High Rate of Multidrug-resistance Among Clinical Isolates of Gram-negative Bacteria in Hospitalized Patients With Febrile Diseases in Central Ethiopia

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Research

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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, empirical treatment is suboptimal. The objective of this study was the characterization of 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

Of 684 study participants, 54.2% were male and median age was 22.0 (IQR: 14-35) years. Blood cultures were positive in 5.4% (n=37). In other clinical samples, cultures were positive in 60.6% (20/33), 20.8% (5/24), and 37.5% (3/8) in swabs/pus, urine and other body fluids, respectively. Of 66 pathogenic isolates, 57.6% (n=38) were GNB, (39.4% (n=26) Gram-positive and 3.0% (n=2) were candida species. Among the isolated GNB, 42.1% (16/38) were *E. coli*, 23.7% (9/38) *K. pneumoniae* and 10.5% (4/38) *P. aeruginosa*.

In total, 27/38 Gram-negative isolates were available for further analysis. Resistance rates were: 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 NDM-1 gene was detected in one *K. pneumoniae* and one *A. baumannii* isolate, which carried an additional OXA-51 gene. Genes for ESBLs were detected in 81.5% (n=22) 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). Clinical treatment data revealed ineffective antimicrobial treatment in 13 of 18 (72.2%) patients.

Conclusion

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

Introduction

Infectious diseases are among the leading causes of morbidity and mortality in many low-income countries such as Ethiopia (1). Bacteremia and sepsis are common and are severe complications of many different infections caused by 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 source of infections (2).

The emerging multi-drug resistance (MDR) in Gram-negative bacteria (GNB) has become a serious public health problem across the world (3). A major driver of resistance in GNB is the horizontal transfer of mobile genetic elements (MGE) carrying genes for extended-spectrum β -lactamases (ESBL) and/or carbapenemases (4). These enzymes hydrolyze penicillins, third generation cephalosporins (3GC) and carbapenems respectively. The most common ESBL genes are CTX-M-type (cefotaximase), TEM (Temoneira), and SHV (sulfhydryl reagent variable), whereas the most prevalent carbapenemase-encoding genes were OXA (oxacillinase), KPC (*Klebsiella pneumoniae*) and MBL (metallo β -lactamase) such as NDM (New Delhi metallo- β -lactamase), IMP (imipenemase) and Verona imipenemase (VIM), which confer resistance to cephalosporins and carbapenems (5, 6).

Despite evidence for high rates of 3GC-reistance among Gram-negative isolates in many African countries of around 50%, 3GCs are among the most commonly used antibiotics for the initial empiric treatment of severe infections and sepsis in sub-Saharan Africa. Data to estimate the mortality associated with antimicrobial resistance (AMR) in sub-Sahara African countries is limited (7). Rapid interventions such as the establishment of antimicrobial stewardship (AMS) programs, strengthening of antimicrobial susceptibility testing (AST) capacity, revision and updating of existing management protocols of infectious diseases and provision of local and national AMR data for common bacterial pathogens should have a high priority. 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 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). There is an increasing need for strategies to enhance prudent use of antibiotics in the country due to the alarming spread of ESBL-producing GNB leading to high rates of infections due to MDR, which are difficult if not impossible to treat (10, 11).

The lack of systematically acquired local data concerning both the causative organisms and common resistance patterns results in ineffective empirical treatment and an unfavorable clinical outcome. Therefore, we assessed the rate and extent of drug resistance among GNB isolated from hospitalized patients with acute infectious diseases at a tertiary hospital in Central Ethiopia and the pertinent drug resistance 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, the secondary and molecular biological investigations were performed in Germany.

A trained study team identified eligible patients. During the study period, all patients ≥ 1 year of age presenting for treatment at the ARTH with fever defined as body temperature $>37.5^{\circ}\text{C}$ according to tympanic measurement were offered inclusion in the study. After written informed consent by patients or legal guardians, blood cultures (BC) 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 treatment guidelines. Socio-demographic 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. 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 minors, from their legal guardians.

