

Original Research Article

Evaluation of CHROMagar ESBL and Double Disk Synergy Test (DDST) for screening of Extended Spectrum Beta-lactamase producing Uropathogens in South-South Nigeria.

Abstract

Background/purpose: 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.

Method: Six hundred and sixty urine samples were collected from pregnant women attending antenatal at General hospital Ikot Ekpene, Eket and Oron. Two hundred and fifty eight isolates were obtained while two hundred and thirty one isolates were ESBL producers. 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 was 35% was recorded. The sensitivity and specificity of DDST was 88% and 89%, respectively. CHROMagar showed an increase in sensitivity and specificity at 48 h with 98% and 99.0%, respectively. 80% of the ESBL producing isolates were multi drugs resistant. The predominant bacterial pathogens were *Enterobacter cloacae* (23%), *Proteus mirabilis* (14%) and *Acinetobacter baumannii* (13.4%). The ESBL producing isolates showed maximum resistance against Cefotaxime (90%), Cefotaxime (91%), Azetronam (95%), Amikacin (68.2%) followed by ofloxacin (70%) while maximum sensitivity was seen for imipenem (90%) and Augmentin (80%). The study demonstrated that CHROMagar was superior and more sensitive than DDST.

Conclusion: It can therefore be recommended for use to detect ESBL production in the absence of PCR.

Key Words: Antibiotic resistance, Extended spectrum beta-lactamase, CHROMagar, DDST

Introduction

Extended spectrum beta-lactamases (ESBLs) are typically plasmid-mediated enzymes that confers resistance to Extended-spectrum beta-lactam antibiotics such as Cefotaxime, Cefotaxime or Azetronam. Extended-spectrum β -lactamases are a subset of beta lactamases that confer resistance to penicillin, cephalosporins and monobactams and are less efficiently antagonized by beta-lactamase inhibitors such as clavulanate, sulbactam and tazobactam [3]. 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. This implies that their proliferation pose serious global health problem if not checked. Beta-lactamases has the ability to open the beta-lactam ring and inactivate the antibiotics, and render them ineffective for treatment [2]. Most ESBLs belong to the CTX-M, SHV (Sulphydryl 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 [37]. 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 [36]. 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 [11].

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 [1]. 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 [2,31].

Extended spectrum beta-lactamases can be readily detected by iodometric, colometric and chromogenic methods [7]. Invitro 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 [8, 9].

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 chromophore 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 chromophore which highlight the micro organism by coloration of the grown colony (Gazin *et al.*, 2012). *E. coli* ESBL produces dark pink to reddish colouration, *Klebsiella*, *Enterobacter* and *Citrobacter* produces a metallic blue colouration while *Proteus* produces a brown halo colour. According to the instruction of the instructions of the manufacturer, any coloured oxidase negative colonies growing on the chromogenic media, should be regarded as presumptive ESBL production isolates.

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.

Materials and Methods

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, 2018. 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 37°C 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.

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 (CLSI, 2012). According to CLSI's guidelines isolates showing inhibition zone size of ≤ 22 mm with Ceftazidime (30µg), ≤ 25 mm with Cefotaxime (30 µg), ≤ 27 mm with Azetronam (30 µg) and ≤ 22 mm with Cefodoxime (10 µg) was identified as potential ESBL producers and shortlisted for confirmation of ESBL production [32]. *E. coli* ATCC 25922 and *S. aureus* 6571 were used as quality control strains.

Double Disk Synergy Test

Double disk synergy test as described by Jarlier *et al.*, [10] was used to confirm ESBL production. Test isolate was swabbed on the surface of Mueller Hinton agar, then placement of a ceftazidime disk close (20 or 30 mm) to an amoxicillin clavulanate disk on a plate inoculated with the test organism. An extension of inhibition of the zone of inhibition around the 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 tested organism.

Inoculation was also done on CHROMagar ESBL, a completely new and innovative chromogenic medium designed specifically for the Screening of Extended Spectrum β -Lactamase-producing Enterobacteria (ESBL) [38]. Incubation was done for 18-24hrs. *Escherichia coli* produced pink to burgundy colouration of β -glucuronidase-producing colonies. *Klebsiella*, *Enterobacter*, *Serratia*, *Citrobacter* (KESC): green/blue to brownish-green colouration of β -glucosidase-producing colonies. *Proteaeae* (*Proteus*, *Providencia*, *Moraganella*) produced dark to light brown colouration.

Comment [u1]: MacConkey

Comment [u2]: 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.

Comment [u3]: of growth inhibition zone

Ethical consideration

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 confidentially.

