PREVALENCE OF EXTENDED BROAD-SPECTRUM B-LACTAMASE PRODUCING BACTERIA IN INDIVIDUALS WITH URINARY TRACT INFECTIONS IN SELECTED HEALTH FACILITIES IN ENUGU METROPOLIS, NIGERIA

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ABSTRACT

Extended Broad-Spectrum Beta-Lactamase (ESBL) is an enzyme produced by some bacteria which is capable of decomposing the third generation cephalosporins. Urinary tract infection (UTI) due to extended spectrum beta-lactamase (ESBL)-producing bacteria has become widespread and resistance patterns vary nationally and internationally from one institution to another. This study examined the prevalence of extended spectrum β-lactamase (ESBL)-producing bacteria in individuals with UTI accessing health care services in two health facilities in Enugu metropolis. One hundred and forty-five (145) bacteria isolate from 133 individuals with positive bacteriuria were subjected to Single Disc and Double Disc Synergy Tests to screen for ESBL. Forty-nine (49) bacteria isolates (*Staphylococcus aureus* (n=5), *Escherichia coli* (n=15), *Klebsiella pneumoniae* (n=16), *Pseudomonas aeruginosa* (n=3), *Enterococcus faecalis* (n=3) and *Proteus mirabilis* (n=7)) were positive for ESBL-producing bacteria accounting for 46.8% prevalence of ESBL-producing bacteria mediated UTI. The prevalence of ESBL producing bacteria induced UTI is huge and portends a great danger to the management of bacterial infections. Antimicrobial susceptibility and resistance surveillance should be institutionalized in management of bacterial infections in order to limit the spread of resistant strains and reduce antibiotics treatment failure.

Keywords: Extended Spectrum β-Lactamase (ESBL); Antibiotic resistance. urinary tract infection, Enugu.

Introduction

Extended Spectrum Beta Lactamase (ESBL) is an enzyme produced by some gram negative and gram-positive bacteria that mediates bacterial resistance against β –lactam antimicrobials (CDC, 2010; Leylabadlo *et al*., 2017). ESBL inactivates or destroys extended-spectrum third generation cephalosporins including ceftazidime, cefotaxime, and ceftriaxone and monobactams especially aztreonam, but not cephamycins such as cefoxitin and cefotetan or carbapenems like meropenem or imipenem (NCCLS, 1999; Leylabadlo *et al*., 2017).

Since the first reports of strains of *Klebsiella* spp resistant to third-generation cephalosporins and the first descriptions of the mechanism of resistance involved, the epidemiological success of Enterobacteriaceae producing extended spectrum b-lactamases (ESBLs) have become a concern in the field of medical bacteriology (Drieux *et. al.*, 2006; Ogefere *et al*., 2015). Bacterial producers of ESBLs include; *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, K. oxytoca, Proteus mirabilis, Salmonella enteric, Neisseria gonorrhoeae, Haemophilus influenza, Kluyvera species, Enterobacter aerogenes, Enterobacter cloacae* (NCCLS, 1999; CDC, 2010; Ogefere *et al*., 2015). Most ESBL-producing bacteria belong to the family *Enterobacteriaceae (*CDC, 2010; Andrew *et. al*., 2017; Chukwunwejim *et. al*., 2018*)*. ESBL is responsible for the increasing antimicrobial resistances worldwide observed in amino and ureido penicillin, oxyimino cephalosporin and monobactams (Omar, 2014). For instance, the increasing resistance to third-generation cephalosporins amongst *E. coli* and *Klebsiella* spp is due to ESBLs (Andrew *et al*., 2017*)*.

ESBL is plasmid-mediated and there are about two hundred different types evolving through point mutations of the classical TEM-1 and SHV-1 β –lactamases (Huizen, 2017) and CTX-M types, which evolved via the escape and mutation of chromosomal β -lactamases from *Kluyvera* spp (e.g., $bla_{\text{TEM/SHV}}$) (Ejaz *et. al.,* 2013), others are mobilized from environmental bacteria (e.g., $bla_{\text{CTX-M}}$) (Overdevest *et. al*., 2011)

Antimicrobial resistance is a threat to the management of infections (*Sievert et al., 2013*). The increasing resistance to the more commonly used antibiotics has made empirical treatment more difficult as resistant microorganisms are more difficult to treat, hence requiring alternative medications or higher doses of antimicrobials (*Dai, 2010*).

