Original article

# Drug resistance patterns of bacterial isolates from infected wounds at Bahir Dar Regional Health Research Laboratory Center, Northwest Ethiopia

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**Abstract**

**Background:** An increased antibiotic resistance of bacterial isolates from wound infections is a major therapeutic challenge. The aim of this study was to identify bacterial isolates associated with wound infection and to determine their current antimicrobial susceptibility profile.

**Methods:** This is a retrospective cross-sectional study in which we analyzed the records of 380 wound swab culture results that have been processed at Bahir Dar Regional Health Research Laboratory Center in the period of 1 January 2013 to 30 December 2015. Swabs from different wound types were collected aseptically and analyzed using standard bacteriological procedures. Antimicrobial susceptibility testing was performed using disc diffusion technique as per the standard protocol. Demographic and bacteriological data were collected using a data extraction sheet. The data were cleaned, entered and analyzed using SPSS version 22.

**Results:** The overall bacterial isolation rate was at 61.6% (234/380). More than half 123 (52.6%) of the isolates were gram positive and 111 (47.4%) were gram negatives. The predominant isolates were *S. aureus* at 100 (42.7%) followed by *E. coli*, 33 (14.1%), *P. aeruginosa*, 26 (11.1%) and *S. pyogenes*, at 23 (9.8%). The proportion of multidrug resistant (MDR) bacterial pathogens was at 54.3%. Out of these, 35 (15.1%) of the isolates were resistant to more than five drugs. The highest resistance rate at (85.9%) was documented for ampicillin by gram-negative isolates. Whereas the highest resistance rate among gram positive isolates was against erythromycin (31.1%). The resistance rate of *S. aureus* for penicillin was at 69.7%.

**Conclusions:** High frequency of mono and multi-drug resistant bacterial pathogens were documented. Thus, an alternative method to the causative agent and antimicrobial susceptibility testing surveillance in areas where there is no culture facility is needed to assist health professionals for the selection of appropriate antibiotics. [*Ethiop. J. Health Dev.* 2016;30(3):112-117]

**Key words:** Wound infection, bacterial isolates, and antimicrobial susceptibility profile.

## Introduction

Exposure of the underlying tissue following a loss of skin integrity due to a range of reasons provides a moist, warm, and nutritious environment that is conducive to microbial colonization and proliferation. Wound infection is one of the health problems that are caused by various types of pathogens (1). Since wound colonization is most frequently poly-microbial, involving different microorganisms that could be potentially pathogenic, any wound is at some risk of getting infected (2, 3).

Reports showed that, *Staphylococcus aureus*, *Pseudomonas spp.*, *Klebsiella* spp, and *E. coli* are the leading bacterial pathogens in wound infection (4, 5, 6). Similar reports have been observed in Ethiopia (4, 7, 8), Nigeria (9), Uganda (10) and Ghana (11).

Inappropriate and continued use of systemic and topical antimicrobial agents has provided the selective pressure that has led to the emergence of antibiotic resistant strains (12).

Recently, alarming reports on the causative agent of wound infection and associated drug resistance pattern in Ethiopia have been reported (4, 7, 8). However, very limited data are available on the kinds of bacterial isolates and their drug resistance profile associated with wound infection in the study area.

Due to the consequential impact of bacterial pathogens involved and increasing antibiotic resistance, local epidemiological information serves as a guide for effective empirical treatment and management of infected wound. Therefore, the present study was conducted to update profile of bacteria identified from wound infections and to describe antimicrobial sensitivity patterns of isolates.

## Methods

***Study design and period***: A retrospective record review of bacteriological culture results of all types of wound swabs referred to Bahir Dar Regional Health Research Laboratory Center (BRHRLC) from 1 January 2013 to 30 December 2015 was conducted. BRHRLC is one of the new state of the art laboratories in Ethiopia established in 1988. It is the technical arm

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of Amhara Regional Health Bureau currently providing specialized services (like, MDR-TB culture and molecular laboratory, real time PCR for HIV exposed infants, trachoma elimination research project and quality assurance service). It is giving referral services to Felege Hiwot Referral Hospital, nearby health centers, private hospitals and clinics.

