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Research Article

# Prevalence of Extended Spectrum Beta Lactamase (ESBL) Producing *Escherichia coli* and *Klebsiella* Species from Buea Regional Hospital Surfaces and Their Susceptibility Pattern to Antimicrobials in Cameroon

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## Abstract

*Escherichia coli* and *Klebsiella* species are pathogens of significant public health interest to which new antibiotics therapies are urgently needed, where they have extended Spectrum of Beta-Lactamase (ESBL) activity. This study was aimed to determine the prevalence of ESBL-production among the environmental isolates of *E. coli* and *Klebsiella* species and their susceptibility to seven antimicrobials at the Buea Regional Hospital (BRH). Samples from surfaces of doors, toilet and hospital beds and tables were collected by swabbing, using sterile hydrophilic cotton swabs and inoculated on EMB. Environmental isolates were tested for ESBL production by using the double disk synergy test by Kirby-Bauer disc diffusion method on Muller-Hinton agar. Susceptibility to antibiotics was performed using the disc diffusion method according to the Clinical Laboratory Standards Institute guidelines. ESBL was observed in 2 isolates giving an overall prevalence of 12.5% (2/16), these two ESBL isolates were *Klebsiella* species. For susceptibility profile, cefepime and cefixime were 100% active against ESBL producing strains. Four *Klebsiella* (33.3%) isolates showed resistance to all the antibiotics tested. The study indicates low prevalence of ESBL producing *Klebsiella* species and *E. coli* from the environment in BRH. However, it gives insight to a foreseeable problem giving the increasing incidence of antibiotics abuse.

**Keywords:** Antimicrobials; Bet-lactamase; Buea; Cameroon; Hospital Surfaces; *Klebsiella*

## Introduction

### Background of the study

Antimicrobial resistance is a serious problem of public health concern and it is a major factor contributing to mortality in individuals especially in immunocompromised persons [1]. Gram- negative bacteria in the family of Enterobacteriaceae produce Extended-spectrum Beta-lactamases (ESBLs) which are a type of Beta Lactamase Enzymes. The production of these enzymes is a common mechanism of bacterial resistance to antibiotics and the ESBL enzymes give the bacteria ability to resist penicillin and cephalosporins of the first, second and third generations as well as aztreonam through hydrolysis of these antibiotics [2]. These enzymes are many and are plasmid mediated and capable of hydrolyzing and inactivating a wide variety of beta-lactam antibiotics [3].

Enterobacteriaceae is a family of bacteria with significant causes of serious infection and many of the bacteria in this family are becoming increasingly resistant to currently available antimicrobials [2].

*Escherichia coli* and *Klebsiella pneumoniae*, are opportunistic pathogens of humans and animals responsible for a wide range of infections such as urinary tract infections, Pneumoniae, wound infections and septicemia [4].

Beta-lactamase producing Enterobacteriaceae has become a global problem. The rate of incidence and type of beta-lactamase enzyme varies in different geographical locations worldwide [5]. Sherchan., *et al*. [2], has reported that extended-spectrum-beta-lactamase (ESBL) producing *E. coli* strains, particularly strains producing CTX-M-type ESBLs, have emerged worldwide and this is predominantly *E. coli* with O antigen type 25 (O25) and sequence type 131 (ST131) is often associated with the CTX-M-15 ESBL.

The spread of microorganisms expressing extended-spectrum beta-lactamase (ESBL) genes was limited to those circulating in hospitals, with most isolates being *Klebsiella pneumoniae*. From the year 2000 however, there has been a sharp increase in the prevalence of ESBL-producing microorganisms worldwide whereby *Escherichia coli* has replaced *K. pneumoniae* as the major carrier of ESBL encoding genes [7].

Enterobacteriaceae are naturally present in the intestinal tract of humans and animal they get to the environment through infected stool or urine samples. In the hospital environment, they can contaminate medical apparatus and devices such as catheters, they can also colonize patients with a prolonged hospitalization, who can become symptomatic or asymptomatic carriers [8].

The increasing emergence and spread of pathogenic gram-negative bacteria, in hospitals is of great concern and continues to challenge infection prevention and hospital epidemiology practice [9]. Spread of gram-negative resistant bacteria from the hospital to the environment by direct person-to-person contact and surfaces is a call for concern. These resistance bacteria have found a permissive niche in the modern hospital environment where a high density of susceptible patients, intense selection pressure for antibiotic resistance and manifold opportunities for transmission intersect [10].