UTIs complicated by ESBL organisms tend to lead to uncertain outcomes and prolong hospitalisation, especially as these organisms tend to be multi-drug resistant (Teklu *et al*., 2019). Although previously these infections were only limited to hospitals, they have found their way into the community (Gharavi *et. al*., 2021).

Managing UTI mediated by ESBL-producing bacteria is difficulty due to the ability of ESBL making therapy with β-lactam antimicrobials ineffective (Chukwunwejim *et. al*., 2018). Resistant microbes being more difficult to treat may result in increased morbidity and mortality, especially amongst UTI patients on intensive care and high-dependency units (*Dai, 2010;* Omar, 2014). The prevalence of ESBL mediated antibiotics resistance among patients with UTI in Enugu is not well known, and there is evidence of treatment failure following administration of some classes of third generation cephalosporin which were hitherto active. Understanding the magnitude of the problem posed by ESBL is a major step in addressing the challenge. Hence, this study aims to determine the prevalence of ESBL producing bacteria in individuals with self-reported UTI attending clinic at ESUT Teaching Hospital and Omniscient Medical Diagnostic Centre, Enugu.

Materials and Methods

Study area

Enugu is a large city in Nigeria, regarded as the oldest urban area in the Igbo speaking area of Southeast Nigeria, and the capital of the same name state. It is located on latitude and longitude 6° 27' 35.8704" N and 7° 32' 56.2164" E. The population is about 725,000 people according to 2006 Nigerian census, and it is a Center for coal mining, business, and education. There are many health facilities, a few bottling companies and palm seed oil producing businesses (www.LatLong.net 2012-2021). The name *Enugu* is derived from the two Igbo words *Énú Ụ́gwụ́* meaning "hill top" denoting the city's hilly geography. It shares boundaries with Anambra on the West, Abia State on the South, Kogi on the North while Benue and Ebonyi on the East. Enugu and Nsukka are its major towns. Enugu was the headquarters of the former East Central State and Eastern Nigeria.

The people of Enugu are typically Ibos. Enugu State has a total of seventeen (17) local government areas. These are Enugu South, Igbo-Eze South, Enugu North, Nkanu, Udi Agwu, Oji-River, Ezeagu, Igbo Eze North, Isi-Uzo, Nsukka, Igbo-Ekiti, Uzo-Uwani, Enugu East, Aninri, Nkanu East and Udenu.

The state is predominantly agricultural with yam tubers, palm produce and rice being their main produce. There is in place an agricultural policy aimed at maximizing its agricultural potentials. Besides coal, new mineral deposits have recently been discovered in Enugu State. These include limestone, iron ore, crude oil, natural gas and bauxite.

3.2 Study Design

This is a cross-sectional and facility-based study in which participants with presumptive urinary tract infection attending clinic in Enugu State University Teaching Hospital and Omniscient Medical Diagnostic Centre were examined for urinary tract infection and bacteria isolates in those with bacteriuria screened for ESBL.

3.3 Study Population

Individuals with presumptive urinary tract infection accessing medical care at Enugu State University Teaching Hospital and Omniscient Medical Diagnostic Centre Enugu between April and August 2019 that had bacteriuria were studied.

3.4 Sample size

A total of 385 patients were studied. The sample size was determined using Cochran's sample size formula by Bartlett *et al.* (2001).

$$n_o = (Z^2pq)/e^2$$

Where:

e = the desired level of precision (i.e. the margin of error) (0.05)
p = the (estimated) proportion of the population which has UTI (0.5)
q = 1 – p.
z = constant depending on confidence level (A 95 % confidence level gives Z values of 1.96)

e = 0.05, p= 0.5, z = 1.96
Substituting the above figures in the formula: $n_o = (Z^2pq)/e^2$

$n_o = ((1.96)^2 (0.5) (0.5)) / (0.05)^2 = 385.$

3.5 Sampling Strategy

Convenient sampling strategy was used to select participants in the study. Urine specimen collection was scheduled for Mondays and Wednesdays in the month of April – August 2019 until the sample size of 385 was reached. The two days were selected conveniently to make for enough time for urine specimen analysis and for space management in the incubator.

