# Title: Healthcare center wastewaters in Burkina Faso: sources of ESBL, AmpC-β-lactamase and carbapenemase producing Escherichia coli and Klebsiella pneumoniae

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## Abstract

### Background

Extended-spectrum β-lactamase (ESBL), plasmid-mediated AmpC-β-lactamase and carbapenemase producing *Escherichia coli* and *Klebsiella pneumoniae* have spread into environment worldwide posing a possible public health threat. However, the prevalence data in low- and middle-income countries are still scarce. The aim of this study was to assess the occurrence of ESBL, AmpC-β-lactamase and carbapenemase producing and multidrug-resistant *E. coli* and K. *pneumoniae* in wastewater collected from healthcare centers in Burkina Faso.

### Methodology

Eighty-four (84) wastewater samples were collected from 5 healthcare centers and bacterial counts on ESBL ChromAgar were performed. *E. coli* and *K. pneumoniae* isolates were identified using API20E. ESBL production was confirmed using the double disc synergy test (DDST). AmpC-β-lactamase detection was performed on Muller Hinton (MH) agar supplemented with cloxacillin (4µg/l). Carbapenemase testing was carried out using O.K.N.V.I. RESIST-5 immunochromatography test.

### Result

*E. coli* and/ or *K. pneumoniae* strains were isolated from 82 wastewater samples (97.6%). In total, 170 strains were isolated, *E. coli* more commonly (64%). Average concentrations of ESBL producing bacteria per hospital varied from $1.10x10^5$ to $5.23x10^6$ CFU/ml. Out of 170 presumptive ESBL producing isolates and 51 presumptive AmpC-β-lactamase producing isolates, 95% and 45% were confirmed, respectively. Carbapenemase production was detected in 10 isolates, 6 were NDM producers, 3 were OXA-48 producers and 1 was NDM and OXA-48 producer. All isolates were multidrug resistant and, furthermore, all of them were resistant to all β-lactams tested. Also, resistance to ESBL inhibitors was common, up to 66% *E. coli* and 62% in *K. pneumoniae.* Amikacin, fosfomycin and nitrofurantoin were the antibiotics for which least resistance was detected.

### Conclusion

This study showed that wastewater from healthcare centers constitutes a reservoir of multidrug-resistant bacteria in Burkina Faso, including those capable of producing carbapenemases, which may disseminate into environment and further back to humans. Therefore, following the microbiological quality of the wastewaters released from healthcare centers is important to include in the future national AMR surveillance program.


## Introduction

The emergence and spread of antimicrobial resistance (AMR) represent a serious threat to human and animal health. In 2014, the number of deaths due to AMR was estimated at 700,000 (1, 2). If no action is taken against AMR, by 2050 this number could reach 10 million per year (1, 2). The economic cost of the AMR will vary from 1.1 to 3.8% of the global GPD (3). The annual shortfall by 2030 was estimated to reach $3.4 trillion (3). Several causes, such as unreasonable use or overuse of antibiotics, have been speculated to favor the emergence and diffusion of resistance genes and multidrug resistant bacteria, (4–9). In low- and middle-income countries (LMICs), socio-economic and behavioral factors, such as poverty, use of poor quality antibiotics, absence of diagnostic tools, absence of antibiotic stewardship policies and uncontrolled use of antibiotics in animals, have been incriminated (5). The persistence of antibiotic residues, non-degraded antibiotics and disinfectants in the wastewaters contribute to selection of resistant bacteria and their wide spread in environment (4–6, 10–14). Multidrug resistant bacteria harboring extended spectrum β-lactamase genes (*bla*TEM, *bla*SHV, *bla*CTX-M) and carbapenemase genes (*bla*OXA-48, *bla*KPC, *bla*NDM, *bla*VIM and *bla*IPM) have been detected in hospital wastewaters from several countries (15–19). Management of healthcare center wastewaters in LMICs is highly insufficient and sometimes the wastewaters are directly discharged into the environment, drainage, rivers, or lakes without any treatment (20–22). Use of this water for various human activities exposes the population to new infections by multidrug resistant bacteria (23, 24)

In Burkina Faso, data on wastewater contamination are patcy, but recent studies have revealed the abundant presence of resistant bacteria in healthcare center effluents,(25, 26). The present study aimed to assess healthcare center wastewaters contamination specifically by ESBL-producing Gram negative bacilli and to perform phenotypic characterization of ESBL-producing *Escherichia coli* (ESBL-Ec) and *Klebsiella pneumoniae* (ESBL-Kp) in wastewaters from hospitals at different levels of the healthcare system in Burkina Faso.