Extended Spectrum Beta-lactamase(β-lactamase) producing enterobacteria have spread across the world but some health authorities are not aware of this problem especially in African countries [11]. Previous studies on the prevalence of ESBL producing enterobactericeae have been carried out in Cameroon on human faecal samples given a prevalence of 54.06% [12]. The prevalence of ESBLs-producing *E. coli* and *Klebsiella species* from the hospital environment is unknown in this region; therefore, this current study aimed at evaluating the prevalence of ESBL-producing *E. coli* and *Klebsiella species* from the environment at the Buea Regional Hospital.

## Materials and Methods

### Sample collection

All samples were collected under septic conditions and samples from surfaces were collected by swabbing using sterile hydrophilic cotton swabs from a demarcated surface area of about 100 $cm^2$. The cotton swabs were humidified using distilled water and numerous to-and-fro rubbing movements were done while rotating the swab in half-turns to ensure maximum use of its surface area after which the swabs were replaced into its protective casing. The surfaces from where samples were collected were door handles, bed sites, cupboard, sinks, tap heads and toilet seats. After collection, each sample was labeled and recorded on a form designed for this purpose. The collection form had the following items; the collection site, the date and time of collection, the name of the collector and other relevant information necessary for interpreting culture results. All samples were immediately carried to the bacteriology laboratory where they were cultured and the microorganisms identified.

### Preparation of culture media

EMB Culture media was prepared following manufacturers procedure. The manufacturers procedures outlined the preparation of 1litter (L) medium. For this study, the media was prepared in small quantities so as to avoid expiry and contamination. Calculations were made using the mass given by the manufacturer, as well as the volume and the new volume (which was to be prepared) to get the amount of culture media that will be enough with the volume of distilled water needed. The culture media was prepared and poured in Petri dishes.

### Bacteriological procedures

In the bacteriology laboratory, each cotton swab was aseptically inoculated into the culture media eosin methylene blue (EMB) and incubated at 37°C for 24 to 48 hours. After incubation, upon growth, the colony colour (green metallic sheen) was noted and gram staining was done to identify gram negative bacteria, fol-

lowed by a subculture on EMB to further isolate the colonies. Those that were gram positive were discarded and those that were gram negative, were subculture and identified using API 20 E test.

### Analytical profile index (API) 20E testing

The API 20E identification test was performed following the manufacturer's instructions. An incubation box (tray and lid) was prepared, and 5 ml of sterile distilled water was distributed into the honey combed wells of the API tray to create a humid atmosphere. The elongated flap of the tray was labelled with the sample number. The strip was then removed from its packaging and placed in the incubation box. A tube containing 5ml of sterile distilled water, labelled with the number assigned to the sample was used to prepare the bacteria suspension as follows.

Using a pipette with sterile pipette tips, a single well isolated colony from the subculture was removed and carefully emulsified in the sterile distilled water in the tube to obtain a homogenous suspension. With the same pipette, the bacteria suspension was distributed into the tubes of the strip with the strip tilted slightly forward and the tip of the pipette placed against the side of the capsule to avoid the formation of bubbles at the base of the tube. The tubes were filled with the bacteria suspension, and some overlaid with mineral oil to create anaerobiosis. The incubation box was then closed and placed in an incubator at 37°c for 18 – 24 hours. After the incubation period, the strip was read by referring to the reading table in the manufacturer's manual. All the spontaneous reactions were recorded on the result sheet, and the tests which required the addition of reagents were also revealed by adding the reagents. The spontaneous tests were; TDA test, revealed by adding a drop of TDA reagent. A reddish brown colour indicated a positive reaction while a yellow colour indicated a negative reaction which was recorded on the result sheet. Also there is the VP test which was revealed by adding one drop each of VP 1 and VP 2 reagent. After 10 minutes, a pink colour indicated a positive reaction while no colour change indicated negative which was also recorded on the result sheet. Lastly for the spontaneous tests was the indole test, revealed by adding a drop of James reagent. A pink colour developed in the whole capsule indicates a positive reaction while no colour change indicated a negative reaction, which was also recorded on the result sheet. In interpreting the results, the identification was obtained with the numerical profile.

To determine the numerical profile on the result sheet the tests were separated into groups of 3 and a value 1, 2 or 4 indicated for each. By adding the values corresponding to positive reactions within each group, a 7-digit profile number is obtained for the API 20E strip. The numerical profile was looked up for in the list of profiles, and the organism pertaining to the numerical profile was noted as the isolate.

After the API 20E kit is inoculated, several colonies from the subculture plate were added to the suspension and homogenized. It adjusted by visual comparison to 0.5 macfarlans standard for antimicrobial susceptibility testing.

It is worthy of notes that the API 20 E strip consists of twenty microtubes containing dehydrated substrates. These tests were inoculated with a bacterial suspension that reconstituted the media. During incubation; metabolism produces colour changes that were either spontaneous or revealed by the addition of reagents.

