From the Department of Gastroenterology, Hepatology and Infectious Diseases at Heinrich Heine University Düsseldorf

Identification and characterization of microbial pathogens of acute infectious diseases in Arsi (IDA) Zone of Ethiopia- IDA study

Dissertation

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"Coming from a developing country is an opportunity if you want to change the world"

"Aus einem Entwicklungsland zu kommen ist eine Chance, wenn man die Welt verändern will".

Publications

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c) Tufa TB, Fuchs A, Feldt T, Galata DT, Mackenzie CR, Pfeffer K, Häussinger D, (2020), CTX-M-9 group ESBL-producing Raoultella planticola nosocomial infection: first report from sub-Saharan Africa, Ann Clin Microbiol Antimicrob, 17;19 (1) 36

d) Früh J, Fuchs A, Tufa TB, Früh L, Hurissa Z, Orth HM, Bode JG, Eberhardt KA, Häussinger D, Feldt T, (2021), Variation of vital signs with potential to influence the performance of qSOFA scoring in the Ethiopian general population at different altitudes of residency: A multisite cross-sectional study, PLoS One, 16 (2): e0245496

e) Tufa TB, Mackenzie CR, Orth HM, Wienemann T, Nordmann T, Abdissa S, Hurissa Z, Schönfeld A, Häussinger D, Pfeffer K, Luedde T, Fuchs A*, Feldt T*, (2022), Prevalence and characterization of antimicrobial resistance among gram-negative bacteria isolated from febrile hospitalized patients in central Ethiopia. Antimicrob Resist Infect Control. 11(1):8.

f) Tufa TB, Fuchs A, Wienemann T, Eggers Y, Abdissa S, Schneider M, Jensen BO, Bode JG, Pfeffer K, Häussinger D, Mackenzie CR, Orth HM, Feldt T, (2020), Carriage of ESBL-producing Gram-negative bacteria by flies captured in a hospital and its suburban surroundings in Ethiopia, Antimicrob Resist Infect Control.;9(1):175

I. Deutsche Zusammenfassung

Akute Infektionskrankheiten (acute infectious diseases, AID) und Sepsis stellen aufgrund des Mangels an angemessener Diagnostik und Therapie schwerwiegende Probleme für das öffentliche Gesundheitswesen in Ländern mit niedrigem und mittlerem Einkommen, dar. Dies wird ohne das Ergreifen geeigneter Maßnahmen in Zukunft so bleiben. Bei fortschreitender antimikrobieller Resistenz (AMR) sind geeignete Antibiotika in den meisten Fällen nur begrenzt verfügbar. Die Kenntnis der Epidemiologie, eine frühzeitige und genaue Diagnose der Krankheiten und eine angemessene Behandlung sind die wichtigsten Voraussetzungen für eine bessere Gesundheitsversorgung. Auf dieser Grundlage hatten wir zum Ziel, das Spektrum, die Epidemiologie und die Resistenzmuster von Krankheitserregern bei Patienten in der Arsi-Zone in Äthiopien systematisch zu untersuchen.

Fieber ist eines der Kardinalsymptome von AID. Wir schlossen Patienten mit Verdacht auf akute fieberhafte Erkrankungen ein und beobachteten den klinischen Verlauf über 28 Tage oder bis zur Entlassung aus dem Krankenhaus, um deren Outcome im Asella Referral and Teaching Hospital (ARTH) zu ermitteln. Dazu führten wir verschiedene Labortests durch, darunter Blutkulturen, Blutausstriche, Schnelltests und Multiplex-PCR für nicht kultivierbare Krankheitserreger. In einer Sub-studie nahmen wir auch gesunde Personen aus verschiedenen Gebieten Äthiopiens auf, um den Normalbereich der Vitalparameter zu bestimmen und mögliche Gründe für die schlechte Eignung des Quick-Sequential-Organ-Failure-Assessment-Scores (qSOFA) zur Identifizierung kritisch kranker Patienten zu untersuchen.

Ergänzende Untersuchungen und Referenztests der lokalen Ergebnisse wurden in Düsseldorf, durchgeführt. Bei allen Isolaten wurden die Identifizierung des Erregers mit der Matrix-Assisted Laser Desorption/Ionization-Time Of Flight Massenspktrometrie (MALDI-TOF) und die lokale Antibiotika-Empfindlichkeitsprüfung (antibiotic susceptibility testing, AST) mit dem VITEK-2 (bioMérieux) bestätigt. Wir wiesen molekulare Resistenzgene nach und konzentrierten uns dabei auf solche, die für β-Laktamasen mit erweitertem Spektrum (ESBL) oder Carbapenamasen in gramnegativen Isolaten kodieren. Bei Patienten, bei denen in den oben genannten Untersuchungen keine Erreger diagnostiziert wurden, führten wir einen Multiplex-PCR-Test auf *Plasmodium* spp., *Borrelia* spp., *Rickettsia* spp., *Leptospira* spp. und Arboviren wie *Pan Flavivirus* und *Chikungunya* durch.

Wir fanden mögliche Erreger bei 14,3 % der Patienten mit akuten fieberhaften Erkrankungen während des Studienzeitraums am ARTH. Von diesen Erregern waren 67,3 % (66/98) mit Hilfe von Kulturen isolierte Bakterien oder Hefen. Ferner wurden 32,7 % nicht kultivierbare Erreger (Plasmodium spp., Borrelia spp. und Rickettsia spp.) mit Hilfe von Multiplex-PCR und Mikroskopie nachgewiesen. Wir berichteten über eine hohe Sterblichkeitsrate (29,4 %) bei Patienten, die nach 28 Tagen einen qSOFA-Score ≥2 aufwiesen. Das erhöhte Sterberisiko stand in signifikantem Zusammenhang mit der Höhe des SOFA- und qSOFA-Scores sowie mit Multi Drug Resistance (MDR)-Infektionen mit gramnegativen Bakterien (16,7 vs. 42,9 %). Die von uns durchgeführten Systematic Review und Metaanalyse bestätigten dies. Bei der normalen Bevölkerung von Asella (2400 m über dem Meeresspiegel) hatten 28 % der Personen einen qSOFA-Score ≥2. Dieser Befund könnte zum Teil dessen schlechte Eignung bei der Erkennung von kritisch kranken Patienten erklären. Die Früherkennung einer Sepsis auf klinischer Basis durch die behandelnden Ärzte war ebenfalls gering (12,4 %).

Aufgrund der hohen Prävalenz von AMR war die empirische Therapie wahrscheinlich bei mehr als zwei Dritteln der Studienteilnehmer unwirksam. Die häufigsten Resistenzgene bei ESBL-produzierenden Bakterien waren *bla*CTX-M-1 und *bla*TEM bei Carbapenem-resistenten Bakterien hingegen *bla*NDM-1. Wir vermuteten, dass Stubenfliegen einer der Risikofaktoren für die Verbreitung von AMR in Krankenhäusern sind. Dabei stellten wir fest, dass auf dem Krankenhausgelände gefangene Fliegen häufiger mit ESBL-produzierenden Bakterien kolonisiert waren als 1,5 km vom Krankenhausgelände in der Stadt Asella gefangene.

Es sind innovative Diagnostik-Ansätze erforderlich, und die bestehenden Methoden müssen zur Verkürzung der Zeit bis zum Ergebniseingang angemessenen Antibiotikatherapie modifiziert werden. Bei der Anpassung von Protokollen zur Früherkennung von Sepsis sollten geografische Lage, Krankheitslast und AST-Profil sowie Gesundheitsinfrastruktur berücksichtigt werden. Neue Methoden wie Metagenomics und Next Generation Sequencing könnten in den kommenden Jahren eine wichtige Ergänzung darstellen.

II. Summary English

Acute infectious diseases (AID) and sepsis are serious public health problems in low- and middle income countries facing a lack of adequate diagnosis and treatment and will remain so in the future unless appropriate action is taken. Due to the spread of antimicrobial resistance (AMR), the availability of appropriate antibiotics is limited in most settings. Knowledge of the epidemiology as well as early and precise diagnosis of the diseases and adequate medication are the most important prerequisites for better healthcare. Therefore, we aimed to systematically investigate the spectrum, epidemiology and resistance patterns of pathogens in patients in the Arsi zone of Ethiopia.

Fever is one of the cardinal symptoms of AID. We enrolled patients suspected of having acute febrile diseases and observed the clinical course for 28-days or until discharge from the hospital in order to determine their outcome at Asella Referral and Teaching Hospital (ARTH). For this purpose, we performed various laboratory tests, including blood cultures, blood smears, rapid tests, and multiplex PCR for non-culturable microbes. In a sub study, we also included healthy individuals from different geographic areas in Ethiopia to determine the normal range of vital signs, to investigate possible reasons for the poor performance of the quick sequential organ failure assessment (qSOFA) score for the identification of critically ill patients.

Complementary investigations and reference testing of local results were performed in Düsseldorf, Germany. For all isolates, identification was confirmed with matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF) and local antibiotic susceptibility testing (AST) was confirmed with VITEK-2 (bioMérieux). We detected molecular resistance genes, focusing on possible genes encoding extended spectrum β-lactamases (ESBL) or carbapenamases in Gram-negative isolates. We performed a multiplex PCR assay in patients without pathogens diagnosis in the above mentioned investigations, targeting *Plasmodium* spp., *Borrelia* spp., *Rickettsia* spp., *Leptospira* spp., and arboviruses such as *Pan Flavivirus* and *Chikungunya*.

We found possible pathogens in 14.3% of the patients with acute febrile diseases examined during study period at ARTH. Of these pathogens, 67.3% (66/98) were bacteria or yeasts isolated by culture method, and 32.7% non-culturable pathogens (*Plasmodium* spp., *Borrelia* spp., and *Rickettsia* spp.,) were detected by multiplex PCR and microscopy. We reported a high mortality rate (29.4%) among patients who had a qSOFA score ≥2 at 28-days. Increased risk of mortality was significantly associated with level of SOFA and qSOFA scores, and multi drug resistance (MDR) Gram-negative bacterial infections (16.7 vs. 42.9%). The systematic review and metaanalysis we conducted confirmed this. When looking at the normal population living in Asella (2400m above sea level), 28% of individuals had a qSOFA score ≥2. This finding may in part explain the poor performance in the identification of critically ill patients. Early detection of sepsis on clinical basis by treating physicians was also low (12.4%).

Due to the high prevalence of AMR, the empirically administered therapy was most likely ineffective in more than two thirds of study participants. The most common resistance genes detected among ESBL-producing bacteria were *bla*CTX-M-1 and *bla*TEM, while *bla*NDM-1 was detected among carbapenem-resistant bacteria. We assumed that houseflies are one of the risk factors for the spread of AMR at hospitals. Once examined, we found that flies caught on hospital campus was more often colonized with ESBL-producing bacteria than flies caught 1.5 km from the hospital compound in Asella town.

Innovative diagnostic approaches are needed and existing methods have to be modified to reduce the time to results of pathogen identification and resistance testing, allowing adequate antibiotic therapy. The adaptation of protocols for the detection of early sepsis onset should consider the geographic location, the burden of the diseases and the AST profile as well as the health infrastructure. Novel diagnostic methods, such as metagenomics and next generation sequencing may be an important complementation of the diagnostic toolkit in the coming years.

III. List of abbreviations

AID	acute infectious diseases
AMR	antimicrobial resistance
ARTH	Asella Referral and Teaching Hospital
AST	antibiotic susceptablity testing
BSIs	bloodstream infections
CR	carbapenemase resistance
cUTI	complicated urinary tract infection
ESBL	extended spectrum β-lactamase
GNB	Gram-negative bacteria
HITM	Hirsch Institute of Tropical Medicine
HHU	Heinrich Heine University
KPC	Klebsiella pneumoniae carbapenemase
LMIC	low- and middle income countries
MALDI-TOF	matrix-assisted laser desorption/ionization-time of flight mass spectrometry
MRSA	methicillin-resistant *S. aureus*
MDW	monocyte Distribution Width
MDR	multi-drug resistance
NDM	New Delhi metallo-β-lactamases
OXA	oxacillinase
PCR	polymerase chain reaction
qSOFA	quick sequential organ failure assessment
RDTs	rapid diagnostic tests
SHV	sulfhydryl reagent variable
SSA	sub-Saharan Africa
SSTI	skin and soft tissue infections
SIRS	systemic inflammatory response syndrome
TEM	Temoneira
VRE	vancomycin-resistant enterococcal

IV. Table of content

1. Introduction

1.1 Burden of Acute Infectious Diseases

Acute infectious diseases (AID) are among the most common illnesses in sub-Saharan Africa (SSA) and present a challenge to clinicians because of the wide range of possible etiologies and expansion of multidrug resistances (MDR). Understanding the local epidemiology and using compressive diagnostic tools, including possible rapid diagnostic tests (RDTs), can guide clinicians in managing patients with suspected AID (1). Recent literature reviews show that bloodstream infections (BSIs) occurring in SSA, including Ethiopia, are often caused by Gram-negative bacteria (GNB), which are MDR and responsible for a large part of BSI- related mortality (2, 3).

In Ethiopian health facilities, a number of AID are underdiagnosed or misdiagnosed. This leads to incorrect medication and causes waste of resources and, in the worst case, loss of life (4). For example, despite low specificity, typhoid and typhus RDTs are commonly used in the diagnostic workup for febrile patients (5). Research results from rural Ethiopia show that febrile illness caused by non-culturable pathogens such *as Borrelia spp*, *Francisella* group, *Bartonella spp* are underdiagnosed (4, 6, 7). Therefore, improving existing laboratory diagnostic services in the country and implementation of new approaches and methods seems crucial for improving health quality or services and for obtaining epidemiological data that support empiric treatment decisions.

The burden of AID is related to host immune status or comorbidities such as HIV- and vaccination status, diabetes mellitus, chronic kidney and heart diseases and malignancies, and also to the pathogenicity of microbes and their antibiograms. In addition, environmental factors such as hygiene, vectors also influence the nature and incidence of the various AID. Therefore, a comprehensive multidisciplinary approach and meaningful data for policy makers are needed to prevent and reduce the incidence and impact of infectious diseases (8).

1.2 Approaches for the early identification of sepsis

The health status of individuals can be determined by vital signs such as respiratory rate (RR), heart rate (HR), blood pressure (BP), and oxygen saturation (SpO2). Deviation of these vital signs from the normal range is an indication of an impending health problem. For example, the

quick Sequential Organ Failure Assessment (qSOFA) score was developed as a tool to identify patients who are at increased risk for a poor outcome when an infection is suspected (9). The three criteria for which one point is awarded each are low systolic blood pressure (BPsys ≤100 mmHg), high respiratory rate (≥22 breaths per minute), or altered mental status (Glasgow Coma Scale (GCS) <15). Thus, those individuals with qSOFA of ≥2 are likely to be septic and at high risk for an adverse outcome (9). Although the qSOFA is easily applicable in any health care setting, it was not commonly used at our study site before the start of this project and therefore, early sepsis detection rate was very low.

The concept of sepsis is poorly established, especially in SSA, and thus, although being associated with high mortality, is often not recognized (10, 11). For the early recognition, diagnosis and quality care of sepsis, reliable and easy-applicable tools are required. Established sepsis scores have either shown limited applicability, as e. g. the sequential organ failure assessment (SOFA) or, as in the case of qSOFA, are significantly compromised in their sensitivity and specificity, as has also been shown (10, 12). The existing studies from SSA suggest a high regional variability and thus probable dependency of local determinants as burden of disease environmental and host factors.

The aim of reducing sepsis mortality can be primarily addressed by early detection and treatment initiation also in the setting of most resource-limited health care settings. Besides controlling the focus of infection, the initiation of an effective antibiotic treatment as early as possible is a key to improve the prognosis. Knowledge on the spectrum of causative pathogens and their resistance profile is thus crucial. It has to be considered, that a significant part of the pathogens causing sepsis is non-culturable and/or fastidious, and therefore may escape detection when conventional diagnostic methods are used.

We attempted to use the qSOFA score to recruit study participants and faced the challenge that the method was unable to distinguish critically ill patients from healthy individuals in Asella (above 2400 m altitude). These criteria, developed in and for high income countries may need to be adapted for countries with limited resources, particular epidemiology of infectious diseases and specific environmental factors as e. g. high altitude. It has to be noted that more than 50% of the country is above 1500 m above sea level. Therefore, we investigated the potential influence of variations within the physiological range of vital signs in the healthy general population living at different altitudes in Ethiopia on qSOFA score performance.

1.3 Antimicrobial resistance, prevalence and risk factors

Bacteria can be resistant to drugs due to the production of enzymes such as β-lactamases and carbapenemases or for other reasons, such as a combination of porin loss and reduced efflux pump permeability. The β-lactamases are classified into four classes from A to D according to the Ambler method, based mainly on the sequencing similarities of the genes (Figure 1) (13). The β-lactamases and carbapenemases encoding genes are most commonly transferred horizontally between GNB isolates. ESBLs are plasmid-mediated β-lactamases that confer resistance to broad-spectrum β-lactum antibiotics, including third- and fourth-generation cephalosporins, acetronam, and extended-spectrum penicillins, while carbapenemases hydrolyze carbapenem antibiotics (14).

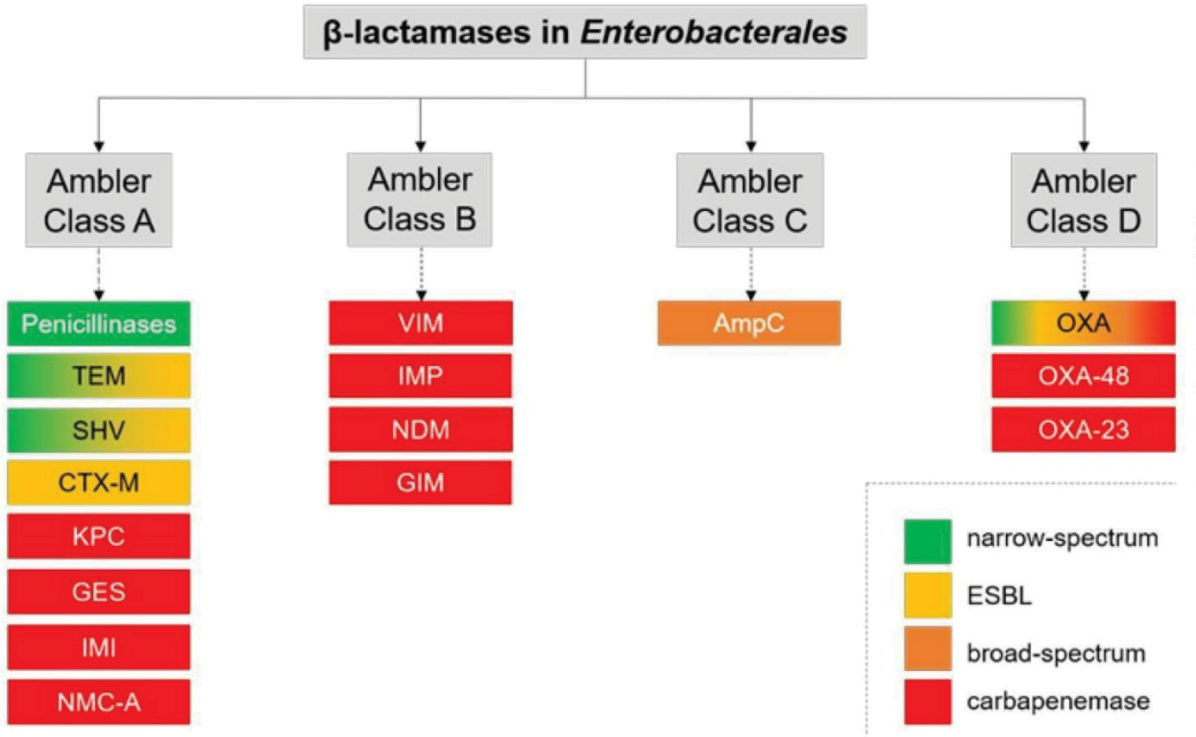

Figure 1. Ambler's classification with examples of main β-lactamases in Enterobacterales drafted by Noster et al. (2021) (13). CTX-M; cefotaximase-Munich; ESBL: extended spectrum β-lactamase GIM: Germany imipenemase; IMP: imipenemase; KPC: *Klebsiella pneumoniae* carbapenemase; NDM: New Delhi metallo-β-lactamases; NMC-A: not metalloenzyme carbapenemase A; OXA: oxacillinase; SHV: sulfhydryl variable lactamase; TEM: Temoniera; "TEM" name came from the patient's name in Greece, Temoniera who was positive of blood culture for a strain of *E. coli*. VIM: Verona integrated-encoded. The name VIM was given due to a new integron-borne Metallo- β-lactamase gene, a carbapenem-resistant *P. aeruginosa* isolated from wound at the Verona University Hospital in Italy in 1997.

Compared to Gram-positive bacteria, AMR was clearly more common in GNB isolates associated with ESBL/carbapenemase resistance (CR) genes in Arsi zone, Ethiopia. CTX-M-1 and TEM enzymes for ESBL and NDM-1 for CR, respectively, were predominantly detected in GNB isolates. SHV enzymes were also frequently detected in *K. pneumonia*. Resistance to non-beta-lactam or other antibiotic classes was very common in ESBL-positive isolates compared with non-ESBL isolates based on the phenotypic AST result. This limits the selection of appropriate antibiotics for the management of GNB infections. In general, the presence of a high prevalence of MDR results in ineffective empiric treatment, probably associated with increased mortality. Local AMR statistics are essential for targeting the use of calculated antimicrobial therapy to reduce sepsis mortality.

Risk factors for the spread of AMR can be related to either the environment or health care facilities, or both. Road and domestic sewage, wastewater, international or national travel, contaminated air or dust, food colonized by bacteria, vectors (e. g. houseflies), bird migration (15), and livestock can all facilitate the transmission of drug-resistant bacteria between humans and the environments. As reported by Doi et al. (2017), ESBL prevalence in the Middle East and Africa (Ethiopia and Kenya) is over 50%, and close contact between livestock and humans may play an important role here. However, in high income countries, pets may also be involved in the spread of ESBL, where close contact between humans and pets is common (16). Using literature, we developed the following conceptual scheme of the ecology of ESBL- or carbapenemse-producing Enterobacterales for tropical regions (Figure 2).

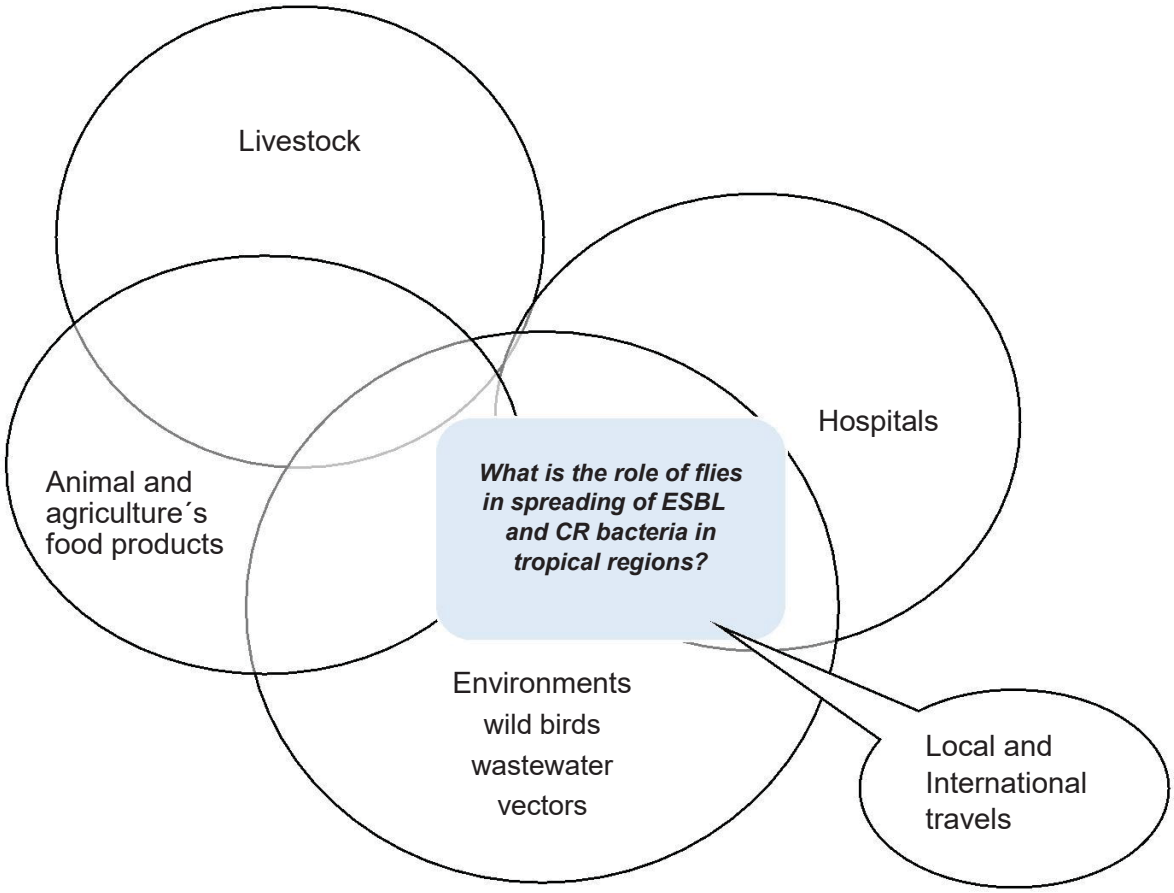

Figure 2. Conceptual scheme of the ecology of ESBL and carbapenemse- producing *Enterobacterales in the tropical regions.*

At health care facilities, level of exposure to broad-spectrum antibiotics, presence of chronic wounds and ulcers, history of having offensive procedures using medical equipment are factors for acquisition of MDR bacterial infections or colonization (17).

Irregular antibiotic use, prolonged hospitalization, poor sanitary practices, houseflies, and frequent travel to other countries are some of the risk factors for the spread of AMR (18, 19). In our study, we found that ESBL-producing GNB were more frequently detected in flies caught in the hospital than in flies caught in the city, which is 1.5 km away from the hospital. The molecular characteristics of the resistance genes detected in the flies are consistent with those we found in the clinical samples (20). Therefore, patient isolation, hand hygiene, and the implementation of antibiotic stewardship alone may not be sufficient to combat the spread of AMR in tropical regions.

1.4 Laboratory infrastructure at Asella

Culture-based microbiological diagnostic methods are currently the standard for pathogen identification in patients with BSIs, although they are time-consuming, insensitive, and in many cases inadequate in specificity. Nevertheless, this method is not available at all in most health care facilities in SSA. Performing high-quality microbiological diagnostics requires a high level of expertise and technical training, as well as well-equipped laboratories and the provision of consumables. For this study, we established a well-equipped microbiology laboratory at the Hirsch Institute for Tropical Medicine (HITM) and expanded the clinical chemistry tests for sepsis diagnosis to include a blood gas analyzer, lactate, C-reactive protein, and renal function tests. However, we transported the plasma and bacterial isolates to Germany for further investigation.

We used molecular methods to identify the most common ESBL enzymes such as CTX-M, SHV, and TEM, as well as carbapenemases like NDM, IMP, VIM, and OXA at the Institute of Medical Microbiology and Hospital Hygiene, University Hospital Düsseldorf, Düsseldorf, Germany. In addition, we performed multiplex PCR assays at the Institute of Virology at Charité - Universitätsmedizin Berlin to detect non-culturable pathogens such as *Borrelia* spp., *Plasmodium* spp., *Rickettsia* spp., and *Leptospira* spp. from plasma of culture-negative participants.

1.5 Treatment and prevention of MDR bacterial infections

1.5.1 MDR Gram-positive bacterial infections

Methicillin resistant *Staphylococcus aureus* (MRSA) strains are considered resistant to all penicillins, cephalosporins, and macrolides (21). These bacteria may remain susceptible to fluoroquinolones, aminogycogides, chloramphenicol, and doxycycline; however, these drugs should not be used alone or as initial treatment for severe MRSA infections. The preferred drugs to treat MRSA infections are glycopeptides like vancomycin and teicoplanin. Linezolid can be used to treat skin and soft tissue infections (SSTI) caused by MRSA (22). Daptomycin is an intravenous antibiotic approved for the treatment of complicated skin infections and bacteremia caused by MRSA (23). Daptomycin should not be used to treat pneumonia because it is inactivated by surfactant. Mupirone can be used for MRSA nasal colonization

eradictions (24). Of note, those antibiotics are expensive and rarely available in health care settings in SSA.

Vancomycin-resistant enterococcal (VRE) are naturally resistant to many antibiotics. The treatment of VRE should consider both the severity of infection and the AST results. Linezolid is the only drug specifically approved for the treatment of VRE BSIs (22). It is useful for patients who require oral or ambulatory therapy, who cannot tolerate glycopeptides, or whose renal function is impaired. Ampicillin in combination with gentamicin or streptomycin may be used to treat enterococcal endocarditis caused by strains susceptible to both agents (25). Uncomplicated urinary tract infections caused by VR could be treated with nitrofurantoin and fosfomycin (26). Tigecycline is effective in vitro against a broad spectrum of Gram-positive and –negative bacteria, anaerobes, and MDR pathogens such as MRSA and VRE. However, there is limited clinical data on its use for the treatment of VRE infections (Table 1).

Table 1. Recommended antibiotics for multidrug resistanct Gram-positive bacteria, based on antimicrobial susceptibility test result.

Source of infection	Preferred treatment	Alternative treatment (first-line options not available or tolerated	Remark	Reference
Methicillin- Resistant *S. aureus* (MRSA)				
UTI-Cystitis	Sulfamethoxazole/trimethropim Fosfomycin			
Pyelonephritis or [1] cUTI	Sulfamethoxazole/trimethropim Fosfomycin	Linezolid		
Bloodstream, intra-abdominal infections, SSTIs, pneumonia	Vancomycin Linezolid			(22)
Vancomycin Resistant *Enterococcus* (VRE)				
UTI-Cystitis	Fosfomycin Nitrofurantoin	*Doxycycline	*not be used as monotherapy	(26)
Pyelonephritis or [1] cUTI	Linezolid Fosfomycin	*Doxycycline	*not be used as monotherapy	(27)
Bloodstream, intra-Abdominal infections, SSTIs, pneumonia	Linezolid, Gentamicin or streptomycin combination with ampicillin			(25, 26)

cUTI: Complicated urinary tract infection

1.5.2 MDR Gram-negative bacterial infections

ESBL is plasmid-mediated β-lactamases that confer resistance to broad-spectrum β-lactam antibiotics, including third- and fourth-generation cephalosporins, acetronam, and extended-spectrum penicillins. These plasmids sometimes encode mutations that confer resistance to other broad-spectrum agents such as aminoglycosides, co-trimoxazoles, and fluoroquinolones, resulting in organisms that are resistant to most broad-spectrum antibiotics.

A major problem with ESBL is their ability to cause treatment failure with cephalosporins, aztreonam and other classes. The emergence of ESBL-producing bacteria is related to the unselective use of cephalosporins. The carbapenems (ertapenem, meropenem, and imipenem) are currently considered the agents of choice for severe infections caused by these pathogens. Piperacillin-tazobactam and cefoperazone-sulbactam may be considered as options for ESBL-producing mild bacterial infections (28).

Carbapenem resistance can be caused by combinations of ESBL or AmpC and porin loss or acquired carbapenemases. Most carbapenemase producers are extremely drug resistant. They are resistant to β-lactam antibiotics, aminogycosides, and β-lactam inhibitor combinations. Therefore, carbapenem-resistant bacterial infections can be treated with reserve antibiotics, such as cefiderocol, polymyxins, colistin, ceftazidime-avibactam, meropenem–vaborbactam and tigecycline (29, 30). A few carbapenemase-producing bacteria are susceptible to other antibiotics such as chloramphenicol, ciprofloxacin, and cotrimoxazole. Depending on local AST results, the combination of these drugs may be used to treat carbapenem-resistant isolates. Here we have tried to show the possible options that can be considered to treat MDR Gram-negative bacterial infections (Table 2).

Table 2. Resistance spectrum of Enterobacteriales according to resistance genes

Detected resistant genes	Penicillin	piperacillin/tazobactam	Cefa-zolin	Ceftriaxon	Cefepim	Ceftaroline	Meropenem	Ceftazidime-avibactam	Fosfomycin	Meropenem –	Plazomicin	Cefiderocol	Polymyxin B colistin
Penicillinase													
ESBL													
Amp-C													
KPC													
NDM-1 or OXA-48													
Activity against non-fermenters[a]													

[a] Non-fermenters refer to Acinetobacter baumannii and Pseudomonas aeruginosa, which can´t catabolize glucose and thus are unable to ferment but positive for ESBL and carbapenamase. Expected activity: green color= active; orange color = possibly active; Red color not or insufficiently active.

Table 3. Recommended antibiotics for ESBL and carbapenemase producing Gram-negative bacteria based on antimicrobial susceptibility test result.

Source of infection	Preferred treatment	Alternative treatment (first-line options not available or tolerated	Remark	Reference
ESBL producing Gram-negative bacteria				
UTI-Cystitis	Nitrofurantoin, TMP-SMX	Amoxicillin-clavulanate, single-dose Aminoglycosides, Fosfomycin Ciprofloxacin*, Levofloxacin, Ertapenem, Meropenem*, Imipenem-Cilastatin*	* Limit for future use and consider toxicity	(31, 32)
Pyelonephritis or [1]cUTI	Ciprofloxacin, Levofloxacin, or TMP-SMX Ertapenem*, meropenem*, Imipenem-Cilastatin*,		* carbapenem: save for future use	
Bloodstream, intra-abdominal infections, SSTIs, pneumonia	Meropenem, Imipenem-Cilastatin, Ertapenem, Ciprofloxacin*, Levofloxacin*, or TMP-SMX*	**Cefepime, **Piperacillin-tazobactam	* if AST is available. ** inferior than carbapenem and influenced by ESBLs	(33, 34)
Carbapenamase producing Gram-negative bacteria				
UTI-Cystitis	Ciprofloxacin, Levofloxacin, TMP-SMX, Nitrofurantoin, or a single-dose Aminoglycoside Meropenem*	**Ceftazidime avibactam, **Meropenem -vaborbactam, Imipenem-cilastatin-relebactam, and Cefiderocol ***Colistin	*Meropenem (S), Ertapenem (R), ** Meropenem (R) *** only when no alternative option	(32, 35)
Pyelonephritis or [1]cUTI	Ceftazidime-avibactam, Meropenem-vaborbactam, Imipenem-cilastatin-relebactam, Cefiderocol *Meropenem	Once-daily Aminoglycosides	*Meropenem (S), Ertapenem (R),	(36, 37)
Bloodstream, intra-abdominal infections, SSTIs, pneumonia	*Meropenem	Ceftazidime-avibactam	*Meropenem (S), Ertapenem (R),	(35)
	Ceftazidime-avibactam, Meropenem-vaborbactam, and Imipenem-cilastatin-relebactam	Cefiderocol *Tigecycline, *Eravacycline	Ertapenem (R), Meropenem (R), *intra-abdominal infections only	(38)
KPC identified	Ceftazidime-avibactam, Meropenem-vaborbactam, and Imipenem-cilastatin-relebactam	Cefiderocol		(39)
Metallo-β-lactamase (i.e., NDM, VIM, or IMP) carbapenemase identified	Ceftazidime-avibactam+aztreonam, Cefiderocol	*Tigecycline, *Eravacycline	*intra-abdominal infections only	(39)
OXA-48-like carbapenemase identified	Ceftazidime-avibactam	Cefiderocol *Tigecycline, *Eravacycline	*intra-abdominal infections only	(39)
Multidrug-resistant *P. aeruginosa* infection				
UTI-Cystitis	Ceftolozane-tazobactam, Ceftazidime-avibactam, Imipenem-relebactam, Cefiderocol, or a single-dose Aminoglycoside	Colistin		(39)
Pyelonephritis or [1]cUTI	Ceftolozane-tazobactam, Ceftazidime-avibactam, Imipenem-cilastatin-Relebactam, and cefiderocol	Once-daily aminoglycosides		
Bloodstream, intra-abdominal infections, SSTIs, pneumonia	Ceftolozane-tazobactam, Ceftazidime-avibactam, or Imipenem-cilastatin-relebactam	Cefiderocol Aminoglycoside monotherapy		
Multidrug-resistant *Acinetobacter baumannii* infection				
UTI-Cystitis	Ampicillin/sulbactam, TMP-SMX, an aminoglycoside	Once-daily aminoglycosides		(40)
Pyelonephritis or [1]cUTI	Ampicillin/sulbactam, TMP-SMX, an aminoglycoside	Once-daily aminoglycosides		(40)
Bloodstream, intra-abdominal infections, SSTIs, pneumonia	high-dose ampicillin/sulbactam an aminoglycoside,	*Tigecycline, *minocycline a polymyxin	*intra-abdominal infections and SSTIs	(40)

1 cUTI: Complicated urinary tract infections are defined as UTIs occurring in association with a structural or functional abnormality of the genitourinary tract, or any UTI in a male patient.

AST: antimicrobial susceptibility test; ESBL: TMP-SMX: trimethoprim-sulfamethoxazole S= susceptible; R: Resistant; SSTIs: skin and soft tissue infections; KPC: *K. pneumoniae* carbapenemase; OXA-48: Oxacillinase-48; NDM: New Delhi Metallo-beta-lactamase; VIM: Verona integron-encoded metallo-beta-lactamase

Because there are few treatment options for patients with MDR bacterial infections, great attention must be paid to containing the spread of these bacteria. Every hospital should improve laboratory detection and reporting of MDR bacteria, hand hygiene, infection prevention, patient isolation, and amicrobial stewardship practices. Improving local and national surveillance of MDR bacteria and rational use of antibiotics can play an important role in preventing the spread of AMR.

