OPEN ACCESS
Freely Available Online

JOURNAL OF PREVENTIVE MEDICINE AND CARE
*ISSN NO: 2474-3585*

Research

**DOI: 10.14302/issn.2474-3585.jpmc-21-3851**

# Antimicrobial Resistance: A Situational Analysis in the Deido Health District, Douala, Cameroon

Charles Njumkeng[1,4], Elvis T. AMIN[1], Denis Zofou[3], Jane-Francis K. T. Akoachere[2], Patrick A. Njukeng[1,2,*]

[1]Global Health Systems Solutions, Douala, Cameroon

[2]Department of Microbiology and Parasitology, University of Buea, Cameroon

[3]Department of Biochemistry and Molecular Biology, University of Buea, Cameroon

[4]Department of Public Health and Hygiene, University of Buea, Cameroon

## Abstract

**Background:** The rapid and ongoing spread of antimicrobial-resistant organisms threatens the ability to successfully prevent, control, or treat a growing number of infectious diseases in developed and developing countries. This study was designed to convey more insight on the profile of antimicrobial resistance and the capacity of laboratories conducting antimicrobial susceptibility testing in Cameroon.

**Methods:** A multicentre cross-sectional study was conducted from October 2019 to March 2020 in the Deido Health District. Laboratories that carry out culture and sensitivity testing within the Deido Health District were identified and assessed to determine their capacity as well as the quality of results from microbiological investigations. Information on antimicrobial susceptibility of various isolates was collected using tablet phones in which the study questionnaires had been incorporated.

**Results:** Gaps identified in antimicrobial susceptibility testing that cut across laboratories included; insufficient standard operating procedures, inadequate records on personnel training and competency assessment, lack of safety equipment such as biosafety cabinet, stock out and non-participation in external quality assurance program. The turnaround time for antimicrobial susceptibility testing ranged from 3 – 7 days. Out of the 1797 samples cultured, 437(24.3%) had at least one isolate. A total of 15 different isolates were identified with *Candida albicans* being the most frequent 178 (40.7%), followed by *Escherichia coli* 80(18.3%). Among the 15 classes of antimicrobial drugs used in this study, the overall resistance of the isolates showed that five classes had class median resistance above 40% (Cephalosporins, Penicillins, Beta-lactam, Macrolides, and Polyenes).

**Conclusion:** This study has shown the need to develop a coordinated national approach to fight antimicrobial resistance. Scaling-up of antimicrobial susceptibility testing will, therefore, require strengthening the microbiology units of laboratory systems as well as ensuring the use of laboratory data for decision making.

**Corresponding author:** Patrick A. Njukeng, Department of Microbiology and Parasitology, University of Buea, Cameroon, Executive Director, Global Health Systems Solutions, Denveur layout, Bonamousadi, P.O, Box. 3918 Douala, Littoral Region, Republic of Cameroon. Email: p3njukeng@gmail.com

**Keywords:** Gram-negative bacteria, Gram-positive, bacteria, Fungi, Mycoplasma, Antimicrobial, resistance, Clinical specimen, Cameroon

**Recieved:** May 27, 2021 **Accepted:** Jun 08, 2021 **Published:** Jun 16, 2021

**Editor:** Padmavathi Kora, Gokaraju Rangaraju Institute of Engineering & Technology, Hyderabad.

## Introduction

Antimicrobial resistance is a major public health problem. The rapid and ongoing spread of antimicrobial-resistant organisms threatens the ability to successfully prevent, control, or treat a growing number of infectious diseases in developed and developing countries [1,2]. Studies have projected that if the current trend continues, by 2050 an estimated 10 million deaths will occur annually as a result of antimicrobial resistance (AMR) [3–5].

WHO underscored in the global action plan the need to continue to raise awareness of AMR through research, surveillance, and monitoring in different countries [6–8]. This is critical for the AMR response system as it: provides data on antimicrobial resistance rate, information to guide clinicians as well as a platform from which AMR reduction strategy can build on [6,9].

The impact of AMR is already overwhelming on several health systems worldwide. In the USA, AMR is estimated to be responsible for more than 2 million of infectious diseases and accounts for about 23,000 annual deaths [10]. Understanding the real situation in Africa has been challenging due to the limited availability of data at the country level [11,12]. The unavailability of data presents significant challenges in the fight against AMR as it creates gaps in the effective surveillance of AMR, standardization of methodologies, and effective data sharing [11,13,14]. The existence of gaps in public health information on AMR is more worrisome given that alteration in resistance mechanisms, the emergence of new resistance, and multidrug-resistant pathogens can only be detected through continuous information gathering. It is, therefore, very certain that the fight against AMR should be founded on the field realities given that accurate data is highly dependent on quality-assured microbiology laboratories [13,15].

Studies on antimicrobial resistance in different parts of Cameroon indicate that antimicrobial drugs top the list of commonly prescribed drugs in hospitals following the high burden of infectious diseases [16,17]. Mindful of the fact that their use has been identified as an important factor for developing and propagating resistance [18], it is important to update the situation of the resistance profile over time to better inform decision-makers. This study was designed to convey more insight on the profile of AMR resistance and the capacity of laboratories conducting antimicrobial susceptibility testing in Cameroon.

## Methods

### *Study Design and Study population*

This was a multicentre cross-sectional study conducted from October 2019 to March 2020 in the Deido Health District. Laboratories that carry out culture and antimicrobial susceptibility testing (AST) within the Deido Health District were identified and assessed to determine their capacity as well as the quality of results from microbiological investigations using the Modified WHO Antimicrobial Resistance Surveillance Questionnaire [16] and Stepwise Laboratory Quality Improvement Process Towards Accreditation (SLIPTA) Checklist [19].

The laboratory staff working on the microbiology bench were trained on how to collect information using a structured questionnaire incorporated in tablet phones. Information on antimicrobial susceptibility of various isolates was collected using tablet phones in which the study questionnaires had been incorporated.

