High Prevalence of Multi-Drug Resistance and Extended Spectrum Beta Lactamase Production in Non-Fermenting Gram-Negative Bacilli in Ethiopia

Adane Bitew

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

ABSTRACT:

BACKGROUND: Emergence of resistance to multiple antimicrobial agents in Non-Fermenting Gram-Negative Bacilli is a major problem to public health, as it limits drug treatment options against infections. The aim of this study was to determine the prevalence of multi-drug resistance and extended spectrum beta lactamase production in Non-Fermenting Gram-Negative Bacilli.

MATERIALS AND METHODS: Different clinical samples were collected and processed following standard procedures. Each sample was then inoculated onto culture media. Identification, drug susceptibility testing, and extended spectrum beta lactamase production of the isolates were carried out by using the VITEK 2 compact system.

RESULTS: Among 996 clinical samples, 135 samples yielded Non-Fermenting Gram-Negative Bacilli of which Pseudomonas and Acinetobacter species were the commonest isolates. The overall drug resistance rates of Non-Fermenting Gram-Negative Bacilli were above 80% against ampicillin (89.6%), ceftazidime (86.9%), nitrofurantoin (85.9%), cefalotin (84.4%), cefoxitin (83.7%), cefazolin (83.0%), and cefuroxime (83.0%). Tobramycin with a resistance rate of 19.3% was the most active antimicrobial agent. Out of 135 isolates, 81.5% were multi-drug resistant of which 13.3% were extensively drug resistant and 10.4% were pandrug resistant. Extended spectrum beta lactamase production was detected in 48.9% of the isolates.

CONCLUSIONS: The spectrum of bacterial species isolated was diverse. The isolates demonstrated high level of drug resistance in different classes of antibiotics. The magnitude of multi-drug resistance and the level of extended spectrum beta lactamase production were high. Hence, further studies on multi-drug resistant and extended spectrum beta lactamase producing Non-Fermenting Gram-Negative Bacilli both in the community and in hospital setting are essential.

KEYWORDS: Multi-drug resistance, carbapenemase, extended spectrum beta lactamase, Non-Fermentative Gram-Negative Bacilli

Introduction

Non-Fermenting Gram-Negative Bacilli (NFGNB) are defined as strictly aerobic and non-spore forming group of bacteria that do not ferment carbohydrates but generate energy required for their metabolic activities by oxidative pathway.3 Intrinsic resistance of NFGNB to the commonly used antiseptics and their ability to survive under a wide range of environmental conditions have aided them to occupy different settings. They have been isolated from soil, aquatic environment, and hospital environments such as anesthesia equipment, sinks, intravenous fluids, and even distilled water. Their survival in different hospital settings facilitated them to transfer from patient to patient through fomites or the hands of medical staff causing hospital acquired infections.2,3

Non-Fermenting Gram-Negative Bacilli are taxonomically diverse group of bacteria that have been considered as commensals or contaminants for many years. However, numerous recent studies revealed that NFGNB are important cause of different types of nosocomial infections, including ventilator-associated pneumonia, septicemia, urinary tract infection, and surgical site infection,4-6 accounting for nearly 15% of all Gram-negative bacilli isolated from these infections.9-15 Immunosuppression, neutropenia, mechanical ventilation, cystic fibrosis, indwelling catheters, and invasive diagnostic and therapeutic techniques have been identified as major risk factors.14,15

Antimicrobial resistance has recently been identified as 1 of the 3 most important problems facing human health by the World Health Organization.16 Frequent isolation of multi-drug resistant (MDR) pathogens in both nosocomial and community-acquired infections further intensified the problem of antimicrobial resistance.17 Acinetobacter baumannii and Pseudomonas aeruginosa have been recognized as the most common and serious MDR pathogens.18

An increase in the incidence of nosocomial infection and the prevalence of MDR in NFGNB have significantly escalated the profile of these emerging opportunistic pathogens.5,19,20 Because of poor laboratory organization (laboratory equipment and supplies), the distribution, prevalence of multi-drug resistance, and the prevalence of extended spectrum beta
Materials and Methods

Study site and period

This study was conducted at Arsho Advanced Medical Laboratory over a period of 1 year from October 2016 to September 2017. Arsho Advanced Medical Laboratory is a private Limited Company located in Bole sub-city, Addis Ababa, Ethiopia. Patients having different clinically confirmed disease manifestations and those with no history of antibiotic drug therapy for not less than 2 weeks prior to their attendance were included in the study.