1.6 Ethical approval and consent to participate

The appropriate ethical review boards of Arsi University College of Health Sciences (reference number A/U/H/S/C/120/6443/2017), the Oromia Regional Health Bureau (reference number BEFO/AHBTFH/1-8/2017), and Düsseldorf University Hospital (reference number 5729) approved the study protocol. The ethical clearance for samples transportation between Ethiopia and Germany was obtained from the National Ethical Review Board of the Ministry of Science and Technology (reference number 310/204/2017). Before inclusion in the study, written informed consent to participate in the study was obtained from each participant or, in the case of children, from their legal guardians. For patients infected with MDR bacteria, we provided the appropriate antibiotic if available in that country. We followed up the participants who developed sepsis for 28 days to determine their progress.

1.7 Aims of Thesis

The aim of this study was to systematically investigate the spectrum, epidemiology and resistance patterns of pathogenic organisms in patients with AID, and to identify pertinent epidemiological, and other determinants of susceptibility and clinical outcome in the Arsi Zone of Ethiopia.

Our main objective to conduct the project was to characterize the spectrum of pathogens causing AID, and in particular rare and previously underdiagnosed pathogens, in patients presenting to the ARTH. For this purpose, we have established microbiology culture facilities, expanded the existing clinical chemistry laboratory, and performed basic diagnostic testing for all participants (n=684) with suspected AID at the HITM. We performed blood cultures and

other appropriate specimens and reported the results as soon as possible. The treating physicians used the local AST results for guiding antibiotic treatment. The local AST results revealed high levels of resistance against commonly used antibiotics, rendering 72.2% of the initiated antibiotic treatments likely to be ineffective. We transported the isolates to Düsseldorf, Germany for identification and confirmation of pathogens and investigation of resistant genes. There were around 20% discrepancies between bacterial species identification performed in Ethiopia and MALDI-TOF confirmation test done in Germany. For instance, three bacteria reported as *Citrobacter* spp. in Ethiopia were identified as *E. coli* by MALDI-TOF and two *Klebsiella* spp. were confirmed as *Raoultella* spp. We reported a *Raoultella planticola* nosocomial infection for the first time in SSA. We also transported the plasma samples and performed multiplex PCR for diagnosis of possible non-culturable pathogens.

We reviewed the literature and performed a meta-analysis to understand the burden of Gram-negative bacterial infections and associated mortality in Ethiopia. We requested an amendment and followed participants who had a systemic inflammatory response syndrome (SIRS) criterion ≥ 2 for 28 days. We followed up only participants who developed sepsis according to qSOFA, SIRS, and SOFA score criteria.

However, we found that existing SIRS or qSOFA criteria are less specific for early detection of sepsis. Therefore, we enrolled 612 adult participants to examine the physiological range of vital signs in the general healthy population at different altitudes (Asella, Adama, and Semera, Ethiopia) for qSOFA score performance.

We did the molecular PCR to characterize the resistance genes of ESBL and carbapenemases in GNB isolates. We found TEM and CTX-M-1 were the most commonly detect ESBL enzymes at the study sites. However, both SHV-group and CTX-M-1 were the most detected in *K. pneumoniae* isolates. Regarding carbapenemases, we only reported a single NDM-1 from one isolated *K. pneumoniae* and a combination of NDM-1 plus OXA-51 from an isolate of *A. baumannii.*

We aimed to investigate the role of flies in the spread of AMR in the hospital and in the community since we observed the presence of flies in the hospital compound. We compared ESBL enzymes types detected in clinical samples with ESBL enzymes detected in bacterial

isolates from flies and found they do have strong similarities. We suggested that flies may be potential risk factors for the spread of AMR in hospitals and communities in tropical regions, and stakeholders should reaffirm the need for a one-health approach to combat the spread of AMR.

In the results section of my dissertation, I present six published papers. The first paper is on a systematic review and meta-analysis of the burden of ESBL-producing Gram-negative bacterial infections in Ethiopia. The second paper is about clinical characterization of sepsis and evaluation of sepsis scores. The third paper addresses an interesting case showing the misidentification of bacteria, AMR and misuse of antibiotics, hospital-acquired infection, and prolonged hospital stays with MDR infection due to Gram-negative bacterial infection. The fourth paper addresses the need for adaptation for the use of qSOFA scoring in the Ethiopian population.The fifth paper addresses the challenges of antimicrobial resistance in Gram-negative bacterial infections in Ethiopia. The sixth or final paper addresses the risk factors for the spread of AMR in hospitals, particularly the role of vectors or houseflies.

2. Own publications

2.1. High rate of extended-spectrum beta-lactamase-producing Gram-negative infections and associated mortality in Ethiopia: a systematic review and meta-analysis, Tufa TB, Fuchs A, Tufa TB, Stötter L, Kaasch AJ, Feldt T, Häussinger D, Mackenzie CR, Antimicrob Resist Infect Control, 9(1)140, (2020)

Tafese B. Tufa's contribution to this paper:

a) Draft the conceptuals of the work
b) Search litiratures
c) Data analysis and draft the manuscript

- We conducted for the first time a review and meta-analysis of the burden of ESBL-producing Gram-negative infections and associated mortality in Ethiopia and reported the high prevalence (50%) of ESBL-producing GNB infections.

- Although there were limited data on the outcome of patients enrolled in the study who were infected with ESBL-producing GNB, mortality was higher among them.

Tufa et al. Antimicrobial Resistance and Infection Control (2020) 9:128
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Antimicrobial Resistance and Infection Control

REVIEW Open Access

High rate of extended-spectrum beta-lactamase-producing gram-negative infections and associated mortality in Ethiopia: a systematic review and meta-analysis

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Abstract

Background: Extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacteria have become a serious threat to global health. Their rapid spread is associated with high mortality due to ineffective antibiotic treatment. To date a regular surveillance of multidrug-resistant (MDR) pathogens in Ethiopia is not established. For this report, published data regarding ESBL-producing bacteria in different health facilities of Ethiopia were reviewed.

Methods: This study collates data from published information on the rates and clinical implications of infection with ESBL-producing Gram-negative bacteria in Ethiopia. A systematic literature search was conducted using PubMed, PubMed Central, Medline, Science Direct and Google scholar from October 2018 to March 2019. Eligible studies were identified by applying quality criteria. The pooled proportion of ESBL-producing Gram-negative bacteria was estimated based on a random effect model. The publication bias and the variation in proportion estimates attributed to heterogeneity were assessed.

Results: Fourteen studies with relevant data were included in the review. In total, 1649 Gram-negative bacteria isolated from 5191 clinical samples were included. The pooled proportion estimate of ESBL-producing Gram-negative bacteria was 50% (95% CI: 47.7–52.5%. Data showed a high level of heterogeneity ($I^2 = 95\%$, $P < 0.01$). ESBL rates varied by species; 65.7% (263/400) in *Klebsiella* spp., 48.4% (90/186) in *Salmonella* spp., and 47.0% (383/815) in *E. coli*. ESBL-encoding genes were reported in 81 isolates: 67 isolates harbored the CTX-M-1 group and 14 isolates TEM. The mortality associated with infections by bacteria resistant to third generation cephalosporins has rarely been investigated. However, two studies reported a mortality of 33.3% (1/3) and 100% (11/11).

(Continued on next page)

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(Continued from previous page)
Conclusions: In this meta-analysis, the pooled prevalence of ESBL-producing pathogens is alarmingly high. Data on mortality rates is scarce. This highlights the need for establishing and upgrading clinical microbiology laboratories in Ethiopia for routine antibiotic susceptibility testing and extended surveillance of multidrug resistance.

Keywords: ESBL, Gram-negative bacteria, Multidrug-resistance, Antimicrobial resistance

Background

Bacterial production of extended-spectrum β-lactamases (ESBL) significantly reduces the efficacy of the most commonly used beta-lactam antibiotics for the empiric therapy of infections caused by putative Gram-negative bacteria [1]. While ESBL enzymes readily hydrolyse penicillins and cephalosporins, they have a far lower affinity for cephamycins and clavulanic acid [1, 2]. These hydrolytic enzymes are encoded by various gene variants. The major groups are TEM (Temoniera), CTX-M (Cefotaximase-Munich), SHV (Sulfhydryl variable), and OXA (oxacillin), all of which have been used for molecular detection of ESBL genes [3–5]. These genes are frequently mobile and located on plasmids and are thus transmitted horizontally [5]. These plasmids often contain mobile elements with resistance genes for additional drug classes such as sulfonamides, aminoglycosides and fluoroquinolones. Thus, bacteria carrying these plasmids are very often multi-drug resistant [6–8].

Gram-negative bacteria with the capacity to produce ESBL have become a serious global health problem, especially in resource-limited settings [9]. The rapid increase of ESBL-producing bacteria is associated with high mortality due to ineffective antibiotic treatment [10]. The management of patients with multidrug-resistant (MDR) bacteria requires well-staffed clinical units, reliable microbiology service and regular interaction between professional groups.

Due to limited financial resources and restricted supply chains, carbapenems are often unavailable in healthcare facilities in developing countries. Thus, the effective treatment of infections caused by ESBL-producing bacteria is limited, contributing to a high mortality [11].

In East Africa considerable variance in the prevalence of ESBL-producing Gram-negative bacteria between 13.4 and 89.0% has been described [12–14]. Understanding the epidemiology of ESBL at a country level is elemental to reinforce effective prevention and control strategies, but systematic surveillance of MDR pathogens in Ethiopia is non-existent. To assess the magnitude of the problem in Ethiopia, data on ESBL-producing bacteria in different regions of Ethiopia were extracted from existing publications, analyzed and summarized in this systematic review.

Methods

To ensure inclusion of relevant information, the study was conducted based on the guideline of the Preferred Reporting Items for Systematic Reviews and Meta Analyses group checklist [15]. The outcomes of interest were the proportion of ESBL-producing bacteria among Gram-negative isolates from samples obtained from human patients in Ethiopia and the associated mortality.

Study area

The study was conducted in Ethiopia; a country situated in the horn of Africa, covering a land area of 1.04 million km^2. With a population of 110,14 million people, Ethiopia is the second most populous nation in Africa following Nigeria [16].

Literature search and eligibility criteria

A systematic literature search was conducted on PubMed, PubMed Central, Medline, Science Direct and Google scholar, which are commonly used medical and biomedical databases in Ethiopia and accessible free of charge through different Ethiopian universities, to identify publications between January 1990 and March 2019 relevant for this review. No chargeable databases or databases without a medical focus were used for the literature search. The search strategy included all articles containing the descriptors. Structured search strategies were developed using the vocabulary terms of each database and targeting the "title" and "abstract" fields. The search was conducted by combining the following medical subject heading terms: "ESBL producing Enterobacteriaceae infections", "Gram-negative infection associated mortality", and "Ethiopia", including all study types and populations. Additional publications were identified in the references of the initially identified articles, including systematic reviews and/or meta-analyses. Citation lists of publications meeting eligibility criteria for this meta-analysis were also reviewed.

In order to identify appropriate publications, the following selection criteria were used: a study had to (i) describe at least one pathogenic bacteria genus among the Gram-negative bacteria, (iii) be conducted on human subjects, (iii) isolate and identify bacteria from clinical specimens, (iv) test isolates for sensitivity to at least amoxicillin plus clavulanic acid and 3rd generation cephalosporins, (v) be conducted in Ethiopia, and (vi) be

published in English. Therefore, reviews and publications not focusing on ESBL-producing Gram-negative bacteria isolated from human samples in Ethiopia and/or not subjected to antimicrobial sensitivity testing [17] were excluded. The titles and abstracts of the search results were reviewed and all publications were included that described cross-sectional, prospective, observational, and/or randomized controlled trials.

The selection of publications followed three steps. First, titles of articles identified by literature search were checked for relevance of the studied topic. Publications, in which testing for ESBL was not performed, did not describe results from Ethiopia or not from clinical human samples and duplicate publications were discarded. Second, abstracts from selected publications were evaluated for the inclusion criteria. Third, the content of the remaining publications was accessed and evaluated for this review. Details of how eligible studies were included in the data synthesis is indicated in the flow diagram of the study selection process (Fig. 1).

Description of studies

Types of studies

All randomized controlled clinical trials and cross-sectional studies investigating the drug susceptibility/resistance pattern of Gram-negative bacteria from Ethiopia published within the given time frame were included in the review.

Antimicrobial susceptibility testing

Within included studies the double disc synergy test (DDST) was used for phenotype detection of ESBL production showing a typical increase in growth inhibition by ceftazidime and cefotaxime in the zone adjacent to amoxicillin + clavulanic acid [18].

Study populations

The participants of included studies were distributed among all age, sex, and Ethiopian ethnic groups. Overall, 1049 published articles were reviewed. Of these, 14 original articles were included.

Outcome measurements

The main outcome measure was the proportion of ESBL-producing bacteria among Gram-negative isolates evaluated by drug susceptibility testing. Based on this, the isolates defined as resistant to the selected drugs were documented. Patient mortality associated with infections with ESBL-producing Gram-negative bacteria was also recorded.

Data extraction and management

Data was collected from included studies with focus on the following characteristics: type of study, study population, antimicrobial sensitivity testing, and characteristic of the laboratory investigation. For data consistency, two researchers extracted data independently. Whenever there was discordance in the data extracted, consensus was reached by double-checking the article. From eligible studies, if available the following data were extracted: first author, year of publication, study period, study site, study design, population size, sample size, type of sample collection, methods used to test for ESBL, antimicrobial substances used for susceptibility testing, gene encoding for ESBL, number of Gram-negative bacteria isolated and tested for ESBL, the proportion of ESBL-producing bacteria among Gram-negative isolates, and mortality associated with ESBL-producing bacterial infection.

Data synthesis and analysis

The mean proportion of ESBL-producing bacteria was calculated, using the sum of the numbers of ESBL-producing Gram-negative bacteria in all studies considered, divided by the sum of the number of Gram-negative bacteria tested for ESBL. The pooled proportion estimates for ESBL-producing Gram-negative bacteria in the general population and their 95% CI were calculated using the random effects model meta-analysis [19].

Heterogeneity between studies was evaluated through the Cochran's Q test (reported as p value) and inverse variance index (I^2) [20].

For each study, the prevalence with corresponding 95% CI and the overall random effects pooled estimate of all the studies were presented.

Data was analyzed using RStudio Version 1.1.456 – © 2009–2018 (RStudio Inc., Boston, MA, USA). A map showing the number of studies and prevalence of ESBL- producing Gram-negative bacteria in different region of Ethiopia was created, using Quantum GIS software version 2.0.1 (Open Source Geospatial Foundation, Boston, USA).

Results

Distribution of articles describing ESBL in Ethiopia

The initial database search returned 1049 abstracts. Of those, 1018 publications were discarded after reviewing their titles. A further 10 articles either described environmental or veterinary samples or ESBL-producing bacteria were not identified and described in the articles. The full text of 21 articles and an additional three articles identified through review articles were scrutinized for eligibility. Of these, 8 were excluded because ESBL phenotypes or genotypes were not clearly described and 2 publications were identified as systematic reviews. Finally, 14 articles fulfilled eligibility criteria and were subjected to meta-analysis [8, 21–33] (Fig. 1).

In total, the 14 articles reviewed described cross-sectional hospital-based studies. Of those, 6 (43.0%) were published from the cities of Addis Ababa, 5 (36.0%) from Jimma, and 1 (7%) from each of the following: Adama, Bahir Dar, and Harar (Fig. 2). All reviewed articles

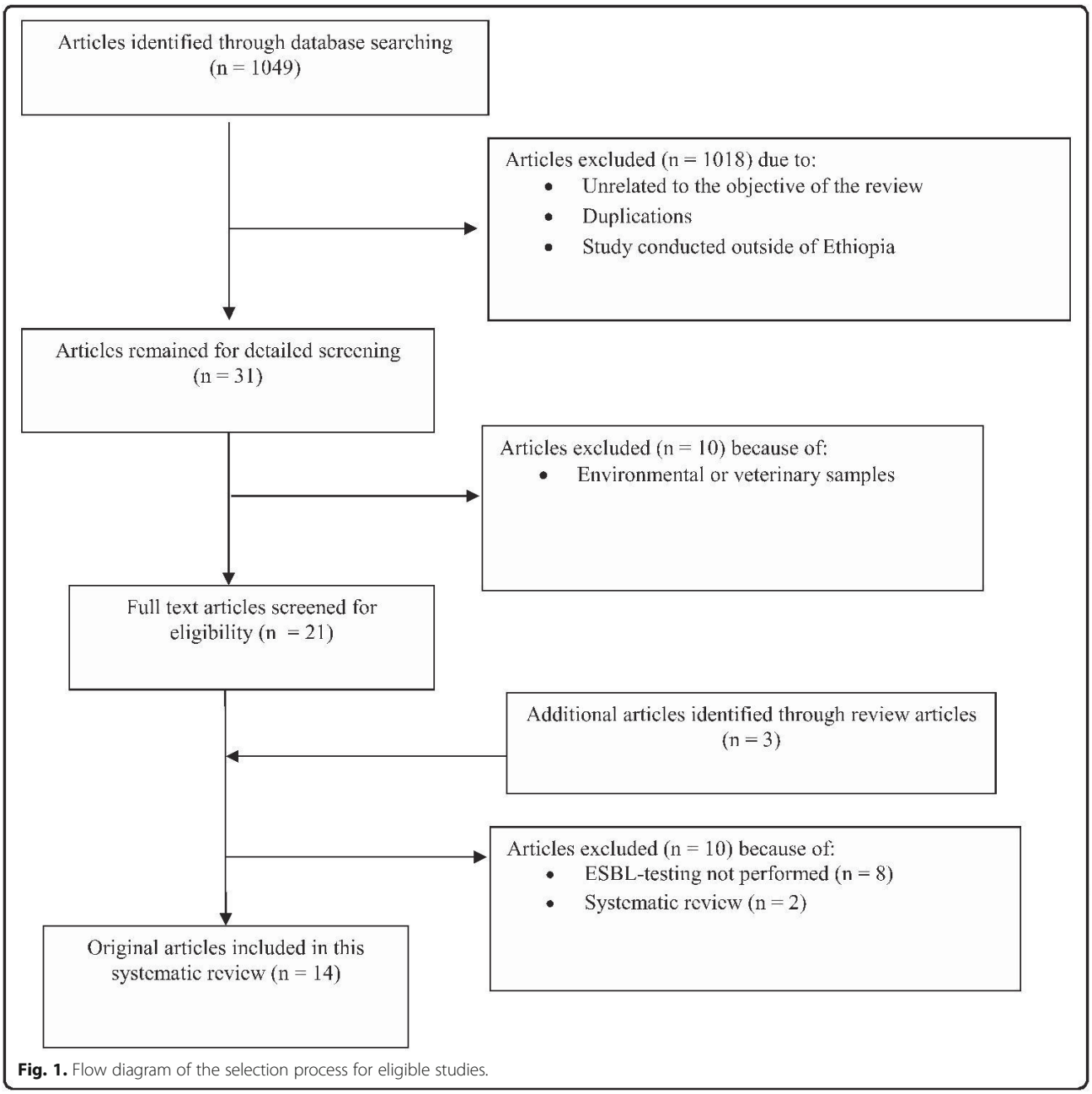

Fig. 1. Flow diagram of the selection process for eligible studies.

included patients attending in-patient and/or outpatient departments. A total of 5191 samples from human study participants were analyzed including urine (n = 1273), stool (n = 1679), swabs (n = 266), sputum (n = 294), blood cultures (n = 192) and body fluids (n = 34) (Table 1).

Laboratory methods used to detect ESBL-producing bacteria

Eleven (78.6%) of the reviewed articles used the double disk synergy test (DDST) alone, while the remaining 3 (21.4%) articles used both DDST and polymerase chain reaction (PCR)-based molecular methods to investigate the proportion of ESBL-producing bacteria.

Selected articles were published from 2005 to 2019. Inclusion of study participants ranged from 2003 to 2017. All data in the included publications were obtained from tertiary hospitals. The highest proportions of ESBL-producing bacteria were reported from the capital city, Addis Ababa (57%) and Bahir Dar, the capital of Amhara Region in the northwestern part of Ethiopia (57%). The lowest proportion was reported from Adama in Oromia Region / central Ethiopia (25%) (Fig. 2).

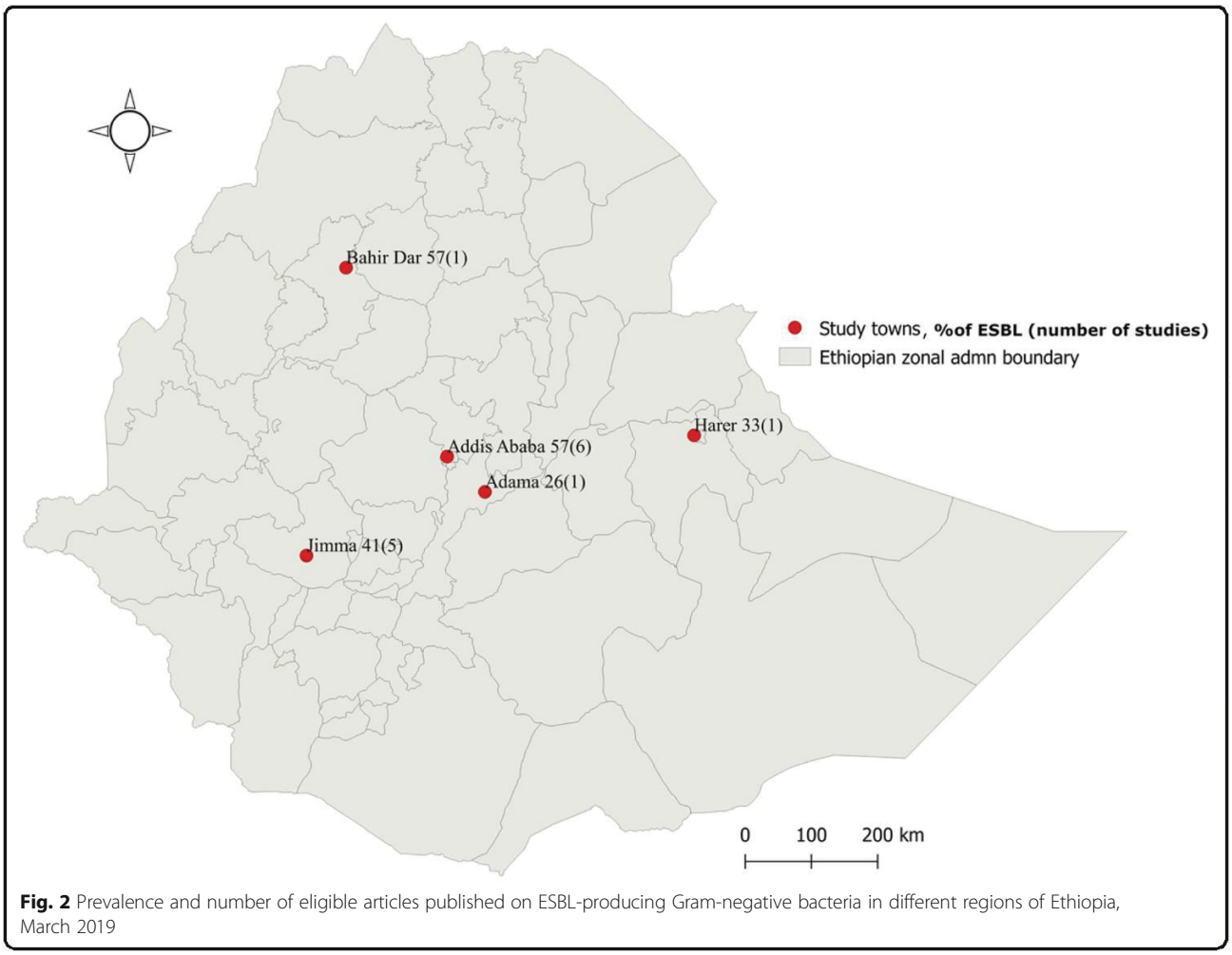

Fig. 2 Prevalence and number of eligible articles published on ESBL-producing Gram-negative bacteria in different regions of Ethiopia, March 2019

As presented in the forest plot (Fig. 3) the pooled proportion of ESBL-producing Gram-negative bacterial isolates from human samples in Ethiopia was 50% (95% CI: 0.48–0.52). Random model methods showed a high level of heterogeneity ($I^2 = 95\%$, $p < 0.01$). Concerning regional differences, the pooled proportion of ESBL-producing Gram-negative bacteria in Addis Ababa was 56.5% (95% CI, 0.53−0.60; $I^2 = 95.2\%$ and $p < 0.0001$) compared to 41% (95% CI, 0.36−0.46; $I^2 = 84.6\%$ and $p < 0.0001$) in the city of Jimma in the southwestern part of Ethiopia.

The studies included were conducted in different clinical settings, study periods, with differing clinical samples and study populations. This might be an influencing factor for the heterogeneity of the results.

In total, 1649 Gram-negative bacteria isolated from 5191 clinical samples were included. The pooled rate of ESBL-producing Gram-negatives was determined to be 50.1% (95% CI: 47.7 –52.5%). Among different species, ESBL production rates were 65.7% ($n = 263$) for *Klebsiella* spp., 62.2% ($n = 33$) for *Enterobacter* spp., 48.4% ($n = 90$) for *Salmonella* spp., 47.0% ($n = 383$) for *E. coli*, 46.8% ($n = 22$) for *Citrobacter* spp., 43.8% ($n = 7$) for *Providencia* spp., 28.3% ($n = 15$) for *Proteus* spp., 17.4% ($n = 4$) for *Pseudomonas aeruginosa*, 9.4% (n = 3) for *Acinetobacter* spp., and 20.8% ($n = 5$) for other Gram-negative bacteria, respectively (Table 2).

Molecular epidemiology of ESBL in Ethiopia

Out of 14 reviewed articles, only three (21.4%) studies, one from Addis Ababa University Black Lion Hospital and two from Jimma University Specialized Hospital, reported on the underlying resistance genes for the detection of ESBL resistance [8, 23, 28]. A total of 81 isolates were molecular-biologically analyzed for genetic resistance mechanism. All of those were positive for ESBL-encoding genes: 82.7% (67/81) carried CTX-M-1 group and 17.3% (14/81) TEM. All CTX-M-1 group ESBL genes reported from included studies were confirmed to be CTX-M-15 genes [8, 23]. Additional genes detected were: one isolate carrying a bla_{OXA} gene from Addis Ababa, two bla_{CTX} of the CTX-M-9 group [23] and three bla_{SHV} genes only expressed in *Enterobacter cloacae* isolated in Jimma [8].

Table 1 Summary of the 14 studies reporting the prevalence of ESBL-producing Gram-negative bacteria in different parts of Ethiopia, 2005–2019

Author, year	Study period	Study area (site)	Sample size	Sample types (Culture specimens)	No. of Gram-negative isolates	No. of ESBL (%)
Abayneh M et al., 2018 [32]	March to June 2016	Jimma	342	urine	74	17 (23.0)
Abera B et al., 2016 [33]	Sept 2013 to March 2015	Bahir Dar	477	blood, urine, pus/swab and body fluids	210	120 (57.1)
Beyene G et al., 2011 [21]	Jan to Aug. 2006	Addis Ababa	1225	stool, blood	113	78 (69.0)
Desta K et al., 2016 [22]	10–20 Dec 2012	Addis Ababa	267	stool	295	151 (51.2)
Eugale T et al., 2018 [23]	April 2013 to March 2014	Addis Ababa	68	Stool	68	12 (17.6)
Gashaw M et al., 2018 [24]	May to Sept. 2016	Jimma	197	urine, swab/pus, blood, and sputum	100	36 (36.0)
Legese et al., 2017 [25]	Jan. to March 2014	Addis Ababa	322	blood and urine	28	22 (78.6)
Mulisa et al., 2015 [26]	April to Aug. 2016	Adama	384	urine, stool, swabs, and body fluids	65	17 (26.0)
Mulualem Y et al., 2012 [27]	Feb. to March 2007	Jimma	359	urine, stool, swabs, and sputum	67	24 (35.8)
Pritsch M et al., 2017 [28]	Jan. 2014 to June 2015	Jimma	224	swabs and body fluids	14	3 (21.4)
Seboxa T et al., 2015 [29]	Aug. 2012 and Oct. 2013.	Addis Ababa	292	blood	20	9 (45.0)
Seid & Asrat, 2005 [30]	Dec. 2003 to Feb. 2004	Harar	384	sputum, urine and pus	57	19 (33.3)
Teklu DS et al., 2019 [31]	Jan. to May 2017	Addis Ababa	426	urine, blood, swabs, and body fluids	426	246 (57.7)
Zeynudin A et al., 2018 [8]	March to Oct. 2014	Jimma	224	urine, swabs, blood and fluids	112	71 (63.4)

Outcome of patients infected with ESBL-producing bacteria

Only two of the analyzed studies reported the mortality rate associated with infections by ESBL-producing pathogens or resistance to 3rd generation cephalosporins. The pooled mortality within these publications was 86% (12/14) [28, 29]. One study from Black Lion Teaching Hospital in Addis Ababa reported a high mortality among patients with Gram-negative bacterial blood stream infection, particularly among patients infected with multi drug resistant bacteria. Twelve out of 20 patients with Gram-negative sepsis died in the hospital. Of those, 91.7% (11/12) were infected with ESBL-producing bacteria [29].

Discussion

Infections caused by ESBL-producing Gram-negative bacteria are increasing at an alarming rate and have

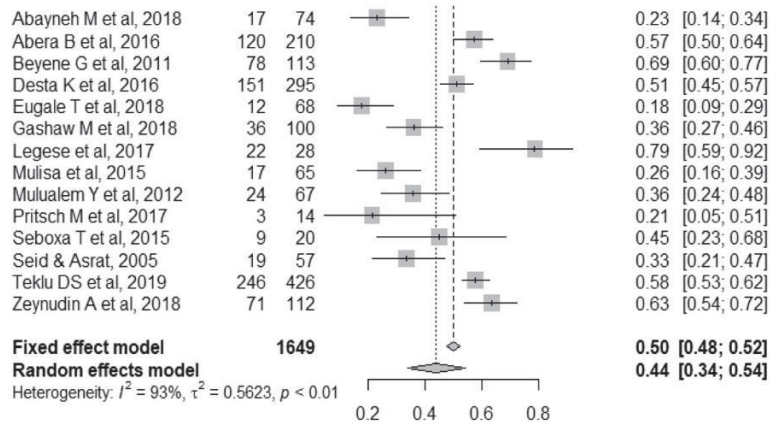

Fig. 3 Forest plot of pooled prevalence of ESBL-producing Gram-negative bacteria in 14 studies, Ethiopia, 2005–2019

Table 2 Species distribution of the pooled proportion of ESBL-producing Gram-negative bacteria

Gram-negative bacteria	No. of studies	No. of isolates	No. of ESBL-positive isolates	ESBL Proportion [95% CI]	I^2 (*p*-value)
Klebsiella spp.	10	400	263	0.66 [.61; .70]	< 0.001
Enterobacter spp.	6	53	33	0.61 [.47; .74]	< 0.001
Salmonella spp.	5	186	90	0.48 [.33; .63]	< 0.001
E. coli	10	815	383	0.47 [.44; .50]	< 0.001
Citrobacter spp.	5	47	22	0.47[.33;,63]	0.001
Providencia spp.	3	16	7	0.44 [.20; .70]	0.011
Proteus spp.	6	53	15	0.28 [.16; .42]	0.053
Pseudomonas aeruginosa	2	23	4	0.17 [.05; .39]	0.386
Acinetobacter spp.	3	32	3	0.09	–
Others[a]	5	24	5	0.09 [.02;.25]	0.142
Total		1649	825	0.25 [.09; .49]	< 0.0001

[a]Others: *Alcaligenes faecalis* (1 ESBL of 3 isolates), *Morganella morganii* (3 ESBL of 12 isolates); *Stenotrophomonas maltophilia (*1 ESBL of 4 isolates), *Escherichia hermanii* (1 ESBL of 1 isolates)

become a serious public health threat worldwide. Summarized data of the burden of ESBL-associated antibiotic resistance is limited in Africa. To our knowledge, this is the first systematic review of data from Ethiopia concerning ESBL-producing bacteria from clinical specimens.

In this study, the pooled prevalence of ESBL-producing Gram-negative bacteria was 50.1%. This prevalence lies in the middle of a wide range between 13.4 to 89.0% reported in other studies from East Africa [13, 14]. Among the different species identified and investigated in the studies, *Klebsiella* spp. were the most frequent ESBL-producing Gram-negative bacteria followed by *Enterobacter* spp., *Salmonella* spp., *E. coli* and *Citrobacter* spp. (Table 2). Similar findings have been reported from Uganda, with ESBL-rates of 52% for *Klebsiella spp.* and 44% for *E. coli* [12]. One recent study from northern Ethiopia also confirmed the highest rate of ESBL-production among Gram-negative isolates from human samples in *K. pneumoniae* [34].

The most common ESBL-producing non-fermenting Gram-negative rods (NFGN) are *Pseudomonas aeruginosa* and *Acinetobacter* spp. [35]. In this review, we identified a relatively low ESBL rate among NFGN bacteria compared with the *Enterobacteriales* of 17.4 and 9.4% for *Pseudomonas aeruginosa* and *Acinetobacter* spp., respectively (Table 2). One study from Jimma, in southwest Ethiopia described a high prevalence of *Acinetobacter* spp., during screening for possible ESBL-production using 3rd generation cephalosporins alone. However, genotyping results confirmed a low proportion of ESBL production in *Acinetobacter* spp. at the study site [8]. This shows that genotypic screening for MDR in NFGN might not be beneficial. In this case, the functional and more general approach of phenotypic resistance testing seems superior.

Molecular ESBL gene detection is not commonly practiced in Ethiopia. From all studies included in this review, gene detection was performed for 81 strains out of the 825 ESBL-producing Gram-negative bacteria determined by DDST. The CTX-M-1 group ESBL genes were most frequently detected, with all of these identified as CTX-M-15, followed by bla_{TEM}. Reports from India showed similar results, in which the prevalence of $bla_{\mathrm{CTX\text{-}M}}$ was highest (82.5%), followed by bla_{TEM} (67.5%) and bla_{SHV} (57.5%) among the ESBL-genes identified from clinical Gram-negative isolates [17]. In the studies included in this review, bla_{SHV} was only detected in three *Enterobacter cloacae* isolates. Only a single isolate carrying a bla_{OXA} gene from Addis Ababa [24] and two isolates expressing CTX-M-9 group genes from Jimma [8] were described. More studies are needed to establish a comprehensive overview of the distribution of ESBL genes in Ethiopia.

Isolates of *Acinetobacter baumannii* carrying the carbapenem resistance gene NDM-1 (New Delhi metallo-beta-lactamase) were reported from Jimma [35]. In East Africa, the NDM-1 gene was first detected from isolated *Acinetobacter baumannii* in Kenya [36]. However, the current data on the prevalence of carbapenem resistance in East Africa ranges from 1% in the Democratic Republic of Congo to 35% in Tanzania [37], although no systematic surveillance is conducted in these countries and thus, as in Ethiopia, the true prevalence is unknown.

Pooled data currently available suggest that infections caused by ESBL-producing bacteria are leading to prolonged hospital stays and are associated with an increased risk of mortality [11, 38]. Even though available data from Ethiopia is very limited concerning the outcome of patients infected with ESBL-producing bacteria, one study from Addis Ababa describes a mortality rate of 100% in 11 patients [29]. In patients with sepsis caused by Gram-negative bacteria, mortality is strongly associated with antibiotic resistance [39, 40]. The absence of timely microbiology reports in patients with Gram-negative bacterial

sepsis compromises successful antimicrobial treatment and possibilities for antimicrobial stewardship. However, even if pertinent resistance patterns would be available earlier, the frequent unavailability and high prices of effective antibiotics greatly contributes to morbidity and mortality associated with these infections.

As a study conducted in Tanzania indicates, inappropriate antibiotic use and two-week fatality rates were significantly higher among patients with septicemia due to ESBL-producing organisms than among those with infections due to non-ESBL producing bacteria [40]. The 30-day and 90-day survival rates of patients infected by MDR bacteria were also significantly lower than patients infected by non-MDR bacteria (58.8% vs. 75.0% at 30 days and 43% vs. 63%, at 90 days) [41]. In a study published in the UK, mortality due to blood stream infections caused by ESBL-producing *E. coli* was significantly higher than from non-ESBL *E. coli* [42]. In countries and regions with high rates of ESBL carrying bacteria, as demonstrated here for Ethiopia, these findings have serious implications for empirical antibiotic prescription practice. In Ethiopia, cephalosporins should be considered ineffective in up to 66% of infections with *Klebsiella* spp. and 47% of cases of *E. coli* infections as was reported in 2007 for ESBL-*E. coli* [42].

In order to guide empiric therapy, continuous surveillance of common resistance patterns of Gram-negative isolates and monitoring of ESBL genes circulating in the population are essential. For this task, clinical microbiology laboratories need to be established, upgraded and maintained. In this review, CTX-M-15 ESBL genes were the most frequently detected ESBL gene. At this time, this gene could be used as target for ESBL screening programs in Enterobacteriales in the country, where high-quality and reliable bacteriological laboratories are still rather rare. In general, the molecular testing for $bla_{\text{CTX-M-1}}$ or $bla_{\text{CTX-M-15}}$ would provide a reliable prediction of overall resistance due to ESBL [43]. In order to reduce the dissemination of antibiotic resistance within the country, infection prevention and control measures and the establishment of antimicrobial stewardship programs should be strengthened.