### Antimicrobial susceptibility testing

*E. coli and Klebsiella* isolates were subjected to antibiotic susceptibility testing by Kirby-Bauer disc diffusion method on Muller-Hinton agar. The turbidity of inoculums suspension was adjusted to 0.5 MacFarland's standard. Then this suspension was inoculated onto Muller-Hinton agar plate by lawn culture. After that various antibiotic discs were placed using sterile forceps and pressed gently to confirm proper contact with medium. The plates were then incubated at 37$^0$C for 24 h. The zone of inhibition was measured and interpreted as per the approved CLSI guidelines [13]. The following antibiotics were used; cefotaxime, ceftriaxone, ceftazidime, cefepime, Cefixime, Cefuroxime and Amoxiclav.

### Detection of ESBL by double disc synergy test (DDST)

The isolated organisms that were screened positive for ESBL production were used in DDST. The turbidity of inoculum suspension was adjusted to 0.5 McFarland's standard. This suspension was inoculated onto Muller-Hinton agar plate by lawn culture as recommended by CLSI. The disc containing Amoxiclav (amoxicillin + clavulanic acid) was placed at center of the plate. Ceftriaxone (CTR), cefepime, cefotaxime, Cefixime, Cefuroxime and ceftazidime (CAZ) discs were placed at aninterdisc distance of 15 mm from the combination disc. The plates were incubated at 37$^0$C for 24 h. After incubation, enhancement of zone of inhibition of the antibiotic discs towards the Amoxycillin/Clavulanic acid discs is indicative of ESBL production. The enhancement is due to inhibition of ESBL by clavulanic acid and subsequent action of the extended spectrum cephalosporins.

### Data management and analysis

Data was entered into excel and exported to SPSS version 22 (Statistical Package for the Social Sciences, Chicago, Illinois) for analysis.

### Ethical and Administrative considerations

This study was authorized by the Regional Delegation of Health and the general supervisor of the Buea Regional hospital. The research was carried out on samples collected from inanimate objects in the hospital environment.

## Results of the Findings

### Locations of sample collection at the BRH

A total of 200 samples were collected from different hospital wards with the highest percentage 55 (27.5%) being collected from the surgical ward, and the least from the blood bank 2 (1.0%) (Table 1).

**Table 1:** Distribution of Environmental Samples from different wards.

| Collection sites | Frequency | Percentage |
|---|---|---|
| Antenatal care (ANC) | 4 | 2.0 |
| Blood bank | 2 | 1.0 |
| Emergency | 24 | 12.0 |
| Female Medical Ward | 39 | 19.5 |
| Haemodialysis unit | 19 | 9.5 |
| Histopathology | 3 | 1.5 |
| Male Medical ward | 34 | 17.0 |
| Paediatric Ward | 16 | 8.0 |
| Surgical Ward | 55 | 27.5 |
| TB treatment unit | 4 | 2.0 |
| Total | 200 | 100.0 |

### Environmental surfaces sampled

A total of 200 samples were collected from six different surfaces. The highest samples were gotten from bed side 54(27%) followed by side cupboards with 51(25.5%) and the least from toilet seat 11(5.5%) (Table 2).

**Table 2:** Frequency distribution of specimens from Different environmental surfaces.

| Surfaces | Frequency | Percentage |
|---|---|---|
| Bed side | 54 | 27.0 |
| Door | 48 | 24.0 |
| Side cupboard | 51 | 25.5 |
| Tap head | 20 | 10.0 |
| Toilet seat | 11 | 5.5 |
| Wash hand basin | 16 | 8.0 |
| Total | 200 | 100.0 |

### Growth pattern on culture plates

Following incubation of EMB plates for 24 hours, most of the plates yielded scanty (1-10 colonies) growth 137(68.5%), while 13(6.5%) had profuse (>50 colonies) growth and 50(25%) had no growth (Figure 1).

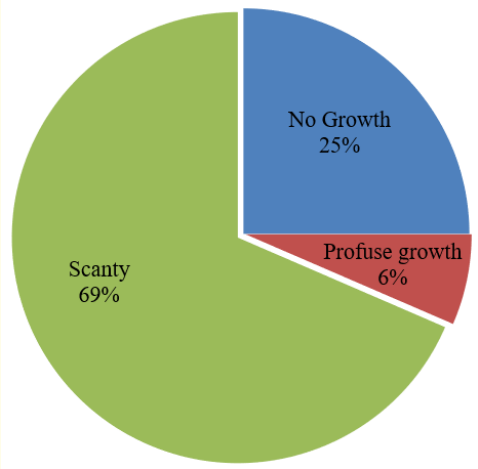


**Figure 1:** Growth pattern on EMB after 24-48 hours incubation.

### Distribution of Growth pattern by surface types

Out of the six surfaces that were swapped and inoculated, some plates yielded no growth, some had scanty growth while others had profuse growth. Door handles had the highest percentage of 44 (91.7%) that yielded no growth. Most of the tap heads 4(20.0%) had profuse growth and the highest with scanty growth was from the side cupboards 50 (98.0%). See table 3 for details.