Statistical Analysis

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

Results

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, 230(35%) were identified as ESBL producers. Among these isolates, the predominant bacterial pathogens were *Enterobacter cloacae* (23%), *Proteus mirabilis* (14%) and *Acinetobacter baumannii* (13.4%). Followed by *Hafnia alvei* (7.3%), *Xanthomonas maltophilia* (4.8%) and *Enterobacter agglomerans* (4.8%). Antimicrobial susceptibility analyses showed that thirty two ESBL producing *A. baumannii* isolates (12.5%) were resistant to quinolone (Ofloxacin 70%) and third generation Cephalosporins (Cefotaxime, 62.5%, Ceftazidime, 90%, and Azetronam and 95%).

Most importantly most isolates were susceptible to carbapenem (Imipenem, 90%), and Augmentin, 80%, aminoglycoside (amikacin) showed 68.2%. 231(35%) isolates were ESBL producing, double disk synergy test detected 143(55%) isolates while CHROM agar ESBL detected 214(82%) isolates. 32.4%(n=214) of isolated Gram negative bacilli were ESBL positive while 2.6% (n=17) of isolated Gram positive bacteria were ESBL positive.

Table 1: Result of Screening And Detection Of ESBL By DDST And ChromAgar ESBL From Oron General Hospital

| Bacterial isolates | CHROMagar ESBL N% | DDST N% | p-value |
|--------------------------|-------------------|---------|---------|
| <i>E. cloacae</i> | 40(41.2) | 15(39) | 0.5195 |
| <i>E. hormaechei</i> | 3(3.1) | 3(77) | |
| <i>E. agglomerans</i> | 2(2.1) | 2(5.1) | |
| <i>Proteus mirabilis</i> | 11(11.3) | 3(77) | |
| <i>Morganella</i> | 3(3.1) | 2(5.1) | |

morganii

| | | |
|--------------------------------|----------------|----------------|
| <i>A. baumannii</i> | 9(9.3) | 2(5.1) |
| <i>Xanthomonas maltophilia</i> | 2(2.1) | 1(2.6) |
| <i>Hafnia alvei</i> | 6(6.2) | 2(5.1) |
| <i>Staphylococcus aureus</i> | - | 2(2.6) |
| <i>Serratia marcescens</i> | 7(7.2) | 2(5.1) |
| <i>Serratia luquefaciens</i> | 1(1) | 1(2.6) |
| <i>Citrobacter sakazakii</i> | 1(1) | 1(2.6) |
| <i>Salmonella subspecies</i> | 6(6.2) | 2(5.1) |
| <i>E. coli</i> | 4(4.1) | 1(2.6) |
| TOTAL | 97(100) | 39(100) |

Table 2: Result of Screening And Detection Of ESBL By DDST And ChromAgar ESBL From Ikot Ekpene General Hospital

| Bacterial isolates | CHROMagar ESBL N% | DDST N% | p value |
|-----------------------------------|-------------------|---------|---------|
| <i>Acinetobacter baumannii</i> | 20(34.4) | 15(25) | 0.5962 |
| <i>Acinetobacter haemolyticus</i> | 5(7) | 5(8.3) | |
| <i>A. iwoffii</i> | 2(3.4) | 2(3.3) | |
| <i>E. coli</i> | 6(10.3) | 5(8.2) | |
| <i>Citrobacter youngae</i> | 1(1.7) | 1(1.7) | |
| <i>Citrobacter freundii</i> | 1(1.7) | 1(1.7) | |
| <i>Citrobacter diversus</i> | 1(1.7) | 3(5) | |
| <i>Hafnia alvei</i> | 4(6.9) | 3(5) | |
| <i>Staphylococcus aureus</i> | - | 15(25) | |
| <i>Enterobacter agglomerans</i> | 1(1.7) | 1(1.7) | |
| <i>Enterobacter cloacae</i> | 2(3.4) | 3(5) | |
| <i>S. maltophilia</i> | 6 (10.3) | 3(5) | |
| <i>Proteus mirabilis</i> | 5(8.6) | 3(5) | |

Comment [u5]: *Stenotrophomonas maltophilia* is the correct term since *Xantomonas* is not currently used

Comment [u4]: *S. maltophilia*

| | | |
|------------------------------|----------------|-----------------|
| <i>Salmonella subspecies</i> | 1(1.4) | 3(5) |
| <i>Klebsiella pneumoniae</i> | 2(3.4) | 1(1.7) |
| TOTAL | 73(100) | 51 (100) |