## Results

# Bacterial concentration in healthcare center wastewaters

The average concentration of bacteria growing on ESBL selective plates from wastewater of each healthcare center varied from $1.10x10^5$ to $5.23x10^6$ CFU/ml. The highest bacterial counts were obtained from wastewater drained from Yalgado Ouédraogo teaching hospital (tertiary level hospital), followed by Koudougou regional hospital center and El Fateh SUKA Clinic (both secondary level healthcare facilities) (Table 1).

Table 1
Prevalence of bacteria from wastewaters of five healthcare centers in Burkina Faso growing on ChromAgar™ ESBL plates.

| Healthcare centers | No. of samples (n = 84) | Average concentration (CFU/ml) |
|---|---|---|
| Yalgado Ouedraogo teaching hospital | 28 | $5.23x10^6$ |
| Koudougou Regional hospital Center | 26 | $3.37x10^6$ |
| El Fateh Suka Clinic | 14 | $3.00x10^6$ |
| Source de Vie medical Center | 6 | $1.10x10^5$ |
| Saint Camille medical Center in Nanoro | 10 | $1.85x10^5$ |

## Prevalence Of Esbl

From the 84 healthcare center wastewater samples, suspected ESBL *E. coli* or *K. pneumoniae* isolates were detected in 82 samples (97.62%). In total, 170 strains were isolated (109 *E. coli* and 61 *K. pneumoniae)*. ESBL test confirmed 160 (95%) isolates (102 *E. coli* and 58 *K. pneumoniae*) to be ESBL positive. Ten isolates were negative but they were resistant to all the β-lactam + ESBL inhibitors tested.

## Prevalence Of Ampc β-lactamase Producers

Isolates which were resistant or intermediately susceptible to cefoxitin (37 ESBL-*Ec* and 14 ESBL-*Kp*) were tested to detect AmpC-β-lactamase production by the phenotypic method. 23 of 51 isolates (45%) were AmpC- β-lactamase producers (Table 2).

Table 2
Prevalence of AmpC-β-lactamase producers among the cefoxitin resistant or intermediately susceptible isolates from the five healthcare centers

| Healthcare centers | *E. coli* | | *K. pneumonia* | | AmpC-β-lactamase producers (%) * |
|---|---|---|---|---|---|
| | tested (n) | AmpC positive (n) | tested (n) | AmpC positive (n) | |
| Yalgado Ouedraogo teaching hospital | 15 | 3 | 4 | 2 | 9.80 |
| Koudougou Regional Hospital Center | 12 | 7 | 5 | 2 | 17.65 |
| El Fateh SUKA Clinic | 5 | 3 | 1 | 0 | 5.88 |
| Source de Vie medical center | 1 | 1 | 0 | 0 | 1.96 |
| Saint Camille medical center (Nanoro) | 4 | 1 | 4 | 4 | 9.8 |
| TOTAL | 37 | 15 | 14 | 8 | 45.09 |

* AmpC-β-lactamase producing *E. coli* and *K. pneumonia* out of the 51 isolates tested.

## Prevalence Of Carbapenemase Producers

Twenty-one isolates resistant or intermediately susceptible to meropenem (15 ESBL-*Ec* and 6 ESBL-*Kp*) were tested to detect carbapenemase production (OXA-48, KPC, NDM, VIM, and IMP). Ten isolates (47.62%) were carbapenemase producers: 6 were NDM producers, 3 were OXA-48 producers, and 1 was NDM and OXA-48 producer. Carbapenemase producing bacteria were detected among wastewater collected from the tertiary and the secondary level healthcare facilities (Table 3).

