**Survey of Extended Spectrum β-lactamase Producing Gram-Negative Bacteria in Lower Respiratory Tract Infections in Kebbi State, Nigeria**

**Abstract**

Extended Spectrum Beta-lactamase (ESBL) producing Gram-negative bacteria are significant public health concern, particularly in developing countries. This study was designed to survey for extended spectrum β-lactamase producing Gram-Negative bacteria in patients with lower respiratory tract infection in Kebbi Sate, Nigeria. Three hundred and fifty (350) sputum samples were collected from patients attending six different hospitals in Kebbi State. The samples were all screened for bacterial pathogens using standard microbiological techniques. The bacterial isolates were identified using conventional biochemical tests and then confirmed using commercial biochemical test kit (MICROBACT) according to manufacturer’s instruction. Antimicrobial susceptibility test was determined using disc diffusion method. The bacterial isolates that were resistance to third generation cephalosporins were confirmed for ESBLs production using double disk synergy test. Out of 350 sputum samples analyzed, 74 (21.1%) were found to be gram-negative bacteria. Also, out of 74 different gram-negative bacterial isolates identified, 50(67.6) were identified to be ESBLs producers. ESBLs productions were seen among *Aeromonas hydrophila* 6(100%), *Pseudomonas aeruginosa* 6(100%), *Burkholderia pseudomallei* 3 (100%) and *Klebsiella pneumoniae* 19 (79.2%). Other ESBL producing bacteria recorded included *Proteus vulgaris 2* (66.7%) *Klebsiella oxytoca* 8(53.3%), *Acinetobacter baumannii* 2(40%) *and Escherichia coli* 4 (33.3%). It was also recorded from this study that ESBLs producing bacteria were detected in all the hospital studied, but highest level of ESBLs production were detected at Sir Yahaya Memorial Hospital (SYMH) and Martha Bamaiyi General Hospital Zuru (MGHZ) with percentage occurrence of 11(22%) respectively. In conclusion, It was observed that high level of ESBL production were detected among *Aeromonas hydrophila* 6(100%), *Pseudomonas aeruginosa* 6(100%), *Burkholderia pseudomallei* 3 (100%) and *Klebsiella pneumoniae* 19 (79.2%). It was also found out that highest level of ESBLs production were detect in SYMH and MGHZ.

**Keywords**: Extended spectrum, B-lactamase, Bacteria, Lower respiratory tract, antibiotics, Resistance

**Introduction**

Lower respiratory tract infections (LRTIs) occur below the level of the larynx, i.e., in the trachea, the bronchi, or in the lung tissue. These includes condition such as tracheitis, bronchitis, bronchiectasis, lung abscess, tuberculosis, pneumonia (WHO, 2003). Lower respiratory tract infection (LRTI) is considered as one of the major public health problems and a leading cause of morbidity and mortality in many developing countries (GBD, 2016; Rakshya *et al*., 2018; Lower Respiratory Infections Collaborators*,* 2018). There were approximately 11.9 million episodes of severe acute lower respiratory infections (ALRI) resulted in hospital admissions in young children worldwide (Nair *et al*., 2013).

The choice of antimicrobial therapy for bacterial LRTIs is relatively straight forward when the etiologic agents and their antibiotic susceptibility patterns are known. However, the clinical presentation is usually not specific enough to make a firm etiologic diagnosis whether in the community or hospital setting (Shah *et* *al*., 2010). In almost all cases, eradication of causative agents requires initiation of antimicrobial therapy before obtaining culture report; however, during the last few years, the increase in antibiotic resistance has compromised the selection of empirical treatment (Jonaidi, 2009) and how to choose an effective antimicrobial agent is a new challenge to the clinicians, as the composition and the resistance to antimicrobial agents of infection pathogens was changing frequently. This trend is presumably due to the empirical administration of antibacterial therapy even before the availability of the culture results (Ahmed, 2013). Various other factors also contribute to the emergence of resistance such as irrational use of antibiotics, transmission of resistant bacteria from patient to patient and from healthcare practitioners to patients and vice versa (WHO, 2012; Mahmoud and Balkhy, 2012).

