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# Prevalence and risk factors of extended spectrum beta-lactamase producing bacteria among patients with lower respiratory tract infections in Benin City, Nigeria

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## ABSTRACT

Lower respiratory tract infection (LRTI) continues to be a major cause of rising morbidity and mortality rates in resource limited settings with drug resistance worsening treatment outcomes. This study was aimed at determining the prevalence and risk factors of extended spectrum beta-lactamase (ESBL) producing bacteria among patients with signs and symptoms of LRTI in Benin City, Nigeria. A total of 489 patients (non-repetitive) presenting with signs and symptoms of LRTI were recruited. Questionnaires were given and filled by participants. Sputum specimens were collected from these patients in sterile wide-mouth containers and sent to the Medical Microbiology Laboratory, University of Benin Teaching Hospital for microbiological analysis. Emergent colonies were identified and antimicrobial susceptibility tests carried out using British Society for Antimicrobial Chemotherapy (BSAC) guidelines. Gram negative rods were screened for ESBL using phenotypic method. ESBL showed high prevalence among *Escherichia coli* (57.1%) while its lowest prevalence was observed for *Pseudomonas aeruginosa* (7.1%). *Klebsiella pneumoniae* recovered from inpatients showed high likelihood of being ESBL positive ($p = 0.0046$). Bacterial strains recovered from inpatients were more likely to be ESBL producing and showed significant association in comparison with outpatients (OR = 3.567; 95%CI = 1.778, 7.153; $p = 0.0005$). ESBL producing bacteria showed a significant relationship with educational ($p < 0.0001$) and occupational status ($p = 0.0135$). Marked level of resistance was shown to antibiotics while the carbapenems showed the highest activity against isolates. The overall prevalence of ESBL producing bacteria was 26.5%. The study advocates caution in the use of carbapenems and harps on prudent use of antibiotics.

**Keywords:** Bacteria, Resistance, Extended spectrum β-lactamase, Sputum.

## INTRODUCTION

Acute lower respiratory tract infections are a significant cause of high morbidity and mortality rates globally with worse outcomes in resource poor settings (Akanbi *et al*., 2009). The burden of LRTIs is highest in areas of low socio-demographic status, populations that depend on solid fuels for cooking and heating, and in malnourished and immunoimpaired populations (Troeger *et al*., 2017). These factors are commonplace in Nigeria and the national burden of LRTI is grossly under-estimated due to poor data management and absence of a national survey (Akanbi *et al*., 2009).

Although majority of LRTI are of bacterial etiology, LRTI can also be caused by fungi, viruses and parasites (Troeger *et al*., 2017). Community-acquired pneumonia (CAP) is most commonly due to *Streptococcus pneumoniae*, atypical bacteria, and respiratory viruses (Horie *et al*., 2018). Hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP) more frequently involve resistant pathogens, such as *Pseudomonas aeruginosa*, Gram-negative Enterobacteriaceae, and methicillin-resistant *Staphylococcus aureus* (MRSA). β-lactam antibiotics are drugs of choice in managing LRTIs of bacterial etiology. However, these drugs including the expanded spectrum cephalosporins are under threat due to the production of extended spectrum β-lactamase (ESBL) enzymes by bacteria (gram negative bacilli) (Paterson and Bonomo, 2005, Ibadin *et al*., 2017).

ESBLs are capable of hydrolyzing the penicillins, the first, second and third generation cephalosporins, and aztreonam thereby inactivating them (Paterson and Bonomo, 2005). They are however inhibited by clavulanate, sulbactam and tazobactam as they bind irreversibly to them (Paterson and Bonomo, 2005). Being of SHV, TEM, OXA, PER and CTX-M types, ESBLs have thus emerged as a major setback in the treatment of clinical infections.

