# Effectiveness of CHROMagar ESBL in Compared to Double Disc Synergy Test (DDST) for the Detection of ESBL Producing Uropathogens

## ABSTRACT

**Background:** Extended spectrum beta-lactamases (ESBL) are enzymes produced by members of the *Enterobacteriaceae* which can hydrolyze the beta-lactam antibiotics like penicillins and cephalosporins and thereby confer antibiotic resistance on strains producing them. Bacterial isolates producing ESBLs have spread to different parts of the world.

Aim: The aim of this study was to evaluate the effectiveness of CHROMagar ESBL in comparison with Double Disc Synergy Test (DDST) for the detection of ESBL producing uropathogens.

**Methods:** The study was carried out within a period of six months at the three secondary health care facilities between July to December, 2022. Six hundred and sixty (660) urine samples were collected from pregnant women attending antenatal at General hospital Ikot Ekpene, Eket and Oron Local Government Area In Akwa Ibom State, Nigeria. Microbact 24E (Oxoid, UK) was used in the identification of bacterial isolates, antibiotic susceptibility test was done using Kirby-Bauer disk diffusion method following CLSI guidelines using commercially available disc (Oxoid Ltd). Double disk synergy test was carried out on the isolates and inoculation was done using CHROMagar ESBL (France).

**Results:** The prevalence of ESBL 92% was recorded. Eighty (80%) of the ESBL producing isolates were multi drugs resistant. The predominant bacterial pathogens were *Enterobacter cloacae* (23%), *Proteus mirabilis (*14%) and *Acinetobacter baumanii* (13.4%). Comparison methods employed for detection of ESBLs in our study showed that confirmed ESBL producers by DDST was (50%) while confirmed ESBL producers by CHROMagar ESBL method was (92 %) with a significant P-value at 0.05

The ESBL producing isolates showed maximum resistance against Ceftazidime (90%), Cefotaxime (91%), Azetronam (95%), Amikacin (68.2%) followed by ofloxacin (70%) while maximum sensitivity was seen for imipenem (90%) and Augumentin (80%). The study demonstrated that CHROMagar was superior and more sensitive than DDST. The research showed that large numbers of Gram-negative bacteria causing community acquired UTIs produce ESBL with most being multi-drug resistant (MDR). Therefore, routine ESBL detection testing and subsequent antibiogram with disk diffusion method could be useful to determine the best treatments for UTI.

**Conclusion:** CHROMagar ESBL seems to be the most reliable method among phenotypic methods for detection of ESBL in the absence of PCR.

*Keywords: Antibiotic resistance; extended spectrum beta-lactamase;* CHROMagar ESBL DDST.

**1. INTRODUCTION**

In spite of the widespread availability of antibacterial drugs, urinary tract infection (UTI) remains one of the major infections in the community and hospital settings. The identification of ESBL producing organisms is difficult for routine diagnostic microbiology laboratories of developing countries without molecular diagnostic facilities. Also screening of ESBL producing organisms by monitoring the decrease in susceptibility to oxyimino-cephalosporin drugs are not a sensitive tool (Nigudgi *et al.,* 2021: Bronson and Barrett, 2001). Extended spectrum beta-lactamases(ESBLs) are typically plasmid-mediated enzymes that confers resistance to Extended-spectrum beta-lactam antibiotics such as Ceftazidime, Cefotaxime or Azetronam (Sridhar, 2015). Beta-lactamases that transmit resistance to penicillin, cephalosporins, and monobactams are known as extended-spectrum β-lactamases. These enzymes are less effectively inhibited by beta-lactamase inhibitors, which include clavulanate, sulbactam, and tazobactam (Bush, 2013). ESBLs enzymes are produced by both Gram positive and Gram negative bacteria but occur predominantly in the family Enterobacteriaceae. Strains resistant to a variety of commonly used antimicrobials produce ESBLs (Coudron, *et al.,* 2003). This implies that their proliferation pose serious global health problem if not checked. Betalactamases has the ability to open the beta-lactam ring and inactivate the antibiotics, and render them ineffective for treatment (Boyd *et al.,* 2004: Uyanga, *et al.,* 2019). Most ESBLs belong to the CTX-M, SHV (Sulfhydryl variable) and TEM (Temoniera) families. Due to the production of multiple enzymes such as the inhibitor-resistant ESBL variants and plasmid-borne AmpC, ESBL phenotypes have become more complex (Glupczynski *et al.,* 2006).The recent resurgence of another group of beta-lactamases, carbapenemase producing bacteria has raised a major public health concern. New Delhi Metallo beta-lactamase (NDM-1) hydrolyses a wide range of beta-lactam antibiotics including carbapenems, which are the last resort of antibiotics for the treatment of infections caused by resistant strain of bacteria [Hosain et al., 2009: Mohanty, 2009: Uyanga *et al.,* 2009). Due to the rising incidence of ESBL harboring microorganisms, there has been a worrisome increase in the use of carbapenems and this can result in pan-resistant organisms (Bradford, 2001).

Hospital and community acquired ESBL producing uropathogens are prevalent worldwide, due to inappropriate use of beta-lactam antibiotics, poor sanitation in hospitals,And unhealthy lifestyles leading to serious infections and raising therapeutic problems (Alasmary, 2021: Ben-Ami *et al.,* 2009).Beta-lactamase may be chromosomal or plasmid borne, inducible or constitutive (Hugo and Russell’s, 2013). ESBLs are often located on plasmids harbouring resistance gene to other antimicrobial classes, resulting in multidrug resistant isolates (Boyd *et al.,* 2004: CLSI, 2012, Uyanga *et al.,* 2019].

