Molecular epidemiology and antimicrobial susceptibility of Pseudomonas spp. and Acinetobacter spp. from clinical samples at Jimma medical center, Ethiopia

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Introduction: Pseudomonas aeruginosa (P. aeruginosa) and Acinetobacter baumannii (A. baumannii) can cause difficult-to-treat infections. We characterized molecular epidemiology of ceftazidime-resistant P. aeruginosa and carbapenem-resistant A. baumannii at a tertiary hospital in Ethiopia.

Materials and methods: Non-fermenting gram-negative bacilli (n = 80) isolated from admitted patients were subjected for species identification by MALDI-TOF. Pseudomonas species resistant to ceftazidime or meropenem, and Acinetobacter species resistant to meropenem, or imipenem were selected for whole genome sequencing. DNA extracted with EZ1 Advanced XL instrument (Qiagen, Hilden, Germany) was sequenced on Illumina (HiSeq2500) using libraries prepared by NEXTRA-kits (Illumina). Raw reads were assembled using SPAdes 3.13.0, and assembled genomes were used to query databases for resistome profile and sequence types.

Result: Among Pseudomonas species isolated, 31.7% (31/101) and 7.3% (3/41) were non-susceptible to ceftazidime, and meropenem, respectively. Carbapenem-resistance was 56.4% (22/39) among Acinetobacter species. Moreover, 92% (12/13) of Pseudomonas species non-susceptible to ceftazidime and/or meropenem, and 89.4% (17/19) of Acinetobacter species encoded multiple resistance genes for at least three classes of antimicrobials. The prevalent β-lactamase genes were blaOXA−48 (53.8%, 7/13), blaCTX−M−15 (23.0%, 3/13) among Pseudomonas species, and blaOXA−11 (57.8%, 11/19) among Acinetobacter. The blaOXA−51-like β-lactamase, blaOXA−69 (63.1%, 12/19) was the most prevalent carbapenemase gene among Acinetobacter isolates. Single isolates from both P. aeruginosa, and A. baumannii were detected with the blaNDM−1. Sequence type (ST)1 A. baumannii and ST274 P. aeruginosa were the prevalent sequence types. A cgMLST analysis of the ST1 A. baumannii
isolates showed that they were closely related and belonged to the international clonal complex one (ICC1). Similarly, ST274 *P. aeruginosa* isolates were clonally related.

**Conclusion:** The prevalence of MDR isolates of *Pseudomonas* and *Acinetobacter* spp. was high. *A. baumannii* isolates were clonally spreading in the admission wards at the hospital. Emergence of bla_{NDM−1} in the intensive care, and surgical wards of the hospital is a severe threat that requires urgent intervention.

**KEYWORDS** ESBLs, carbapenemase, bla_{CTX−M−15}, bla_{GES−11}, bla_{NDM−1}, *P. aeruginosa*, *A. baumannii*, Ethiopia

**Introduction**

*Pseudomonas aeruginosa* (*P. aeruginosa*) and *Acinetobacter baumannii* (*A. baumannii*) are among the main causes of nosocomial infections (De Oliveira et al., 2020). They belong to the group of bacteria known as “ESKAPE” (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *Enterobacter* species). Moreover, these group of bacteria are difficult to treat in most cases (Bergogne-Bérézin and Towner, 1996; De Oliveira et al., 2020; Ma et al., 2020).

The prevalence of antimicrobial resistant sub-populations of these strains has rapidly increased over the last few decades (Wong et al., 2017; Horcajada et al., 2019). Carbapenemase-producing *A. baumannii* (CRAB) and *P. aeruginosa* were listed top two of the three critical priority pathogens for which new antimicrobials are urgently needed (WHO, 2017). Several *Acinetobacter* and *Pseudomonas* species were previously reported from different clinical samples from both animal, and human infections (Chusri et al., 2014; Wong et al., 2017; Agnese et al., 2018). Many of them were resistant to multiple classes of antibiotics primarily by several intrinsic resistance mechanism they encode, and secondly by acquired resistance mechanisms (Bello-López et al., 2020; Meng et al., 2020). Studies have also shown that multidrug-resistant strains of *P. aeruginosa* and *A. baumannii* were the main drivers of hospital-acquired infections (Djahmi et al., 2014; Eichenberger and Thaden, 2019; Kazmierczak et al., 2020). A recent review of global epidemiology of carbapenemase-producing Gram-negative bacteria reported that carbapenemase-producing *P. aeruginosa* (CRPA) were associated with high mortality and morbidity among hospitalized patients with pneumonia and bloodstream infections in the United States (Brink, 2019). Though regional variations are common, colonization by carbapenem-resistant *A. baumannii* increased the risk of acquisition of bloodstream infection four-fold (Munoz-Price et al., 2016; Bassetti et al., 2017). In low-income countries like Ethiopia, comprehensive microbiological data is lacking.