Participants included in this study were individuals of all ages and sex who visited any of the three (Deido District Hospital, St Padre Pio Hospital and Daniel Muna Memorial Clinic) main hospitals in the Deido Health District for culture and sensitivity tests between October 2019 and March 2020.

### *Antimicrobial Susceptibility Testing*

Antimicrobial susceptibility of the isolates was determined using the disc diffusion technique on Mueller Hinton agar for bacteria isolates and Sabouraud dextrose agar for fungi isolates as described in the guidelines of the Clinical and Laboratory Standard Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints[20,21]

After inoculating the isolates and placing the antimicrobial discs, the plates were incubated for 24h against Staphylococcus isolates and 16–18h for other isolates. The diameters of the zones of complete inhibition (as qualified with the eye) were measured, the diameter of the antimicrobial disk was also measured.

The measured diameter was compared to the critical values of each antimicrobial disc to determine whether the target isolate was sensitive, resistant or intermediate. Mindful of the difficulty in obtaining commercial control strains in the country to be used for daily routine, the laboratories had established in house controls that are derived from commercial controls. Control tests were performed with *Staphylococcus aureus and E. coli* in house derived strains.

For mycoplasma susceptibility testing, the specimen was inoculated into the Mycoplasma Susceptibility kit (Autobio Diagnostics Co.,Ltd., Zhengzhou, China) within an hour as described by the manufacturer's guidelines. If there is a growth, *Urealpasma urealyticum* and *Mycoplasma hominis* metabolizes urea and arginine, respectively. This changes the colour of the culture medium (from yellow to red). The kit was incubated at 37°C for 48 h after which susceptibility or resistance bacterial were determined with the aid guidelines of Clinical and Laboratory Standards Institute.

*Data Collection*

Data on the laboratory capacity and quality of culture and antimicrobial susceptibility testing was collected with the modified WHO Antimicrobial Resistance Surveillance Questionnaire and the SLIPTA Checklist. A structured questionnaire was used to collect demographic information, patient history concerning the usage of antimicrobials. Furthermore, the questionnaire was also designed to capture information on the type of specimen that was cultured, the isolate, and the drugs to which the microbe was resistant as well as the turnaround time for culture and antimicrobial susceptibility testing. Since the questionnaire was incorporated into a tablet phone, an email address was created which was used for weekly backup of the data set.

*Data Analysis*

Data was collected with a questionnaire designed using Epi Info Data software. The data set was exported from Epi Info to excel spreadsheet. Missing variables or discrepancies in data were corrected from the medical records of patient. The data was then exported and analyzed using SPSS version 20 (IBM, Chicago, IL). Descriptive statistics such as the number of microbes isolated, type of specimen cultured were expressed as proportions. The overall resistance of the isolates to a class of antimicrobial agents was calculated as median resistance and inter quarter range. The resistance rate of a specific group of the isolate to the antimicrobial agent was also calculated as median resistance. Comparison of the proportion of antimicrobial resistance between groups was assessed with the chi-square test and the threshold for statistical significance set at $p < 0.05$.

*Ethical Considerations*

Ethical approval was obtained from the Faculty of Health Sciences Institutional review board of the University of Buea (N0: 2019/941-01/UB.SG.IRB.FHS). Administrative authorization was obtained from the Littoral Regional Delegation of Public Health and the Deido Health District. Written consent was obtained from the participants after the purpose of the study was orally explained to them. Since the questionnaire was incorporated into the tablet phones, passwords were given for each tablet to avoid unauthorized accessto the database

## Results

*Laboratory Antimicrobial Susceptibility Testing Capacity*

From the audit results using WHO Antimicrobial Resistance Surveillance and SLIPTA checklist, the methods in use for culture, identification, and antimicrobial susceptibility testing in the laboratories were kit-based (only for mycoplasma) and conventional methods. With respect to personnel, the laboratories were headed by qualified microbiologists and Quality Assurance Officers were present in all the laboratories. The laboratories reported not to be using control strains but they had established in-house quality control isolates. All the laboratories had a backup system for power. The laboratories were using either the "CLSI or the EUCAST" guide for the interpretation of AST.

On the other hand, gaps identified which cut across the three laboratories included; insufficient standard operating procedures, inadequate records on personnel training and competency assessment, lack of safety equipment such as biosafety cabinet, stock out, non-participation in external quality assurance programs, and audits were not regularly performed. The study findings also showed that there are no structures

in place to oversee AMR activities in the facilities and at district levels, hence non-utilization of laboratory AST data to inform authorities. Another gap that cut across these laboratories was the non-usage of quality indicators to measure performance.

*Characteristics of the Study Population*

A total of 1797 samples were cultured in the three laboratories during the study period among which, 424 (23.6%) were female samples. The samples were collected from individuals aged 1 to 94 years, with a mean age of 19.84 years (SD 14.9). The majority of the participants 1199 (66.7) were within the age group 20 – 40 years. Most of the specimens cultured were, urine 731(40.7%) follow by vagina smear 642(35.7%) while the CSF was 2(0.1%). It took the laboratories 3 to 7 days to give out culture and susceptibility testing results. It is important to note that 1164(64.7%) of results were given within 3 working days while 16(0.9%) took more than 5 days (Table 1).

Out of the 1797 samples cultured, 437(24.3) had at least one isolate. A total of 15 different isolates were identified with *Candida albicans* being the most frequent 178 (40.7%), mainly isolated from virginal smears followed by *Escherichia coli* 80 (18.3%) isolated predominantly from urine cultures. Only one *Neisseria gonorrhoeae* was isolated from a urethra smear during the study period. Overall the greatest number of isolates was from virginal smears 198 (45.3%) followed by urine 116 (26.5%) and stool 81(18.5%) (Table 2).