Extended spectrum beta-lactamases can be readily detected by iodometric, colometric and chromogenic methods (Thomson and Sanders, 1992). *In vitro* detection of ESBL has faced many challenges due to the fact that many strains are susceptible to broad spectrum beta-lactam such as Cefotaxime and Ceftriazone (Katsanis *et al.,* 1994: Jarlier *et al.,* 1988: Uyanga *et al.,* 2019).

Commercial available chromogenic media such as CHROMagar(Paris, France) have been used to detect ESBL production. Chromogenic culture media is a rapid culture based methods used for detection of ESBL and presumptive organism identification. The media has a chromogenic enzyme substrate as a detection system. Chromogenic substrates consist of chromophor which is linked to an enzyme-recognizing part such as carbohydrate, amino acids or phosphate. Specific enzymes produced by the target micro-organism will cleave to the chromogenic substrate liberating the chromophor which highlight the micro organism by coloration of the grown colony (Ongut et al., 2014: Uyanga *et al*., 2019). *E .coli* ESBL produces dark Pink to reddish and colouration, Klebsiella, Enterobacter Citrobacter produces a metallic blue colouration while Proteus produces a brown halo colour. According to the instruction of the manufacturer, any coloured oxidase negative colonies growing on the chromogenic media, should be regarded as presumptive ESBL producing isolates (Uyanga *et al*., 2019).

In the study described here, we evaluate the effectiveness of CHROMagar ESBL in comparison with Double Disc Synergy Test (DDST) for the detection of ESBL producing uropathogens from pregnant women attending antenatal at general hospital Ikot Ekpene, Eket and Oron, Akwa Ibom State, Nigeria.

**2. MATERIALS AND METHODS**

**2.1 Sample Collection**

The study was carried out within a period of six months. A total of 660 urine samples were collected from pregnant women attending antenatal at the three secondary health care facilities between July to December, 2022. Ethical approval was obtained from Ministry of Health, Akwa Ibom State. All pregnant women who were not on any antibiotics and willing to participate were included in the studies, while those on any antibiotic therapy were excluded from the studies .

Mid stream clean- catch urine samples were collected and inoculated on MacConkey and CHROMagar ESBL and incubated at 37ºC for 24 hours. They were examined for growth and colony counts yielding bacterial growth of 105 /ml of urine were taken to be significant. Samples were Gram stained and also subjected to Microbact 24E identification (Uyanga *et al*., 2019).

**2.2 Antimicrobial Susceptibility Testing**

Antibiotic susceptibility was determined by the disk diffusion method on Mueller-Hinton agar (Oxoid, UK) according to Clinical and Laboratory Standard Institute(CLSI) guidelines. ESBL-producing isolates were screened using double-disk synergy test in accordance with CLSI guidelines [17]. According to CLSIs guidelines isolates showing inhibition zone size of < 22mm with Ceftazidime (30µg), < 25mm with Cefotaxime (30 µg), <27mm with Azetronam (30 µg) and <22 mm with Cefodoxime (10 µg) was identified as potential ESBL producers and shortlisted for confirmation of ESBL production (Florence *et al.,* 2020). *E. coli* ATCC 25922 and *S. aureus* 6571 were used as quality control strains (Uyanga *et al*., 2019).

**2.3 Double Disk Synergy Test**

Double disk synergy test as described by Jarlier *et al* (1988) was used to confirm ESBL production. Isolates to be tested were swabbed on the surface of the MullerHinton agar and a ceftazidime disk was placed close (20 to 30 mm to the amoxicillin-clavulanate disk already placed. An extension of growth inhibition zone peripheral disk towards the centrally placed amoxicillin-clavulanate disk indicates ESBL production. This extension occurred because the clavulanic acid present in the Augmentin disc inactivated the ESBL produced by the organism (Uyanga *et al*., 2019).

Innoculation was also done on CHROMagar ESBL, a completely new and innovative chromogenic medium designed specifically for the Screening of Extended Spectrum ß-Lactamase-producing Enterobacteria (ESBL) (Gazin *et al.,* 2012) Incubation was done for 18-24hrs. *Escherichia coli* produced pink to burgundy colouration of ß-glucuronidase-producing colonies *Klebsiella, Enterobacter, Serratia, Citrobacter* (KESC):green/blue to browny-green colouration of ß-glucosidase-producing colonies. *Proteeae(Proteus, Providencia, Moraganella)* produced dark to light brown colouration (NCCL, 2019).

**2.4 Statistical Analysis**

The SPSS statistical package version (18.0) was used for statistical analysis. A p-value <0.05 was considered as statistically significant.