Majority of clinical infections are due to LRTI (Khan *et al*., 2014). Previous studies have shown risk factors

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for ESBL to include previous administration of a third generation cephalosporin, prolonged hospital stays and use of invasive medical devices (Paterson and Bonomo, 2005). Differences in study populations, selection of cases, selection of controls and sample size may have led to differing and conflicting risk factors as observed in literature (Paterson and Bonomo, 2005). In our region however, data is scarce on the risk factors and prevalence of ESBL-producing bacteria causing LRTI in the hospital and community setting. This study was therefore aimed at determining the prevalence and risk factors of ESBL producing bacteria among patients with signs and symptoms of LRTI in Benin City, Nigeria.

## MATERIALS AND METHODS

### Study Area:

The study was conducted at the University of Benin Teaching Hospital in Benin City, Nigeria. UBTH is a tertiary hospital with 700 beds and 20 wards, serving the specialist healthcare needs of Edo State and 6 to 10 other neighboring States.

### Study Design:

The study was cross-sectional.

### Study Population:

A total of 489 patients (non-repetitive) presenting with signs and symptoms of LRTI were recruited for this study. These included patients admitted in wards (inpatients) and attending outpatient clinics in University of Benin Teaching Hospital. Informed consent was obtained from patients or parents/guardians of children before specimen collection. Questionnaires were also given to patients. Ethical approval was sought from the Ethical Committee of University of Benin Teaching Hospital. This was approved with Number: ADM/E 22/A/VOL. VII/1489.

### Specimen Collection and Sample Processing:

Sputum specimens were collected where appropriate from these patients in sterile wide-mouth containers and sent to the Medical Microbiology Laboratory, University of Benin Teaching Hospital for microbiological analysis. Specimens were processed within two hours after collection. Specimens were thereafter cultured on blood, chocolate and MacConkey agar plates respectively. Films were made from the sputum specimens and stained by Gram's method. Emergent colonies were identified using standard microbiological techniques (Barrow and Feltham, 2003). Isolates that were Gram negative rods were identified using the Microbact 24E (OXOID, U.K) identification system.

### Disc Susceptibility Test

Antimicrobial susceptibility tests were performed using antibacterial discs namely ciprofloxacin (5 µg), ofloxacin (5 µg), levofloxacin (5 µg), sulfamethoxazole-trimethoprim (25 µg), cefotaxime (30 µg), cefuroxime (30 µg), cefixime (5 µg), ceftriaxone-sulbactam (30/15 µg), gentamicin (10 µg), imipenem (10 µg) and meropenem (10 µg) (all from Axiom, India), using the British Society for Antimicrobial Chemotherapy (BSAC) method (Andrew, 2009).

Bacterial isolates which showed resistance to ≥ 3 classes of antibacterial agents were deemed multi-drug resistant (MDR).

### ESBL DETECTION

The presence of ESBL was detected in all bacterial isolates as previously described in the double disc synergy test (DDST), using 30µg ceftazidime and cefotaxime antibacterial drugs (all from Abtek, UK) (Livermore and Brown, 2001). The presence of ESBL was inferred as positive if there was an expansion of the zone of inhibition between the ceftazidime and amoxicillin–clavulanate disc, cefotaxime and amoxicillin–clavulanate disc or both. The positive control strain *Klebsiella pneumoniae* ATCC 700603 was included.

## RESULTS

Out of 489 patients presenting with signs and symptoms of LRTI in this study, a total of 245 (50.1%) were culture positive for sputum (non-MTB). This comprised of Gram positive cocci which included *S. aureus* - 27 (11.0%), Coagulase negative staphylococci - 9 (3.8%), *S. pneumonia* -3 (1.2%), and Gram negative rods which included *Haemophilus influenzae*- 21 (8.6%), Enterobacteriaceae -167 (68.2%) and oxidase positive rods - 18 (7.3%). ESBL showed high prevalence among *E. coli* (57.1%) while its lowest prevalence was observed for *P. aeruginosa* (7.1%). The findings were however not statistically significant ($p = 0.1031$) (Table 1). *Klebsiella pneumoniae* recovered from inpatients showed high likelihood of being ESBL producing than the same organism from outpatients ($p = 0.0046$). Also bacterial strains recovered from inpatients were more likely to be ESBL producing and showed significant