Table 3
Prevalence of carbapenemase producers among the meropenem resistant isolates from the five healthcare centers

| Healthcare centers | *E. coli* | | *K. pneumoniae* | |
|---|---|---|---|---|
| | tested (n) | carbapenemase positive (n) | tested (n) | carbapenemase positive (n) |
| Yalgado Ouédraogo teaching Hospital | 8 | 1 OXA-48<br>1 OXA-48 + NDM | 4 | 2 OXA-48<br>1 NDM |
| Koudougou Regional hospital Center | 4 | 1 NDM | 1 | 1 NDM |
| El Fateh SUKA Clinic | 3 | 2 OXA-48<br>1 NDM | 0 | 0 |
| Source de Vie medical center | 0 | 0 | 0 | 0 |
| Saint Camille medical center (Nanoro) | 0 | 0 | 1 | 0 |

## Resistance To Antibiotics

All the isolates from the ESBL selective plates (109 *E. coli* and 61 *K. pneumoniae* isolates) were tested against 31 antibiotics representing different antibiotic categories (Table 4). All the isolates were multidrug resistant. All the isolates (100%) were resistant to aminopenicillins (ampicillin, piperacillin) and cephalosporins except cefoxitin. In case of the ESBL inhibiting combination antibiotics, 65.42% and 65.74% of E. *coli* and 61.67% and 45.76% of *K. pneumoniae* were resistant to amoxicillin + clavulanic acid and to piperacillin + tazobactam, respectively.

Table 4
Antibiotic resistance of ESBL producing *E. coli* and *K. pneumoniae* strains.

| Antibiotic group | Antibiotics (concentration in µg) | Resistance and susceptibility to the antibiotic | | | |
|---|---|---|---|---|---|
| | | E. coli | | K. pneumoniae | |
| | | Resistance n (%) | Susceptible n (%) | Resistance n (%) | Susceptible n (%) |
| Penicillin, Penicillin and inhibitors | Ampicillin (10) | 97(100) | 0(0) | 61(100) | 0(0) |
| | Piperacillin (100) | 97(100) | 0(0) | 60(100) | 0(0) |
| | Amoxicillin + acid clavulanic (30) | 71(65.74) | 37(34.26) | 37(61.67) | 24(38.13) |
| | Piperacillin + Tazobactam (110) | 70(65.42) | 37(34.58) | 27(45.76) | 32(54.24) |
| Cephalosporin | Cefazolin (30) | 97(100) | 0(0) | 60(100) | 0(0) |
| | Cefuroxime (30) | 95(100) | 0(0) | 56(100) | 0(0) |
| | Ceftriaxone (30) | 95(100) | 0(0) | 60(100) | 0(0) |
| | Ceftazidime (30) | 95(100) | 0(0) | 60(100) | 0(0) |
| | Cefepime (30) | 95(100) | 0(0) | 55(98.21) | 1(1.79) |
| | Cefoxitin (30) | 43(40.57) | 63(59.43) | 14(23.73) | 45(76.27) |
| Monobactam | Aztreonam (30) | 92(94.85) | 5(5.15) | 53(94.64) | 3(5.36) |
| Carbapenem | Meropenem (10) | 17(15.74) | 91(84.26) | 5(8.19) | 55(91.81) |
| | Imipenem (10) | 22(20.75) | 84(79.25) | 5(8.19) | 55(91.81) |
| | Ertapenem (10) | 35(32.71) | 72(67.29) | 11(18.33) | 49(81.67) |
| Aminoglycosides | Gentamycin (10) | 46(44.66) | 57(55.34) | 31(5082) | 30(49.18) |
| | Amikacin (30) | 7(6.93) | 94(93.7) | 8(13.11) | 53(86.89) |
| | Tobramycin (10) | 74(71.15) | 30(28.85) | 35(57.37) | 26(42.63) |
| | Kanamycin (30) | 65(71.43) | 26(28.57) | 35(77.77) | 10(22.23) |
| Macrolides | Azithromycin (15) | 68(68.69) | 31(31.31) | 21(35.59) | 38(64.41) |
| Quinolones, Fluoroquinolones | Ciprofloxacin (5) | 98(95.15) | 5(4.85) | 56(91.80) | 5(8.20) |
| | Ofloxacin (5) | 59(67.05) | 28(32.95) | 11(24.44) | 34(75.56) |
| | Levofloxacin (5) | 71(71.72) | 18(28.28) | 32(53.33) | 28(46.67) |
| | Pefloxacin | 58(100) | 0(0) | 57(93.44) | 4(6.56) |
| | Nalidixic acid (30) | 99(94.29) | 6(5.71) | 40(88.89) | 5(11.11) |
| | Norfloxacin (30) | 68(80.95) | 16(19.05) | 30(50.0) | 30(50.0) |
| Cyclins | Tetracycline (30) | 80(86.02) | 13(13.98) | 36(78.26) | 10(21.74) |
| | Doxycycline (30) | 70(67.31) | 34(32.69) | 37(60.66) | 24(39.34) |
| Sulfonamides | Sulfamethoxazole (50) | 73(93.59) | 5(6.41) | 23(100) | 0(0) |
| | Sulfamethoxazole + trimethoprim (25) | 94(89.52) | 11(10.48) | 52(88.14) | 7(11.86) |
| Nitrofurans | Nitrofurantoin (300) | 42(40) | 63(60) | 24(40) | 36(60) |
| Phosphonic acid | Fosfomycin (200) | 12(11.43) | 93(88.57) | 36(61.02) | 23(38.98) |