The worldwide increase in infections caused by extended-spectrum beta-lactamase (ESBL) and AmpC producing Enterobacteriaceae (ESBL-E) is a concern. Surveillance is extensive in Europe, North America, and Asia. Yet, there is no summarizing surveillance in Africa. (Hertz *et al*., 2019). Resistance to antibiotics most especially third generation cephalosporins has assumed a worrisome dimension globally. Genes conferring this resistance which are mediated by enzymes known, as extended spectrum beta-lactamases (ESBLs) are now wide spread among several Enterobacteriaceae species. (Kpoda *et al.,* 2018). ESBLs include a group of beta-lactamases that hydrolyze the extended-spectrum cephalosporins, the penicillins, and monobactams, but not cephamycins and carbapenems (Khalifa *et al*., 2019), and are inhibited by beta-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam. However, the extent of the problem of ESBL is still probably underestimated due to inadequate or ineffective detection in some clinical settings (Gashaw *et al*., 2018). Prior studies have reported the poor therapeutic outcome when patients with infection due to ESBL producers are treated with antibiotics to which the organisms are resistant (Founou *et al*., 2019; Khalifa *et al*., 2019). This, therefore, made ESBL producers difficult to treat because such strains are resistant to multiple antimicrobial agents, thereby limiting therapeutic options, which may lead to therapeutic failure (Khalifa *et al*., 2019). It has also been reported that the problem of ESBL-producing organisms is more severe in developing countries (Gashaw *et al*., 2018).

In developing countries including Nigeria, treatment of LRTI is made usually empirically in which the etiologic agent is rarely identified. So, identifying the most common bacterial pathogens from patients with LRTI, drug resistance profile would be valuable to reduce morbidity and mortality as a result of the disease (Temesgen *et al*., 2019). While studies have been conducted in various regions of Nigeria, data specific to Kebbi State remain scarce. Therefore, this study was conducted to provide data on ESBL producing Gram-negative bacteria in LRTIs in Kebbi State, Nigeria.

**MATERIALS AND METHODS**

**Study Area**

This study was conducted in Kebbi State which is located on latitude 11.6781° N and longitude 4.0695° E, the state is bounded by Sokoto State to the north and east, Niger State to the south, and Benin Republic to the west (Kebbi State, 2022). The major ethnic groups are Hausa and Fulani, other ethnic groups include Dakarkari, Zabarmawa, Dukkawa and Kambari. Kebbi State have a total land area of 36,129 sq. km. Agriculture is the main occupation of the people especially in rural areas. Crops produced are mainly grains. Animal rearing and fishing are also common. The state has the total population of 3,256,541 people as projected from the 2006 census (NPC, 2022).

**Study Population**

The study population included both male and female patients from all age group that presented the clinical evidence of lower respiratory tract infection such as fever, rigors (chills), fatigue (feeling overtired), anorexia (loss of appetite), diaphoresis (sweating), dyspnea (shortness of breath), productive cough and pleuritic chest pain (Ashby and Turkington, 2007) as diagnosed by the attending physician at the General out Patient Department (GOPD) of the selected hospitals in Kebbi State.

**Study Design**

This is a Cross-sectional and hospital-based study where samples/data were obtained from a representative subset of a population in the selected hospitals in Kebbi State at a specific point in time.

**Sampling Technique**

Stratified sampling and purposive technique were employed for this study until the sample size was completed i.e., the entire population in Kebbi State were grouped into more relatively homogenous sub-groups or strata and the samples were then purposively collected from each stratum proportionately.

**Ethical Clearance**

Ethical approval (MOH/KSREC/VOL1/57) was obtained from the Ministry of Health ethical review committee in Kebbi State. Informed consent both oral and written was obtained from all the participants while ascents were obtained from parents in case of children. All data were stored anonymously and was handled only by the investigator and authorized personnel.