Blood culture

From every child and adult participant, between approximately 5 mL and 10-20 mL of blood were drawn, and inoculated into an aerobic blood culture bottle (in-house production for the first 200 participants and thereafter commercially available blood culture bottles were used). The culture was incubated at 37°C for a maximum period of five days. After 24 hours and at the end of the incubation period, Gram staining and sub-culturing on blood, MacConkey, and chocolate agar in candle jar or a 5% CO_2 enriched atmosphere were performed. Biochemical identification tests were subsequently done based on the Gram-staining result. Due to limited resources, only one aerobic culture was performed per patient. Regular quality controls for sensitivity were performed by incubation of standard laboratory strains of different bacteria.

Urine Culture

In participants with possible urinary tract infection, mid-stream urine specimens were collected using sterile urine cups and 1 μL of the urine was inoculated onto blood and MacConkey agar. After 24 hours 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 appropriate to the participants' complaints, swabs or aspiration of other body fluid were collected using appropriate sterile sampling devices (swabs, sterile syringes etc.) and cultivated on blood and chocolate agar in 5% CO_2 enriched atmosphere. The isolates were further processed following the local laboratory's SOPs.

Antimicrobial susceptibility testing

Following the identification of Gram-negative bacteria, Kirby-Bauer AST was performed after cultivation on Mueller Hinton Agar. The procedure and interpretation followed the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations. The microbiological result was reported to the treating physicians at the 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 mass spectrometry (MALDI-TOF, Vitek[®] MS, bioMérieux, Marcy-l'Étoile, France) and the local AST result was confirmed by VITEK[®] 2 (bioMérieux).

ESBL and carbapenemase genes detection

After identification and AST, DNA of the GNB 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.

Bacterial strains with suspected production of ESBL or carbapenemase according to the AST result were investigated by PCR. 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) and an in-house PCR protocol established for the detection of the carbapenemase genes *bla*_{NDM-1} and *bla*_{OXA-51}, as described by Wendel et al. (13, 14) (Table1).

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 mean, median and standard division were used for continuous variables. Differences were considered statistically significant at $p < 0.05$.

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

Primer	Sequence (5'–3')	Amplicon size	Reference
bla _{SHV} (F)	AGCCGCTTGAGCAAATTAAAC	786 bp	12
bla _{SHV} (R)	GTTGCCAGTGCTCGATCAGC		
bla _{TEM} (F)	CATTTCCGTGTCGCCCTTATTC	846 bp	12
bla _{TEM} (R)	CCAATGCTTAATCAGTGAGGC		
bla _{CTX-M-1} (F)	CGTCACGCTGTTGTTAGGAA	781 bp	12
bla _{CTX-M-1} (R)	ACGGCTTTCTGCCTTAGGTT		
bla _{CTX-M-2} (F)	CTCAGAGCATTGCGCGCTCA	843 bp	12
bla _{CTX-M-2} (R)	CCGCCGCAGCCAGAATATCC		
bla _{CTX-M-9} (F)	GCGCATGGTGACAAAGAGAGTGCAA	876 bp	12
bla _{CTX-M-9} (R)	GTTACAGCCCTTCGGCGATGATTC		
bla _{NDM-1} (F)	CGG CAT CACCGA GAT TGC	732 bp	133
bla _{NDM-1} (R)	CACCGA CAT CGC TTTTGGT		
bla _{OXA-51} (F)	TGT CTAAGGAAGTGAAGCGTG	112 bp	14
bla _{OXA-51} (R)	AACTGTGCCTCTTGCTGAG		

Resistance gene	Base pairs	Bacterial species	ATCC number
SHV-group	780	<i>K. pneumoniae</i>	NRZ-02718
TEM-group	860	<i>K. oxytoca</i>	NRZ 09574
CTX-M-1 group	688	<i>E. coli</i>	NRZ-04944
CTX-M-2 group	404	<i>E. coli</i>	NRZ-09082
CTX-M-9 group	561	<i>E. coli</i>	NRZ 00552
NDM-1	180	<i>K. pneumoniae</i>	JS-37
OXA-51	112	<i>Acinetobacter baumannii</i>	DMBF-4

F, forward; R, reverse; bp, base pairs.

