

## ORIGINAL ARTICLE

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**DETERMINATION OF THE ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF EXTENDED SPECTRUM BETA LACTAMASE (ESBL) PRODUCING AND THE NON-ESBL PRODUCING STRAINS OF *ESCHERICHIA COLI***

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## ABSTRACT

**Background:** The extended spectrum beta lactamases producing bacteria are bacteria of great concern among Gram negative bacilli. *Escherichia coli* stand out as major carrier of this enzyme. The appropriate control of this resistance pattern depends on using the antimicrobial regimen of best choice. Therefore the value of the susceptibility profile of organism harboring this enzyme cannot be overemphasized.

**Objectives:** To determine the antimicrobial susceptibility of extended spectrum beta lactamases (ESBL) producing and the non-ESBL producing strains of *Escherichia coli* from clinical isolates of *Escherichia coli* in University of Maiduguri Teaching Hospital.

**Methodology:** Confirmed variants of *Escherichia coli* were screened and confirmed for ESBL possession. Subsequently, modified Kirby Bauer method was utilized to test for antibiotic susceptibility using the commercially available Oxoid single disc for some major antibiotics.

**Results:** A total of 172 strains of *Escherichia coli* were identified during the study period. Out of this number; 131 were identified as ESBL positive while a total of 41 were ESBL negative. The highest sensitivity for both the ESBL positive and ESBL negative strains of *Escherichia coli* was observed with Imipenem followed closely by Gentamicin.

**Conclusion:** The study reveals narrow choice of antibiotics for the ESBL positive isolates of *Escherichia coli* although Imipenem antibiotic still retains its sensitivity.

**Key words:** Cephalosporins, Resistance, Maiduguri, Nigeria.

**LA DETERMINATION DE LA MODELE DE SENSIBILITE ANTIMICROBIEN BETA LACTAMASE A SPECTRE ETENDU (BLSE) SOUCHES PRODUCTRICES ET NON - SOUCHES PRODUTRICES DE BLSE D'*ESCHERICHIA COLI*.**

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#### RESUME :

**Contexte :** Les bêta lactamases à spectre étendu produisant bactérie sont des bactéries de grande inquiétude parmi les bacilles à Gram négatif. *Escherichia coli* se démarque en tant que porteur de cette enzyme. Le contrôle approprié de cette modèle de résistance dépend d'usage de régime antimicrobien de la meilleur choix. Donc, la valeur du profil de sensibilité d'organisme hébergeant cette enzyme ne peut être soulignée.

**Objectifs :** Déterminer la sensibilité antimicrobienne des bêta lactamases à spectre étendu (BLSE) souches productrices et non productrices de BLSE d'*Escherichia coli* des isolats cliniques d'*Escherichia coli* à l'Université hôpital d'enseignement, Maiduguri.

**Méthodologie :** Des variantes confirmées d'*Escherichia coli* ont été examinées et confirmées pour la possession de BLSE. Ensuite, méthode Kirby Bauer modifiée a été utilisée pour analyser la sensibilité antibiotique en utilisant le disque unique Oxoid disponible dans le commerce pour des antibiotiques majeurs.

**Résultats :** Un total de 172 souches d'*Escherichia coli* ont été identifiées au cours de la période d'étude. Sur ce nombre, 131 étaient identifiées en tant que BLSE positifs alors qu'un total de 41 étaient BLSE négatifs. La plus haute sensibilité pour les souches BLSE positifs et les souches BLSE négatifs d'*Escherichia coli* était observées avec imipenème suivi étroitement par Gentamicine.

**Conclusion :** L'étude révèle un choix limité des antibiotiques pour les isolats BLSE positifs d'*Escherichia coli* bien que l'antibiotique imipenème conserve encore sa sensibilité.

**Mots - clés :** Céphalosporines, Résistance, Maiduguri, Nigeria.

#### INTRODUCTION

Extended spectrum beta lactamases are plasmid mediated enzymes that are capable of conferring bacterial resistance to the penicillins, first, second and third generation cephalosporins and aztreonam. (1) They do this by hydrolysis of these antibiotics but they are inhibited *in vitro* by beta lactamase inhibitors such as clavulanic acid. (1) The beta lactams are the most commonly used antimicrobial drugs accounting for almost 50% of antibiotic use. (2)

ESBLs have been reported worldwide in many different genera of *Enterobacteriaceae* and *Pseudomonas aeruginosa*. (3) However, they are more common in *Klebsiella pneumoniae* and *Escherichia coli*. (4) Carbapenems are the drugs of first choice in most infections due to ESBL producers but they need to be used judiciously to remain efficacious. (5)

This study aims to determine the antimicrobial susceptibility pattern of the ESBL and the non ESBL producing strains of *Escherichia coli* in our locality with the aim of providing a rationale antibiotic profile.

