Multi-drug resistance and nosocomial infections of Acinetobacter baumannii and Pseudomonas aeruginosa among patients hospitalized at Felegehiwot Referral Hospital, Northwest Ethiopia: A cross-sectional study

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Abstract

Background: Multi-drug resistant (MDR) Acinetobacter baumannii and Pseudomonas aeruginosa are major causes of nosocomial infections globally. They are the current World Health Organization critical priority pathogens for resistance and discovery of new antibiotics. However, there is paucity of data on nosocomial infections caused by such superbugs in Ethiopia. Therefore, this study determined the magnitude and profile of nosocomial MDR Acinetobacter baumannii and Pseudomonas aeruginosa infections among patients hospitalized at Felegehiwot referral hospital, Northwest Ethiopia.

Methods: A cross-sectional study was conducted at Felegehiwot referral hospital from April 1 to July 31, 2018. A total of 238 patients presumptive for blood stream, urinary tract and surgical site nosocomial infections were enrolled using convenient sampling technique. Either blood, urine and wound swab specimens were collected and processed using standard bacteriological procedures. A. baumannii and P. aeruginosa isolates were identified using standard bacteriological techniques and confirmed by automated Vitek2 Compact. Antimicrobial susceptibility testing on isolates was performed using the disk diffusion technique. The results interpreted as per the standard zone sizes of Clinical and Laboratory Standards Institute. Chi-square test was done to determine associations among variables. P.value < 0.05 was considered statistical significant.

Results: The median age of participants was 29 years. Overall, 20(8.4%) of patients had nosocomial MDR A. baumannii and P. aeruginosa infections. The proportion of nosocomial MDR blood stream, urinary tract and surgical site infections were 13(8.9%), 5(8.3%) and 2(6.3%), respectively. The mean age of patients with nosocomial infection was significantly lower (24.9 years) than their counter parts (29.6 years) (P=0.035). All isolates of nosocomial infections were from patients with intravenous catheterization. The frequency of nosocomial MDR A. baumannii infection was 9(3.8%) and for P. aeruginosa
nosocomial infections was 11(4.6%). A.baumannii and P.aeruginosa isolates were 100% MDR. MDR A.baumannii isolates were 100% resistant to ampicillin and piperacillin. MDR P.aeruginosa isolates was 100% resistant to ampicillin, piperacillin, cefotaxime and ceftriaxone. On the other hand, A.baumannii isolates showed 36.4% and 44.5% resistance against ciprofloxacin and meropenem while P.aeruginosa isolates revealed 33.3% and 45.5% resistance against Pseudomonas aeruginosa.

Conclusions: Health care associated MDR A.baumannii and P.aeruginosa infections are critical problems in the study area. Therefore, urgent focused interventions required to contain the spreading of MDR NIs. Treatment of NIs for patients on health care should be guided by antimicrobial susceptibility testing.

Background

Nosocomial infection (NI) is an infection occurring in a patient at the time of care in a hospital that was not manifest or incubating during admission but developed after 48 hours of hospitalization [1]. The hospital environment contains a large number of immunocompromised individual’s and patients with diverse bacterial pathogens and normal flora [2, 3]. NI accounted 7% - 10% prevalence in the world [3]. According to 2014 World Health Organization (WHO) report, 15% of all the hospitalized patients suffered from NIs [4]. Surgical-site, blood stream and urinary tract infections are the most frequently reported types of NIs [2, 5].

Nosocomial infections due to multi-drug resistant (MDR) bacteria are the major issue to global health. It is reported as the leading causes of NIs in the world [2, 6, 7]. NIs with MDR organisms are very difficult for treatment and are main causes of morbidity, mortality, prolonged hospitalization and high health care costs [8, 9]. The situation is true and urgent in Ethiopia.

The non-fermentative gram negative bacilli Acinetobacter baumannii (A.baumannii) and
*Pseudomonas aeruginosa* (*P. aeruginosa*) have emerged as serious particular concern [10, 11]. They are among the most common and serious MDR pathogens documented along with *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Enterobacter* spp., and abbreviated as ESKAPE pathogens [11, 12].

Multi-drug resistant *A. baumannii* and *P. aeruginosa* survive in the hospital environment, transmitted easily between patients through the hands of health care workers in the health care setting [6, 11]. Earlier findings elsewhere in the world reported that *A. baumannii* and *P. aeruginosa* commonly possess inherent resistance to antimicrobial agents through reduced permeability of the outer membrane, efflux pump systems, enzymatic inactivation and biofilm formation [12, 13]. Thus, *A. baumannii* and *P. aeruginosa* are often resistant to almost all β-lactams, aminoglycosides and quinolones [12, 14].