Table 3: Result of Screening And Detection Of ESBL By DDST And ChromAgar ESBL From Eket General Hospital

| Bacterial isolates | CHROMagar ESBL N% | DDST N% | p-value |
|------------------------------|-------------------|----------------|---------|
| <i>E. cloacae</i> | 15(25) | 14(32) | 0.5962 |
| <i>A. baumannii</i> | 2(3) | 1(2.3) | |
| <i>A. haemolyticus</i> | 1(1.6) | 1(2.3) | |
| <i>A. iwofii</i> | 1(1.7) | 1(2.3) | |
| <i>E. agglomeran</i> | 8(13.3) | 7(16) | |
| <i>E. hormaechei</i> | 1(1.7) | 1(2.3) | |
| <i>P. gresoviae</i> | 1(1.7) | 1(2.3) | |
| <i>P. mirabilis</i> | 16(27) | 8(18) | |
| <i>Morganella morganii</i> | 2(3.3) | 1(2.3) | |
| <i>S. maltophilia</i> | 4(6.7) | 3(6.8) | |
| <i>Hafnia alvei</i> | 7(11.6) | 4(9.1) | |
| <i>Staphylococcus aureus</i> | - | 1(2) | |
| TOTAL | 60(100) | 44(100) | |

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 $P < 0.05$.

Sensitivity = $(TP/TP + FN) \times 100$; Specificity = $(TN/TN + FP) \times 100$.

Discussion

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

The prevalence of ESBL in this research was 35%. Much higher (58%) prevalence of ESBL producers in urinary isolates of gram negative bacilli was observed in India (Grude, Tveta and Kristiansen, 2001; Bell *et al.*, 2002). Also, Ezeanya *et al.*, reported a higher prevalence of 61% from their studies (Ezeanya *et al.*, 2017).

In our study, 32.4 % (n=214) of isolated Gram negative bacilli were ESBL positive while 2.6% (n=17) of isolated Gram positive bacteria were ESBL positive. The predominant bacterial pathogens were *Enterobacter cloacae* (23%), *Proteus mirabilis* (14%) and *Acinetobacter baumannii* (13.4%). Followed by *Hafnia alvei* (7.3%), *Xanthomonas maltophilia* (4.8%) and *Enterobacter agglomerans* (4.8%). In contrast to our results, in the study of Hosain Zadeegan *et al.* 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, of nine isolated *Acinetobacter* spp. strains, 2 (0.9%) were ESBL-positive [19]. These values are lower than the rates in our study. The frequency of ESBL in this study agrees with the study of Adham *et al.*, who reported a sensitivity of (38.4%) [21].

Increasing resistance to broad spectrum cephalosporins due to the production of β -lactamases have been reported from different countries (Bouchillon *et al.*, 2002; Khanfar *et al.*, 2009). 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.*, 2002). 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 [12]. The differences in sensitivity results in DDST be due to the fact that optimal substrate profile varies from one ESBL enzyme to another [35]. DDST an easy procedure with subjective interpretation of result [39].

The distance between antibiotic discs affects the sensitivity of DDST. Studies by Ho *et al.* [16] 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

Comment [u6]: *Stenotrophomonas maltophilia* is the correct term since *Xantomonas* is not currently used.

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.

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 [39]. 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 [13,14].

In this study, it was observed that CHROMagar ESBL had 99% specificity which is significantly higher than that of DDST with specificity of 85%. The specificity of CHROMagar ESBL in this study was higher than previous studies whose studies revealed a specificity of 95.7% and 93.0% respectively [33,34]. The sensitivity of CHROMagar in this study correlates with the study Ezeanya, *et al.* Who reported a sensitivity of 97.8% [29] Vercauteren *et al.* (Vercauteren *et al.*, 1999) reported a sensitivity of 96.9%. Also Ravi *et al.* reported 94.89% sensitivity and 75.91% specificity for DDST [39].

The inclusion of Cefpodoxime 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 [17].

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 still susceptible to these drugs [22] Adham *et al.*, observed 98.8% 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.

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 [22,25].

Amoxicillin/clavulanic acid as an alternative treatment to carbapenems for infections involving ESBL-producing organisms remains debated [23].

In this study, a high rate of susceptibility (80%) was observed for Amoxicillin/clavulanic acid. In a Randomized controlled trial conducted by Yu Bin Seo, it was reported that Piperacillin/tazobactam (TZP) which is also a beta-lactamase inhibitor like Amoxicillin/clavulanic acid is effective in the treatment of UTI caused by ESBL-*E. coli* when the in vitro test indicates susceptibility [24] 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 [5]. Differences in susceptibility patterns of organisms and frequency of infection between hospitals and communities make knowledge of local prevalence and resistance data extremely important [30]. 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.