3.6.2 Urine sample collection

The participants were advised on how to collect a 'Clean catch' mid-stream urine. The specimens were coded to ensure anonymity and confidentiality with demographic details of the patients

regarding age and gender properly recorded before transported to Omniscient Medical Diagnostic Centre, Enugu, Enugu State for further processing.

3.6.2.1 Urine culture

The urine specimens were cultured on Blood agar, MacConkey agar and Cysteine, Lactose, and Electrolyte Deficient (CLED) agar using streaking method (Ochei and Kolhatkar, 2000). The culture was incubated at 37^{o}C for 24 hours and the plate read. UTI isolates were subjected to gram staining and biochemical tests for identification.

3.6.2.2 Identification of Isolates

Gram staining and a combination of conventional biochemical testing techniques including catalase and coagulase tests for *S. aureus,* indole test for suspected *E. coli,* citrate and malonate utilization tests for suspected *K. pneumoniae,* and oxidase test for suspected *P. aeruginosa,* Bile Resistance test for *E. faecalis*, and motility test for *P. mirabilis* were carried out.

Characterization of ESBL-producing isolates

Isolates were characterized for ESBL production using Single Disk method following the method described by CLSI (2011). To screen all the isolates for the production of ESBL enzymes, single antibiotic disks comprising cefotaxime (30 µg) and ceftazidime (30 µg) were placed aseptically at a distance of 30 mm apart on Mueller-Hinton agar plate previously inoculated with standardized inoculum of the test bacterium. Once the disk was placed on the medium, the plates were allowed in refrigerator for 30 minutes for pre-diffusion of the antibiotics in order to get more prominent zones (as the lower temperature will curb the growth of the bacterium but not affect the diffusion of the antibiotic) before incubating for 18-24 hrs at 37^{o}C. After incubation, the zones of inhibition were measured and recorded to the nearest millimetre using a meter rule. ESBL production was inferred or suspected if any of the test bacteria showed reduced susceptibility or is resistant to any one of the third generation cephalosporins (cefotaxime and ceftazidime) as per the breakpoints of CLSI.

ESBL production was confirmed in the isolates by the double disk synergy test (DDST) method as previously described CLSI (2011). DDST was performed as a standard disk diffusion assay on MH agar plates. Standardized bacterial suspension was aseptically inoculated by swabbing on the MH agar plates. Amoxycillin- clavulanic acid disc (20/10 µg) was placed at the centre of the plate, and cefotaxime (30 µg) and ceftazidime (30 µg) discs were each placed at a distance of 15 mm (centre to centre) from the amoxycillin-clavulanic acid disc. Once the disks were placed on the medium, the plates were allowed in refrigerator for about 30 minutes for pre-diffusion of the antibiotics in order to get more prominent zones before incubating for 18-24 hrs at 37^{o}C. ESBL production was

confirmed phenotypically when a difference of ≥5 mm increase in the inhibition zone diameter for the zones of inhibition of the cephalosporins (cefotaxime and ceftazidime) tested alone and in combination with amoxicillin-clavulanic acid was observed.

3.7 Inclusion and Exclusion Criteria

All adult individuals with suspected UTI whose willingness and consent were obtained were included, while those who refused to participate by not giving consent as well as female patients menstruating were excluded.

3.8 Ethical Considerations

Ethical permission for the research was obtained from Enugu State University Teaching Hospital Ethical Committee. The three ethical principles namely; safety, privacy and confidentiality of participant's information were maintained. The guidelines on the conduct of biomedical research by the Council of International Organization of Medical Sciences (CIOMS) and that of the International Conference on Harmonization – Good Clinical Practice (ICH-GCP) were followed on the course of the research.

3.9 Statistical Analysis

The data were analysed by SPSS 23 software. To compare qualitative variables, chi-square test was applied. Level of significance was $P < 0.05$.

Results

Prevalence of ESBL-producing bacteria mediated UTI among studied participants in Enugu metropolis

Out of 133 urine samples that were positive for bacterial urine test, 49 isolates were ESBL-producing accounting for ESBL-mediated UTI prevalence rate of 46.8%. *Staphylococcus aureus* (n=5), *Escherichia coli* (n=15), *Klebsiella pneumoniae* (n=16), *Pseudomonas aeruginosa* (n=3), *Enterococcus faecalis* (n=3) and *Proteus mirabilis* (n=7) were implicated as shown in Figure 1.