association in comparison with outpatients (OR = 3.567; 95%CI = 1.778, 7.153; p = 0.0005) (Table 2 and 3). In relation to socio-demographic factors, than 10 showed the highest prevalence of ESBL-producing bacteria (50.0%), the findings were however not statistically significant (p = 0.2358). Patients without formal education showed the highest prevalence of ESBL-producing bacteria (81.1%), the observations were statistically significant (p <0.0001). There was also a significant relationship between traders and infection with ESBL-producing bacteria (p = 0.0135) (Table 4).

gender was unassociated with ESBL producing bacteria causing LRTI (p = 0.9356). Children less

The antimicrobial susceptibility pattern of bacterial isolates is shown on Figure 1. Imipenem and meropenem showed high activity against ESBL producing strains while the cephalosporins (cefuroxime, cefotaxime and cefixime) showed markedly poor activity. ESBL negative strains showed high susceptibility to the fluoroquinolones, imipenem and meropenem. Forty-one (85.4%) ESBL producing strains were MDR while 44 (32.1%) of ESBL negative strains were MDR.

Table 1: Distribution of ESBL among GNB recovered from patients with LRTI in Benin.

| **Organism** | **No of isolates recovered (% of total)** | **ESBL positive (%)** |
|---|---|---|
| *Citrobacter* spp | 4 (2.2) | 2 (50.0) |
| *Escherichia coli* | 14 (7.6) | 8 (57.1) |
| *Enterobacter* spp | 43 (23.2) | 13 (30.2) |
| *Klebsiella pneumoniae* | 82 (44.3) | 22 (28.0) |
| *Hafnia alvei* | 2 (1.1) | 0* |
| *Proteus mirabilis* | 2 (1.1) | 0* |
| *Proteus vulgaris* | 2 (1.1) | 0* |
| *Providencia* spp | 6 (3.2) | 0* |
| *Shigella* spp | 1 (0.5) | 0* |
| *Serratia* spp | 4 (2.2) | 1 (25.0) |
| *Acinetobacter* spp | 7 (3.8) | 1 (14.3) |
| *Alkaligenes* spp | 4 (2.2) | 0* |
| *Pseudomonas aeruginosa* | 14 (7.6) | 1 (7.1) |
| Total | 185 | 48 (26.5) |

ESBL- Extended spectrum beta lactamase, ESBL vs isolates: p = 0.1031. *= not included in statistical analysis

Table 2: Distribution of ESBL enzymes among GNB in patients with LRTI in relation to source of patient.

| **Organism** | **Inpatients (n = 54)** | **Outpatients (n = 131)** | **p** |
|---|---|---|---|
| *Citrobacter* spp | 1 (1.9) | 1 (0.8) | 0.5151 |
| *Escherichia coli* | 5 (9.3) | 3 (2.3) | 0.0852 |
| *Enterobacter* spp | 5 (9.3) | 8 (6.1) | 0.6554 |
| *Klebsiella pneumoniae* | 12 (22.2) | 10 (7.6) | 0.0046 |
| *Serratia* spp | 1 (1.9) | 0* | 0.6462 |
| *Acinetobacter* spp | 0* | 1 (0.8) | 0.5197 |
| *Pseudomonas aeruginosa* | 0* | 1 (0.8) | 0.5197 |
| Total | 24 (44.4) | 24 (18.3) | 0.0005 |

*= not included in statistical analysis

Table 3: Frequency of ESBL detection among GNB recovered from patients in relation to source of patients.

| **Clinical isolates** | **Number of GNB Tested** | **ESBL positive (%)** | **OR** | **95% CI** | **p** |
|---|---|---|---|---|---|
| **Inpatient** | 54 | 24 (44.4) | 3.567 | 1.778, 7.153 | 0.0005 |
| **Outpatient** | 131 | 24 (18.3) | | | |