***Data collection***: This is a paper based bacteriological laboratory registration record review in which we have extracted a total of 380 wound swab culture laboratory reports using data extraction sheet. We considered all records documented during the stated time period. Patient's demographic data (age and sex), types of isolated bacteria from wound swab culture and antimicrobial resistance profiles of the isolates were retrieved. All patient records having the above variables were included for analysis.

***Specimen collection, culture and bacterial tests***: All types of wound samples were collected using sterile cotton swabs dipped in normal saline as per the standard microbiological procedures (13). Wound swabs were inoculated on sheep blood agar and MacConkey agar plates (Oxoid, UK). The samples were streaked in four quadrants of the plate using 5 mm diameter sterile wire loop to get pure colonies. Then, sheep blood agar plates were incubated in 5% $Co_2$ at 37ºc. Similarly, MacConkey agar plates were incubated at 37°C. Finally, the plates were examined for bacterial growth after 24 hours (8).

Bacterial isolates were characterized using colony morphology, gram stain and using a panel of biochemical tests based on the gram reaction [for gram positives; catalse, cuagulase, bastracin, and for gram negatives: glucose and lactose fermentation, sulfide-indole-motility, cimon's citrate, urease lysine iron agar tests] carried out following standard microbiological methods (14).

***Antimicrobial susceptibility testing***: Antimicrobial susceptibility testing was performed on Mueller Hinton agar using Kirby-Bauer disk diffusion method (15). Morphologically identical pure bacterial colonies from overnight cultures were suspended in 5ml nutrient broth and incubated for 4-6 hours at 37ºc. The turbidity of the suspension was equilibrated to match with 0.5 McFarland standards. Then, the bacterial suspensions were seeded on the surface of the Mueller Hinton agar using a sterile cotton swab. The antibiotic disks were placed on the surface of inoculated agar and incubated at 37ºC for 18-24 hours. After incubation, the diameters of the discs growth inhibition zone was measured and interpreted as per the standard protocol (16). The antimicrobials tests were obtained from Oxoid Ltd. (England).

Discs used for gram-positive isolates with their respective concentrations include: ciprofloxacin (5 µg), cotrimoxazole (25 µg), tetracycline (30 µg), clindamycin (30 µg), chloramphenicol (30 µg), erythromycin (15 µg), penicillin (10IU), and oxacillin (30 µg). Similarly, the following discs were employed for gram negatives: ampicillin (10 µg), ciprofloxacin (5 µg), cotrimoxazole (25 µg), pepracillin (100 µg), gentamicin (10 µg), penicillin (10IU), ceftriaxone (30 µg), chloramphenicol (30 µg), ceftazidime (30 µg), and amoxicillin-clavulanic acid (Augmentine) (30 µg).

The antibiotic susceptibility pattern was interpreted based on clinical and laboratory standard institute (CLSI, 2014) (16). The standard reference strains of *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) were used for quality control of culture and antimicrobial susceptibility testing.

***Statistical analysis***: The generated data were cleaned, entered and analyzed using statistical software for social sciences version 22 (*IBM Corp, Released 2011: IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp*). The results were summarized using descriptive statistics including frequencies and mean. Odds ratio and its 95% confidence interval (CI) was considered to compare the proportion of bacterial isolates with patients' demographic information and differences were considered significant when *p*-value was less than 0.05.

***Ethical considerations***: Permission and ethical clearance was obtained from Amhara Regional Health Bureau Institutional Review Board (ARHBIRB), located in Bahir Dar Regional Health Research Laboratory Center to exploit the recorded laboratory data for research purpose. No patient identity, like name was used and thus confidentiality was maintained.

## Results

***Socio-demographic characteristic of patients and types of bacterial isolates***: In this study, a total of 380 wound swab specimens were analyzed. Out of these, 234 (61.6%) were positive for bacteriological culture. More than half, 195 (51.3%) wound swab specimens were collected and analyzed from male patients with a male to female ratio of 1.1:1. The age of patients was ranged from 4 months to 76 years (median age 39.2 years).