*Overall Antimicrobial Resistance in the Study Population*

Among the 15 classes of antimicrobial drugs used in this study, the overall resistance of the isolates showed that 5 classes had class median resistance above 40% (Cephalosporins, Penicillins, Beta-lactam, Macrolides and Polyenes). Polyenes had the highest median resistance with Amphotericin B having an overall resistance rate of 89.6 (81.7 – 94.9). The median resistance for Beta-lactam 62.2% with oxacillin being the most resistant in the group 73.0% (95% CI 60.3 – 83.4). On the other hand, the least resistance was observed among the aminoglycosides with a class median of 12.8% with amikacin having the lowest resistance in the group (6.5%, 95% CI 0.8 – 21.4) (Table 3).

For the resistance of the various isolates to the different antimicrobials tested against, coagulase-negative *Staphylococcus (CoNS), Streptococcus spp., Pseudomonas aeruginosa* and *Serratia liquefaciens* had median resistance rate ≥50%. The highest median resistance rate was observed for *Pseudomonas aeruginosa* with a median resistance rate of 71.0% (IQR, 45.6 – 89.5). The least overall resistance was observed among *Citrobacter* isolates 17.0% (IQR 0.0 – 61.8), followed by *Ureaplasma urealyticum* and *Klebsiella* species each with a median resistance rate of 33.0% (Table 4).

*Resistance Rates of Gram-Positive Bacteria to Antimicrobials*

Among the cephalosporins, cefixime showed 100% resistance to *Staphylococcus aureus and CoNS* while cefuroxime showed no resistance to these two groups of bacterial. Among penicillins, ampicillin showed a resistance rate of at least 50% to all gram-positive isolates while cloxacillin (9.1%), amoxicillin-clavulanic acid (33.3%) piperacillin (21.4%) and amoxicillin (11.1%) were the resistance to *Staphylococcus aureus*, CoNS, *Enterococcus spp* and *Streptococcus* respectively. Among the Beta-lactam, *Staphylococcus aureus* and *Enterococcus spp* showed 100% resistance to oxacillin. With respect to the Quinolones, *Staphylococcus aureus*, *Enterococcus spp,* and *Streptococcus* showed 100% resistance to nalidixic acid while perfloxacin was the least resistant to *Staphylococcus aureus*, and *Streptococcus* with a resistance of 11.1% and 28.6% respectively. *Streptococcus* also had 100% resistance to ciprofloxacin. Most of the tetracyclines showed 100% resistance.

Generally, among the 10 classes of drugs tested against the gram-positive bacteria in the study, only clarithromycin (a quinolone) and rifampicin (an antimycobacterial) showed resistance rate with a significant difference across the different category of gram-positive bacteria ( p-value 0.044 and 0.0001 respectively) (Table 5)

*Resistance Rates of Gram-Negative Bacteria to Antimicrobials*

*Escherichia coli* showed high resistance of above 70% to ampicillin, amoxicillin-clavulanic acid, oxacillin, nalidixic acid, vancomycin, and trimethoprim while it was least resistant to netilmicin (4.2%) followed by

Table 1. Description of the study participants according to sex, age, and specimen and turnaround time in Deido Health District

| Variable | Category | Frequent (%) N= 1797 |
|---|---|---|
| Sex | Male | 424(23.6) |
| | Female | 1,373(76.4) |
| | <5 | 92(5.1) |
| | 5-10 | 88(4.9) |
| | 11-19 | 126 (7.0) |
| | 20-40 | 1199 (66.7) |
| | 41-60 | 205(11.4) |
| | >60 | 87(4.8) |
| | Mean | 29.84 (SD,14.9, ) |
| | Range | 94 |
| Specimen | US | 55(3.1) |
| | VS | 642(35.7) |
| | Urine | 731(40.7) |
| | Stool | 301(16.8) |
| | Semen | 11(0.6) |
| | Wound | 20(1.1) |
| | CSF | 2(0.1) |
| | Vulva | 24(1.3) |
| | Others body fluid | 11(0.6) |
| Duration of Culture results | 3days | 1164(64.7) |
| | 4-5day | 617(34.3) |
| | >5days | 16(0.9) |
| | Mean | 3.54 (SD 0.8) |
| | Range | 3- 7 |

*US: Urethra smear, VS: Vagina Smear, CSF: Cerebrospinal Fluid, SD: standard Deviation*

Table 2. Distribution of Clinical specimen and isolated pathogens in the Deido Health District

| Isolate | Frequency (%) N = 437 | Specimen |
|---|---|---|
| *Staphylococcus aureus* | 26(5.9) | US (8), VS (5), Urine (9), Semen (1), Wound (3) |
| *Enterococcus spp.* | 10(2.3) | Urine (10) |
| *Enterobacter spp* | 11(2.5) | Vulva (1), wound (2) Urine (4) VS (4), |
| *Neisseria gonorrhoeae* | 1(.2) | US (1) |
| *Escherichia coli* | 80(18.3) | VS (17), wound (2) Urine (58) Stool (1), Vulva (1), |
| *Klebsiella spp.* | 22(5.0) | VS (8), Urine (14) |
| *Salmonella spp.* | 2(.5) | Stool (2) |
| *Pseudomonas aeruginosa* | 8(1.8) | Urine (2), Wound (6) |
| *Ureaplasma urealyticum* | 16(3.7) | VS (13), US (2) Semen (1) |
| *Mycoplasmas hominis* | 24(5.5) | VS (4), US (19) Urine (1) |
| *Serratia liquefaciens* | 12(2.7) | VS (1), wound (1) Urine (9), Other body fluid (1), |
| *Citrobacter* | 11(2.5) | VS (6), Urine (5) |
| *Stretococcus* | 12(2.7) | VS (1), US (1) Semen (1) Urine (1) |
| CoNS | 24(5.5) | VS (19), US (1) Semen (1) Urine (3) |
| *Candida albicans* | 178(40.7) | VS (97), Stool (79), Vulva (2), |
| Total | 437(100.0) | VS (198), US (17), Urine (116), Stool (81), Semen (5), Wound (12), Valve (5) other body fluid (1) |