The classical phenotyping methods commonly used in low-income countries cannot reliably define mechanism of resistance in both *Pseudomonas* and *Acinetobacter* species. Lack of sufficient standardized genotypic methods for detection and tracking of multidrug-resistant or extensively drug-resistant isolates was one of the challenges to understand epidemiology of antimicrobial resistance in sub-Saharan African countries (Eichenberger and Thaden, 2019). In most cases, global reports on antimicrobial resistance lack data from African countries. Despite discrepancies in availability of data, there is sufficient overall evidence that carbapenemase-producing Gram-negative bacilli have become a threat to global health. Rapid detection and tracking of any ongoing spread of resistant strains is necessary.

We aimed to analyze the phenotypic and molecular characteristics of *Pseudomonas* species and *Acinetobacter* species isolated from clinical samples at Jimma Medical Center (JMC), a tertiary hospital in Ethiopia.

**Materials and methods**

**Isolation, identification, and selection of strains**

As part of a large epidemiological study, a total of 1,087 clinical samples (urine, stools, wound secretions, and sputum) were collected from patients with suspected infections seeking medical care from June to October 2016 at JMC, Ethiopia. *Pseudomonas* species and *Acinetobacter* species were isolated on MacConkey agar and sheep blood agar. Species identification was performed by MALDI-TOF (Bruker Daltonik GmbH, Bremen, Germany) at Karolinska University Hospital (KUH), Clinical Microbiology laboratory, and a full panel of
| Patient ID | Age | Sex | Inpatient/Outpatient | Current diagnosis | *Current antibiotic | Specimen | Underlying disease | Pseudomonas species |
|------------|-----|-----|----------------------|-------------------|---------------------|----------|-------------------|-------------------|
| I020       | 28  | M   | Inpatient            | Surgical site infection | CRO, MET           | Wound swab | Surgical incision | P. aeruginosa     |
| I032       | 28  | M   | Inpatient            | Urinary tract infection | CRO, MET           | Urine     | Trauma            | P. aeruginosa     |
| I038       | 30  | M   | Inpatient            | Urinary tract infection | CRO                | Urine     | Severe head injury | P. putida        |
| I043       | 17  | M   | Inpatient            | Urinary tract infection | CRO                | Urine     | Aspiration pneumonia | P. aeruginosa |
| M019       | 70  | M   | Inpatient            | COPD               | CRO, VAN           | Sputum    | Cor pulmonale     | P. aeruginosa     |
| M030       | 60  | M   | Inpatient            | Community-acquired pneumonia | CRO       | Sputum    | **COPD           | P. aeruginosa     |
| M074       | 50  | M   | Inpatient            | COPD               | VAN, CIP           | Sputum    | Asthma            | P. aeruginosa     |
| M119       | 40  | M   | Inpatient            | Pneumonia          | CRO                | Sputum    | Post TB fibrosis  | P. aeruginosa     |
| M304       | 40  | M   | Inpatient            | Community-acquired pneumonia | No       | Sputum    | Post TB fibrosis  | P. aeruginosa     |
| M334       | 35  | F   | Inpatient            | Severe community-acquired pneumonia | CRO       | Sputum    | No                | P. aeruginosa     |
| M521       | 60  | F   | Outpatient           | Community-acquired pneumonia | CRO       | Sputum    | **T2DM           | P. aeruginosa     |
| P014       | 14  | M   | Inpatient            | Wound infection    | AUG                | Wound swab | No                | P. aeruginosa     |
| P109       | 4   | F   | Inpatient            | Diarrhea           | AMox, GENT        | Stool     | SAM, pneumonia    | P. aeruginosa     |
| S007       | 32  | M   | Inpatient            | Necrotizing fasciitis | No                | Wound swab | No                | P. aeruginosa     |
| S010       | 5   | M   | Inpatient            | Wound infection    | CAF, CLO          | Wound swab | Trauma            | P. aeruginosa     |
| S011       | 7   | M   | Inpatient            | Wound infection    | AMP, CAF          | Wound swab | Trauma            | P. aeruginosa     |
| S017       | 18  | M   | Inpatient            | Necrotizing fasciitis | CRO, MET          | Wound swab | No                | P. aeruginosa     |
| S019       | 60  | M   | Inpatient            | Foot ulcer         | CRO, MET          | Wound swab | Diabetes mellitus | P. aeruginosa     |
| S020       | 30  | M   | Inpatient            | Wound infection    | CRO, MET          | Wound swab | Trauma            | P. aeruginosa     |
| S036       | 17  | F   | Inpatient            | Wound infection    | No                | Wound swab | Unstable pelvis fracture | P. aeruginosa |
| S047       | 10  | M   | Inpatient            | Wound infection    | AMP                | Wound swab | Chronic osteomyelitis | P. aeruginosa |
| S048       | 15  | M   | Inpatient            | Wound infection    | AMP, CAF          | Wound swab | Fracture of femoral shaft | P. aeruginosa |
| S077       | 3   | M   | Inpatient            | Wound infection    | AMP, GENT         | Wound swab | Colostomy, imperforation | P. aeruginosa |
| S116       | 50  | F   | Inpatient            | Wound infection    | AMOX, MET         | Wound swab | Uterine cancer     | P. aeruginosa     |
| S129       | 77  | M   | Inpatient            | Wound infection    | CRO, VAN          | Wound swab | Amputation of leg  | P. aeruginosa     |
| S133       | 45  | M   | Inpatient            | Wound infection    | CRO, MET          | Wound swab | Neck injury trauma | P. aeruginosa     |
| S114       | 25  | F   | Inpatient            | Acute kidney infection | CRO, MET          | Urine     | Rib fracture       | P. aeruginosa     |
| S155       | 21  | F   | Inpatient            | Wound infection    | AMP, CAF          | Wound swab | Infected skin graft | P. aeruginosa     |
| S174       | 60  | M   | Inpatient            | Wound infection    | CAE, AMP          | Wound swab | Infected fracture site | P. aeruginosa |
| S192       | 75  | M   | Inpatient            | Urinary tract infection | CRO               | Urine     | **BOO             | P. putida        |
| S195       | 21  | F   | Inpatient            | Wound infection    | CRO, MET          | Wound swab | Skin graft infection | P. aeruginosa     |
| S198       | 57  | M   | Outpatient           | Wound infection    | CLO, CAF          | Wound swab | Left femoral fracture | P. aeruginosa |