#### METHODOLOGY

**Study Area:** The study was carried out in the department of Medical Microbiology and Parasitology University of Maiduguri Teaching hospital from January to June, 2014.

**Study Design:** Descriptive, Observational and Cross-sectional in design

**Sample Size:** A total of 172 strains of *Escherichia coli* were isolated during the study period

**Clinical Specimen:** The isolates were obtained from the following specimens; wound swabs, wound biopsies, aspirates, urine, cerebrospinal fluid, blood culture, sputum, ear swabs and eye swabs that were submitted to Medical Microbiology Department of University of Maiduguri Teaching Hospital (UMTH) for routine analysis.

**Sampling Method:** Non-probability, convenient sampling was used. All specimens that yielded the growth of *Enterobacteriaceae* during the study period were utilized.

**Bacterial Culture and Preliminary Identification:** The specimens were inoculated on MacConkey agar. They were then incubated at 18-24 hours under aerobic atmosphere at 37 °C. Any isolate with the typical morphology of *Escherichia coli* is picked for additional studies. The morphology of *Escherichia coli* on MacConkey is that of a lactose fermenter; producing pink colored colonies that are 1-4 mm in diameter and slightly mucoid. (6) Gram staining and motility testing was done. *Escherichia coli* are Gram negative rods and motile.

**Bacterial Confirmation:** Suspected isolates of *Escherichia coli* were confirmed by the Microbact Gram negative identification system 24E™ (Oxoid) according to the manufacturer's instructions.

**ESBL Screening:** Isolates were screened for ESBL production by using disc diffusion of cefotaxime (CTX) and ceftazidime (CAZ) placed on inoculated plates containing Muller Hinton agar according to Clinical and Laboratory Standard Institutes (CLSI) recommendations. (7)

**ESBL Confirmation:** Double disk synergy test was performed by placing ceftazidime (30 µg) and cefotaxime (30 µg) at a distance of 20 mm (centre to centre) from a disc containing amoxicillin (20 µg) plus clavulanate (10 µg); (augmentin; 30 µg). Positivity for ESBL production was interpreted if there is a ≥ 5mm diameter for either antimicrobial agent tested in combination with clavulanic versus its diameter when tested alone as recommended by CLSI. (7)

**Susceptibility Testing:** The modified Kirby Bauer method was utilized. Antibiotic sensitivity testing was done using the commercially available Oxoid single disc comprising of ampicillin(10µg), amoxicillin/ clavulanic acid(10/20 µg), ciprofloxacin(5µg), trimethoprim/ sulphamethoxazole(1.25/23.75µg), gentamicin(10µg), ceftazidime(30µg), cefotaxime(30µg) and imipenem(10µg). The test was carried out on Mueller Hinton agar according to CLSI guidelines.(7)

**Ethical Consideration:** The study protocol was reviewed and approved by the Ethical Review Committee of UMTM

**Data Analysis:** Data analysis was carried out using the Microsoft Excel, computer software.

## RESULTS

A total of 172 isolates of *Escherichia coli* were identified during the study period. Out of this number 60 were screened as ESBL positive while 112 were screened as ESBL negative. However, following the confirmatory testing; 41 were identified as ESBL producers while 131 were identified as ESBL negative. The distribution of the various isolates based on the specimen is as shown in Table 1.

The antimicrobial susceptibility profile of the 131 ESBL negative *Escherichia coli* were as shown in Figure 1.

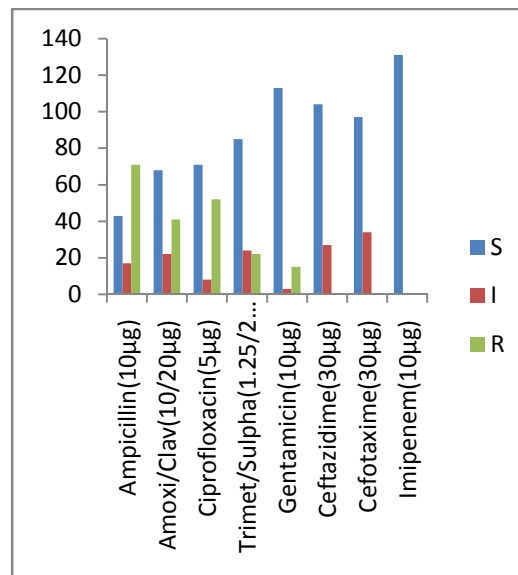
All the 131(100%) isolates were sensitive to imipenem. Gentamicin was the second most sensitive antimicrobial agent with 113(86%). The highest resistance of 71(54%) was observed with ampicillin, followed by ciprofloxacin with 52(40%).