Table 3. Overall activity of antimicrobial to the isolates in the study Deido Health District

| Antimicrobial Class | Antimicrobial | Resistance (95%CI) | Class Median Resistance (IQR) |
|---|---|---|---|
| Cephalosporins | Cefuroxime | 50.0 (23.0 – 77.0) | 51(40-53) |
| | Cefotaxime | 50.6 (39.1 – 62.1) | |
| | Ceftazidime | 50.8 (37.5 – 64.1) | |
| | Ceftriaxone | 30.1 (20.5 - 41.2) | |
| | Cefixime | 54.7(41.7 – 67.2) | |
| Penicillins | Amoxicillin | 37.5(24.9 – 51.5) | 49(39- 68) |
| | Ampicllin | 65.7(55.6 – 74.8) | |
| | Piperacillin | 40.5(29.6 – 52.1) | |
| | Cloxacillin | 48.6(36.4 – 60.8) | |
| | Amoxiclav | 71.0 (61.5 – 79.4) | |
| Beta-lactam | Aztreonam | 51.4 (34.0 – 68.6) | 62.2 |
| | oxacillin | 73.0 (60.3 – 83.4) | |
| Quinolones | Ciprofloxacin | 37.7(26.3 – 50.2) | 39(36.8- 49.3) |
| | Norfloxacin | 39.2 (25.8 – 53.9) | |
| | Ofloxacin | 55.9(45.2 – 66.2) | |
| | Nalidixic acid | 42.9 (24.5 – 62.8) | |
| | Levofloxacin | 32.0(19 – 46.7) | |
| | Perfloxacin | 33.3 (23.2 – 44.7) | |
| Macrolides | Josamycin | 31.6 (12.6 – 56.6) | 42(33 - 52) |
| | Erythromycin | 33.9 (22.1 – 47.4) | |
| | Clarithromycin | 42.2 (29.9 – 55.2) | |
| | Roxithromycin | 57.9(33.5 – 79.7) | |
| | Azithromycin | 62.1 (42.3 – 79.3) | |
| Aminoglycosides | Gentamicin | 17.6 (10.4 – 27.0) | 12.8(ð) |
| | Amikacin | 6.5 (0.8 – 21.4) | |
| | Netilmicin | 12.8 (6.8 – 21.2) | |
| Glycopeptide | Vacomycin | 48.4 (30.2 – 66.9) | - |
| Tetracyclines | Tetracycline | 31.3(11.0 – 58.7) | 27.1 |
| | Doxycycline | 27.1 (15.3 – 41.8) | |
| | Monocycline | 38.5 (23.4 55.4) | |
| Antifolate | Trimethoprim | 75.0 (53.3 – 90.2) | - |
| Nitrofurans | Nitrofurantoin | 49.3 (36.8 – 1.8) | - |
| Antimycobacterial | Rifampicin | 59.4 (46.4 - -71.5) | - |
| | Chloramphenicol | 23.8 (8.2 – 47.2) | - |
| Antifungals | | | |
| Azoles | Fluconazole | 56.4 (46.2 – 66.3) | 31(16 - 62) |
| | Itraconazole | 9.5 (1.2 – 30.4) | |
| | Econazol | 21.5 (15.4 – 28.8) | |
| | Ketoconazole | 55.8 (47.6 – 63.7) | |
| | Miconazole | 10.4(6.2 – 16.1) | |
| | Clotrimazole | 41.0(30.0- 52.7) | |
| Polyenes | Nystatin | 68.7 (57.6 – 78.4) | 79.2 (ð) |
| | Amphotericin B | 89.6 (81.7 – 94.9) | |
| Allyl amines | Terbinafine | 50.0 (29.1 – 70.9) | - |
| Others | Grisefulvin | 31.3 (`6.1 – 50.0) | - |

Table 4. Overall isolates resistance to the various antimicrobials they were tested against in Deido Health District

| Isolate | Median resistance (IQR) |
|---|---|
| *Staphylococcus aureus* | 41.5 (12.8 – 80.0) |
| CoNS | 50 (11.0 – 73.0) |
| *Enterococcus spp.* | 29(0.0 – 57.5) |
| *Streptococcus spp.* | 61.0( 2.75 – 82.3) |
| *Escherichia coli* | 43.5(29 – 54.8) |
| *Klebsiella spp.* | 33.0 (0.0 – 60.0) |
| *Pseudomonas aeruginosa* | 71.0(45.6 – 89.5) |
| *Serratia liquefaciens* | 50.0(14.0 - 100) |
| *Citrobacter* | 17.0(0.0 – 61.8) |
| *Urealpasma urealyticum* | 33.0(0.0 – 51.0) |
| *Mycoplasma hominis* | 40.0(20.3 – 59.0) |
| Candida albicans | 43.50(19.75 – 70.0) |

Table 5. Antimicrobial resistance among gram-positive bacteria in Deido Health District