Different clinical samples from sputum, urine, wound, cerebrospinal fluid, ear, and nasal blood were collected and processed following standard procedures. Only 1 clinical sample was collected from each patient as per the request of the physicians. Specimens collected from each patient were inoculated onto culture media and incubated at appropriate temperature and period according to standard protocols related to each sample. Samples were cultured as soon as possible. In cases where a delay in culturing is an avoidable appropriate transport media were used.

Identification and drug susceptibility testing

Species characterization and antimicrobial susceptibility testing were performed with the VITEK 2 automated system (bioMérieux, France) using the AST-GN72 cards, in accordance with the manufacturer's instructions by inoculating pure isolates of bacterial pathogen and controls into AST-GN72 cards. In the case of mixed culture, only significant bacterium was used for further study. The ESBL phenotype was determined with the VITEK 2 automated system using the ESBL test panel with 6 wells containing 3 third-generation cephalosporins, alone (ceftepin, cefotaxime, and ceftazidime) and in combination with clavulanic acid (CA) as per the instruction of the manufacturer. Growth in each well was quantitatively measured by means of an optical scanner. Final results were investigated using version 4.0 software, an advanced expert system (AES) designed to evaluate the results produced by the VITEK 2 system. The relative reduction in growth in wells having cephalosporin plus CA compared with those containing the cephalosporin alone was considered positive for ESBL production. Strains were noted as ESBL-negative whenever phenotypic interpretations other than ESBLs were suggested by the AES. Quality control strains, P aeruginosa ATCC 27853, was included in each run. The isolates were categorized as MDR, extensively drug resistance (XDR), and pandemic drug resistance (PDR), as per the definition of Magiorakos et al.

Ethics and consent to participate

The study was carried out after the approval of the Institutional Review Board (IRB) of Department of Medical Laboratory Sciences (DRERC/246/16/MLS and Arsho Advanced Medical Laboratory Private Limited Company; AAML RERC/12/7/16). Data collection was started after obtaining written informed consent from study subjects, and assent form was completed and signed by parents or guardians for those study subject \( \leq 16 \) years of age. All the information obtained from the study subjects were coded to maintain confidentiality.

Results

Of a total of 996 clinical samples processed, NFGNB were recovered in 135 samples. Among the isolates, 78 (57.8%) were Pseudomonas species and 47 (34.8%) were Acinetobacter species. The remaining 10 (7.4%) isolates were represented by bacteria belonging to 6 different genera of NFGNB. P. aeruginosa (61; 45.2%) and A. baumannii (28; 20.7%) were the 2 most frequently isolated NFGNB (Table 1).

The antimicrobials tested and the percentage of antimicrobial resistance rates of NFGNB are depicted in Table 2. The overall resistance rates of NFGNB were above 80% against ampicillin (89.6%), cefuroxime axetil (88.9%), nitrofurantoin (85.9%), cefalotin (84.4%), cefoxitin (83.7%), cefazolin (83.0%), and cefturoxime (83.0%). Tobramycin with a resistance rate of 19.3%, gentamicin with a resistance rate of 23.7%, piperacillin/tazobactam combination with a resistance rate of 25.9%, and cefepime with a resistance rate of 25.9% were better active antimicrobial agents. Pseudomonas aeruginosa showed less resistance rates against tobramycin (6.6%), gentamicin (13.1%), piperacillin/tazobactam combination (16.4%), cefepime (19.7%), ciprofloxacin (19.7%), levofloxacin (23.0%), and ceftazidime (27.9%). The resistance rates of the pathogen toward the other antimicrobial agents tested were over 85%. Tobramycin and gentamicin were consistently the most active agents against other Pseudomonas species. These species were 100% susceptible to the 2 antimicrobial agents. Acinetobacter baumannii had high resistance rates against all antimicrobial agents tested. The lowest resistance rate for A. baumannii was observed against tobramycin (ie, 57.1%). Acinetobacter baumannii was equally resistant to ceftazidime, piperacillin/tazobactam combination, gentamicin, ciprofloxacin, and...
levofloxacin, that is, 67.9%. Other NFGNB isolates were better sensitive to the antimicrobial agents tested than the isolates of *Pseudomonas* and *Acinetobacter* species.