Nosocomial isolates of MDR *A. baumannii* and *P. aeruginosa* complicated the treatment of infections and had adverse effect on clinical outcomes and increases patient treatment costs [15]. Factors such as antimicrobial drug overuse, prescription of drugs without susceptibility testing, self-medication and long duration of hospitalization are reported to the occurrence of MDR [8, 16]. However, there is a scarcity of data on the burden of nosocomial MDR and *A. baumannii and P. aeruginosa* infections in Ethiopia in general and study area in particular. Unavailability of local antibiogram data linked with self-drug prescription by patients and poor awareness on antimicrobial resistance are also big issues. Thus, the study aimed at determining the proportion of nosocomial MDR *A. baumannii* and *P. aeruginosa* infection among patients clinically presumptive for NIs at Felegehiwot Referral Hospital (FHRH), Ethiopia.

**Methods**
Study design, period and setting

A cross-sectional study was conducted from April 1 to July 31, 2018 at FHRH, Bahir Dar, Northwest Ethiopia. Bahir Dar is a capital city of the Amhara National Regional State, located approximately 565 kilometers northwest of Addis Ababa. FHRH is a tertiary hospital that provides services for 5 to 7 million people. It has 684 health care and 166 administrative workers. The hospital has 466 beds for inpatient services. FHRH consists of 13 different wards. Bacteriological procedures were conducted at FHRH microbiology laboratory and further confirmatory identification of isolates and screening of MDR made using VITEK2 compact at Amhara Public Health institute (APHI), Ethiopia. All patients admitted at FHRH and clinically presumptive for either of nosocomial urinary tract, post-operative surgical site and blood stream infection after 48 hours of admission during the study period were the study population.

Sample size and sampling

The sample size for NIs was determined using Epi info version 3.5.1 (public domain software, www.cdc.gov) by considering 95% confidence level and marginal error (5%). A proportion of 0.83 MDR NIs taken from previous study in other parts of South East Ethiopia [16]. Thus, the total sample size was 238. All patients who were clinically presumptive for nosocomial blood stream, urinary tract and post-operative surgical site nosocomial infections were included conveniently until the required sample size was achieved.

Variables

Nosocomial MDR A.baumannii and P. aeruginosa infections were the dependent variables while demographic variables (age, sex, residence, educational status, occupation) and clinical data on co-morbidity, urinary catheterization, intravenous catheterization, duration of catheterization, previous history of antibiotics, previous history of surgery,
duration of hospitalization, wards of patients hospital and duration of operation were the independent variables.

Inclusion and exclusion criteria

All patients who had either clean and clean-contaminated operations or other medical reasons admitted in different wards of FHRH and developed clinical evidences of nosocomial infection after 48 hours of admission included during the study period. However, patients who had re-operation, contaminated and dirty operations and developed clinical evidences of infection within 48 hours of admission were excluded from the study.

Data collection

Information on demographic variables was collected from each participant by face-to-face interview using a structured questionnaire. Clinical data related to co-morbidities, hospitalization, surgery and use of antibiotics were collected by reviewing patient’s medical record and in consultation with the respective physician. Clinical specimens (blood, urine and wound swab) were collected as soon as nosocomial infection was reported following the bacteriological standard procedures (Rods, 2014).

Wound swab collection and processing

Two wound samples from each participant were collected aseptically by sterile cotton swabs dipped in normal saline using Levine method [17]. After, the wound was cleaned with normal saline moistened sterile gauze, the swab taken by rotating the sterile cotton tipped applicator in a 1cm² area of cleaned wound for five seconds without contaminating with skin commensals. Specimens were transported to FHRH microbiology laboratory within 20 minutes of collection. The wound swabs were inoculated on MacConkey agar (MAC) and Blood agar (BA) (Oxoid, UK) at a time. Both plates were incubated at 37 °C and
examined for visible bacterial growth after 48 hours of incubation.

**Blood sample collection and processing**

As per the standard protocol, 10, 2 and 1ml of venous blood, respectively were collected from adult, children and neonates presumptive for blood stream infection [18]. Following cleaning of the site of blood collection with 70% alcohol and 2% tincture iodine, two blood samples were collected from two different sites of peripheral vein of each febrile patient using two bottles of blood sample within 30 minutes difference. The collected blood samples were inoculated directly to 5 - 10ml Tryptic Soya broth blood culture medium bottle (Oxoid, UK) and transported to FHRH microbiology laboratory. All blood culture broths were incubated aerobically at 37 °C and regular subcultures were done after 1, 2 and then 3 days later daily up to 7 days of incubation. Subcultures were made on BA and MAC (Oxoid, UK). All of the inoculated agar plates were incubated at 37ºc. Finally, plates were examined for bacterial growth after 24 hours.