Sex Distribution of ESBL isolates in individuals with presumptive urinary tract infections in Enugu metropolis

Results showed that the prevalence of ESBL-mediated UTI in females was 65.3% ($^{31}/_{49}$) while males had a prevalence of 34.7% ($^{18}/_{49}$) as shown in Figure 2. *E. faecalis* was found only in females.

Age distribution of ESBL-producing bacteria in patients with self-reported urinary tract infections in Enugu metropolis

Participants in 25 – 34years and >64 years age groups had prevalence of ESBL-mediated UTI accounting for 32.7% and 26.5% of the infection respectively (Figure 3). Age group 15-24years had prevalence of 2%, 35-44years had 8%, 45-54years had 3%, while 55-64years had 7% respectively (Figure 3).

Distribution of ESBL-producing bacteria in individuals with presumptive urinary tract infections in Enugu metropolis based on the occupation

Infection due to ESBL-producing bacteria was 38.8% among civil servants, 28.6% in business people, 10.2% among students, 8.2% in the unemployed and 14.3% among farmers respectively (Table 1).

ESBL-producing bacteria in individuals with presumptive urinary tract infections in Enugu metropolis based on Educational levels

The prevalence of ESBL-producing bacteria mediated UTI was 34.7% among participants with tertiary level of education, 13.0% among secondary school students, 14% in those with primary level of education, and 4.2% for those with non-formal education respectively. Figure 4 presents the prevalence of ESBL-producing bacteria mediated UTI among individuals with presumptive UTI in two selected health facilities in Enugu metropolis.

Discussion

From figure 1, the prevalence of ESBL enzymes among the participants with positive bacteria urine test was 46.8%. Similar studies have been carried out in some countries. For instance, Latifpour *et. al.* (2016) and Inies *et. al*. (2012) reported a prevalence rate of 30% in Korea, 36% in Pakistan, 68% in India, 20% in Algeria, 29% in Spain, 28.4% in Taiwan, 5–10% in Madrid and 44% in the USA respectively. The prevalence of 46.8% ESBL-producing bacteria observed in this study is not consistent with the prevalence of ESBL- producing bacteria reported for countries like Korea and Parkistan. However, higher prevalence rate was obtained in India, while the values for Algeria (20%), Spain (29%) and Taiwan (28.4%) were lower (Rizyi *et. al*., 2011; Inies *et. al*., 2012; Latifpour *et. al.*, 2016). The reason for the differences in prevalence rate reported in this study and those of other locations could be attributed to either the development of evidence-based protocols and guidelines as well as the implementation of surveillance programs such as Study for Monitoring Antimicrobial Resistance Trends (SMART) in some countries or the non-existence of such interventions in others (Rizyi *et. al.*, 2011; Inies *et. al*., 2012). Other factors responsible for the emergence of resistant strains and transfer of resistant genes to other bacteria leading to the variation in ESBL prevalence include; increased and prolonged duration of hospitalization and treatment procedures as well as frequent use of urinary catheters in hospitals as well as indiscriminate and arbitrary use of beta-lactam drugs for community-acquired infections which differ from one country to another (Latifpour *et. al.*, 2016).

ESBL gene expression was noted among *S. aureus, E. coli*, K. *pneumoniae, P. aeruginosa, E. faecalis* and *P. mirabilis*. However, there was a significant difference ($p < 0.05$) in the occurrence of ESBL isolates among bacteria uropathogens causing UTI in the present study. For instance, 5(26.3%) of *S. aureus*, 15(28.8%) of *E. coli*, 16(35.6%) of *K. pneumoniae*, 3(25.0%) of *P. aeruginosa*, 3(33.3%) of *E. faecalis* and 7(87.5%) of *P. mirabilis* were ESBL producing. Findings of this study for *E. coli* and *K. pneumonia* was consistent with ESBL prevalence for *Escherichia coli (*25.84%, 37.11% and 47%*) and K. pneumoniae* (100% and 37%) reported in a similar study by Rizyi *et. al*. (2011) while studying the prevalence of Antimicrobial Resistance in Urinary Tract Infections During Pregnancy. Mitu, *et. al.* (2019) in a similar extended spectrum beta-lactamase (ESBL) and AmpC beta-lactamase producing bacteria in urinary tract infection patients prevalence study in Bangladesh reported similar results. The result however did not agree with the prevalence of 73% observed in a similar study by Inies *et. al.* (2012) in a tertiary care hospital in Madrid. Among the ESBL producing bacteria isolates in this study, expression of ESBL was more by *Proteus mirabilis* as 87.5% of the organism produced ESBL. This observation did not agree with the findings of