| Antimicrobial Class | Antimicrobial | Isolated bacteria Resistance (%) | | | | P Value |
|---|---|---|---|---|---|---|
| | | *Staphylococcus aureus* | CoNS | *Enterococcus spp.* | *Streptococcus spp* | |
| Cephalosporins | Cefuroxime | 0 | 0 | - | | |
| | Cefotaxime | 18.2 | 66.7 | - | | 0.227 |
| | Ceftazidime | - | - | 50 | | |
| | Ceftriaxone | 75 | 33.3 | 33.3 | | 0.510 |
| | Cefixime | 100 | - | 100 | - | - |
| Penicillin | Amoxicillin | 33.3 | | 0 | 11.1 | 0.411 |
| | Ampicllin | 50.0 | 60.0 | 50.0 | 100 | 0.895 |
| | Piperacillin | 21.4 | 0 | 40 | 0 | 0.234 |
| | Cloxacillin | 9.1 | | 50.0 | 66.7 | 0.131 |
| | Amoxicillin clavulanic acid | 50.0 | 33.3 | 60 | 60 | 0.125 |
| Beta-lactam | Aztreonam | 80 | | 66.7 | | 0.315 |
| | oxacillin | 100 | 68.8 | 100 | 62.5 | 0.418 |
| Quinolones | Ciprofloxacin | 0 | 75 | 0 | 100 | 0.282 |
| | Norfloxacin | 100 | 0 | 0 | | |
| | Ofloxacin | 80.0 | 87.5 | 0 | 83.3 | 0.071 |
| | Nalidixic acid | 100 | 73.3 | 100 | 100 | |
| | Levofloxacin | - | 0 | - | - | - |
| | Perfloxacin | 11.1 | 50 | 0 | 28.6 | 0.063 |
| Macrolides | Erythromycin | 55.6 | 35.3 | - | 50.0 | 0.351 |
| | Clarithromycin | 33.3 | 60.0 | 0 | 80 | 0.044 |
| | Azithromycin | 100 | 80.0 | 0 | 100 | 0.145 |
| Aminoglycosides | Gentamicin | 20.0 | 50 | 0 | 66.7 | 0.199 |
| | Amikacin | 50.0 | 50.0 | 0 | - | 0.287 |
| | Netilmicin | 22.2 | 11.1 | 33.3 | 14.3 | 0.741 |
| Glycopeptide | Vacomycin | 25 | - | - | - | - |
| Tetracyclines | Tetracycline | 10.0 | 100 | 0 | 0 | 0.348 |
| | Doxycycline | 0 | 0 | 100 | 0 | 0.122 |
| | Monocycline | | | | | |
| Antifolate | Trimethoprim | 0 | 35.7 | - | 0 | 0.298 |
| Nitrofurans | Nitrofurantoin | 57.1 | 73.3 | 0.0 | 62.5 | 0.133 |
| Antimycobacterial | Rifampicin | 91.7 | - | 25.0 | 0.0 | 0.00001 |

ceftriaxone and perfloxacin 11.4% and 11.8% respectively. *Klebsiella spp* was less resistant to netilmicin (5.9%) and pefloxacin (16.7%) while it showed resistance above 70% to cefuroxime, cefotaxime, amoxicillin-clavulanic acid, oxacillin and rifampicin.

*Pseudomonas aeruginosa* showed high resistance of above 70% to cefuroxime, cefotaxime, cefixime, piperacillin, cloxacillin, amoxicillin-clavulanic acid, oxacillin, ofloxacin, azithromycin, antifolate and chloramphenicol, whereas, its lowest resistance wastto ceftriaxone (20.0%) while it showed no resistance to ampicillin, pefloxacin and amikacin (Table 6).

*Serratia liquefaciens* resistance to ceftazidime, amoxicillin-clavulanic acid, oxacillin, ofloxacin, nalidixic acid, perfloxacin, trimethoprim, and rifampicin was above 70% while it was not resistant to aztreonam, levofloxacin, gentamicin, amikacin, and nitrofurantoin. *Citrobacter* showed 100% resistance to trimethoprim, oxacillin, nalidixic acid, and perfloxacin. (Table 6).

*Mycoplasma Resistance to Antimicrobial Drug*

For the resistance of *Ureaplasma urealyticum* and *Mycoplasma hominis* to the various antimicrobials, no statistical significance difference was noted. *Ureaplasma urealyticum* showed no resistance to oxacillin, perfloxacin, erythromycin, vancomycin, and nitrofurantoin while *Mycoplasma hominis* showed no resistance to only ciprofloxacin. *Ureaplasma urealyticum* highest resistance was to Clarithromycin (63.6%) while *Mycoplasma hominis* showed 100% resistance to trimethoprim (Table 7).

Among the antifungal agents, the highest resistance of *Candida albicans* was observed against Amphotericin B (88.8%). Fluconazole, ketoconazole, and nystatin showed resistance above 50%. *Candida albicans* showed the least resistance to Miconazole (9.8%) (Fig 1)

**Discussion**

This study was designed to provide insight on the profile of AMR resistance and the capacity of antimicrobial susceptibility testing laboratories in the Deido Health District. It is worth noting that, the laboratories involved in this study are located within an urban setting were more the 60% of the Cameroon health workforce is concentrated. It has been proven that Antimicrobial resistance is a great threat in treating infectious diseases and it is increasing the cost of medical care [22,23] However, the lack of information on the situation has been a setback in steaming the fight against this threat. Therefore, understanding the true picture of AMR is very critical and important in a country like Cameroon and other developing countries where there are weak or no systematic guidelines for antibiotic usage.

Quality laboratory diagnosis is paramount for containing and enhancing the appropriate usage of antimicrobials [24,25]. Some of the gaps identify in the laboratory testing included insufficient standard operating procedures, inadequate records on personnel training and competency assessment, lack of safety equipment such as biosafety cabinet, stock out, non-participation in external quality assurance program and audits were not regularly performed. These gaps epitomize the fact that little attention has been put in place to support testing. Funding to support the fight against AMR is insufficient, especially in developing countries. Previous publications have attributed the scarcity information on AMR in the Central Africa Region to lack of established national or regional AMR surveillance systems, inadequate laboratory capacity, insufficient resources, weak infrastructures and insufficient standard operating procedures [3,4].

This study showed that it took at least 3days for a culture result to be released. This can be justified by the fact that the laboratories only use conventional identification and AST techniques. The automated methods and point of care kit that provide faster results are not in use in these laboratories.

The findings in this study indicate that there is a need for more commitment to improving laboratory capacities of hospitals in Cameroon. There have been limited commitment by the Government on health care in Cameroon over the past years with only 4.7% of the national GDP currently allocated to health care with just about 8% of this budget allocated to improving health infrastructure and laboratory capacity [26].