This review is limited by the fact that available literature does not permit a meta-analysis of adjusted mortality associated with infections caused by ESBL-producing bacteria, as only two of the 14 included studies reported the results of patient outcome. Thus, only crude mortality but neither attributable mortality, nor causality were described. Therefore, this meta-analysis highlights the deficiencies of the existing literature to calculate adverse outcome attributable to ESBL-associated resistance. Because of possible clinical implications it would be interesting to describe the proportion of ESBL-positive Enterobacterales in the different clinical specimen (blood, urine. Etc.). However, it was not possible to list proportions of ESBL-producing isolates in different clinical specimen, because it was not stated respective of clinical samples in most articles included into this study. Since ESBL genotypes were only reported in two studies, the pooled prevalence of infections due to ESBL phenotype in Ethiopia was analyzed.. Clinical Laboratory Standards Institute (CLSI) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines were used to interpret AST results by all investigators of studies included in this review.

Conclusion and recommendations

In this meta-analysis, the pooled prevalence of phenotypic ESBL production among Gram-negative isolates from human samples is remarkably high. Despite the scarcity of data, infections caused by ESBL-producing bacteria are very likely resulting in an increased mortality. In resource-limited settings, double-disk synergy test can be implemented for screening of ESBL production. The CTX-M-1 group is the predominantly detected ESBL genotype with all of the detected genes confirmed to be CTX-M-15 genes. The CTX-M-1 group or CTX-M-15 gene could therefore be targeted for rapid genetic ESBL screening in the country, thus providing early essential information for decisions on appropriate and effective treatment. In order to provide physicians with urgently needed guidance for antimicrobial therapies according to detected antimicrobial resistance patterns, establishment, upgrading and maintaining of clinical microbiology laboratories in the country capable of reliable double-disk synergy testing are essential. The possibility of ESBL gene detection for routine surveillance is desirable. National and regional treatment guidelines should be based upon MDR surveillance to effectively treat patients and prevent the spread of resistance genes in hospitals and communities. Emphasis for production of antibiotic substances in the country or import from abroad should consider recent MDR surveillance data.

Abbreviations

AST: Antimicrobial susceptibility testing; CI: Confidence interval; CLSI: Clinical Laboratory Standards Institute; CTX-M: Cefotaximase-Munich; DDST: Double disc synergy test; ESBL: Extended spectrum beta-lactamase; EUCAST: European Committee on Antimicrobial Susceptibility Testing; MDR: Multidrug-resistant; NDM: New Delhi metallo-beta-lactamase; NFGN: non-fermenting Gram-negative rods; OXA: Oxacillin; PCR: polymerase chain reaction; SHV: Sulfhdryl variabl; TEM: Temoniera

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Authors'contributions

Tafese Beyene Tufa: Conception and design, acquisition and analysis of the data, drafting of the manuscript. Andre Fuchs: Conception and design, analysis of the data, drafting and revision of the manuscript. Takele Beyene Tufa. Design, acquisition and analysis of the data, drafting of the manuscript. Loraine Stoetter, Drafting and revision of the manuscript. Achim J. Kaasch.

Analysis and interpretation of data, drafting of the manuscript. Torsten Feld: Critical revision of manuscript and approval of final version to be published. Dieter Häussinger: Critical revision of manuscript and approval of final version to be published. Colin R. Mackenzie: Interpretation of data, critical revision of the manuscript and approval of final version to be published.

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All data generated or analysed during this study are included in this published article.

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Tafese B. Tufa's contribution to this paper:

a) Draft the coceptuals of the work
b) Data collection
c) Perform the laboratory activities
d) Data analysis and draft the manuscript

- In Ethiopia, there was little data on the diagnosis and treatment of sepsis in adults. Therefore, we conducted a study on adult sepsis for the first time in our hospital and found that the early detection of sepsis is very low (12.4%).
- Neither qSOFA nor SOFA are sensitive and specific enough to detect sepsis in Asella (at 2400 m above sea level), and adaptation to local settings is urgently needed.

  We have reported also high mortality among MDR Gram-negative bacteremia.

PLOS ONE

RESEARCH ARTICLE

Clinical and microbiological characterization of sepsis and evaluation of sepsis scores

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Abstract

Background

Despite the necessity of early recognition for an optimal outcome, sepsis often remains unrecognized. Available tools for early recognition are rarely evaluated in low- and middle-income countries. In this study, we analyzed the spectrum, treatment and outcome of sepsis at an Ethiopian tertiary hospital and evaluated recommended sepsis scores.

Methods

Patients with an infection and ≥2 SIRS criteria were screened for sepsis by SOFA scoring. From septic patients, socioeconomic and clinical data as well as blood cultures were collected and they were followed until discharge or death; 28-day mortality was determined.

Results

In 170 patients with sepsis, the overall mortality rate was 29.4%. The recognition rate by treating physicians after initial clinical assessment was low (12.4%). Increased risk of mortality was significantly associated with level of SOFA and qSOFA score, Gram-negative bacteremia (in comparison to Gram-positive bacteremia; 42.9 versus 16.7%), and antimicrobial regimen including ceftriaxone (35.7% versus 19.2%) or metronidazole (43.8% versus 25.0%), but not with an increased respiratory rate (≥22/min) or decreased systolic blood pressure (≥100mmHg). In Gram-negative isolates, extended antimicrobial resistance with expression of extended-spectrum beta-lactamase and carbapenemase genes was common. Among adult patients, sensitivity and specificity of qSOFA score for detection of sepsis were 54.3% and 66.7%, respectively.

Conclusion

Sepsis is commonly unrecognized and associated with high mortality, showing the need for reliable and easy-applicable tools to support early recognition. The established sepsis scores were either of limited applicability (SOFA) or, as in the case of qSOFA, were significantly impaired in their sensitivity and specificity, demonstrating the need for further evaluation and adaptation to local settings. Regional factors like malaria endemicity and HIV prevalence might influence the performance of different scores. Ineffective empirical treatment due to antimicrobial resistance is common and associated with mortality. Local antimicrobial resistance statistics are needed for guidance of calculated antimicrobial therapy to support reduction of sepsis mortality.

1. Introduction

1.1. Introduction

Infectious diseases remain a leading cause for morbidity and mortality in low and middle income countries, in particular in sub-Saharan Africa (SSA) [1]. Here, in contrast to the declining mortality due to Human Immunodeficiency Virus (HIV) infection, malaria and tuberculosis in the past decade, the mortality attributed to severe bacterial infections plays an increasing role. The intensive and longstanding programs to curb the mortality of the above-mentioned diseases were finally successful, whereas bacterial diseases and sepsis have not been in the focus of attention and funding until recently. Furthermore, antimicrobial resistance is increasing and contributing to the rise in mortality in the light of limited treatment options. Regarding sepsis in SSA, awareness is limited [2] and the WHO Global Burden of Diseases Reports do not list sepsis as specific cause of death. Due to various reasons, diagnostic criteria for recognition and prognostic evaluation of sepsis are often not systematically applied in the clinical routine and it has therefore to be assumed that a high number of cases remain unrecognized and unreported. Therefore, interventions and investigations focusing on sepsis treatment do not cover the full extent of the problem and alone may not be sufficient to solve the problem of high sepsis mortality. Tools for early recognition of sepsis as the SIRS score proved ineffective and SOFA and qSOFA scoring have recently been proposed as clinical tools for sepsis recognition [3]. Early recognition of patients at risk is necessary to allocate limited resources and to achieve improved treatment outcomes in resource-limited countries.

In 2017, the international Surviving Sepsis Campaign (iSSC) published revised guidelines for the diagnosis and treatment of sepsis and septic shock after publication of the Sepsis-3 definition in 2016, a renewed definition of sepsis as life-threatening organ dysfunction caused by a dysregulated host response to an infection [3, 4]. With this publication, the SOFA (Sepsis-related Organ Failure Assessment) score and, for use in adult patients only, the qSOFA (quick SOFA) score were introduced for early recognition of organ dysfunction and prognostic assessment of sepsis (see Tables 1 and 2). However, these scores have been extracted from large cohorts of patients, which have mostly been investigated in high income countries without major resource limitations in the health care system and with no relevant causes for sepsis apart from bacterial infections [5, 6]. The diagnostic and prognostic value of these sepsis scores have yet to be evaluated for the setting of SSA, where tropical diseases like malaria substantially contribute to the total disease burden [7].

The availability of systematic data regarding the epidemiology of sepsis, the spectrum of causing organisms and their resistance patterns, as well as the evaluation of management and

Table 1. SOFA score.

SOFA score	0	1	2	3	4
Respiration					
PaO$_2$/FIO$_2$ (mmHg)	>400	<400	<300	<200	<100
SaO$_2$/FIO$_2$		221–301	142–220	67–141	<67
Coagulation					
Platelets (x10^3/mm^3)	>150	<150	<100	<50	<20
Liver					
Bilirubin (mg/dl)	<1.2	1.2–1.9	2.0–5.9	6.0–11.9	>12.0
Cardiovascular					
Hypotension (MAP in mmHg)	No hypotension	<70			
Dopamine or norepinephrine (μg/kg/min)			Dopamine ≥ 5 or dobutamine (any)	Dopamine > 5 or norepinephrine ≥ 0.1	Dopamine > 15 or norepinephrine > 0.1
CNS					
Glasgow Coma Scale	15	13–14	10-Dec	06-Sep	<6
Renal					
Creatinine (mg/dl) urine output (ml/d)	<1.2	1.2–1.9	2.0–3.4	3.5–4.9 or <500	>5.0 or <200

A score ≥2 is considered positive, and associated with an increased mortality risk according to [8]

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outcome of patients with sepsis in SSA is limited. The impact of antimicrobial resistance (AMR) and malaria epidemiology, as well as optimal treatment modalities for sepsis on the clinical outcome remain unclear [10]. Available data on resistance patterns of Gram-negative isolates from countries in SSA is alarming. A severe bacterial infection caused by multi-resistant bacteria has a poor prognosis considering the limited choice of antimicrobial treatment regiments [11, 12].

1.2. Sepsis in sub-Saharan Africa

Because of the uncertainties described above, established guidelines for treatment of sepsis from developed countries cannot simply be transferred to SSA and treatment of sepsis often remains suboptimal [13, 14]. This problem is intensified by limitations of resources at a local health care level and the lack of specially trained health care workers (HCW), intensive care units, and adequate microbiology laboratories. The sparse existing data indicate that sepsis-associated morbidity and mortality are of great importance in countries across SSA, and even in maximum care facilities in high-income countries, sepsis and septic shock are associated with a high case fatality rate [15]. Therefore, there is an urgent need for improved medical care for patients with sepsis and septic shock especially in SSA [16].

Table 2. qSOFA score.

Criteria	Positive Finding
Respiratory rate	>22 /min
Altered cognition (GCS)	<15
Systolic blood pressure	≥100 mmHg

Each criterion is rated with 1 point if within given range, a score ≥2 is considered positive, and associated with an increased mortality risk according to [9]

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Data from the iSSC suggests that blood culture diagnostics in patients with sepsis is associated with an increased survival rate [17, 18]. In one study performed in Uganda, sepsis was associated with a high mortality rate of >30% and risk factors for mortality were impaired level of consciousness and malnutrition [19]. Another study from Uganda in women with postpartum infections showed extensive AMR in 80% of the isolated Gram-negative bacteria. In the investigated patients, empiric antimicrobial treatment was effective in only 14% of cases [20]. Other investigators also described alarming rates of AMR among bacterial strains isolated from patients with sepsis, and associated risk of failing empiric antimicrobial therapies across different countries in SSA [21–24].

1.3. Local situation in Ethiopia

To date, in the Ethiopian setting no specific diagnosis and treatment guidelines for patients with sepsis exist. Scoring systems for early recognition of sepsis patients like the fast and easy applicable qSOFA score [9] are not regularly implemented in standard care. Measured against the international treatment guidelines published by the iSSC, suboptimal patient care with delayed initiation of antimicrobial treatment and insufficient fluid resuscitation is likely. To our knowledge, no systematic study on recognition and treatment of sepsis in adults has been conducted in Ethiopia.

1.4. Objectives

This study aims to evaluate the feasibility, reliability and performance of the iSSC-recommended clinical scores SOFA and qSOFA, to investigate recognition and management of sepsis, as well as treatment outcome and risk factors for unfavorable outcome in the setting of a teaching and referral hospital in Central Ethiopia.

2. Materials and methods

2.1. General information

This cross-sectional prospective observational study was performed for the duration of 10 months (March 2017 to April 2018) at the Asella Teaching and Referral Hospital (ATRH). The ATRH is situated at around 2,400 m above sea level and serves as referral hospital for a population of approximately 4 million people of the Arsi and Bale zones in the eastern Ethiopian highlands.

2.2. Ethical considerations

Ethical approval has been obtained by the institutional ethical review board of Arsi University in Asella, Ethiopia, the National Ethical Review Board of the Ethiopian Ministry of Science and Technology in Addis Ababa, Ethiopia, and the Ethics Committee of the Faculty of Medicine, Heinrich-Heine-University, Du¨sseldorf, Germany. Written informed consent was obtained from all participating patients or legal guardians (if appropriate) before inclusion. The study was conducted according to the principles of the Declaration of Helsinki.

2.3. Inclusion

During the study period, patients ≥1 year of age admitted to the ATRH during usual business hours (Monday to Friday) with signs of infection according to the appraisal of the treating physician were screened for SIRS (Systemic Inflammatory Response Syndrome) criteria by the study team (see Table 3). The primary diagnosis documented during the admission process was recorded and could also be of non-infectious nature (e. g. decompensated heart failure), if

Table 3. SIRS criteria.

Criteria	Positive finding
Body temperature	>38˚C or <36˚C
Heart rate	>90/min
Respiration rate	Respiratory rate >20/minute (or $PaCO_2$ <32 mmHg)
White blood cell count	>12,000/mm^3 or <4,000/mm^3 or the presence of >10% immature neutrophils

Each criterion is rated with 1 point if within given range, a score ≥ 2 is considered positive [25].

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signs of infection were also present at the same time. White blood cell count (WBC), body temperature, heart rate and respiratory rate were recorded.

Patients fulfilling ≥2 SIRS criteria in screening investigation by the study team were given the option to take part in the study. Once informed consent was obtained, SOFA score and, in participants ≥18 years, qSOFA score were assessed, peripheral venous blood cultures and a blood sample for blood smear microscopy were drawn. An arterial blood gas analysis was performed to measure lactate levels and PaO_2 for SOFA score, using the i-STAT®1 analyzer with i-STAT® CG4+ cartridges (Abbott Laboratories, Chicago, USA). Admission diagnoses, vital signs and antimicrobial treatment were recorded. Socioeconomic data and medical history were documented upon admission using a standardized questionnaire. Patients with clinical evidence of an infection and with ≥2 points in the SOFA score were diagnosed with sepsis according to the sepsis-3 definition by the iSSC [26] (see Fig 1).

2.4. Follow-up

In patients diagnosed with sepsis according to SOFA score, clinical data and treatment details (e.g. antimicrobial therapy) were recorded daily until discharge, death or referral. After 48 (±24) hours, the SOFA score, SIRS criteria and, in patients ≥18 years, the qSOFA score were reassessed. Information on 28-day mortality was collected from the patient's file or by phone interview, if patients were already discharged.

2.5. Microbiological procedures

One set of commercially available blood culture bottles (aerobe and anaerobe bottles, BacT/ALERT®, bioMerieux, Marcy-l'E toile, France) was used for blood culturing. Due to limited resources and supplies, no additional blood culture bottles were collected. Regular quality controls for sensitivity were performed by incubation of standard laboratory strains of different bacteria.

All inoculated blood cultures were investigated by Gram staining and light microscopy 24 and 120 hours after inoculation and sub-cultured on Blood, MacConkey and Chocolate Agar in 5% CO_2 enriched atmosphere if Gram staining showed bacterial growth. Subsequent species identification and antimicrobial sensitivity testing (AST) with standardized antibiotic disks on Mueller Hinton Agar were performed. AST results were interpreted following Clinical & Laboratory Standards Institute (CLSI) guidelines. If Gram staining and subcultures 5 days after inoculation of the blood culture failed to show bacterial growth, the cultures were considered negative.

For quality control, sample colonies of all isolated bacterial strains were exported to the Institute of Medical Microbiology and Hospital Hygiene of the Du¨sseldorf University Hospital in Du¨sseldorf, Germany. Species identification was reassessed by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS) and AST were

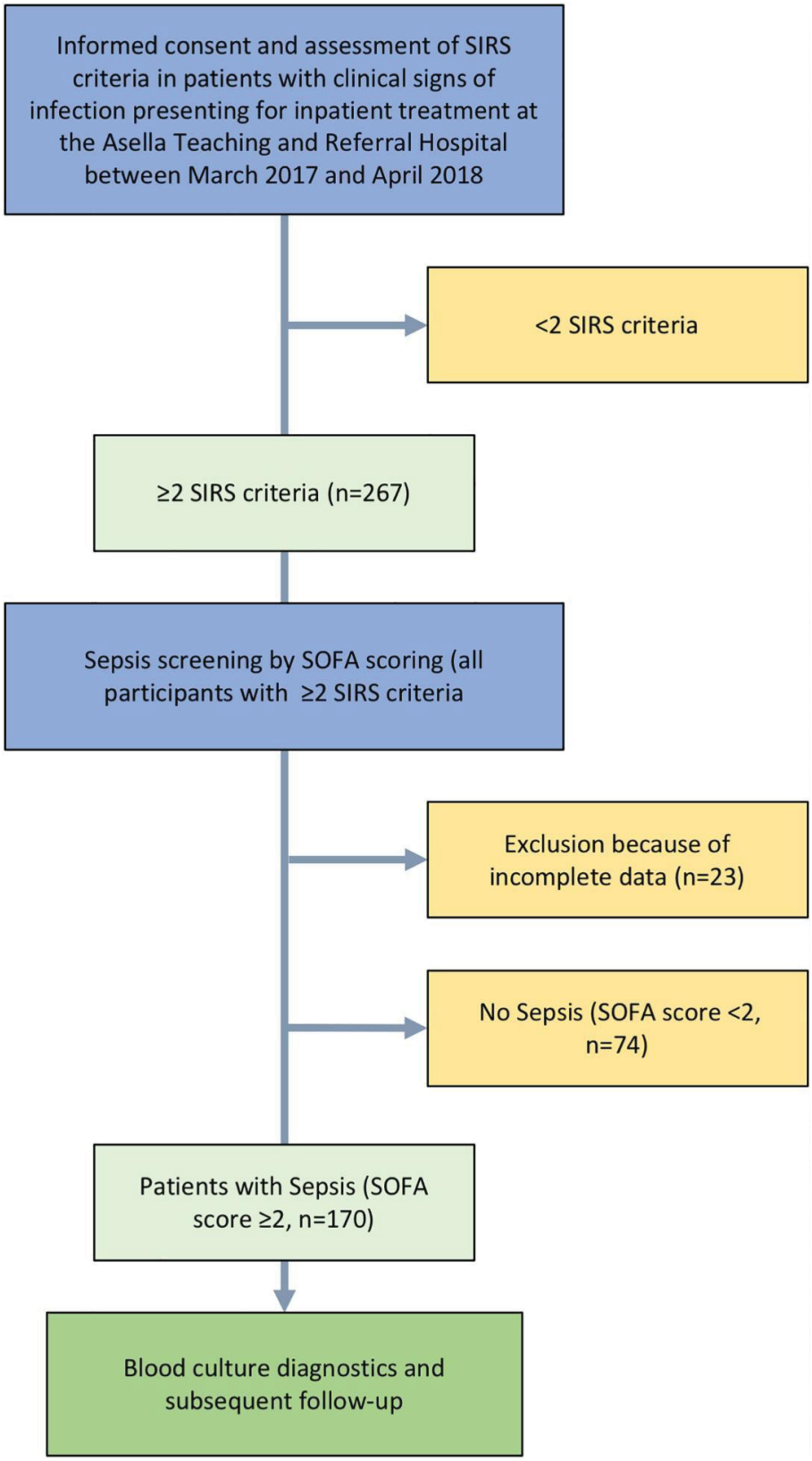

Fig 1. Flow chart of study procedures.

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repeated by standardized laboratory procedures. Interpretation of AST results followed EUCAST (European Committee on Antimicrobial Susceptibility Testing) guidelines.

Identification of bacterial resistance genes was performed by quality-controlled molecular biological analysis. After DNA extraction, bacterial strains with suspected production of extended-spectrum beta-lactamases (ESBL) or carbapenemases were investigated by PCR, following the protocols described by Strauß et al. for identification of the beta-lactamase $bla_{\text{CTX-M}}$, bla_{SHV} and bla_{TEM} genes and an in-house PCR protocol established for the detection of carbapenemases (bla_{VIM}, bla_{NDM}, bla_{KPC}, bla_{IMP}, bla_{GES}, bla_{OXA}) as described by Wendel et al. [27, 28].

In addition, blood smear light microscopy with a 100x oil immersion objective after Giemsa staining was performed from blood samples drawn at study inclusion to investigate for infections caused by hemoparasites.

2.6. Data handling and statistical analysis

Statistical analysis of data was performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA). Frequency and percent were used to describe qualitative variables; for quantitative variables, mean and standard deviation were used if data were normally distributed. Median and interquartile range (IQR) were used, if data were non-normally distributed, as assessed by Shapiro Wilk test. Pearson's chi-squared test and Fisher's exact test were used for the analysis of differences between groups. P-values <0.05 were considered statistically significant.

3. Results

3.1. Inclusion and general information

A total number of 267 patients with clinical signs of infection and $\geq$2 positive SIRS criteria were screened for possible sepsis by SOFA scoring. Twenty-three patients were excluded from further analysis because of incomplete data. In 170 (69.7%, 30 minors [<18 years] and 140 adults [$\geq$18 years]) of the remaining 244 patients eligible for analysis, the SOFA score was $\geq$2, indicating sepsis. In particular, the SOFA score was <2, 2, 3, 4, and $\geq$5 in 30.3% (n = 74), 25.8% (n = 63), 15.6% (n = 38), 9.8% (n = 24) and 18.4% (n = 45), respectively (Fig 2). Overall, median SOFA score at screening was 2 (IQR 1–4). Follow-up investigations were performed in patients with sepsis only.

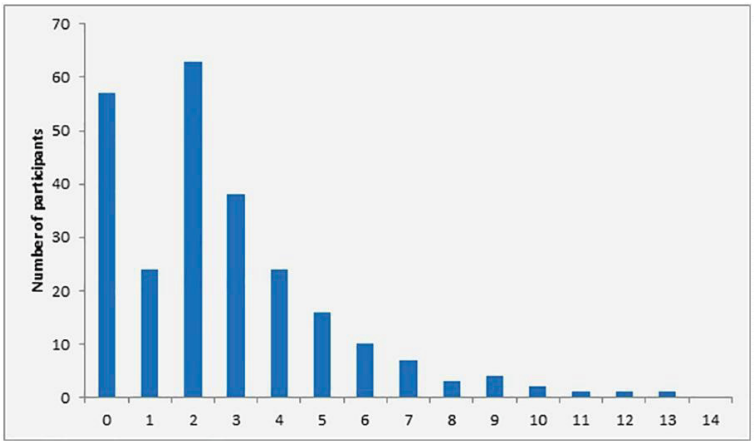

Fig 2. Distribution of SOFA score at screening among participants with $\geq$2 SIRS criteria (n = 170).

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Within the group of patients with sepsis (n = 170), the median age was 30 years (range 1–90 years, IQR 18–46), 48.8% were female and 23.5% were classified as underweight by the treating physician. In total, 30 study participants were minors (i.e. 1–17 years, median age 14 years [IQR 8–16]), and thus excluded from analyses involving the qSOFA score. The remaining 140 adult patients (median age 35 years [IQR 23–50]) were eligible for further analysis involving the qSOFA score.

At least one chronic disease (e.g. diabetes mellitus, hypertension, cardiac insufficiency or HIV infection) was present in 21.8% (n = 37) of participants. In particular, 11.8% (n = 20) were HIV-infected and chronic wounds were present in 15.3% (n = 26) of participants (Table 4). According to initial assessment by the treating physician at admission, 32.9% (n = 56) of the participants with sepsis (SOFA score ≥2) were diagnosed with respiratory infections, 10.0% (n = 17) with central nervous system infections, and 10.0% (n = 17) with gastrointestinal or hepatobiliary infections. In 20.0% (n = 34) of patients, the focus of infection was unclear. The primary diagnosis documented during admission was non-infectious (e. g. cardiovascular complications) in 12.4% of patients (n = 21), who showed concomitant signs of infection according to the appraisal of the treating physician and thus fulfilled the inclusion criteria (Fig 3).

3.2. Outcome of sepsis and risk factors for mortality

Overall, the 28-day mortality of patients with sepsis was 29.4% (n = 40) in 136 patients for whom this information was available. Mortality in patients with SOFA score of 2, 3, 4 and ≥ 5 was 10.4%, 13.8%, 42.1% and 57.5%, respectively. Among adult study participants with sepsis and complete data on 28 day mortality and qSOFA, [n = 110], the 28-day mortality was 0%, 17.4%, 44.4% and 66.7% in patients with qSOFA score of 0, 1, 2 or 3 at inclusion, respectively (Fig 4). Overall, the 28-day mortality in patients with qSOFA ≥2 was 46.7% (28/60). The mortality rate according to SIRS criteria at inclusion was 31.0%, 28.0% and 31.3% in patients with 2, 3 or 4 positive SIRS criteria, respectively (n = 170, Fig 5).

The association between level of SOFA score and increase in mortality rate was statistically significant ($p<0.001$). Twenty-eight-day mortality was also significantly associated with qSOFA scores ≥2 upon inclusion (p = 0.001), but not with the number of positive SIRS criteria (p = 0.923).

Table 4. Demographic and anamnestic characteristics of study population (n = 170).

Variables	% (n)
Gender	
Male	51.2 (87)
Female	48.8 (83)
Age group	
<18	17.6 (30)
18–65	72.9 (124)
>65	9.4 (16)
Chronic disease present	21.8 (37)
Known HIV infection	11.8 (20)
Chronically open wounds	15.3 (26)
Nutritional status (approximation)	
Obese	4.7 (8)
Normal weight	64.1 (109)
Underweight	23.5 (40)
Unknown	7.6 (13)

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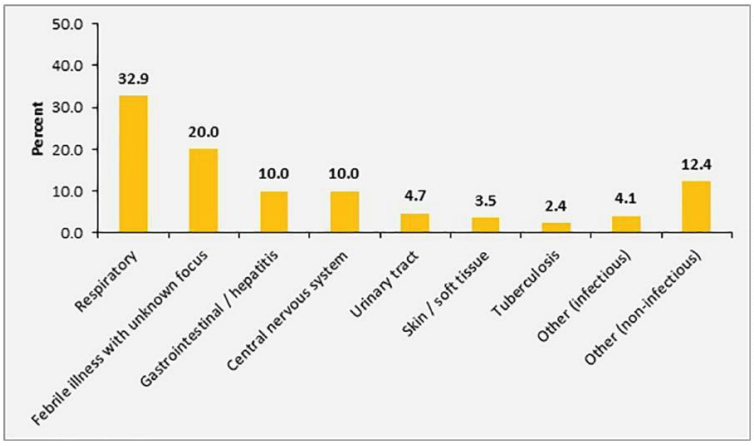

Fig 3. Sources of infection / main diagnosis in patients with SOFA score ≥2 (n = 170).

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Higher age (>65 years) was associated with increased mortality (60.0% vs. 25.6%, p = 0.022). There was no significant association between 28-day mortality and focus of infection, admission diagnosis, chronic diseases, HIV-status, gender or nutritional status. In addition, there was no significant difference in mortality rate between patients with positive vs. sterile blood cultures upon admission (40.0% vs. 28.1%, p = 0.25).

3.3. Follow-up

Data of follow-up assessment of SIRS, SOFA and qSOFA (only participants ≥18 years) 48 hours after admission were available for 53 of 170 participants (31.2%). Of those, 26.4% (14/53) had died before the follow-up investigation. In participants with complete follow-up data, the SOFA-score decreased in 53.8% (n = 21), remained steady in 28.2% (n = 11) and increased in 17.9% (n = 7) of investigated patients. The mortality rate of patients with failure to decrease in level of SOFA score at follow-up was significantly higher compared to patients with decreasing trend (50.0% versus 19.0%, p = 0.041). For SIRS and for qSOFA score (only participants ≥18 years), this association was not statistically significant (Table 5).

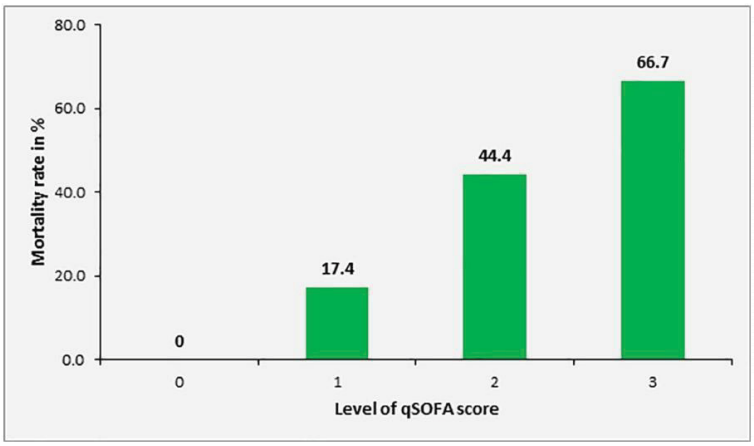

Fig 4. Mortality rate of study participants with sepsis (SOFA ≥2) according to qSOFA score at inclusion (patients ≥18 years, n = 140).

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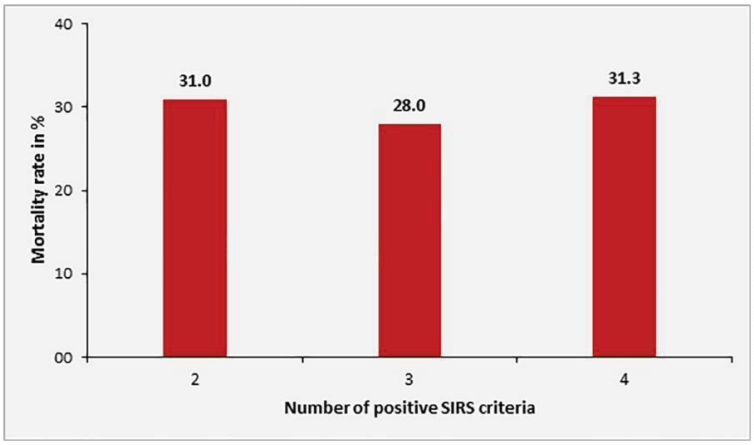

Fig 5. Mortality rate of sepsis patients according to number of positive SIRS criteria at inclusion (n = 170).

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3.4. Recognition of sepsis

According to review of chart documentation, only 12.4% (21/170) of the study participants with sepsis were diagnosed with sepsis or septic shock during the routine clinical assessment by health care workers (HCWs) of the ATRH. In particular, 12.7%, 7.9%, 8.3% and 17.8% of patients with SOFA score of 2, 3, 4 and ≥5, respectively were recognized as patients with sepsis by the hospital's HCWs. There was no difference in sepsis recognition rate in the subset of adult patients (n = 140) with elevated qSOFA score (≥2) in comparison to those with qSOFA score <2 (14.5% versus 7.8%, p = 0.167). In patients with positive blood cultures, the sepsis recognition rate was 11.1% (2/18) compared with 12.5% (19/152) in patients with negative blood cultures (p = 0.611). There was no difference in mortality rate in patients diagnosed with sepsis compared with those with missed diagnosis of sepsis (28.6 versus 29.5%, p = 0.61).

3.5. Respiratory rate and systolic blood pressure, as components of qSOFA score, in adult patients with sepsis

Since respiratory rate and blood pressure are highly age-dependent and considering that the qSOFA score has not been evaluated in pediatric populations, the following analyses were only performed in the subgroup of adult sepsis patients (n = 140). The elevation of the respiratory rate ≥22 /min and the decrease of the systolic blood pressure ≥100 mmHg are decisive components of the qSOFA score, corresponding to one point each. The score is considered positive once two points are reached (see Table 2). At the study site, 2,400 m above sea level, the median respiratory rate was 29.5 /min (IQR 24–36), and 89.3% of participants (n = 125) had a respiratory rate of ≥22 /min. However, there was no significant difference regarding the rate

Table 5. Mortality rate according to the trend of the different scores at follow-up investigation after 48 ± 24 hours.

	Mortality rate (in %)		
Score	Decrease at follow-up	Equal or higher at follow-up	p-value
SOFA	19.0	50.0	**0.044**
qSOFA	14.3	42.3	0.171
SIRS	26.7	37.5	0.367

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of patients with increased respiratory rate and level of SOFA score (89.7% of patients with SOFA 2–3 and 88.7% of patients with SOFA ≥4; p = 0.528).

The median systolic blood pressure in adult participants was 101.5 mmHg (IQR 90.3–116.0). In 50.0% (n = 70) the systolic blood pressure at inclusion was ≥100 mmHg. There was no significant difference with regard to a reduced systolic blood pressure ≥100 mmHg between patients with SOFA score 2–3 and SOFA score ≥4 (20.5% versus 32.3%; p = 0.83). The mortality rate was not associated with the presence of an elevated respiratory rate (≥22 /min: 31.6%; <22 /min: 41.7%, p = 0.346) or a reduced systolic blood pressure (≥100 mmHg: 29.1%; >100 mmHg: 36.4%, p = 0.271).

3.6. Microbiological results

3.6.1. Blood culture isolates. Before blood cultures were drawn, 71.4% (n = 120) of patients had received antimicrobial treatment (see below). The blood cultures yielded positive results in 10.6% (n = 18) and the blood culture positivity rate did not differ between minors and adults (13.3% [4/30] versus 10.0% [14/140], p = 0.395). Coagulase-negative staphylococci (CONS) were isolated in 5.3% (n = 9). Because of a high suspicion for contamination, all blood cultures (n = 9) showing growth of CONS were considered negative. There was no significant difference in blood culture positivity rate in patients with previous administration of antibiotic treatment at the time of sampling compared to patients without antibiotic treatment (9.2% versus 13.7%, p = 0.269).

Gram-positive cocci (GPC) and Gram-negative rods (GNR) were isolated in the same proportion of 44.4% (n = 8) each. In 11.1% of cases (n = 2), *Candida* spp. were isolated. The most common bacterial isolates were *Staphylococcus aureus* (n = 7, 38.9%) and *Escherichia coli* (n = 4, 22.2%). For detailed results of microbiological culturing, see Table 6.

3.6.2. Blood culture positivity and sepsis scores. There was no significant association between a positive blood culture result and SOFA or qSOFA (only participants ≥18 years) scores, or number of SIRS criteria at inclusion. However, blood cultures from patients with SOFA score ≥5 yielded positive results in 17.8% compared to 8.1% in patients with SOFA score 2–4 (p = 0.201). In our cohort, a SOFA score of ≥5 was significantly associated with Gram-negative bacteremia (11.1% versus 2.4%, p = 0.028), but not with Gram-positive bacteremia or candidemia.

3.6.3. Bacterial isolate and mortality rate. Overall, the mortality rate in patients with positive blood culture was 40.0% compared to 28.1% in patients with sterile blood cultures (p = 0.34). The mortality rate was 100% (2/2) in patients with *Candida* sepsis, 42.9% (3/7) in patients with Gram-negative sepsis and 16.7% (1/6) in patients with Gram-positive sepsis

Table 6. Isolates from blood cultures at study inclusion.

Isolate	Number	Percentage
Gram-positive cocci	8	44.4
Staphylococcus aureus	7	38.9
Enterococcus faecalis	1	
Gram-negative rods	8	44.4
Escherichia coli	4	22.2
Klebsiella pneumoniae	2	11.1
Acinetobacter baumannii	1	
Pseudomonas aeruginosa	1	
Yeasts	2	11.1
Candida spp. (*C. albicans* and C. tropicalis)	2	11.1

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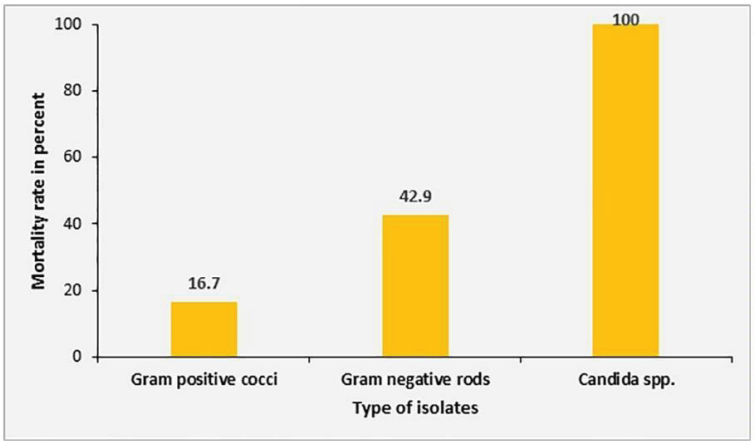

Fig 6. Mortality rate according to different isolates from blood cultures.

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(Fig 6). However, none of these differences were significant due to the small numbers in each subgroup.

3.6.4. Blood film investigation. Blood film microscopy from 169 available samples revealed two cases of *Plasmodium vivax* malaria, but no cases of *P. falciparum* malaria were detected. Both patients survived until end the end of follow-up.