(Continued)
TABLE 1A (Continued)

| Patient ID | Age | Sex | Inpatient/ outpatient | Current diagnosis | *Current antibiotic | Specimen | Underlying disease | Pseudomonas species |
|------------|-----|-----|-----------------------|-------------------|--------------------|----------|-------------------|---------------------|
| S209       | 18  | F   | Inpatient             | Wound infection   | CRO                | Wound swab | Burn wound        | P. aeruginosa       |
| S248       | 20  | F   | Inpatient             | Contaminated wound| CRO                | Wound swab | No                | P. aeruginosa       |
| S288       | 50  | M   | Inpatient             | Pneumonia         | No                 | Sputum    | Abdominal mass    | P. aeruginosa       |
| S319       | 40  | M   | Inpatient             | Urinary tract infection| No             | Urine     | BPH               | P. aeruginosa       |
| S325       | 37  | M   | Inpatient             | Wound infection   | CAF, AMP           | Wound swab | Compound distal fracture | P. aeruginosa |
| S328       | 60  | M   | Inpatient             | Pneumonia         | CRO, MET           | Sputum    | 3rd degree burn   | P. aeruginosa       |
| S332       | 30  | M   | Inpatient             | Wound infection   | CRO, MET           | Wound swab | 2nd degree burn   | P. fulva            |
| S356       | 30  | M   | Inpatient             | Wound infection   | CRO, MET           | Wound swab | Infected palate of right knee | P. aeruginosa |
| S371       | 64  | M   | Inpatient             | Surgical site infection | CLO, CAF           | Wound swab | Laparotomy         | P. aeruginosa       |

*Current antibiotics: CRO, ceftriaxone; Met, metronidazole; VAN, vancomycin; CLO, cloxacillin; CAF, chloramphenicol; CIP, ciprofloxacin; AMP, ampicillin; GENT, gentamicin.

**Underlying diseases: COPD, congestive obstructive pulmonary disease; T2DM, type-2 diabetes mellitus; BOO, Bladder outlet obstruction; BPH, Benign prostatic hyperplasia.

Antimicrobial susceptibility testing was performed by using the EUCAST 2021 v11 guideline. 1

**Antimicrobial susceptibility testing**

All *Pseudomonas* species and *Acinetobacter* species isolated were subjected to disk-diffusion susceptibility testing. Antibiotic discs of ceftazidime, meropenem, piperacillin-tazobactam, gentamicin, amikacin, ciprofloxacin was used for *Pseudomonas* species. Similarly, all *Acinetobacter* isolates were tested by using meropenem, imipenem, gentamicin, amikacin, ciprofloxacin, trimethoprim-sulfamethoxazole. Then, isolates with reduced susceptibility to ceftazidime and/or meropenem for *Pseudomonas* spp., and meropenem or imipenem for *Acinetobacter* spp. were selected for antimicrobial susceptibility testing using the newer antimicrobials (cefiderocol, ceftazidime-avibactam, ceftolozane-tazobactam, and imipenem-relebactam for *Pseudomonas* spp., and cefiderocol, meropenem, imipenem, and imipenem-relebactam for *Acinetobacter* spp. using microbroth dilution technique), and whole genome sequencing (WGS). Patients’ clinical data like admission, presence of underlying chronic illnesses, current use of antibiotics, and other factors was collected using a structured questionnaire.

DNA was quantified using Qubit™ 3.0 (Massachusetts, United States) and library preps were performed using NEXTRA-kit (Illumina) and sequenced using HiSeq2500 (Illumina). Raw reads were assembled using SPAdes ver. 3.13.0, and the assembled draft genomes were used for querying different databases, MLST-typing 2.0, hosted at center for genomic epidemiology, and detection resistome profile by using ResFinder 4.1. 2, 3 Epidemiologic analysis of relatedness between the isolates, and to other international isolates was performed by the minimum spanning tree using the isolate genomes deposited at the public domain for *A. baumannii* at 4, and *P. aeruginosa* at. 5 The Genome sequences were deposited at the NCBI, SRA database (Bioproject number: PRJNA593604, Biosample accession: SUB11593554).