**3. RESULTS**

**3.1 Description of the Bacterial Isolates**

During the study period, a total of 660 urine samples from pregnant women were processed. Out of the 660 samples, A total of 231 (92%) out of the 252 uropathogenic isolates were found to be ESBL producers, the predominant bacterial pathogens were *Enterobacter cloacae* (23%), *Proteus mirabilis (*14%) and *Acinetobacter baumanii* (13.4%). Followed by *Hafnia alvei* (7.3%), *Stenotrophomonas maltophilia* (4.8%) and *Enterobacter agglomerans* (4.8%).Antimicrobialsusceptibility analyses showed that thirty two ESBL producing *A. baumanii* isolates (12.5%) were resistant to quinolone (Ofloxacin 70%) and third generation Cephalosporins (Cefotaxime, 62.5%, Ceftazidime, 90%, and Azetronam 95%).

Most isolates were sensitive to Imipenem (90%), Augumentin (80%), and Aamikacin (68.2%). ESBL production was detected in (35%) of the isolates. Double disk synergy test detected (50%) of the isolates, while CHROM agar ESBL detected (92%) of the isolates. Gram negative ESBL producing bacteria were 32.4% while Gram positive were 2.6%

**3.2 Statistical Analysis**

Chi-square was used to determine if a significant difference existed between results from both procedures. Where a significant difference exists it was interpreted as P ˂ 0.05.

**Table 1. Result of Confirmed ESBL producers detected by Chrom agar ESBL versus DDST**

**From Oron, Ikot Ekpene and Eket Local Government Area**

|  |  |  |  |
| --- | --- | --- | --- |
| **Bacterial isolates** | **Confirmed ESBL producers detected by Chrom agar ESBL (%)** | **Confirmed ESBL producers detected by DDST (%)** | **P-value** |
| *E. cloacae* | 58 (23) | 1. 12.6) | 0.05962 |
| *E. coli* | 44 (17.4) | 25 (9.9) |  |
| *K. pneumonia* | 34 (13.4) | 16 ( 6.3) |  |
| *H. alvei* | 17 (6.7) | 8 (3.2) |  |
| *S. aureus* | 17 (6.7) | 11 (4.4) |  |
| 1. *haemolyticus* | 6 (2.4) | 1. (1.9) |  |
| 1. *Iwoffi* | 3 (1.2) | 2 (0.8) |  |
| *S. subspecies* | 7 (2.7) | 4 (1.6) |  |
| *M. morganii* | 5 (1.9) | 2 (0.8) |  |
| *E. hormaechei* | 4 (1.6) | 2 (0.8) |  |
| *E. agglomerans* | 11 (4.36) | 6 (2.6) |  |
| *C. sakazaki* | 1 (0.4) | 1 (0.4) |  |
| *S. luquefaciens* | 1 (0.4) | 1 (0.4) |  |
| *E. gresoviae* | 1 (0.4) | 1(0.4) |  |
| *S. marcescens* | 7 (2.8) | 3 (1.2) |  |
| *S. maltophilia* | 12 (4.8) | 3 (1.2) |  |
| *C. youngae* | 1 (0.4) | 1 (0.4) |  |
| *C. diversus* | 1 (0.4) | 1 (0.4) |  |
| *C. freundii* | 1 (0.4) | 1(0.4) |  |
| Total | 231 (92) | 125 (50) |  |

**4. DISCUSSION**

In this study, we present ESBLs producing isolates from Akwa Ibom State, Nigeria. The isolates showed resistance to third generation cephalosporins, quinolone and aminoglygoside and showed sensitivity to Imipenem and Augmentin

The study was carried out to evaluate the performance of Double Disc Synergy Test (DDST) and CHROMagar ESBL) in screening for ESBL among isolates from urine samples of pregnant women attending antenatal in the three study areas. Among clinical isolates, the prevalence of ESBL greatly varies in Geographical areas and worldwide (Uyanga *et al*., 2019).

**Table 4. Antibiotic susceptibility profile of ESBL producing *Acinetobacter baumanii* from Ikot Ekpene, Eket and Oron General Hospital**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Antimicrobials** | **Ikot Ekpene** | **R(%)** | **Eket** | **R**  **(%)** | **Oron =9** | **R**  **(%)** |
| **µg** | **n=20 S(%)** |  | **n=3 S(%)** |  | **S(%)** |  |
| CTX(30) | 2(10) | 18(90) | 0 | 3(100) | 9(100) | 0 |
| OFX (5) | 19(95) | 1(5) | 1(33) | 2(67) | 7(78) | 2(22) |
| CAZ(30) | 0 | 20(100) | 1(33) | 2(67) | 5(56) | 4(44) |
| ATM (30) | 0 | 20(100) | 0 | 3(100) | 6(67) | 3(33) |
| IPM (30) | 19(95) | 1(5) | 1(33) | 2(67) | 9(100) | 0 |
| AUG (30) | 20(100) | 0 | 1(33) | 2(67) | 9(100) | 0 |
| AK(30) | 12(60) | 8(40) | 1(33) | 2(67) | 5(56) | 4(44) |

*Key:CTX- cefotaxime, OFX-oflxacin, CAZ-Ceftazidime, ATM-Azetronam, IPM-Imipenem, Aug-Augumentin, AK-Amikacin*

**Table 5. Antibiotic susceptibility profile of *Enterobacter cloacae* from Ikot Ekpene, Eket and Oron General Hospital**

|  |  |  |
| --- | --- | --- |
| **Antimicrobials µg** | **n=65 S (%)** | **R(%)** |
| CTX(30) | 16(25) | 49(75) |
| OFX (5) | 18(28) | 47(72) |
| CAZ(30) | 16(25) | 49(75) |
| ATM (30) | 15(23) | 50(77) |
| IPM (30) | 56(74) | 9 (26) |
| AUG (30) | 48 (74) | 17(26) |
| AK(30) | 8(12) | 57 (87) |