Higher proportion of wound infection was documented among the participants in the age group of 0-10 years at 59 (84.3%) followed by 21-30 years at 50 (53.2%) although there was no significant difference among the different age groups ($p>0.05$). Moreover, the proportion of bacterial isolation from males was at 131 (67.2%) and from females was at 103 (55.7%). Sex of patient's was found significantly associated with wound infection [OR: 1.63; 95%CI: (1.07- 2.47), *P* value: 0.021] (Table 1).

Table 1: **Distribution of participants (n=380) with infected wound by age and sex at Bahir Dar Regional Health Research Laboratory Center, Northwest Ethiopia, January 2013 to December 2015.**

| Variables | n (%) of Culture positives | n (%) of Culture negatives | OR (95%CI) | p-value |
|---|---|---|---|---|
| **Age in years** | | | | |
| 0-10 | 59 (84.3) | 11 (15.7) | 3.2 (0.66 – 15.46) | 0.144 |
| 11-20 | 40 (54.8) | 33 (45.2) | 0.7(0.16- 3.17) | 0.678 |
| 21-30 | 50 (53.2) | 44 (46.8) | 0.7(0.15- 3.01) | 0.613 |
| 31-40 | 42 (59.2) | 29 (40.8) | 0.9(0.19- 3.92) | 0.855 |
| 41-50 | 20 (54.1) | 17 (45.9) | 0.7(0.14- 3.39) | 0.663 |
| 51-60 | 18 (66.7) | 9 (33.3) | 1.2(0.23- 6.18) | 0.828 |
| >60 | 5 (66.7) | 3 (33.3) | 1.00 | |
| **Sex** | | | | |
| Female | 103 (55.7) | 82 (54.3) | 1.00 | |
| Male | 131 (67.2) | 64 (32.8) | 1.63 (1.07- 2.47) | 0.021 |
| **Total** | 234 (61.6) | 146(38.4) | | |

The distribution pattern of isolates identified from wound is summarized in Figure 1, where *S. aureus*, at 100 (42.7%) was the predominant isolate followed by *E. coli* at 33 (14.1%), *P. aeruginosa* at 26 (11.1%) and *S. pyogenes* at 23 (9.8 %). Gram-positive cocci and gram-negative rods constituted 123 (52.6%) and 111(47.4%), respectively. Whereas 226 (96.6%) showed single infection the rest at 8 (3.4%) had mixed bacterial infection. The frequency of mixed isolates on wound infections is indicated in Table 2.

***Antimicrobial susceptibility profile of the isolates***

In this study, 85.9% of gram-negative isolates were found resistant to ampicillin, followed by augmentin (58.8%), and co-trimoxazole (52.3%) (Table 3). *P. aeruginosa* showed a resistance at 73.1% to ceftazidim and 50% to pepracillin. The two-gram positive isolates, *S. aureus* and *S. pyogenes*, showed resistance to erythromycin at 31.1% followed by tetracycline at (27%) and co-trimoxazole at (17%) (Table 4). All tested isolates of *S. pyogenes* were 100% sensitive to penicillin.

Table 2: **Frequency of mixed wound infections at Bahir Dar Regional Health Research Laboratory Center, Northwest Ethiopia, January 2013 to December 2015.**

| Mixed isolates | Frequency |
|---|---|
| Proteus species and *S. aureus* | 3 |
| Proteus species and Citrobacter | 1 |
| *P.aeruginosa* and *S. aureus* | 1 |
| *P. aeruginosa* and *K. pneumonia* | 1 |
| *P.aeruginosa* and *E.coli* | 1 |
| *S. aureus* and *K. pneumonia* | 1 |