In this study, a total of 1797 samples were received by the laboratories for culture from the three hospitals within the study period. This number of cultures done within 5months seems small because

Table 6. Antimicrobial resistance among isolated gram-negative bacteria in Deido Health District

| Antimicrobial Class | Antimicrobial | Isolated bacteria Resistance (%) | | | | | P-Value |
|---|---|---|---|---|---|---|---|
| | | *Escherichia coli* | *Klebsiella spp.* | *Pseudomonas aeruginosa* | *Serratia liquefaciens* | *Citrobacter* | |
| Cephalosporins | Cefuroxime | 42.9 | 100 | 100 | - | - | 0.582 |
| | Cefotaxime | 50.0 | 71.4 | 85.7 | 40.0 | 25.0 | 0.158 |
| | Ceftazidime | 48.7 | 0 | 50.0 | 80.0 | 50.0 | 0.277 |
| | Ceftriaxone | 11.4 | 57.1 | 20.0 | 33.3 | 0 | 0.025 |
| | Cefixime | 43.9 | 50.0 | 85.7 | 50.0 | 66.7 | 0.420 |
| Penicillins | Amoxicillin | 33.3 | 60.0 | - | 66.7 | 60.0 | 0.796 |
| | Ampicllin | 74.5 | 25.0 | 0.0 | 14.3 | 16.7 | 0.011 |
| | Piperacillin | 40.0 | 62.5 | 80.0 | 40.0 | 16.7 | 0.111 |
| | Cloxacillin | 55.0 | 45.5 | 100 | 66.7 | 42.9 | 0.977 |
| | Amoxiclav | 81.2 | 100 | 83.3 | 88.9 | 50.0 | 0.705 |
| Beta-lactam | Aztreonam | 53.8 | 50.0 | 66.7 | 0 | 0 | 0.192 |
| | oxacillin | 100 | 100 | 100 | 100 | 100 | |
| Quinolones | Ciprofloxacin | 38.7 | 0 | 33.3 | 20.0 | 0 | 0.183 |
| | Norfloxacin | 28.6 | 25.0 | 50.0 | 60.0 | 42.9 | 0.846 |
| | Ofloxacin | 48.4 | 50.0 | 100 | 100 | 0 | 0.462 |
| | Nalidixic acid | 71.4 | 0 | - | 100 | 100 | 0.362 |
| | Levofloxacin | 40.0 | 0.0 | 60.0 | 0.0 | 0.0 | 0.249 |
| | Perfloxacin | 11.8 | 16.7 | 0 | 100 | 100 | 0.111 |
| Macrolides | Erythromycin | 50.0 | 0.0 | - | - | 0.0 | 0.415 |
| | Clarithromycin | 0 | 0 | - | - | 0 | - |
| | Azithromycin | - | - | 100 | - | - | |
| Aminoglycosides | Gentamicin | 10.9 | 33.3 | 50.0 | 0 | 0 | 0.052 |
| | Amikacin | 0 | 0 | 0 | 0 | - | 0.755 |
| | Netilmicin | 4.2 | 5.9 | 50.0 | 25.0 | 0 | 0.200 |
| Glycopeptide | Vacomycin | 100 | 40.0 | - | - | - | 0.090 |
| Tetracyclines | Tetracycline | | | | | | - |
| | Doxycycline | 40.0 | 0.0 | - | - | 66.7 | 0.090 |
| Antifolate | Trimethoprim | 79.6 | - | 80.0 | 100 | 100 | 0.853 |
| Nitrofurans | Nitrofurantoin | 28.6 | 0.0 | - | 0.0 | 0.0 | 0.094 |
| Antimycobacterial | Rifampicin | 54.2 | 71.4 | - | 100 | 0.0 | 0.016 |
| | Chloramphenicol | - | - | 75.0 | - | 0.0 | 0.171 |

Table 7. Mycoplasma resistance to the antimicrobial drug in Deido Health District.

| Antimicrobial Class | Antimicrobial | Isolated bacteria Resistance (%) | | P Value |
|---|---|---|---|---|
| | | *Urealpasma urealyticum* | *Mycoplasma hominis* | |
| Beta-lactam | Aztreonam | | | |
| | oxacillin | 0.0 | 56.2 | 0.471 |
| Quinolones | Ciprofloxacin | 50.0 | 0.0 | 0.264 |
| | Ofloxacin | 33.3 | 40.0 | 0.554 |
| | Levofloxacin | 28.6 | 40.0 | 0.647 |
| | Perfloxacin | 0.0 | 63.2 | 0.376 |
| Macrolides | Josamycin | 35.7 | 20.0 | 0.516 |
| | Erythromycin | 0.0 | 21.1 | 0.798 |
| | Clarithromycin | 63.6 | 27.3 | 0.128 |
| | Roxithromycin | 57.1 | 60.0 | 0.912 |
| | Azithromycin | 53.8 | 60.0 | 0.895 |
| Glycopeptide | Vacomycin | 0.0 | 47.1 | 0.516 |
| | Doxycycline | 33.3 | 20.0 | 0.675 |
| | Monocycline | 37.5 | 39.1 | 0.499 |
| Antifolate | Trimethoprim | - | 100 | |
| Nitrofurans | Nitrofurantoin | 0.0 | 52.6 | 0.376 |

these health facilities are secondary level health facilities in the most populated town in Cameroon They can be partially accounted for by the fact that national and local treatment guidelines in many resources limited countries still emphasize on empirical treatment as reported by a study in Tanzania with similar findings [27]. However, ensuring adherence to antimicrobial therapy guidelines formulated using evidence-based generated data will go a long way to reducing the burden of antimicrobial resistance. Urine specimens contributed to over 40% of the specimen cultured and this could be attributed to a previously reported high prevalence of urinary tract infections of over 54% in some parts of Cameroon [28]

The most frequently isolated pathogens were *Candida albicans* (40%) predominantly from vagina smear. This was followed by *E. coli* isolated from urine culture. These findings could be explained by the fact that most of the samples were from women with a majority within the reproductive age. Candida vaginitis has also been reported to be common among women within the reproductive age [29]. With regards to the resistance pattern to antimicrobials, the highest resistance of Candida albicans was noted with Amphotericin B while miconazole had the least resistance. Even though Amphotericin B resistance is unexpectedly high and contrary to many other studies, the susceptibility of *Candida albicans* to miconazole has been widely reported despite the rising trend of resistance to all Azoles [30,31].