Results

**Clinical and demographic characteristics of the patient**

From a total of 1,087 non-repeat clinical samples collected during the study period, non-duplicate, non-fermenting Gram-negative bacilli that belong to either *Pseudomonas* spp. or *Acinetobacter* spp. were isolated from 80 patients. Most of these patients, 73.7% (59/80) were male, and 26.3% (21/80) were female. Ninety percent (72/80) of these patients were

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1. www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_11.0_Breakpoint_Tables.pdf

2. https://cge.food.dtu.dk/services/MLST/

3. https://cge.food.dtu.dk/services/ResFinder/

4. https://pubmlst.org/bigsdb?db=pubmlst_abumannii_isolates&query=genomes=1

5. https://pubmlst.org/organisms/pseudomonas-aeruginosa
| Patient ID | Age | Sex | Inpatient/ outpatient | Current diagnosis                     | *Current antibiotic | Specimen | Underlying disease              | Bacterial species |
|------------|-----|-----|-----------------------|---------------------------------------|---------------------|----------|-------------------------------|------------------|
| I027       | 45  | F   | Inpatient             | Aspiration pneumonia                  | CRO, MET            | Sputum   | Stroke/hemiparalysis           | A. baylyi        |
| I030       | 28  | F   | Inpatient             | Skin infection                        | No                  | Wound swab | No                            | A. baumannii     |
| M029       | 22  | M   | Inpatient             | Urinary tract infection               | CRO                 | Urine    | Retroviral infection           | A. baumannii     |
| M037       | 60  | F   | Inpatient             | COPD                                  | No                  | Sputum   | No                            | A. baumannii     |
| M135       | 70  | F   | Outpatient            | Community-acquired pneumonia          | CRO                 | Sputum   | No                            | A. junii         |
| M212       | 25  | M   | Inpatient             | Pneumonia                             | CRO, VAN            | Sputum   | Disseminated tuberculosis      | A. calcoaceticus |
| M217       | 35  | M   | Inpatient             | Community-acquired pneumonia          | No                  | Sputum   | **COPD                        | A. schindleri    |
| M328       | 50  | F   | Outpatient            | Severe community-acquired pneumonia   | No                  | Sputum   | **T2DM                        | A. baumannii     |
| M344       | 17  | M   | Outpatient            | Pneumonia                             | No                  | Sputum   | Electrical burn                | A. junii         |
| M431       | 50  | F   | Inpatient             | Pneumonia                             | CRO                 | Sputum   | Rt&Lt femoral fracture         | A. baumannii     |
| P015       | 30  | M   | Inpatient             | Wound infection                       | CLO, CAF            | Wound swab | Compound fracture of right tibia | A. baumannii     |
| P038       | 35  | M   | Outpatient            | Wound infection                       | AMP, CAF            | Wound swab | Infected fracture site         | A. baumannii     |
| S073       | 15  | M   | Inpatient             | Wound infection                       | CRO                 | Wound swab | Chronic osteomyelitis          | A. baumannii     |
| S082       | 45  | M   | Inpatient             | Wound infection                       | CIP                 | Wound swab | Femoral fracture               | A. baumannii     |
| S126       | 40  | M   | Inpatient             | Wound infection                       | CRO, MET            | Wound swab | Surgical site infection        | A. baumannii     |
| S130       | 30  | M   | Inpatient             | Wound infection                       | CRO, MET            | Wound swab | 3rd deg. burn                 | A. baumannii     |
| S147       | 30  | F   | Inpatient             | Wound infection                       | CRO                 | Wound swab | Colostomy                     | A. baumannii     |
| S161       | 50  | M   | Inpatient             | Wound infection                       | AMP, CAF            | Wound swab | No                            | A. parvus        |
| S165       | 8   | M   | Inpatient             | Wound infection                       | CAF, CLO            | Wound swab | No                            | A. baumannii     |
| S167       | 40  | M   | Inpatient             | Wound infection                       | CRO, MET            | Wound swab | Scalp abscess                  | A. baumannii     |
| S170       | 27  | M   | Inpatient             | Wound infection                       | CRO, MET            | Wound swab | No                            | A. baumannii     |
| S171       | 32  | M   | Inpatient             | Wound infection                       | CRO, MET            | Wound swab | Retroviral infection           | A. baumannii     |
| S176       | 25  | F   | Inpatient             | Necrotic wound infection              | CRO, MET            | Wound swab | No                            | A. baumannii     |
| S209       | 50  | M   | Outpatient            | Pneumonia                             | CRO, MET            | Sputum   | No                            | A. baumannii     |
| S210       | 25  | M   | Inpatient             | Wound infection                       | CRO                 | Wound swab | No                            | A. baumannii     |
| S212       | 40  | M   | Inpatient             | Wound infection                       | CRO, MET            | Wound swab | Compound fracture left leg     | A. baumannii     |
| S217       | 48  | M   | Inpatient             | Pneumonia                             | CRO, MET            | Sputum   | colostomy/post-operation      | A. baumannii     |
| S219       | 39  | M   | Inpatient             | Wound infection                       | No                  | Wound swab | Tibia-fibular fracture        | A. baumannii     |
| S226       | 24  | M   | Inpatient             | Urinary tract infection               | No                  | Urine    | Urethral stricture             | A. haemolyticus  |
| S247       | 40  | M   | Outpatient            | Wound infection                       | AMP, CAF            | Wound swab | Infected incision site        | A. baumannii     |
| S260       | 18  | F   | Inpatient             | Wound infection                       | CRO                 | Wound swab | Burn wound                    | A. baumannii     |
| S267       | 30  | F   | Inpatient             | Wound infection                       | CRO, MET            | Wound swab | Surgical site infection       | A. baumannii     |
| S270       | 26  | M   | Inpatient             | Wound infection                       | CRO, MET            | Wound swab | Chronic osteomyelitis          | A. baumannii     |
| S275       | 48  | M   | Inpatient             | Wound infection                       | No                  | Wound swab | Compound fracture of femur    | A. baumannii     |
| S294       | 70  | M   | Inpatient             | Urinary tract infection               | CRO                 | Urine    | Bladder outlet obstruction    | A. baumannii     |