**Table 3:** Distribution of growth on each surface.

| Surface | N | No Growth | Profuse growth | Scanty |
|---|---|---|---|---|
| Bed side | 54 | 6 (11.1) | 3 (5.6) | 45 (83.3) |
| Door handle | 48 | 44 (91.7) | 0 (0.0) | 4 (8.3) |
| Side cupboard | 51 | 0 (0.0) | 1 (2.0) | 50 (98.0) |
| Tap head | 20 | 0 (0.0) | 4 (20.0) | 16 (80.0) |
| Toilet seat | 11 | 0 (0.0) | 2 (18.2) | 9 (81.8) |
| Wash hand basin | 16 | 0 (0.0) | 3 (18.8) | 13 (81.3) |

## Colonial characterestics and Gram reaction of EMB positive cultures

Most of the positive cultures (81.3%) on EMB medium, presented as small, pink in colour, slightly raised colonies followed by (8.0%) large, pink-purple in colour, mucoid, regular margin colonies (Figure 2).

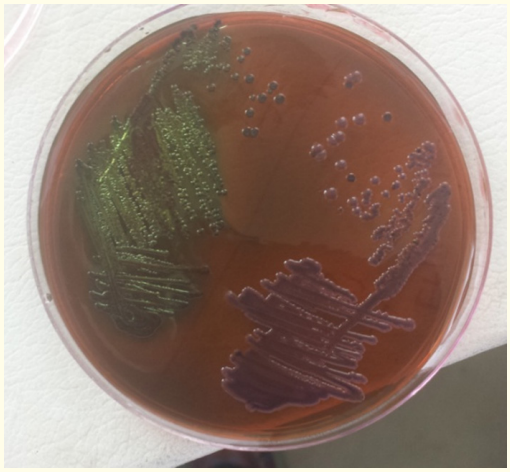

**Figure 2:** Colonies on EMB culture plate showing profuse growth.

Out of a total of 150 samples with growth, 133(88.7%) were gram positive coccimorphotype and 17(11.3%) were gram negative rods. All the isolates that had small, pink in colour, slightly raised colonies were gram positive cocci, similarly all large, pink-purple in colour, mucoid, regular margin and colonies with green metallic sheen colour were gram negative rods (Table 4).

## Biochemical characteristics of gram negative rods isolates

Of the 17 gram negative isolates that were further identified using Analytical Profile Index 20E, 12(70.6%) were *Klebsiella* species,

**Table 4:** Description of isolates on EMB culture.

| Colonial morphology | n (%) | Gram negative rod | Gram positive cocci |
|---|---|---|---|
| Large, green metallic sheen, slightly raised colonies | 5 (3.3) | 5(100) | 0 (0.0) |
| Large, pink-purple, mucoid, circular colonies | 12 (8.0) | 12 (100) | 0 (0.0) |
| Small, black, slightly raised colonies | 7 (4.7) | 0 (0.0) | 7 (100) |
| Small, pink, flat colonies | 4 (2.7) | 0 (0.0) | 4 (100) |
| Small, pink, slightly raised colonies | 122 (81.3) | 0 (0.0) | 122 (100) |
| Total | 150 (100) | 17 (11.3) | 133 (88.7) |

4(23.5%) were *E. coli* and 1(5.9%) was *Raotella planticola* (Figure 3).

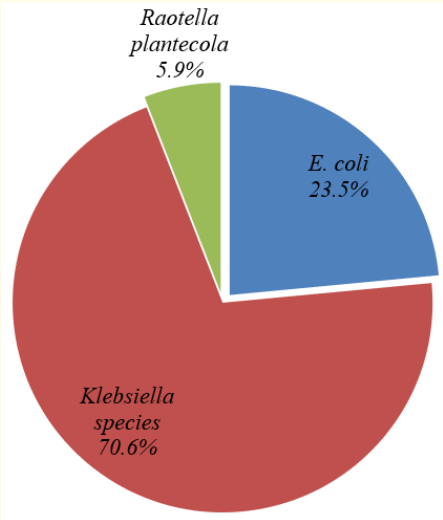


**Figure 3:** Gram negative bacteria Isolates.

## Biotypes distribution

Biotyping of *Klebsiella pneumoniae* environmental isolates using API 20E revealed 3 different biotypes as in Table 5. The most prevalent was Kp I (5205773), which occurred at a prevalence of 6(37%), followed by Kp II (5215773), with a prevalence of 4(25%). The lowest detected biotype was Kp III (5214773) which occurred at a prevalence of 2(12.5%). The differences in the biotypes are due to their reaction on URE and Voges-Proskauer (VP). Kp I shows a negative result for *Urease* while urease is positive for Kp II and Kp III. Kp III is different from Kp I and II in that Kp III is negative for Voges-Proskauer (VP), while KpI and II are positive for VP.