*Key:CTX- cefotaxime, OFX-oflxacin, CAZ-Ceftazidime, ATM-Azetronam, IPM-Imipenem, Aug-Augumentin, AK-Amikacin*

The prevalence of ESBL in this research was 92%%. A prevalence of 50%, 92% and 95% prevalence of ESBL producers in urinary isolates of Gram negative bacilli was observed by other researchers (Getie *et al.,* 2023: Bell *et al.,* 2002, Alghamdi *et al.,* 2023). Also, Fares *et al.,* reported a moderate prevalence of 59.5% from their studies (Fares *et al.,* 2023).

In our study, the predominant bacterial pathogens were *Enterobacter cloacae* (23%), *Proteus mirabilis (*14%) and *Acinetobacter baumanii* (13.4%). Followed by *Hafnia alvei* (7.3%), *Stenotrophomonas maltophilia* (4.8%) and *Enterobacter agglomerans* (4.8%) (Uyanga *et al*., 2019). Getie *et al.* (2023)reported that the proportion of extended spectrum beta lactamase among Gram-negative isolates was 50% in contrast to our results, in the study of Hosain Zadegan et al. (2009) 23.5% of isolated Gram-negative microorganisms (53 of 222 isolates) were ESBL producers with the most frequent isolates being *K. pneumoniae* (8.9%), *E. coli* (4.4%), and *P. aeruginosa* (4.4%); also, Alghamdi reported a prevalence of 85% for *E. coli* (Alghamdi *et al.,* 2023). These values are lower than the rates in our study. However, the rate of ESBL-producing uropathogenic bacteria in Nigeria has been constantly increasing, Onanuga *et al*., (24%) and Jesumirhewe *et al*. (40.5%) have documented an increasing rate of ESBL producers from asymptomatic bacteriuria cases ([Onyango](https://www.scirp.org/Journal/articles.aspx?searchCode=Hellen+A.++Onyango&searchField=authors&page=1" \t "_blank) *et al.,* 2018: Jesumirhewe *et al*., 2020). These differences in prevalent rate may be due to geographical area, time, and the diagnostic technique used.

Increasing resistance to broad spectrum cephalosporins due to the production of β-lactamases have been reported from different countries (Lin *et al.,* 2022: Uyanga *et al.,* 2019). The development and use of simple screening tests that are suitable for routine use in the clinical microbiology laboratory is a critical step towards large-scale monitoring of these enzymes (Migliavacca *et al.,* 2022). Due to the outcome of the antibiotic Susceptibility test result, the ESBL producing isolates were subjected to ESBL screening using Double Disc Synergy Test (DDST) and CHROMagar ESBL. DDST is described as a reliable technique for ESBL detection (Ongut *et al.,* 2014). The differences in sensitivity results in DDST be due to the fact that optimal substrate profile varies from one ESBL enzyme to another (Asad *et al.,* 2017). DDST an easy procedure with subjective interpretation of result (Hugo and Russell’s 2013: Uyanga *et al.,* 2019).

**Table 6. Antibiotic susceptibility profile of ESBL producing *Proteus mirabilis* the three study area**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Antimicrobials** | **n=5 S(%)** | **R**  **(%)** | **Ikot** | **Ekpene** | **R**  **(%)** | **Eket** | **R**  **(%)** |
| **µg** |  |  | **n=11 S(%)** | |  | **n=16 S(%)** |  |
| CTX(30) | 0 | 5(100) | 10(91) |  | 1(9) | 8(50) | 8(50) |
| OFX (5) | 5(100) | 0 | 10(91) |  | 1(9) | 9(56) | 7(44) |
| CAZ(30) | 0 | 5(100) | 6(55) |  | 5 (45) | 4 (25) | 12(75) |
| ATM (30) | 0 | 5(100) | 1(9) |  | 10(91) | 10(62) | 6(38) |
| IPM (30) | 4(80) | 1(20) | 10(91) |  | 1(9) | 10(62) | 6(38) |
| AUG (30) | 4(80) | 1(20) | 5(45) |  | 6(55) | 5(31) | 11(69) |
| AK(30) | 2(40) | 3(60) | 2(18) |  | 8(72) | 11(69) | 5(31) |

*Key:CTX- cefotaxime, OFX-oflxacin, CAZ-Ceftazidime, ATM-Azetronam, IPM-Imipenem, Aug-Augmentin, AK-Amikacin*

**

**Fig. 1. Culture plate of showing a clear extension of the edge of the inhibition zone of cephalosporin using Co-amoxiclav Disc on Mueller-Hinton agar was interpreted as positive for ESBL production**

****

**Fig. 2. Culture plate of clinical isolate of *Escherichia coli* from urine showing distinctive pink colony colouration on CHROMagar ESBL agar was interpreted as positive for ESBL production**



**Fig. 3. Culture plate of clinical isolate of *Acinetobacter baumanii* from urine showing distinctive colourless to cream colony colouration on CHROMagar ESBL agar was interpreted as positive for ESBL production**

****

**Fig. 4. Culture plate of clinical isolate of *E. clocae* from urine showing blue colonies on CHROMagar ESBL**

The distance between antibiotic discs affects the sensitivity of DDST. Studies by Ho et al. (2000) revealed the sensitivity of DDST to be 83.8% at a single interdisc width of 30mm. Their study also showed an increase in sensitivity to 97.9% by decrease in the interdisc width to 20 mm. In this study, sensitivity and specificity of DDST was 91.3% and 89.5% respectively at 24 h which was the same at 48h at a single interdisc width of 15mm. DDST can detect both Gram positive and Gram negative ESBL producing bacteria while CHROMagar is only limited to Gram negative organisms (Uyanga *et al.,* 2019).

