

## Phenotypic Detection of Extended Spectrum $\beta$ -lactamase-producing Bacteria from Selected Hospital Contact Surfaces

Lawal Garba<sup>1\*</sup>, Nune M. Chiroma<sup>1</sup>, Jessica Justine<sup>1</sup>, I. Yusuf<sup>2</sup>, A.B. Inuwa<sup>2</sup> and Abdulrahman Idris<sup>3</sup>

<sup>1</sup>Department of Microbiology, Faculty of Science, Gombe State University, PMB 127, Tudun Wada Gombe, Gombe State, Nigeria.

<sup>2</sup>Department of Microbiology, Faculty of Life Sciences, Bayero University, Kano, PMB 3011, Kano State, Nigeria.

<sup>3</sup>Department of Microbiology, Kaduna State University, P.M.B. 2339, Kaduna, Kaduna State Nigeria.

\*Corresponding author:

Lawal Garba  
Department of Microbiology,  
Faculty of Science,  
Gombe State University,  
PMB 127, Tudun Wada Gombe,  
Gombe State,  
Nigeria.  
Email: [lagarpak@gmail.com](mailto:lagarpak@gmail.com)

### HISTORY

Received: 20<sup>th</sup> Jan 2020  
Received in revised form: 30<sup>th</sup> March 2020  
Accepted: 1<sup>st</sup> April 2020

### KEYWORDS

phenotypic detection  
ES $\beta$ L  
Gram-negative bacteria  
drug resistance  
contact surfaces

### ABSTRACT

Community and hospital acquired infections caused by drug resistant organisms especially the extended spectrum  $\beta$ -lactamase producers among members of the family Enterobacteriaceae have been on a global increase. Such infections could lead to an increased healthcare costs, morbidity and mortality. The Extended Spectrum  $\beta$ -lactamases are enzymes produced by some bacteria to inactivate group of  $\beta$ -lactam antibiotics such as penicillin and its derivatives as well as broad spectrum cephalosporins including monobactams. The enzymes have been the key source of multidrug resistance among many Gram-negative bacteria. Previous studies conducted on Extended Spectrum  $\beta$ -lactamase (ES $\beta$ L) in Gombe Specialist Hospital focused more attention on the occurrence of the ES $\beta$ L producing bacteria in clinical samples. This study aimed at phenotypic detection of ES $\beta$ L producing bacteria from some selected contact surfaces in the same hospital for the first time to the best of our knowledge. One hundred and fifty (150) samples were collected from contact surfaces such as door handles, wheelchairs, ATM machines etc. using sterile swab sticks. The samples were cultured on nutrient and MacConkey agar plates to isolate bacterial contaminants. The bacterial isolates were identified using standard procedures. Gram negative isolates were screened for the potential ES $\beta$ L producers using CLSI breakpoint and phenotypically confirmed using double disc synergy test (DDST). The results obtained confirmed high ES $\beta$ L producers among *E. coli* with 20 (44.40%) followed by *K. pneumoniae* with 15 (33.30%) and then *P. mirabilis* having the least confirmed isolates. In conclusion, the ES $\beta$ L producers detected from the contact surfaces suggest the adoption of more personal and environmental hygiene practices to limit the spread of drug resistant organisms and possible outbreak of nosocomial infections.

### INTRODUCTION

Contaminated hospital contact surfaces such as equipment, water supplies and medicine have been described as the common sources of infection outbreaks due to drug resistant pathogens. General guidance aimed to preventing and controlling contamination has been made available by healthcare departments, specialist societies and manufacturers. However, the role played by contaminated environmental surfaces towards the development of healthcare related infections is worrisome

[1]. Hospital environment plays a crucial role in the transmission of drug resistant organisms which increase the risk of acquiring nosocomial infections associated with a high morbidity and mortality [2]. Antibiotic resistance due to pathogenic bacteria has been on the increase due to a number of factors such as versatility of genetic system of microorganisms especially under stress condition of control agent(s). Bacteria develop resistance to antibiotics through several mechanisms such as mutation and exchange of genetic materials using plasmids, transposons and chromosomes which collectively alter the cell membrane of the

target cells to prevent the entry of control agents or simply by developing substitute enzymes which are entirely not the drug target or produce drug inactivating enzymes such as ESβLs [3-4].