Quality control

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

Table 2: Control strains used for resistance gene analysis

Results

In total, 684 study participants were included; 54.2% were male and the median age was 22 (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 3. Sociodemographic characteristics of study participants

Socio-demographic 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 before 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: out-patients department

Bacterial cultures

The overall bacterial detection rate across all sample materials was 12.1% (83/684). In 9.6% (n=66) the isolated bacterium was considered a pathogen and in 2.5% (n=17) coagulase-negative staphylococci (CoNS) were isolated and considered as 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 rate in other clinical samples was

60.6% (20/33), 20.8% (5/24), and 37.5% (3/8) in swabs/pus, urine, and other body fluids, respectively. Out of 12 cultured cerebrospinal fluid samples, only one sample revealed growth of a pathogen (*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) Gram-positive and 3% (n=2) *Candida* species. *S. aureus* and *E. coli* were the most prevalent isolates among Gram-positive and Gram-negative isolates, respectively. Among the 38 Gram-negative isolates, 42.1% (n=16) were *E. coli*, 23.7% (n=9) *K. pneumoniae*, and 10.5% (n=4) *P. aeruginosa* (for further details see Table 4).

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

Bacterial species	Number	%
<i>E. coli</i>	16	42.1
<i>K. pneumonia</i>	9	23.7
<i>P. aeruginosa</i>	4	10.5
<i>Salmonella typhi</i>	2	5.3
<i>Enterobacter spp.</i>	2	5.3
<i>A. baumannii</i>	1	2.6
<i>N. meningitides</i>	1	2.6
<i>R. ornithinolytica</i>	1	2.6
<i>R. planticola</i>	1	2.6
<i>Serratia spp.</i>	1	2.6

As indicated in Table 5, among all clinical samples bacterial growth was identified significantly more often among patients with skin and soft tissue infections (SSTI) ($p<0.001$). Although not statically significant, samples from patients with a leukocyte count $>12,000$ or <4000 cells / μ L at the time of sampling tended to be more likely to reveal bacterial growth ($p=0.07$). On the other hand, blood cultures from patients with the diagnosis of acute febrile illness with unknown source were least likely to reveal bacteremia compared with other symptoms ($p=0.005$). Being HIV-positive did not have any impact on blood culture positivity rate ($p=0.85$). Equally the C-reactive protein (CRP) had no predictive value concerning blood culture positivity (blood culture positive versus blood culture negative, CRP mean 71.7 mg/L vs. 64.6 mg/L; $p=0.20$).

Table 5. Culture positivity rate according to clinical diagnosis of source of infection and laboratory parameters among study participants blood

Infectious focus / laboratory parameters	Culture positive (N=66)	Culture negative (N=618)	p-value
	% (n)	% (n)	
Pneumonia / RTI (n=165)	19.7 (13)	24.6 (152)	0.38
Urinary tract 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 illness with unknown source (n=264)	22.7 (15)	40.3 (249)	0.005
Sepsis* (n=32)	4.5 (3)	4.7 (29)	0.96
HIV sero-positivity (n=58)	9.1 (6)	8.4 (52)	0.85
Leukocytes count $>12,000$ or <4000 / μ L (n=218)	42.4 (28)	30.7 (190)	0.07
	Mean	Mean	
CRP in mg/L	71.7	64.6	0.20

RTIs: Respiratory tract infection, SSTI = skin and soft tissue infection; GITI= gastrointestinal tract infection, CRP=C-reactive protein

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

Regarding 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.