**Urine sample collection and processing**

Clean-catch mid-stream urine was collected from catheterized and non-catheterized patients presumptive for urinary tract infection. From catheterized patients, 5 milliliters (ml) of catheterized urine transferred to a sterile container after cleansing the outlet of catheter. For non-catheterized patients, the same amount of urine sample was collected by the patient using sterile screw-capped and wide-mouth container after careful instruction. Then, the urine was delivered to FHRH Microbiology Laboratory within 20 minutes. Urine samples were inoculated on MAC and BA (Oxoid, UK) using calibrated wire loop that can deliver 0.001 ml of urine. All agar plates were incubated aerobically at 37 °C for 24 hours and observed for bacterial growth. Blood agar colonies were counted using colony counter and checked for significant bacteriuria. Culture from catheterized and non-catheterized patients that grew ≥ 10² CFU/ml and 10⁵ CFU/ ml, respectively was taken as
a significant bacteriuria, respectively. For heterogeneous colonies, sub-culturing of individual distinct colonies was performed to ensure pure cultures.

**Identification of bacterial isolates**

Preliminary identification of bacteria was done based on gram reaction, colony morphology and pigment formation. A series of enzymatic tests (catalase and oxidase), carbohydrate fermentation test (triple sugar iron (TSI)), protein tests (Lysine decarboxylase (LDC) and indole), and motility tests were performed on colonies of pure culture for identification of *A. baumannii* and *P. aeruginosa* isolates [18]. Non-fermentative grape-like colonies with blue-green pigment production on culture media, motile, oxidase and catalase positive, LDC and indole negative isolates were considered as *P. aeruginosa*. On the other hand, non-motile, gram negative short rods, oxidase, indole and LDC negative, catalase positive and non-glucose fermenter isolates were considered as *A. baumannii*. All *A. baumannii* and *P. aeruginosa* suspected isolates were also further confirmed by an automated Vitek2 Compact (BioMérieux, France).

**Antimicrobial Susceptibility testing**

Antimicrobial susceptibility testing was carried out for each isolates of *A. baumannii* and *P. aeruginosa* on Muller Hinton agar (MHA) (Oxoid, UK) by Kirby - Bauer disk diffusion technique [19]. All *A. baumannii* and *P. aeruginosa* isolates were tested against the following classes of antibiotics: Penicillin (ampicillin (10μg)), piperacillin (100μg)), β-lactam/β-lactamase inhibitor combination (amoxicillin-clavulanic acid (20/10μg)), cephalosporin (ceftazidime (30μg), cefotaxime (30μg) and ceftriaxone (30μg)), aminoglycosides (gentamycin (10μg)), fluoroquinolones (ciprofloxacin (10μg)), Folate pathway inhibitor (trimethoprim-sulphamethoxazole(1.25/23.75μg)), carbapenem (meropenem (10μg)) and tetracycline (30μg) (Oxoid, England). A 0.5 McFarland standard was used to standardized the turbidity of the inoculums suspension. Standard inoculums
of each isolate were inoculated by streaking the swab over the entire sterile agar surface within 15 minutes after adjusting the turbidity of the inoculums suspension. The antimicrobial disks were placed on the lawn of bacterial isolates using sterile forceps and incubated aerobically at 37°C for 18–24 hours. The diameter of zone of inhibition measured using caliper. The results were interpreted using the standard zone sizes of the Clinical and Laboratory Standard Institute (CLSI, 2017) guidelines [20]. All intermediate readings were taken as resistant during data entry.

**Quality control**

The prepared questionnaire was checked for its completeness and validity prior to the data collection. All the standard operating procedures (SOPs) were strictly followed at all stages of microbiological analysis. Reference strains of *P. aeruginosa* ATCC27853 and *E. coli* ATCC 25922 were used for quality control of antimicrobial susceptibility testing. MDR *A. baumannii* and *P. aeruginosa* isolates were confirmed by using Vitek2 compact. A standardized bacteriological procedure was followed to maintain correct laboratory results. At regular intervals and whenever a new batch of strain or reagent is prepared, standard strains of *P. aeruginosa* ATCC27853 and *E. coli* ATCC 25922 were used as positive controls. The sterility of the media was checked by incubating the media overnight before its use. The data were checked for completeness and representativeness prior to entry.

**Data analysis**

Data were checked, entered and analyzed using Statistical Package for Social Science 23 (IBM Corp Released 2011.IBM SPSS statistics. Armonk, NY: IBM Corp). Descriptive statistics were used to describe relevant variables. Chi-square test, Fishers exact test and Independent samples T Test was obtained to determine association between dependent and independent variables. P-value of < 0.05 was considered statistical significant.

**Results**
Demographic characteristics

A total of 238 patients with clinical evidence of nosocomial infection (BSI, UTI and SSI) were enrolled in the study. Of them, 129 (54.2%) were males. The majority (21.4%) of participants were found in the age group of > 51 years with median age of 29 years. One hundred twenty six (52.9%) of the study participants were from urban settings. Data on occupation showed that majority (39.1%) of participants were government employee. Table1 depicts the demographic characteristics of the study participants.