The ESβL are used by some bacteria to hydrolyse group of β-lactam antibiotics such as penicillin and its derivatives as well as broad spectrum cephalosporins including monobactams [4]. These enzymes have been the key source of multidrug resistance among Gram-negative bacteria and could be categorised into diverse classes like Sulphydril variable gene (SHV-2) and Temoniera gene (TEM-3) representing the notable ones reported in Germany as far back as 1982 and 1987, respectively. Subsequent subtypes were derived from the SHV-2 and TEM-3. Other relevant and widely spread ESβL phenotype reported globally in *Escherichia coli* are referred to as cefotaximases (CTX-M). Five (5) main groups of certain CTX-M containing over 100 various CTX-M types have been phenotypically confirmed [5-6]. Many enteric Gram-negative rods such as *Morganella morganii*, *Escherichia coli*, and *Klebsiella pneumoniae* are known to produce ESβLs as established by previous reports [4,7]. The worldwide distribution of *E. coli* strains that produce CTX-M class of extended-spectrum β-lactamases has become worrisome as a result of new microbiological and epidemiological features [8]. Surely, *E. coli* remains a commensal organism in the gastrointestinal tract of human beings and has been the most commonly detected organism in different community and hospital settings. Therefore, an increased detection of multidrug resistant *E. coli* could result into overuse of the available potent antibiotics like carbapenems and probably the emergence of carbapenem-resistant organisms [9].

According to a report by Ibrahim *et al.* [10], a co-production of carbapenemase and ESβL was detected from both *E. coli* and *K. pneumoniae* isolated from different clinical samples. Other study has confirmed high occurrence of ESβL producing *E. coli* and *K. pneumoniae* from clinical samples [11] while another report showed that *E. coli* and *K. pneumoniae* were among the multidrug resistant bacteria detected from various surfaces of health care environment [2]. In this study, we report the detection of ESβL producing bacteria from some contact surfaces in Gombe State Specialist Hospital for the first time to the best of our knowledge.

## MATERIALS AND METHODS

### Sample Collection

A total of one hundred and fifty (150) samples were collected from different contact surfaces such as door handles, wheelchairs, stretchers, sinks, and ATM machine in Gombe State Specialist Hospital, Gombe State, Nigeria. The samples were brought to Department of Microbiology, Gombe State University for analysis.

### Isolation and Identification of Bacteria

The samples were cultured on MacConkey agar and nutrient agar plates prepared according to manufacturer's instruction and incubated for 24 h at 37 °C. Cultural and morphological characteristics of the bacterial colonies were studied and identified the Gram negative isolates using some biochemical tests such as indole test, urease test, citrate utilization test, catalase test, triple sugar ion test, and motility test according Cheesbrough, 2006 [12] as described by Garba *et al.* [13].

### Standardization of Inoculum

The Gram-negative isolates were cultured on Mueller Hinton Agar plates and incubated for 24 h at 37 °C. Following the incubation, a few loopful of colonies were dissolved in sterile normal saline (3-4 ml) using sterile wire loop to match 0.5 McFarland standards for sensitivity test according to Cheesbrough, 2006 [12] as quoted previously [5,13].

### Screening of Isolates Based on Clinical Laboratory

#### Standard Institute (CLSI) breakpoint test

Sensitivity of the standardized inocula of the isolates to Cefotaxime (CTX 30 µg, Oxoid England) and Ceftazidime (CAZ 30 µg, Oxoid England) discs was investigated on prepared Mueller Hinton agar plates as described in Kirby Bauer methods and suggested by NCCLS, 1999. Sterile swap sticks were immersed into test tubes containing the test organism. Excess fluid was removed by pressing the sticks at the wall of each tube prior to inoculating the Mueller Hinton agar plates by streaking the surface of the media evenly in three directions. The plates were left to stand for 3-5 min for the surface of the agar to dry. Sterile forceps was used to place the Oxoid discs on the inoculated plates. Within 30 min of disc application, the plates were incubated aerobically for 24 h at 37 °C after which zones of growth inhibition were recorded.