The antimicrobial susceptibility profile of the 41 ESBL positive *Escherichia coli* were as shown in Figure 2. The highest sensitivity of 37(90%) was observed with imipenem, followed by gentamicin with 27(66%). However, the highest resistance was observed with ceftazidime and cefotaxime with 41(100%) and 35(85%) respectively.

**TABLE 1: DISTRIBUTION OF THE 172 ISOLATES OF *ESCHERICHIA COLI* BASED ON SPECIMEN ISOLATED**

Specimen	No	%
Swabs	39	22.7
Urine	68	39.5
Blood	18	10.5
CSF	16	09.3
Pus	31	18.0
<b>Total</b>	<b>72</b>	<b>100</b>

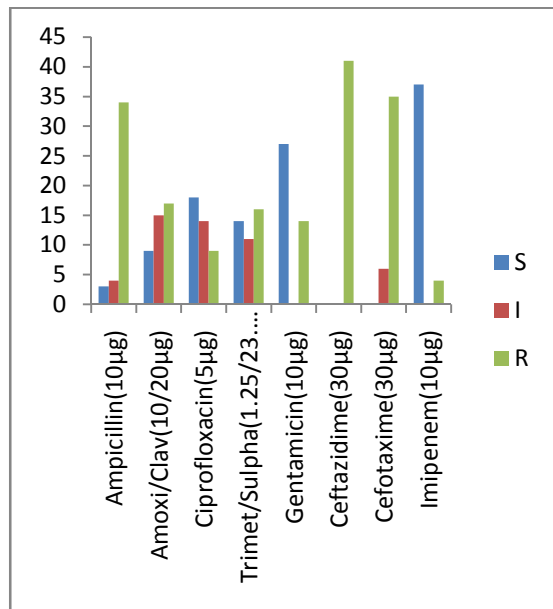
**FIGURE 1: SUSCEPTIBILITY PATTERN OF ESBL NEGATIVE STRAINS OF *ESCHERICHIA COLI* (N=131)**



**Legend:** Amoxi/Clav = Amoxicillin/Clavulanic acid; Trimet/Sulpa = Trimethoprim/Sulphamethoxazole

**S = Sensitive; I = Intermediate; R = Resistant**

**FIGURE 2: SUSCEPTIBILITY PATTERN OF ESBL POSITIVE STRAINS OF ESCHERICHIA COLI (N=41)**



**Legend:** Amoxi/Clav = Amoxicillin/Clavulanic acid; Trimet/Sulpa = Trimethoprim/Sulphamethoxazole

**S = Sensitive; I = Intermediate; R = Resistant**

## DISCUSSION

This study recorded a low resistance to imipenem but a high resistance to ampicillin and ciprofloxacin for the ESBL negative strains of *Escherichia coli*. This implies that imipenem are effective for the treatment of the ESBL positive strains but ampicillin and ciprofolaxacin are ineffective and may result in treatment failure if used. Nwadioha and colleagues reported a similar finding, (8) although it's in contrast to the work of Olanitola where ESBL negative *Escherichia coli* were found to have low resistance to ciprofloxacin and amikacin. (9)

This study recorded a low resistance of imipenem, gentamicin and ciprofloxacin but a high resistance of ceftazidime, cefotaxime and ampicillin for the ESBL positive strains of *Escherichia coli*. The finding is in agreement with the work of Kadar *et al* (10) in 2005, where 89% of the ESBL producers were susceptible to imipenem and meropenem. Even though, a different finding was noted by Okesola and Ori (11) in a study, to determine the susceptibility of carbapenems (imipenem and meropenem) and amikacin against the

ESBL-producing *Klebsiella* isolates. The finding of a high sensitivity to imipenem is likely due to the fact that this antibiotic is expensive and not commonly prescribed hence selection for resistance is minimal compared to the other readily available antimicrobials. The clinical significance of the finding is that ampicillin and cephalosporins are not likely to be successful in treating the infection caused by ESBL positive isolates of *Escherichia coli*. However, trimethoprim/sulphamethoxazole can be used with caution when treating empirically for the urinary isolates of *Escherichia coli* as it has a relative sensitivity for ESBL positive *Escherichia coli* from this study.

Carbapenem (imipenem) class antibiotic was the most reliably effective empirical therapies for infection with these organisms, even though worrisome fact of resistance to imipenem of 9.8% (4/41) for ESBL positive *Escherichia coli* was observed.