Multi-drug resistant profile of NFGNB and the prevalence of ESBL producing isolates are shown in Table 3. Out of 135 isolates of NFGNB, 110 (110/135; 81.5%) were MDR, of which 18 (18/135; 13.3%) were XDR and 14 (14/135; 10.4%) were PDR. Among 78 isolates of *Pseudomonas* species, 65 were MDR, of which 7 and 4 isolates were XDR and PDR, respectively. Among the isolates of *P. aeruginosa*, 56 (56/61; 91.8%) were MDR, of which 11 and 10 isolates were XDR and PDR, respectively. Our finding was similar with the findings of Malini et al. and Rit et al.

The overall drug resistance profile of NFGNB against cephalosporins was very high. Except for the extended beta lactam cephalosporins, cefepime and ceftazidime, the drug resistance rates of NFGNB against the 7 cephalosporins extend from 68.9% for ceftriaxone to 88.9% for cefuroxime axetil. The overall drug resistance profile of NFGNB against the commonly prescribed drugs in Ethiopia such as ampicillin (89.6%), nitrofurantoin (95.9%), tetracycline (68.1%), and trimethoprim-sulfamethoxazole (63.7%) was also high. A notable observation was that the majority of NFGNB showed a relatively reduced resistance pattern against aminoglycosides (gentamicin and tobramycin), fluoroquinolone (ciprofloxacin and levofloxacin), and piperacillin/tazobactam combination. The ability of NFGNB, in particular *P. aeruginosa* and *A. baumannii*, to quickly adapt to selective changes in environmental pressures, upregulation of the intrinsic resistance mechanisms, and

### Table 1. Distribution of NFGNB isolated from different clinical samples (n = 135).

| SPECIES                     | URINE | WOUND | BLOOD | CSF | EAR | NASAL | NO. OF ISOLATES (N, %) |
|-----------------------------|-------|-------|-------|-----|-----|-------|------------------------|
| *Pseudomonas aeruginosa*    | 5     | 25    | 6     | 5   | 12  | 8     | 61 (45.2)              |
| *Pseudomonas fluorescens*   | 1     | 1     | 1     | 1   | –   | –     | 4 (3.0)                |
| *Pseudomonas putida*        | –     | –     | 5     | –   | 1   | –     | 6 (4.4)                |
| *Pseudomonas luteola*       | 2     | 3     | –     | –   | 2   | –     | 7 (5.2)                |
| *Acinetobacter baumannii*   | 8     | 7     | 3     | 5   | 4   | 1     | 28 (20.7)              |
| *Acinetobacter calcoaceticus* | 1   | 2     | 3     | 1   | 1   | 1     | 9 (6.7)                |
| *Acinetobacter ursingii*    | –     | –     | 3     | –   | –   | –     | 7 (5.2)                |
| *Cupriavidus pauculus*      | –     | –     | –     | 1   | –   | –     | 1 (0.7)                |
| *Stenotrophomonas maltophilia* | –    | 1     | –     | –   | –   | –     | 1 (0.7)                |
| *Methylobacterium rhodesianum* | –   | –     | 1     | –   | –   | –     | 1 (0.7)                |
| *Moraxella nonliquefaciens*  | 1     | –     | –     | –   | –   | –     | 1 (0.7)                |
| *Sphingomonas paucimobilis*  | –     | –     | 2     | 2   | –   | –     | 4 (3.0)                |
| *Burkholderia cepacia*      | –     | –     | 1     | –   | 1   | –     | 2 (1.5)                |

No of species per sample (n, %) 19 (14.1) 40 (29.6) 28 (20.7) 13 (9.6) 25 (18.5) 10 (7.4) 135 (100)

Abbreviations: CSF, cerebrospinal fluid; NFGNB, Non-Fermenting Gram-Negative Bacilli.