Rate of nosocomial infection and frequency of bacterial isolates

The overall prevalence of the combined nosocomial MDR A. baumannii and P. aeruginosa infection was 20 (8.4%). Of them, the proportion of BSI, UTI and SSI were 13 (8.9 %), 5 (8.3 %) and 2 (6.3%), respectively. The proportion of nosocomial MDR A. baumannii and P. aeruginosa infection were 11 (4.6 %) and 9 (3.8%), respectively. P. aeruginosa accounted for 6.3%, 4.8% and 3.3% of nosocomial SSI, BSI and UTI, respectively while, MDR A. baumannii causes 5% and 4.1% of nosocomial UTI and BSI, respectively (Table 2).

Participants with NIs had lower mean of age (24.9 years) than those without NIs (29.6 years) and the difference was statistical significant (P = 0.035). Highest (15.4%) NIs rate was found in age groups < 10 years. The proportion of NI was 13 (11.4%) in those participant with co-morbidity. All isolates of NIs were from patients with intravenous catheterization (Table 3). Duration of operation was significantly higher in those patients with confirmed NIs (180 minutes) than their counterparts (155 minutes) (P = 0.04). Moreover, the duration of catheterization was higher in those with confirmed NIs (13.6 days) than their counter parts (11.3 days). However, the difference was not statistical significant (P = 0.25) (Table 3).

Multi-drug resistance profiles of A. baumannii and P.
Aeruginosa isolates

A. baumannii and P. aeruginosa isolates were resistant to three to six antibiotics from different classes. All isolates of A. baumannii (9) and P. aeruginosa (11) were MDR (100%). Among this, 3 (33.3%) isolates of A. baumannii and 4 (36.4%) isolates of P. aeruginosa showed resistance to antibiotics from six different classes, respectively. Three (33.3%) isolates of A. baumannii and 1 (9.1%) isolates of P. aeruginosa were resistant against antibiotics from 4 different classes, respectively. Moreover, 3 (33.3%) of isolates of A. baumannii and 6 (54.5%) of P. aeruginosa isolates were resistant against antibiotics from three different classes (Table 4).

Antimicrobial resistance profiles of A. baumannii and P. aeruginosa

A. baumannii isolates showed 100% resistance against ampicillin and piperacillin. P. aeruginosa isolates showed 100% resistance against ampicillin, amoxicillin-clavulanic acid, piperacillin, cefotaxime, ceftriaxone and trimethoprim-sulphamethoxazole. A. baumannii isolates showed resistance 88.9% to amoxicillin-clavulanic acid, ceftriaxone and cefotaxime and also 77.8% to tetracycline and ceftazidime. However, P. aeruginosa isolates revealed a high resistance rate to ceftazidime (63.6%) and tetracycline (90.6%). A. baumannii isolates showed low level resistance rate to ciprofloxacin (44.5%) and meropenem (33.3%). Similarly, P. aeruginosa isolates showed low level of resistance against ciprofloxacin (36.4%) and meropenem (45.5%) (Table 5).

Discussion

Antibiotic resistant nosocomial infections are becoming serious health care problem in ICU and other areas of hospital care, leading to high rate of morbidity and mortality [6, 21]. The epidemiological and antimicrobial resistance profiles of NIs showed variations among hospitals around the globe. Many of the infections are caused by bacteria that are
resistant to multiple antibiotics [21, 22]. This study showed the proportion of NIs due to two MDR non-fermentative gram negative bacilli among patients hospitalized in different wards of a referral hospital.

In the present study, 8.4% of patients were infected with nosocomial MDR *A. baumannii* and *P. aeruginosa*. This indicated that MDR *A. baumannii* and *P. aeruginosa* infections are the major health problem in the clinical area in Ethiopia. High patient load, overcrowding, poor infrastructure, poor infection control practices of the hospital and differences in trained medical staff for aseptic procedures might be the possible explanations. This finding was coherent with reports in Tikur Anbessa Hospital, Ethiopia (8.12%) [22], Uganda (7.39%) [23], Morocco (7 - 8%) [24], Italy (9.3%) [25] and Gaza city (6.9%) [26]. However, it was higher compared to reports from Hiwot Fana Hospital, Ethiopia (0.5%) [27], Gabon (5.7%) [28], China (0.78%) [29] and Indonesia (3.5%) [30]. In contrast, the overall nosocomial MDR *A. baumannii* and *P. aeruginosa* infections in the present study was lower than studies done in Nigeria (12.5%) [31] and Ghana (23.5%) [32]. This might be due to variation in sample size, clinical site of infection, age of patients, hospital setting, duration of hospitalization, patients exposure to high risk devices or surgical procedures, microbiological methods employed for detection and screening of MDR resistant strains.

In this study, the proportion of nosocomial MDR *A. baumannii* and *P. aeruginosa* surgical site infection (6.3%) was comparable with reports from Tikur Anbessa Hospital (6.6%), Ethiopia [22] and Ghana (8.5%) [32]. However, it was lower than studies from Nigeria (11.1%) [31], Morocco (70.9%) [24], Southeast China (28.5%) [33], Heraklion (20.1%) [34], Pakistan (19.5%) [35]. This could be the difference in age of study participants as the present study included any age groups of patients, type of surgery and handling of surgical equipments.