**Inclusion Criteria**

All consenting patients with clinical sign and symptoms of LRTI such as fever, chills, rigors, fatigue, anorexia, diaphoresis, dyspnea, productive cough (Ashby and Turkington, 2007), as diagnosed by the attending physician and those who have not taken antibiotic two weeks prior to sample collection were included into this study. The sputum samples were graded by Bartlett’s grading system. Only those samples for which the score is >0 were included in the study i.e., sputum samples with squamous cells less than 10/low power field were included (Rosón *et al*., 2000).

**Exclusion Criteria**

Patients who did not give their consent or those that took antibiotic two weeks prior to sample collection were excluded from this study. Sputum sample with more than 10 squamous epithelial cells per low power field were excluded in the study (Rosón *et al*., 2000).

**Sample Collection**

Early morning sputum specimens were collected aseptically from patients (350) attending the selected Hospitals in Kebbi State after obtaining an approval from the ethical review committee. All patients were instructed on how to collect the sputum samples aseptically, i.e., they were asked to cough deeply into a well-labeled sterile, leak proof, wide mouthed container, with tight fitting cover, which was taken to the laboratory for analysis.

**Culture of the Sputum Samples**

The sputum samples were cultured on chocolate agar, sheep blood agar (5%), and MacConkey agar plates (oxoid). On the Chocolate agar, optochin disks were placed at secondary inoculation to screen for *S. pneumoniae*. The chocolate agar plates were incubated in an incubator (5% CO2) at 37 ºC for 24 hours while blood agar and MacConkey agar were incubated in an aerobic atmosphere at 37ºC for 24 hours (Borkot *et al*., 2016). Isolated colonies, such as colonies that were large, round, golden yellow surrounded by zones of clear beta-haemolysis on blood agar, large mucoid colonies on CLED agar and round non-fermenting colonies with or without fluorescent greenish colour were sub-cultured for purification and thereafter preserved on nutrient agar slants and stored in a refrigerator (4ºC) for subsequent analysis.

**Antimicrobial Susceptibility Pattern of the Isolated Bacteria**

Antimicrobial susceptibility test was done using disc diffusion method. The disc diffusion method that was presented in this study, was a modification of the Kirby Bauer technique that has been carefully standardized by CLSI (2017).

**Screening and Confirmatory Test for ESBLs**

Isolates showing resistance to 3rd generation cephalosporin, namely Ceftazidime, Cefotaxime and Ceftriaxone were tested with the CLSI confirmatory test using double disc synergy test, in this method, synergy between a disc of Augmentin (Amoxycillin and clavulanic acid) and third generation cephalosporins was detected. The clavulanate in Augmentin disc diffuses through the agar and inhibits the β–lactamases surrounding third generation cephalosporin disc. Discs containing 30μg of ceftazidime, cefotaxime and ceftriaxone were placed over inoculated Mueller-Hinton agar plates 20 mm apart from centrally placed amoxicillin-clavulanic acid disc. Following overnight incubation at 37°C, diameter of zone of inhibition was measured. Extension of the edge of the zone inhibition of ceftazidime, cefotaxime and ceftriaxone disc on the side exposed to the disc containing amoxicillin-clavulanic acid was positive for ESBL (CLSI, 2017).