Omeregie *et. al.* (2010) in a similar study on urinary tract infections among the elderly in Benin City, in which *Escherichia coli* was the major ESBL-producer.

In this study, both gram-negative bacteria (*E. coli, K. pneumonia, Ps. aeruginosa and P. mirabilis*) and gram-positive bacteria (*S. aureus* and *E. faecalis*) in the ratio of 2:1 were involved in expression ESBL gene. This is consistent with the result obtained by Nwachukwu *et al.* (2018) in a similar study on the prevalence of urinary tract infections in pregnant women in Onitsha, in which more gram negatives than gram positive bacteria were implicated in ESBL production. ESBL expression by gram negatives more than the gram positives could be attributed to the differences in the genetic make-up of these two groups of bacteria with gram negative bacteria having higher chances of acquisition of ESBL gene from the environment through their thin cell wall than gram positive bacteria with thick cell wall, changes in the structure of TEM and SHV enzymes among other factors (Latifpour *et. al.,* 2016; Mitu *et. al.,* 2019). ESBL *Bla* genes are abundant in the Enterobacteriaceae family, and study has implicated *E. coli*, a gram-negative bacterium, as the carrier of the $bla_{\text{CTX-M-15}}$ which serves as a reservoir from where the gene could be transferred to other gram-negative bacteria (Overdevest *et. al.,* 2011).

Some risk factors have been identified that predispose individuals to community-associated ESBL infections (Chukwunwejim *et. al.,* 2018). These include, but not limited to; old age, being female, diabetes mellitus, previous antibiotic usage, recurrent urinary tract infections, and prior instrumentation to urinary tract (Yousefipour *et al.,* 2019). The prevalence of ESBL was higher (65.3%) in females than 34.7% in males (see figure 2). This agrees with the result of Latifpour *et. al.* (2016) in a similar study assessing the prevalence of Extended-Spectrum Beta-Lactamase-producing *K. pneumoniae* in which 65% prevalence was recorded for females while males had 34.7% with all the *E. faecalis* (100%) and 71.4% of *P. mirabilis* producing ESBL in females against none and 28.6% in males respectively. The reason for this is unclear, but some studies suggest that females are more at risk of developing infection by uropathogens due to their anatomical structure (Gharavi *et al*., 2021). However, according to Odoki *et. al*. (2019) and Nwachukwu *et. al*. (2018), gender (female) and marital status (married) had statistically significant relationships with UTI. This could be attributed to sexual activities and hormonal profile associated with age, gender and marital status all of which are important predictors of UTI.

Age groups 25 – 34 years and >64 years showed ESBL prevalence of 32.7% and 26.5% respectively as shown in Figure 3. *E. faecalis* (66.7%) and P. *mirabilis* (42.9%) were involved in UTI among those in the age groups of 25 – 34 years, while *E. coli* (40%) and *K. pneumoniae* (31.3%) were

common among those in the age group >64 years. There was a significant difference ($p<0.05$) in positive ESBL-producing bacteria urine test based on age with participants in the age group of 26-30 years showing a higher prevalence ($p=0.03$).

Civil servants recorded 38.8% prevalence of ESBL mediated UTI. Wadepohl *et al.*, (2020) reported that the occupational exposure might be a possible route of transmission as previous studies were able to demonstrate increased rates of colonization with gentamicin-resistant *E. coli* in U.S. poultry workers compared with community references. Participants with tertiary level of education had 34.7% prevalence of ESBL mediated UTI as presented in Figure 4. Although no study had dwelt on the association between educational status and prevalence of ESBL producing bacteria. Other factors of importance in UTI are malnutrition, poor hygiene and low socio-economic status, which are common in rural settings (Iseghohi *et al*., 2020), and may have association with Educational status. Iseghohi *et al*. (2020) however reported that factors such as patient's residence, tribe, level of education, marital status, circumcision, pregnancy, hypertension, HIV, abortion, sexual intercourse had no correlation with UTI but can contribute to reinfection.