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 has a high sensitivity and specificity making it reliable for ESBL detection. This medium allows for easy differentiation of different bacteria based on colony colouration.

Table 4 Antibiotic Susceptibility profile of ESBL producing *Acinetobacter baumannii* from Ikot Ekpene, Eket and Oron General Hospital

| Antimicrobials | Ikot Ekpene | Eket | Oron |
|----------------|-------------|------|------|
| | | n=3 | =9 |

| µg | n=20 | | S(%) | R(%) | S(%) | R(%) |
|---------|-------|--------|------|--------|--------|------|
| | S(%) | R(%) | | | | |
| 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) |

| | | | | | | |
|----------|-------|-------|-------|-------|--------|--------|
| 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-ofloxacin, CAZ-Ceftazidime, ATM-Azetronam, IPM-Imipenem, Aug-Augmentin, AK-Amikacin

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

Table 5:Antibiotic susceptibility profile of *Enterobacter cloacae* from Ikot Ekpe, 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) |



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

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

Table6:Antibiotic susceptibility profile of ESBL producing *Proteus mirabilis* the three study Area

| Antimicrobials µg | n=5 | Ikot Ekpe n=11 | | Eket n=16 | | |
|----------------------|--------|-------------------|--------|--------------|--------|--------|
| | | S(%) | R(%) | R(%) | S(%) | R(%) |
| 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) |

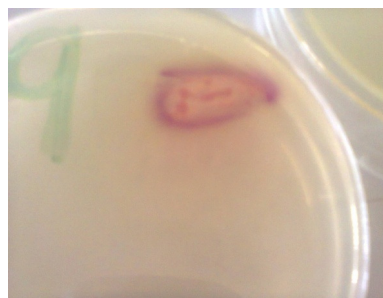


Fig. 2 Culture plate of clinical isolate of *Escherichia coli* from urine showing distinctive pink colony colouration on CHROMagar ESBL agar was interpreted as positive for ESBL production



Fig. 3 Culture plate of clinical isolate of *Acinetobacter baumannii* 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. cloacae* from urine showing blue colonies on CHROMagar ESBL