High resistance rates were detected against aminoglycoside, quinolone, and fluoroquinolone antibiotic categories. Indeed, in the aminoglycoside family, resistance rates recorded were up to 71.43% in *E. coli* and 77.77% in *K. pneumoniae* against kanamycin. Isolates were more susceptible to amikacin since only 6.93% of resistance in *E. coli* and 13.11% in *K. pneumoniae* were recorded (Table 4). The resistance rates reported against quinolones and fluoroquinolones varied from 67.05–100% in *E. coli* and from 24.44–93.44% in *K. pneumoniae* (Table 4).

In case of carbapenems, 17 *E. coli* (15.74%) and 5 *K. pneumoniae* (8.19%) isolates were resistant to meropenem.

Other families of antibiotics commonly used in hospitals in Burkina Faso include cyclins, 86.02% *E. coli* and 78.26% *K. pneumoniae* isolates were resistant to tetracycline. In case of sulfonamides, 88.14% of *K. pneumoniae* isolates were resistant to Sulfamethoxazole + trimethoprim and 100% to

sulfamethoxazole (Table 4).

Azithromycin, an antibiotic widely used in Burkina Faso for Covid19 patient treatment (27, 28), was inactive for 68.69% of *E. coli* isolates and for 35.59% of *K. pneumoniae* isolates (Table 4)

## Discussion

β-lactams are widely used in treating patients in healthcare in Burkina Faso, but nowadays bacteria are often highly resistant to these antibiotics. Therefore, we isolated *E. coli* and *Klebsiella pneumoniae* strains from ESBL selective ChromAgar plates inoculated with healthcare center wastewaters to determine their susceptibility to other antibiotics available. Over 97% of the 84 wastewater samples analyzed contained ESBL producing *E. coli* and/or *K. pneumoniae*. The concentrations of ESBL producing Gram-negative bacteria in the healthcare center wastewaters were high, but, our results are comparable to those published in previous studies elsewhere (15, 29–31). For instance, concentrations up $10^7$ CFU/ml of ESBL, CARB and OXA producing Enterobacteriaceae were reported from hospital wastewaters in Slovenia and Austria (15). Among our samples, the wastewaters collected from the tertiary and secondary level healthcare centers were the most contaminated with ESBL producers, possibly because these hospitals receive more patients, generally referred from a district level healthcare. Also, antibiotics are used more in terms of both quantity and diversity in tertiary and secondary level hospitals.