Conclusion

Antimicrobial resistance is a global threat to the management of infectious diseases and the contribution of extended Spectrum Beta Lactamase (ESBL) is huge. In this study, ESBL-producing bacteria is responsible for 46.8% positive bacteria urine test with both gram-negative and gram-positive bacteria playing role. The frequency of isolation of ESBL-producing *K. pneumonia* was higher than the other isolates, but *P. mirabilis* exhibited higher likelihood of ESBL production. Females, those in the age group 25 – 34 years, married, civil servants and those with tertiary level of education were more likely to have ESBL mediated UTI than others.

Further ESBL surveillance of bacteria uropathogen in Enugu metropolis is necessary to fully understand the size of the problem of ESBL-mediated antimicrobial resistance. Investigations should be carried out to verify the role of location and characteristics of individual in the prevalence in ESBL-producing bacteria mediated UTI. Identification of resistance genes involved in ESBL-mediated antimicrobial resistance is recommended.

Table

Table 1: Occupational distribution of ESBL-producing bacteria in individuals with presumptive urinary tract infections in Enugu metropolis based on the occupation

Occupations	**Number with ESBL-producing bacteria (n=49)**
Civil servant	19(38.8%)
Business people	14(28.6%)
Student	5(10.2%)
Unemployed	4(8.2%)
Farming	7(14.3%)

Figures

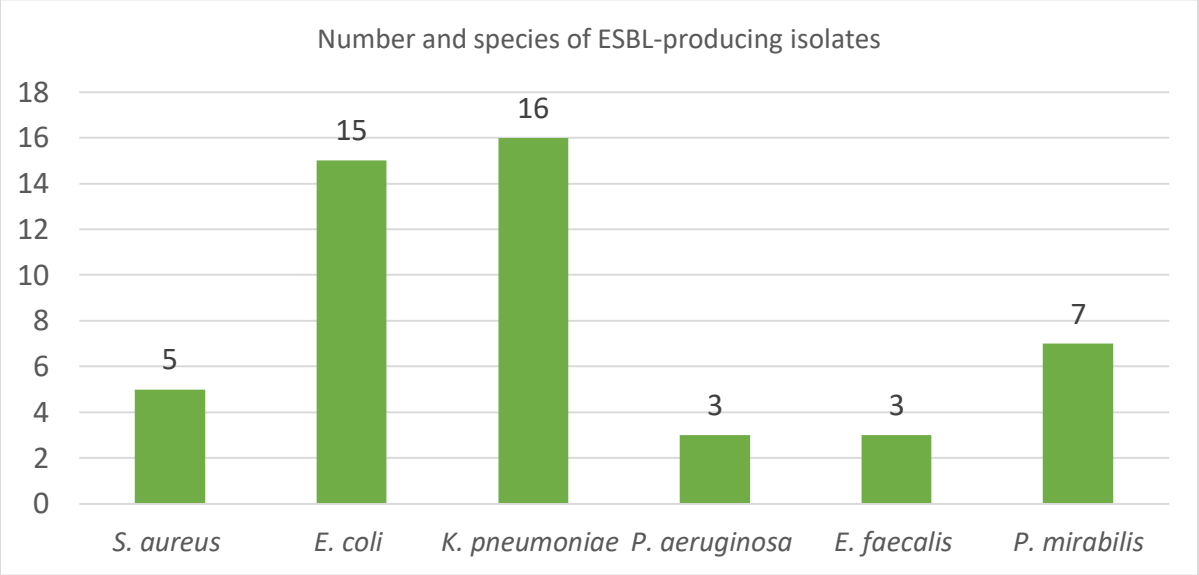

Figure 1: Number and species of ESBL-producing isolates among the studied participants