The proportion of nosocomial urinary tract infection linked with MDR *A. baumannii* and
*P. aeruginosa* (8.3%) isolates in the present study was consistent with a study conducted in Kenya (9%) [36]. However, it was lower than studies in Morocco (52.83%) [24], Nigeria (12.5%) [31], Gabon (26%) [28], USA (16%) [37], Indonesia (16.5%) [30], Pakistan (32%) [35]. In contrast, the proportion of MDR nosocomial UTI in the present study was lower than a study in Vietnam (4%) [28]. This could be due to differences among study participants in terms of age, catheterization and hospitalization.

The prevailing proportion of nosocomial BSI in this study (8.9%) was comparable with studies done in Nigeria (8.6%) [31] and USA (10%) [37]. However, it was higher than studies from Indonesia (3.5%) [30] and Pakistan (2.4%) [35]. In contrast, it was lower than reports from Morocco (70.3%) [24], Gabon (20%) [28], Southeast China (46.1%) [33] and Tunis (48%) [38]. The observed difference might be due to non-sustainable infection control practices in hospitals, difference in use of invasive medical devices and procedures, hospital type and diverse nature of study participants.

In this study, the proportion of nosocomial MDR *A. baumannii* infection (3.8%) was in agreement with studies conducted in Uganda (2.39%) [23], Ghana (4%) [32] and Gabon (5.7%) [28]. However, it was lower compared with findings from Sodo Ethiopia (15.3%) [15], Morocco (7%) [24], Gaza city (6.9%) [26] and Thailand (17.3%) [39]. In contrast, it was higher than findings from Uganda (0.95%), Italy (0.52%) [25] and China (0.42%) [29]. The variations in this report might be due to host, microbial and environmental factors.

The proportion of nosocomial MDR *P. aeruginosa* infection (4.6%) in the present study was comparable with a study in Uganda (5%) [23]. However, it was lower than findings from other parts of Ethiopia (11.1% - 66.7%) [15, 22, 27, 40], Ghana (19.5%) [32], Morocco (8%) [41], India (76.8%) [42], Thailand (9.6%) [39] and Italy (8.7%) [25]. In contrast, it was higher compared to a study in China (0.36%) [29]. The variations in this report might
be due to host, microbial and environmental factors.

In this study all isolates of MDR *P. aeruginosa* were resistant for ampicillin and amoxicillin-clavulanic acid. This was consistent with reports from Tikur Anbessa hospital, Ethiopia (22) and Southeast China [33] where 87.5% and 100% resistance levels against ampicillin and amoxicillin-clavulanic acid, respectively were noticed. In this study, all isolates of *P. aeruginosa* revealed 100% resistance against piperacillin. This was significantly higher than studies from Italy (25%) [25], Vietnam (17.7%) [43], Southeast China (12%) [33], Turkey (28.7%) [44] and Taiwan (66.8%) [45]. This might be associated with differences in the number of MDR strains of *P. aeruginosa* and patient type. The frustrating level of resistance against piperacillin antibiotic is an alarm for treatment to be guided with antimicrobial susceptibility testing hence so far, piperacillin was not prescribed in the study area (FHRH).

In this study, high levels of resistance to cephalosporins (cefotaxime (63.6%) and ceftazidime (100%)) were obtained against *P. aeruginosa* isolates. This was coherent with studies in Uganda [23], India [42] and Taiwan [45] where 71 - 77% resistance against ceftazidime reported. Moreover, 70.8% and 92.8% level of resistance against cefotaxime documented in Sodo, Ethiopia [15] and Southeast China [33], respectively. On the other hand, low level of resistance against ceftazidime reported in Tikur Anbessa Hospital (12.5%) and Sodo, Ethiopia (29.1%) [15, 22], Italy (31%) [25] and Vietnam (22.1%) [43]. The highest level of resistance against third generation cephalosporins might be linked with excessive use, mis and inappropriate use of these antibiotics in the study hospital that drives selective pressure and emergence of MDR.

In the present study, *P. aeruginosa* isolates showed 45.5% levels of resistance against meropenem. This was coherent with studies in Sodo, Ethiopia (41.7%) [15], Southeast China (36.6%) [33], India (54%) [42] and Vietnam (40%) [43]. However, higher level of
resistance against meropenem was documented in Taiwan (73.2%) [45], Pakistan (86.4%) [35] and Saudi (81.8%) [46]. The relatively lower proportion resistance against meropenem in the present study might be due to the absence of meropenem prescription practice for patients in the study hospital. In contrast, lower level of resistance against meropenem was reported in Uganda (14%) [23] and Turkey (20.4%) [44]. This could be due to variation in the availability of meropenem in each localities, prescription difference, misuse and inappropriate use of antibiotics.

In this study, all isolates of MDR A. baumannii were resistance against ampicillin. This was parallel with studies conducted in Tikur Anbessa Hospital (88.2%), Ethiopia [22] and Southeast China (100%) [33]. Moreover, high level (88.9%) of MDR A. baumannii isolates resistance to amoxicillin clavulanic acid in the present study was comparable with earlier studies in Nigeria [47] and Southeast China [33], where all isolates of A. baumannii were resistant against amoxicillin-clavulanic acid.