In addition to being ESBL producers, many of the isolates characterized in this study were also AmpC-β-lactamase (23 positive out of 51 isolates tested) and carbapenemase (10 positive out of 21 isolates tested) producers. Two types of carbapenemases, OXA-48 and NDM, were detected. Previously, using a metagenomics approach, presence of several carbapenemase genes (*bla*VIM, *bla*IMP, *bla*NDM and *bla*OXA-48) was reported in the wastewaters of some of the same hospitals in Burkina Faso (26). The resistance rate to carbapenems in our study was 15.74% to meropenem, 20.75% to imipenem and 32.71% to ertapenem. Our results differ from the results recently reported from Burkina Faso, where imipenem was the only carbapenem tested and no resistance to it was detected (25, 32). Occurrence and eventual spread of the carbapenem-resistant bacteria into the environment is of a particular concern since carbapenems are currently the antimicrobials of last resort in healthcare.

Wastewaters originating from healthcare centers present a public health concern in Burkina Faso and other countries, where they are discharged directly into the environment or into municipality wastewater channels without any prior treatment (20, 22). Furthermore, in LMICs, hospital wastewater may be used for irrigation of vegetable crops (5). Indeed, ESBL producing bacteria have been isolated on lettuce leaves in Burkina Faso (32). Common intestinal carriage of these bacteria can increase their prevalence in patients visiting healthcare centers, where presence of these bacteria leads to therapeutic treatment complications, prolonged patient hospitalizations and increased hospitalization costs, as well as higher mortality and morbidity (33).

The high level of resistance to the commonly used antibiotics has been reported also by other research groups in West Africa (34, 35). In Nigeria, full resistance to cefotaxime, cefpodoxime, sulfonamide and ertapenem was reported among ESBL producing *E. coli* isolated from wastewater originating from a healthcare facility (35). Likewise, in Côte d'Ivoire, ESBL producing *E. coli* and *K. pneumoniae* isolated from hospital wastewaters were reported to be fully resistant to amoxicilline + clavulanic acid, cefotaxime, ceftriaxone and ceftazidime, *E. coli* were also fully resistant and *K. pneumoniae* 62.5% resistant to ciprofloxacin, 87% resistant to nalidixic acid and cefepime, and furthermore, 76.5% of *E. coli* and 50% of *K. pneumonia* were resistant to gentamycin (34). The high resistance level of bacteria in wastewaters from healthcare centers is the consequence of antimicrobials misuse in hospitals, the discharge at high concentrations of not metabolized antibiotics and antibiotic residues into hospital wastewater, and the fecal contamination by patients (7, 22, 36–38). Furthermore, the high concentration of bacteria in these wastewaters offers an increased chance for horizontal transfer of resistance genes between bacteria (30–32, 37).

Amikacin, fosfomycin and nitrofurantoin were the antibiotics against which we recorded low resistance rates. Also in Mexico, a low resistance rate to amikacin among carbapenemase-producing *Klebsiella* spp. isolated from hospital wastewater was reported recently (40). These antibiotics, mostly used for urinary tract infection treatment, represent a major therapeutic option in case of infection with ESBL producing bacteria.

## Conclusion

This study shows that wastewaters from healthcare facilities represent a reservoir of multidrug-resistant bacteria in Burkina Faso. Wastewaters collected from the healthcare centers representing tertiary and secondary level of the healthcare system were the most contaminated. The ESBL producing *E. coli* and *K. pneumoniae* isolates were resistant to all commonly used antibiotics in Burkina Faso, such as β-lactams, β-lactams combined with ESBL inhibitors (amoxicillin + clavulanic acid and piperacillin + tazobactam), quinolones, fluoroquinolones, aminoglycosides, sulfonamides, cyclins, and macrolides. Only amikacin and fosfomycin showed good activity against these bacteria.