3.6.5. Antimicrobial resistance. Concerning GPC, 71.4% of the isolated strains of *Staphylococcus aureus* were resistant to co-trimoxazole and 28.6% to clindamycin. None of the isolated *Staphylococcus aureus* strains were *methicillin resistant Staphylococcus aureus* (MRSA). Antimicrobial resistance in the isolated Gram-negative bacterial isolates was much more common. *Enterobacterales* were frequently resistant to aminopenicillins combined with beta-lactamase-inhibitors (83.3%), 3rd generation cephalosporins (66.7%), quinolones (66.7%) and sulfonamides (83.5%). Resistance to aminoglycosides (33.3%) and carbapenems (n = 1) was less frequent but still considerably high. There were only two isolated strains of non-fermenting GNR, of which one displayed a high level of antibiotic resistance due to the production of two different carbapenemases (see below, Table 7). Production of extended-spectrum beta-lactamases was found in 75% (6/8) and carbapenemases (NDM-1 and OXA-51) in 25% (2/8) of the isolated GNR. In only one multi-resistant Gram-negative bacterial strain, none of the resistance genes tested were detected (Table 8). The ESBL-genes $bla_{\text{CTX-M-1}}$ and bla_{TEM} were the most commonly isolated resistance genes.

3.7. Antimicrobial treatment

The majority of patients (70.0%; 119/170) included in this study received antimicrobial treatment at the time of inclusion. All initial antimicrobial treatments were administered empirically. In 74.8% (89/119) of patients receiving antimicrobial treatment, more than one substance was administered. There was no difference in the number of antimicrobial substances used for treatment in patients diagnosed with sepsis by the treating physicians at the ATRH compared to patients without diagnosis of sepsis (≥2 antimicrobial substances, 52.4% versus 52.3%, p = 0.592). In addition, there was no significant difference in the actual SOFA score between patients treated with combined antimicrobial treatment and patients receiving antimicrobial monotherapy (SOFA score ≥4, 46.1% versus 34.6%, p = 0.085). However, the qSOFA score was positive (i.e. ≥2) significantly more often in patients receiving combined antimicrobial therapies compared to patients receiving monotherapy (63.9% versus 44.1%,

Table 7. Rate of resistance to different antibiotic classes among the bacterial isolates.

Antibiotic class (tested substance)	Resistant isolates in % (n)			
	Gram-positive cocci		Gram-negative rods	
	S. aureus (n = 7)	*Enterococcus faecalis* (n = 1)	*Enterobacterales* (n = 6)	Non-fermenting Gram-negative rod (n = 2)
Benzylpenicillin (penicillin G)	85.7 (6)	1	*nt*	100 (2)
Isoxazolylpenicillines (oxacillin)	0 (0)	1	*nt*	100 (2)
Aminopenicillin (ampicillin or amoxicillin)	*nt*	1	83.3 (5)	100 (2)
Aminopenicillin + beta-lactamase-inhibitor (BLI) (clavulanic acid or sulbactam)	0 (0)	*nt*	83.3 (5)	100 (2)
Ureidopenicillin (piperacillin)	*nt*	*nt*	83.3 (5)	100 (2)
Ureidopenicillin + BLI (piperacillin/tazobactam)	0 (0)	*nt*	50 (3)	50 (1)
1st generation cephalosporin (cefazolin or cefalexin)	0 (0)	*nt*	*nt*	100 (2)
2nd generation cephalosporin (cefuroxime or cefoxitin)	0 (0)	*nt*	66.7 (4)	100 (2)
3rd generation cephalosporins (cefotaxime or ceftazidime)	0 (0)	*nt*	66.7 (4)	100 (2)
Carbapenems (imipenem or meropenem)	0 (0)	*nt*	16.7 (1)	50 (1)
Quinolones (ciprofloxacin or moxifloxacin)	0 (0)	*nt*	66.7 (4)	100 (2)
Glycopeptide (vancomycin)	0 (0)	0 (0)	*nt*	*nt*
Sulfonamides (trimethoprim-sulfamethoxazole)	71.4 (5)	100 (1)	83.3 (5)	100 (1)≥
Aminoglycosides (amikacin or gentamicin)	0 (0)	*nt*	33.3 (2)	50 (1)
Lincosamides (clindamycin)	28.6 (2)	*nt*	*nt*	*nt*
Macrolides (azithromycin or clarithromycin)	*nt*	*nt*	*nt*	*nt*
Tetracyclines (tetracycline)	42.9 (3)	*nt*	*nt*	100 (2)
Oxazolidinones (Linezolid)	0 (0)	0 (0)	*nt*	*nt*
Glycylcyclines (tigecycline)	0 (0)	0 (0)	0 (0)	50 (1)
Epoxide antibiotics (fosfomycin)	0 (0)	*nt*	*nt*	*nt*
Lipopetide antibiotics (daptomycin)	0 (0)	*nt*	*nt*	*nt*

Nt, not tested

≥, not tested in *Pseudomonas* isolates

https://doi.org/10.1371/journal.pone.0247646.t007

p = 0.015). Ceftriaxone was the most commonly used substance as part of the antimicrobial treatment regimen (87.4%, n = 104), followed by metronidazole (30.2%, n = 36) plus vancomycin (22.7%, n = 27) (Fig 7).

Table 8. Resistance genes in isolated Gram-negative bacteria.

Bacterial isolate	ESBL			Carbapenemase	
	CTX-M-1	TEM	SHV	NDM-1	OXA-51
E. coli	+	-	-	-	-
E. coli	+	+	-	-	-
E. coli	-	+	-	-	-
E. coli	-	-	-	-	-
K. pneumoniae	+	+	+	-	-
K. pneumoniae	+	+	+	+	-
Pseudomonas aeruginosa	+	+	-	-	-
Acinetobacter baumannii	-	-	-	+	+
Overall percentage	**62,5**	**62,5**	**25,0**	**25,0**	**12,5**

ESBL, extended spectrum beta-lactamase

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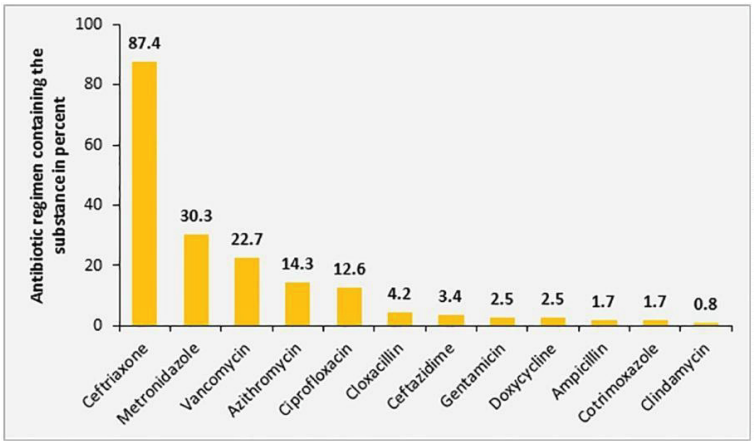

Fig 7. Distribution of antimicrobial substances used for empiric treatment among study participants with sepsis (n = 170).

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A significant proportion of patients with sepsis according to SOFA scoring did not receive any initial antibiotic treatment (30.0%, n = 51). Regarding mortality rate, there was no significant difference in study participants with sepsis receiving any antimicrobial treatment compared to those without antibiotic treatment (32.7% [32/98] versus 21.1% [8/38], p = 0.13). Higher mortality rates were significantly associated with an antimicrobial regimen including ceftriaxone (35.7% versus 19.2%, p = 0.03) and metronidazole (43.8% versus 25.0%, p = 0.037), but not with regimen including vancomycin (31.6% versus 29.1%, p = 0.508).

The mortality rate in patients receiving multiple antimicrobials was 34.7% in comparison to 29.6% in patients with single-agent antimicrobial treatment (p = 0.410), although there was no significant difference in the frequency of combined antibiotic treatment regimen regarding the attributed SOFA score (48.8% in patients with SOFA 2–4 versus 62.2% in patients with SOFA score $\geq$5, p = 0.085).

According to performed AST, at least one of the empirically prescribed antimicrobial substances was tested to be active against the isolated pathogen in only 27.8% (5/18) of cases. Isolated pathogens were susceptible to the administered treatment with ceftriaxone in 44.4% (4/9), to ciprofloxacin in 50% (1/2) and to vancomycin in 33.3% (1/3). Metronidazole was frequently utilized as part of combined treatment regimen; however, no anaerobic bacteria were isolated. All patients, in whom the isolated pathogen was sensitive to at least one of the empirically administered antimicrobials survived. The mortality rate in patients, in whom none of the antibiotics administered was effective against the isolated organism was 55.6% (p = 0.098).

3.8. Evaluation of qSOFA as a clinical sepsis score

At study inclusion, the qSOFA score of adult participants with sepsis (n = 140) was 0, 1, 2 and 3 in 4.3%, 41.4%, 49.3% and 5.0%, respectively. The sensitivity of qSOFA for the recognition of sepsis was 54.3% (76/140) using a SOFA score $\geq$2 as a gold standard. In patients with a higher SOFA score (i.e. SOFA score $\geq$4) compared to patients with a SOFA score of 2 or 3, the sensitivity was significantly higher (72.6% versus 39.7%, p<0.001, compare Fig 8).

The specificity of qSOFA for the diagnosis of sepsis was 66.7%; 36 out of 54 adult patients without sepsis had a qSOFA score <2 at study inclusion. The positive and negative predictive value of qSOFA for the diagnosis of sepsis were 80.4% and 36.0%, respectively. The area under the ROC curve was 0.601.

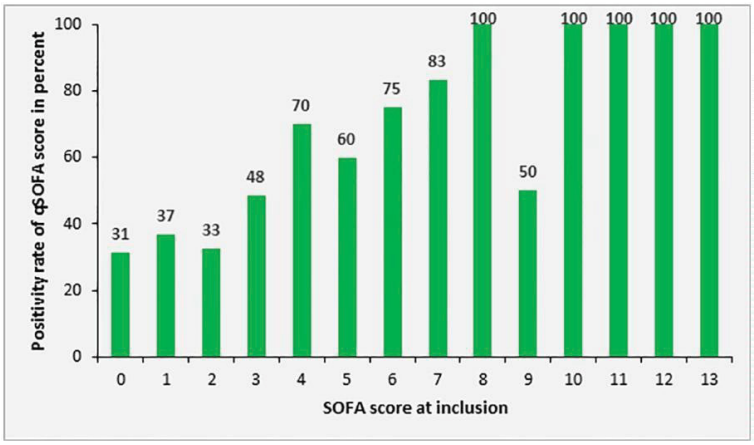

Fig 8. Rate of adult patients with positive qSOFA (≥2) according to SOFA score at study inclusion (n = 140).

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4. Discussion

Early recognition of sepsis is essential for optimal treatment and outcome. Therefore, reliable and practical tools are needed in order to achieve this important goal under specific local conditions. The primary objective of this study was to evaluate the feasibility and performance of SOFA and qSOFA scores at the ATRH, a tertiary hospital situated at an altitude of 2,400 m in Central Ethiopia. For preselection of appropriate study participants, SIRS criteria were applied because they are easily and quickly applicable and highly sensitive for recognition of sepsis despite limited specificity [29, 30], followed by SOFA scoring for confirmation of sepsis, as introduced in the sepsis-3 definition by the iSSC [26].

Sepsis was found to be highly prevalent and yet underdiagnosed by the hospital's medical staff at the study center according to chart documentation, without consideration of the score results. The 28-day mortality rate in our study (29.4%) was considerably higher than mortality rates of 17–18% described from other sepsis cohorts from high income countries [31, 32], but comparable to those described for other low- and middle-income countries [33, 34]. To analyze the quality of sepsis treatment was not part of this investigation. However, restricted diagnostic and therapeutic possibilities, limited capacities of intensive care units and lack of intensive care training are all likely to contribute to the high mortality rate.

As expected, the mortality rate increased significantly with severity of the disease, as indicated by elevation of the SOFA and qSOFA scores, with the latter being calculated in adult participants only. Measurement of the SOFA score, as a gold standard for the diagnosis of sepsis in this study, is complex and requires appropriate laboratory equipment and resources. Although the sensitivity and specificity of the qSOFA score for the diagnosis of sepsis deviated from values published in the literature, it appears a suitable tool to guide the risk-adapted allocation of restricted medical resources, such as intensive care unit capacities or the use of expensive broad-spectrum antimicrobial substances, in resource-limited settings.

The overall recognition rate of sepsis by the treating physicians of the ATRH was strikingly low and sepsis scores are not routinely utilized in the clinical setting. It is noteworthy that there were no significant differences in mortality, rate of positive blood cultures or antibiotic treatment between patients diagnosed with sepsis and patients with a missed sepsis diagnosis. A possible explanation is that these patients may have been recognized as seriously ill by treating physicians without establishing the formal diagnosis of sepsis. However, we are convinced that the correct and standardized detection of sepsis could contribute to an optimized management.

Despite the global emergence of AMR, local AMR statistics and antibiotic susceptibility data for patients in resource-limited settings are largely unavailable in resource-limited settings and thus antimicrobial treatment regimens are mostly prescribed purely empirically. In this study cohort, empirical antimicrobial treatment did not prove beneficial regarding mortality. This observation most likely reflects the extensive AMR, rendering most antimicrobial treatment regimens administered within this cohort ineffective. The overall blood culture positivity rate within the studied population was low. The sensitivity of the blood cultures may be limited, because only one set of blood cultures was investigated, as this is common practice at the study center due to lack of reliable supply and high costs of disposable bottles [35]. There is also widespread reluctance in the local population including HCWs to provide the relatively high blood volume required for optimal sensitivity of blood cultures, due to the common belief that even small amounts of blood loss are associated with a health hazard. Furthermore, a preselection of resistant bacteria might have occurred, since 71.4% of patients had received antibiotics before blood culture sampling.

There was a trend towards a higher mortality rate in patients with bacteremia compared to patients with sterile blood cultures, without reaching statistical significance due to the small case numbers. Among the isolated pathogens, GPC and GNR were isolated in equal proportions, with some cases of candidemia.

Among patients with positive blood cultures, the mortality rate was highest in patients with candidemia and noticeably higher in patients with Gram-negative sepsis compared to patients with Gram-positive sepsis. The probability of receiving effective empirical treatment might play a role for this observation, being higher in Gram-positive pathogens, compared to Gram-negative and fungal pathogens. Due to the high altitude of the study site at 2400 m above sea level, malaria did not play a decisive role as a trigger for sepsis in this cohort in contrast to other sepsis cohorts from SSA. No cases of *P. falciparum* malaria were diagnosed and *P. vivax* malaria did not lead to mortality.

Among GNR, AMR against multiple antibiotics was common. Regarding GPCs, MRSA was not isolated from any patient. Nevertheless, AMR against sulfonamides and lincosamides, as frequently used antimicrobial substances at the study site, was present in relevant proportions. In GNR, the two ESBL genes $bla_{\text{CTX-M-1}}$ and bla_{TEM} were most frequently detected. Despite the rare application of carbapenems as part of antimicrobial treatment regimens at the ATRH, pathogens carrying carbapenemases (NDM-1 and OXA-51) were isolated in 25% of cases. These findings are consistent with other studies describing the prevalence of ESBL-genes and carbapenemases in Gram-negative isolates from Ethiopia [36, 37].

In our cohort, more than two thirds of the patients had received some kind of empiric antimicrobial treatment at the time of inclusion. Of note, despite the low recognition rate of sepsis and septic shock, almost three quarters of applied regimens comprised at least two substances from different antimicrobial classes. Adult patients with more severe illness according to qSOFA score were more likely to receive a combination antimicrobial therapy. In light of the limited sensitivity of blood cultures and according to susceptibility testing, the empirically administered antimicrobial therapy proved effective only in about one quarter of cases. More than half of the patients (56%) receiving an ineffective antibiotic regimen died, whereas all patients receiving at least one effective substance as part of their treatment survived until end of follow-up. Ceftriaxone was the most commonly used antimicrobial substance but the rate of resistance to ceftriaxone in isolated GNR was high. This finding may explain why the use of ceftriaxone was associated with increased mortality. Likewise, metronidazole treatment was associated with higher mortality and no pathogens susceptible to metronidazole were isolated. However, in the light of the reduced sensitivity of blood cultures, these results must be interpreted with caution.

The specificity of qSOFA score for diagnosing sepsis in the subgroup of adult patients was especially limited in the study population. However, the sensitivity for diagnosis of sepsis or septic shock of 54.3% within our cohort of patients with ≥2 positive SIRS criteria was considerably higher compared to previously published sensitivities ranging between 16.3–32%. On the other hand, the specificity of 66.7% was significantly lower compared to the published range between 96.1–98% [38–40]. In the light of the high proportion of patients with a markedly elevated respiratory rate, the high altitude of the study center with correspondingly lower partial pressure of oxygen might potentially influence the performance of the qSOFA score. It is known, that altitude adaptation mechanisms can affect respiratory rate, oxygen saturation and blood pressure [41], and therefore alter the normal ranges for these parameters. Since large populations of around 389 million people live in altitudes above 1500 m [42], e. g. more than 8 million at >2300 m in Mexico City, further research on the influence of physiological adaptation mechanisms and reference values for these populations is warranted.

Compared to the national prevalence of people living with HIV/AIDS of 1.4%, the prevalence of HIV-infected patients of 11.8% within the study population was remarkably higher, as also previously reported from other sepsis cohorts from SSA [33, 43].

Almost one quarter of the study participants was considered underweight. Data from the general population for a comparison were not available, although clinical signs of malnutrition and low body mass index (<20 kg/m^2) are common in the general population [44]. Malnourishment has not been evaluated as independent risk factor in other sepsis cohort investigations from SSA, but severe malnourishment seems to be independently associated with increased mortality [45]. In our study, we found no association between mortality rate and known HIV infection or nutritional status.

5. Conclusions

Sepsis is common and associated with high mortality in the study center, but remains underdiagnosed in clinical practice. The SOFA score was associated with mortality but is invasive and elaborate to assess and thus not suitable for routine use in LMIC. The easily applicable qSOFA score showed a poor performance for diagnosis of sepsis in comparison with other sepsis cohorts, having a higher sensitivity and markedly lower specificity in this cohort. The reasons for this finding warrant further investigation, including the role of adaptation mechanisms to high altitude.

Blood culture diagnostics proved cumbersome, requiring rigorous quality control mechanisms. Gram-negative bacteremia was significantly associated with a high mortality rate, with Gram-negative isolates showing a high rate of extensive AMR and an ineffectiveness of empirically administered antibiotic therapy.

Our results indicate the need for comprehensive evaluation of existing clinical tools for the diagnosis of sepsis in resource-limited settings and describe some of the options for optimization. Furthermore, it should be considered that diagnosing sepsis is only one of many aspects necessary for the adequate management of this complex clinical syndrome. Apart from the knowledge on the spectrum and resistance pattern of bacterial pathogens, improved treatment options for patients with severe sepsis and multi-organ failure are required. Finally, comprehensive capacity building and research activities are essential in order to raise awareness of sepsis, to understand the specific characteristics of sepsis and to identify and investigate potential intervention opportunities.

Supporting information

S1 Data.
(SAV)

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2.3. CTX-M-9 group ESBL-producing *Raoultella planticola* nosocomial infection: first report from sub-Saharan Africa, Tufa TB, Fuchs A, Feldt T, Galata DT, Mackenzie CR, Pfeffer K, Häussinger D, Ann Clin Microbiol Antimicrob, 17;19 (1) 36, (2020)

Tafese B. Tufa's contribution to this paper:

a) Draft the case
b) Perform the laboratory activities
c) draft the manuscript

- We have reported a case of nosocomial infection with *Raoultella planticola* of group CTX-M-9 producing ESBL, the first report from sub-Saharan Africa.
- We summarized the case to show the importance of MALDI-TOF for the correct identification of bacteria and AST for the selection of an appropriate antibiotic.

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CASE REPORT Open Access

CTX-M-9 group ESBL-producing *Raoultella planticola* nosocomial infection: first report from sub-Saharan Africa

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Abstract

Background: *Raoultella* are Gram-negative rod-shaped aerobic bacteria which grow in water and soil. They mostly cause nosocomial infections associated with surgical procedures. This case study is the first report of a *Raoultella* infection in Africa.

Case presentation

We report a case of a surgical site infection (SSI) caused by *Raoultella planticola* which developed after caesarean section (CS) and surgery for secondary small bowel obstruction. The patient became febrile with neutrophilia (19,157/μL) 4 days after laparotomy and started to develop clinical signs of a SSI on the 8th day after laparotomy. The patient continued to be febrile and became critically ill despite empirical treatment with ceftriaxone and vancomycin. *Raoultella* species with extended antimicrobial resistance (AMR) carrying the CTX-M-9 β-lactamase was isolated from the wound discharge. Considering the antimicrobial susceptibility test, ceftriaxone was replaced by ceftazidime. The patient recovered and could be discharged on day 29 after CS.

Conclusions: *Raoultella planticola* was isolated from an infected surgical site after repeated abdominal surgery. Due to the infection the patient's stay in the hospital was prolonged for a total of 4 weeks. It is noted that patients undergoing surgical and prolonged inpatient treatment are at risk for infections caused by *Raoultella*. The development of a SSI caused by *Raoultella planticola* with extended AMR has to be assumed to be a consequence of ineffective antibiotic utilization. The presented case advices that rare bacteria as *Raoultella* should be considered as potential cause of nosocomial SSI with challenging treatment due to high levels of AMR.

Keywords: *Raoultella planticola*, Nosocomial infection, Antimicrobial resistance, Extended spectrum β-lactamases, ESBL, CTX-M-9 group, Africa, Ethiopia

Background

Raoultella are Gram-negative rod-shaped aerobic bacteria growing in water and soil. They can also be detected in the human gastrointestinal tract (GIT) or upper respiratory tract (URT) and are a rare cause of mostly nosocomial infections in humans. They were defined as a new genus in the family of *Enterobacteriaceae* in 2001, based on gene sequences of its 16S rRNA and *rpoB* gene [1]. *Raoultella* can grow at wide range of temperature (4 °C to 44.5 °C) and do not produce gas from lactose at 44.5 °C. All *Raoultella* isolates are resistant to ampicillin due to the over expression of chromosomally encoded class-A β-lactamase [2].

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Raoultella planticola, R. ornithinolytica, *R. terrigena*, and *R. electrica* are medically relevant *Raoultella* species [3–6], with *R. planticola and R. ornithinolytica* currently being most commonly reported from clinical samples. Factors contributing to the pathogenesis of diseases caused by the genus of *Raoultella* share similarities with those of *Klebsiella* and include lipopolysaccharides, polysaccharide capsules, fimbriae, siderophores [7], toxins [8], hydrolytic enzymes, and bacteriocins [9]. *Raoultella* species are also able to form biofilms [10]. In contrast to *Klebsiella* species, *Raoultella* species harbour histidine decarboxylase, enabling the bacteria to produce histamine. This information might be used for species differentiation [11, 12].

Following phenotypic and biochemical microbiological methods only, *Raoultella* species are most likely being underreported due to the difficult differentiation from *Klebsiella* species. Over recent years, the identification rate has improved by increased utilization of matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) [13].

Raoultella planticola is not well known as human pathogen. Literature search revealed 87 reported cases of *R. planticola*-related infections. Here, bloodstream infections (32 cases), urinary tract infection (16 cases), and pneumonia (11 cases) are most frequent. Among abdominal foci of *Raoultella*-infections, 5 cases of cholangitis, 3 cases of pancreatitis, 3 cases of cholecystitis, 3 cases of surgical site infection (SSI), 2 cases of secondary bacterial peritonitis, and a single case of enterocolitis have been described. Antimicrobial resistance (AMR) of *Raoultella* causing human infections has not been analysed systematically. However, *Raoultella* species harbouring extended spectrum β-lactamase (ESBL) and carbapenemase genes have been reported [14–17]. Most of the cases were reported in Europe and the USA. To our best knowledge, this report is the first description of an SSI caused by *R. planticola* with multidrug resistance was MDR) in Africa.

Case presentation

Initial presentation

A 17-year-old previously healthy pregnant woman presented to Asella Teaching and Referral Hospital (ATRH) delivery ward in Asella, Central Ethiopia. Upon admission she appeared healthy, without any signs of infection or life-threatening disease. Caesarean section (CS) was indicated due to posterior cephalic position of the child and large fetal size. Lower uterine transverse CS was performed and a healthy male neonate delivered.

Development of surgical site infection

For the first three days after delivery, the patient recovered well from her surgery. However, on the 4th day she developed cramping abdominal pain, constipation with clinical signs of ileus and an elevated body temperature (T) of 37.8 °C. After physical examination and abdominal X-ray revealed signs of small bowel obstruction, emergency laparotomy was performed. Intra-operative findings were a purulent peritonitis due to a volvulus of the cecum with formation of a gangrene. Peritoneal drainage and lavage and a right hemicolectomy with primary ileotransverse anastomosis were performed. The postoperative course for the first days was uneventful.

On her 5th day after laparotomy (and 9th after CS) the patient developed shortness of breath with mild hypotension, tachycardia, tachypnea and fever (blood pressure (BP) 110/70 mmHg, pulse rate (PR) 108/min, RR 40/min, T 38.5 °C). Breath sounds were clear with good bilateral air entry. Abdominal examination revealed passage of faecal matter from the surgical site. Complete blood count (CBC) showed leucocytosis (21.5 $\times$ 10_3/µl) with an increased fraction of neutrophils (89.1%). Platelet count and haemoglobin level where within normal range. There was no growth in a blood culture for a total incubation period of 5 days (1 bottle, local production).

Further course and treatment

The patient was diagnosed with a suspected intestinal anastomotic leak and empiric parenteral antibiotic treatment was started according to local guidelines with 1 g ceftriaxone plus 1 g vancomycin daily. Re-laparotomy on the same day revealed intraperitoneal pus and faeces due to an anastomotic dehiscence with perforation of the distal ileum about 50 cm from the previous anastomosis. After dissection of the insufficient anastomosis, resection of necrotic intestine and peritoneal lavage, re-anastomosis and closure of the abdominal cavity were performed.

On the 6th day post re-laparotomy (11 days post first laparotomy and 15 days after CS), the patient transferred to intensive care unit and she developed purulent discharge from the surgical site. At this time, a wound swab was taken for microbiological diagnostics. The culture revealed growth of *Raoultella* species and the previous antibiotic treatment was adjusted according to the drug susceptibility test result (see Table 1) by replacement of ceftriaxone with ceftazidime. After 7 days of parenteral antibiotic treatment with this new regimen (ceftazidime 1 g three times daily and vancomycin 1 g once daily) the patient developed frequent watery diarrhoea and bilateral lower extremity swelling. Because of suspected *Clostridium difficile* enteritis (diagnostic tests for *Clostridium*

Table 1 Results of antimicrobial susceptibility testing of *Raoultella planticola* isolated strain

Name of antimicrobial substance	Kirby–Bauer disc diffusion test[a]		VITEK® 2 result	
	Diameter (mm)	EUCAST interpretation	MIC	EUCAST interpretation
Piperacillin	0	R	≥ 128	R
Piperacillin/tazobactam	21	S	8	S
Cefotaxime	0	R	8	R
Ceftazidime	22	S	≤ 1	S
Cefepime	22	I	≤ 1	S
Aztreonam	Not tested		2	I
Imipenem	26	S	0.5	S
Meropenem	27	S	≤ 0.25	S
Amikacin	20	S	≤ 2	S
Gentamicin	9	R	≥ 16	R
Tobramycin	12	R	8	R
Moxifloxacin	Not tested		2	R
Tigecycline	Not tested		1	S
Ciprofloxacin	17	R	1	R
Fosfomycin	Not tested		≤ 16	S
Colistin	Not tested		≤ 0.5	S
Trimethoprim/sulfamethoxazole	Not tested		≥ 320	R

MIC minimum inhibitory concentration; *R* resistant, *S* sensitive, *I* intermediate

[a] Results of antimicrobial susceptibility testing (AST) was done by using disc diffusion method at Asella, Ethiopia whereas VITEK was performed at institute of Medical Microbiology and Hospital Hygiene, Düsseldorf, Germany. Both results were interpreted by using European Committee on Antimicrobial Susceptibility Testing (EUCAST) version: 08.01

difficile are not available), intravenous antibiotics were discontinued and the patient was started on oral metronidazole. Along with easing of the diarrhoea, the patient recovered and could be discharged in good condition on the 29th day after CS. In general the case was summarized by (Fig. 1).

Microbiology results

During the patient's stay in the hospital, one blood culture and one wound swab from the surgical site were sent for microbiological culture. Despite the intraoperative finding of purulent peritonitis upon first laparotomy, no intraoperative swabs were ordered. The blood culture remained sterile after an incubation period of 5 days. The swab taken from the SSI 15 days after CS and before

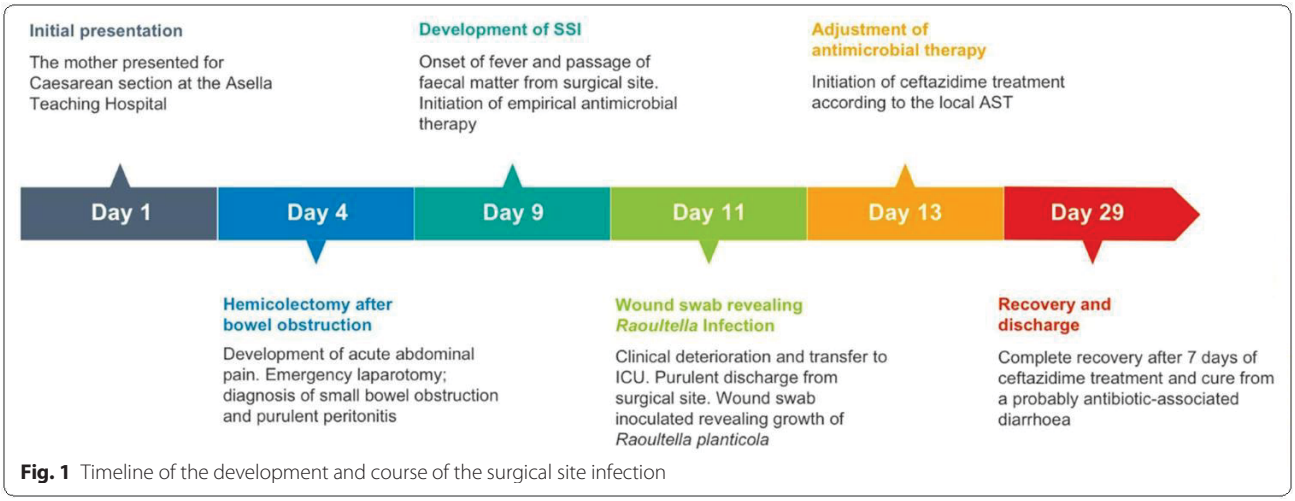

Fig. 1 Timeline of the development and course of the surgical site infection

the 2nd laparotomy was positive for Gram-negative rod-shaped bacteria. According to biochemical identification tests performed on site in Ethiopia, the isolated bacteria were identified as *Klebsiella oxytoca* (oxidase and methyl red negative; lactose, urease, citrate and indole positive). The isolate was exported to the Institute of Medical Microbiology and Hospital Hygiene at Heinrich Heine University Düsseldorf, Germany for confirmation, further identification and antimicrobial susceptibility testing (AST)". Using MALDI-TOF (VITEK®-MS, bioMérieux, Marcy-l'Étoile, France), the bacteria was re-classified as *Raoultella planticola* with a likelihood of 99.9%.

The AST was done using Kirby–Bauer disc diffusion and VITEK methods. The results of the disc diffusion test and the VITEK® 2 (bioMérieux) investigation are described in (Table 1). For molecular resistance gene detection, polymerase chain reactions with primers described in (Table 2) were performed [18].

CTX-M-9 group and TEM ESBL coding genes were detected and the isolated strain was identified as MDR (Fig. 2). However, ESBLs from the groups CTX-M-1, CTX-M-2 or the SHV β-lactamase were not detected.

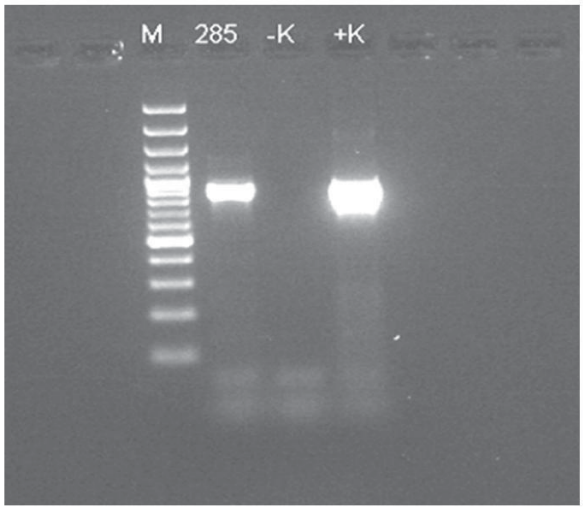

Fig. 2 Picture of *bla*CTX-M-9 ESBL positive result. *bla*CTX-M-9 ESBL gene detected in the isolated *R. planticola* strain (with M = DNA ladder; −K = negative control; + K = positive control; 285 = code given for patient's sample

Discussion

Cases of infections caused by *Raoultella planticola* including infections with abdominal foci have been reported from different countries [19]. In addition, the isolation of *Raoultella* species as the causative agent of human infections containing ESBL and also carbapenemase genes have previously been reported. It is assumed that, to date, *Raoultella* species are often misdiagnosed as *Klebsiella* species in the context of restricted diagnostic capacities in many African laboratories. Therefore, and to our best knowledge, the described hospital-acquired SSI caused by an ESBL-producing strain of *R. planticola* is the first such case reported in Africa. The bacterial strain was isolated from a SSI after CS complicated by cecal volvulus with secondary peritonitis due to a breakdown of the primary anastomosis. Due to secondary infection the patient's overall stay in the hospital was prolonged.

It is noted that being immunocompromised, surgical procedures, long-term antibiotic therapy and prolonged stays in hospital have been described as risk factors to develop *Raoultella* infections [17, 20]. In our case report, the patient's gastrointestinal tract has probably been colonized with *R. planticola* and the leakage of intestinal luminal contents and gut flora after anastomosis insufficiency was the likely route of the infection. It appears likely that, selection of resistant bacteria by the previous empirical antibiotic therapy with ceftriaxone, which was ineffective against identified *R. planticola*.

Cases of *Raoultella* infections can occur in many organ systems (e.g. urinary tract, gastrointestinal tract, respiratory tract) or at surgical sites. Bacteraemia, osteomyelitis, meningitis, cerebral abscess, mediastinitis, pericarditis, conjunctivitis, mandibular osteomyelitis and otitis caused by *Raoultella* have also been reported [20, 21]. In general, the most common microorganisms isolated from SSIs after small bowel surgery are aerobic Gram-negative enteric bacteria [14].

Regarding the management of intra-abdominal infections, therapy should focus on adequate source control and appropriate adjustment of antimicrobial therapy to individual patient factors. Empiric antimicrobial therapy

Table 2 Oligonucleotide sequences of the primer pairs for molecular resistant genes detection

Primer	Sequence (5′–3′)	Amplicon size (bp)
bla_{SHV} (F)	AGCCGCTTGAGCAAATTAAAC	786
bla_{SHV} (R)	GTTGCCAGTGCTCGATCAGC	
bla_{TEM} (F)	CATTTCCGTGTCGCCCTTATTC	846
bla_{TEM} (R)	CCAATGCTTAATCAGTGAGGC	
$bla_{CTX-M-1}$ (F)	CGTCACGCTGTTGTTAGGAA	781
$bla_{CTX-M-1}$ (R)	ACGGCTTTCTGCCTTAGGTT	
$bla_{CTX-M-2}$ (F)	CTCAGAGCATTCGCCGCTCA	843
$bla_{CTX-M-2}$ (R)	CCGCCGCAGCCAGAATATCC	
$bla_{CTX-M-9}$ (F)	GCGCATGGTGACAAAGAGAGTGCAA	876
$bla_{CTX-M-9}$ (R)	GTTACAGCCCTTCGGCGATGATTC	

Possible extended spectrum β-lactamases (ESBLs) coding genes were screened by using conventional polymerase chain reaction (PCR)

F forward, *R* reverse, *bp* base pairs

is essential [22], however inappropriate antibiotic therapy may result in unfavourable outcome and in the selection of bacterial AMR bacteria. As seen in the described case, health care-associated infections caused by multi-resistant bacteria have to be considered which lead to the necessity of complex multidrug regimens [23]. For the selection of empirical antibiotic treatment, a length of hospital stay of 5 days has moderate specificity and very high sensitivity for predicting the presence of MDR bacteria [24].

Due to the high likelihood of infections caused by resistant bacteria, guidelines for empirical treatment of SSIs recommend utilization of broad spectrum antimicrobials (e.g. carbapenems) [25] which are frequently not available in resource-limited settings like Ethiopia. In the absence of carbapenems, piperacillin/tazobactam could be an option for the empiric treatment of high-risk intra-abdominal infections [26, 27], which lead to high rates of mortality [28].

The initial empirical treatment in the described case was a combination of vancomycin and ceftriaxone. It was shown to be ineffective according to the subsequently available AST results (see Table 1). The patient only recovered after the antibiotic treatment was adapted according to the AST, despite the local miss-identification of the causing pathogen as *Klebsiella* species. If AST results are not or not yet available, the initiation of antimicrobial treatment with substances active against most *Enterobacteriaceae* should be considered for critically-ill patients with secondary intra-abdominal infections [29]. In general, regional and local susceptibility profiles of common bacterial isolates should be available and considered before initiation of empiric antibiotic treatments. This becomes increasingly important because of rising resistance of Gram-negative bacteria circulating in the communities. Local guidelines considering common regional AMR patterns should be implemented and updated for the management of SSI and intra-abdominal infections.