(Continued)
admitted to different wards in the hospital [surgical ward (n = 52), intensive care unit (n = 6), pediatric ward (n = 3), and medical ward (n = 11)]. Clinical samples collected include urine for urinary tract infections, sputum for lower respiratory tract infections, wound swab for wound infections/abscess, and stools for diarrhea. Most of these patients were admitted to the hospital for other underlying diseases and developed infection after admission, and most of them were treated with locally available antimicrobial therapy. Among Pseudomonas isolates 68.2% (28/41) were from surgical ward, followed by 17.0% (7/41) from medical ward, and 9.7% (4/41) from the intensive care unit (Table 1A). Similarly, 69.2% (27/39) of the Acinetobacter species were isolated from the surgical ward and 20.5% (8/39) from the medical ward (Table 1B).

Species diversity and antimicrobial susceptibility

Species diversity
From a total of 41 isolates of Pseudomonas spp., 92.6% (38/41) were P. aeruginosa, and 7.3% (3/41) were another Pseudomonas spp. Among 39 isolates of Acinetobacter spp. (n = 39), 79.5% (31/39) were A. baumannii, and 20.5% (8/39) were other Acinetobacter spp. [A. junii which account for 5.1% (2/39), and A. baylyi, A. lwofii, A. calcoaceticus, A. haemolyticus, A. parvus, and A. schindleri each account for 2.5% (1/39)].

Antimicrobial susceptibility pattern
Among isolates of Pseudomonas spp., 31.7%, (13/41) were non-susceptible to ceftazidime, and 7.3% (3/41) to meropenem. Among isolates of Acinetobacter spp., 56.4% (22/39) were carbapenem-resistant. Most of the ceftazidine-resistant Pseudomonas spp. were also resistant to piperacillin-tazobactam 84.6% (11/13), ciprofloxacin 30.7% (4/13), ceftazidime-avibactam 46.1% (6/13) and ceftolozane-tazobactam 53.8%

(7/13). However, most of these isolates were susceptible to cefiderocol 84.7% (11/13), imipenem-relebactam 92.3% (12/13), and all Pseudomonas isolates were susceptible to amikacin (Table 2A). Most of the Acinetobacter isolates were resistant to meropenem, imipenem, and imipenem-relebactam 54.5%, (12/22). However, a lower rate of resistance was detected to ciprofloxacin 18.1% (4/22), gentamicin 27.2% (6/22), amikacin 18.1% (4/22), and cefiderocol 9.1% (2/22) (Table 2B).

Resistome profile of Pseudomonas and Acinetobacter species

Resistome profiling showed that both Pseudomonas and Acinetobacter spp. encoded multiple β-lactamase genes. The blaOXA−48, blaOXA−50 and blaCTX−M−15 genes were most common among Pseudomonas isolates, and the blaOXA−51-like blaOXA−69, blaOXA−66, blaOXA−91, blaOXA−180 and blaGES−11 were the most common among Acinetobacter isolates (Table 3A).

Moreover, two isolates, P. aeruginosa (n = 1) and A. baumannii (n = 1) carrying the blaNDM−1 carbapenemase gene were detected. Furthermore, the resistome profile shows that resistance genes to the antimicrobial classes of aminoglycosides, fluoroquinolones, and trimethoprim were prevalent among these two isolates (Tables 3A,B).

Molecular epidemiology using cgMLST

Epidemiologic typing using the seven gene multi-locus sequence typing demonstrated that ST274 (23.0%, 3/13) among P. aeruginosa and ST1 (MLST-Pasteur) A. baumannii (63.1%, 12/19) were the most prevalent sequence types (Tables 3A,B). A cgMLST analysis showed that the ST1 A. baumannii isolates were highly similar with no allelic differences between them. Most of the Acinetobacter isolates belonged to the international
TABLE 2A  Antimicrobial susceptibility pattern of ceftazidime resistant *Pseudomonas* spp. isolated at JMC, Ethiopia.