Table 3: **Antimicrobial resistance patterns of gram negative bacterial isolates from wound swabs at Bahir Dar Regional Health Research Laboratory Center, Northwest Ethiopia, January 2013 to December 2015.**

| Antimicrobial agents | *E. coli* | | *P. aeruginosa* | | *K. pn* | | *Proteus* pp. | | *Citrobacter* | | *Enterobacte* | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | #*T* | %R | #*T* | %R | #*T* | %R | #*T* | %R | #*T* | %R | #*T* | %R | |
| **Ampecillin** | 33 | 93.9 | ND | ND | 20 | 75 | 22 | 77.3 | 5 | 100 | 5 | 100 | 73 (85.9) |
| **Augmentine** | 33 | 72.7 | ND | ND | 20 | 50 | 22 | 54.5 | 5 | 40.0 | 5 | 40 | 50 (58.8) |
| **CAF** | 26 | 19.2 | 3 | 0 | 18 | 44.4 | 22 | 68.2 | 5 | 60.0 | 5 | 0 | 31(39.2) |
| **Ceftriaxone** | 24 | 25.0 | ND | ND | 18 | 11.1 | 18 | 44.4 | 5 | 40.0 | 5 | 40 | 20 (28.6) |
| **Ciprofloxacin** | 33 | 45.4 | 26 | 19.2 | 20 | 20.0 | 22 | 22.7 | 5 | 0 | 5 | 0 | 34 (19.1) |
| **Gentamycin** | 33 | 54.5 | 23 | 30.4 | 18 | 61.1 | 22 | 22.7 | 5 | 0 | 50 | 0 | 41(38.7) |
| **Pepracillin** | ND | ND | 26 | 50.0 | ND | ND | ND | ND | ND | ND | ND | ND | 13 (50) |
| **Ceftazidime** | ND | ND | 26 | 73.1 | ND | ND | ND | ND | ND | ND | ND | ND | 19 (73.1) |
| **Cotrimoxazole** | 30 | 76.6 | 9 | 33.3 | 20 | 40.0 | 17 | 41.2 | 3 | 100 | 5 | 0 | 44 (52.3) |
| **Amikacin** | 11 | 18.2 | 26 | 7.7 | 4 | 0 | 8 | 12.5 | 5 | 0 | 5 | 0 | 4 (8.5) |

Key: # T: Number of isolates tested against each antimicrobial agent
%R: Percent of isolates resistance to the respective antimicrobial agent
ND: Not done

Table 4: **Antimicrobial resistance patterns of gram positive isolates from wound swabs at Bahir Dar Regional Health Research Laboratory Center, Northwest Ethiopia, January 2013 to December 2015**.

| Isolates | Pattern | Antimicrobial agents (number of tested isolates with % resistance) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | ERT | TTC | P | CAF | OXA | CLN | CPN | SXT |
| ***S. aureus*** (n=100) | #T | 96 | 98 | 98 | 74 | 95 | 100 | 67 | 94 |
| | %R | 31.3 | 27.6 | 69.7 | 0 | 18.9 | 7 | 7.5 | 17 |
| ***S. pyogenes*** *(n=23)* | #T | 23 | 13 | 16 | 6 | ND | 23 | 20 | 7 |
| | %R | 30.4 | 23.1 | 0 | 0 | - | 0 | 5 | 28.6 |
| ***Total (n=123)*** | #T | 119 | 111 | 114 | 80 | 95 | 123 | 87 | 101 |
| | %R | 31.1 | 27 | 59 | 0 | 18.9 | 5.7 | 6.9 | 17.8 |

**Key**: # T: number of isolates tested against each antimicrobial agent, %R: percent of isolates resistance to the respective antimicrobial agent. Not all isolates were tested against for all kinds of dicks, due to shortage/stock out of antibiotic.
ERT: Erythromycin, TTC: Tetracycline, P: Penicillin, CAF: Chloramphenicol, OXA: Oxacillin, CLN: Clindamycin, CPN: Ciprofloxacin, SXT: Cotrimoxazole

Antimicrobial resistance pattern of the isolates for more than one antimicrobial agents were documented among *Citrobacter* spp, Proteus spp. *E. coli* and *K. pneumonia* at (100%), (85.7%), (78.8%) and (60%), respectively. About 127 (54.3%) of the bacterial isolates were found resistant to two or more drugs. However, resistances to more than five Antimicrobial agents were documented in 35 (15.1%) of the isolates (Table 5).