There were significant differences (P ˃ 0.001) among ESBL-producing isolates that emerged from the three general hospitals. The differences could be attributed to the fact that DDST is a technically easy procedure with subjective interpretation of result ,while CHROMagar ESBL Agar gives an advantage of easier detection of ESBL-producing Gram negative as well as other members of the Enterobacteriaceae family due to its chromogenic properties. Results are easier to interpret as it employs colony coloration technique (Naas *et al.,* 2007; Ravi *et al*., 2011: Prabha *et al.,* 2016; Uyanga *et al*., 2019).

Comparison methods employed for detection of ESBLs in our study showed that confirmed ESBL producers by DDST was (50%) while confirmed ESBL producers by CHROMagar ESBL method was (92 %) with a significant P-value at 0.05. This is not in accordance with the findings of Prabha *et al*. who reported that comparison methods employed for detection of ESBLs in their study showed DDST confirmed 29% of the isolates while CHROMagar ESBL method confirmed 35% ESBL producers (Ugbo *et al*., 2020). Elsewhere in the eastern part of Nigeria, Ezeanya *et al*. reported that confirmed ESBL producers by DDST method was 91.3% while 97.8% ESBL producers was confirmed by Brilliance ESBL agar (Ezeanya *et al.,* 2017).

The detection of ESBL producers among isolates as observed in this study is not in line with the work of Ugbo *et al*. where they stated that confirmed ESBL producers by brilliance agar and DDST was 100% respectively (Ugbo *et al*., 2020). The variance in DDST’s sensitivity tests may be due to the fact that it is a technologically less straight forward method with subjective interpretation of the test (Ravi *et al.,* 2011). The distance between antibiotic discs affects sensitivity. The sensitivity results of ESBL detection using CHROMagar ESBL however, has been rather challenging due to the need for color-based differentiation of several genera of *Enterobacteriaceae* or Gram-negative bacteria (GNB) harboring ESBLs, an indicator β-lactam(s) that provides possible coverage of the ESBL spectrum, as these enzymes vary widely in resistance profiles that is made complex by the presence of multiple β-lactamases in the same organism (Gazin *et al.,* 2012; Aurilio *et al.,* 2022).

The inclusion of Cefodoxime in CHROMagar ESBL rather than Cefotaxime and Ceftazidime could attribute for its higher sensitivity over DDST. Thus, performance of CHROMagar ESBL agar in this study justifies claims that Cefpodoxime is the best substrate for screening all ESBL types in clinical specimens (Ravi *et al*., 2012).

The carbapenems (Imipenem, Ertapenem and Meropenem) are still the first line agents in treatment of serious infection with ESBL-producing organisms as >98% of ESBL-producing organisms stillsusceptible to these drugs (Guet et al., 2017). Alasmary *et al*., (2021) observed 99% susceptibility for Imipenem while in our study, we observed 90% susceptibility for Imipenem.

In this study, we found that about 68.2% of ESBL producing uropathogens were susceptible to amikacin, however, a poor susceptibility for cefotaxime (90%), ceftazidime (91%), Ofloxacin (70%) was observed. Also, 70% of ESBL-producing uropathogens were sensitive to Augmentin. Aminoglycosides displays bactericidal concentration-dependent killing action and are active against ESBL producing bacteria (Uyanga *et al*., 2019). In a Spanish study published in 2014, aminoglycosides were used in the treatment of carbapenems-resistant Klebsiella infection showing a statistically significant reduction in mortality (Guet *et al.,* 2017: Bell *et al*., 2002).

Amoxicillin/clavulanic acid as an alternative treatment to carbapenems for infections involving ESBL-producing organisms remains debated (Seo *et al.,* 2017). In this study, a high rate of susceptibility (80%) was observed for Amoxicillin/clavulanic acid. In a Randomized controlled trial conducted by YuBin Seo, it was reported that Piperacillin/tazobactam(TZP) which is also a beta-lactamse inhibitor like Amoxicilin/clavulanic acid is effective in the treatment of UTI caused by ESBL-*E. coli* when the in vitro test indicates susceptibility (Gonzalez *et al.,* 2015). Hence this antibiotic may be used as an alternative treatment to carbapenems for pyelonephritis caused by ESBL-producing uropathogens.

The variations of resistance to antibiotics can be explained in part based on different local antibiotic practices (Datta *et al.,* 2004). Differences insusceptibility patterns of organisms and frequency of infection between hospitals and communities make knowledge of local prevalence and resistance data extremely important (Rossolini *et al.,* 2008). This has direct bearing on choice of empiric therapy. Our research showed that large numbers of Gram-negative bacteria causing community acquired UTIs produce ESBL with most being multi-drug resistant (MDR). Therefore, routine ESBL detection testing and subsequent antibiogram with disk diffusion method could be useful to determine the best treatments for UTI.