In this study all isolates of A. baumannii were resistant to piperacillin. This was comparable with studies conducted in Morocco (75 - 95%) [24, 41], Southeast Asia (83.7%) [33] and Italy (81) [25]. In present study, A. baumannii isolates revealed 77.8% and 88.9% resistance against ceftazidime and cefotaxime, respectively. The finding was comparable with studies done in Tikur Anbessa Hospital [22], Morocco [24], Tanzania [48], Southeast China [33], Italy [25] and Vietnam [43], where resistance against ceftazidime and cefotaxime reported in 71 - 97.1% and 54.4—100% of isolates, respectively.

In the present study, 33.3% of A. baumanii isolates showed resistance against meropenem. The finding was relatively similar with studies conducted in Sodo, Ethiopia [15], Tanzania [48] and Nigeria [47] where, 30.2% - 40% resistance rate against meropenem reported. However, in Vietnam [43] and Saudi [46], resistance against meropenem were reported in 40 and 90.5% of A. baumanii isolates, respectively.
The resistance level of MDR isolates of *A. baumannii* to ciprofloxacin (44.5%) in the present study was lower than studies done in Tikur Anbessa Hospital (70.6%) and Sodo Ethiopia (88.4%) [15, 22], Uganda (78%) [23], Morocco (78-80%) [24], Nigeria (100%) [47], Southeast China (89.6%) [33] and Italy (84%) [25]. The low resistance rate against ciprofloxacin and meropenem in the present study might be due to the high price and unavailability of the drugs in the hospital.

In this study, all isolates of *A. baumannii* were MDR (100%). This finding was consistent with studies conducted in Sodo, Ethiopia (81.4%) [15], Tanzania (100%) [48] and Ghana (100%) [26]. However, it was higher than reports from Uganda (40%) [23], Morocco (77.5%) [24], Pakistan (22.7%) [35], Saudi Arabia (71.8%) [49] and Italy (54%) [25].

In the present study, all isolates of *P. aeruginosa* were MDR (100%) which is comparable with earlier studies in Sodo, Ethiopia (83.3%) [15], Ghana (100%) [26] and India (76.8%) [42]. However, it was significantly higher than earlier studies in Uganda (38%) [23], Saudi Arabia (28.2%) [49] and Italy (20%) [25]. The overall MDR proportion of *A. baumannii* and *P. aeruginosa* isolates in the study area is very alarming and needs urgent intervention and strict adherence to infection control practices to contain them.

The high MDR proportion observed in two non-fermenter gram negative bacilli in this study is probably related to the contaminations and cross transmission of this bacteria from hospital environment [15], hands of healthcare workers, frequent use of broad spectrum antibiotics, inherent resistance nature to many antimicrobial agents and the ability of pathogens persist in the environment, and on medical devices for a long period of time [6]. Moreover, intrinsic nature of the bacteria, high prescription practice of common antibiotics and third generation cephalosporins and use of drugs outside the hospital might contribute for the high resistance rate of *A. baumannii* and *P. aeruginosa* different classes of antibiotics.
The highest proportion of nosocomial infection due to MDR non-fermentative gram negative bacilli infection among the lower age groups in the present study is consistent with earlier studies elsewhere [28, 50, 51]. On the other hand, in the present study all NIs observed among patients with intravenous catheterization. The rate of nosocomial infections was also significantly higher among patients who had prolonged time of operation than their counter parts. This was consistent with previous study in Tikur Anbessa Hospital, Ethiopia (22) and India [42]. This might be due to the high rate of exposure of patients to the two MDR pathogens from the hospital environment, health care professionals, multiple invasive device and cross-contamination among patient’s procedures. This study was limited to participants admitted in hospital but nosocomial infection that arose after discharge was not detected.

Conclusions

Alarming proportion of nosocomial MDR A. baumannii and P. aeruginosa infection obtained in the study area. All isolates of the non-fermentative gram negative bacilli were MDR for atleast three antibiotics from different classes. Therefore, urgent intervention towards nosocomial infection prevention practices required. Moreover, treatment of patients on care should be guided with antimicrobial susceptibility testing.

Declarations

Ethics approval and consent to participate

Ethical clearance was secured from Institutional Review Board (IRB) of College of Medicine and Health Sciences, Bahir Dar University. Permission letter was obtained from the Amhara Public Health Institute and FHRH prior to data collection. All the study participants were informed about the purpose of the study. Written informed consent was obtained
from each study participants and guardian for participants under 16 years old before
clinical examination and sample collection. Information obtained in the course of the study
was kept confidential. Participants who were positive for the pathogen reported to
physicians for treatment and any other care.