ESBL- Extended spectrum beta lactamase, GNB- Gram negative bacilli

Table 4: Distribution of ESBL enzymes in relation to socio-demographic factors of patients

| Factor | Division | Number of GNB (n = 185) | ESBL positive (%) | p |
|---|---|---|---|---|
| Gender | Male | 78 | 20 (25.6) | 0.9356 |
| | Female | 107 | 28 (26.2) | |
| Age (yrs) | 0-10 | 6 | 3 (50.0) | 0.2358 |
| | 11-20 | 14 | 4 (28.6) | |
| | 21-30 | 22 | 5 (22.7) | |
| | 31-40 | 31 | 10 (32.3) | |
| | 41-50 | 32 | 9 (28.1) | |
| | 51-60 | 37 | 7 (18.9) | |
| | 61-70 | 23 | 2 (8.7) | |
| | ≥ 71 | 20 | 8 (27.3) | |
| Educational status | No Formal | 16 | 13 (81.3) | < 0.0001 |
| | Primary | 35 | 9 (25.7) | |
| | Secondary | 65 | 9 (25.7) | |
| | Tertiary | 69 | 17 (24.6) | |
| Occupation | Business/Trading | 51 | 20 (39.2) | 0.0135 |
| | Artisan | 22 | 1 (4.5) | |
| | Civil/Public Servant | 22 | 2 (9.1) | |
| | Teacher/Lecturer | 10 | 2 (20) | |
| | Unemployed | 13 | 2 (15.4) | |
| | Student | 36 | 13 (36.1) | |
| | Others | 31 | 8 (25.8) | |

ESBL- Extended spectrum beta lactamase, GNB- Gram negative bacilli

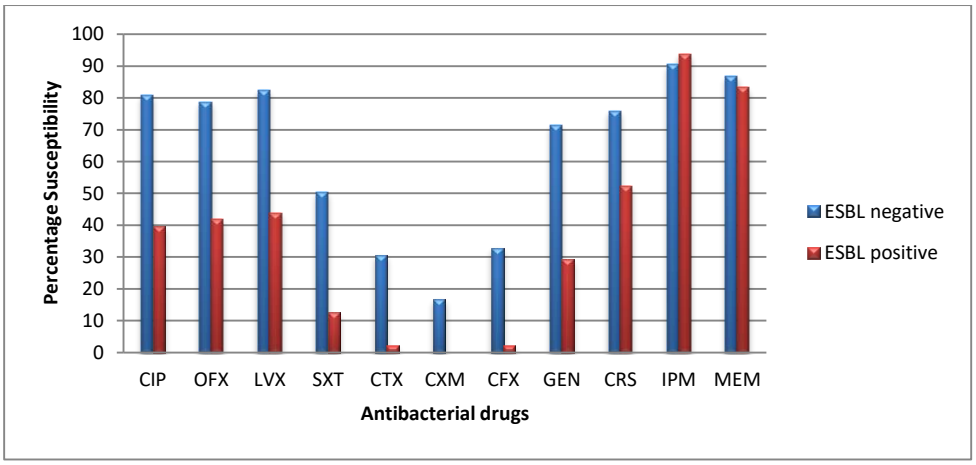


Figure 1: Antimicrobial susceptibility pattern of bacterial isolates in relation to ESBL production.

**DISCUSSION**

In the present study, the prevalence of ESBL producing bacteria was 26.5%. The value is slightly lower than a previous study in Benin which reported 33.9% (Ibadin *et al*., 2017), and strikingly lower than another in South-western Nigeria where the prevalence of ESBL from bacteria recovered from sputum specimens was 50.1% (Abike *et al*., 2018). ESBL prevalence has been shown to vary according to geographical location and over time (Khan *et al*., 2014). This may explain our findings. Also, *E. coli* showed a high likelihood of being ESBL producing and showed the highest prevalence (57.1%). This observation is comparable with a south-western study where *E. coli* showed the highest prevalence (66.7%) (Abike *et al*., 2018). In previous Indian and Nepali studies, *E. coli* and *K. pneumoniae* showed the highest prevalence with 36.36 and 42.2% respectively (Mishra *et al*., 2015; Vijay and Delala, 2016). *E. coli* and *K. pneumoniae* are prominent bacteria of medical significance that are ESBL producers and in hospital settings, are resistant to various antibiotics (Paterson and Bonomo, 2005).