Also biotyping of *E. coli* environmental isolates was done using API 20E which revealed just one biotype being Ec1 (5044573). All *E. coli* samples isolated were found to be the same biotype present on different surfaces.

| Code | ONPG | ADH | LDC | ODC | CIT | H2S | URE | TDA | IND | VP | GEL | GLU | MAN | INO | SOR | RHA | SAC | MEL | AMY | ARA | | Biotype |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B9 | + | - | + | - | - | - | - | - | + | - | - | + | + | - | + | + | + | + | + | + | 5044573 | *Ec1* |
| B14 | + | - | + | - | - | - | - | - | + | - | - | + | + | - | + | + | + | + | + | + | 5044573 | *Ec1* |
| C16 | + | - | + | - | - | - | - | - | + | - | - | + | + | - | + | + | + | + | + | + | 5044573 | *Ec1* |
| Sink1 | + | - | + | - | - | - | - | - | + | - | - | + | + | - | + | + | + | + | + | + | 5044573 | *Ec1* |
| OP sink | + | - | + | - | + | - | - | - | - | + | - | + | + | + | + | + | + | + | + | + | 5205773 | *Kp I* |
| T7 | + | - | + | - | + | - | - | - | - | + | - | + | + | + | + | + | + | + | + | + | 5205773 | *Kp I* |
| MTS2 | + | - | + | - | + | - | - | - | - | + | - | + | + | + | + | + | + | + | + | + | 5205773 | *Kp I* |
| T1 | + | - | + | - | + | - | - | - | - | + | - | + | + | + | + | + | + | + | + | + | 5205773 | *Kp I* |
| T9 | + | - | + | - | + | - | - | - | - | + | - | + | + | + | + | + | + | + | + | + | 5205773 | *Kp I* |
| DTS4 | + | - | + | - | + | - | - | - | - | + | - | + | + | + | + | + | + | + | + | + | 5205773 | *Kp I* |
| Sink2 | + | - | + | - | + | - | + | - | - | + | - | + | + | + | + | + | + | + | + | + | 5215773 | *Kp II* |
| B7 | + | - | + | - | + | - | + | - | - | + | - | + | + | + | + | + | + | + | + | + | 5215773 | *Kp II* |
| B16 | + | - | + | - | + | - | + | - | - | + | - | + | + | + | + | + | + | + | + | + | 5215773 | *Kp II* |
| C7 | + | - | + | - | + | - | + | - | - | + | - | + | + | + | + | + | + | + | + | + | 5215773 | *Kp II* |
| A27B1 | + | - | + | - | + | - | + | - | - | - | - | + | + | + | + | + | + | + | + | + | 5214773 | *KpIII* |
| T15 | + | - | + | - | + | - | + | - | - | - | - | + | + | + | + | + | + | + | + | + | 5214773 | *KpIII* |
| F. Sink 2 | + | - | + | - | + | - | + | - | + | + | - | + | + | + | + | + | + | + | + | + | 5255773 | *Rp* |

**Table 5:** Biotype distribution of isolates from environmental samples.

Ec: *E. coli*, Kp: *Klepsiella pneumonia*; Rp: RaotellaplantecolaBiochemical tests: ONPG- Ortho-Nitrophenyl-β-galactoseside, ADH- arginine dihydrolase, LDC- lysine decarboxylase, ODC- ornithine decarboxylase, CIT- Citrate, H2S- Hydrogen sulfide, URE- Urease, TDA- Tryptophan deaminase, IND- Indole, VP- Voges-Proskauer, GEL- glucose, MAN- mannose, INO- inositol, SOR- sorbitol, RHA- rhamnose, SAC- sucrose, MEL- melibiose, AMY- amygdalin, ARA- arabinose.