From both clinical and natural environment, MDR strains of *R. planticola* and *R. ornithinolytica* were reported [30, 31]. In recent years, infections with *Raoultella* strains producing ESBL from the families TEM, SHV, and CTX-M have been described [32]. The ability to produce ESBL was also detected in *Raoultella* strains isolated from the hospital environment. From different clinical samples, AmpC β-lactamase-producing *R. ornithinolytica* strains have been isolated [33]. Emerging organisms like *Raoultella* species are likely to escape routine identification or be disregarded as insignificant contaminants despite their potential to cause infections which are complicated to manage due to MDR [34]. Limitations in diagnostic microbiological capacities might lead to delayed identification of emerging pathogens and extended AMR patterns, resulting in suboptimal patient care. In the light of increasing AMR among pathogens worldwide but also in resource-limited settings, strengthening of microbiological facilities and thus improved infection surveillance and control will lead to better hospital hygiene and infection control practices, which are needed for optimal outcome and containment of spreading resistance genes.

Conclusions

This report shows evidence of an infection due to a MDR strain of *R. planticola* causing a SSI and intra-abdominal infection with the presence of the CTX-M-9 group ESBLs and is the first description of such an infection in Africa. Surgical treatment with intestinal leakage was the likely route of the infection caused by *R. planticola*. The source of the bacteria might be from gastrointestinal colonization as consequence of ineffective antibiotics utilization or from the hospital environmental. The absence of diagnostic facilities, limited awareness of treating physicians for the importance of microbiological culturing and AST, restricted availability of antibiotic substances for treatment of infections caused by MDR bacteria and lack of data for common local AMR patterns in resource limited settings is jeopardizing the success rate of empirical antibiotic treatment. These insufficiencies become more severe in patients with hospital-acquired infections and prolonged stay in a hospital. In general, the presented case serves to increase the awareness that rare bacterial species like *Raoultella* should be considered as a potential cause of nosocomial SSI with a high rate of AMR. This leads to the necessity of up-to-date guidelines for the management of intra-abdominal infections and for regular microbiological investigation of suitable clinical specimen in nosocomial infections.

In settings where colonization with MDR *Enterobacteriaceae* is common, surveillance and screening for colonization with MDR bacterial strains before invasive procedures and implementation or strengthening of antibiotic stewardship programs would contribute to successful patient care and should be considered.

Abbreviations

AMR: Antimicrobial resistance; AST: Antimicrobial susceptibility testing; ATRH: Asella Teaching and Referral Hospital; BP: Blood pressure; CS: Caesarean section; ESBL: Extended spectrum β-lactamase; MDR: Multi-drug resistant; PR: Pulse rate; RR: Respiratory rate; SSI: Surgical site infection; T: Body temperature.

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Authors' contributions
TBT: Design, Acquisition and interpretation of data, drafting of the manuscript. AF: Conception and design, acquisition and interpretation of data, drafting and revision of the manuscript. TF: Accountability for the published work, conception and design, interpretation of data, critical revision of the manuscript. DTG: Analysis and interpretation of data, drafting of the manuscript. Colin Mackenzie: Interpretation of data, accountability for microbiological results, critical revision of the manuscript. KP: Accountability for microbiological results, critical revision of the manuscript. DH: Critical revision of manuscript and approval of final version to be published. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analysed during this study are included in this published article [and its additional information files].

Ethics approval and consent to participate
Not applicable.

Consent for publication
The patient consented to participation and publication. The consent form (written in the patient's mother tongue, Amharic) is available upon request.

Competing interests
The authors declare that they have no competing interests.

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Tafese B. Tufa's contribution to this paper:

a) Draft the coceptuals of the work
b) Data collection
c) write the manuscript

- During our second work (2.2, page 24-45), we faced an obstacle in enrolling participants due to the low specificity of the qSOFA score in Asella (at 2400 m above sea level).
- To understand the reason for this, we evaluated the qSOFA score in the healthy population (without signs of acute infection) at different altitudes of residence and found qSOFA score >1 in Asella (28%), which was significantly higher than in Adama (1600 m above sea level) (8%; $p<0.001$) and Semara (400 m above sea level) (15.1%; $p = 0.005$) among healthy individuals.
- Therefore, adjustment of existing scores using local standard values could be helpful for reliable risk assessment.

PLOS ONE

RESEARCH ARTICLE

Variation of vital signs with potential to influence the performance of qSOFA scoring in the Ethiopian general population at different altitudes of residency: A multisite cross-sectional study

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Abstract

Introduction

The physiological range of different vital signs is dependent on various environmental and individual factors. There is a strong interdependent relationship between vital signs and health conditions. Deviations of the physiological range are commonly used for risk assessment in clinical scores, e.g. respiratory rate (RR) and systolic blood pressure (BP_{sys}) in patients with infections within the quick sequential organ failure assessment (qSOFA) score. A limited number of studies have evaluated the performance of such scores in resource-limited health care settings, showing inconsistent results with mostly poor discriminative power. Divergent standard values of vital parameters in different populations, e.g. could influence the accuracy of various clinical scores.

Methods

This multisite cross-sectional observational study was performed among Ethiopians residing at various altitudes in the cities of Asella (2400m above sea level (a.s.l.)), Adama (1600m a.s.l.), and Semara (400m a.s.l.). Volunteers from the local general population were asked to complete a brief questionnaire and have vital signs measured. Individuals reporting acute or chronic illness were excluded.

Results

A positive qSOFA score (i.e. $\geq$2), indicating severe illness in patients with infection, was common among the studied population (n = 612). The proportion of participants with a

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positive qSOFA score was significantly higher in Asella (28.1%; 55/196), compared with Adama, (8.3%; 19/230; p<0.001) and Semara (15.1%; 28/186; p = 0.005). Concerning the parameters comprised in qSOFA, the thresholds for RR (≥22/min) were reached in 60.7%, 34.8%, and 38.2%, and for BP_{sys} (≥100 mmHg) in 48.5%, 27.8%, and 36.0% in participants from Asella, Adama, and Semara, respectively.

Discussion

The high positivity rate of qSOFA score in the studied population without signs of acute infection may be explained by variations of the physiological range of different vital signs, possibly related to the altitude of residence. Adaptation of existing scores using local standard values could be helpful for reliable risk assessment.

Introduction

The normal range of vital signs depends on various environmental and individual factors, and there is a strong interdependent relationship between vital signs and health condition [1, 2]. Deviations of physiological parameters, including respiratory rate (RR) and blood pressure (BP), from the normal range are used in several clinical scores, e.g. the qSOFA (quick sequential organ failure assessment) score. The qSOFA score has been developed as a tool for the identification of patients who are at greater risk for a poor outcome among patients with suspected infection outside the intensive care unit (ICU) [3]. It uses three criteria, assigning one point for low systolic blood pressure (BP_{sys} ≥100 mmHg), high respiratory rate (≥22 breaths per min), or altered mentation (Glasgow coma scale (GCS) <15). Thus, the score ranges from 0 to 3 points and patients with 2 or more qSOFA points are likely to be septic and are at high risk for an unfavorable outcome [3]. Key advantages of the qSOFA are the easy and universal availability of the comprised parameters in clinical settings. A limited number of studies evaluating the performance of sepsis scores in low-resource health care settings have shown inconsistent results with mostly poor discriminative power and high variability across different study sites and settings [4–10]. Previous studies conducted elsewhere have mostly found lower BP, higher resting heart rate (HR) and lower peripheral oxygen saturation (SpO_2) in people living at high altitudes compared to lowlanders [11–13], although some studies have also documented a rise in BP with increasing altitude [14, 15].

Divergent physiological ranges of the applied vital signs in different populations could be a reason for heterogeneity of the performance of vital sign-dependent clinical scores as the qSOFA score in different populations. A possible cause of such heterogeneities may be attributed to adaptation mechanisms of the local population to higher altitudes. Thus, we investigated the potential influence of variations within the physiological range of vital signs in the general healthy population residing at different altitudes in Ethiopia on the performance of the qSOFA score.

Materials and methods

The study has been approved by the appropriate Ethical Review Committee (ERC) of the College of Health Sciences, Arsi University, Asella, Ethiopia (project number: A/CHS/RC/72/18). All volunteer healthy participants gave verbal consent after study procedures were thoroughly explained in local language by the study team before data acquisition. The method of verbal

informed consent was approved by the Ethical Review Board, considering the high rate of illiteracy and that no invasive procedures were performed. The data were analyzed anonymously. For this multisite cross-sectional observational study, we selected three study sites in Ethiopian cities at different altitudes. Asella was the study site located at the highest altitude (2400 m a.s.l.). Second, we aimed for a study site at the lowest possible altitude within the country, inhabited by a population with similar descent. Since Ethiopia is a landlocked country with large shares of highlands and the areas with the lowest altitude in the country are barren, hostile deserts where hardly any people live, no inhabited area at sea level was available. Therefore, Semara, as one of the cities of the country located at the lowest altitude (400 m a.s.l.), was selected as study site. Third, for comparison at mid-level altitude, the city of Adama (1620 m a.s.l.) was chosen. In order to investigate comparable populations at the respective study centers and therefore avoid selection errors, we selected urban centers at the respective altitudes as study sites. Reliable data on socioeconomic differences between the study sites were not available.

The study was conducted between December 2018 and March 2019 among adult alert volunteers with a minimum age of 16 years (Fig 1). The selection of volunteers was randomly made among pedestrians on the street at each study site. Participants were included for measurements during daytime for two subsequent days in order to achieve a sufficiently large sample size. A mobile medical unit was set up in a tent in a busy area downtown at each of the study sites and random passers-by were invited to have their vital signs checked and to participate in our investigation. Apart from the age limit and the residency in the assigned area, no other initial study eligibility criterion was applied. This approach of random selection of subjects in a busy area of the respective city, populated by local people who pursue their ordinary activities was chosen in order to achieve inclusion of a representative cross-section of the local population. The physiological parameters like body temperature (T), BP_{sys}, RR, SpO_2 and HR were measured non-invasively with medical infrared thermometers, photo optic finger clip pulse oximeters, and aneroid sphygmomanometers. Height and weight were measured from which body mass index (BMI) was calculated by two trained nurses. Prior to these measurements, the volunteers were asked to come to rest in a designated waiting area for not less than 5 minutes, which is in conformity with official recommendations and international guidelines [16, 17]. To avoid measurement errors in pulse oximetry, the measurement was performed only on clean fingers without nail polish. The participants were asked to rest their arm during the measurement. In addition, data on socio-demographic background, current health condition, as well as chronic diseases were collected using a standardized questionnaire. In order to rule out potential influences on vital parameters by medical conditions, participants with acute or previously known chronic illnesses were excluded a posteriori. The qSOFA was calculated using the cutoffs of $\geq$22/min for RR, and $\geq$100 mmHg for (BP_{sys}). Since all participants were fully conscious and responsive during the study procedures, the GCS, assessing mental alteration, was graded unimpaired (15 points) in all participants. Continuous variables were expressed as median (interquartile range, IQR). Post-hoc sample size calculation and power analysis were performed using the approach by Cohen, J. and the R-Package "pwr" [18]. Multigroup comparisons were done using the Kruskal-Wallis Test or one-way ANOVA. Additionally, pairwise comparisons between group levels at the different study sites were performed and adjusted for multiple comparisons using the false discovery rate approach. Categorical variables were compared using either the $\chi 2$ test or the Fisher exact test, as appropriate. To evaluate the association of the place of residency with the qSOFA score, a multiple ordinal regression model was used and adjusted for other covariates. An alpha of 0.05 was determined as the cutoff for significance. All statistical analyses were performed using R (version 3.6.3, R Foundation for Statistical Computing, Vienna, Austria).

Fig 1. Geographical position of the three study sites in Ethiopia. The original map was downloaded from https://www.cia.gov/static/c383432a1174420f80c37d230bdfc5ee/Ethiopia_Physiography.jpg and modified.

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Results

A total of 612 participants were included in the final analysis. Forty-nine Participants (7.4% of the original study collective) were excluded prior to the final data analysis due to an acute (0.9%) or previously known chronic illnesses (6.5%), primarily arterial hypertension and lung diseases, including tuberculosis (see Fig 2). The mean age of included participants was 31.5 ±12.8 years and 30.6% of them were female (Table 1). All participants were fully alert and oriented and GCS was graded 15/15.

For details on vital parameters see Table 2 and Fig 3. Interestingly, the majority of vital parameters differed significantly across the sites (Table 2). The median respiratory rate was significantly higher in Asella compared with the other sites (22 [IQR 20–24], 20 [IQR 18–22], and 21 [IQR 19–23] /min in Asella, Adama, and Semara, respectively), whereas the median systolic blood pressure was lower in participants from Asella (110 [IQR 100–113], 110 [IQR 100–120], and 110 [IQR 100–120] mmHg in Asella, Adama, and Semara respectively). With

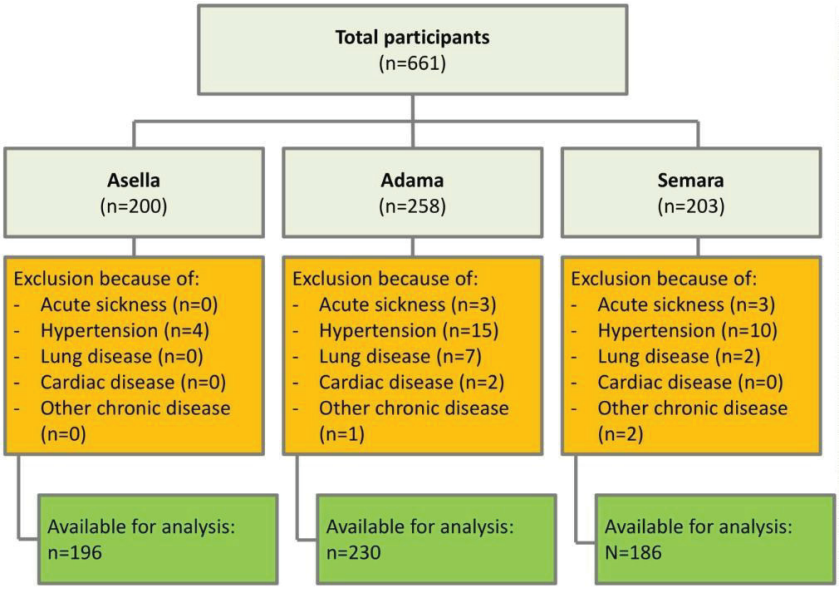

Fig 2. Flow chart of inclusions and exclusions at the different study sites. A total of 661 participants were recruited. After screening for any sickness or disease, the remaining participants used for the analysis was 612.

https://doi.org/10.1371/journal.pone.0245496.g002

regard to the qSOFA, RR threshold was reached in 60.7% (77/196), 34.8% (80/230) and 38.2% (71/186) in Asella, Adama and Semara, and BP threshold was reached in 48.5% (95/196), 27.8% (64/230) and 36.0% (67/186), respectively. Remarkably, in Asella, at high altitude, the median RR in the analyzed healthy population reached the qSOFA score threshold (Fig 3). As presented in Table 3, across all sites 16.7% (102/612) of participants scored 2 points in the qSOFA score ($RR \geq 22$ and $BP_{sys} \geq 100$ mmHg). In particular, the qSOFA score reached 2 points in 28.1% (55/196) of participants in Asella, in 8.3% (19/230) of participants in Adama and 15.1% (28/186) of participants in Semara (see Fig 4). Notably, the distribution of the qSOFA score in Asella was significantly different from Adama and Semara ($p<0.001$, Table 3 and Fig 4). Also, when adjusting for the covariates age, sex and BMI in the multiple ordinal regression model, Asella as area of residency was significantly associated with an elevated

Table 1. Demographic parameters.

		Total (n = 612)	Asella (n = 196)	Adama (n = 230)	Semara (n = 186)	p-value (group differences)
Age in years	Mean ± SD	31.5 ± 12.7	26.7 ± 10.6	36.0 ± 13.4	31.1 ± 12.1	<0.001
Sex	male, n (%)	425 (69.4)	125 (63.8)	148 (64.3)	152 (81.7)	<0.001
	female, n (%)	187 (30.6)	71 (36.2)	82 (35.7)	34 (18.3)	
Ethnicity	Oromo, n (%)	293 (47.9)	155 (79.1)	132 (57.4)	6 (3.2)	<0.001
	Amhara, n (%)	179 (29.2)	21 (10.7)	52 (22.6)	106 (57.0)	
	Afar, n (%)	58 (9.5)	0 (0)	0 (0)	58 (31.2)	
	Gurage, n (%)	24 (3.9)	7 (3.6)	17 (7.4)	0 (0)	
	Tigray, n (%)	14 (2.3)	3 (1.5)	8 (3.5)	3 (1.6)	
	Wolayita, n (%)	8 (1.3)	0 (0)	2 (0.9)	6 (3.2)	
	Silete, n (%)	7 (1.1)	4 (2.0)	3 (1.3)	0 (0)	
	Somali, n (%)	1 (0.2)	0 (0)	0 (0)	1 (0.5)	
	Other, n (%)	17 (2.8)	2 (1.0)	10 (4.3)	(5 (2.7)	
	not stated, n (%)	11 (1.8)	4 (2.0)	(2.6)	1 (0.5)	

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Table 2. Physiological parameters at the different study sites.

	Asella, 2400 m a.s.l. (A)	Adama, 1620 m a.s.l. (B)	Semara, 400 m a.s.l. (C)	p-value (group differences)	p-value (Single group comparisons)		
					A|B	A|C	B|C
Temperature in ˚C, median (IQR)	36.7 (36.5–36.9)	36.5 (36.3–36.8)	36.6 (36.1–37.0)	<0.001	<0.001	0.009	0.555
Heart rate in /min, median (IQR)	80 (72–90)	81 (73–90)	83 (74–91)	0.233	0.90	0.21	0.21
Systolic blood pressure in mmHg, median (IQR)	110 (100–113)	110 (100–120)	110 (100–120)	<0.001	<0.001	0.004	0.017
02 saturation in %, median (IQR)	96 (95–97)	97 (96–98)	98 (98–99)	<0.001	<0.001	<0.001	<0.001
Respiratory rate in /min, median (IQR)	22 (20–24)	20 (18–22)	21 (19–23)	<0.001	<0.001	<0.001	0.022
Body mass index in kg/m^2, median (IQR)	21.7 (19.4–23.6)	24.05 (20.8–26.7)	20.6 (18.6–23.1)	<0.001	<0.001	0.009	<0.001

BMI: Body Mass Index

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qSOFA score compared to the location Adama (aOR 3.26 [2.20–4.86], p<0.001, Table 4), whereas no difference between Semara and Adama was observed. Furthermore, male gender was significantly associated with a lower qSOFA score (p = 0.004).

Discussion

Within the cohort of healthy adult volunteers, the high proportion of individuals reaching the cutoff for a positive qSOFA score was surprising. The qSOFA score has been developed as an easy-to-use bedside score in order to quickly identify individuals at risk of a poor outcome among patients with an infection [19]. In previous studies, positive qSOFA criteria showed a similar prognostic significance compared with more complex tools as the SOFA, MEDS (Mortality in Emergency Department Sepsis) or APACHE II (Acute Physiology And Chronic Health Evaluation II) scores [20]. The specificity to predict mortality among patients with an infection of the qSOFA score in sub-Saharan African cohorts was reported to be 82% (95% Confidence Interval [CI] 76–88) and 81% (95% CI 78–85) while sensitivity was much lower (55% [95% CI 23–83] and 44% [95% CI 33–55], respectively) [5, 7].

All study participants were alert pedestrians, were not apparently mentally altered, as assessed during study procedures, and did not report to suffer from chronic illness. Thus, all patients were considered to have an unimpaired GCS of 15. The RR was significantly higher

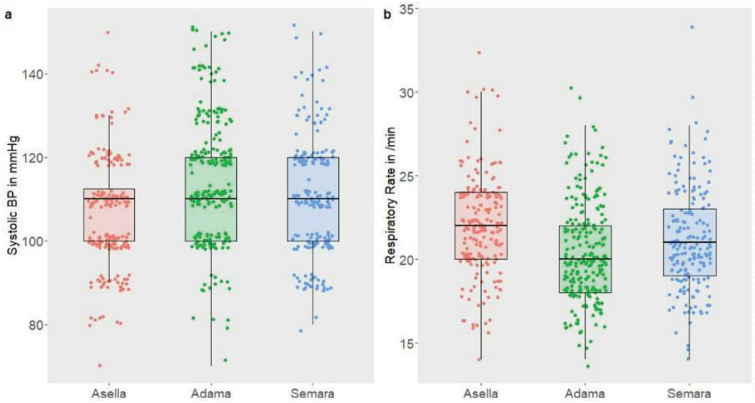

Fig 3. Scatter plots of the vital parameters systolic blood pressure in mmHg (a) and respiratory rate in breaths/min (b) according to areas of residency. Individual measurements of participants are represented by colored dots, and medians as horizontal lines of box plots.

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Table 3. Proportions of the qSOFA at the different study sites.

qSOFA-score, n (%) group	Total (n = 612)	Asella (n = 196) (A)	Adama (n = 230) (B)	Semara (n = 186) (C)	p-value (group differences)	p-value (Single comparisons)		
						A|B	A|C	B|C
0	218 (35.6)	37 (18.9)	105 (45.7)	76 (40.9)	<0.001	<0.001	<0.001	0.089
1	292 (47.7)	104 (53.1)	106 (46.1)	82 (44.1)				
2	102 (16.7)	55 (28.1)	19 (8.3)	28 (15.1)				

qSOFA: Quick Sequential Organ Failure Assessment

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and the SpO_2 significantly lower in Asella, the site situated at the highest altitude a.s.l., whereas there was no significant difference between the sites at lower altitudes. Although the rate of positive qSOFA was also remarkably high at those sites, we postulate that the high altitude of 2400 m a.s.l. might contribute to the high rate of unspecific positive qSOFA score values. As it has been suggested by other authors [5], an adaption of existing scores to various settings might be necessary to improve the performance. Alternatively, to improve the impaired performance of clinical scores at different altitudes, a constant conversion factor could possibly be derived from future cohort analyses.

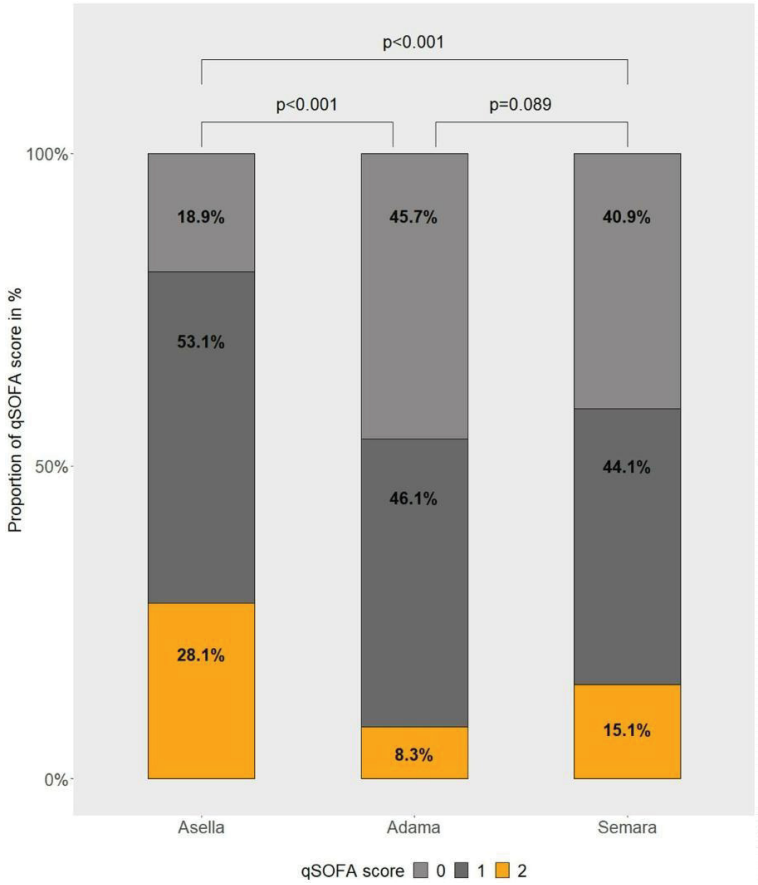

Fig 4. Proportions of qSOFA categories according to study site.

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Table 4. Factors associated with the qSOFA at the different study sites.

	qSOFA		
Covariates	aOR	95% CI	p-value
Age in years	0.99	(0.98–1.01)	0.247
Female	1		
Male	0.61	(0.43–0.86)	0.004
BMI in kg/m^2	0.98	(0.94–1.02)	0.295
Adama	1		
Asella	3.26	(2.20–4.86)	<0.001
Samara	1.32	(0.89–1.96)	0.171

qSOFA: Quick Sequential Organ Failure Assessment, aOR: adjusted Odds Ratios, CI: Confidence Interval, BMI: Body Mass Index

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The finding of a positive qSOFA score in the normal population was mostly common in Asella, located at 2400 m a.s.l. It has been shown elsewhere, that the qSOFA can be a reliable predictor of mortality, also in resource-limited settings [6, 21, 22] but perhaps not at extreme elevations. Our hitherto deviating results can only partly be explained by comparatively high altitudes in Ethiopia, since also at lower altitude, in Semara at 400 m a.s.l., a positive qSOFA was frequently found and was present in 15.1% of the local population. However, the median RR in the normal healthy population at Asella reached the threshold of the qSOFA score and even though the median BP_{sys} was 110 mmHg at all three sites, there was a significant difference in the IQR between the three sites, being lowest in participants from Asella. These findings explain the high rate of healthy individuals reaching a positive qSOFA. On the other hand, body temperature and HR, both parameters not included in the qSOFA score, showed no or hardly any differences between the study sites at different altitudes.

A limited specificity of the score in the studied population has already been described in previous studies conducted in countries with limited resources in Sub-Saharan Africa [5, 10, 23, 24] and may be partially explained by deviations of the standard values of different vital signs like RR or BP_{sys} although optimal thresholds remain uncertain. Our results support previous findings questioning the accurate applicability of the qSOFA not only in resource-limited settings, but also in more developed settings [8, 25, 26]. With a high proportion of positive qSOFA criteria in a normal population, the score fails to serve as specific tool for the identification of septic patients at risk for adverse outcomes.

Our findings might be limited due to the fact that our data was recorded as a single cross-sectional assessment and do not reflect physiological variation of the parameters within individuals. Nevertheless, also vital parameters used to calculate sepsis scores are usually assessed once at a certain time point in clinical settings. The analysis of multiple individuals within our cohort reduces the risk for selection bias. Since all study procedures were performed using volunteers, the results could be influenced by volunteer bias. However, since the sampling methods did not differ between the study sites, this bias cannot explain the apparent differences between the different study groups. No extrinsic motivation in form of any compensation was offered for participants. The conducted post-hoc power analysis indicated a sufficiently large group size to test the study objective.

Participants reporting any form of chronic disease were excluded from further analysis to rule out the possibility of changes in vital signs caused by illness. However, Patel et al. were able to show that self-reporting leads to limitations in the reliability of chronic disease detection [27]. Thus, among the participants classified as healthy in this study, there may have been

individuals potentially suffering from a chronic disease. Since this limitation applies equally to all study centers, no distortion of the study results in the comparison of the study centers is to be expected.

The European Society of Cardiology, the American Heart Association and others suggest having a patient rest for 5 minutes before measuring BP [16, 17, 28]. This approach was followed during our study. However, there are other data which indicate that a longer resting time of 10 or even 25 minutes might be necessary for reliable stabilization of BP [29, 30]. To circumvent a resulting error, no volunteers apparently exhausted by physical activity were included and the same procedure was followed at all study sites.

Also, the differences among demographic parameters such as age, gender and BMI between the study sites have to be considered as possible limitation of the study, but for this very reason the ordinal regression model was adjusted using these parameters and it was shown that Asella as place of residence is associated with a higher probability of a false positive qSOFA score, regardless of age, gender and BMI. Possibly, this finding could also be confounded by different socio-economic and environmental conditions (e.g. climate, air pollution) at the three study sites with Adama as a metropolis, Asella as a major district town and Semara as rather remote city. The varying BMI at the different study populations could reflect the respective economic strength and might be interpreted as an indication of different lifestyles of the population at the study sites. However, this assumption is based on personal observations and reliable data to support this hypothesis are insufficient.

Conclusion

Our study indicates that the applicability of the qSOFA score or other clinical scores based on examination of the vital signs BP and RR may be adversely affected by shifts in the range of normal values of the vital signs, e.g. as an adaptation mechanism for altitude. As it has previously been suggested by the international Sepsis-3 Task force, the qSOFA needs further investigation and validation especially in resource-limited health care settings [19]. High altitude might potentially be a relevant factor, since large populations of around 389 million people live in altitudes above 1.500 m, especially in Mexico, South America, the South-Central Asian Highlands, and Eastern Africa (Kenya, Ethiopia) [31]. Adaption of scores based on physiological parameters, as the qSOFA, according to local variances could improve the performance of these scores.

Supporting information

S1 Dataset. Minimal dataset.
(XLSX)

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Author Contributions

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Methodology: Jonas Fru¨h, Andre Fuchs, Tafese Beyene Tufa, Zewdu Hurissa, Torsten Feldt.

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Tafese B. Tufa's contribution to this paper:

a) Draft the coceptuals of the work
b) Perform the laboratory activities
c) Data analysis and draft the first manuscript

- Blood cultures were positive in only 5.4% of febrile hospitalized patients, and GNB were the most frequently (57.6%) isolated bacteria.
- Of the isolated GNB, 81.5% were ESBL-producing bacteria, and the TEM group (77.2%) and the CTX-M-1 group (68.2%) were the most frequently detected ESBL enzymes.
- Based on the local in vitro AST results, empirical treatment initiated in 72.2% of patients was likely ineffective.

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Antimicrobial Resistance and Infection Control

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Prevalence and characterization of antimicrobial resistance among gram-negative bacteria isolated from febrile hospitalized patients in central Ethiopia

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Abstract

Background: Infectious diseases are among the leading causes of death in many low-income countries, such as Ethiopia. Without reliable local data concerning causative pathogens and antimicrobial resistance, empiric treatment is suboptimal. The objective of this study was to characterize gram-negative bacteria (GNB) as pathogens and their resistance pattern in hospitalized patients with infections in central Ethiopia.

Methods: Patients ≥ 1 year of age with fever admitted to the Asella Referral and Teaching Hospital from April 2016 to June 2018 were included. Blood and other appropriate clinical specimens were collected and cultured on appropriate media. Antibiotic susceptibility testing (AST) was performed using the Kirby–Bauer method and VITEK® 2. Species identification and detection of resistance genes were conducted using MALDI-ToF MS (VITEK® MS) and PCR, respectively.

Results: Among the 684 study participants, 54.2% were male, and the median age was 22.0 (IQR: 14–35) years. Blood cultures were positive in 5.4% (n = 37) of cases. Among other clinical samples, 60.6% (20/33), 20.8% (5/24), and 37.5% (3/8) of swabs/pus, urine and other body fluid cultures, respectively, were positive. Among 66 pathogenic isolates, 57.6% (n = 38) were GNB, 39.4% (n = 26) were gram-positive, and 3.0% (n = 2) were Candida species. Among the isolated GNB, 42.1% (16/38) were Escherichia coli, 23.7% (9/38) Klebsiella pneumoniae and 10.5% (4/38) Pseudomonas aeruginosa.

In total, 27/38 gram-negative isolates were available for further analysis. Resistance rates were as follows: ampicillin/sulbactam, 92.6% (n = 25); cefotaxime, 88.9% (n = 24); ceftazidime, 74.1% (n = 20); cefepime, 74.1% (n = 20); gentamicin, 55.6% (n = 15); piperacillin/tazobactam, 48.1% (n = 13); meropenem, 7.4% (n = 2); and amikacin, 3.7% (n = 1). The bla_{NDM-1} gene was detected in one K. pneumoniae and one Acinetobacter baumannii isolate, which

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carried an additional bla_{OXA-51} gene. The ESBL enzymes were detected in 81.5%(n=22) of isolates as follows: TEM, 77.2%(n=17); CTX-M-1 group, 68.2%(n=15); SHV group, 27.3%(n=6); and CTX-M-9 group, 9.1%(n=2). Based on the in vitro antimicrobial susceptibility results, empiric treatment initiated in 13 of 18 (72.2%) patients was likely ineffective.

Conclusion: We report a high prevalence of ESBL-producing bacteria (81.5%) and carbapenem resistance (7.4%), with more than half of GNB carrying two or more ESBL enzymes resulting in suboptimal empiric antibiotic therapy. These findings indicate a need for local and national antimicrobial resistance surveillance and the strengthening of antimicrobial stewardship programs.

Keywords: Antimicrobial resistance, CTX-M-1, TEM, NDM-1, ESBL, Carbapenemase, Sub-Saharan Africa

Introduction

Infectious diseases are among the leading causes of morbidity and mortality in many low-income countries, such as Ethiopia [1]. Bacterial sepsis is a severe complication of different infections caused by gram-negative bacteria (GNB), leading to high rates of mortality. Pneumonia, bacteremia, urinary tract infection, intra-abdominal infection and skin and soft tissue infection are the major GNB sources of infections [2].

The emerging multidrug resistance (MDR) in GNB has become a serious public health problem worldwide [3]. A major driver of resistance in GNB is the horizontal transfer of mobile genetic elements carrying genes for extended-spectrum β-lactamases (ESBL) and/or carbapenemases [4]. These enzymes hydrolyze penicillins, third-generation cephalosporins (3GCs) and carbapenems. The most common ESBLs are cefotaximase (CTX-M-type), temoniera (TEM), and sulfhydryl reagent variable (SHV). Furthermore, the most prevalent carbapenemases are oxacillinases (OXA), namely, OXA-23, OXA-24/40, OXA-48, OXA-51, OXA-58, OXA-143, and OXA-235; Klebsiella pneumoniae carbapenemase (KPC); metallo-beta-lactamases (MBL), such as the New Delhi metallo-β-lactamase (NDM); imipenemase (IMP); and Verona imipenemase (VIM). These enzymes confer resistance to cephalosporins and carbapenems [5, 6].

Despite evidence for high rates of 3GC resistance among gram-negative isolates in many African countries, 3GCs are among the most commonly used antibiotics for the initial empiric treatment of severe infections and sepsis in sub-Saharan Africa (SSA). Data to estimate the mortality associated with antimicrobial resistance (AMR) in SSA are limited [7]. Implementation of antimicrobial stewardship (AMS) programs, improvement of antimicrobial susceptibility testing (AST) capacity, and provision of local and national AMR data for common bacterial pathogens should be given attention in these regions. Continued extensive empiric use of 3GC in the absence of AST might lead to a further decrease in antimicrobial activity and therefore increase the burden on the resource-limited health care systems of the countries affected [8]. This development can also be observed locally, and previous investigations at the study site show that the effectiveness of the limited choice of available antimicrobials is diminished due to the high rate of ESBL-producing GNB [9]. Due to the increase in ESBL-producing GNB-associated infections that are difficult or impossible to treat under local circumstances, there is a growing need for strategies to improve the prudent use of antibiotics in this country [10, 11].

The lack of systematically acquired local data concerning both the causative organisms and common resistance patterns likely results in ineffective empiric treatment and an unfavorable clinical outcome. Therefore, we assessed the rate and extent of drug resistance among GNB isolated from hospitalized patients with infectious diseases at a tertiary hospital in central Ethiopia and characterized the AMR genes.

Materials and methods

Study design and inclusion

This prospective operational research was conducted from April 2016 to June 2018 at the Asella Referral and Teaching Hospital (ARTH), a tertiary hospital in the town of Asella, located in the central part of Ethiopia. The bacterial cultures were performed in Ethiopia, and secondary and molecular biological investigations were performed in Germany.

During the study period, all patients ≥1 year of age presenting for treatment at ARTH with fever defined as body temperature >37.5 °C according to tympanic measurement were offered inclusion in the study (Fig. 1). The eligible patients who fulfilled the inclusion criteria were identified by a trained study team. After written informed consent was obtained from the patients or legal guardians, blood cultures (BCs) were drawn from all participants. Previously initiated antibiotic treatment was not an exclusion criterion. In addition to BC, appropriate clinical samples according to the patient's symptoms and the treating physician's decision were collected for further microbiological investigation, according to local standard operating procedures (SOPs) and national

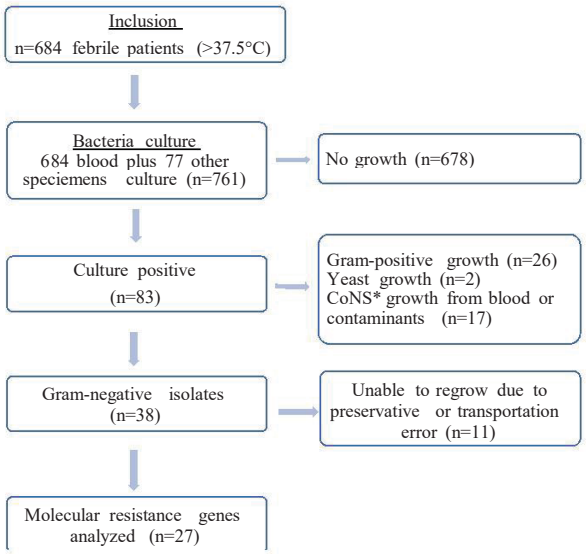

Fig. 1 Flow diagram of participants' inclusion.*CoNSgrowth was only considered in blood samples and interpreted as contaminants since we only used one set of blood culture bottles

treatment guidelines. Sociodemographic and clinical data were collected by using a standardized questionnaire.