Table 5: **Multiple drug resistance (MDR) patterns of isolates from wound swab at Bahir Dar Regional Health Research Laboratory Center, North West Ethiopia, January 2013 to December 2015.**

| Bacterial isolates | Multiple drug resistance patterns of isolates, n (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | R0 | R1 | R2 | R3 | R4 | ≥R5 | Total | MDR* |
| *S. aureus* | 27 (27) | 25 (25) | 20 (20) | 19 (19) | 5 (5) | 4 (4) | 100 (100) | 48 (48) |
| *E. coli* | 2 (6.1) | 5 (15.2) | 0(0) | 8 (24.2) | 6 (18.2) | 12 (36.2) | 33 (100) | 26 (78.8) |
| *P. aeruginosa* | 6 (22.2) | 6 (22.2) | 8 (29.6) | 2 (7.4) | 2(7.4) | 3 (11.1) | 27 (100) | 15 (55.5) |
| *S. pyogenes* | 13 (56.5) | 7 (30.4) | 3 (13) | 0 (0) | 0 (0) | 0 (0) | 23 (100) | 3 (13) |
| *Proteus* spp | 0(0) | 3 (14.3) | 6 (28.6) | 2 (9.5) | 2 (9.5) | 8 (38.1) | 21 (100) | 18 (85.7) |
| *K. pneumoniae* | 2(10) | 6 (30) | 3 (15) | 1 (5) | 0 (0) | 8 (40) | 20 (100) | 12 (60) |
| *Citrobacter* spp | 0(0) | 0 (0) | 0 (0) | 5 (100) | 0 (0) | 0 (0) | 5 (100) | 3 (100) |
| *Enterobacter* spp | 0(0) | 3 (60) | 0 (0) | 2 (40) | 0 (0) | 0 (0) | 5( 100) | 2 (40) |
| Total | 50 (21.5) | 55 (23.7) | 40 (17.2) | 37(15.9) | 15 (4.5) | 35 (15.1) | 234 (100) | 127 (54.3) |

Key: R1-R5 = Number of antimicrobial class in which a given isolate was resistant.
MDR= Resistant to two or more antibiotics.

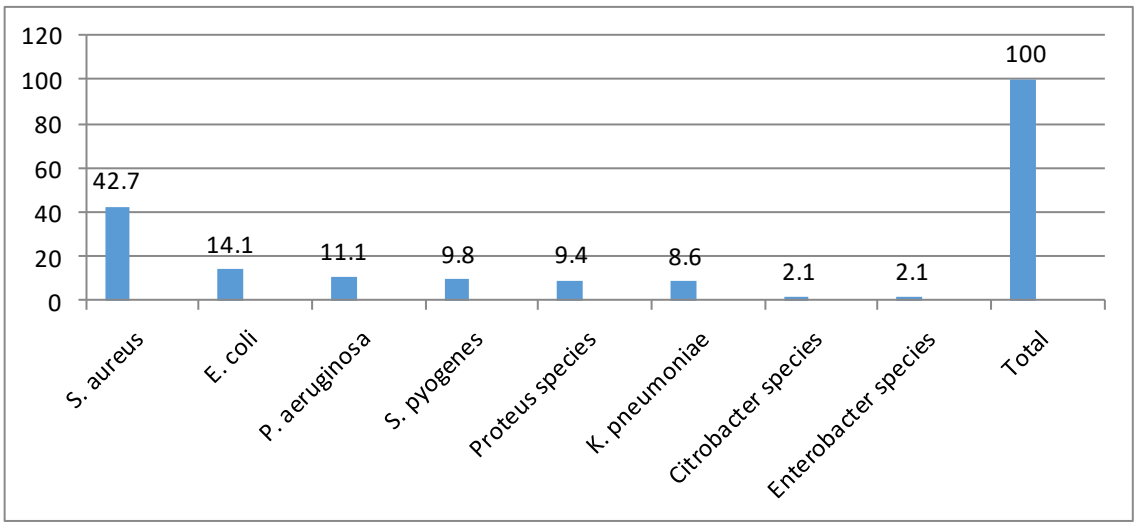


Figure 1: Percentage distribution of bacterial isolates from wound infections at Bahir Dar Regional Health Research Laboratory Center, North West Ethiopia, January 2013 to December 2015.