Table 6. Culture positivity rate among the different clinical samples

Clinical sample	Culture negative	Culture positive	
		Gram-negative isolate % (n)	Gram-positive isolate % (n)
All samples (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)

Antibiotic 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: ampicillin/sulbactam 93.3% (n=21), cefotaxime 88.9% (n=24), ceftazidime 74.1% (n=20), cefipime 74.1% (n=20), ciprofloxacin 70.4% (n=19), and gentamicin 63.0% (n=17). Resistance rates against rarely used antibiotics was 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 (Table7).

Resistance genes

The overall frequencies of ESBL and carbapenemase genes 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 gene was detected in the isolated GNB. The different ESBL were characterized as 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 from 5/6 (83.3%) of the *K. pneumoniae* isolates, whereas SHV-group was only detected 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 empirical 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 an 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 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, empirical antibiotic treatment and AST result according to Kirby-Bauer disc diffusion test are summarized below (Table 9).

Table 7. Antibiotic resistance rate of Gram-negative isolates

Bacterial species	Ampicillin	Ampicillin/sulbactam	Piperacillin	Piperacillin/tazobactam	Cefotaxime	Ceftazidime	Cefepime	Meropenem	Amikacin	Gentar
<i>Escherichia coli</i> (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)
<i>Klebsiella pneumoniae</i> (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)
<i>Pseudomonas aeruginosa</i> (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)
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)
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)

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

Table 8. Characterization and frequency of detected ESBL and carbapenemase genes among the Gram-negative isolates

Bacterial species	Resistance genes % (n)							
	ESBL genes					CP genes		
	All ESBL	CTX-M-1 group	TEM-group	SHV-group	CTX-M-9 group	All CP	NDM-1	OXA-51
<i>E. coli</i> (n=13)	92.3 (12)	69.2 (9)	76.9 (10)	7.7 (1)	0	0	0	0
<i>K. pneumoniae</i> (n=6)	100 (6)	83.3 (5)	50.0 (3)	83.3 (5)	0	16.7 (1)	16.7 (1)	0
<i>P. aeruginosa</i> (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)
All GNB (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)

*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
CP, carbapenemase

Table 9. Clinical-evaluation, Kirby-Bauer AST result and empirical treatment of participants with *E. coli* or *K. pneumoniae*

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

Discussion

To date, infectious disease 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 distribution of AMR remains limited. In this study, we found a high rate of MDR in GNB isolated from febrile patients. As previously described from Uganda, MDR GNB are the main cause of sepsis in febrile cancer patients with more than 50% of the 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 blood cultures was low, which might partially be explained by the fact that the causative pathogen was non-cultivable in a proportion of patients (haemoparasites, viruses, or non-cultivable bacteria). Similar findings were reported from South Africa (18). In patients with febrile

illness with unknown source of infection in comparison to other clinical diagnoses, the likelihood of positive blood cultures was lowest. The highest yield of bacterial cultures was reported from swabs in patients with SSTI. There was a trend of a decreased or elevated leucocyte count or an increased CRP level to being associated with 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 strengthens a previous report from Italy that CRP level alone is not sufficient to predict blood culture positivity (19). In this time of wide-spread AMR and the associated risk of failing antibiotic therapies, blood culture diagnostics are essential to guide 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 blood cultures (20, 21).

In our study, the resistance rates of isolated GNB against commonly used antibiotics was high, severely confining the effectiveness of aminopenicillins in combination with betalactamase-inhibitors or 3GC for empirical treatment of infections possibly caused by GNB. The lowest resistance rates were found for the carbapenem 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 virtually not applied at all (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 genes 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 world-wide and many variants associated with clinically relevant functional heterogeneity have been described. Thus, co-expression of different ESBL types is more common among GNB which harbor CTX-M-type enzymes (26).

Both, CTX-M-1 group and SHV-group were abundantly detected in *K. pneumoniae*, whereas in *E. coli* the SHV-group was detected only in 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 a *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 general, this finding is not surprising since the *bla*_{NDM-1} carbapenemase gene in *K. pneumoniae* and *A. baumannii* have been commonly reported from other eastern African countries like Kenya, Uganda (29), Egypt (30) and Sudan (31).