**Discussion**

Non-Fermenting Gram-Negative Bacilli, once considered to be contaminants, emerged as important opportunistic pathogens. Their frequent isolation from patients with nosocomial infections has demonstrated their pathogenic potential in health care institution. The isolation rates of NFGNB, however, are not uniform. The isolation rate of NFGNB in the present study was 13.6%. Lower isolation rates of NFGNB than the present study have been reported in studies conducted in Brazil (2.2%), in Karnataka (4.5%), and in India (10.0%). On the contrary, higher isolation rates of NFGNB have been reported in studies carried out in Saudi Arabia (16%) by Eltabawy et al., in India (21.8%) by Sidhu et al., and again in India (45.9%) by Vijaya et al. Among 14 species of NFGNB, *P. aeruginosa* and *A. baumannii* were the most common isolates accounting for 45.2% and 20.7% of the total isolates, respectively. Our finding was similar with the findings of Malini et al. and Rit et al.

The overall drug resistance profile of NFGNB against cephalosporins was very high. Except for the extended beta lactam cephalosporins, cefepime and ceftazidime, the drug resistance rates of NFGNB against the 7 cephalosporins extend from 68.9% for ceftriaxone to 88.9% for cefuroxime axetil. The overall drug resistance profile of NFGNB against the commonly prescribed drugs in Ethiopia such as ampicillin (89.6%), nitrofurantoin (95.9%), tetracycline (68.1%), and trimethoprim-sulfamethoxazole (63.7%) was also high. A notable observation was that the majority of NFGNB showed a relatively reduced resistance pattern against aminoglycosides (gentamicin and tobramycin), fluoroquinolone (ciprofloxacin and levofloxacin), and piperacillin/tazobactam combination. The ability of NFGNB, in particular *P. aeruginosa* and *A. baumannii*, to quickly adapt to selective changes in environmental pressures, upregulation of the intrinsic resistance mechanisms,
Table 2. Percentage antimicrobial resistance profile of NFGNB (n = 135).