Consent for Publication
Not applicable

Availability of data and materials
The finding of this study is generated from the data collected and analyzed based on the
stated methods and materials. All the data are already found in the manuscript and there
are no supplementary flies. The original data supporting this finding will be available at
any time upon request

Competing interests
The authors declared that no competing interest exists.

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Authors’ Contributions
HM: Conceptualized and designed the study, performed the laboratory investigation.
Collected the data, analyzed the data and drafted the manuscript. WM: Conceptualized
and designed the study, supervised the laboratory investigation and data collection,
analyzed the data, wrote, revised and critically edited the manuscript. FB: Conceptualized
the study, supervised the data collection and revised the manuscript. All authors have
read and approved the manuscript, and ensure that this is the case.
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Tables

Table 1: Demographic characteristics of patients clinically presumptive for nosocomial infection at FHRH, 2018 (n = 238).
Demographic variables | Number | Percent
---|---|---
**Sex** | | 
Female | 109 | 45.8  
Male | 129 | 54.2
**Age (in years)** | | 
<1 | 31 | 13.0  
1-10 | 34 | 16.4  
11-20 | 29 | 12.2  
21-30 | 37 | 15.7  
31-40 | 25 | 10.5  
41-50 | 21 | 8.8  
>51 | 51 | 21.4
**Residence** | | 
Urban | 126 | 52.9  
Rural | 112 | 47.1
**Occupation** | | 
Farmer | 23 | 15.2  
House wife | 31 | 20.5  
Government employee | 59 | 39.1  
Private employee | 27 | 17.9  
Other work | 11 | 7.3  
Unemployed | 87 | 36.6
**Education** | | 
Under school age | 49 | 20.6  
Illiterate | 52 | 27.5  
Elementary completed | 60 | 31.7  
Highschool completed | 11 | 5.8  
Diploma | 36 | 19.0  
Degree and above | 30 | 15.9

Key: Unemployed: Below school age + students; Other work: daily laborer and merchant

Table 2: Proportion of nosocomial MDR *Acinetobacter baumannii* and *Pseudomonas aeruginosa* infection in patients clinically presumptive for nosocomial blood stream, urinary tract and surgical site infection at FHRH, 2018 (n = 238).

| Type of MDR isolates | Rate of nosocomial infection N (%) | 
|---|---|---|
| | Urinary tract infection (n = 60) | Surgical site infection (n = 32) | Blood stream infection (n = 146) |
| *Acinetobacter baumannii* | 3 (5) | 0 (0) | 6 (4.1) |
| *Pseudomonas aeruginosa* | 2 (3.3) | 2 (6.3) | 7 (4.8) |
| Total | 5 (8.3) | 2 (6.3) | 13 (8.9) |

Table 3. Distribution of nosocomial MDR *A.baumannii* and *P.aeruginosa* infection in different variables of study participants clinically presumptive for nosocomial infection at FHRH, 2018 (n = 238).

| Variables | Confirmed rate of nosocomial infection |
|---|---|
| | Positive N (%) | Negative. N (%) | Total. N (%) |
| Sex | | | |
| Female | 9(8.3) | 100(91.7) | 109(45.8) |
|                       | Male  | Female | Total |
|-----------------------|-------|--------|-------|
| **Age**               |       |        |       |
| < 1                   | 5(16.1)| 26(83.9)| 31(13.0) |
| 1-10                  | 5(14.7)| 29(85.3)| 34(16.4) |
| 11-20                 | 1(3.4) | 28(96.6)| 29(12.2) |
| 21-30                 | 2(4.3) | 45(95.7)| 47(19.7) |
| 31-40                 | 2(8)   | 23(92) | 25(10.5) |
| 41-50                 | 0(0)   | 21(100)| 21(8.8) |
| >51                   | 5(9.8) | 46(90.2)| 51(21.4) |
| **Residence**         |       |        |       |
| Urban                 | 9(7.1)| 117(92.9)| 126(52.1) |
| Rural                 | 11(9.8)| 101(90.2)| 112(47.1) |
| **Education**         |       |        |       |
| Non-educated          | 3(5.8)| 49(94.2)| 52(27.5) |
| Educated              | 8(5.8)| 129(94.2)| 137(31.1) |
| Below school age      | 9(18.4)| 40(81.6)| 49(20.6) |
| **Type of NI**        |       |        |       |
| UTI                   | 5(8.3)| 55(91.7)| 60(25.2) |
| SSI                   | 2(6.3)| 30(93.8)| 32(13.4) |
| BSI                   | 13(8.9)| 133(91.1)| 146(61.1) |
| **Occupation**        |       |        |       |
| Farmer                | 0(0)  | 23(100)| 23(15.2) |
| House wife            | 4(12.9)| 27(87.1)| 31(20.5) |
| Government employee   | 5(8.5)| 54(91.5)| 59(39.1) |
| Private employee      | 0(0)  | 27(100)| 27(17.9) |
| Other                 | 1(9.1)| 10(90.9)| 11(7.3) |
| Unemployed            | 10(11.5)| 77(88.5)| 87(36.6) |
| **Ward of patients hospital** |     |        |       |
| Surgical              | 4(8.5)| 43(91.5)| 47(19.7) |
| Non-surgical          | 16(8.4)| 175(91.6)| 191(80.0) |
| ICU                   | 5(9.1)| 50(90.9)| 55(23.1) |
| Non- ICU              | 15(8.2)| 168(91.8)| 183(76.0) |
| **Underlying disease**|       |        |       |
| Yes                   | 13(11.4)| 101(88.6)| 114(47.1) |
| No                    | 7(5.6)| 117(94.4)| 124(52.1) |
| **Urinary Catheterization** |     |        |       |
| Yes                   | 9(7.4)| 112(92.6)| 121(50.1) |
| No                    | 11(9.4)| 106(90.6)| 117(49.9) |
| **IV-Catheterization** |     |        |       |
| Yes                   | 20(9.0)| 201(91.0)| 221(92.1) |
| No                    | 0(0)  | 17(100)| 17(7.1) |
| **Previous antibiotics** |     |        |       |
| Yes                   | 14(9.0)| 141(91.0)| 155(65.5) |
| No                    | 6(7.2)| 77(92.8)| 83(34.9) |
| **Previous surgery**  |       |        |       |
| Yes                   | 5(10.2)| 44(89.8)| 49(20.6) |
| No                    | 15(7.9)| 174(92.1)| 189(79.1) |
| **Total**             | 20(8.4)| 218(91.6)| 238(101) |
| **Mean Age of participants** | 24.9 | 29.6 |
| Duration of hospitalization (days) | 14.5 | 15.7 |
|-----------------------------------|------|------|
| Duration of operation (Minutes)    | 180  | 155  |
| Duration of catheterization (Days) | 13.6 | 11.3 |