ESBL–producing *Klebsiella pneumoniae* was more likely to be implicated in LRTI among patients admitted than out-patients in this study. The predilection of ESBL for *K. pneumoniae* is not clearly understood as over 75% of ESBL studies are on this bacterium (Paterson and Bonomo, 2005, Falagas and Karageorgopoulos, 2009). However, they are frequently associated with nosocomial outbreaks, especially in ICU settings and risk factors for acquiring infection due to this strain include senior, critical, or immunocompromised statuses (Falagas and Karageorgopoulos, 2009). Furthermore, the pathogenicity of this bacterium is largely due to the production of a polysaccharide-rich cell surface that provides protection from the inflammatory response (Lawlor *et al*., 2005). Also, when ESBL producing strains are implicated in pneumonia among patients admitted in wards, it may worsen treatment outcomes, prolong hospital stays and may be a source of spread or outbreak in wards (Paterson and Bonomo, 2005; Falagas and Karageorgopoulos, 2009).

In this study, inpatients with symptoms of LRTI were 3.5 times more likely to be infected with an ESBL producing organism than outpatients. Previous studies around the world have observed that being admitted is a risk factor for colonization and infection with ESBL producing bacteria (Paterson and Bonomo, 2005; Falagas and Karageorgopoulos, 2009; Troeger *et al*., 2017). Also, in the study site, third generation cephalosporins like ceftriaxone and most β-lactam antibiotics are administered empirically, during emergencies and as antibiotic coverage during surgery. This may have over time exerted selective pressure, leading to the production of ESBL enzymes by opportunistic bacteria (Ibadin *et al*., 2017).

Although patients above 60 years have been previously shown to be more prone to infection with ESBL-producing organisms (Falagas and Karageorgopoulos, 2009), age did not significantly affect the prevalence of ESBL-producing bacteria. This study however outlines risk factors for ESBL in our setting to include no formal education and trading. Though reasons are not clear, patients without formal education may take drugs without compliance and may purchase antibiotics over the counter when they feel ill. Antibiotic abuse is rife in our setting and over-the counter purchase is rampant (Ibadin *et al*., 2017). Also, traders come across persons from various backgrounds, cultural practices including carriers of ESBL-producing organisms. Poor hygiene and antibiotic abuse may play contributory roles in infection. More studies that explore in detail the role of socio-demographic factors in ESBL prevalence in our region are however required. Majority of ESBL strains were MDR (85.4%), with no activity against the third generation cephalosporins. The fluoroquinolones were also poorly effective against these strains. This finding was not too surprising as plasmid bearing ESBL genes have been known to carry fluoroquinolone, trimethoprim/sulfamethoxazole and aminoglycoside resistant genes (Paterson and Bonomo, 2005). The carbapenems also showed high activity against ESBL producing strains as over 80% of MDR strains were susceptible to either imipenem or meropenem. This finding is comparable with previous studies (Ibadin *et al*., 2017). The carbapenems are drugs of last resort in our setting and are expensive. This may have preserved the drug from abuse in comparison to the fluoroquinolones and other cephalosporins which are easily purchased over-the-counter. Our study therefore recommends caution in the use of carbapenems in order to minimize resistance to this class of antimicrobials.

**CONCLUSION**

The prevalence of ESBL producing strains was 26.5%. Patients having symptoms of LRTI and admitted in wards showed significant association with ESBL producing bacteria with *K. pneumonia* being more likely to be the infecting organism. Risk

factors for ESBL included an absence of formal education and trading. The carbapenems showed high activity against ESBL-producing bacteria. The study harps on prudence in the use of antibiotics.

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