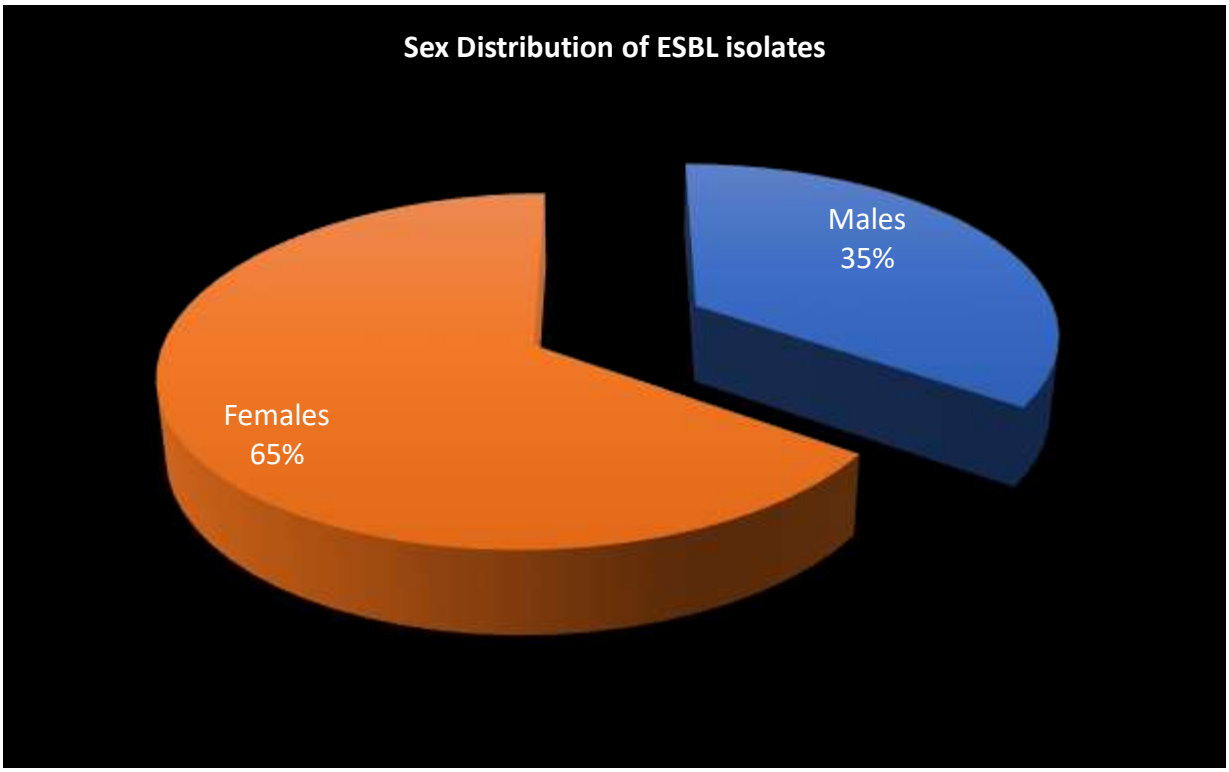

Figure 2: Sex Distribution of ESBL isolates in patients with self-reported urinary tract infections in Enugu metropolis