## Material And Methods

# Study design, study sites and sampling

A prospective study was carried out in 5 healthcare centers in Burkina Faso representing different healthcare system levels. The samples were collected from the Yalgado Ouédraogo teaching hospital in Ouagadougou (university hospital, tertiary level care), Koudougou regional hospital center in Koudougou and El Fateh SUKA Clinic in Ouagadougou (secondary level care), Source de Vie medical center in Ouagadougou and Saint Camille medical center in Nanoro rural area (primary level).

Three healthcare centers had a sewer system, Yalgado teaching hospital, Koudougou regional hospital center and Saint Camille medical center. Yalgado teaching hospital sewers connected to the city sewage system leading to the city's wastewater treatment plant. Wastewater from the hospital is discharged into the general sewage without any prior treatment. Koudougou regional hospital center has a chemical treatment device. The treated wastewater is discharged into the municipality channel, which is connected to a backwater in the town. Source de Vie medical center and El Fateh SUKA Clinic do not have a sewer system and their wastewater is collected in septic tanks. The management of wastewater in these two healthcare centers and in Saint Camille medical center in Nanoro are not clearly documented. As a rule, there is no wastewater treatment plants in rural areas in Burkina Faso, instead, the wastewaters are directly discharged into the environment without any treatment.

We collected wastewater samples from several sites along the sewers from the healthcare centers with the sewer system and from septic tanks from the healthcare centers without a sewer system. Two rounds of sampling were done, 1) October to December 2019 and 2) October 2020 to March 2021. A total of 84 wastewater samples were collected (Table 1). One liter of wastewater was collected in a sterile glass bottle. The samples were immediately placed in a cooler containing ice blocks and transported to analysis in the microbiology lab of the Nanoro clinical research unit (CRUN) within 12 hours.

## Bacterial Count, Isolation, And Identification

Two dilutions were prepared for each sample (1/10 and 1/100) using sterile 0.9% physiological saline water. Following the WHO Tricycle instructions (41), 100 µl of each dilution was inoculated on ESBL selective agar plates (ChromAgar™ ESBL), which were incubated at 35 ± 2°C for 24 hours. A positive control was carried out for all samples by inoculating a non-selective CLED agar plate with 100µl of the sample. After incubation,, all visible bacterial colonies on the plates were counted, and the results were expressed in colony-forming units per milliliter of wastewater (CFU/ml). Only a plate of one dilution from each sample was considered for the bacterial count.

The agar plates were also inspected for different morphotypes of bacteria, according to the manufacturer's instructions (ChromAgar™ ESBL). Red or pink colonies were considered to be *E. coli*, and blue, green, or blue-green the KESC group (*Klebsiella, Enterobacter, Serratia and Citrobacter*). Five colonies of the same morphotype of *E. coli* or the KESC group were picked for purification on eosin methylene blue agar (EMB). The purified isolates were identified using the API20E (Biomérieux, France).

## Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of 170 presumptive ESBL producing isolates (109 *E. coli* and 61 *K. pneumoniae* isolates) was performed using the disk diffusion method on Muller Hinton (MH) agar. Thirty-one antibiotic discs were tested (Table 4) and the results were interpreted according to the American Clinical and Laboratory Standards Institute (CLSI) 2021 guidelines.

## Extended Spectrum β-lactamase (Esbl) Confirmation

ESBL confirmation testing was carried out on MH agar using the double disc synergy test (DDST) between a 3rd generation cephalosporin (ceftriaxone or ceftazidime, C3G), a 4th generation cephalosporin (cefepime, C4G) and amoxicillin + clavulanic acid (AMC) following the CLSI 2021 guidelines. The result was interpreted as positive when there was a visible synergy inhibition zone between C3G-AMC-C4G (Fig. 1).

## Phenotypic Ampc-β-lactamase Testing

The 51 isolates (37 *E. coli* and 14 *K. pneumoniae*) which were resistant or intermediately susceptible to cefoxitin were tested for the AmpC-β-lactamase production. A bacterial suspension prepared with fresh colonies (McFarland 0.5) was inoculated on to entire surface of the MH agar supplemented with cloxacillin at 4µg/l and a disk of cefoxitin was placed on the plate. The test was positive if the inhibition zone diameter around cefoxitin disc was ≥ 18 mm.