| ANTIMICROBIAL AGENTS | AM | AMC | TZP | CZ | CF | CXMA | CXM | FOX | CPD | CRO | CAZ | PEP | TM | GM | LEV | CIP | FT | TE | SXT |
|-----------------------|----|-----|-----|----|----|------|-----|-----|-----|-----|-----|-----|----|----|-----|----|----|----|----|
| P. aeruginosa (n = 61) | 98.4 | 90.2 | 16.4 | 91.8 | 91.8 | 96.7 | 95.1 | 93.4 | 93.4 | 91.8 | 27.9 | 19.7 | 6.6 | 13.1 | 23.0 | 19.7 | 91.8 | 91.8 | 88.5 |
| P. fluorescens (n = 4) | 25.0 | 0 | 0 | 50 | 50 | 50 | 0 | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 0 | 0 |
| P. putida (n = 6) | 100 | 66.7 | 0 | 83.3 | 100 | 100 | 100 | 100 | 83.4 | 16.7 | 0 | 16.7 | 0 | 0 | 66.7 | 33.3 | 100 | 50.0 | 50.0 |
| P. luteola (n = 7) | 71.4 | 14.3 | 0 | 57.1 | 57.1 | 42.9 | 42.9 | 57.1 | 42.9 | 14.3 | 14.3 | 0 | 0 | 0 | 14.3 | 14.3 | 57.1 | 14.3 | 0 |
| A. baumannii (n = 28) | 100 | 92.9 | 67.9 | 92.9 | 96.4 | 100 | 100 | 100 | 92.9 | 89.3 | 67.9 | 96.7 | 96.7 | 96.4 | 67.9 | 67.9 | 96.4 | 71.4 | 64.3 |
| A. calcoaceticus (n = 9) | 100 | 88.9 | 44.4 | 100 | 100 | 100 | 100 | 100 | 88.9 | 77.8 | 66.7 | 44.4 | 44.4 | 44.4 | 77.8 | 77.8 | 100 | 55.6 | 55.6 |
| A. ursingii (n = 7) | 42.9 | 28.6 | 0 | 57.1 | 71.4 | 42.9 | 42.9 | 42.9 | 42.9 | 14.3 | 14.3 | 14.3 | 14.3 | 14.3 | 28.6 | 14.4 | 42.9 | 57.1 | 71.4 | 42.9 | 57.1 |
| C. pauculus (n = 1) | 0 | 0 | 0 | 100 | 66.7 | 33.3 | 33.3 | 33.3 | 66.7 | 0 | 33.3 | 0 | 0 | 0 | 33.3 | 33.3 | 100 | 0 | 33.3 |
| S. maltophilia (n = 1) | 100 | 0 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| M. rhodesianum (n = 1) | 100 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| M. nonliquefacens (n = 1) | 100 | 100 | 0 | 0 | 100 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 | 0 |
| S. paucimobilis (n = 4) | 75 | 0 | 50 | 25 | 25 | 25 | 25 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 50 | 25 | 0 |
| B. cepacia (n = 2) | 100 | 100 | 0 | 100 | 100 | 100 | 100 | 100 | 100 | 50 | 0 | 0 | 0 | 0 | 50 | 100 | 100 | 50 |
| Total | 89.6 | 74.1 | 25.9 | 83.0 | 84.4 | 88.9 | 83.0 | 83.7 | 79.3 | 68.9 | 33.3 | 25.9 | 19.3 | 23.7 | 36.3 | 34.8 | 85.9 | 68.1 | 63.7 |

Abbreviations: AM, ampicillin; AMC, amoxicillin/clavulanic acid; CAZ, ceftazidime; CF, cefalotin; CIP, ciprofloxacin; CPD, ceftodoxime; CRO, ceftriaxone; CXM, cefuroxime; CXMA, cefuroxime axetil; CZ, cefazolin; FOX, cefoxitin; FT, nitrofurantoin; GM, gentamicin; LEV, levofloxacin; PEP, cefepime; SXT, trimethoprim/sulfamethoxazole; TE, tetracycline; TM, tobramycin; TZP, piperacillin/tazobactam.
and acquisition and transferring of drug resistance genes through mobile genetic elements such as plasmids and transposons could be possible explanation for an elevated overall drug resistance prevalence rates against different category of drugs. Drug resistance genes so acquired are known to facilitate bacteria to produce beta lactamase enzymes particularly of ESBLs that confer resistance to the majority of beta lactam antibiotics. ESBLs producing Gram-negative bacteria have also been identified to have additional resistance mechanisms to other categories of antimicrobials such as phenicols, sulfonamides, fluoroquinolones, tetracyclines, and aminoglycoside.30

As far as species-specific antimicrobial resistance rates are concerned, the drug reissuance rate of P. aeruginosa ranged from 6.6% to 98.4%. Antimicrobial agents in antimicrobial classes of fluoroquinolone (ciprofloaxine and levofloxaolin) and aminoglycosides (tobramycin and gentamicin) were better active against the isolates of P. aeruginosa of which tobramycin was the most active drug with a resistance rate of 6.6%. Our finding was similar with that of Manikandan and Amsath31 who reported 6% resistance rate of P. aeruginosa against tobramycin. Of a panel of 9 cephalosporins tested, the 2 extended beta lactam cephalosporins, cefepime and ceftazidime, with resistance rates of 19.7% and 27.9% were better active than the remaining antimicrobial agents within the class. The resistance rates of the pathogen to the first-generation cephalosporins ranged from 96.7% for cefuroxime axetil to 91.8% for cefazolin and cephalexin. Our observation was consistent with those of Manikandan and Amsath31 and Lu et al.32 In contrast to our result, a study conducted in Egypt by Hassuna et al33 reported that 86% and 72% of the isolates of P. aeruginosa have been resistant to the extended beta lactams, ceftazidime and cefotaxime, respectively. Similarly, a study from Tehran demonstrated that P. aeruginosa was 100% resistant to cefepime, ceftazidime, ceftriaxone, and ciprofloxacine.34 Among the penicillin plus beta lactam inhibitor combinations, the piperacillin/tazobactam combination was better active with a resistance rate of 16.4%. The susceptibility rates of the bacterium were extremely poor for the old generation antimicrobial agents such as tetracycline, SXT (trimethoprim/sulfamethoxazole), ampicillin, and nitrofurantoin. Our result in this regard was similar with the findings of Manikandan and Amsath31 who reported 90% resistant rates of P. aeruginosa to both amoxicillin and ampicillin. The overall resistance rates of P. putida and P. luteola were also high except for tobramycin, gentamicin, and piperacillin/tazobactam combination in which both species were 100 susceptible. On the contrary, P. fluorescens showed a highly reduced resistance to almost all drugs.

