Original Research Article

Prevalence of Extended-Spectrum Beta-Lactamase Mediated Urinary Tract Infections in a Tertiary Hospital in Enugu, South East, Nigeria

Abstract

Extended-Spectrum Beta-Lactamase (ESBL) is a class of plasmid-mediated diverse, complex and rapidly evolving enzymes which hydrolyzes penicillins, monobactams and broad-spectrum cephalosporins. There is increasing prevalence of ESBL-producing bacteria globally with higher prevalence reported in developing countries. The present study was designed to determine the prevalence and antimicrobial sensitivity pattern of ESBL producing bacteria isolates in patients with Urinary Tract Infection (UTI) in Enugu.Urine samples were collected from a total of 284 subjects with self-reported UTI In the Enugu State University of Science and Technology Teaching Hospital, Enugu State, Nigeria. Samples were analyzed for the presence of UTI, antimicrobial resistance pattern of ESBL-producing bacteria using standard methods. Data was analyzed by descriptive statistics using SPSS version 23 (IBM Corp Armok, NY). A total of 145 strains of S. aureus (n= 19). E.coli (n=52), K. pneumonia (n=45), P. aeruginosa (n =12), E. faecalis (n=9) and P. Mirabilis (n=8), were isolated from 132 subjects giving a UTI prevalence rate of 34.6%. Forty-nine (49) ESBL-producing strains comprising S. aureus (n=5), E. coli (n=15),K. pneumonia (n=16), S. aeruginosa (n=13), E. faecalis (n=8) and P. mirabilis (n=7) were implicated in UTI accounting for 46.8%ESBL-producing bacteria mediated UTI. There is a high prevalence of ESBL-producing bacteria induced UTI. This portends a great danger to the management of bacterial infections.

Keywords: Urinary Tract Infection, Extended Spectrum Beta-Lactamase, Prevalence, Enugu.

Introduction

Extended-Spectrum Beta-Lactamase (ESBL) is a class of plasmid-mediated diverse complex and rapidly evolving enzyme which hydrolyze penicillins, monobactams and broad-spectrum cephalosporins(1). Therapeutic options for treatment of infections due to ESBL producing bacteria are limited (2). The current drug of choice for the treatment of infections caused by ESBL-producing bacteria are the carbapenems but the use of carbapenems has also been associated with the emergence of resistant organisms (3).There is increasing prevalence of ESBL-producing bacteria globally with higher prevalence reported in developing countries. This has been reported to be due to inadequate or ineffective detection in some clinical settings, self-medication, indiscriminate use of antibiotics and lack of monitoring for microbial drug resistance (4,5).This is a concern to both clinicians and patients. In Enugu, reports on available literature have described different epidemiological prevalence of ESBL producing bacteria in urinary tract infections (6).

The inclusion and exclusion criteria of isolates have been suggested as an important factor which may over or underestimate the ESBL prevalence rates in UTI (7). The present study was designed to determine the prevalence of ESBL-producing bacterial isolates in patients with UTI in Enugu.

Materials And Methods

Study Design

This is a cross-sectional and single facility-based study conducted on patients with urinary tract infections in the Enugu State University of Science and Technology Teaching Hospital, Parklane, Enugu State, Nigeria.

Study Area

This study was carried out in Enugu State, South east, Nigeria. Enugu State is made up of three (3) senatorial zones namely Enugu East, Enugu West and Enugu North. The senatorial zones are divided into seventeen (17) local Government Areas comprising 450 communities. The State takes its name from its capital and largest city, Enugu. It has

an area of 7,161km^2 with a population of 3,267,837 comprising mainly the Igbo tribe of the south eastern Nigeria; about 50% of which lives in rural areas. It lies between longitudes 6^{0}30'E and 6^{0}55'E and latitudes 5^{0}15'N and 7^{0}15'E.Enugu State University of Science and Technology Teaching Hospital is accessed by people from different socio-economic backgrounds being located at the centre of the city. The hospital serves as referral centre for different primary and secondary healthcare facilities in the state, hence its choice for the present study (8).

Ethical Considerations

Ethical clearance for the study was obtained from the Enugu State University of Science and Technology Teaching Hospital Ethics Committee. Informed consent was obtained from each patient after educating on the objective of the study before recruitment as a subject.

Sample Size

The sample size was determined using the simple proportion method and a prevalence of 12.3%(1).

$$n = \frac{Z^2 P((1-P)}{D^2}$$

Where n = sample size
Z = 1.96 for confidence level at 95%
P = prevalence rate at 12.3%
D = 0.05 for marginal error at 5%

The sample size was calculated as 165.

Sampling Technique

Convenient sampling strategy was used to select participants for the study. Urine sample collection was scheduled for Mondays and Wednesdays in the month of April – May, 2019 until 385 samples were collected. The two days were selected conveniently to make for enough time for urine specimen analysis with emphasis on creating space in the incubator for incubation. The participants were advised on how to collect a "clean catch" mid-stream urine.