#### Phenotypic Detection of ESβL Production using Double Disc Synergy Test

The suspected ESβL producing isolates from the CLSI breakpoint test were subjected to double disc synergy test for confirmed detection of ESβL according to standard method described by Jarlier *et al* [14]. In this test, standardized inocula of Gram-negative bacteria were swabbed onto Mueller Hinton agar plates. A susceptibility disc of Amoxicillin-Clavulanate commonly known as Augmentin (AMC 30 µg, Oxoid England) was placed at the centre of each plate and discs containing cefotaxime and ceftazidime were placed 15 mm alongside the central Augmentin disc. After 30 min of pre-incubation time at room temperature to enable pre-diffusion of the antibiotics into the medium, the plates were incubated aerobically for 24 h at 35 °C after which zones of inhibition were read.

## RESULTS

### Isolation and identification of bacteria

A total of one hundred and fifty (150) bacteria have been isolated from different hospital contact surfaces as described in the methodology section. The bacteria were identified based on cultural characteristics, Gram staining and biochemical tests. According to Gram staining reactions, the bacteria were separated into sixty (60) Gram positive and ninety (90) Gram negative. The Gram-negative isolates were identified as *Escherichia coli* (35), *Klebsiella pneumoniae* (29), and *Proteus mirabilis* (26).

### Occurrence of Suspected ESβL-Producing Bacteria

The ninety (90) Gram negative bacteria comprising of *Escherichia coli* (35), *Klebsiella pneumoniae* (29), and *Proteus mirabilis* (26) have been screened for ESβL production using CLSI breakpoint. Eighty (80) isolates of these bacteria were found to be positive for the screening test with *E. coli* having higher number of suspected positive isolates (30), followed by *K. pneumoniae* (26) and then *P. mirabilis* (24) (Fig. 1).

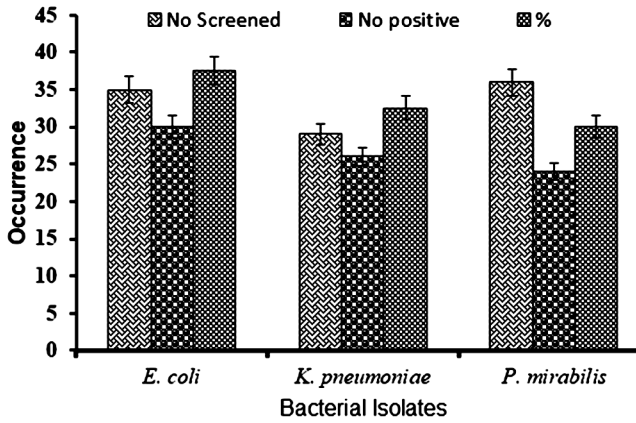


Fig. 1. Suspected ESBL Producers among the Isolates Based on CLSI.

**Phenotypic confirmation of ESBL-Production among the Gram-negative Bacteria**

The eighty (80) positive bacterial isolates from the screening tests have been subjected to phenotypic confirmation using double disc synergy test as described in the methodology. The confirmatory test revealed that forty five (45) isolates were confirmed ESBL producers with *E. coli* having the highest number of confirmed cases corresponding to 20 (44.40%) followed by *K. pneumoniae* with 15 (33.30%) and then *P. mirabilis* having the least confirmed isolates with 10 (22.20%) as shown in Fig. 2. A suspected ESBL producing organisms is confirmed positive when it produces an increased zone of growth inhibition of  $\geq 5$  mm with amoxicillin/ clavulanic acid (Augmentin) over any of the screening antibiotics placed centre to centre on agar plates in the double disc synergy test (DDS) as demonstrated in Fig. 3.

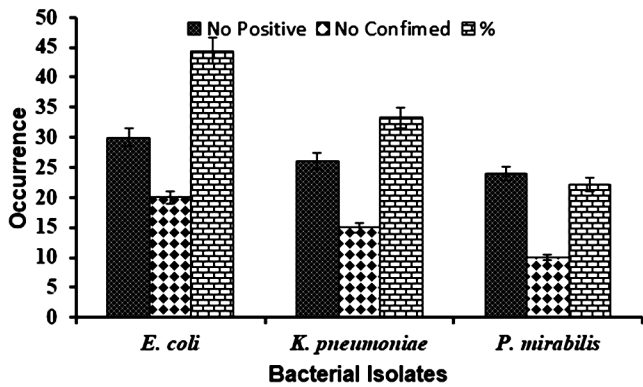


Fig. 2. Confirmed Phenotypic Detection of ESBL Production among the bacteria.