### Table 3. Prevalence of multi-drug resistance and ESBL production in NFGNB against 8 antimicrobial categories.

| SPECIES             | R0 | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | N (%) MDR | N (%) XDR | N (%) PDR | ESBL (%) |
|---------------------|----|----|----|----|----|----|----|----|----|-----------|-----------|-----------|----------|
| P. aeruginosa (n=61)| 2  | 1  | 2  | 1  | 0  | 1  | 46 | 4  | 4  | 56 (91.8) | 6 (9.8)   | 4 (6.6)   | 40 (51.3) |
| P. fluorescens (n=4)| 2  | 0  | 2  | 0  | 0  | 0  | 0  | 0  | 0  | 0 (0.0)   | 0 (0.0)   | 0 (0.0)   | –        |
| P. putida (n=6)     | 0  | 0  | 0  | 0  | 2  | 3  | 1  | 0  | 0  | 6 (100)   | 1 (16.7)  | 0 (0.0)   | 1 (1.3)  |
| P. luteola (n=7)    | 2  | 1  | 1  | 2  | 0  | 1  | 0  | 0  | 0  | 3 (42.9)  | 0 (0.0)   | 0 (0.0)   | 1 (1.3)  |
| A. baumannii (n=28)| 0  | 1  | 1  | 5  | 0  | 0  | 7  | 13 | 26 (92.9)| 10 (35.7) | 7 (25.0)  | 21 (44.7) |
| A. calcoaceticus (n=9)| 0 | 0 | 0 | 1 | 3 | 1 | 0 | 4 | 9 (100) | 0 (0.0) | 3 (33.3) | 2 (4.3) |
| A. iwofii (n=7)     | 0  | 2  | 0  | 3  | 1  | 0  | 1  | 0  | 0  | 5 (71.4)  | 1 (14.3)  | 0 (0.0)   | 1 (2.1)  |
| A. ursingi (n=3)    | 0  | 1  | 1  | 1  | 0  | 0  | 0  | 0  | 0  | 1 (33.3)  | 0 (0.0)   | 0 (0.0)   | –        |
| C. pauculus (n=1)   | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 (0.0)   | 0 (0.0)   | 0 (0.0)   | –        |
| S. maltophilia (n=1)| 1  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 (0.0)   | 0 (0.0)   | 0 (0.0)   | –        |
| M. rhodesianum (n=1)| 0  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 1 (100)   | 0 (0.0)   | 0 (0.0)   | –        |
| M. nonliquefaciens (n=1)| 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 (100) | 0 (0.0) | 0 (0.0) | – |
| S. paucimobilis (n=4)| 3  | 0  | 0  | 0  | 1  | 0  | 0  | 0  | 0  | 1 (25.0)  | 0 (0.0)   | 0 (0.0)   | –        |
| B. cepacia (n=2)    | 0  | 0  | O  | 0  | 1  | 0  | 0  | 0  | 1  | 1 (50.0)  | 0 (0.0)   | 0 (0.0)   | –        |
| Total               | 10 | 7  | 7  | 10 | 14 | 6  | 48 | 11 | 22 | 110 (81.5%)| 18 (13.3%)| 14 (10.4%)| –        |