| Bacterial species | Type of antimicrobials used for antimicrobial susceptibility testing |
|-------------------|---------------------------------------------------------------|
|                   | Ceftazidime | Meropenem | Imipenem | Piperacillin-tazobactam | Ciprofloxacin | Amikacin | Cefiderocol | Ceftazidime-avibactam | Ceftolozane-tazobactam | Imipenem-relebactam |
| *Pseudomonas aeruginosa* (n = 10) | S (%) | 0 (0.0%) | 7 (70) | 8 (80) | 0 (0) | 7 (70) | 10 (100) | 9 (90) | 5 (50) | 6 (60) | 9 (90) |
| R (%) | 10 (100) | 3 (30) | 2 (20) | 10 (100) | 3 (30) | 0 (0) | 1 (10) | 5 (50) | 4 (40) | 1 (10) |
| Other *Pseudomonas* species (n = 3) | S (%) | 1 (32.4) | 1 (32.4) | 3 (100) | 1 (32.4) | 1 (32.4) | 2 (66.6) | 3 (100) | 2 (66.6) | 2 (66.6) | 3 (100) |
| R (%) | 2 (66.6) | 2 (66.6) | 0 (0) | 2 (66.6) | 1 (32.4) | 0 (0) | 1 (32.4) | 1 (32.4) | 3 (100) | 0 (0) |

S, susceptible; R, resistant, other *Pseudomonas* species [*Pseudomonas putida* (n = 2), *Pseudomonas fulva* (n = 1)].

TABLE 2B Antimicrobial susceptibility pattern of carbapenem resistant *Acinetobacter* species isolated at JMC, Ethiopia.

| Bacterial species | Type of antimicrobials used for antimicrobial susceptibility testing |
|-------------------|---------------------------------------------------------------|
|                   | Meropenem | Imipenem | Imipenem-relebactam | Cefiderocol | Ciprofloxacin | Trimethoprim/sulfamethoxazole | Gentamicin | Amikacin |
| *Acinetobacter baumannii* (n = 22) | S (%) | 5 (27.8) | 9 (50) | 9 (50) | 17 (94.4) | 14 (77.8) | 12 (66.7) | 15 (83.3) |
| R (%) | 13 (72.2) | 9 (50) | 9 (50) | 1 (5.6) | 4 (22.2) | 6 (33.3) | 6 (33.3) | 3 (16.7) |
| Other *Acinetobacter* spp. (n = 4) | S (%) | 1 (25) | 1 (25) | 1 (25) | 4 (100) | 1 (25) | 0 (0) | 4 (100) |
| R (%) | 3 (75) | 3 (75) | 3 (75) | 0 (0) | 3 (75) | 4 (100) | 0 (0) | (100) |

S, susceptible; R, resistant, Other *Acinetobacter* spp. (n = 4): *A. calcoaceticus*, *A. baylyi*, *A. junii*, and *A. lwoffi*. 
clonal complexes, ICC1 (includes ST1) \((n = 12)\) and ICC2 \((n = 2)\) (Table 3A).

**Discussion**

In this study, nearly all isolates of both *Pseudomonas* and *Acinetobacter* spp. were from patients admitted to the hospital for more than 72 h. Nosocomial acquisition of MDR isolates of *Pseudomonas* spp. and *Acinetobacter* spp. is worrisome. The situation is complicated by limited availability of antimicrobial agents, lack of prescription guidelines, and insufficient standard routine microbiology laboratory services to support antibiotic selection. In such cases, the safety of patients admitted to the hospital can be severely compromised.

The prevalence of ESBL-producing strains among *P. aeruginosa*, and carbapenem non-susceptible isolates among *A. baumannii* strains was high in the present study. Previous phenotypic studies from Ethiopia also show that MDR strains of *Pseudomonas* and *Acinetobacter* were prevalent (Motbainor et al., 2020; Birhanie Fiseha et al., 2021). However, most of these studies did not describe the genotypes and resistome profile of the isolates, and mechanism of resistance is difficult to compare between studies. So far, to our knowledge, there is no report of a genotypic study on the prevalence of ESBL-producing *P. aeruginosa* in Ethiopia. Generally, there are limited studies conducted on carbapenemase-producing *P. aeruginosa* in Africa. The few reports available are from northern Africa and mainly from Egypt (Osei Sekyere and Reta, 2020; Rizk et al., 2021). In northern Africa, the prevalence ranges from 0 to 96% (Gaballah et al., 2018). A finding from Uganda (7.4%) was comparable to the present study (Aruhomukama et al., 2019). But, one study from South Africa (51.0%) reported a higher prevalence of ceftazidime resistant *P. aeruginosa* compared to the present study (Hosu et al., 2021). The phenotypic studies from Ethiopia, and other African countries showed higher prevalence as compared to the present study.

In Ethiopia, a high prevalence of carbapenem-resistant *Acinetobacter* spp. was reported from one previous phenotypic study (Ayenew et al., 2021), which is comparable to the prevalence of carbapenem resistance herein. On the other hand, a systematic review and meta analysis on carbapenemase producing *P. aeruginosa* and *A. baumannii* in Africa showed that the lowest prevalence of carbapenemase-producing *A. baumannii* was 4.7% \((n = 21)\), and the highest prevalence was 100% \((n = 7)\) (Kindu et al., 2020). Studies conducted on *P. aeruginosa* and *A. baumannii* strains from Africa were limited to small sample sizes and were mainly phenotypic studies, which makes the comparison with genotypic studies difficult (Kindu et al., 2020; Osei Sekyere and Reta, 2020; Ayenew et al., 2021; Mekonnen et al., 2021). However, most available studies including a study for hospital environment (Solomon et al., 2017) reported higher prevalence of the MDR *P. aeruginosa* and *A. baumannii* in Ethiopia, calling for the application of genotypic methods to studies on mechanisms of resistance and spread.