Ethical approval and consent to participate

The appropriate ethical review boards of Arsi University (reference number A/U/H/S/C/120/6443/2017), the Oromia Regional Health Bureau (reference number BEFO/AHBTFH/1-8/2017), and Düsseldorf University Hospital (reference number 5729) approved the study. Ethical clearance for sample transportation between Ethiopia and Germany was obtained from the National Ethical Review Board of the Ministry of Science and Technology (reference number 310/204/2017). Before inclusion in the study, written informed consent to participate in the study was obtained from each participant or, in the case of minors, from their legal guardians.

Blood culture

Approximately 5 ml of blood from each child and 10–20 ml from each adult participant was collected and inoculated into an aerobic blood culture bottle. For the first 200 participants, we used in-house-produced blood culture bottles, and for the remaining participants, we used commercially available blood culture bottles (BacT/ALERT culture media bottles, bioMérieux, Marcy-l'Étoile, France). In both cases, we used the same incubator and did not find a significant difference in the positivity rates except for slightly more contaminants when using the in-house-produced blood culture bottles. The cultures were incubated at 37 °C for a maximum period of five days. After 24 h and at the end of the incubation period, Gram staining and subculturing on blood, MacConkey, and chocolate agar in a candle jar or a 5% CO_2-enriched atmosphere were performed. Biochemical identification tests were subsequently performed based on the gram staining results. For GNB identification, the biochemical identification tests performed were oxidase, lactose, indole, mannitol, urease, triple sugar iron, citrate, and lysine decarboxylase, whereas for gram-positive bacteria, catalase, coagulase and hemolysis tests were used. Only one aerobic culture was performed per patient for in-house blood culture bottles, and a single pair (one aerobic and one anaerobic culture media bottle) was performed for BacT/ALERT blood culture bottles. Regular quality controls for sensitivity were performed by incubation of ATCC 25922 (Escherichia [E.] coli), ATCC 700603 (Klebsiella [K.] pneumoniae), ATCC 27853 (Pseudomonas [P.] aeruginosa), ATCC 747 (Acinetobacter [A.] baumannii) and ATCC 25923 (Staphylococcus [S.] aureus).

Urine culture

In participants with possible urinary tract infection, midstream urine specimens were collected using sterile urine cups, and 1 µL of urine was inoculated onto blood and MacConkey agar. We used a semiquantitative urine culture method to interpret the results for female participants. Hence, after 24 h of incubation at 37 °C, bacterial colonies were further processed if the colony count was $\geq 10^5$ CFU/mL.

Swabs and other body fluid culture

If the patient had abscesses or any wounds, swabs or aspirates of other body fluids were collected using appropriate sterile sampling devices (swabs, sterile syringes, etc.) and cultivated on blood and chocolate agar in a 5% CO_2-enriched atmosphere. The isolates were further processed following the local laboratory's SOPs.

Antimicrobial susceptibility testing

Following the identification of GNB, Kirby–Bauer AST was performed after cultivation on Mueller–Hinton agar. The procedure and interpretation followed the European Committee on Antimicrobial Susceptibility Testing (EUCAST) version 7.1 recommendations. The microbiological results were reported to the treating physicians at ARTH as soon as possible to ensure appropriate patient care.

Confirmation of species identification and antimicrobial susceptibility test

Bacterial isolates were preserved at −80 °C in Microbank® vials (Pro-Lab Diagnostics Inc., Toronto, Canada). Subsequently, samples of all isolates were exported to

Germany for confirmation of species identification and AST as well as molecular biological analysis of resistance mechanisms. Species identification was confirmed by using matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (Vitek® MS, bioMérieu), and the local AST result was confirmed by VITEK® 2 (bioMérieux). There were no significant discrepancies between the Kirby–Bauer and VITEK AST results. However, some discrepancies between the species identification according to the biochemical tests performed in Ethiopia and the MALDI-TOF results occurred. For instance, 81.5% (22/27) of GNB isolates were correctly identified by biochemical tests performed in Ethiopia compared with the MALDI-TOF results.

ESBLand carbapenemase gene detection

After identification and AST, GNB DNA was extracted by suspending a pure colony grown on MacConkey agar in 200 µL of Tris–EDTA pH 8.0 buffer. The suspension was then heated at 95 °C for 20 min, followed by centrifugation at 10,000 rpm for 10 min. Then, 150 µl of the supernatant was transferred into a new tube and stored at −20 °C until PCR testing could be performed.

All available GNB isolates were investigated by PCR (Fig. 1). We used the PCR protocols described by Strauß et al. for identification of the β-lactamase bla_{CTX-M}, bla_{SHV} and bla_{TEM} genes [12] for all available GNB isolates and an in-house PCR protocol established for the detection of the carbapenemase genes bal_{IMP-1}, bal_{VIM-1}, bal_{VIM-2}, bla_{GIM-1}, bla_{OXA-23}, $bla_{OXA-24/40}$, bla_{OXA-58}, bla_{NDM-1} and bla_{OXA-51}, as described by Wendel et al. [13, 14] (Table 1) for strains that were resistant to imipenem/meropenem according to the AST results.

Data analysis

Data were entered and analyzed using IBM SPSS Statistics for Windows version 25 (IBM Corp., Armonk, NY, USA). Fisher's exact and Pearson's chi-squared tests were used for the analysis of quantitative variables, and the mean, median and standard deviation were used for continuous variables. Differences were considered statistically significant at $p<0.05$.

Quality control

For quality control, the control strains listed in Table 2 were used during the resistance gene analysis.

Results

In total, 684 study participants were included; 54.2% were male, and the median age was 22 years (interquartile range: 14–35 years). More than half of the participants were from rural areas, and 167 (24.4%) could not read or write (Table 3).

Table 1 Oligonucleotide sequences of the primer pairs used for molecular resistance gene detection

Primer	Sequence (5′–3′)	Amplicon size (bp)	References
bla_{SHV}(F)	AGCCGCTTGAGCAAATTAAAC	786	[12]
bla_{SHV}(R)	GTTGCCAGTGCTCGATCAGC		
bla_{TEM}(F)	CATTTCCGTGTCGCCCTTATTC	846	[12]
bla_{TEM}(R)	CCAATGCTTAATCAGTGAGGC		
$bla_{CTX-M-1}$(F)	CGTCACGCTGTTGTTAGGAA	781	[12]
$bla_{CTX-M-1}$(R)	ACGGCTTTCTGCCTTAGGTT		
$bla_{CTX-M-2}$(F)	CTCAGAGCATTCGCCGCTCA	843	[12]
$bla_{CTX-M-2}$(R)	CCGCCGCAGCCAGAATATCC		
$bla_{CTX-M-9}$(F)	GCGCATGGTGACAAAGAGAGT GCAA	876	[12]
$bla_{CTX-M-9}$(R)	GTTACAGCCCTTCGGCGATGA TTC		
bla_{NDM-1}(F)	CGGCATCACCGAGATTGC	732	[13]
bla_{NDM-1}(R)	CACCGACATCGCTTTTGGT		
bla_{OXA-51}(F)	TGTCTAAGGAAGTGAAGCGTG	112	[14]
bla_{OXA-51}(R)	AACTGTGCCTCTTGCTGAG		

F forward, R reverse, bp base pairs

Table 2 Control strains used for resistance gene analysis

Resistance gene	Base pairs	Bacterial species	ATCCnumber
SHVgroup	780	Klebsiellapneumoniae	NRZ-02718
TEMgroup	860	Klebsiellaoxytoca	NRZ09574
CTX-M-1group	688	Escherichiacoli	NRZ-04944
CTX-M-2group	404	Escherichiacoli	NRZ-09082
CTX-M-9group	561	Escherichiacoli	NRZ00552
NDM-1	180	Klebsiellapneumoniae	JS-37
OXA-51	112	Acinetobacterbaumannii	DMBF-4

Bacterial cultures

The overall bacterial detection rate across all sample materials was 10.9% (83/761). In 8.7% (n = 66), the isolated bacterium was considered a pathogen, and in 2.2% (n = 17), coagulase-negative staphylococci (CoNS) were isolated and considered clinically irrelevant because of the high likelihood of contamination. The overall blood culture positivity rate was 5.4% (37/684), among which 51.4% (n = 19) were GNB. The overall detection rates in other clinical samples, namely, swabs/pus, urine, and other body fluids, were 60.6% (20/33), 20.8% (5/24), and 37.5% (3/8), respectively. Out of 12 cultured cerebrospinal fluid samples, only one revealed growth of the pathogen Neisseria meningitidis (N. meningitidis). Overall, 66 pathogenic bacteria were isolated from a total of 761 different clinical samples. Of those, 57.6% (n = 38) were GNB, 39.4% (n = 26) were gram-positive, and 3% (n = 2) were Candida species. Staphylococcus

Table 3 Sociodemographic characteristics of study participants

Sociodemographic characteristics		Numbers	Percentage (%)
Sex	Male	371	54.2
Age	1–15	190	27.8
	16–30	286	41.8
	31–45	110	16.1
	46–60	51	7.5
	> 60	47	6.9
Area of residence	Rural	396	57.9
	Urban	288	42.1
Educational status	Illiterate	167	24.4
	Literate	434	63.5
	Children below school age	83	12.1
Patient settings	Internal medicine ward	129	18.9
	Pediatric ward	131	19.2
	Surgical and Gynecology wards	47	6.9
	Emergency OPD	347	50.7
	ICU	30	4.4

ICU intensive care unit, OPD outpatient department

Table 4 Frequencies of isolated GNB isolates from different clinical samples from the study participants (n = 38)

Bacterial species	Number	Percentage (%)
Escherichia coli	16	42.1
Klebsiella pneumoniae	9	23.7
Pseudomonas aeruginosa	4	10.5
Salmonella typhi	2	5.3
Enterobacter spp.	2	5.3
Acinetobacter baumannii	1	2.6
Neisseria meningitidis	1	2.6
Raoultella ornithinolytica	1	2.6
Raoultella planticola	1	2.6
Serratia spp.	1	2.6

aureus and E. coli were the most prevalent isolates among the gram-positive and gram-negative isolates, respectively. Among the 38 g-negative isolates, 42.1% (n = 16) were E. coli, 23.7% (n = 9) were K. pneumoniae, and 10.5% (n = 4) were P. aeruginosa (for further details see Table 4).

As indicated in Table 5, among all clinical samples, bacterial growth was identified significantly more often among patients with skin and soft tissue infections (SSTIs) ($p < 0.001$). Although not statistically significant, samples from patients with a leukocyte count > 12,000 or < 4000 cells/μL tended to be more likely to reveal bacterial growth ($p = 0.07$). On the other hand, BCs from patients with a diagnosis of acute febrile illness of unknown source were least likely to verify bacteremia compared with other symptoms ($p = 0.005$). Being HIV positive had no statistically significant effect on the blood culture positivity rate ($p = 0.85$). □ere was no statistically significant difference between the mean value of C-reactive protein (CRP) in positive and negative BCs (mean CRP 71.7 mg/L vs. 64.6 mg/L; $p = 0.20$).

Regarding the culture positivity rate among different clinical samples, there was no significant difference between the positivity rates of gram-positive or gram-negative isolates. See Table 6 for details.

Antimicrobial susceptibility testing

In total, 27 gram-negative isolates were available for susceptibility testing with VITEK® 2. Among those, the resistance rates against commonly used antibiotics at the study site were as follows: ampicillin/sulbactam, 93.3% (n = 21); cefotaxime, 88.9% (n = 24); ceftazidime, 74.1% (n = 20); cefepime, 74.1% (n = 20); ciprofloxacin, 70.4% (n = 19); and gentamicin, 63.0% (n = 17). □e resistance rates against rarely used antibiotics were 48.1% (n = 13) for piperacillin/tazobactam and 7.4% (n = 2) for meropenem. Only one case of amikacin resistance was detected among all 27 isolates (Table 7).

Resistance genes

The overall frequencies of ESBL and carbapenemase detection among the isolated GNB were 81.5% (22/27) and 7.4% (2/27), respectively. In 55.6% of cases (n = 15), more than one ESBL enzyme was detected in the isolated GNB. The different ESBL enzymes were characterized as

Table 5 Culture positivityrate according to clinicaldiagnosis, source of infection and laboratory parameters

Infectious focus/laboratory parameters	Culture positive (N=66) % (n)	Culture negative (N=618) % (n)	p value
Pneumonia/RTI(n=165)	19.7(13)	24.6(152)	0.38
Urinarytract infections (n=30)	7.6(5)	4.4(25)	0.20
Meningitis/encephalitis (n=57)	6.1(4)	8.6(53)	0.48
GITI/hepatitis(n=43)	6.1(4)	6.3(39)	0.94
SSTIs(n=52)	28.8(19)	5.3(33)	<0.001
Acute febrile illnesswith unknown source (n=264)	22.7(15)	40.3(249)	0.005
Sepsis*(n=32)	4.5(3)	4.7(29)	0.96
HIVseropositivity(n=58)	9.1(6)	8.4(52)	0.85
Leukocytecount >12,000or<4000/µL(n=218)	42.4(28)	30.7(190)	0.07
	Mean	Mean	
CRPin mg/L	71.7	64.6	0.20

RTIs respiratory tract infections, SSTI skin and soft tissue infection, GITI gastrointestinal tract infection, CRP C-reactive protein

According to the clinician's diagnosis, regardless of the focus of the infection

Table 6 Culture positivity rates among the different clinical samples

Clinical sample	Culture negative	Culture positive	
		Gram-negative isolate % (n)	Other isolates* % (n)
Allsamples (n=761)	91.3(695)	5.0(38)	3.7(28)
Blood*(n=684)	94.6(647)	2.9(20)	2.5(17)
Urine(n=24)	79.2(19)	12.5(3)	8.3(2)
Swab or pus of infected skin lesion or abscess (n=33)	33.3(11)	36.4(12)	24.2(8)
CSF(n=12)	91.7(11)	8,3(1)	-
Other body fluids (n=8)	62.5(5)	25.0(2)	(1)

*Gram-positive bacteria (n=26) and two Candida spp. microbes (yeasts) isolated from blood were considered

TEM (n=17, 77.3%), CTX-M-1 group (n=15, 68.2%), SHV group (n=6, 27.3%) and CTX-M-9 group (n=2, 9.1%). Both CTX-M-1-type and SHV-type were detected in 5/6 (83.3%) of the K. pneumoniae isolates, whereas the SHV group was detected only in 1/13 of the E. coli isolates. In E. coli, only the CTX-M-1 group was common (see Table 8). Regarding carbapenemases, a single bla_{NDM-1} from one isolated K. pneumoniae and a combination of bla_{NDM-1} plus bla_{OXA-51} from an isolate of A. baumannii were detected.

Effectiveness of empiric antibiotic treatment

The results of the locally performed Kirby–Bauer disc diffusion test were available from 25 study participants with E. coli and K. pneumoniae isolates. Eighteen of these 25 study participants received empirically initiated antibiotic treatment at the time of sampling. The Kirby–Bauer AST revealed high levels of resistance against commonly used antibiotics, rendering 72.2% (13/18) of the initiated antibiotic treatments likely to be ineffective. In particular, 72.0% (18/25) of the isolated GNB were resistant to 3GC, 60.0% (15/25) to fluoroquinolones and 48.0% (12/25) to gentamicin. The results of the initial clinical evaluation, empiric antibiotic treatment and AST results according to the Kirby–Bauer disc diffusion test are summarized below (Table 9).

Discussion

To date, infectious diseases are one of the most common causes of morbidity and mortality in resource-limited settings, such as Ethiopia [1], but the availability of epidemiological data about causative pathogens and the distribution of AMR remains limited. In this study, we found a high rate of MDR in GNB isolated from febrile patients. As previously described in Uganda, MDR GNB is the main cause of sepsis in febrile cancer patients, with more than 50% of sepsis episodes being caused by E. coli infections [15]. Similar to these findings and to the findings of Wasihun et al. (2015) and Moges et al. (2021) from northern Ethiopia [16, 17], E. coli was the most prevalent isolated GNB in our study.

The culture positivity rate of 5.4% from BCs was low, which might partially be explained by the fact that the causative pathogen was noncultivable in a proportion of patients (hemoparasites, viruses, or noncultivable bacteria). Similar findings were reported in South Africa [18]. In patients with febrile illness with

Table 7 Antibiotic resistance rate of Gram-negative isolates

Bacterial species	Ampicillin	Ampicillin/sulbactam	Piperacillin	Piperacillin/tazobactam	Cefotaxime	Ceftazidime	Cefepime	Meropenem	Amikacin	Gentamicin	Ciprofloxacin
Escherichia coli (n = 13)	92.3% (12/13)	92.3% (12/13)	92.3% (12/13)	69.2% (9/13)	84.6% (11/13)	84.6% (11/13)	84.6% (11/13)	0% (0/13)	0% (0/13)	53.8% (7/13)	23.1% (3/13)
Klebsiella pneumoniae (n = 6)	100% (6/6)	100% (6/6)	100% (6/6)	50.0% (3/6)	100% (6/6)	100% (6/6)	100% (6/6)	16.7% (1/6)	16.7% (1/6)	83.3% (5/6)	50.0% (3/6)
Pseudomonas aeruginosa (n = 4)	-	-	50.0% (2/4)	0% (0/4)	100% (4/4)	50.0% (2/4)	50.0% (2/4)	0% (0/4)	0% (0/4)	25.0% (1/4)	75.0% (3/4)
Other (n = 4)	75.0% (3/4)	75.0% (3/4)	75.0% (3/4)	25.0% (1/4)	75.0% (3/4)	25.0% (1/4)	25.0% (1/4)	25.0% (1/4)	0% (0/4)	50.0% (2/4)	75.0% (3/4)
Total (n = 27)	91.3% (21/23)	91.3% (21/23)	85.2% (23/27)	48.1% (13/27)	88.9% (24/27)	74.1% (20/27)	74.1% (20/27)	7.4% (2/27)	3.7% (1/27)	63.0% (17/27)	70.4% (19/27)

Others: *Raoultella ornithinolytica* (n = 1); *Raoultella planticola* (n = 1); *Salmonella* Typhi (n = 1); *Acinetobacter baumannii* (n = 1)

Table 8 Characterization and frequency of detected ESBLand carbapenemase enzymes among the Gram-negative isolates

Bacterial species	Resistance genes % (n)							
	ESBLenzymes					CP enzymes		
	Any ESBL	CTX-M-1group	TEM-group	SHV-group	CTX-M-9group	Any CP	NDM-1	OXA-51
E.coli(n=13)	92.3(12)	69.2(9)	76.9(10)	7.7(1)	0	0	0	0
K.pneumoniae (n=6)	100(6)	83.3(5)	50.0(3)	83.3(5)	0	16.7(1)	16.7(1)	0
P.aeruginosa (n=4)	50.0(2)	25.0(1)	50.0(2)	0	0	0	0	0
Other* (n=4)	75.0(3)	0	50.0(2)	0	50.0(2)	25.0(1)	25.0(1)	25.0(1)
AllGNB(n=27)	81.5(22)	55.6(15)	63.0(17)	22.2(6)	7.4(2)	7.4(2)	7.4(2)	3.7(1)

CP carbapenemase

*Other isolates: R. planticola (n=1) and R. ornithinolytica (n=1): both positive for CTX-M-9 group; Salmonella Typhi (n=1): no ESBL- or CPE-production; and A.
baumannii (n=1): positive for OXA-51 and NDM-1

an unknown source of infection in comparison to other clinical diagnoses, the likelihood of positive BCs was lowest. The highest yield of bacterial cultures was reported from swabs in patients with SSTIs. There was a tendency toward an association between a decreased or elevated leucocyte count or an increased CRP level and blood culture positivity. However, these associations were not significant, and the predictive value of these parameters to guide blood culture diagnostics is insufficient. Thus, our finding confirms a previous report from Italy that CRP level alone is not sufficient to predict blood culture positivity [19]. In this time of widespread AMR and the associated risk of failing antibiotic therapies, blood culture diagnostics are essential to guide the management of bacterial infections. If the broad application of blood culture diagnostics is not possible due to resource limitations, the application of other parameters, such as procalcitonin or monocyte distribution width (MDW), could help to guide the rational use of BCs [20, 21].

In our study, the resistance rates of isolated GNB against commonly used antibiotics were high, severely confining the effectiveness of aminopenicillins in combination with beta-lactamase inhibitors or 3GC for empiric treatment of infections possibly caused by GNB. The lowest resistance rates were found for the meropenem and amikacin. These results are consistent with the results of other recently published data from different parts of Ethiopia, in particular from Addis Ababa [11, 22], Jimma [23] and Bahir Dar [10]. The different resistance rates to certain antibiotics reflect the frequency of antibiotic prescriptions. While aminopenicillins and 3GCs are applied very frequently, carbapenems are hardly used due to their high cost and limited availability. Of note, while the resistance rate for amikacin was very low, many of the isolated GNB were resistant to gentamicin. This difference might be explained by the frequent application of gentamicin at the study center. In contrast, amikacin is almost never applied [24, 25].

Our data revealed overall frequencies of ESBL and carbapenemase production of 81.5% and 7.4% among the isolated GNB, respectively. Of note, all isolates of K. pneumoniae were ESBL-positive. These findings are consistent with recently published data from other parts of Ethiopia, where K. pneumoniae has also been shown to be the most common ESBL-expressing pathogen, followed by E. coli [10, 11, 22].

Regarding the characterization of ESBL enzymes among the gram-negative isolates in our study, TEM-type and CTX-M-1-type were most common, followed by SHV-type and, least frequently, CTX-M-9-type. CTX-M-2 and CTX-M-8/25 were not detected at all. Compared to TEM-type and SHV-type enzymes, CTX-M-type enzymes are more widely disseminated worldwide, and many variants associated with clinically relevant functional heterogeneity have been described. Thus, coexpression of different ESBLtypes is more common among GNB, which harbors CTX-M-type enzymes [26].

Both the CTX-M-1 group and SHV group were abundantly detected in K. pneumoniae, whereas in E. coli, the SHV group was detected in only 8% of the isolates (Table 8). This finding matches the report by Ogutu et al. that SHV-type is the predominant ESBL enzyme in K. pneumoniae and TEM-type is the most prevalent in E. coli [27].

A single bla_{NDM-1} carbapenemase gene in a K. pneumoniae isolate and bla_{NDM-1} plus bla_{OXA-51} carbapenemase genes in an A. baumannii isolate were detected. The expression of bla_{NDM-1} in an A. baumannii isolate has previously been reported from the southwestern part of Ethiopia [28], but to our knowledge, no case of bla_{NDM-1} presence in K. pneumoniae has been reported to date. In

Table 9 Clinical-evaluation,Kirby-BauerASTresult and empirical treatment of participants with E.colior K.pneumoniae

Participant No	Bacterial isolate	Resistance against			Clinical diagnosis	Empirical antibiotic treatment	Effectiveness of antibiotic treatment
		3GC	Fluoroquinolones	Gentamicin			
20	E.coli	Yes	Yes	No	UTI	Ciprofloxacin	Ineffective*
56	E.coli	Yes	No	Yes	RTI	Ceftriaxone,cloxacillin	Effective
258	E.coli	No	Yes	Yes	AFI	None	–
259	E.coli	Yes	Yes	Yes	AFI	None	–
264	E.coli	Yes	No	No	SSTI	Ceftriaxone,metronidazole	Ineffective
272	E.coli	Yes	Yes	Yes	SSTI	Cloxacillin	Ineffective
275	E.coli	Yes	No	No	SSTI	Ceftriaxone,metronidazole	Ineffective
351	E.coli	Yes	Yes	Yes	SSTI	Ceftriaxonemetronidazole	Ineffective
411	E.coli	Yes	Yes	Yes	AFI	None	–
423	E.coli	No	Yes	Yes	RTI	None	–
432	E.coli	Yes	Yes	Yes	RTI	Azithromycin,ceftriaxone	Ineffective
440	E.coli	Yes	Yes	Yes	UTI	Ceftriaxone	Ineffective
483	E.coli	Yes	Yes	No	AFI	Azithromycin,ceftriaxone	Ineffective
503	E.coli	No	No	No	RTI	None	–
568	E.coli	No	No	No	AFI	None	–
639	E.coli	Yes	No	No	UTI	None	–
42	K.pneumoniae	No	No	No	AFI	Ceftriaxone,gentamicin	Effective
59	K.pneumoniae	No	No	No	RTI	Ceftriaxone,metronidazole	Effective
64	K.pneumoniae	No	Yes	No	Meningitis/ encephalitis	Ceftriaxone	Effective
278	K.pneumoniae	Yes	Yes	Yes	SSTI	Ceftriaxone,cloxacillin	Ineffective
314	K.pneumoniae	Yes	Yes	Yes	SSTI	Ceftriaxone,vancomycin	Ineffective
332	K.pneumoniae	Yes	Yes	Not	AFI,sepsis	Ceftriaxone,gentamicin	Effective
545	K.pneumoniae	Yes	Yes	Yes	UTI	Tuberculostatic treatment	Ineffective
677	K.pneumoniae	Yes	Not	No	RTI	Ceftazidime,vancomycin	Ineffective
681	K.pneumoniae	Yes	Yes	No	RTI	Ceftriaxone,vancomycin	Ineffective

3GC 3rd generation cephalosporin, UTI urinary tract infection, RTI respiratory tract infection, AFI acute febrile illness (febrile disease with unknown source), SSTI skin and soft tissue infection

*Ineffective, high likelihood of empiric treatment failure based on in vitro AST result

general, this finding is not surprising since the bla_{NDM-1} carbapenemase genes in K. pneumoniae and A. baumannii have been commonly reported from other eastern African countries, such as Kenya, Uganda [29], Egypt [30] and Sudan [31].

In this study, most of the antibiotics initiated by the treating physicians for empiric treatment in the participating patients were ineffective according to the AST. However, these data have to be interpreted with caution since most microbiological culturing was performed after initiation of empiric antibiotic therapy, and evaluation of the clinical success rate of the empirically initiated antibiotic therapies was not part of this investigation. Nevertheless, antibiotic resistance impairing the success of empiric antibiotic therapies seems alarmingly common, and local epidemiological resistance data should be taken into account before the initiation of antibiotic therapy [32]. An adaptation of the local empiric antibiotic therapy strategy could be all the more necessary, as the excessive use of 3GC could be one of the main reasons for the spread of ESBL-producing bacteria [33].

Selectively utilizing antibiotics based on the AST result is not only favorable for optimal treatment success but also plays a major role in combating the spread

of MDR bacteria. Adequate microbiological culturing before initiation of empiric antibiotic treatment is necessary to enable AST-based antibiotic therapy. As resources are limited and the supply of laboratory materials is unreliable at the study site, as at many other sites in low-income countries, it may not always be possible to perform comprehensive microbiological testing. In such cases, at least surveillance studies with the subsequent establishment of resistance statistics should be carried out to enable calculated antibiotic therapies adapted to the local resistance status. This might help to reduce the imprudent use of antibiotics [8].

A limitation of this study might be the impaired sensitivity of BCs, since for the first 200 participants, a single locally prepared blood culture bottle was inoculated, and subsequently, only one set of standard commercially available blood culture bottles was used for blood culturing. The limited availability and high cost of commercially available blood culture bottles prevent the regular use of such products on site. Molecular resistance testing was not possible for all gram-negative isolates due to loss upon storage and transport. Our study did not investigate whether infections caused by ESBL- or carbapenemase-producing GNB were associated with reduced success of antibiotic therapies or increased mortality.

Conclusion

In this study, conducted in a tertiary hospital in Ethiopia, we isolated GNB from patients with infectious diseases with high rates of MDR, including 3GC resistance, which is the most commonly used drug class for empiric antibiotic treatment. Based on local AST results, empiric treatment initiated in 72.2% of patients was likely ineffective. As a cause of widespread drug resistance, we found a high prevalence of various ESBL enzymes, with TEM- and CTX-M-1-types predominating. More than half of the gram-negative isolates harbored two or more ESBL genes. In addition, carbapenemases were detected in 7.4% of gram-negative isolates, despite the limited availability and infrequent use of carbapenems in the country. These findings underscore the need for regular microbiological testing of appropriate specimens before initiating empiric antibiotic treatment wherever possible. Therefore, there is an urgent need to strengthen AMS, take appropriate measures to regulate antibiotic use and monitor the emergence of resistant bacteria. In this study, we reported a low positivity rate (5.4%) for BCs, indicating the need for a new diagnostic approach such as plasma cell-free DNA sequencing or the use of MDW- or PCT-guided blood culture to improve the positivity rate.

Abbreviations

3GC:3Rd generation cephalosporin; AST:Antimicrobialsusceptibility testing; BC:Blood cultures; CP:Carbapenemase; CTX-M:Cefotaximase-Munich;EDTA: Ethylene diamine tetraacetic acid; ESBL:Extended-spectrum β-lactamase; EUCAST:European Committee on AntimicrobialSusceptibilityTesting;GNB: Gram-negative bacteria; IMP:Imipenemase metallo-β-lactamase; KPC:K. pneumoniae Carbapenemase; MBL:Metallo-beta-lactamase; MDR:Multidrug resistance; MDW:Monocyte distribution width; NDM:New Delhi metallo-β-lactamase; OXA:Oxacillinase;PCR:Polymerase chain reaction; SHV:Sulfhydryl variable;SOPs:Standard operating procedures; TEM:Temoniera;VIM:Verona imipenemase.

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Authors' contributions

TBT:Conception and design, performance of laboratory investigation acquisition, analysis,and interpretation of data, drafting of the manuscript and approval of the manuscript for publication. CM:Interpretation of data, critical revision of the manuscript and approval of the manuscript for publication. HMO:Conception and design, revision of the manuscript and approval of the manuscript for publication. TW:Supervision of the laboratory investigations, interpretation of the results and approval of the manuscript for publication. TN:Conception and design, revision of the manuscript and approval of the manuscript for publication. SA:Performance of laboratory investigations and approval of the manuscript for publication. ZH:Interpretation of data, revision of the manuscript and approval of the manuscript for publication. AS:Conception and design, revision of the manuscript and approval of the manuscript for publication. MB:Conception and design, revision of the manuscript and approval of the manuscript for publication. DH:Interpretation of data, critical revision of the manuscript and approval of the manuscript for publication. KP: Interpretation of data, criticalrevision of the manuscript and approval of the manuscript for publication. TL:Criticalrevision of the manuscript and approval of the manuscript for publication. AF:Conception and design, acquisition and interpretation of the data, revision of the manuscript and approval of the manuscript for publication. TF:Conception and design, interpretation and analysis of data, criticalrevision of the manuscript and approval of the manuscript for publication. Allauthors read and approved the final manuscript.

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Availability of data and materials

Allrelevant data generated or analyzed during this study are included in this article.

Declarations

Ethics approval and consent to participate

The appropriate ethical review boards of ArsiUniversity,Ethiopia (reference number A/U/H/S/C/120/6443/2017),the Oromia Regional Health Bureau, Ethiopia (reference number BEFO/AHBTFH/1-8/2017),and DüsseldorfUniversity Hospital,Germany (reference number 5729) approved the study. Ethical clearance for sample transportation between Ethiopia and Germany was obtained from the National EthicalReviewBoard of the Ethiopian Ministryof Science and Technology (reference number 310/204/2017). Beforethe start of the study procedures, written informed consent to participate in the study was obtained from each study participant or, in the case of minors, from their legal guardians.

Consent for publication

The informed consent form signed by each study participant or, in the case of minors, the legal guardians contained consent for publication of anonymized data.

Competing interests
The authors declare that they have no competing interests.

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Tafese B. Tufa's contribution to this paper:

a) Draft the coceptuals of the work
b) Perform the laboratory activities
c) Data analysis and draft the first manuscript

- As described in our work 2.5 (page 67-79), the prevalence of ESBL-producing bacteria among clinical isolates was very high (81.5%). Therefore, for the first time, we conducted a study to determine the role of flies in the spread of AMR in the hospital.
- The flies caught on hospital site were colonized with high proportion of ESBL-producing bacteria (67%), whereas ESBL-producing bacteria colonization was very low (2%) in flies collected from a butcher located 1.5 km from the hospital.
- The flies may be a relevant factor in the spread of MDR microbes in hospitals or hospitals surrounding`s in tropical region.

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Antimicrobial Resistance and Infection Control

RESEARCH Open Access

Carriage of ESBL-producing Gram-negative bacteria by flies captured in a hospital and its suburban surroundings in Ethiopia

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Abstract

Background: Local data from the Asella Teaching and Referral Hospital in the town of Asella, Ethiopia reveal a high prevalence of extended-spectrum β-lactamase- (ESBL) producing Gram-negative bacteria (GNB) in clinical isolates. To investigate a possible route of transmission, we determined the proportions ESBL-producing GNB in isolates from flies caught in the hospital and in the town of Asella.

Methods: Flies were collected in August 2019 from the neonatal intensive care unit (NICU), the orthopedic ward, the hospital's waste disposal area, and from a butchery situated 1.5 km from the hospital. After trapping, the flies were macerated and suspended in sterile normal saline. The suspensions were inoculated on MacConkey agar and incubated overnight. Species identification and antimicrobial susceptibility testing were performed using Vitek®-MS and VITEK® 2.

Results: In total, 103 bacterial isolates were obtained from 85 flies (NICU: 11 isolates from 20 flies, orthopedic ward: 10 isolates from 12 flies, waste disposal area: 37 isolates from 26 flies, butchery: 45 isolates from 27 flies). The proportions of ESBL-producing bacteria among isolates obtained from flies collected in the hospital compound were significantly higher (82%, 90%, and 57% in NICU, orthopedic ward and waste disposal area, respectively) compared to flies collected outside of the hospital compound (2% (@1/45) in the butchery) ($p \leq 0.001$). The proportion of ESBL was 67% (6/9) among Raoultella spp. 67% (4/6) among Kluyvera spp., 56% (5/9) among Enterobacter spp., 50% (5/10) among E. coli, and 44% (8/18) among Klebsiella spp. Of the 40 ESBL-genes detected, 85% were CTX-M-like, 83% TEM-like, 23% SHV-like, and 2% CTX-M-2-like. ESBL-producing bacteria showed higher rates of resistance against ciprofloxacin (66% vs. 5%), gentamicin (68% vs. 3%), piperacillin-tazobactam (78% vs. 5%), and trimethoprim-sulfamethoxazole (88% vs. 16%), compared to non-ESBL-producing bacteria.

Conclusion: A high proportion of ESBL was identified in isolates from flies caught in the hospital compound compared with isolates of flies collected at a distance of 1.5 km from the hospital. Flies can be potential vectors for transmission of multidrug-resistant (MDR) bacteria within hospitals. Further studies are needed to determine the source of MDR colonization in flies and possible impact of MDR for nosocomial infections.

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Keywords: Flies,Colonization,Multidrug resistance, Transmission,One health, Africa,Antimicrobialresistance

Introduction

The flies have been proposed to be a potential vector for communicable diseases and multidrug resistance (MDR) in hospitals, particularly in developing countries [1, 2]. Flies can transmit MDR microorganisms in the three ways: mechanical translocation, regurgitation (bio-enhanced transmission) and defecation, in which MDR bacteria may become a part of the gut flora of flies thus carrying bacteria for the life span of the fly and contaminating their environment via feces and/or regurgitation. If flies play a role in AMR transmission, current hospital hygiene programs, focusing on patient isolation, hand hygiene, and antimicrobial stewardship programs, may not be sufficient to address the expansion of antimicrobial resistance (AMR), especially in resource limited, and possibly fly-abundant, settings [1, 3].

The expansion of MDR due to extended spectrum β-lactamase- (ESBL) production in Gram-negative bacteria (GNB) has become an emerging threat to antibiotic treatment success in resource limited settings. ESBLs are enzymes, encoded by genes often found on mobile genetic elements, which mainly include class A β-lactamases, such as CTX-M-type, TEM, and SHV and they confer resistance to the penicillin and cephalosporin antibiotic classes. CTX-M-type β-lactamases are the most abundantly found ESBLenzymes worldwide [4].

Ceftriaxone and ceftazidime are the most commonly used antibiotic substances for the treatment of blood stream infections caused by GNB at the study site. Local data from the Asella Teaching and Referral Hospital (ATRH) reveal a high prevalence of ESBL-producing GNB in clinical isolates, hampering the efficacy of empiric antibiotic therapy [5]. To our knowledge, there are no reports on the colonization rate of flies with ESBL-producing bacteria in Ethiopian hospitals and the possible implications for the spread of AMR. A prospective study was therefore initiated to investigate the colonization of flies with ESBL-producing GNB at the ATRH compound and in Asella town.

Methods and materials

The flies were collected in August 2019 in the ATRH's neonatal intensive care unit (NICU), the orthopedic ward, the hospital's waste disposal area, and in a butchery located 1.5 km from the hospital. The flies were trapped with non-toxic retail flycatchers (Profissimo® Giftfreier Fliegenfänger, Germany) and stored in 2 ml of sterile normal saline within the same day.

For further analysis in this study, only animals displaying essential taxonomic morphologic criteria of flies such as size, shape and color were selected.