## Carbapenemases Detection Test

The isolates that were resistant or intermediately susceptible to meropenem were tested for carbapenemase production.with the immunochromatographic test O.K.N.V.I. RESIST-5 (CORIS BioConcept, Belgium), according to the manufacturer's instructions. A total of 21 isolates were tested for the five main carbapenemases (OXA-48-like, KPC, NDM, VIM, IMP) within 15 minutes.

## Abbreviations

AMC: amoxicillin + clavulanic acid; AMR: antimicrobial resistance; CAZ: ceftazidime; CFU: colony forming unit; CLED: Cystine Lactose Electrolyte Deficient; CLSI: Clinical and Laboratory Standards Institute; CTX: cefotaxime; CRUN: clinical research unit of Nanoro; C3G: third generation cephalosporin; C4G: fourth generation cephalosporine; DDST: double disc synergy test; EMB: eosin methylene blue; ESBL: Extended-spectrum β–lactamases; KESC: *Klebsiella Enterobacter Serratia* and *Citrobacter*; ITM: Institute of Tropical Medicine; MH: Muller Hinton;

## Declarations

### Ethics approval and consent to participate

This study received approval from the health research committee of Burkina Faso (N°153-12-2018/ CE-RS). Authorizations were obtained from all hospitals

### Consent for publication

Not applicable

### Availability of data and materials

All data generated or analyzed during this study are included in this published article

### Competing interests

The authors declare that they have no competing interests.


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### Authors' contributions

ZG, IOJB, KH, LS, and NB conceived and designed the study. ZG, NOM, collected samples. ZG, NOM, PPAV, MAB, IK, ALWT performed bacterial isolation and antimicrobial susceptibility test. HT Contributed for the reagents/materials/analysis tools. ZG, IOJB, HMN, KH and NB were the major contributors in writing the manuscript. All authors read and approved the final manuscript.


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## Figures

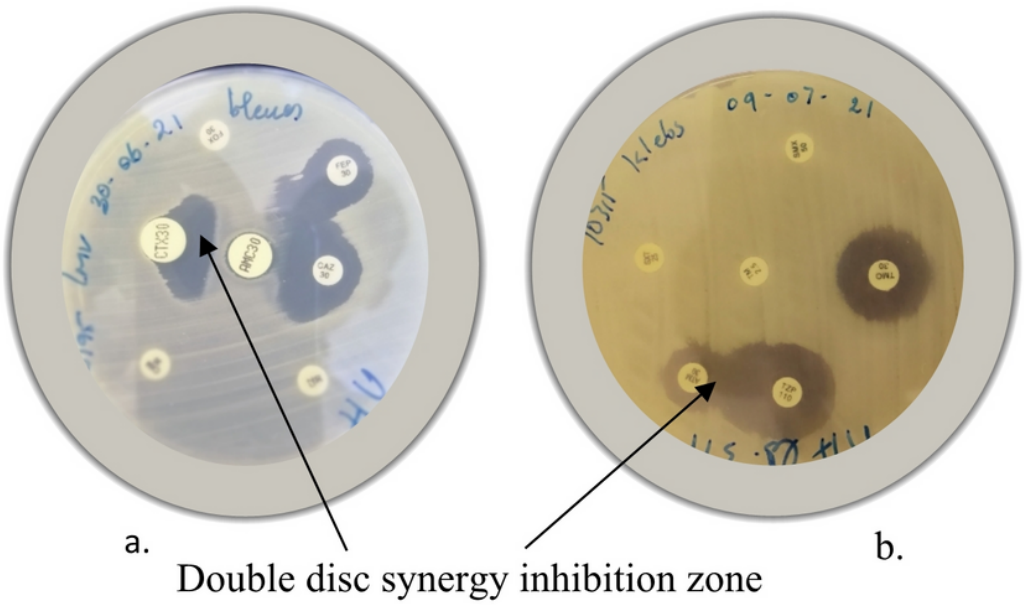


**Figure 1**

ESBL confirmation test for a *K. pneumoniae* strain showing a double disc synergy inhibition zone.