**5. CONCLUSION**

ESBL continues to pose a serious public health threat as it receives attention from the general public, policy makers and clinical microbiologist. Results from our study revealed that CHROMagar ESBL was able to detect more ESBL producing Isolates compared to DDST. This medium allows for easy differentiation of different bacteria based on colony colouration.

**CONSENT**

As per international standard written and informed participant consent has been collected and preserved by the authors.

**ETHICAL APPROVAL**

Ethics committee of Akwa Ibom State Ministry of Health, provided ethical clearance for the study.Ref:MH/PRS/99/VOL.IV/200. Participants’ privacy and confidentiality have been assured (no names have been used, only serial numbers were used) and all data and results have been handled and treated confidently.

Competing interests

Authors have declared that no competing interests exist.

**REFERENCES**

1. Alasmary, M. Y. (2021). Antimicrobial Resistance Patterns and ESBL of Uropathogens Isolated from Adult Females in Najran Region of Saudi Arabia. *Clinical Practical* 14;11(3):650-658
2. Alghamdi, S. A. A., Mir, S. S., Alghamdi, F. S., Al Banghali, MAMMA, and Almalki S. S. R.(2023). Evaluation of Extended-Spectrum Beta-Lactamase Resistance in Uropathogenic *Escherichia coli* Isolates from Urinary Tract Infection Patients in Al-Baha, Saudi Arabia. Microorganisms. 21;11(12):2820.
3. Asad, U. K., Lubna, M. and Raffaelle, Z. (2017). Structure Genetics and World wide Spread of New Delhi Metallo-Beta-lactamase (NDM-1): A threat to public Health. *BMC Microbiology.*17:101.
4. Aurilio, C., Sansone P, Barbarisi, M., Pota, V., Giaccari, L. G., Coppolino, F., Barbarisi, A., Passavanti, M. B. and Pace, M. C. (2022). Mechanisms of Action of Carbapenem Resistance. Antibiotics (Basel). 21;11(3):421.
5. Bell, J.M., Turnidge ,J. D., Gales, A. C., Pfaller, M. A. and Jones, R. N. (2002). Sentry APAC and Study Group, Prevalence of extended spectrum beta-lactamase (ESBL)- producing clinical isolates in the Asia coli and *Klebsiella pneumoniae* at an Indian tertiary care hospital: Plasmid-mediated cefoxitin resistance. 22. (1998—99). *Diagn Microbiol. Infect Dis.*; 42:193-8.
6. Ben-Ami, R., Rodríguez-Baño, R. and Arslan, H. J, (2009). A multinational survey of risk factorset al. A multinational survey of risk factors for infection with extended-spectrum beta-lactamase-producing Enterobacteriaceae in nonhospitalized patients. *Clin Infect Dis.* 49:682.
7. Bouchillon, S. K., Johnson, B. M., Hoban, D. and Bradford, P. A.( 2004). Determining Incidence of extended spectrum Beta-lactamase producing Enterobacteriaceae vancomycin resistant Enterococcus facium and methicillin resistant *Staphylococcus aureus* in 38 centers from 17 countries. The PEARLS study 2001-2002.International Journal of Antimicrobial Agents. 24(92):119-24.
8. Boyd, D. A, Tyler, S., Christianson, S., McGeer, A., Muller, M. P., Willey, B. M. (2004) Complete nucleotide sequence of a 92-kilobase plasmid harboring the CTX-M-15 extended-spectrum beta-lactamase involved in an outbreak in long term-care facilities in Toronto, Canada. *Antimicrob Agent Chemother.* 48:3758-64.
9. Bradford, P. A. (2001) Extended Spectrum β-lactamases in the 21st century: Characterization, epidemiology and detection of this important resistance threat. *Clin Microbiol Rev.*14(4):933-951.
10. Bronson, J. J. and Barrett, J. F. (2001). Quinolone, everninomycin, glycylcycline, carbapenem, lipopeptide and cephem antibacterials in clinical development. Current medicinal chemistry. 8(14):1775-93.
11. Brown, A. O., Brien, S., Cocks, S., Beares, J. and Dimmer, S. A.(2011). Laboratory evaluation of chromogenic screening media for the detection of ESBL producing bacteria. United Kingdom: Oxoid Ltd.;1.
12. Bush, K. (2013). The ABCD’s of β-lactamase Nomenclature. *J Infect Chemother.* 19:549-59.
13. Clinical and Laboratory Standard Institute (2012) Performance Standard for Antimicrobial susceptibility Testing 22nd informational supplement, M100-S20.Wayne, P A:Clinical and Laboratory Standard Institute;
14. Coudron, P. E., Hanson, N. D., and Climo, M. W.(2003). Occurrence of extended spectrum and AmpC β-FOX-5 and ACT-1 AmpC b-lactamases. in bloodstream isolates of Klebsiella FOX-5 and ACT-1 AmpC b-lactamases. *J. Clin Microbiol*.41:772-7.