The antibiotic susceptibility of the ESBL positive isolates of *Escherichia coli* revealed a limited group of effective antibiotics for the treatment of infections caused by this organism. The multi drug resistance observed in ESBL positive isolates might reflect the fact that, ESBLs have been associated with co-resistance to other agents including trimethoprim-sulphamethoxazole, gentamicin and ciprofloxacin. (12)

Cephalosporins, frequently used against *Enterobacteriaceae*, were widely recommended and abused in the past decade. (13) This study revealed the already documented resistance of ESBLs to cephalosporins in addition to likely quinolones co-resistance. This happens because some of the patients had exposure to both cephalosporins and quinolones or both resistances could be simultaneously adopted by plasmid-mediated mechanisms. (14) Therefore these agents cannot be use for empirical treatment of infections due to ESBLs producing organisms.

**CONCLUSION:** The study revealed a pan resistance of ESBL positive *Escherichia coli* isolates to the beta lactam agents in addition to the likely quinolone co-resistance. However, Imipenem retain its sensitivity for the ESBL positive isolates. In view of this drug resistance the practice of routine ESBL testing along with the use of the appropriate antibiotic following a conventional antibiogram would be useful for all cases which will help in the proper treatment of the patient and also prevent further development of bacterial drug resistance.

## REFERENCES

1. Johann DD, Pitout DK, Kevin BL. Extended spectrum beta lactamases producing *Enterobacteriaceae*: an emerging public health concern. *Lancet Infect Dis* 2008; 8: 159-166.
2. Coque T.M, Baquero F, Canton R. Increasing Prevalence of ESBL. *Eurosurveillance* 2008; 13: 347-349.
3. Falagas ME, Karageorgopoulos DE. Extended spectrum beta lactamase producing organisms. *J Hosp Infect* 2009; 73(4): 345-354.
4. Agrawal P, Ghosh A, Kumar S, Basu B, Kapil K. Prevalence of extended spectrum beta lactamases among *Escherichia coli* and *Klebsiella pneumoniae* isolates in tertiary care hospital. *Indian j Pathol Microbiol* 2008; 51: 139-142.
5. Mody RM, Erwin DP, Summers AM, Carrero HA, Selby EB, Ewell AJ, *et al.* Ertapenem susceptibility of extended spectrum beta lactamases producing organisms. *Ann of Clin Microbiol Antimicrob* 2007; 6:6.
6. Farmer JJ. Manual of Clinical Microbiology. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, and Tenover RH, editors. 3rd ed. Washington DC: Lange Publishers; 1999. *Enterobacteriaceae*: Introduction and Identification; p. 442-50
7. CLSI. Performance standards for antimicrobial susceptibility testing: twenty-first informational supplement. CLSI document M100-S21. Wayne, PA: Clinical and laboratory standard institute; 2011.
8. Nwadioha SI, Nwokedi EE, Jombo GTA, Kashibu E, Alao OO. Antimicrobial susceptibility pattern of uropathogenic bacterial isolates from community and hospital acquired urinary tract infections in a Nigerian tertiary hospital. *Internet J Infect Dis* 2010; 8(1): 10-15.
9. Olonitola OS, Olayinka AT, Inabo HI, Shaibu AM. Production of extended spectrum beta-lactamases of urinary isolates of *Escherichia coli* and *Klebsiella pneumoniae* in Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. *Int J Biol Chem Sci* 2007; 1(2); 181-185.
10. Kader AA, Angamathu K. Extended spectrum beta lactamases in urinary isolates of *Escherichia coli*, *Klebsiella pneumoniae* and other Gram negative bacteria in a hospital in eastern province, Saudi Arabia. *Saudi Med J* 2005; 26(6): 956-969.
11. Okesola AO, Oni AA. Prevalence of extended spectrum beta lactamases producing *Klebsiella* in a tertiary care hospital in South West Nigeria. *Int J Pharm Biomed Sci* 2012; 3(4): 148-151.
12. Pitout JD. Multi resistant *Enterobacteriaceae*: new threat of an old problem. *Expert Rev Anti Infect Ther* 2008; 6:657-669.
13. Khongwong M, Chatgaew C, Chaita S. Risk factors of extended-spectrum beta lactamases producing *Enterobacteriaceae* bacteremia in Thai emergency department: a retrospective case-control study. *Asian Biomed* 2011; 5(1): 129-138.
14. Oktem IM, Gulay Z, Bicmen M, Gur D. HITIT Project Study Group: A prevalence in extended-spectrum beta-lactamases-positive *Enterobacteriaceae* isolates from Turkey. *Jpn J Infect Dis* 2008; 61:13-17.