Among the different pathogens isolated, *Pseudomonas aeuroginosa* demonstrated the highest resistance to most antimicrobials, followed by streptococcus spp and *Escherichia coli*. This was similar to previous reports [28,32] as these infections are frequently treated with cephalosporins and penicillins that also demonstrated the highest level of resistance. Cephalosporins and penicillins are the most common antimicrobials sold over the counter in most pharmacies as well as by roadside vendors without any formal quality control [33]. For the Mycoplasmas, both *Ureaplasma urealyticum* and *Mycoplasma hominis* demonstrated increasing resistance to Clarithromycin and Trimethoprim. However, this is explained by the fact that the biological characteristic of Mycoplasmas have been reported to result in the ineffectiveness of several Other substances (sulfonamides, trimethoprim, rifampin, polymyxin, nalidixic acid, linezolid, and some others) [34]. Furthermore, despite fluoroquinolones, and macrolides being considered the most effective anti-mycoplasma agents, there have also been recent reports of treatment failure, rising resistance rates due to repeated mutations [29].

On the other hand, it was also found that, among the gram-positive bacteria isolates, *Staphylococcus aureus* showed the highest resistance to the cephalosporins and Beta-lactam and quinolones antimicrobials included in the study. Methicillin-resistant *Staphylococcus aureus* has also been among the frequently reported gram-positive isolate with resistance to common antimicrobials and has been designated a public health threat [10]. The high resistance rate could be because gram-positive pathogens generally exhibit an immense genetic repertoire to adapt and develop resistance to virtually all antimicrobials clinically available. Furthermore, Methicillin-resistant *Staphylococcus aureus* has been reported to be resulting from nosocomial infections exposed to a variety of antimicrobials [35].

## Conclusion

The antimicrobial resistance situation in the Deido Health District is preoccupying as is the case with other developing countries. Apart from aminoglycosides, pathogens showed an antibiotic class median resistance over 25% of the various antimicrobial agents. Despite the importance of testing in the fight against AMR, the laboratory turnaround time remains long and the laboratories are under-equipped and the quality of AST is seriously affected.

This study has shown there is a need to develop a coordinated national approach by Cameroon's ministry of public health to fight AMR with much priority on antimicrobial susceptibility testing. Scaling-up AST testing will, therefore, require strengthening the microbiology units of laboratory systems and ensuring the use of laboratory data for clinical decision-making.

## Declarations

## Acknowledgments

The authors express sincere gratitude to the Faculty of Science University of Buea, Cameroon, the directors and laboratory staff of the hospitals involved,

all the study participants and the littoral Regional Delegation of Public Health

**Funding Sources**

This study was funded by the corresponding author and also received support from the faculty of Science University of Buea, Cameroon. However, the results and conclusions made in this publication are made by the authors and may not represent the official position of the faculty of Science University of Buea, Cameroon

**Abbreviations**

AMR: antimicrobial resistance

AST: antimicrobial susceptibility testing

CLSI: Clinical and Laboratory Standard Institute

CSF: Cerebrospinal fluid

EUCAST: European Committee on Antimicrobial Susceptibility Testing

IRB: Institutional review board

SLIPTA: Stepwise Laboratory Quality Improvement Process towards Accreditation

US: Urethral Smear

VS: Vagina Smear

WHO: World Health Organization

**Availability of Data and Materials**

All relevant data are included in this manuscript

**Ethics Approval**

This study protocol was reviewed and approved by the Faculty of Health Sciences Institutional Review Board (IRB) of the University of Buea, Cameroon (N0: 2019/941-01/UB.SG.IRB.FHS).

**Competing Interests**

The authors declare that they have no competing interests.

**Consent for Publication**

Not applicable

**Author's Contributions**

PAN conceived, designed and supervised the study implementation, CN conceived, designed, coordinated the study, analyzed the data and drafted the paper, ETA designed, coordinated the study and participated in drafted the paper, JKTA reviewed and corrected the study proposal and the final manuscript write up and DZ contributed in developing the manuscript. All authors read and approved the final manuscript

**References**

1. WHO. WHO | Antimicrobial resistance: global report on surveillance 2014 [Internet]. Antimicrobial resistance: global report on surveillance 2014. 2016. Available from: http://www.who.int/antimicrobial-resistance/publications/surveillancereport/en/%0Ahttp://www.who.int/drugresistance/documents/surveillancereport/en/
2. Hillier S, Roberts Z, Dunstan F, Butler C, Howard A, Palmer S. Prior antibiotics and risk of antibiotic-resistant community-acquired urinary tract infection: A case-control study. Vol. 60, Journal of Antimicrobial Chemotherapy. 2007. p. 92–9.
3. Africa Centre for Disease Control. Africa CDC Framework for Antimicrobial Resistance, 2018-2023. Africa Centre for Disease Control. 2018. p. 1–24.
4. Achiangia Njukeng P, Ebot Ako-Arrey, D, Tajoache Amin E, Njumkeng C, Sevidzem Wirsiy F. Antimicrobial Resistance in the Central African Region: A Review. Vol. 03, Journal of Environmental Science and Public Health. 2019.
5. WHO. Antimicrobial resistance in SEARO [Internet]. 2014. Available from: https://www.who.int/southeastasia/health-topics/antimicrobial-resistance
6. World Health Organization (WHO). Global Antimicrobial Resistance Surveillance System. Who. 2015;36.
7. Tsutsui A, Suzuki S. Japan nosocomial infections surveillance (JANIS): A model of sustainable national antimicrobial resistance surveillance based on hospital diagnostic microbiology laboratories. Vol. 18, BMC Health Services Research. 2018.
8. WHO. WHO | Global action plan on AMR [Internet]. Who. 2016. Available from: http://www.who.int/antimicrobial-resistance/global-action-plan/en/
9. Jorak A, Keihanian F, Saeidinia A, Heidarzadeh A, Saeidinia F. A cross sectional study on knowledge, Attitude and practice of medical students toward antibiotic resistance and its prescription, Iran. Vol. 8,

Advances in Environmental Biology. 2014. p. 675–81.