15. Datta, P., Thakur, A., Mishra, B., Gupta, V. (2004). Prevalence of clinical strains resistant to various β-lactamase in a Tertiary care Hospital in India. Jpn J Infect Dis. 57(4):146-149.
16. Ezeanya, C. C., Agbakoba, N. R., Ejike, C. E., Okwelogu,S. I.(2017). Evaluation of a Chromogenic Medium for the Detection of ESBL with Comparison to Double Disk Synergy Test, *British Journal of Medicine and Medical Research*. 21(12):1-11.
17. Fares, R., Debabza, M., Mechai, A.(2023). Detection and prevalence of extended spectrum β -lactamases production among Enterobacteriaceae isolated from urinary tract infections. *Biosystems Diversity*.14(2), 163–169.
18. Florence, Z. U., Emmanuel, O. E., Emmanuel, O. N., and Inimfon A I.(2020). Molecular characterization of extended spectrum beta-lactamase from enterobacter cloacae, *E. coli* and *Klebsiella pneumoniae* from Pregnant Women in South-south Nigeria. International Journal of Microbiology and Biotechnology. 5(2):48-54.
19. Gazin, M., Paasch, F., Goostens, H, Malhotra-Kumar S. (2012; ) Current trends in culture based and molecular detection of extended spectrum β-lactamase harbouring and Carbapenem – resistant Entero-bacteriaceae. *J Clin Microbiol.* 50(4):1140-1146.
20. Getie M, Gebre-Selassie S, Getu Y, ( 2023). Bacterial profile and extended spectrum beta lactamase screening of urinary tract infection among asymptomatic and symptomatic pregnant women attending antenatal care in ALERT Hospital, Addis Ababa, Ethiopia. SAGE Open Medicine. 11
21. Getie, M., Gebre-Selassie, S., Geto, Y., Birara, S., Tiruneh, C., Abebaw, A., Akelew, Y., Abeje, G. And Enkobahry, A. (2023). Bacterial profile and Extended spectrum Beta-lacamase screening of Urinary tract Infection among asymptomatic and symptomatic pregnant women attending antenatal care in ALERT hospital Addis ababa, Ethoipia. *SAGE OPEN MED*, 11: 20503121231197587.
22. Glupczynski, Y., Berhin, C., Bauraing, C., Bogaerts, P. (2006). Evaluation of a New Selective Chromogenic agar Medium for Detection of Extended-spectrum Beta-lactamase Producing Enterobacteriaceae. J clin Microbiol. 45(2):501-5.
23. Gonzalez PM, Torre CJ, Rivera EF, Pontes MA, Lopez CL, et al. Gentamicin therapy for sepsis due to carbapenem-resistant and colistin-resistant Klebsiella pneumoniae. JAntimicrob Chemother.2015;70:905-913.
24. Guet RH, Tomini E, Emirian A, Join LO, The spread of CTX-M-type extended- Lécuyer H, et al. Piperacillin/tazobactam spectrum beta-lactamases.Clin. Microbiol as an alternative antibiotic therapy to carbapenems in the treatment of urinary tract infections due to extended-spectrum β-lactamase-producing Enterobacteriaceae: An in silico pharmacokinetic study. Int J Antimicrob Agents. 2017;1:62-
25. Ho PL, Tsang DN, Que TL, Ho M, Yuenk Y. (2000). Comparison of screening methods for detection of extended spectrum beta - lactamase and their prevalence among *E.coli* and Kleshiella spp. in Hong Kong. APMIS.;108(3):237-240.
26. Hosain Z., H., Ramazanzadeh, R.and Hasany, A.(2009). Cross-sectional study of extended spectrum beta-lactamase producing gram-negative bacilli from clinical cases in Khorramabad, Iran. *Iran J Microbiol*. 1:16–9.
27. Hugo, Russell’s (2013). Pharmaceutical Microbiology, 8th edition.
28. Husna A, Rahman MM, Badruzzaman ATM, Sikder MH, Islam MR, Rahman MT, Alam J, Ashour HM. Extended-Spectrum β-Lactamases (ESBL): Challenges and Opportunities. Biomedicines. 2023 30;11(11):2937.
29. Irajian G, Jazayeri-Moghadas A, Beheshti A. Prevalence of extended-spectrum beta-lactamase positive and multidrug Resistance pattern of Escherichia coli and Klebsiella pneumonia isolates, Semnan, Iran. Iran J Microbiol. 2009;1:49–53.
30. Jarlier V, Nicolas M-H, Fournier G, Philippon A.( 1988) Extended broad-spectrum b-lactamases conferring transferable resistance to newer b-lactam agents in Enterobacteriaceae: Hospital prevalence and susceptibility patterns. Rev. Infect. Dis.;10:867–878.

### Jesumirhewe, C., Springer, B., Allerberger, F. and Ruppitsch, W. (2020) Whole Genome Sequencing of Extended Spectrum β-lactamase Genes in Enterobacteriaceae isolates from Nigeria. *PLoS ONE* ,15(4): e0231146.