**Discussion**

The overall bacterial isolation rate in the present study was at 61.6%. However, relatively higher rate of isolation at 87.3% (4) and 70.2% (7) was reported elsewhere in Ethiopia. This disparity might be due to the differences in wound swab collection techniques, as it needs careful cleaning of the wound surface before sample collection to avoid skin contaminants like coagulase negative staphylococcus (17, 18).

The isolation rate of gram-positive cocci was at 52.6% in this study compared to other findings reported by Kibret *et al.* in 2011 at 43.4% (19). The possible explanation for such disparity might be due to methodological differences in the identification of the isolates.

In our study, the isolation rate of *S. aureus* was at 42.7%. A number of findings reported previously on wound infection in Ethiopia and elsewhere in the world also indicated that *S. aureus* was the predominant isolate (3, 4, 7, 8, 15, 20, 21). This may be because it is an endogenous source of infection and infection with *S. aureus* may also be due to contamination of the wound from the environment, like from surgical instruments and health professionals. When there is disruption of natural skin barrier, *S. aureus*, which is a common bacterium on surfaces, could easily contaminate wounds and eventually cause infection. Moreover, the 2nd and 3rd predominate isolates in our study were *E.coli* at (14.1 %) and *P. aeruginosa* at (11.1%), respectively. Similar findings were reported in another study (17).

In this study, higher proportion of patients in the age group of 0-10 years was more affected than other groups. There was no significant difference among the different age groups ($p$>0.05). This finding is in agreement with another study from Nigeria (6). Bacterial isolation from wound swab (to have infected wound) was 1.63 times more likely among males than females [OR: 1.63; 95%CI: (1.07- 2.47), *P* value: 0.021]. This might be related with better habit of cleanliness among females than males. This is also in agreement with a report from Gondar (7).

We have documented relatively higher drug resistance rates among gram negatives to ampicillin, augmentin (amoxicillin/clavulanate) and co-trimoxazole. Similar findings were reported in Ethiopia (4). In this study, amikacin and ciprofloxacin were found to be the most effective antimicrobial agents against gram-negative bacterial isolates. However, chloramphenicol and ampicillin were found to be more effective against gram positives. This is comparable with previous studies conducted in Southwest Ethiopia (4). Similar pattern of results were also documented elsewhere in the world (1, 4, 17, 22). Moreover, majority of the isolates showed resistance to more than one drug. Previous reports in Ethiopia demonstrated comparable results (4, 7, 8, 19).

In this study, 21.5% of the isolates were sensitive to all antibiotics tested and 23.7% were found to be resistant to only one antibiotic. Similarly, we documented that 54.7% of the isolates were resistant to two or more antimicrobials and 35(15.1%) were resistant to more than five antibiotics. This implies that antimicrobial resistance rates among common bacterial pathogens is continue to evolve and appear to be increasing to many commonly used agents from time to time (3). However, our results showed inconsistency with those reported by other scholars (7, 23). The potential differences in the rate might be due to differences in the study population. The study population of the previous studies included hospitalized patients in which higher multi drug resistant strains are expected.

Due to the retrospective nature of this study, authors are unable to present detailed clinical data of patients to identify predictors of all forms of wound infection and antimicrobial resistance. This calls for the improvement in documentation and keeping of medical records of patients properly. Moreover, shortage of antibiotic discs hinders to present the whole antimicrobial sensitivity picture of all isolates for each available disc. However, our study is one of the few researches that provide updated information concerning bacteriology of wound infection and this could be useful for further studies.

***Conclusions***:

High frequencies of bacterial isolates were identified from wound. The predominant isolates were *S. aureus* followed by *E. coli* and *P. aeruginosa.* Most of the isolates were found to be resistant to commonly used drugs. Hence, it is essential to exercise periodic surveillance of antimicrobial susceptibility testing, and proper management of wound infection to avoid the emergence and spread of drug resistance bacterial strains.

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