**RESULTS AND Discussion**

**Table 1. Resistant Pattern of the Isolated Bacteria in Patients with LRTIs in Kebbi State**

|  |  |  |  |
| --- | --- | --- | --- |
| S/N | Bacterial isolates | No. of Isolates | Resistance Pattern |
| 1 | *Klebsiella pneumoniae* | 24 | Β-lactam antibiotics (CXM, CAZ, CRO, CFM, CTX), Sulfonamides (SXT), Macrolide (AZM) |
| 2 | *Klebsiella oxytoca* | 15 | Β-lactam antibiotics (CXM, CAZ, CRO, CFM, CTX), Sulfonamides (SXT), Macrolide (AZM) |
| 3 | *Escherichia coli* | 12 | Β-lactam antibiotics (CRO, CFM, CTX, PRL), Sulfonamides (SXT), Aminoglycoside (CN), fluoroquinolones (CIP) |
| 4 | *Aeromonas hydrophila* | 6 | Β-lactam antibiotics (CXM, CAZ, CRO, CFM, CTX), Sulfonamides (SXT), Aminoglycoside (CN) |
| 5 | *Acinetobacter baumannii* | 5 | Β-lactam antibiotics (CXM, CAZ, CRO, CFM, CTX), Macrolide (AZM), Aminoglycoside (CN) |
| 6 | *Pseudomonas aeruginosa* | 6 | Β-lactam antibiotics (CAZ, CRO, CTX), Macrolide (AZM), Sulfonamides (SXT) |
| 7 | *B. pseudomallei* | 3 | Β-lactam antibiotics (CXM, CAZ, CRO, CFM, CTX, PRL), Sulfonamides (SXT), Aminoglycoside (CN), fluoroquinolones (CIP) |
|  | **TOTAL** | **74** |  |

MDR- Multidrug-resistance, AML- Amoxicillin, OB- Cloxacillin, CXM- Cefixime, CAZ- Ceftazidine, CRO- Ceftriaxone, CFM- Cefuroxime, CTX- Cefotaxime, PRL- Piperacillin, SXT- Trimethoprim Sulphametaxazole, AZM- Azithromycin, E- Erythromycin, VA- Vancomycin, CN- Gentamycin, CIP- Ciprofloxacin

**Table 2. Frequency of Occurrence of ESBLs Producers among Bacterial Isolates of LRTIs in Kebbi State**

|  |  |  |  |
| --- | --- | --- | --- |
| S/N | Bacterial isolates | No of Isolated Bacteria | ESBL Producers (%) |
| 1 | *Klebsiella pneumoniae* | 24 | 19(79.2) |
| 2 | *Klebsiella oxytoca* | 15 | 8(53.3) |
| 3 | *Escherichia coli* | 12 | 4(33.3) |
| 4 | *Aeromonas hydrophila* | 6 | 6(100) |
| 5 | *Acinetobacter baumannii* | 5 | 2(40.0) |
| 6 | *Pseudomonas aeruginosa* | 6 | 6(100) |
| 7 | *B. pseudomallei* | 3 | 3(100) |
| 8 | *Proteus vulgaris* | 3 | 2(66.7) |
|  | **TOTAL** | **74** | **50(67.6)** |

ESBL- Extended spectrum β-lactam

**Table 3. Distribution of ESBL Producing Bacteria in Patients with LRTIs in Some Hospitals in Kebbi State**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| S/N | Bacterial Isolates  | No. of isolates | SYMHNo.(%) | KMCNo.(%) | ABGHJNo.(%) | GHANo.(%) | GHYNo.(%) | MBGHZNo.(%) |
| 1 | *Klebsiella pneumoniae* | 19 | 6(31.6) | 5(26.3) | 1(5.3) | 2(10.5) | 2(10.5) | 3(15.8) |
| 2 | *Klebsiella oxytoca* | 8 | 2(25) | 0(0) | 2(25) | 0(0) | 2(25) | 2(25) |
| 3 | *Escherichia coli* | 4 | 1(25) | 1(25) | 0(0) | 1(25) | 0(0) | 1(25) |
| 4 | *Aeromonas hydrophila* | 6 | 1(16.7) | 0(0) | 0(0) | 4(66.7) | 1(16.7) | 0(0) |
| 5 | *Acinetobacter baumannii* | 2 | 0(0) | 0(0) | 1(50) | 0(0) | 1(50) | 0(0) |
| 6 | *Pseudomonas aeruginosa* | 6 | 0(0) | 0(0) | 0(0) | 0(0) | 2(33.3) | 4(66.7) |
| 7 | *B. pseudomallei* | 3 | 0(0) | 0(0) | 0(0) | 1(33.3) | 1(33.3) | 1(33.3) |
| 8 | *Proteus vulgaris* | 2 | 1(50) | 1(50) | 0(0) | 0(0) | 0(0) | 0(0) |
|  | **Total** | **50** | **11(22)** | **7(14)** | **4(8)** | **8(16)** | **9(18)** | **11(22)** |