1. Katsanis GP, Spargo J, Ferraro MJ, Sutton L, Jacoby GA.( 1994). Detection of Klebsiella pneumoniae and Escherichia coli strains producing extended-spectrum b-lactamases. J. Clin. Microbiol.;32:691–696.
2. Khanfar HS, Bindyna KM, Senok AC, Botta GA. Extended Spectrum betalactamase (ESBL) in Escherichia coli and Klebsiella pneumonia: Trends in the Hospitals and Community settings. J Infect Dev Ctries. 2009;1:3(4)295-
3. Lin, X., Kück, U. Cephalosporins as key lead generation beta-lactam antibiotics. Appl Microbiol Biotechnol. 2022;106:8007–8020.
4. Migliavacca, N. R, Pollin ES. Prevalence of Extended-Spectrum Beta-lactamase, AmpC and MBL in a tertiary care hospital. J Antimicrob Chemother, 2002;1:22-29.
5. Mohanty, S, Gaind R, Ranjan R, Deb M.(2009) Use of cefepime-clavulanate ESBL Etest for Detection of Extended spectrum beta-lactamase in AmpC co-producing bacteria*. J Infect Dev Ctries.*4:24-29.
6. Naas T, Oxacelay C, Nordmann P. (2007). Identification of CTX-M-type extendedspectrum-β-lactamase genes using real time PCR and Pyro sequencing. Antimicrob Agents Chemother.;51: 223-230.
7. National Committee for Clinical Laboratory Occurrence and detection of AmpC β-lactamases among Escherichia coli, Klebsiella pneumoniae and Standards. Performance standards for antimicrobial susceptibility testing; Ninth informational supplement (M100-S9). Wayne, PA, USA: National Committee for Clinical Laboratory Standards; 1999.
8. Nigudgi A, Hajare V, Biradar S, Anandkumar H. (2021) Evaluation of different phenotypic diffusion methods in the identification of extended spectrum beta lactamase producing uropathogenic Escherichia coli. Indian Journal of Microbiology Research;8(3):243–248.
9. Ongut G, Dalogle AE, Bayson BO, Daglar D, Ogunc D, Sekercioglu AO. (2014) Evaluation of a Chromogenic medium for detection of extended spectrum beta-lactamase producing Escherichia coli and Klebsiella pneumoniae strains. Clin Lab.;60:1213-1215.
10. [Onyango](https://www.scirp.org/Journal/articles.aspx?searchCode=Hellen+A.++Onyango&searchField=authors&page=1), H. A., [Ngugi](https://www.scirp.org/Journal/articles.aspx?searchCode=Carolyne++Ngugi&searchField=authors&page=1), C., [Maina](https://www.scirp.org/Journal/articles.aspx?searchCode=John++Maina&searchField=authors&page=1), J. and [Kiiru](https://www.scirp.org/Journal/articles.aspx?searchCode=John++Kiiru&searchField=authors&page=1), J. (2018). Urinary tract infection among pregnant Women at Pumwani Maternity Hospital, Nairobi, Kenya: Bacterial Etiologic Agents, Antimicrobial Susceptibility Profiles and Associated Risk Factors, *Advances in Microbiology,* 8(3): 175-187.
11. Pfaller, M.A. and Segreti, J. (2006). Overview of the epidemiological profile and laboratory detection of extended spectrum β-lactamases. Clin Infect Dis. 42(4): S153-163.
12. Prabha, R; Joshy M. E and Swapna, M (2016). Phenotypic detection of Extended Spectrum Beta- Lactamase producing uropathogens using DDST, PCT, Chrom agar and E-test–A comparative study. International Journal of Current Microbiology and Applied Science, 5(4): 565-577.
13. Ravi, S., Namratha, W. and Chandraskhar, M. R.(2011). Comparison of Disc Diffusion Method for the Detection of Extended-Spectrum Beta Lactamase-Producing Enterobacteriaceae. *Journal of Laboratory Physicians*. 3(1):33-36.
14. Ravi, S. G. and Namratha, W. N. and Chandrasekhar, M. R. (2011). Comparison of Disc diffusion methods for the Detection of extended -spectrum beta lactamase Producing Entero-bacteriaceae. *J Lab Physican*, 3(1):33-36.
15. Rossolini GMD’, Andrea MM, Mugnaili C. (2008). The spread of CTX-M-type extendedspectrum beta-lactamases.*Clin. Microbiol infect.* 14:33-41.
16. Seo Y, Lee J, Kim Y, Lee S, Lee J, et al.(2017). Randomized controlled trial of piperacillintazobactam, cefepime and ertapenem for the treatment of urinary tract infection caused by extended-spectrum betalactamase- producing Escherichia coli. BMC Infect Dis. 17:404.
17. Sridhar Rao P. N. (2015). Extended Spectrum Betalactamases- A Comprehensive Review, Pp.18.Available: Www.Microrao.Com.
18. Thomson, K. S. and Sanders, C. C. ( 1992) Detection of extended-spectrum b-lactamases in members of the family Enterobacteriaceae: Comparison of the double-disk and three-dimensional test. Antimicrob. Agents Chemother.;36:1877–1882.
19. Ugbo EN, Anyamene CO, Moses IB, Iroha IR, Babalola OO, Ukpai EG, Chukwunwejim CR,Egbule CU, Emioye AA, IgweOF, UgaduIO. (2020). Prevalence of blaTEM, blaSHV, and blaCTX-M genes among Extended Spectrum Beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae of Cinical Origin. Elsevier.;21(2): 56-31.
20. Uyanga, F. Z., Ekundayo, E. O,.,Nwankwo, E. O, Inimfon ,A. I. ( 2019) Evaluation of CHROMagar ESBL and double disk synergy test (DDST) for screening of extended spectrum beta-lactamase producing uropathogens in South-South Nigeria. Journal of Advances in Microbiology.;17(4):1-11.