After maceration in sterile saline the suspensions were inoculated on MacConkey agar and incubated at 37 °C for 18–24 h. All colonies growing on MacConkey agar were isolated and sub-cultured for species identification. All phenotypically different colonies obtained from one fly were subcultured. The isolates were preserved at − 81 °C in the Microbank® vials (Pro-Lab Diagnostics Inc., Toronto, Canada) and transported to Germany for identification using MALDI-ToF-MS (VITEK®-MS, bioMérieux, Marcy-l'Étoile, France) and antimicrobial susceptibility testing (AST) with VITEK® 2 (bioMérieux) and Kirby-Bauer for confirmation of some results by VITEK 2 (performed at the Institute of Medical Microbiology and Hospital Hygiene, Heinrich Heine University Düsseldorf, Germany). All results were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) version 2018 V8.0. All bacteria positive in the VITEK ESBLphenotype screening were subjected to molecular detection using PCR as detailed below.

After identification, the bacterial DNA was prepared by producing a suspension of a pure colony from MacConkey agar in 200 µL of Tris–EDTA pH 7.5. The suspension was then heated at 95 °C for 10 min, followed by centrifugation at 10,000 rpm for 2 min. Then 150 µl the supernatant was transferred to the new 1.5 ml tube and was stored at − 20 °C until PCR testing.

Identification of bacterial resistance genes was performed by PCR of ESBL-gene sequences common to groups of ESBL types. Bacterial strains with suspected production of ESBL were investigated by PCR, following the protocols described by Strauß et al. for identification of the β-lactamase (bla) CTX-M, bla_{SHV} and bla_{TEM} genes [6].

The frequency of ESBLgenes detected in isolates from flies' colonization was compared with the frequency of ESBLgenes previously detected in clinical isolates from patients with acute infectious diseases or sepsis from the same hospital (unpublished data). Escherichia coli and Klebsiella pneumonia were the most common GNB isolated from blood, urine and wound swabs which were used as the clinical isolates to compare the proportion of ESBL frequency with GNB colonized flies. The same method was followed for identification and AST result interpretation for the bacteria isolated from clinical samples and flies.

IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA) was used for statistical analysis.

Results

A total of 103 bacterial isolates were obtained from 85 flies (NICU: 11 isolates in 20 flies, orthopedic ward: 10 isolates in 12 flies, waste disposal area: 37 isolates in 26 flies, butchery: 45 isolates in 27 flies). Klebsiella spp., and Proteus spp. were among common pathogenic bacteria isolated in the butchery. However, nearly half of the bacteria isolated from flies caught at the butchery in Asella town were not commonly pathogenic for humans. The frequency of ESBL-production among isolated bacteria from flies caught at the different study sites was variable (see Table 1).

Table 1 Frequency of ESBL-producing Gram-negative bacteria in 85 flies caught in at ATRH and Asella town, Ethiopia

Site	Isolates (n)	Rate of ESBL-n (%)
NICU	11	9 (82%)
Orthopedic ward	10	9 (90%)
Waste disposal area	37	21 (57%)
Butchery	45	1[a] (2%)

[a] We found only a single Escherichia coli ESBL producing from the butchery

The proportion of ESBL producing bacteria among GNB isolates from flies was 9 (82%), 9 (90%), and 21 (57%) in NICU, orthopedic ward and waste disposal area in the hospital's compound, respectively. Only one isolate with ESBL-carriage was identified in flies from the butchery. Overall, the proportion of ESBL-producing bacteria colonized flies in the hospital compound was 39 (67%). The different colonization rates with ESBL of flies trapped inside and outside the hospital compound was statistically significant ($p \leq 0.001$) (see Fig. 1).

The proportion of ESBL expression was 67% (6/9) in Raoultella spp., and 67% (4/6) in Kluyvera spp., 56% (5/9) in Enterobacter spp., 50% (5/10) in E. coli and Citrobacter spp. and 44% (8/18) in Klebsiella spp., respectively (Table 2).

Among bacteria carrying ESBL isolated from flies in this investigation, 85% (n = 34) carried CTX-M-like, 83% TEM-like (n = 33), 23% SHV-like (n = 9) and 1 CTX-M-2-like genes. CTX-M-9- and CTX-M-8/25-like genes were not detected. The comparison of the detection frequency of the different major ESBL genes of GNB isolated from blood, urine and wound swab samples between 2016 and 2019 from the same hospital (own data, not published) and of bacterial isolates from flies shows clear similarities

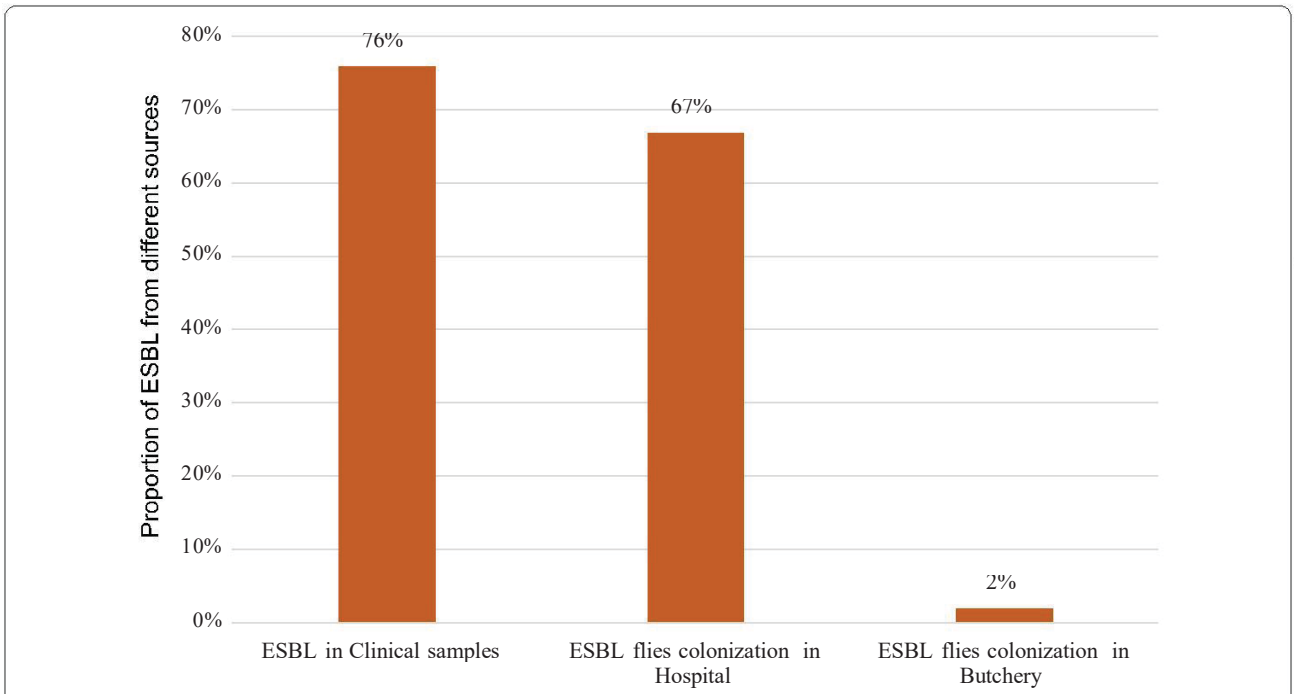

Fig. 1 Comparison of ESBL frequency in clinical samples and flies colonization. Source of clinical samples data: ESBL in clinical isolates of Gram-negative bacilli at Asella teaching and referral hospital, central Ethiopia (2016 to 2019). The proportion of ESBL flies colonization in hospital was significantly different compared with ESBL flies colonization in butchery (67% vs. 2%; P value < 0.001)

Table 2 Proportion of ESBL expression among GNB isolates from flies trapped from hospital compound and butchery

Bacterial species	ESBLpositive n (%)	ESBLnegative n (%)	Total n
Raoultellaspp.	6 (67%)	3 (33%)	9
Kluyveraspp.	4 (67%)	2 (33%)	6
Enterobacterspp.	5 (56%)	4 (44%)	9
Escherichiacoli	5 (50%)	5 (50%)	10
Citrobacterspp.	5 (50%)	5 (50%)	10
Klebsiellaspp.	8 (44%)	10 (56%)	18
Providenciaspp.	2 (29%)	5 (71%)	7
Proteusspp.	1 (13%)	7 (87%)	8
Moellerellawisconsensis	1 (10%)	9 (90%)	10
Others[a]	3 (30%)	7 (70%)	10

[a] Others (one isolate each): Comamonas testosteroni, Pantoea agglomerans, and Rahnella aquatilis (ESBLexpression); Aeromonas hydrophila, Cedecea davisae, Hafnia alvei, Leclerciaadecarboxylata, Lelliottiaamnigena, Serratia liquefaciens, and Yokenellaregensburgei (no ESBLexpression); Even though we found four isolates of Acinetobacter spp. from flies, the proportion of ESBLwas not analyzed in this study. As described in (Fig. 1) above, the proportion of ESBLwas near to similar in GNBisolated from clinical samples and flies caught in hospital compound. However, it was extremely low 1 (2%) in flies caught in butchery

(Table 3). However, CTX-M-9 was only detected from clinical specimens and CTX-M-2 was only detected from isolates from flies.

Among isolated bacteria, phenotypical AMR against non- β-lactam antibiotics used for treatment of other ESBL-producing bacteria was very high. ESBL-producing bacteria showed a higher rates of AMR against ciprofloxacin (66% vs. 5%, $p<0.001$), gentamicin (68% vs. 3%, $p<0.001$), piperacillin-tazobactam (78% vs. 5%, $p<0.001$), and trimethoprim-sulfamethoxazole (88% vs. 16%, $p<0.001$) compared to non ESBL-producing bacteria (Fig. 2).

Discussion

Recently, flies were recognized as potential vectors for AMR in hospital and non-hospital environments [7, 8]. In the study center, the proportion of ESBL-producing GNB isolated from clinical samples was very high (Fig. 1). We conducted this study in order to compare the colonization of flies with ESBL-producing GNB at various locations inside and one location with high density of flies outside of the hospital compound. We found a high proportion of ESBL-producing bacteria among isolates from flies collected inside the hospital compared to the near absence of ESBLgenes in bacterial isolates from flies collected 1.5 km away from the hospital. The ESBL proportion was highest at the NICU and at the orthopedic ward, and only slightly lower at the hospital waste disposal area (Table 1). Our findings could partly be explained by exposition of bacteria to different antibiotics in the environment of the hospital or more likely by the accumulation of resistant bacteria in and on flies in the patients' environment. Similar to our findings, a study conducted in Iran shows that bacterial isolates from houseflies in a hospital compound had a significantly higher frequencies of antimicrobial resistance against various antibiotics, than bacteria isolated from houseflies in non-hospital environment [7]. A study conducted in Berlin, Germany showed that the prevalence of ESBL in flies trapped from two different residential areas differed (0% vs. 18%) [9]. According to this study, the distribution of ESBL-producing bacteria among flies in certain geographical locations is not uniform.

In our study, the proportion of ESBL-producing GNB was very high among common pathogenic bacteria like *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., Citrobacter spp., and *Raoultella* spp. compared with opportunistic bacteria. Kluyvera spp. are opportunistic bacteria with the highest rate of ESBL-production (Table 2). Half of the bacteria isolated from the butchery in Asella town were not commonly pathogenic bacteria. This different distribution of bacteria colonizing the isolated flies might also influence the proportion of ESBL isolates from different sites based on pathogenicity of the bacteria and their exposure to cephalosporin antibiotics [10].

In this study, the most frequently detected resistance genes in confirmed ESBL-producing GNB colonized flies were CTX-M-1-like gene, followed by TEM-like gene and SHV-like gene, respectively. The frequency and characterization of ESBL genes of clinical samples and flies isolates showed similarities (Table 3) [11] and also similar findings reported by Boulesteix et al. [12] from

Table 3 Comparison of frequency and characterization of ESBLgenes from clinical isolates (n = 32) and isolates from flies (n = 40)

	ESBLgenes					
	Total	CTX-M-1 n (%)	TEM n (%)	SHV n (%)	CTX-M-9 n (%)	CTX-M-2 n (%)
ESBLin clinicalisolates	32	26 (81%)	22 (69%)	7 (22%)	2 (6%)	0
ESBLin isolates from flies	40	34 (85%)	33 (83%)	9 (23%)	0	1 (3%)

ESBL,extended spectrum β-lactamases; CTX-M,cefotaximase-Munich; SHV,sulfhdryl variable; TEM,Temoniera

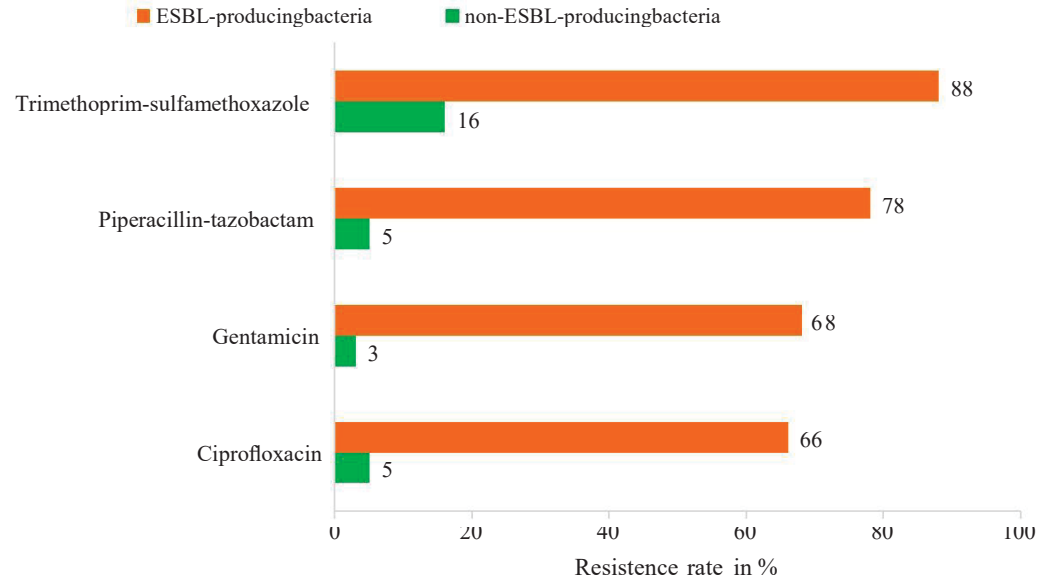

Fig. 2 Resistance rate among ESBL-(n = 40) and non-ESBL-producing(n = 62) Gram-negative bacteria isolated from flies

Dakar, Senegal. This suggests that flies may acquire the bacteria from the hospital environment. Similar findings were reported by Fotedar et al. [13]; however, to clearly identify the source of the ESBL-producing bacteria on flies needs further investigation. On the basis of our results, no statement can be made on the question of whether flies can be considered as vectors for MDR bacteria. In this context, however, it is interesting to note that Rahuma et al. [14] reported earlier that flies may be potential vectors for the transmission of MDR bacteria from hospitals to surrounding communities. This may suggest that flies colonized with ESBL-producing bacteria found in the hospital compound can possibly spread AMR to surrounding residential areas or restaurants, thereby endangering public health [15]. As described in Table 2, not only well-known pathogenic bacteria but also opportunistic bacteria can carry clinically relevant resistance genes and enhance the spread of AMR in the community.

Published investigations from Ethiopia demonstrate that MDR GNB commonly express the $bla_{CTX\text{-}M\text{-}1}$ gene encoded in ESBLs and the $bla_{NDM\text{-}1}$ gene in carbapenemase-producing bacteria [5, 16–18]. For molecular detection of ESBL, $bla_{CTX\text{-}M\text{-}1}$ ESBL gene can be used as target gene by either conventional PCR or a loop-mediated isothermal amplification (LAMP) technique, which is rapid, effective and affordable to detect the presence of $bla_{CTX\text{-}M\text{-}1}$ ESBL gene in RLS like Ethiopia [19].

In this study, the susceptibility to non-β-lactam antibiotics such as ciprofloxacin, gentamicin, and trimethoprim-sulfamethoxazole was significantly lower in ESBL-producing bacteria compared to ESBL-negative bacteria ($p < 0.001$). This finding is an indicator for the limited options of appropriate antibiotic therapy regimen for ESBL-producing bacterial infection management. Poudel et al. [20] also reported high rates of resistance of E. coli and K. pneumoniae isolated from flies against tetracycline and ampicillin due to emergence of ESBL-producing strains. This might probably be caused by plasmid-mediated mobile resistance genes such as quinolone-resistance (qnr) genes, aminoglycoside acetyltransferase (aac), dfr (trimethoprim resistance) and sul (sulfamethoxazole resistance) genes, being more frequent in ESBL-producing bacteria compared to ESBL-negative bacteria [21–24]. However, the identification of other resistance genes than the described ESBL genes was not part of this investigation.

In tropical regions where poor hospital hygiene is common, hand hygiene and patient isolation or implementation of antimicrobial stewardship programs may not be sufficient to control the expansion of AMR. Our findings can be considered as indicators for a possible dissemination of antimicrobial resistance inside and outside of hospital compounds and to the nearest environments by flies. Therefore, to tackle the expansion of ESBL-producing bacteria, fly-control measures in critical areas of the hospitals might be essential [7, 15]. In order to inhibit further expansions of ESBL-carrying bacteria from hospitals to residential areas, environmental and health professionals and municipality administration should work together and strengthen a one health approach. Future AMR prevention and control protocols may consider

screening of flies for AMR and eradication-measures to control the population density of flies at health care facilities in tropical regions [20]. As distribution of ESBL genes in clinical samples and flies caught in the hospital show comparable results, flies might be used as an indicator organism for ESBL-prevalence in hospital facilities.

Our study has certain limitations. Fly species identification was not performed and we recognize that the different species Musca sorbens and Musca domestica have a different life style and thus may be involved in bacterial transmission to different degrees. Nevertheless, an identification of the different species in this study is unlikely to have an impact on the results in general. ESBL-colonization in the community was reported as low, but flies were sampled from a single butchery only. Even though the colonization with ESBL to be common in flies at the hospital, the source of the ESBL was not addressed. The study design also fails to point out whether the external organs like legs and mouth or the gut of the flies are more involved in carrying ESBL-producing bacteria, a factor with possible impact for the transmission of the bacteria. The role of flies in transmission of nosocomial infections and the source of ESBL-producing bacteria in the hospital needs further investigation.

A further limitation is the use of non-selective media for the screening process in flies and the fact that we tested phenotypically different isolates obtained from one fly (in some case we found more than one bacteria from a single fly). This procedure was chosen in order to harmonize the study protocol with diagnostics in the clinical setting. In consequence, our data reflect the proportion of ESBLamong GNB isolates from flies, but not the prevalence of ESBL-carriage among flies, which might have been higher, if a selective screening approach was used. However, even with the non-selective culture technique used, ESBL-producing bacteria were detected in a major proportion of flies.

Conclusions and recommendations

A high proportion of flies trapped within the hospital compound were colonized with ESBL-producing bacteria, whereas ESBL-production was nearly absent among flies collected in a butchery 1.5 km away from the hospital. The flies may be a relevant factor in the spread of MDR microbes in hospitals or hospitals surroundings. Antibiotic susceptibility to ciprofloxacin, gentamicin, and trimethoprim-sulfamethoxazole was lower in ESBL-producing bacteria compared with ESBL-negative bacteria which can limit treatment options. Antimicrobial resistance prevention and control protocols should consider the role of flies in hospitals in tropical regions. Our findings warrant the need of a one health approach to minimize the spreading of MDR strains to the environment. Further studies are needed to determine the role of flies as vectors for MDR nosocomial infections.

Abbreviations

aac: Aminoglycoside acetyltransferase; AMR: Antimicrobialresistance; AST: Antimicrobialsusceptibility testing; ATRH: AsellaTeaching and ReferralHospital; CTX-M: Cefotaximase-Munich; dfr: Dihydrofolate reductases; EDTA: Ethylene diamine tetraacetic acid; ESBL: Extended spectrum β-lactamase; EUCAST: European Committee on AntimicrobialSusceptibilityTesting; GNB: Gram-negative bacteria; LAMP: Loop-mediated isothermal amplification; MALDI-TOF: Matrix-assistedlaser desorption/ionization time-of-flight mass spectrometry; MDR: Multidrug-resistant; NDM: New Delhi metallo-β-lactamase; NICU: Neonatal intensive care unit; PCR: Polymerase chain reaction; qnr: Quinolone-resistance; RLS: Resource limited settings; sul: Sulfamethoxazole resistance gene; SHV: Sulfhydrylvariable; TEM: Temoniera.

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Authors' contributions

Tafese Beyene Tufa: Conception and design, acquisition and analysis of the data, interpretation of data, drafting of the manuscript and approval of the manuscript for publication. Andre Fuchs: interpretation of data, revision of the manuscript and approval of the manuscript for publication. Tobias Wienemann: Analysis of the data, performance of laboratory investigations and approval of the manuscript for publication. YannikEggers: Interpretation of data, drafting of the manuscript and approval of the manuscript for publication. SileshiAbdissa: Performance of laboratory investigations and approval of the manuscript for publication. Marlen Schneider: Interpretation of data, revision of the manuscript and approval of the manuscript for publication. Björn-ErikOle Jensen: Conception and design, criticalrevision of the manuscript and approval of the manuscript for publication. Johannes G. Bode: Interpretation of data, criticalrevision of manuscript and approval of the manuscript for publication. KlausPfeffer: Interpretation of data, criticalrevision of the manuscript and approval of the manuscript for publication. Dieter Häussinger: Interpretation of data, criticalrevision of manuscript and approval of the manuscript for publication. ColinMackenzie: Interpretation of data, criticalrevision of the manuscript and approval of the manuscript for publication. Hans Martin Orth: Conception and design, drafting of the manuscript and approval of the manuscript for publication. Torsten Feldt: Interpretation of data, criticalrevision of the manuscript and approval of the manuscript for publication. Allauthors read and approved the final manuscript.

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Availability of data and materials

Alldata generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

Localethical clearance from ArsiUniversityand national ethical clearance from Ethiopian MinistryScience and Technology was obtained for transportation of bacteria isolates from Ethiopia to Germany.

Consent for publication

For bacteria isolated from clinicalsamples written informed consent of patients was granted.

Competing interests

The authors declare that they have no competing interests.

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Publisher's Note

3. Discussion

AID and sepsis are among the leading causes of morbidity and mortality in SSA, although most are preventable and treatable. We initiated this study because we have observed that febrile illnesses ranked the third among diseases in the local hospital admission register. The treatment of patients with suspected AID are challenging with existing resources. The major factors for unfavourable outcome are a) unknown aetiologies, b) lack of local epidemiologic data on possible pathogens and AST, c) lack of basic laboratory diagnosis, d) lack of microbiology result logistic system and e) difficulties to guide medical training. The overall, comprehensive burden of infectious diseases had not been previously studied in this region. Therefore, this study is the first to provide comprehensive data on common causes of AID with their AMR profile. We have also identified non-culturable pathogens using multiplex PCR.

Regular accurate epidemiological data of infectious diseases are important for appropriate medication and control of the diseases during this genomic sequencing era. They are important not only for the patient management, but also for the population to combat transmission of the diseases (41). To this end, regional and national surveys and good documentation of aetiologies and resistance patterns are critical in order to update the existing health system by policy makers (42). This also needs to be supported by rigorous data and well-designed studies. We understand that this requires both resources and technology, which is a major problem in low-income countries such as Ethiopia, where a wide range of these diseases occurs. Applying empirical management in the absence of epidemiological data of pathogens and effective antibiotic therapy to the specific disease is almost considered as a double-blind decision. Therefore, the generation of cumulative, meaningful data through national and international collaboration should be prioritized to solve resource constraints and provide better health services. This is the main reason for which we have diagnosed a broad spectrum of infectious diseases with our German partner.

Infectious disease epidemiological data may vary from region to region based on geographic location or other contributing factors such as prevalence of HIV, non-communicable diseases, population culture and lifestyle, personal and environmental sanitations, malnutrition, immunization coverage, immigration, climate changes and the country's health care system (43, 44). In this study, we focused on BSIs and acute febrile illnesses and offered blood cultures and blood smears to all participants. We also performed a multiplex PCR in culture- negative patients targeting *Plasmodium* spp., *Borrelia* spp., *Rickettsia* spp., *Leptospira* spp., and arboviruses such as *Pan Flavivirus* and *Chikungunya*, and we did not detect any of *Leptospira*, *other Flavivirus*,

and Chikungunya (Figure 3). We found a total of 66 pathogenic bacteria in blood and other clinical specimens using culture and 32 non-culturable pathogens (*Plasmodium* spp., n=13; *Borrelia* spp, n=12; and *Rickettsi*a spp., n=7) by multiplex PCR or blood smear. The total percentage of patients in whom infectious agents were detected was 14.3%. A limitation in this study is that we did not include sputum cultures and specific testing for tuberculosis, invasive fungal infections, and other viral infections.

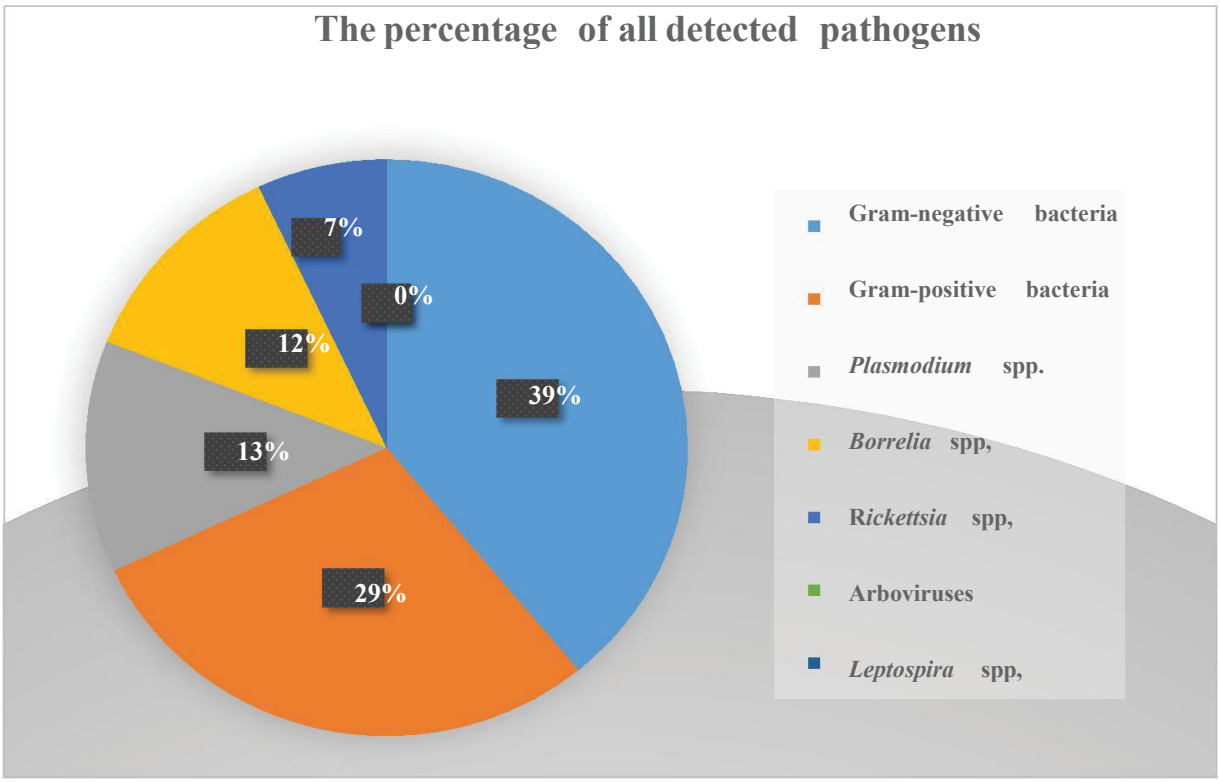

Figure 3. Pathogenic organisms (n=98) detected from febrile patients at Asella Referral and Teaching Hospital, Ethiopia.

Of the three pathogens detected by multiplex PCR in blood culture-negative individuals, 88% (22/25) causative agents of malaria and relapsing fever were diagnosed by blood smear. Relapsing fever, which is transmitted by lice, is known to be endemic in East African countries such as Somalia, Eritrea, and Ethiopia and is sometimes detected in Europe in immigrants from these countries (45). The main gaps we found are that since Asella is located at high altitude (2400 m above sea level), the blood smear test may not be used in all patients with febrile illness. The second important point is that diseases caused by *Rickettsiae* spp. may be underdiagnosed or misdiagnosed, although the diseases are easily treatable. As a solution, a species-specific serology test or a molecular test is needed at the study area.

On the other hand, according to our findings, the rate of positive blood cultures was low (5.4%) and some commonly expected bacteria were not isolated. The blood culture positivity rate is lower than reported from east Africa by Maze 2018, who found that bacterimia among febrile patients was 10.4% (1), but similar to a recent report from southern part of Ethiopia by Shimelis T, et al., (2020) (46). For example, only a single Salmonella bacterium was isolated withs standard blood culture in our study. We could not get Brucella spp growth. However, in the study area, it is common for febrile patients with suspected typhoid or brucellosis to be treated empirically with antibiotics. This finding can be used as a basis for further studies and allows clinicians to guide empirical therapy decisions. The positivity rate of blood cultures could be enhanced by the selection of patients being eligible for blood culture diagnostics. The use of other parameters such as procalcitonin or monocyte distribution width (MDW) could help guide the rational use of blood culture (47).

Based on our results, appropriate rapid tests and new molecular techniques such as next generation sequencing of cell free DNA and RNA can be a valuable complementation to the standard diagnostic methods in order to provide baseline data, covering a broad range of infectious diseases. The innovative molecular techniques have the potential to contribute to the region-specific understanding of febrile events by detecting bacteria, fungi, viruses, and parasites in a non-targeted diagnostic approach (48).

In another way, the emergence of AMR continues and health care facilities should implement AST to provide a standard infectious diseases management and combat the spread of AMR (49). In our study, resistance rates of isolated GNB to common antibiotics was high, and as consequences severely limiting the efficacy of aminopenicillins in combination with beta-lactamase inhibitors or 3GC for empiric treatment of infections possibly caused by GNB. The lowest resistance rates were shown for Gram- positive bacteria and for the antibiotics meropenem and amikacin. These results are consistent with those of other recently published data from different parts of Ethiopia, particularly Addis Ababa (50, 51), Jimma (52) and Bahir Dar (53). The different resistance rates to certain antibiotics also reflect the frequency of antibiotic prescriptions. While aminopenicillins and 3GCs are applied very frequently, carbapenems are hardly used due to their high cost and limited availability. Of notice, while resistance rate against amikacin was very low, many of the isolated GNB harbored gentamicin resistance, another antibiotic of the same class of aminoglycosides. This difference might be explained by the frequent application of gentamicin at the study center. In contrast, amikacin is virtually not applied at all (54, 55).

Our data revealed overall frequencies of ESBL and CR production of 81.5% and 7.5% among the isolated GNB, respectively. Of notice, all isolates of *K. pneumonia* were ESBL-positive. These findings are consistent with recently published data from other parts of Ethiopia, where *K. pneumoniae* has also been shown to be the most common ESBL-expressing pathogen, followed by *E. coli* (50, 51, 53, 56).

Regarding the characterization of ESBL enzymes among the Gram-negative isolates in our study, TEM-type and CTX-M-1-type were most common, followed by SHV-type and least frequently CTX-M-9-type. CTX-M-2 and CTX-M-8/25 were not detected at all. Compared to TEM-type and SHV-type, CTX-M-type enzymes are more widely disseminated in the world and many variants which are associated with functional diversity with clinical relevance have been described. Thus, co-expression of different ESBL types are more common among GNB which harbor CTX-M-type enzymes (57).

Both, CTX-M-1 group and SHV-group were abundantly detected in *K. pneumonia,* whereas in *E. coli* SHV-group was detected only in 8% of the isolates. This finding matches the report by Ogutu et al. that SHV-type is the predominant ESBL enzymes in *K. pneumoniae and* TEM-type is the most prevalent in *E. coli* (58).

A single $bla_{\text{NDM-1}}$ CR encoding gene in a *K. pneumoniae* isolate and $bla_{\text{NDM-1}}$ plus $bla_{\text{OXA-51}}$ CPE encoding genes in an *A. baumannii isolate were detected*. The expression of a $bla_{\text{NDM-1}}$ in an *A. baumannii* isolate has previously been reported from the southwestern part of Ethiopia (59), but to our knowledge to date, not case of an expression of $bla_{\text{NDM-1}}$ in *K. pneumonia* was reported. In general, this finding is not surprising since $bla_{\text{NDM-1}}$ CR encoding gene expression in *K. pneumonia and A. baumannii* have been commonly reported from other eastern African countries like Kenya, Uganda (60), Egypt (61) and Sudan (62).

In contrast to GNB, MDR was less common among Gram-positive isolates in the study area. Among the Gram-positive isolates, the prevalence of MRSA was 5% in our study. The resistance profile of 19 *S. aureus* isolates was 95% and 68% to penicillin and trimethoprim-sulfamethoxazole, respectively according to VITEK-2 results (Figure 3). Compared with the literature in high-income countries (63), the 68% resistance of *S. aureus* to trimethoprim-sulfamethoxazole is exceptionally high, and similar results have been reported

from other parts of Ethiopia (64, 65). This warrants further investigation, as it may be related to the intensive use of this antimicrobial agents for prophylaxis in HIV-infected patients with low CD4 counts. Nevertheless, ineffective empirical treatment, which was 72.2% in the case of GNB, was not quantified in the study area for Gram-positive isolates.

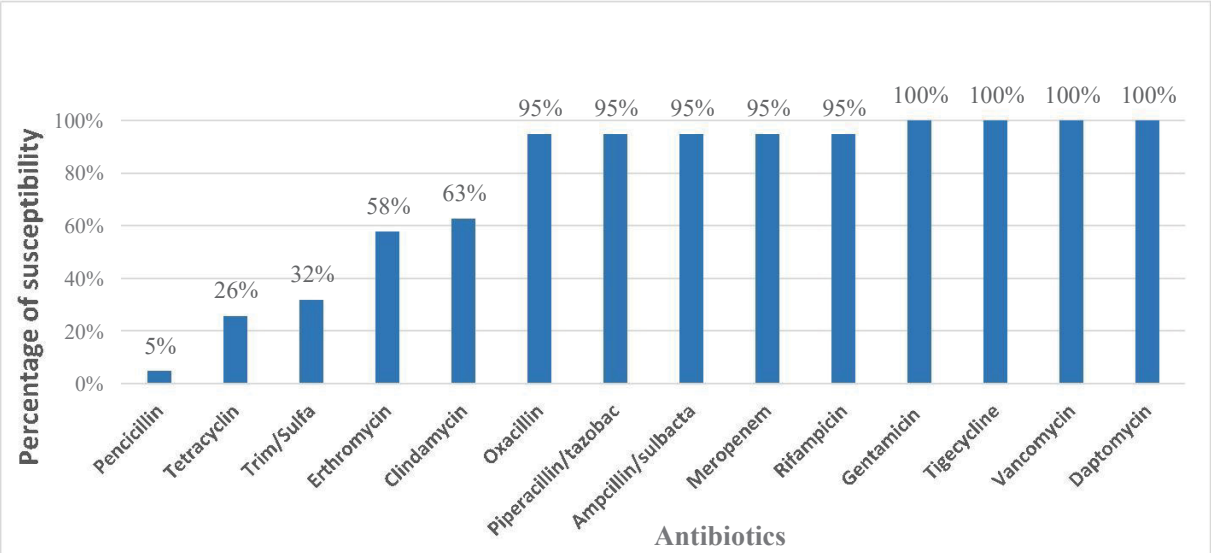

Figure 3 Antimicrobial susceptibility test (VITEK2) result of *Staphylococcus aureus* (n=19) isolates from febrile patients at Asella Referral and Teaching Hospital, Ethiopia.

In this study, most of the antibiotics initiated by the treating physicians for empirical treatment in the participating patients were ineffective according to AST. However, these data have to be interpreted with caution since mostly microbiological culturing was performed after initiation of empirical antibiotic therapy and evaluation of the success rate of the empirically initiated antibiotic therapies was not part of this investigation. Nevertheless, antibiotic resistance endangering the success of empirical antibiotic therapies seems alarmingly common and local epidemiological resistance data should be taken into account before initiation of antibiotic therapy (66). These results also indicate a possible need to adapt the strategy for empirical antibiotic therapy. An adaption could be all the more necessary as the excessive use of 3GC could be one of the main reasons for the spread of ESBL-producing bacteria (67).

Selectively utilizing antibiotics based on the AST result is not only favorable for optimal treatment success, it also plays a major role in combating the spread of MDR bacteria. Adequate microbiological culturing before initiation of empirical antibiotic treatment is necessary to enable AST-based antibiotic therapy. As resources are limited and supply of laboratory materials is unreliable at the study site as at many other sites in low-income countries, it may not always

be possible to perform comprehensive microbiological testing. In such cases, at least surveillance studies with the subsequent establishment of resistance statistics should be carried out to enable calculated antibiotic therapies adapted to the local resistance status. This might help to reduce the imprudent use of antibiotics (68).

Conclusions and recommendations

This dissertation is an attempt to describe and address the extensive burden of AID in Arsi Zone, Asella, Ethiopia. Including a case study, literature review and meta-analysis related to the project, we have so far published six articles in peer reviewed international journals and shared papers to authorized bodies addressing the existing challenges.