## Drug susceptibility testing of Gram Negative Isolates

All the isolates were examined for resistance to 7 antibacterial agents including both β-lactam antibiotics and non β-lactam antibiotics (figure 4). Among the isolate highest resistance for *Klebsiella species* was observed against Augmentin10 (83.3%) followed by Ceftazidime with 8(66.7%), and the lowest resistance was observed in Cefepime and Cefixime with (33.3%) each. While the highest number of resistance for *E. coli* was seen in Augmentin 4(100%) and the lower number of isolates showed resistance against Cefepime 0(0.0%), Cefixime 1(25.0 %) and Cefotaxime 1(25.0%). Varying degrees of resistance were noticed with the various groups of antimicrobial drugs used as shown in table 6.

**Table 6:** Antibiotic susceptibility profile of the isolated *E. coli* and *Klebsiella* species.

| Antimicrobials | | *E. coli* (n = 4),n (%) | *Klebsiella* species (n = 12), n (%) |
|---|---|---|---|
| Cefepime (FEP) | S | 3 (75.0) | 8 (66.7) |
| | I | 1 (25.0) | 0 (0.0) |
| | R | 0 (0.0) | 4 (33.3) |
| Cefixime (CFM) | S | 2 (50.0) | 6 (50.0) |
| | I | 1 (25.0) | 2 (16.7) |
| | R | 1 (25.0) | 4 (33.3) |
| Cefotaxime (CTX) | S | 1 (25.0) | 3 (25.0) |
| | I | 2 (50.0) | 3 (25.0) |
| | R | 1 (25.0) | 6 (50.0) |
| Ceftriaxone (CRO) | S | 2 (50.0) | 5 (41.7) |
| | R | 2 (50.0) | 7 (58.3) |
| Ceftazidime (CAZ) | S | 2 (50.0) | 4 33.3) |
| | R | 2 (50.0) | 8 (66.7) |
| Cefuroxime (CXM) | S | 2 (50.0) | 1 (8.4) |
| | I | 0 (0.0) | 4 (33.3) |
| | R | 2 (50.0) | 7 (58.3) |
| Augmentin (AMC) | S | 0 (0.0) | 2 (16.4) |
| | R | 4 (100.0) | 10 (83.3) |

## Antibiotic Resistance pattern for *Escherichia coli* isolates

From the study, of the 4 *E. coli* that were isolated, three different resistance patterns were observed (table 7). Two *E. coli* species were resistant for just one antibiotic presenting a similar pattern with the highest percentage of 2(50) while the others showed varying resistance to different antibiotics. AMC was resistant for all the isolates.

## Antibiotic Resistance pattern for 12 *Klebsiella* species isolated from the environment

Out of 12 *Klebsiella species* isolated from the environment 6 different resistance patterns where observed with the highest percentage of 4 (33.3) and the least being 1(8.3). 4 different isolates were resistance to all the antibiotics, while 4 isolates were resistant to just 1 antibiotic and the rest of the isolates showed varying resistance to the different antibiotics (Table 8).

**Table 7:** Antibiotic Resistance Pattern for 4 *Escherichia coli* isolated from the hospital environment.

Key: Cefixime (CFM), Cefotaxime (CTX), Ceftriaxone (CRO), Ceftazidine (CAZ), Cefuroxime (CXM), Augmentin (AMC).

| Number of Antibiotics | Resistance Pattern | Number of Resistant isolates n (%) |
|---|---|---|
| 1 | AMC | 2 (50) |
| 4 | CRO, CAZ, CXM, AMC | 1 (25) |
| 6 | CFM, CTX, CRO, CAZ, CXM, AMC | 1 (25) |

**Table 8:** Antibiotic Resistance pattern for 12 *Klebsiella* species isolated from the environment.

Key: Cefepime (FEP),Cefixime (CFM),Cefotaxime (CTX),Ceftriaxone (CRO), Ceftazidine (CAZ),Cefuroxime (CXM), Augmentin (AMC).

| Number of Antibiotics | Resistance Pattern | Number of Resistant isolates, n (%) |
|---|---|---|
| 1 | AMC | 4 (33.3) |
| 2 | CTX, CAZ | 1 (8.3) |
| 3 | CRO, CAZ,CXM | 1 (8.3) |
| 4 | CRO, CAZ, CXM, AMC | 1 (8.3) |
| 5 | CTX, CRO, CAZ, CXM, AMC | 1 (8.3) |
| 7 | FEP, CFM, CTX, CRO, CAZ, CXM, AMC | 4 (33.3) |

## Prevalence of ESBLs and non ESBL among Escherishia coli and Klebsiella species from Environmental sources

Two (12.5%) of the 16 environmental isolates were ESBL-producers. Of these, none was *E. coli* and 2(16.7%) were *K. species* while 14(87.5%) were non-ESBL. Of these, 4(100%) *E. coli* was non ESBL and 10(83.3%) *Klebsiella species* was non ESBL as shown in table 9.