References

1. Ben-Ami, R., Rodríguez-Baño, R, Arslan, H, J., et al. (2009). A multinational survey of risk factors for infection with extended-spectrum beta-lactamase-producing enterobacteriaceae in nonhospitalized patients. *Clin Infect Dis*; 49:682.
2. Boyd, D. A., Tyler, S, Christianson, S., McGeer, A., Muller, M. P., Willey B. M. et al (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
3. Bush, K. (2013). The ABCD's of β -lactamase Nomenclature. *J Infect Chemother*, 19:549-59.
4. 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.
5. Datta, P., Thakur, A., Mishra, B. and 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.
6. Sridhar Rao, P. N. (2015). Extended Spectrum Betalactamases-A Comprehensive Review, www.microrao.com Pp.18
7. 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
8. Katsanis, G. P., Spargo, J. Ferraro, M. J. Sutton, L. and G. A. Jacoby. (1994). Detection of Klebsiella pneumoniae and Escherichia coli strains producing extended-spectrum b-lactamases. *J. Clin. Microbiol.* 32:691-696.
9. Jarlier, V., Nicolas, M.-H. Fournier, G. and 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.
10. 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 Enterobacteriaceae. *J Clin Microbiol.* 50(4):1140-1146.
11. 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.
12. Ongut, G., Dalogle, A. E., Bayson BO, Daglar D, Ogunc D, Sekercioglu, A. O.(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.
 13. Naas, T. Oxacelay, C., Nordmann, P.(2007). Identification of CTX-M-type extended-spectrum-β-lactamase genes using real time PCR and Pyro sequencing. *Antimicrob Agents Chemother.* 51:223-230.
 14. Ravi, S. G., Namratha, W. N., Chandrasekhar, M. R.(2011). Comparison of Disc diffusion methods for the Detection of extended -spectrum beta lactamase Producing Enterobacteriaceae. *J Lab Physican.*; 3(1):33-36.
 15. Ho, P. L., Tsang, D. N., Que, T. L., Ho, M. and Yuenk. and Y.(2000) Comparison of screening methods for detection of extended spectrum beta -lactamase and their prevalence among *E. coli* and *Klebsiella spp.* in Hong Kong. *APMIS.*108(3):237-240.
 16. 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.
 17. Vercauteren, E. Descheemaeker, P. Ieven, M., Sanders, C. C. and Goossens, H.(1997). Comparison of screening methods for detection of extended-spectrum beta-lactamases and their prevalence among blood isolates of *Escherichia coli* and *Klebsiella spp.* in a Belgian Teaching Hospital. *J Clin Microbiol.*;35(9):2191–2197.
 18. Hosain Zadegan, H., Ramazanadeh, 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.
 19. Irajian, G. Jazayeri-Moghadas, A. and Beheshti A.(2009). Prevalence of extended-spectrum beta-lactamase positive and multidrug Resistance pattern of *Escherichia coli* and *Klebsiella pneumoniae* isolates, Semnan, Iran. *Iran J Microbiol.*;1:49–53.
 20. Adham, A. T., Amna, S. Ahmad, J., and Yusuf, D.(2018) Prevalence and Risk Factors of Extended Spectrum Beta-Lactamase-Producing Uropathogens among UTI Patients in the Governmental Hospitals of North West Bank: A Cross-Sectional Study *J Infect Dis Preve Med* , 6:2
 21. Fernando, M. Luke, W., Miththinda, J., Wickramasinghe, R., Sebastampillai, B., et al. (2017) Extended spectrum beta lactamase producing organisms causing urinary tract infections in Sri Lanka and their antibiotic susceptibility pattern-A hospital based cross sectional study. *BMC Infect Dis* 17: 138.
 22. Guet, R. H, Tomini, E., Emirian, A., Join, L. O, Lécuyer H, et al. (2017) Piperacillin/tazobactam 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* 1: 62-66.
 23. Seo, Y., Lee, J., Kim, Y., Lee, S., Lee, J. et al. (2017) Randomized controlled trial of piperacillin-tazobactam, cefepime and ertapenem for the treatment of urinary tract infection caused by extended-spectrum beta-lactamase-producing *Escherichia coli* . *BMC Infect Dis* 17: 404
 24. Gonzalez, P. M, Torre, C. J, Rivera, E. F, Pontes, M. A, Lopez, C. L, et al. (2015) Gentamicin therapy for sepsis due to carbapenem-resistant and colistin-resistant *Klebsiella pneumoniae*. *JAntimicrob Chemother* 70: 905-913.
 25. Bell, J. M., Turnidge, J.D. Gales, A.C. Pfaller, M.A. Jones, R.N. SENTRY APAC and Study Group, (2002).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.
 26. 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, P.A., USA: National Committee for Clinical Laboratory Standards, 1999.
 27. Grude, N., Tveten , Y. and Kristiansen, B.E. (2001). Urinary tract infections in Norway: Bacterial Etiology lactamases in bloodstream isolates of *Klebsiella pneumoniae*:isolates harbor plasmid-mediated and susceptibility, a retrospective study of clinical isolates. *Clin. Microbiol. Infect*, 7: 543-7.
 28. Ezeanya, C. C. Agbakoba, N. R. Ejike, C. E and 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 & Medical Research 21(12): 1-11.
 29. Shahid, M., Malik, A., Akram, M. Agrawal, L.M. Khan, A.U. and Agrawal, M. (2008). Prevalence of extended spectrum beta-lactamase (ESBL)- producing clinical isolates in the 2008.Prevalent phenotypes and antibiotic resistance in *Escherichia coli* and *Klebsiella pneumoniae* at an Indian tertiary care hospital: plasmid-mediated cefoxitin resistance Intl. J. Infectious Dis., 12: 256-264.

30. Rossolini, G. M., D' Andrea, M. M. and Mugnaili, C. (2008) The spread of CTX-M-type extended-spectrum beta-lactamases. *Clin. Microbiol Infect* 2008;14:33-41.
31. 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.
32. Brown, A., O' Brien, S., Cocks, S., Beares, J., Dimmer, S. A. (2011). laboratory evaluation of chromogenic screening media for the detection of ESBL producing bacteria. United Kingdom: Oxoid Ltd.1
33. Huang, T. D., Bogaerts, P., Berhin, C., Guisset, A. and Glupczynski, Y. (2010). Evaluation of Brilliance ESBL agar, a novel chromogenic medium for detection of ESBL-producing Enterobacteriaceae. *J Clin Microbiol*; 48(6):2091-2096.
34. Wong, B. A. (2001). Therapeutic Challenges associated with extended spectrum beta-lactamase producing *E. coli* and *Klebsiella pneumoniae*. *Pharmacotherapy* 21:583-92.
35. Asad, U. K., Lubna, M. and Raffaele, 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.
36. Mohanty, S. Gaiind, R. Ranjan, R. and 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
37. Glupczynski, Y., Berhin, C., Bauraing, C. and 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.
38. 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.

UNDER PEER REVIEW