Table 4. Multi-drug resistance profile of A. baumannii and P. aeruginosa isolates from patients clinically presumptive for nosocomial infection at FHRH, 2018 (n=20)

| Bacterial isolate       | Antiibiogram Profile                                      | Antibiotic Class |
|-------------------------|-----------------------------------------------------------|------------------|
| A. baumannii (n = 9)    | (AMP,AMC,CAZ,PIP,CIP,CN,SXT,MEM)                          | 6                |
|                         | (AMP,AMC,CAZ,PIP,CN,SXT)                                  | 4                |
|                         | (AMP,AMC,CAZ,PIP,CIP,CN)                                  | 4                |
|                         | (AMP,AMC,PIP,CN,SXT)                                      | 3                |
|                         | (AMP,CAZ,PIP,SXT)                                         | 3                |
| P.aeruginosa (n = 11)   | (AMP,AMC,CAZ,PIP,CIP,CN,SXT,MEM)                          | 6                |
|                         | (AMP,AMC,CAZ,PIP,CIP,SXT)                                 | 4                |
|                         | (AMP,AMC,PIP,CN,SXT)                                      | 3                |
|                         | (AMP,AMC,CAZ,PIP,SXT)                                     | 3                |

Key: AMP: ampicillin, AMC: amoxicillin-clavulanic acid, PIP: piperacillin, CAZ: ceftazidime, CIP: ciprofloxacin, CN: gentamicin, MEM: meropenem and SXT: trimethoprim sulphamethoxazole, R3,4,6,: resistance to 3,4,6 antibiotic drug classes.

Table 5: Antimicrobial resistance profiles of A. baumannii and P. aeruginosa isolates from participants presumptive for nosocomial infection at FHRH, Bahir Dar, Northwest Ethiopia, April to July, 2018

| Antimicrobials                | A.baumannii | P.aeruginosa |
|------------------------------|-------------|--------------|
| Ampecillin                   | # T: 9 | 9 (100) | # T: 11 | 11 (100) |
| Amoxicillin-clavulanic acid  | # T: 9 | 8 (88.9) | # T: 11 | 11 (100) |
| Piperacillin                 | # T: 9 | 9 (100) | # T: 11 | 11 (100) |
| Cefotaxime                   | # T: 9 | 8 (88.9) | # T: 11 | 11 (100) |
| Ceftriaxone                  | # T: 9 | 8 (88.9) | # T: 11 | 11 (100) |
| Ceftazidime                  | # T: 9 | 7 (77.8) | # T: 11 | 7 (63.6) |
| Ciprofloxacin                | # T: 9 | 4 (44.5) | # T: 11 | 4 (36.4) |
| Gentamicin                   | # T: 9 | 8 (88.9) | # T: 11 | 6 (54.5) |
| Meropenem                    | # T: 9 | 3 (33.3) | # T: 11 | 5 (45.5) |
| Tetracycline                 | # T: 9 | 7 (77.8) | # T: 11 | 10 (90.9) |
| Trimethoprim sulphamethoxazole | # T: 9 | 6 (66.7) | # T: 11 | 11 (100) |

Total 
#T: Number of isolates tested,  R%: Percentage of resistant isolates