AID and sepsis are common causes of morbidity and mortality in our study site, but remain underdiagnosed in clinical practice. The SOFA score was associated with mortality but is invasive and elaborate to assess and thus not suitable for routine use in LMIC. Our study indicates that the applicability of the qSOFA score or other clinical scores based on examination of the vital signs systolic BP and RR may be adversely affected by shifts in the range of normal values of the vital signs, e.g. as an adaptation mechanism for high altitude of residency. The qSOFA needs further investigation and validation especially in resource-limited health care settings.

The sensitivity of blood culture diagnostics was low in this study and also in line with the literature, and may not be sufficient to diagnose AID at the study site, although the knowledge on pathogens and their resistance patterns are very important for implementing antimicrobial stewardship and medical training, especially in the light of increasing resistance. Of the total 98 pathogenes detected in our study, one third was non- culturable organisms such as *Plasmodium* spp, *Borrelia* spp, and *Rickettsia* spp, which were detected by multiplex PCR from patients' plasma.

We found a high AMR rate, especially in GNB isolates, and 81.5% of them were ESBL positive. Mortality rate was significantly higher in patients with Gram-negative bacterial infections, and 72.2% of empirically initiated antibiotics for these patients were likely ineffective based on local AST results. Antibiotic susceptibility to other antibiotics like ciprofloxacin,

gentamicin, and trimethoprim-sulfamethoxazole was lower in ESBL-producing bacteria compared with ESBL-negative bacteria, which can further limit treatment options. As underlying cause for extended drug resistance, we found a high prevalence of different ESBL enzymes with predominance of TEM- and CTX-M-1-types, and NDH-1 for CR in study area.

The sources and transmission modes of ESBL or CR producing bacteria comprise hospital environmental, gastrointestinal colonization as consequence of ineffective antibiotics utilization and health care providers themselves and hospital hygiene measures are an important component in the fight against AMR. The absence of diagnostic facilities may limit the information of treating physicians about the resistance profile of pathogens. These insufficiencies may lead to more severe consequences in patients with hospital-acquired infection and prolonged stay in a hospital. In resource-limited settings like Ethiopia with confined capacities for regular microbiological investigation, local and national programs for MDR surveillance are important tools to reduce the futile use of antibiotics and improve the treatment outcome of calculated antibiotic therapies.

Recommendations

- Our findings point to the need for a comprehensive assessment of existing clinical tools for the diagnosis of sepsis in resource-limited settings and to develop options for optimization.
- Comprehensive capacity building and research activities are essential to raise awareness of sepsis, to understand its specific features, and to identify potential intervention options.
- Adapting scores based on physiologic parameters such as qSOFA to local differences could improve the performance.
- Blood cultures have a low positivity rate for the diagnosis of BSI and do only cover a limited spectrum of pathogens causing sepsis; so molecular diagnostic techniques such as next-generation sequencing could be important for the complementation of the diagnostic toolbox in the near future.
- The introduction of new strategies such as MDW or procalcitonin guided blood culture could help enable the resource-efficient use of microbiological culture techniques.
- Strengthening antimicrobial stewardship and appropriate strategies to regulate antibiotic uses and monitor the emergence of resistant bacteria are needed immediately.

- Local, updated MDR surveillance data should be considered when antibiotics are prescribed for pateints.
- In facilities where colonization with MDR Enterobacteriaceae is common, intensive surveillance and implementation of antibiotic stewardship, as well as screening for colonization with MDR bacterial strains, should be considered e. g. prior to invasive procedures.
- Antibiotic resistance prevention and control protocols should consider the role of flies in hospitals in tropical regions to prevent transmission e. g. during surgical procedures. A One Health approach within AMR control programs ensures that the spread of MDR bacterial strains in the environment is minimized.

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5. Appendix

5.4. Participant Information Sheet

Annex I a: Participant Information Sheet for adults English version

Date _____

Title: **Identification and characterization of microbial pathogens acute infectious diseases in Arsi Zone of Ethiopia.**

Background: Potentially treatable infections remain the leading cause of death in Ethiopia. No sufficient epidemiological data on causative organisms of infections and their resistance profile to antimicrobial agents or data on determinants of susceptibility and individuals risk factors for unfavorable outcome of infectious diseases is available from Ethiopia or the Arsi Zone in particular. Since facilities for bacterial culturing or molecular biological identification of pathogens are not yet widely established in Ethiopia, some pathogens causing infections in Ethiopia might not have been described before. Existing data suggests high prevalence for resistant bacterial strains. Enhancing knowledge about the epidemiology of causative pathogens of infections such as infections of the skin, the respiratory tract, the urinary, sepsis or acute febrile illness will enable physicians in Ethiopia to administer empirically targeted antimicrobial therapies to their patients, increase patient safety and lower the mortality of infectious diseases, respectively.

Objective of the Study: To systematically investigate the epidemiology of pathogenic organisms causing acute infections and their resistance profile to antimicrobial substances causing different infections in Arsi Zone and to investigate related existing risk factors.

Organizations: Hirsch Institute of Tropical Medicine, Asella, Ethiopia, in collaboration with the College of Health Sciences, Arsi University, Asella, Ethiopia, Heinrich-Heine University Düsseldorf, Germany, and the German Federal Institute for Risk Evaluation in Berlin, Germany, will conduct the study.

Procedure: Experts in the area willcollect blood specimen from the acute infectious suspected patients. The number of specimen (blood) needed to be obtained may increase from the usual since repeated blood cultures and samples of serum and blood are needed to identify the causing organisms. The blood cultures willbe collected periodically, to increase the probability of identifying the pathogen.

Participation: The procedure is to be carried out after getting your consent to participate. Consenting to this study includes consenting to sample export to specialized laboratories in Germany and to the conduction of genetic investigations. All volunteer patients, fulfilling inclusion criteria, will be included.

Risks Associated with Sample Collection: You will experience minor pain during certain specimen collection blood although that is not incurred because of participation in this study. However, you will be treated accordingly. Risk of side effects of blood collection is extremely rare and includes infection or damage of nerves at the puncture site. The volume of the blood sample is small and insignificant concerning effects on your health.

Benefit: Participation in this study may and should end in identifying the pathogen currently causing you to feel ill. Identification of the pathogen allows your treating physician to perform a more goal directed and individualized therapy, which raises the probability of a successful treatment outcome.

Compensation: You will receive the results of the tests performed with your samples (only if samples showed positive findings) through your physician. However, you will not be treated free of charge if your tests reveal bacterial or other infections. No compensation for elapsed time can be offered.

Export of samples for further investigation: In the case locally performed testing on the samples obtained from you reveals results, which raise further scientific interest (e.g. unusual patterns of antibiotic resistance, suspected new strains of certain bacteria), the sample or isolated bacterial strains might be exported to Germany for further investigation after storage at the Hirsch Institute of Tropical Medicine until the transport is performed. In case your microbiological test results do not reveal evidence for bacterial growth or other scientific issues are being raised serum and EDTA blood samples will be stored at the Hirsch Institute and exported to a cooperating laboratory in Germany for further investigations, which cannot be performed in Ethiopia. After all planned investigations are performed in the cooperation laboratories in Germany all samples are being destroyed. The consent to sample export may be revoked at any time through contacting the Hirsch Institute (see contact details below).

Confidentiality: From medical ethics point of view and research ethics, every part of your personal information will be kept confidential. Information to be collected and variables expressing your identity will be coded and also be kept confidential. The responsible person to link your variables (important for your follow up and treatment) with the code number is the principal investigator. However, other researchers can access your clinical information, which is coded without revealing your identity. The result of testing and revealed personal information will be used only for the mentioned purpose. For treatment purposes results of performed tests will be revealed to your treating physicians.

Sharing the results: Eventually, the results of performed testing, devoid of your identity, will be reported through publication in a medical journal or by other means. Have no suspicion on the confidentiality of your information, even at this time.

Right to refuse or withdraw: It is your right to agree or to refuse to participate in this study. Withdrawal from the study is also possible, at any time and without the necessity to give any reasons. Withdraw or refusing to participate in this study will not have any impact on your normal diagnostic or medical follow up.

You can address problems or questions through anyone of the addresses given bellow.

Contact Addresses:

1. Tafese Beyene Tufa, Hirsch Institute for Tropical Medicine (HITM) and College of Health Sciences (CoHS), Arsi University, Asella, Ethiopia, Tel: +25191177189; E-mail: tafeseb.tufa@yahoo.com

2. **Dr. Andreas Schönfeld**, Institute Coordinator, Hirsch Institute of Tropical Medicine, College of Health Sciences, Arsi University, Asella, Ethiopia. Phone: **+251(0)926 735 856;** E-mail: **whitm-asella@med.uni-duesseldorf.de**

3. **Mr. Hailu Fikadu**, chair of IRB of Arsi Universit College of Sciences, Arsi University, Asella, Ethiopia. Phone: **+251911 71 7608;** E-mail: hailufekadu18@yahoo.com

Annex I b: Participant Information Sheet for children English version

Date _____

Title: Identification and characterization of microbial pathogens acute infectious diseases in Arsi Zone of Ethiopia

Background: Potentially treatable infections remain the leading cause of death in Ethiopia. No sufficient epidemiological data on causative organisms of infections and their resistance profile to antimicrobial agents or data on determinants of susceptibility and individuals risk factors for unfavorable outcome of infectious diseases is available from Ethiopia or the Arsi Zone in particular. Since facilities for bacterial culturing or molecular biological identification of pathogens are not yet widely established in Ethiopia, some pathogens causing infections in Ethiopia might not have been described before. Existing data suggests high prevalence for resistant bacterial strains. Enhancing knowledge about the epidemiology of causative pathogens of infections such as infections of the skin, the respiratory tract, the urinary, sepsis or acute febrile illness will enable physicians in Ethiopia to administer empirically targeted antimicrobial therapies to their patients, increase patient safety and lower the mortality of infectious diseases, respectively.

Objective of the Study: To systematically investigate the epidemiology of pathogenic organisms causing acute infections and their resistance profile to antimicrobial substances causing different infections in Arsi Zone and to investigate existing risk factors for infectious diseases in the investigated population.

Organizations: Hirsch Institute of Tropical Medicine, Asella, Ethiopia, in collaboration with the College of Health Sciences, Arsi University, Asella, Ethiopia, Heinrich-Heine University Düsseldorf, Germany, and the German Federal Institute for Risk Evaluation in Berlin, Germany, will conduct the study.

Procedure: Experts in the area willcollect blood specimen from the acute infectious suspected patients. The number of specimen (blood) needed to be obtained may increase from the usual since repeated blood cultures and samples of serum and blood are needed to identify the causing organisms. The blood cultures willbe collected periodically, to increase the probability of identifying the pathogen.

Participation: The procedure is to be carried out after getting your consent to participate. Consenting to this study includes consenting to sample export to specialized laboratories in Germany and to the conduction of genetic investigations. All volunteer families, whose child fulfillinginclusion criteria, allow their child to be included into the study.

Risks Associated with Sample Collection: Your child may feel minor pain during 5ml of blood although that is not incurred because of participation in this study. However, your child will be treated accordingly. Risk of side effects of blood collection is extremely rare and includes infection or damage of nerves at the puncture site. The volume of the blood sample is small less than 5ml and insignificant concerning effects on your health.

Benefit: Participation in this study may and should end in identifying the pathogen currently causing your child to feel ill. Identification of the pathogen allows the treating physician to perform a more goal directed and individualized therapy, which raises the probability of a successful treatment outcome.

Compensation: Your child will receive the results of the tests performed with the samples (only if samples showed positive findings) through your physician. However, your child will not be treated free of charge if his/her tests reveal bacterial or other infections. No compensation for elapsed time can be offered.

Export of samples for further investigation: In the case locally performed testing on the samples obtained from your child reveals results, which raise further scientific interest (e.g. unusual patterns of antibiotic resistance, suspected new strains of certain bacteria), the sample or isolated bacterial strains might be exported to Germany for further investigation after storage at the Hirsch Institute of Tropical Medicine until the transport is performed. In case your child`s microbiological test results do not reveal evidence for bacterial growth or other scientific issues are being raised serum and EDTA blood samples will be stored at the Hirsch Institute and exported to a cooperating laboratory in Germany for further investigations, which cannot be performed in Ethiopia. After all planned investigations are performed in the cooperation laboratories in Germany all samples are being destroyed. The consent to sample export may be revoked at any time through contacting the Hirsch Institute (see contact details below).

Confidentiality: From medical ethics point of view and research ethics, every part of your child`s personal information will be kept confidential. Information to be collected and variables expressing your identity will be coded and also be kept confidential. However, other researchers can access your child`s clinical information, which is coded without revealing his /her identity. The result of testing and revealed personal information will be used only for the mentioned purpose. For treatment purposes results of performed tests will be revealed to the treating physicians.

Sharing the Results: Eventually, the results of performed testing, devoid of your identity, will be reported through publication in a medical journal or by other means. Have no suspicion on the confidentiality of your information, even at this time.

Right to refuse or withdraw: It is your right to agree or to refuse to allow your child to be participated in this study. Withdrawal from the study is also possible, at any time and without the necessity to give any reasons. Withdraw or refusing to participate in this study will not have any impact on your child normal diagnostic or medical follow up.

You can address problems or questions through anyone of the addresses given bellow.

Contact Addresses:

1. Tafese Beyene Tufa, Hirsch Institute for Tropical Medicine (HITM) and College of Health Sciences (CoHS), Arsi University, Asella, Ethiopia, Tel: **+25191177189**; E-mail: tafeseb.tufa@yahoo.com

2. **Dr. Andreas Schönfeld**, Institute Coordinator, Hirsch Institute of Tropical Medicine, College of Health Sciences, Arsi University, Asella, Ethiopia. Phone: **+251(0)926 735 856;** E-mail: **whitm-asella@med.uni-duesseldorf.de**

3. **Mr. Hailu Fikadu**, chair of IRB of Arsi Universit College of Sciences, Arsi University, Asella, Ethiopia. Phone: **+251911 71 7608;** E-mail: hailufekadu18@yahoo.com

5.5. Informed Consent Form

Annex IIa: Participant Information Sheet for Adults Afaan Oromoo version

Guyyaa _____

Mata-duree: Goostaa fi amala jeermoota nama irratti dhukkubaa sardaa naannoo Godina Arsi keessatti baa`ina fiduu danda`aan**.**

Seensa: Biyya keenya keessatti dhukkubni yaalamanii qorchaan fayyuu danada`aman gaaggamsa guddaa fidaa jiru. Ragaan jarmoota dhukkuba kana namatti fiidaniifi qorchoonni isaan ittiin waal baraniiran hinqoratamane. Kunis kan ta`uudhaaf danada`e waanta biyya keenya keessatti adeemsi jarmoota kana biqilchaan qortaan akkasumas meeshalee laboratoriiti gadifageenya qorchuu tjaajjilan waanta hinbaballanneef. Garuu ragaan tokko tokkoo jarmoonni kun akka isaan qorcha nutti fayyaadamaa jiru faana akka waal-baraniiran agarsiisa. Hubannoo fi faca`insa dhukkuba jamoota adda addaan dhufu kana beekuun hakiimota keenya akka isaani dEnhukkuba kana sirritti yaalaniif baayyee barbaachisaadha.

Kaayyoo Qorannoo kanaay: Bifa qindaa`aa ta`een, goostaa fi amala jeermoota nama irratti dhukkubaa sardaa naannoo Godina Arsi keessatti baa`ina fiduu danda`aan fi waantoota dhukkuba kanaaf nama saaxilani qorachuu ta`a.

Dhaabbilee: Jidduu galeessa qorannoo HITM kan asallatti argamu, Yuunivarsitii Arsii Kooleejii Fayyaa, Yuuniversitii Dosildoroof kan biyya Jarmaniitti argamu, Jidduu Galeessa qorannoo biyyolessa kann Berliinitti argamu, kan Jarmaniitti argamani.

Adeemsa: namoonni naannoo qorannoo kana irraatti muxannoo gaha ta`e qaban dhiiga dhukkubstoota dhiibeekaniin shekamani irraa dhiiga ni fudhatu. Dhiiga fudhatame keessa yoo jarmoonni jirataniif ni ni guddatu; yoo hinguddannee ta`e immoo gara biyyaa Jarmaniitti ni geeffama dhiigni hafe.

Hirmootonni: qorannoo kana keessatti osoo hinhirmaatiin, dorsa hirmaatoonni feedhiin isaanii ni gaafatama. Dhiigni isaanii gareen akka gara biyya Jarmaniitti ergamu illee feedhiin isaanii dursee ni gaaffatama.

Miidhaa dhiiga fudhachuu: Yeroo dhiigni fudhatamu dhukkubiin xiqqoon sitti dhagamuu ni danda`a. Garuu dhukkubiin kun miidhaa biroo gara biraa tokkollee siirran hingahu. Hami dhiigaa sirraa fudhatamu 20ml yoo baayyate, baayyee xiqqaadha.

**Bu`aa/Faayidaa**: qorannoo kana keessatti hirmaachuun kee jarmoonni dhukkuba baayyee sardaa kana sitti fidaan yoo jiraatan sirritti gad-fageenyaan qoratamanii yaala baayyee gaarii ta`e argachuu dandeessa.

Keennaa: qorannoo kana keessatti hirmaachuu keetiif keennaa adda ta`e siif kennam tokko illee hin jiru. Garuu ba`aan dhiigakee yoo jarmaoonni keessati argaman gara hakiima sii yaala jiruuttu siif ergama.

Dhiiga gara biyya alaatti qorannoo dabalataaf erguu: Yoo bu`aan dhiigakee qoratamee
Asallaatti beekamuu baate qorannoo adda addaaf gara biyyaa Jarmaanitti dhiignikee ykn jarmoonni dhiigakee keessatti mula`atan ni ergama. kanaafis haal gaariin jidduu galaeessa qorannoo HITM keessaatti saampiliin nii kuufama. Kunis qorannooo adda addaa kan biyyaa keenya keessati hinjirree ykn hinhojetamin akka biyyaa Jarmaniti hojetamuuf karaa seera qabeessan ni ergama. Kan irraa hafe garuu ni barbadaa`a.

Icciitii: Akka seera oogummaa fayyaatti ykn immoo qorannoo fayyaatti odeeffannoon waa`ee keessani icciitidhaan ni eegama. Oddeeffannoo waa`ee keessani hundi lakkoomsa dhoksaadhan qabama. kanaafis dura bu`aan qorataa kana itti gafatammuummaa qaba. Qorattooni biraan garuu, waa`eeke tokkoo illee baruu hindanada`an. Bu`aan dhiigakee fi malloottoonni dhukkubakee hakiimnikee yaala barbaachisaa ta`e siif kennuuf itti dhima nibaha.

Bu`aa dabarsuu: qorannoo kuni hojjetamee ergaa xummuramee booda, eenyummaa kee osoon hin ibsiin maxxasa adda addaa irratti maxxasamuu ni mala.

Mirgaa Hirmaachuu diduu ykn dhaabuu: Hirmaachuun, hirmachuu dhiisuun ykn immoo dhaabuun yoom illee mirga guutuu qabda.

Rakkoo ykn Gaaffii yoo qabatan, Teessoo armaan gidiitti nu qunamuu ni dandeessu.

Teessoo:

1. Tafese Beyene Tufa, HITM. kooleejii fayyaa, Yuuniversitii Arsii, Asallaa, Itiyoopiyaa. Lakk. bilbilaa: +251911771893; E-mail: tafeseb.tufa@yahoo.com
2. **Dr. Andreas Schönfeld,** HITM. kooleejii fayyaa, Yuuniversitii Arsii, Asallaa, Itiyoopiyaa. Lakk. bilbilaa:.
   M. phone: **+251(0)926 735 856;** E-mail: **whitm-asella@med.uni-duesseldorf.de**
3. **Obboo Haayiluu Fikaduu**, walitqabaa boordii qorannoo kooleejii fayyaa, Yuuniversitii Arsii, Asallaa, Itiyoopiyaa. Lakk. bilbilaa: **+251911 71 7608;** E-mail: hailufekadu18@yahoo.com

Annex IIb: Participant Information Sheet for children Afaan Oromoo version

Guyyaa _____

Mata-duree: Goostaa fi amala jeermoota nama irratti dhukkubaa sardaa naannoo Godina Arsi keessatti baa`ina fiduu danda`aan**.**

Seensa: Biyya keenya keessatti dhukkubni yaalamanii qorchaan fayyuu danada`aman gaaggamsa guddaa fidaa jiru. Ragaan jarmoota dhukkuba kana namatti fiidaniifi qorchoonni isaan ittiin waal baraniiran hinqoratamane. Kunis kan ta`uudhaaf danada`e waanta biyya keenya keessatti adeemsi jarmoota kana biqilchaan qortaan akkasumas meeshalee laboratoriiti gadifageenya qorchuu tjaajjilan waanta hinbaballanneef. Garuu ragaan tokko tokkoo jarmoonni kun akka isaan qorcha nutti fayyaadamaa jiru faana akka waal-baraniiran agarsiisa. Hubannoo fi faca`insa dhukkuba jamoota adda addaan dhufu kana beekuun hakiimota keenya akka isaani dEnhukkuba kana sirritti yaalaniif baayyee barbaachisaadha.

Kaayyoo Qorannoo kanaay: Bifa qindaa`aa ta`een, goostaa fi amala jeermoota nama irratti dhukkubaa sardaa naannoo Godina Arsi keessatti baa`ina fiduu danda`aan fi waantoota dhukkuba kanaaf nama saaxilani qorachuu ta`a.

Dhaabbilee: Jidduu galeessa qorannoo HITM kan asallatti argamu, Yuunivarsitii Arsii Kooleejii Fayyaa, Yuuniversitii Dosildoroof kan biyya Jarmaniitti argamu, Jidduu Galeessa qorannoo biyyolessaa kann Berliinitti argamuu, kan Jarmaniitti argamani.

Adeemsa: namoonni naannoo qorannoo kana irraatti muxannoo gaha ta`e qaban dhiiga dhukkubstoota dhiibee kaniin shekamani irraa dhiiga ni fudhatu. Dhiiga fudhatame keessa yoo jarmoonni jirataniif ni guddatu; yoo hinguddannee ta`e immoo gara biyyaa Jarmaniitti ni geeffama dhiigni hafe.

Hirmootonni: qorannoo kana keessatti osoo daa`imnni kee akka hirmaatu hingodhiin, dursa maatiinn feedhii qabachuu qabu ykn feedhiin isaanii ni gaafatama. Dhiigni daa`imakee gareen akka gara biyya Jarmaniitti ergamu illee feedhiin maatii dursee ni gaaffatama.

Miidhaa dhiiga fudhachuu: Yeroo dhiigni fudhatamu dhukkubiin xiqqoon mucaakeetti i dhagamuu ni danda`a. Garuu dhukkubiin kun miidhaa biroo gara biraa tokkollee irraan hingahu. Hami dhiigaa irraa fudhatamuu 5ml yoo baayyate, baayyee xiqqaadha.

**Bu`aa/Faayidaa**: qorannoo kana keessatti hirmaachuun daa`imakee jarmoonni dhukkuba baayyee sardaa kana fidaan yoo jiraatan sirritti gad-fageenyaan qoratamanii yaala baayyee gaarii ta`e argachuu dandeessa.

Keennaa: qorannoo kana keessatti daa`imnikee hirmaachuusaatiif keennaa adda ta`e isaaf kennamu tokko illee hin jiru. Garuu ba`aan dhiiga isaa yoo jarmaoonni keessati argaman gara hakiima isaati yaala jiruuttu siif ergama.

Dhiiga gara biyya alaatti qorannoo dabalataaf erguu: Yoo bu`aan dhiigakee qoratamee

Asallaatti beekamuu baate qorannoo adda addaaf gara biyyaa Jarmaanitti dhiignikee ykn jarmoonni dhiigakee keessatti mula`atan ni ergama. kanaafis haal gaariin jidduu galaeessa qorannoo HITM keessaatti saampiliin nii kuufama. Kunis qorannooo adda addaa kan biyyaa keenya keessati hinjirree ykn hinhojetamin akka biyyaa Jarmaniti hojetamuuf karaa seera qabeessan ni ergama. Kan irraa hafe garuu ni barbadaa`a.

Icciitii: Akka seera oogummaa fayyaatti ykn immoo qorannoo fayyaatti odeeffannoon waa`ee keessani icciitidhaan ni eegama. Oddeeffannoo waa`ee keessani hundi lakkoomsa dhoksaadhan qabama. kanaafis dura bu`aan qorataa kana itti gafatammuummaa qaba. Qorattooni biraan garuu, waa`eeke tokkoo illee baruu hindanada`an. Bu`aan dhiigakee fi malloottoonni dhukkubakee hakiimnikee yaala barbaachisaa ta`e siif kennuuf itti dhima nibaha.

Bu`aa dabarsuu: qorannoo kuni hojjetamee ergaa xummuramee booda, eenyummaa kee osoon hin ibsiin maxxasa adda addaa irratti maxxasamuu ni mala.

Mirgaa Hirmaachuu diduu ykn dhaabuu: Hirmaachuun, hirmachuu dhiisuun ykn immoo dhaabuun yoom illee mirga guutuu qabda.

Rakkoo ykn Gaaffii yoo qabatan, Teessoo armaan gidiitti nu qunamuu ni dandeessu.

Teessoo:

1. Tafese Beyene Tufa, HITM. kooleejii fayyaa, Yuuniversitii Arsii, Asallaa, Itiyoopiyaa. Lakk. bilbilaa: +251911771893; E-mail: tafeseb.tufa@yahoo.com

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Annex III a: Informed Consent Form for adults

Participant CONSENT FORM

Name: _____ **Study-No.:**_____

Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information to enable the prospective participant to make an informed decision on whether to participate or not.

NAME: _____

DATE: _____ SIGNATURE: _____

Statement of person giving consent:

I have read the information on the study "**Identification and characterization of microbial pathogens acute infectious diseases in Arsi Zone of Ethiopia**" or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction. I understand that my participation is voluntary (optional) and not a requirement for further treatment of my illness. For this study I explicitly allow the investigator's delegate to obtain blood and serum for the investigation. I also understand explicitly that samples taken from my body and/or bacterial strains isolated from samples might be used for genetic testing and might be transported abroad for further investigations but nonetheless every sample obtained will be discarded and not used for any other purpose than consented to within this study. I understand no monetary benefits will arise by participating in this study. I know enough about the purpose, methods, risks and benefits of the research study to judge that I want to take part in it and without being externally forced to do so I consent in participating in this study with my signature below. I understand that I may freely stop being part of this study at any time.

Name:_____DATE:_____SIGNATURE/ THUMBPRINT: _____

WITNESSES NAME: _____

Annex III b: Informed Consent Form for children

Participant CONSENT FORM

Name: _____ **Study-No.:**_____

Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information to enable the prospective participant to make an informed decision on whether to participate or not.

NAME: _____

DATE: _____ SIGNATURE: _____

Statement of person giving consent:

I have read the information on the study "Identification and characterization of microbial pathogens acute infectious diseases in Arsi Zone of Ethiopia" or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction. I understand that my child participation is voluntary (optional) and not a requirement for further treatment of her/his illness. For this study, I explicitly allow the investigator's delegate to obtain blood and serum for the investigation from my child. I also understand explicitly that samples taken from my child body and/or bacterial strains isolated from samples might be used for genetic testing and might be transported abroad for further investigations but nonetheless every sample obtained will be discarded and not used for any other purpose than consented to within this study. I understand no monetary benefits will arise by allowing my child to be participated in this study. I know enough about the purpose, methods, risks and benefits of the research study to judge that my child wants to take part in it and without being externally forced to do so I consent in making decision to allow my child to participate in this study with my signature below. I understand that I may freely stop my child being part of this study at any time.

LEGAL GUARDIAN SIGNATURE: _____

LEGAL GUARDIAN NAME: _____

DATE: _____SIGNATURE/THUMB PRINT: _____

Annex IVa: Informed Consent Form adults (Afan Oromo Version)

Ragaa Uunkaa Hirmaatottaa

Maqaa: _____ Lakkk**:**_____

Jechaa nama ragaa fudhatee:

Ani guutummaatti waa`ee qorannoo kanaa _____ yoo fedha isaa ykn ishee ta`e akka hirmaataniif oddeeffanno gahaa ta`e kenneera.
Maqaa: _____
Guyyaa: _____ Mallattoo: _____

Jechaa namaa ragaa kennuu:

Ani oddeeffannoo waa`ee qorannoo "Goostaa fi amala jeermoota nama irratti dhiibee dhukkubaa naannoo Godina Arsi keessatti baa`ina fiduu danda`aan" dubbiiseera ykn akkaataa ani hubachuu danda`uutti naaf dubbifameera. Dabalatanis haasaa taasisuudhan, oddeeffannoo qubsa ta`e argadheera. Qorrannoo kana keessatti hirmaachunis feedhii irrati qofaa akka ta`e sirritti hubadheera. Qorannoo kanaafis kan oolu dhiigni 20ml akka naarraa barbaachisu hubadheera. Naamunaa narraa fudhatemu kun ykn jermoonni nakeessati mul`atan gad-fageenya akka qoratamaniif gara biyya Jarmanitti geessamuu akka danda`an natt beeksifameera. Walumaagalatti ani oddeeffannoo gaha ta`e waa`ee kaayyoo, miidhaa fi faayidaa qorannoo kana dhiibbaa tokko malee hubadhee irrattii hirmaachuuf feedhii qabachukoo mallattookoo armaan gadiittin mirkannessa. Yeroo barbaaddetti dhaabuu akkan danda`us hubadheera.

Maqaa: _____Guyyaa: _____Mallattoo/ashaara qubaa: _____

Maqaa nama ragaa ta`ee: _____

Mallattoo maatii (daa`immaniif qofa): _____

Annex IVb: Informed Consent Form children (Afan Oromo Version)

Ragaa Uunkaa Hirmaatottaa

Maqaa: _____Lakkk.**:**_____

Jechaa nama ragaa fudhatee:

Ani guutummaatti waa`ee qorannoo kanaa _____ yoo fedha isaa ykn ishee ta`e akka hirmaataniif oddeeffanno gahaa ta`e kenneera.

Maqaa: _____

Guyyaa: _____ Mallattoo: _____

Jechaa namaa ragaa kennuu:

Ani oddeeffannoo waa`ee qorannoo "Goostaa fi amala jeermoota nama irratti dhukkubaa sardaa naannoo Godina Arsi keessatti baa`ina fiduu danda`aan" dubbiiseera ykn akkaataa ani hubachuu danda`uutti naaf dubbifameera. Dabalatanis haasaa taasisuudhan, oddeeffannoo qubsa ta`e argadheera. Qorrannoo kana keessatti hirmaachunis feedhii irrati qofaa akka ta`e sirritti hubadheera. Qorannoo kanaafis kan oolu dhiigni 5ml akka daa`ima koorraa barbaachisu hubadheera. Dhiiga irraa fudhatemu kun ykn jermoonni keessati mul`atan gad-fageenya akka qoratamaniif gara biyya Jarmanitti geessamuu akka danda`an natti beeksifameera. Walumaagalatti ani oddeeffannoo gaha ta`e waa`ee kaayyoo, miidhaa fi faayidaa qorannoo kana dhiibbaa tokko malee hubadhee daa`imnikoo irrattii akk hirmaatu feedhii qabachukoo mallattookoo armaan gadiittin mirkannessa. Yeroo barbaaddetti daa`imakoo qorannoo kana keessaa baasu akkan danda`us hubadheera.

Maqaa maatii: _____
Mallattoo maatii: _____Guyyaa: _____

Mallattoo/ ashaara qubaa: _____
Maqaa nama ragaa ta`ee: _____

5.6. Questioners

Questionnaire / Participant's history sheet

Reporting hospital department

☐ Internal Medicine ward
☐ Pediatrics ward
☐ Surgical ward ☐ Gynecology ward
☐ EOPD ☐ Adult OPD
☐ ART ☐ pediatric OPD
☐ Other, specify:

Patient Information

Reporting Hospital: ☐ Asella Teaching Hospital / ☐Bekoji District Hospital

Name / Father's name:

Age: Gender: ☐ Male / ☐ Female

Marital Status : a. ያላገባ/ች/Single/qofaa

b. ያገባ/ች/ Married / kan heerumte

c. የፈታ/ች/ Divorced / kan hiikte

d. ባል/ሚስት/የሞተበት/ባት/ Widowed / kan dhirsii duu'e

Children:

Total Number:

Children under 1 Year of Age:

Card #

Address:

Region: Zone:

Woreda: Kebele:

Phone 1: Area of residence: ☐ rural / ☐ urban

Whose no is it?/mine, spouse's, parent's, neighbor's, relative's/

Study number (filled by HITM):

Diagnosis upon study inclusion/ Gosa Dhukkubaa

☐ Respiratory tract infection
☐ Urinary tract infection
☐ Meningitis / Encephalitis
☐ Gastroenteritis
☐ Infection of the skin or wound infection
☐ Acute febrile illness
☐ And / or sepsis
☐ Other, specify:

Have you left the above mentioned Residence in the last 4 weeks?

☐ Yes, specify Where:

Region: Zone:

Woreda:

☐ No

Occupation

☐ Farmer/ገበሬ
☐ Governmental or private employee/የመንግስት/ የግልስራ
☐ Merchant/ነጋዴ
☐ Student (school, college, university)/ተማሪ
☐ Unemployed /ስራየለኝም
☐ Housewife/የቤትእመቤት
☐ Other, specify:/ሌላ፡ይገለፅ

Duration of Symptoms

☐ ≤ 1 week ☐ 2-4 weeks
☐ 1-2 weeks ☐ > 4 weeks

Symptoms upon study inclusion

☐ Fever / body temperature ≥ 37,5°C
☐ Fever / body temperature ≥ 38,5°C
☐ Heart rate > 90 bpm (adults only)
☐ BP systolic < 90 mmHg
☐ Respiratory rate > 20 / min

Type of infection

☐ Community acquired
☐ Nosocomial (3 days after discharge or 30 days after surgery)

Type of sample acquired for analysis

☐ Sputum
☐ Urine (midstream)
☐ Swab or pus collection of infected skin lesion or abscess
☐ CSS
☐ Serum and EDTA blood (for each patient!)
☐ Venous blood cultures
☐ no blood cultures acquired (if no fever)

Previous / current antibiotic treatment

☐ No current antibiotic treatment upon inclusion
☐ Last antibiotic treatment > 2 weeks ago
☐ Currently on antibiotic treatment, specify:

☐ Last antibiotic treatment ≤ 2 weeks ago, specify:

☐ Substance 1:

☐ Substance 2:

☐ Duration of treatment:

☐ Substance:

☐ Dosage:

☐ Duration of treatment:

Have you ever had contact with these animals? / ከሚከተሉትእንስሳትጋርንክኪነበርዎት?/

a) Dog/ዉሻ/saree
b) Cat/ድመት/Adurree
c) Cattle/ከብቶች/Lowwan
d) Sheep/በጎች/Hoolaa
e) Camels/ግመሎች/Gaala
f) Horses/ፈረሶች/ Farda
g) Goat/ፍየሎች/ Re'ee
h) Monkey/ጦጣ/ Qamalee
i) Rodents/አይጦች/ Hantuta
j) Other/ሌላ.....

Were there any insect or tick bites in the last 4 weeks? /ከዚህበፊትነብሳትነድፎዎትወይም መዥገርነክሶት□□□□?/

a) Yes, Specify/አዎ/eeyyee; ይገለፅ.....
   1. Mosquito/ የወባትንኝ
   2. Fly/ዝንብ
   3. Flee /ቁንጫ
   4. Louse/ቅማል
   5. Tick/መዥገር

   When .....

b) No/አይ/ lakki

If from malaria area: use of bed nets

a) Yes/□□/ eeyyee
b) No/ □□/ lakki

When did fever start? ትኩሳቱመቼነዉ የጀመርዎት?

a) <3 day
b) 3 - 15 days
c) >15 days

Do you have close contact or work with soil? በስራወይም በሌላአጋጣሚ አፈርየመንካትእድሉ አለዎት?

a) Yes/□□/eeyyee
b) No/□□□□□/ lakki

Do you consume raw milk or meat? □ፊ ወተትወይም ስጋይመገባሉ?

a) Yes/□□/eeyyee
b) No/□□□□□/ lakki

What is the source of water that you are using? /የንፁህዉሃ አቅርቦትአለዎት?/

a) Tap water/የቧንቧዉሀ
b) River/የወንዝ
c) Spring/የምንጭ
d) Protected Well/የተጠበቀጉድጓድ
e) Other, specify/ሌላ፡ ይገለፅ.....

Do you have any chronic disease (e. g. TB, HIV, Diabetes, Cancer)? /ሀኪም የነገሮትየቆየህመም አለ?/

a) Yes, Specify/አዎ/ eeyyee ፣ይገለፅ/.....
b) No /የለም/ lakki

If yes do you take any medication for this? / መልስዎ አዎ ከሆነ፡- የሚወስዱት መድሀኒትአለ?

a) Yes, specify/አዎ/eeyyee ፡ይገለፅ.....
b) No /አይደለም/ lakki

Where do you sleep (bed/floor)/□□ □□□□ □□□□□? /

a) Bed /□□□□□/
b) Floor/□□□ □□/

Literacy Level

a) Literate
b) Illiterate

Do you always wash your hands after using the toilet? /ሁልጊዜሽንትቤት ከተጠቀሙ በኋላእጅዎን በሳሙና ይታጠባሉ?

a) Yes /አዎ/eeyyee
b) No/አይደለም/ lakki

Have you had surgery or trauma in the last month? /ባለፈዉአንድወርየገጠሞትአደጋ(ድብደባ/ የመኪና) ወይም የተሰራሎትቀዶጥገናነበር?

a) Yes /አዎ/eeyyee
b) No/አይደለም/ lakki

Name of interviewer	Date	Signature

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