Fig. 3. Confirmation of ESBL production using Double Disk Synergy Test (DDST). Standardised inoculant of *Escherichia coli* (a), *Klebsiella pneumoniae* (b) and *Proteus mirabilis* (c) were swabbed on Mueller Hinton Agar plates and placed Ceftazidime and Cefotaxime discs 5 mm centre to centre from the central Augmentin disc. The plates were incubated for 24 hours at 37 °C and recorded the zones of growth inhibition. The experiments were conducted in replicates and calculated the average.

**DISCUSSION**

Nosocomial infections constitute the key public health issue due largely to their high incidence, severity, as well as economic and social costs coupled to difficulty of their control. World Health Organization (WHO) shows that about 8.7% of patients acquire nosocomial infections, with over 1.4 million people estimated to have hospital related infectious complications globally. In general, nosocomial infections may originate from the patient’s own normal flora (endogenous source of infection), or may be from pathogens acquired from hospital personnel, the hospital environment such as air, water, surfaces or even other patients [2,15].

Contamination of contact surfaces may be determined by certain characteristics whether the surface is smooth, porous or rough. It could also be characterised by the physical state of such surface being it wet, dry, new or old. Contaminated surfaces represent the major ecological niche of bacteria capable of producing biofilms. These organisms could last on hospital surfaces for quite long periods of time ranging from a few days to about ninety (90) days. Several reports have confirmed hospital environments as a reservoir for contamination with multidrug-resistant bacteria [16]. Environment plays a significant role in transmitting multidrug resistant organisms which suggests an increased risk of acquiring nosocomial infections. Moreover, resistance to potent antimicrobial agents has escalated the rate of morbidity and mortality due to hospital acquired infections [17]. Detection of Extended Spectrum  $\beta$ -lactamases (ESBL) particularly from hospital contact surfaces is very essential for deterrence of spread of ESBL producers through cross-transmission as well as for epidemiological reasons [8].

In this study, forty-five (45) Gram negative bacteria have been isolated from hospital contact surfaces and phenotypically confirmed to be Extended Spectrum  $\beta$ -lactamase producers using Double Disc Synergy Test. As shown in Fig. 2, *E. coli* had the highest percentage of occurrence corresponding to 44.40% followed by *K. pneumoniae* with 33.30% while the least occurrence was observed with *P. mirabilis* having just 22.20%. Although the incidence of ESBL-producing organisms differs from one institution to another, previous reports suggest that *E. coli* and *K. pneumoniae* represent the most commonly isolated ESBL-producing bacteria [11, 6, 10]. The detection of *K. pneumoniae* and *E. coli* from the contact surfaces may suggest some level of health concerns. *K. pneumoniae* has been shown to be very virulent due to its possession of a capsular material made up of a fibrillous structure that shield the bacterial cell surface and hinder the penetration of a drug into the bacterial cells. Moreover, the attachment of somatic O-antigens to host cells through fimbrial or non-fimbrial ways contributes to the virulence of this bacterium making it so resistant to third generation cephalosporins [4].

The three ESBL-producing bacteria detected in this study are members of the family Enterobacteriaceae. Several reports showed that most Gram-negative bacteria isolated from various hospital surfaces and confirmed as ESBLs producers are members of this family. In line with our findings, Chaoui *et al.* [2] published that the predominant bacterial contaminants isolated from different health care environmental surfaces were members of the family Enterobacteriaceae out of which 32.3% were confirmed to be ESBL producers. Another study showed that of the total bacterial contaminants isolated from hospital medical equipment, 25.93% were observed to be ESBL- producing bacteria [18].

## CONCLUSION

ESBL producing bacteria have been phenotypically detected from different contact surfaces in Gombe state specialist hospital. The bacteria detected include *E. coli* with highest percentage of occurrence followed by *K. pneumoniae* and then *P. mirabilis*. This may suggest the possible transmission of nosocomial infections caused by these bacteria which are resistant to most potent antibiotics and at the same time leading to treatment failure and high cost of infections management.