## Distribution of ESBL and Non ESBL on different environmental surfaces

The percentage distribution of ESBLs and non ESBLs among bacteria isolated from environmental samples was also examined in this study (Tables 10). Among Environmental isolates, non ESBL were most prevalent in bed sides while the least prevalence was observed in side cupboard and ESBLs producers were most prevalent in tap head and wash hand basin (50.0%).

**Table 9:** Phenotypic Expression of ESBLs and Non ESBL.

| S/N | Number of Isolates | Number of Isolates screened | Non ESBL n (%) | Number of positive ESBLs, n (%) |
|---|---|---|---|---|
| 1 | *Escherichia coli* | 4 | 4 (100) | 0(0.0) |
| 2 | *Klebsiella species* | 12 | 10 (83.3) | 2(16.7) |
| | Total | 16 | 14 (87.5) | 2 (12.5) |

**Table 10:** Distribution of ESBL and Non ESBL on different environmental surfaces.

| Environmental Surface | Non ESBL | | ESBL | |
|---|---|---|---|---|
| | *Kleb. sp.* n=12/16 (75.0%) | *E. coli*, n=4/16 (25.0%) | *Kleb. sp.* n=2/2 (100%) | *E. coli* n= 0 (0.0%) |
| Bed side | 3 (25) | 2 (50) | 0 (0.0) | 0 (0.0) |
| Door handle | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Side cupboard | 1 (8.3) | 1 (25) | 0 (0.0) | 0 (0.0) |
| Tap head | 4 (33.3) | 0 (0.0) | 1 (50) | 0 (0.0) |
| Toilet seat | 2 (16.7) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Wash hand basin | 2 (16.7) | 1 (25.0) | 1 (50.0) | 0 (0.0) |
| total | 12 (100) | 4 (100) | 2 (100) | 0 (0.0) |

### Distribution of Isolates in different wards of the hospital

Out of 12 *Klebsiella* isolates the highest percentage 3(25.0) was found in the Male Medical and the surgical wards while the lowest percentage of 2(16.7) was found in emergency unit, female Medical Ward and haemodialysis center each. *For E. coli* the highest percentage was observed in the surgical ward and the least in the female medical ward. See table 11 for details.

**Table 11:** Distribution of Isolates in different wards of the hospital.

| Ward | *Klebsiella species* N = 12 | *E. coli* N = 4 |
|---|---|---|
| ANC | 0(0.0) | 0(0.0) |
| Blood bank | 0(0.0) | 0(0.0) |
| Emergency | 2 16.7 | 0(0.0) |
| Female Medical Ward | 2 (16.7 | 1 (25.0) |
| Haemodialysis center | 2 (16.7 | 0(0.0) |
| Histopathology | 0(0.0) | 0(0.0) |
| Male Medical ward | 3 (25.0) | 0(0.0) |
| Paediatric Ward | 0(0.0) | 0(0.0) |
| Surgical Ward | 3 (25.0) | 3(75.0) |
| TB treatment unit | 0 (0.0) | 0(0.0) |
| Total | 12 (100) | 4 (100) |

## Discussion and Conclusions

### Discussion

This study reveals that out of 200 samples collected from the hospital environment, a total of 16 isolates (8.0%) were obtained with 12 being *Klebsiella* species (6.0%) which was the most prevalent and 4 being *E. coli* (2.0%) showing least prevalence. This is in contrast with that of Gundugan [4], which shows that *E. coli* is most prevalent in the environment than Klebsiella. This could suggest that Klebsiella species have the ability to survive in the environment for longer period than *E. coli*. These 16 isolates were obtained from different wards and different surfaces in the hospital.

The result of this study shows that none of the door handles were contaminated with either *Klebsiella* species or *E. coli*, this is in agreement with a previous report by Muzslay., *et al*. [15]. This might be due to the fact that the number of patients who touch the door handles are few and are not contaminated. Surgical wards displayed the highest level of contamination in this study with both *E.coli* and *Klebsiella* species.

In our findings, surgical wards and the male medical wards had the highest percentage of contamination; this is in agreement with

Engda., *et al*. [16]. This may be due to differences in the number of patients attending each section of the wards and their health status.

According to this study a total of four isolates all being *Klebsiella* species were resistant to all seven antibiotics used. The resistance displayed by all the isolates to the various antibiotics tested is worrisome, especially resistance that was demonstrated to three or more of the antibiotics, an indication of multi- drug resistance.