 P- Value = 0.005

SYMH- Sir Yahaya Memorial hospital Birnin Kebbi, KMC- Kalgo Medical Centre ABGHJ- Aisha Buhari General Hospital Jega, GHA- General Hospital Argungu MBGHZ- Martha Bamaiyi General Hospital Zuru

Antimicrobial susceptibility test performed on 108 bacterial isolates in the present study showed that, most of the isolates were susceptible to piperacilin (51%), trimethoprim sulphamethoxazole (61%), Azithromycin (70%), Ciprofloxacin (71%) and Gentamycin (74%), in order of ranking, these are supported by the findings of El-Mahmood *et al*., (2010), Taura *et al*., (2013) and a study in Kathmandu, Nepal (Rakshya *et al*., 2018) where it was documented that most of the isolates were susceptible to piperacillin, trimethoprim sulphamethoxazole, Azithromycin, Ciprofloxacin and Gentamycin. High resistance was recorded in almost all the βeta-lactam antibiotics tested such as Ceftriaxone (63%), Cefuroxime (70%), Cefotaxime (71%), Ceftazidime (75%), Oxacillin (87%) and Amoxicillin (93%). High resistance was also recorded among macrolide (Erythromycin) and Glycopeptide (Vancomycin). This finding correlate with the work carried out by Barkot *et al*., (2016) in Bangladesh. The resistance pattern recorded could generate new disease burden within the population.

*Klebsiella pneumoniae* was the second most predominant bacteria isolated from patients with clinical evidence of LRTI in this location followed by *Klebsiella oxytoca*. Out of 24 isolates of *Klebsiella pneumoniae* and 15 isolates of *Klebsiella oxytoca*, recorded in this study, 19(38%) and 8(16%) were ESBLs producers respectively. They demonstrated an alarming resistance among βeta-lactam antibiotics such as cefuroxime, ceftazidime, ceftriaxone, cefixime, cefotaxime except piperacillin. While Ciprofloxacin and gentamycin demonstrated high activity among *Klebsiella pneumoniae* and *Klebsiella oxytoca* isolates followed by Azithromycin and trimethoprim sulphamethaxazole, this finding is similar to the work of Rakshya *et al*., (2018) in Nepal and Abayomi *et al*., (2020) in Ogbomoso, Nigeria.

The activity of βeta-lactam antibiotics on *Eschericia coli* was moderate except that high resistance were recorded on cefuroxime. Out of 12 (33%) *Eschericia coli* isolated in this study, only 4 were ESBLs producers, this finding agrees with the work in Ogbomoso, Nigeria in which out of 107 *Eschericia coli* isolated only 16 (14%) were ESBLs producers (Abayomi *et al*., 2020). Most of the *Escherichia coli* isolates were susceptible to gentamycin and trimethoprim sulphamethoxazole followed by Azithromycin and Ciprofloxacin.

*Pseudomonas aeruginosa* demonstrated 100% resistance to all βeta-lactam antibiotic tested except piperacillin and most of the isolates were found to be susceptible to Ciprofloxacin, Gentamycin and Azithromycin. The resistance to all antibiotics tested by *Pseudomonas aeruginosa* as presented in this study is of public health concern with associated treatment failure, this could be attributed to the ability of *P. aeruginosa* to produce biofilms in the lungs and also intrinsic resistance display by the organism to many antibiotics due to the nature of it cell wall. *P. aeruginosa* is a common pathogen in the lungs of those with cystic fibrosis (CF) and is associated with frequent pulmonary exacerbations and high morbidity and mortality (Sriramulu, 2013). The lungs of patients with CF can harbour this organism for decades. With increasing levels of *P. aeruginosa* drug resistance, treatment of pulmonary exacerbations can be increasingly difficult over time (Atkin *et al*., 2018).