The multiple genetic variants of antibiotic resistance observed among both *Pseudomonas* and *Acinetobacter* spp. pose a huge challenge on the limited therapeutic options available to low-income countries. Among *Acinetobacter* spp., the presence of the blagES−11 (ESBL-genotype and weak carbapenemase) and the OXA-51-like (blaOXA−66 and blaOXA−69) carbapenemases encoding genes is a serious threat. The OXA-51-like intrinsic carbapenemase encoding ST1 *A. baumannii* Isolates were reported from India (Rose et al., 2021), but isolates from the present study encoded additionally the weakly carbapenem hydrolyzing blagES−11 gene. The blagES−11 ESBL-genotypes, and the blaOXA−51-like carbapenemases have not been previously reported from Ethiopia, and the present study is to our knowledge the first report of blaOXA−51-like and blagES−11 from Ethiopia. Generally from Africa, only one study from Tunisia has documented clinical isolates of *A. baumannii* encoding the blagES−11 (Chihi et al., 2016). The emergence of blanDM−1 encoding isolates of *A. baumannii* at surgical ward, and *P. aeruginosa* at intensive care unit can severely compromise the safety of vulnerable patients admitted to the hospital. Moreover, detection of two isolates encoding the blanDM−1 which showed resistance to the newer antimicrobials, *A. baumannii* for (cefiderocol and imipenem-relebactam), and *P. aeruginosa* for (cefpiderocol, ceftazidime-avibactam, cefotolozane-tazobactam) compromises the already limited treatment alternatives for vulnerable groups of patients at this hospital.

Sequence typing showed that both *P. aeruginosa* and *A. baumannii* strains were polyclonal. But, since a large proportion of *A. baumannii* isolates were ST1, the spread of *A. baumannii* isolates at this hospital might also be to some extent clonal. A previous study conducted at the same hospital had identified three strains of *A. baumannii* encoding blanDM−1, and all of them belonged to the ST597. Furthermore, cgMLST analysis of *A. baumannii* with other international isolates in pubmlst showed that isolates in the current study were distinct from isolates from other African countries (Figure 1).\(^6\) However, these isolates were clustered with isolates from other countries like United States, and Brazil. Though isolates from this study were polyclonal, the most prevalent isolates were those that belong to the international cluster, CC1 and CC2.

Although *A. baumannii* is a well-known major cause of nosocomial infections, knowledge of its genomic epidemiology

\(^6\) https://pubmlst.org/organisms/acinetobacter-baumannii
## TABLE 3A Sequence types and resistance genes observed among carbapenem-resistant Acinetobacter species isolated at Jimma Medical Center, Ethiopia (n = 19).

| Isolate ID | STs | Carbapenemase | ESBL and other β-lactamases | Aminoglycosides | Trimethoprim | Sulfonamides | Tetracycline | Macrolides |
|------------|-----|---------------|-----------------------------|-----------------|--------------|--------------|--------------|------------|
| B027AB*    | ST2 | blaOXA−68     | blaGSE−11                   | aac(6′)-Ib3, aac(6′)-Ib-cr | dfrA7         | sul1         |              |            |
| B030AB     | ST1 | blaOXA−69     | blaGSE−11                   | aac(6′)-Ib3, aac(6′)-Ib-cr | dfrA7         | sul1         |              |            |
| M217AB     | ST1 | blaOXA−69     | blaGSE−11                   | aac(6′)-Ib3, aac(6′)-Ib-cr | dfrA1, sul1   |              |              |            |
| M328AB     | 164 | blaOXA−91     | blaCARB−5, blaCARB−49       | aac(6′)-Ib3, aac(6′)-Ib-cr |              |              |              | tet(39)    |
| P015AL     | NA  |               | blaCTX−M−15, blaOXA−1       | aac(6′)-Ib-cr   |              |              |              | tet(A)     | mdf(A), mph(A), mph(D) |
| S126AB     | ST2090 | blaOXA−250/180 |               |                |              |              |              |            |
| S130AB     | ST85 | blaNDM−1, blaOXA−94 |               |                |              |              |              |            |
| S161AB     | ST2 | blaOXA−66     | blaTEM−10                  | aac(3)-Ia, aph(6)-I-d | sul1         |              |              | mph(E) mrr(E) |
| S167AB     | ST1 | blaOXA−69     | blaGSE−11                   | aac(6′)-Ib-cr, aac(6′)-Ib3 | dfrA7         | sul1         |              |            |
| S170AB     | ST164 | blaOXA−91 | blaCARB−5, blaCARB−16       |              |              |              |              | tet(39)    |
| S171AB     | ST1 | blaOXA−69     | blaGSE−11                   | aac(6′)-Ib3, aac(6′)-Ib-cr | dfrA7         | sul1         |              |            |
| S176AB     | ST1 | blaOXA−69     | blaGSE−11                   | aac(6′)-Ib3, aac(6′)-Ib-cr | dfrA7         | sul1         |              |            |
| S209AB     | ST2 | blaOXA−66     | aac(3)-Ia, aph(6)-I-d, aadA1 |              | sul2         |              |              | tet(B)     |
| S212AB     | ST1 | blaOXA−69     | blaGSE−11                   | aac(6′)-Ib3, aac(6′)-Ib-cr | dfrA7         | sul1         |              |            |
| S270AB     | ST1 | blaOXA−69     | blaGSE−11                   | aac(6′)-Ib3, aac(6′)-Ib-cr | dfrA7         | sul1         |              |            |
| S275AB     | ST1 | blaOXA−69     | blaGSE−11                   | aac(6′)-Ib3, aac(6′)-Ib-cr | dfrA7         | sul1         |              |            |
| S296AB     | ST1 | blaOXA−69     | blaGSE−11                   | aac(6′)-Ib3, aac(6′)-Ib-cr | dfrA7         | sul1         |              |            |
| S315AB     | ST1 | blaOXA−69     | blaGSE−11                   | aac(6′)-Ib3, aac(6′)-Ib-cr | dfrA7         | sul1         |              |            |
| S327AB     | ST1 | blaOXA−49     |               |                |              |              |              |            |
### Table 3B