## REFERENCES

1. Otter JA, Yezli S, Salkeld JA, French GL. Evidence that contaminated surfaces contribute to the transmission of hospital pathogens and an overview of strategies to address contaminated surfaces in hospital settings. *Am J Infect Control*, 2013;41(5): S6-S11.
2. Chaoui L, Mhand R, Mellouki F, Rhallabi N. Contamination of the Surfaces of a Health Care Environment by Multidrug-Resistant (MDR) Bacteria. *Int J Microbiol*, 2019.
3. Garba L, Lawan HS, Puma HU, Abdullahi MM, Yusuf I, Mukhtar MD. Phytochemical Screening and in vitro Bacteriostatic Effects of *Syzigium aromaticum* (Clove) Extracts on Clinical Bacterial Isolates. *J Biochem Microbiol Biotechnol*, 2019; 7(1): 5-9.
4. Rout S, Dubey D, Panigrahy R, Padhy RN. Surveillance of extended-spectrum  $\beta$ -lactamase producing bacteria in an Indian teaching hospital. *J Taibah Univ Med Sci*, 2014; 9(4): 274-281.
5. Shu'aibu I, Hadiza JA, Yusha'u M, Kabiru MY, Ahmad MM, Lawal G, Khairiyya M. Assessment of foods and drinks for the presence of extended spectrum beta lactamase (ESBL) producing bacteria in Gombe Metropolis, Nigeria. *Adv Sci Lett*, 2018;24(5): 3646-3651.
6. Garba L, Yusha'u M, Abdullahi MM, Abubakar MU, Inuwa AB, Isa S, Adamu MT. Effectiveness of double discs synergy test in the confirmation of extended spectrum  $\beta$ -lactamase (ESBL) Production. *J Biochem Microbiol Biotechnol*, 2018; 6(2): 15-18.
7. Rath S, Dubey D, Sahu MC, Debata NK, Padhy RN. Surveillance of ESBL producing multidrug resistant *Escherichia coli* in a teaching hospital in India. *Asian Pac J Trop Dis*, 2014;4(2):140-149.
8. Garrec H, Drieux-Rouzet L, Golmard JL, Jarlier V, Robert J. Comparison of nine phenotypic methods for detection of extended-spectrum  $\beta$ -lactamase production by Enterobacteriaceae. *J clinical microbiology*, 2011; 49(3): 1048-1057.
9. Nordmann P, Cuzon G, Naas T. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. *Lancet Infect Dis*, 2009; 9(4), 228-236.
10. Ibrahim Y, Sani Y, Saleh Q, Saleh A, Hakeem G. Phenotypic detection of extended spectrum beta lactamase and carbapenemase co-producing clinical isolates from two tertiary hospitals in Kano, North West Nigeria. *Ethiopian J Health Sci*, 2017; 27(1): 3-10.
11. Garba L, Yusha'u M. Detection of Extended-Spectrum  $\beta$ -Lactamases among Gram Negative Isolates from Gombe Specialist Hospital Using Disc Replacement Method. *Bayero J Pure Appl Sci*, 2012; 5(1): 109-112.
12. Cheesbrough M. District laboratory practice in tropical countries. Cambridge University Press, 2006.
13. Garba L, Yusha'u M, Yerima A. Antibacterial activity of ethanol extract of *Phoenix dactylifera* leaves against some Gram-negative bacterial isolates. *Greener J. Biol. Sci*, 2013; 3:238-243.
14. Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended broad-spectrum  $\beta$ -lactamases conferring transferable resistance to newer  $\beta$ -lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. *Clin Infect Dis*, 1988; 10(4): 867-878.
15. World Health Organization. Rapid guidance on the decommissioning of Ebola care facilities (No. WHO/EVD/Guidance/Strategy/15.1). World Health Organization, 2015.
16. Caselli E, Arnoldo L, Rognoni C, D'Accolti M, Soffritti I, Lanzoni L, Mazzacane S. Impact of a probiotic-based hospital sanitation on antimicrobial resistance and HAI-associated antimicrobial consumption and costs: a multicenter study. *Infect Drug Resist*, 2019; 12: 501.
17. Chemyal RF, Simmons S, Dale JC, Ghantaji SS, Rodriguez M, Gubb J, Stibich M. The role of the healthcare environment in the spread of multidrug-resistant organisms: update on current best practices for containment. *Therapeutic advances in infectious disease*, 2014;2(3-4): 79-90.
18. Bennani Mechita N, Razine R, Saadi A, Lahlou L, Kasouati J, Sabai Idrissi K, Mrabet M. Evolution of Extended Spectrum Beta-Lactamase enterobacteriaceae in the Hospital Cheikh Zaid from 2009 to 2011. *Int J Epidemiol*, 2015; 44(1):268-269.