Abbreviations: ESBL, extended spectrum beta lactamase; MDR, multi-drug resistant; NFGNB, Non-Fermenting Gram-Negative Bacilli; PDR, pandemic drug resistance; XDR, extensively drug resistance.
R0: no antibiotic resistant; R1: resistant to 1 antimicrobial category; R2: resistant to 2 antimicrobial categories; R3: resistant to 3 antimicrobial categories; R4: resistant to 4 antimicrobial categories; R5: resistant to 5 antimicrobial categories; R6: resistant to 6 antimicrobial categories, R7: resistant to 7 antimicrobial categories, R8: resistant to 8 antimicrobial categories.
Acinetobacter baumannii, the second most commonly isolated bacterium, had the highest rates of resistance to most antimicrobial agents than other NFGNB. None of the tested antimicrobial agents achieved susceptibility rates above 45% of which the least resistance rates were demonstrated against cefepime and tobramycin. The resistance rates of gentamicin, ciprofloxacin, levofloxacin, ceftazidime, and pipercillin/tazobactam combination were 67.9%. The pathogen was 100% resistant to ampicillin, cefuroxime axetil, cefuroxime, and cefoxitin. Our findings was in line with the findings of Lu et al32 who reported a resistance rate of 77% to 100% to cephalosporins, greater than 80% to fluoroquinolone and greater than 75% to pipercillin/tazobactam combination. A. calcoaceticus and A. lwofii depicted more or less similar resistance pattern as A. baumannii.

Multi-drug resistant in NFGNB has emerged as a main cause of health-care-related infections particularly in patients whose immune system is compromised by underlying diseases. This was evident by the present study in which out of 135 isolates of NFGNB, 110 (81.5%) were MDR in which P. aeruginosa accounted for 56 (91.8%) of the isolates. The prevalence rate of MDR strains of the bacterium observed in our study was higher than the prevalence rates documented by Gales et al33 (Canada, 3.3%), Gill et al35 (India, 50%), and Saderi and Owlia36 (Tehran, 54.5%). Among the MDR strains of the bacterium, 6 (9.8%) and 4 (6.6%) of them were noted as XDR and PDR, respectively. Lower (2.3%) and higher (33% and 63%) prevalence rates of XDR isolates of the pathogen than the present report were documented by Gill et al35, Saderi and Owlia36 and Hasanin et al37 respectively. However, none of these studies reported PDR. The prevalence of MDR in A. baumannii was also very high. Out of 28 isolates of the bacterium, 26 (92.9%) were MDR, whereas 24 NFGNB (36.4%) were ESBL producers, 42 NFGNB (63.6%) were Pseudomonas species, whereas 24 NFGNB (36.4%) were Acinetobacter species. Among ESBL producing Pseudomonas species, 95.2% (40/42) were represented by P. aeruginosa. Similarly, among ESBL producing species of Acinetobacter species, 87.5% (21/24) were accounted by A. baumannii. ESBL production rate in P. aeruginosa and A. baumannii in our study was higher than ESBL production rates reported in Turkey by Vahaboglu et al43 in Korea by Yong et al44 in India by Sinha et al45 and in Ethiopia by Solomon et al.46 Species of Cupriavidus, Stenotrophomonas, Methylobacterium, Moraxella, Sphingomonas, and Burkholderia are emerging as potential human pathogens causing life-threatening bloodstream infections.

Conclusions
Isolation of large number of NFGNB, high prevalence of multi-drug resistance, and ESBL phenotypes warrant for further study in this field, including the consequences of colonization with MDR and ESBL producing NFGNB, both in the community and in hospital setting.

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Author Contributions
Research design, experimental work, data analysis and write up of the work were carried out by Adane Bitew.

ORCID iD
Adane Bitew https://orcid.org/0000-0003-1470-479X

Data Availability Statement
All the data are found in the manuscript and there are no supplementary files because we did not collect images and videos, and our study does not include sequencing and structure.

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