10. CDC. Biggest Threats and Data | Antibiotic/ Antimicrobial Resistance | CDC [Internet]. 2019. 2019. p. 1. Available from: https://www.cdc.gov/drugresistance/biggest-threats.html%0Ahttps://www.cdc.gov/drugresistance/biggest_threats.html
11. Wangai FK, Masika MM, Lule GN, Karari EM, Maritim MC, Jaoko WG, et al. Bridging antimicrobial resistance knowledge gaps: The East African perspective on a global problem. Vol. 14, PLoS ONE. 2019.
12. Tadesse BT, Ashley EA, Ongarello S, Havumaki J, Wijegoonewardena M, González IJ, et al. Antimicrobial resistance in Africa: A systematic review. BMC Infect Dis. 2017;17(1):1–17.
13. O 'neill J. Tackling Drug-Resistant Infections Globally: an Overview of Our Work the Review on Antimicrobial Resistance. 2016;(March).
14. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Sponcer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: A microbiological and molecular biological study. Vol. 16, The Lancet Infectious Diseases. 2016. p. 161–8.
15. Xavier BB, Lammens C, Ruhal R, Malhotra-Kumar S, Butaye P, Goossens H, et al. Identification of a novel plasmid-mediated colistinresistance gene, mcr-2, in Escherichia coli, Belgium, june 2016. Vol. 21, Eurosurveillance. 2016.
16. Cookson BD. Nosocomial antimicrobial resistance surveillance. J Hosp Infect. 1999;43(SUPPL. 1).
17. E.D. C, D.N. A, J.-F.K.T. A. Prescribing patterns and associated factors of antibiotic prescription in primary health care facilities of Kumbo East and Kumbo West Health Districts, North West Cameroon [Internet]. Vol. 13, PLoS ONE. 2018. p. 196861. Available from: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L620962927%0Ahttp://dx.doi.org/10.1371/journal.pone.0193353
18. Malik B, Bhattacharyya S. Antibiotic drug-resistance as a complex system driven by socio-economic growth and antibiotic misuse. Vol. 9, Scientific Reports. 2019.
19. Clinical F, Laboratories PH. S tepwise L aboratory Quality I mprovement P rocess T owards A ccreditation ( SLIPTA ) Checklist For Clinical and Public Health Laboratories. 2015;(September 2008).
20. Brown DFJ, Wootton M, Howe RA. Antimicrobial susceptibility testing breakpoints and methods from BSAC to EUCAST. Vol. 71, Journal of Antimicrobial Chemotherapy. 2016. p. 3–5.
21. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests: Approved standard - Eleventh edition. Vol. 32. 2012. 1–58 p.
22. Laxminarayan R, Chaudhury RR. Antibiotic Resistance in India: Drivers and Opportunities for Action. Vol. 13, PLoS Medicine. 2016.
23. Dadgostar P. Antimicrobial resistance: implications and costs. Vol. 12, Infection and Drug Resistance. 2019. p. 3903–10.
24. Okeke IN, Peeling RW, Goossens H, Auckenthaler R, Olmsted SS, De Lavison JF, et al. Diagnostics as essential tools for containing antibacterial resistance. Vol. 14, Drug Resistance Updates. 2011. p. 95–106.
25. Mendelson M, Røttingen JA, Gopinathan U, Hamer DH, Wertheim H, Basnyat B, et al. Maximising access to achieve appropriate human antimicrobial use in low-income and middle-income countries. Vol. 387, The Lancet. 2016. p. 188–98.
26. Nkoutchou H, Arrey WH. Report on Cameroon's 2019 budget. 2019.
27. Antimicrobial resistance pattern: a report of microbiological cultures at a tertiary hospital in Tanzania | BMC Infectious Diseases | Full Text [Internet]. Available from: https://bmcinfectdis.biomedcentral.com/articles/10.1186/s12879-016-2082-1
28. Akoachere JFTK, Yvonne S, Akum NH, Seraphine EN. Etiologic profile and antimicrobial susceptibility of community-acquired urinary tract infection in two Cameroonian towns. Vol. 5, BMC Research Notes. 2012.
29. Muller EE, Mahlangu MP, Lewis DA, Kularatne RS. Macrolide and fluoroquinolone resistance-associated mutations in Mycoplasma genitalium in Johannes-

burg, South Africa, 2007-2014. Vol. 19, BMC Infectious Diseases. 2019.

30. Ghaddar N, Anastasiadis E, Halimeh R, Ghaddar A, Dhar R, Alfouzan W, et al. Prevalence and antifungal susceptibility of Candida albicans causing vaginal discharge among pregnant women in Lebanon. Vol. 20, BMC Infectious Diseases. 2020.
31. Zaidi KU, Mani A, Thawani V, Mehra A. Total Protein Profile and Drug Resistance in Candida albicans Isolated from Clinical Samples . Vol. 2016, Molecular Biology International. 2016. p. 1–6.
32. Richard P, Flochl R Le, Chamoux C, Pannier M, Espaze E, Richt H. Pseudomonas aeruginosa outbreak in a burn unit: Role of antimicrobials in the mergence of multiply resistant strains. Vol. 170, Journal of Infectious Diseases. 1994. p. 377–83.
33. Ekambi GAE, Ebongue CO, Penda C, Nga EN, Mpondo EM, Moukokoid CEE. Knowledge, practices and attitudes on antibiotics use in Cameroon: Self-medication and prescription survey among children, adolescents and adults in private pharmacies. Vol. 14, PLoS ONE. 2019.
34. Chernova OA, Medvedeva ES, Mouzykantov AA, Baranova NB, Chernov VM. Mycoplasmas and their antibiotic resistance: The problems and prospects in controlling infections. Vol. 8, Acta Naturae. 2016. p. 24–34.
35. Rice LB. Antimicrobial resistance in gram-positive bacteria. Am J Med. 2006 Jun;119(6 Suppl 1):S11-9; discussion S62-70.