In the antimicrobial susceptibility test, one isolates of ESBL producing *Klebsiella* was 100% resistant to ceftazidime, ceftriaxone and amoxicillin with clavulanic acid which is similar to that reported by Engda., *et al*. [16]. None of the isolates tested was susceptible to all the antimicrobial drugs used in this study. This is an issue because many clinicians fall back on the quinolones for the treatment of gram- negative pathogens in the face of multidrug resistance [14]. All the ESBL producers were 100% susceptible to cefipime and cefixime.

The most challenging condition in the management of infectious diseases associated with ESBL producing Entrobacteriaceae are the development of multiple drug resistance (Resistant to three or more drugs) [16]. However, the current study showed a high frequency of multiple antibiotics resistance to commonly used antibiotics which is similar to the report of Engda., *et al*. [16], in Ethiopia. This might be due to inappropriate use of antimicrobials, lack of laboratory diagnostic tests, and failure of patient adherence to their medication.

ESBL-producers are frequently resistant to advance generation cephalosporins. However, in the present study some ESBL producing isolates showed susceptibility to advance generation cephalosporins (cefotaxime, ceftazidime, and ceftriaxone). This is in harmony with the result previously published by Shaikh., *et al.* [17]. This could happen due to the incidental presence of various other mechanisms of resistance and counter resistance in a given bacterium.

The existence of ESBL producing *E. coli* and *Klebsiella* is of serious concern since the pathogens have the capacity to acquire resistance to a wide range of clinically important antibiotics. This may lead to horizontal gene transfer among bacteria thus disseminating resistance in the environment.

From our study, detection of ESBL production showed that 12.5% (2/16) of the environmental isolates were considered as potential producers of ESBLs, which is higher than 3.1% reported by Muzslay., *et al*. [15], from environmental isolates. In another report, Chinedu. [14], (45.1%) showed a contrasting situation with prevalence of ESBL which is higher than that of the present study. These variations might be due to difference in geographical area, study period as well as the sample size.

The result of this study suggests that the environmental contamination is higher in ESBL producing *Klebsiella* species than ESBL producing *E. coli*. ESBL positivity was found to be 0.0% (0/4) of *E. coli* and 16.6% (2/12) of *Klebsiella* species this is line with Afifi. [18], who reported 15.4% and 19.8% respectively. This could suggest that ESBL producing *Klebsiella* has a higher ability to spread and /or persist in the environment than ESBL producing *E. coli. Klebsiella* species are known to form biofilms which may be a way of surviving during long periods in the environment [18].

In this study ESBL was prevalent in tap heads and wash hand basins from toilets; this is in line with the results reported by Afifi [18]. This observation might be due to the fact that, toilets being the site for urination and defecation, gives a greater chance to the number of patients who touch the wash hand basin and the tap heads. However, this observation might also highlight the quality of surface cleaning at the hospital and the efficacy of the disinfectants used. This result confirms the serious role of the hospital environment in the transmission and spread of infections.

## Conclusions

Most of the isolates in this study were found to be resistant against three or more antibiotics. This study showed that antibiotics such as cefepime and cefixime are very effective in environmental isolates. Conversely, resistance to β-lactam; ceftriaxone, ceftazidime, cefuroxime and Augmentin were observed.

Generally, the study indicates low prevalence of ESBL producing *Klebsiella* species and *E. coli* from the environment in BRH. However, it gives insight to a foreseeable problem giving the increasing incidence of antibiotics abuse. ESBL producing gram negative bacteria have been responsible for numerous outbreaks of infection worldwide and therefore, pose a challenge to efficient infection control. So, it is essential to identify ESBLs as a routine in the hospitals.

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## Authors' Contributions

This work was conceived, Supervised and written by Dr Ndaleh from the beginning to the end. Dr Dilonga supervised the sample collection and Laboratory work to ensure quality at the Buea Regional Hospital. He also contributed in reading the manuscript to correct some errors. Ebonlo Pamela and Ngwa Fabrice did the laboratory work at the Buea Regional Hospital where the data were collected and used to realize this piece of work. In summary, all authors contributed in one way or the other to make this work what it is.

## Conflict of Interest

We certify that we have participated and contributed sufficiently for the completion of the manuscript and have agreed to have our name listed as contributors. We have accepted to be represented by the corresponding author before the IMC journal of Medical Science with respect to submission of this manuscript titled Prevalence of Extended Spectrum Beta Lactamase (ESBL) Producing Escherichia Coli and Klebsiella Species from Hospital Surfaces and Their Susceptibility Pattern to Antimicrobials at the Buea Regional Hospital South West Region, Cameroon. There is no conflict of interest between and among author and co-authors.

## Ethical Disclosure

This work did not involve any human or animal subject and samples were collected from inanimate surfaces and no clinical trial was involved. Therefore no ethics was needed for the work.

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