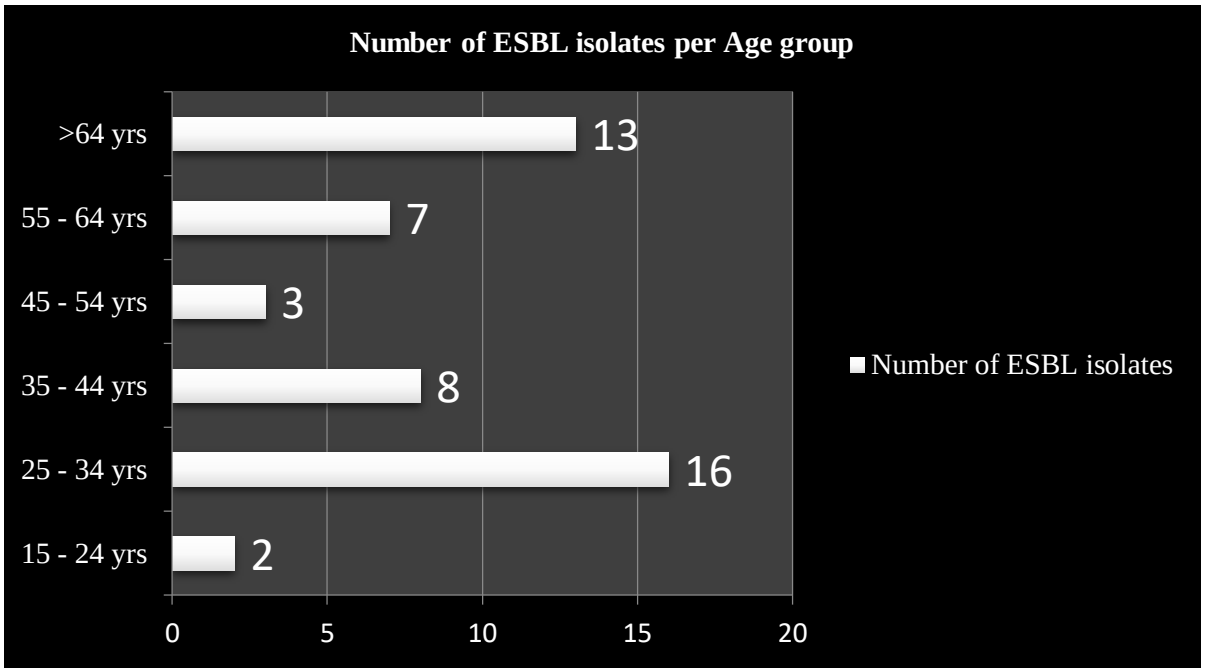

Figure 3: Age Distribution of ESBL producing bacteria in individuals with presumptive urinary tract infections in Enugu metropolis

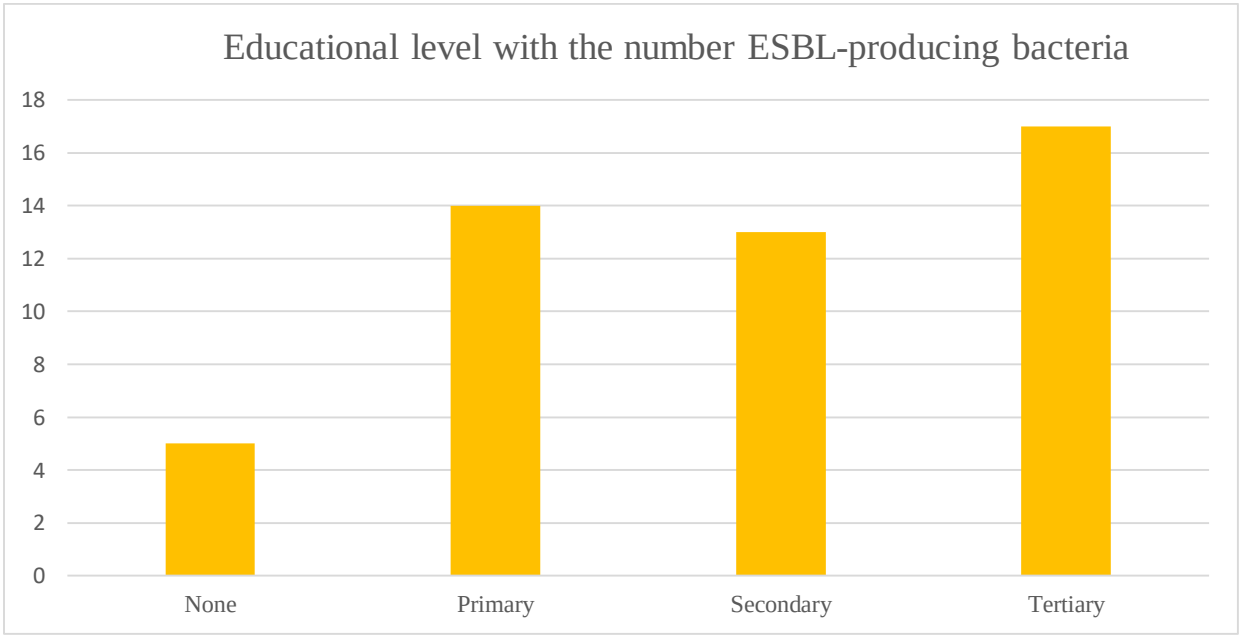

Figure 4: Distribution of ESBL-producing bacteria in individuals with presumptive urinary tract infections in Enugu metropolis based on educational level of the participants

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