Sequence types and resistance genes observed among ceftazidime-resistant *Pseudomonas* spp. isolated at Jimma Medical Center, Ethiopia (n = 13).

| Strain ID | STs | Carbapenemase | β-lactamase genes | Aminoglycosides | Fluoroquinolones | Sulfonamides | Phenics | Fosfomycin |
|-----------|-----|----------------|-------------------|-----------------|-----------------|-------------|---------|-----------|
| I020PA*   | 11  | `blaOXA-486, blaPAO` |
| I032PA    | 2948| `blaOXA-10, blaOXA-30` |
| I038PP*   | NA | `blaCTX-M-15, blaOXA-1` |
| M019PA    | 1228| `blaVE-1, `blaOXA-46, `blaOXA-10` |
| M119PA    | 274 | `blaOXA-486, blaPAO` |
| M304PA    | 274 | `blaOXA-486, blaPAO` |
| S114PP*   | NA | `blaCTX-M-15, `blaOXA-1` |
| S116PA    | 500 | `blaOXA-486, blaPAO` |
| S155PA    | 274 | `blaOXA-486, blaPAO` |
| S248PA    | 840 | `blaOXA-486, blaPAO` |
| S332PF**  | NA | `blaPER-1, `blaOXA-486` |
| S356PA    | 646 | `blaOXA-486, blaOXA-40, blaOXA-50, blaTEM-1B` |
| S047PA    | 244 | `blaOXA-50` |

*A. baumannii; PA, Pseudomonas aeruginosa; PP**, Pseudomonas putida; PF**, Pseudomonas fulva.*
and availability of reliable data regarding the genetic basis of antibiotic resistance is limited in low-income countries. Similarly, *P. aeruginosa* isolates were found to be polyclonal, and different from a collection of isolates other African counties found in pubmlst (see text footnote 5) (Figure 2).

The current study may serve as a baseline regarding local spread of international clones and alert clinicians and other health workers, researchers, and public health policy makers to the problem. Implementation of strict infection prevention and control strategies, and antimicrobial stewardship programs are highly desirable in the admission wards where the international clones are spreading. Furthermore, despite limitation of resources, the added value of next generation sequencing is in understanding the dynamics and mechanisms of spread of MDR bacterial clones.
Conclusion

The prevalence of MDR isolates is high among both in clinical isolates of *Pseudomonas* species and *Acinetobacter* species at Jimma medical center. Emergence of the *blaNDM−1* in clinical isolates of *P. aeruginosa* and *A. baumannii* strains is worrisome. However, the susceptibility of *P. aeruginosa* strains to amikacin, cefiderocol, imipenem-relebactam and cefotaxime-tazobactam, and *A. baumannii* strains to amikacin and cefiderocol is important to consider as alternative options to carbapenems. The use of next generation sequencing is important to understand the mechanism of resistance and spread of resistant clones such as ICC1, and ICC2 *A. baumannii* strains detected at this hospital.

Data availability statement

The genome sequences were deposited at the NCBI, SRA database (PRJNA593604, Biosample accession: SUB11593554).

Ethics statement

The study obtained ethical approval from Addis Ababa University Institutional Review Board (AAU-IRB), Armauer Hansen Research Institute – ALERT Hospital Institutional Review Board (AHRI-ALERT-IRB), and Ethiopian National Ethics Review Committee (NERC). Patients were informed about the study and given written consent to participate in the study.

Author contributions

TS contributed to design, data acquisition, data analysis, and drafting of the manuscript. DA and YW contributed to data acquisition and write-up. AA contributed to the design, data acquisition, and revision of the manuscript. CG contributed to the overall design, data acquisition, supervision, drafting, and writing of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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