*Burkholderia pseudomallei* demonstrated 100% resistance to all the antibiotics tested except that, one of the isolates was susceptible to gentamycin and two isolates were susceptible to ciprofloxacin. *Burkholderia pseudomallei* is the causative agent of melioidosis, an often-fatal disease with a predicted global burden of 165,000 cases per year and 89,000 deaths worldwide (Limmathurotsakul *et al*., 2016). Regions of melioidosis endemicity, including Southeast Asia and northern Australia, account for up to 40% (Limmathurotsakul *et al*., 2010) and 10% (Currie *et al*., 2010) case fatality rates, respectively. Transmission routes include percutaneous inoculation, inhalation, and ingestion of contaminated soil and water (Limmathurotsakul *et al*., 2013). *B. pseudomallei* is intrinsically resistant to many antibiotics, including penicillin, ampicillin, and first and second generation cephalosporins (Wiersinga *et al*., 2018). Melioidosis is predicted to be endemic in Nigeria, a country with the highest estimated annual incidence, mortality, and disease burden in Africa, partly explained by its suitable environment and large population (Limmathurotsakul *et al*., 2016; Birnie *et al*., 2019; Birnie *et al*., 2022). Clinical evidence of melioidosis in Nigeria is scarce and based only on traveler-associated cases in the United Kingdom and reports from Nigeria presuming the presence of *B. pseudomallei* (Salam *et al*., 2011; Adejobi *et al*., 2021; Osunla *et al*., 2021; Birnie *et al*., 2022). According to a report of Savelkoel *et al*. (2019) as reported by CDC, 2023, the systematic confirmation of the environmental presence of *B. pseudomallei* and *B. thailandensis* across multiple statesin Nigeria was documented. The highest *B. pseudomallei* positivity rates were identified in the southeastern states (Ebonyi andEnugu), but also *B. pseudomallei* was identified Northwestern state (Birnin Kebbi, Kebbi State). The study also highlightsthe probability of unrecognized melioidosis in Nigeriaand warrant the attention of health workers and publichealth officials (CDC, 2023).

*Aeromona hydrophila* also shows resistance to almost all the antibiotics tested except Gentamycin and Azithromycin, this is consistent with the findings in Taiwan (Chao *et al*., 2013). This study also found out that, approximately 67% of the patients with *Aeromonas* infection had various underlying diseases, such as diabetes mellitus and hypertension. Similar findings have been reported in a number of case reports, Nagata *et al*., (2011) described a case of *A. hydrophila* pneumonia in a 75-year-old woman with colon cancer who died of the disease, Ye *et al*., (2010) reported on a patient with severe pneumonia due to drug-resistant A. *caviae*, and Murata *et al*., (2001) reported on a case of fulminant *A. hydrophila* pneumonia in a patient with chronic renal failure and liver cirrhosis. The morbidity and mortality rates associated with *Aeromonas* pneumonia were relatively high (Chao *et al*., 2013). Therefore, physicians should be aware that immunocompromised patients of advanced age are at risk of developing *Aeromonas* pneumonia.

**Conclusion**

The overall incidence of ESBLs among the bacterial pathogens of LRTI was 67.6%. the bacterial species implicated were *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *B. pseudomallei*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Klebsiella oxytoca*, *Acinetobacter baumanii* and *Escherichia coli*. It was also found out that most of the isolates were resistance to the B-lactam antibiotics tested. Therefore, treatment of LRTI cause by bacteria with B-lactam antibiotics should be discourage due to high level of resistance exhibited by the isolated bacteria in this location.

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