In this study, most of the antibiotics initiated by the treating physicians for empirical treatment in the participating patients were ineffective according to the 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 clinical success rate of the empirically initiated antibiotic therapies was not part of this investigation. Nevertheless, antibiotic resistance impairing the success of empirical antibiotic therapies seems alarmingly common and local epidemiological resistance data should be taken into account before 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 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 (8).

A limitation of this study might be an impaired sensitivity of blood cultures, since only one set of locally prepared 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. The 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 performed at a tertiary hospital in Ethiopia, we isolated GNB from patients with infectious diseases with high rates of MDR including 3GC-resistance, which are the most commonly used substances for empirical antibiotic treatment. Thus, 72% of the initiated antibiotic therapies in this cohort should be considered ineffective according to AST. As an underlying cause for extensive drug resistance, we found a high prevalence of different ESBL enzymes with predominance of TEM- and CTX-M-1-types. More than half of Gram-negative isolates carried two or more resistance ESBL genes. In addition, carbapenemases were detected in as many as 7.4% of the Gram-negative isolates, despite the limited availability and rare application of carbapenems in the country. These findings emphasize the need for regular microbiological investigation of appropriate specimen before initiation of empirical antibiotic treatment wherever possible. In resource-limited settings like Ethiopia with confined capacities for regular microbiological investigation, local and national

programs for MDR surveillance might be helpful to reduce the futile use of antibiotics and improve the treatment outcome of calculated antibiotic therapies. The implementation of new strategies like MDW- or PCT-guided culturing could help to enable the resource-saving use of microbiological culture methods. Strengthening of antimicrobial stewardship programs and appropriate policies to regulate the use of antibiotics and to monitor the emergence of resistant bacteria is thus needed without delay.

Declarations

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Ethics approval and consent to participate

The appropriate ethical review boards of Arsi University, 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 Düsseldorf University Hospital, Germany (reference number 5729) approved the study. The ethical clearance for sample transportation between Ethiopia and Germany was obtained from the National Ethical Review Board of the Ethiopian Ministry of Science and Technology (reference number 310/204/2017). Before the start of 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 signed by each study participant or, in the case of minors, from their legal guardians, contained the consent for publication of anonymized data.

Availability of data and material

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

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Tafese Beyene Tufa: Conception and design, performance of laboratory investigations acquisition, analysis, and interpretation of data, drafting of the manuscript and approval of the manuscript for publication

Colin Mackenzie: Interpretation of data, critical revision of the manuscript and approval of the manuscript for publication

Hans Martin Orth: Conception and design, revision of the manuscript and approval of the manuscript for publication

Tobias Wienemann: supervise the laboratory investigations, interpret the results and approval of the manuscript for publication

Tamara Nordmann: Conception and design, revision of the manuscript and approval of the manuscript for publication

Sileshi Abdissa: Performance of laboratory investigations and approval of the manuscript for publication

Zewdu Hurissa: Interpretation of data, revision of the manuscript and approval of the manuscript for publication

Andreas Schönfeld: Conception and design, revision of the manuscript and approval of the manuscript for publication

Matthias Bosselmann: Conception and design, revision of the manuscript and approval of the manuscript for publication

Dieter Häussinger: Interpretation of data, critical revision of the manuscript and approval of the manuscript for publication

Klaus Pfeffer: Interpretation of data, critical revision of the manuscript and approval of the manuscript for publication

Tom Luedde: critical revision of the manuscript and approval of the manuscript for publication

André Fuchs: Conception and design, acquisition and interpretation of the data, revision of the manuscript and approval of the manuscript for publication

Torsten Feldt: Conception and design, interpretation and analysis of data, critical revision of the manuscript and approval of the manuscript for publication

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Figures

Figure 1

Flow diagram of participants' inclusion