do not appear dark.  
<sup>c</sup>*P. mendocina* is starch hydrolysis negative, CDC Vb-3 is starch hydrolysis positive.  
<sup>d</sup>CDC Group O-1 is OF glucose weak or delayed, OF maltose-negative, Group O-2 is OF maltose-, sucrose- and usually glucose-positive.  
 +, 90% or more strains positive; -, 90% or more strains negative; V, 11%-83% of strains positive; numbers in parentheses are percentage of strains giving positive reaction; NA, results not available; KIA, Kligler's Iron Agar.  
 Instructions for interpreting table using shaded boxes are provided under heading "Steps to Follow" on p. 392.  
 All of these pigments develop only as the culture ages. Most strains of *Chryseobacterium* sp. and *Sphingobacterium* sp. are also pigmented (yellow); however, these organisms are excluded from this table because they are nonmotile (see Tables 7-27 and 7-28).

**TABLE 7-32** Identification of Acetamide-Positive Nonfermenters<sup>a</sup> (Oxidase +, Motility +, Fluorescein -, 10% Lactose -)

Organism	Arginine	OF Dextrose	OF Fructose	OF Mannitol	Urease	Nitrate to Gas	Nitrate Reduced	Nitrite to Gas	Malonate <sup>b</sup>	Orange Indole
<i>Pseudomonas aeruginosa</i>	+	+	V (89)	V (68)	V (66)	V (60)	V (74)	NA	NA	-
<i>Burkholderia cepacia</i> complex	-	+	+	+	V	-	V (37)	NA	NA	-
<i>Achromobacter xylosoxidans</i>	-	+	V (9)	-	-	V (69)	+	NA	NA	-
<i>Delftia acidovorans</i>	-	-	+	+	-	-	+	NA	NA	+
<i>Oligella ureolytica</i>	-	-	-	-	++	V (58)	+	V (63)	-	-
<i>Achromobacter denitrificans</i>	-	-	-	-	V (31)	+	+	+	+	-
<i>Achromobacter piechaudii</i>	-	-	-	-	-	-	+	-	+	-
<i>Alcaligenes faecalis</i>	-	-	-	-	-	-	-	+	+	-
<i>Bordetella hinzii</i>	-	-	-	-	-	-	-	-	+	-
<i>Bordetella avium</i>	-	-	-	-	-	-	-	-	-	-

<sup>a</sup>Data obtained from references 367, 438, and 1109.

<sup>b</sup>Data for alkalization of malonate from reference 62.

+, 90% or more strains positive; -, 90% or more strains negative; V, 11%-89% of strains positive; ++, strong positive reaction; NA, results not available; numbers in parentheses are percentage of strains giving positive reaction; R, resistant; S, susceptible. Instructions for interpreting table using shaded boxes are provided under heading "Steps to Follow" on p.392

**TABLE 7-29** Screening Tests for Species Identification of Oxidase-Positive, Motile Nonfermenters

				-	+	Fluorescence (Pseudo F agar under UV light)
				-	+	10% Lactose
			-	+		Acetamide
		-	+			Lysine (Moeller)
	-	+				Pigment on blood agar
	-	+				Gas from nitrate or nitrite
-	+					OF dextrose

## 12 Declaration

Declaration I, the undersigned agree to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports as per terms and conditions of the research publications office.

*M.Sc. candidate:*

**Megfira Redwan (B.Sc.)**

Signature: \_\_\_\_\_

Date of submission: \_\_\_\_\_

This thesis has been submitted with our approval as advisors.

*Advisors:*

**Regasa Diriba (M.Sc.):**

**Dr. Melese Hailu (PhD):**

Signature: \_\_\_\_\_

Signature: \_\_\_\_\_

Date: \_\_\_\_\_

Date: \_\_\_\_\_

Place: \_\_\_\_\_

Place: \_\_\_\_\_

Addis Ababa, Ethiopia