Urine Culture

The urine specimens were cultured on Blood Agar, MacConkey Agar and Cysteine Lactose Electrolyte Deficient (CLED) Agar using streaking method (10).The culture was incubated at 37^{o}C for 24 hours and the plates read. The isolates pathogens in the samples were subjected to gram staining and biochemical tests for identification.

Identification of Isolates

Gram staining and a combination of conventional biochemical testing techniques including Catalase and Coagulase tests for suspected S. aureus, Indole test for E. coli, Citrate and Malonate utilization tests for K. pneumonia, Oxidase test for P. aerugnosa , Bile Resistance test for E. faecalis and Motility test for P. Mirabilis were carried out (11).

Antimicrobial Susceptibility Test

Antimicrobial susceptibility of the isolates was carried out using the modified Kirby-Bauer Disk Diffusion method (12).Mueller Hinton (MH) Agar (Oxoid, UK) was prepared according to the manufacturer's instructions and 10ml transferred into 90mm diameter sterile Petri dishes making a depth of 4mm. The surface was lightly and uniformly inoculated using a sterile cotton wool swab in three directions while rotating the plate to ensure even distribution. Prior to inoculation. The swab stick was dipped into bacterial suspension having visually equivalent turbidity to 0.5 Mcfarland standards. Excess liquid from the sterile cotton-wool swab was dipped in the bacterial suspension which was removed by turning the swab stick against the side of the tube. The plates were covered and allowed to dry on the bench before applying the disc. Antibiotic discs were placed on the agar plate within 15 minutes of inoculation of isolates. The plates were allowed in refrigerator for about 30 minutes for pre-diffusion of the antibiotics once the disks were placed on the medium 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 18-24hrs at 37^{o}C. Inoculated plates were incubated at 37^{o}C for 24hrs. Plates were read on the next day by taking measurement of zone of inhibition using a meter rule. The E. coli A TCC 25922 was used as a negative control. Antibiotic discs used include cefotaxime (30mg), ceftazidime (30mg), ceftriazom (30mg), imipenem (10mg), meropenem (30mg), tetracycline (30mg), erythromycin (15mg), cefpodoxime (10mg), amoxicillin-clavulani acid (20/10mg), sulphamethoxazole-trimethoprim (25mg), ciprofloxacin (5mg) and gentamicind (10mg) (Oxoid, UK).

Characterization of ESBL-Producing Bacteria

Isolates were characterized for ESBL production using the Single Disk method (13). Single antibiotic disks of third generation cephalosporins comprising cefotaxime (30mg) and ceftazidine (30mg) were placed aseptically at a distance of 30mm apart on Mueller Hinton Agar plate previously inoculated with standardized inoculums of the test bacterium. 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 18-24hrs at 37^{o}C. After incubation, the zones of inhibition were measured and recorded to the nearest millimeter using a meter rule. The ESBL production was suspected if any of the test bacteria showed reduced susceptibility and is resistant to any of the third generation cephalosporins (cetotaxime and ceftazidime).

Confirmation of ESBL-Producing Bacteria

The ESBL production was confirmed in the isolates by the Double Disk Synergy Test (DDST) method (14). The Double Disk Synergy Test was performed as a standard disk diffusion assay on Mueller Hinton Agar plates. Standardized bacteria suspension was aseptically inoculated on the agar plates. Amoxicillin clavulanic acid disc (20/10mg) was placed at the centre of the plate and cefotaxime (30mg) and ceftazidime (30mg) were placed at a distance of 15mm (centre to centre) from the amoxicillin-clavulanic acid disc. The plates were allowed in refrigerator for about 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 18-24hrs at 37^{o}C. The ESBL-producing bacteria was confirmed phenotypically when a difference of $\geq$ 5mm 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.

Data Analysis

Data obtained was analyzed using IBM Statistical Package for Social Sciences (SPSS) for Window Version 23 of IBM Corporations, Armok,NY.Data was presented as descriptive statistics

Results

Six (6) different species of bacteria were isolated from 133 participants occurring either single or as a mixed growth. Bacteria species isolated include 18(13.5%) of Staphylococcus aueus, 42(31.6%) of Escherichia coli, 33(24.8%) of klebsiella pneumonia, 11(8.3%) Pseudomonas aeruginosa, 9(6.8%) of Enterococcus faecalis and 8(6.0%) of Proteus mirabilis as well as 10(7.5%) of Klebsiella pneumonia + Escherichia coli, 1(0.8%) of Staphylococcus aureus + Escherichia coli and 1(0.8%) of Pseudomonas aeruginosa + klebsiella pneumonia. Therefore, a total of 145 bacterial pathogens belonging to the above listed 6 genera were isolated from 133 participants with UTI. One hundred and twenty one (121) isolates were from 121 participants who had UTI due to a single bacterial specie and 24 isolates from 12 participants with UTI due to mixed infection caused by two different bacteria species. Figure (1) shows the number of bacteria species isolated.

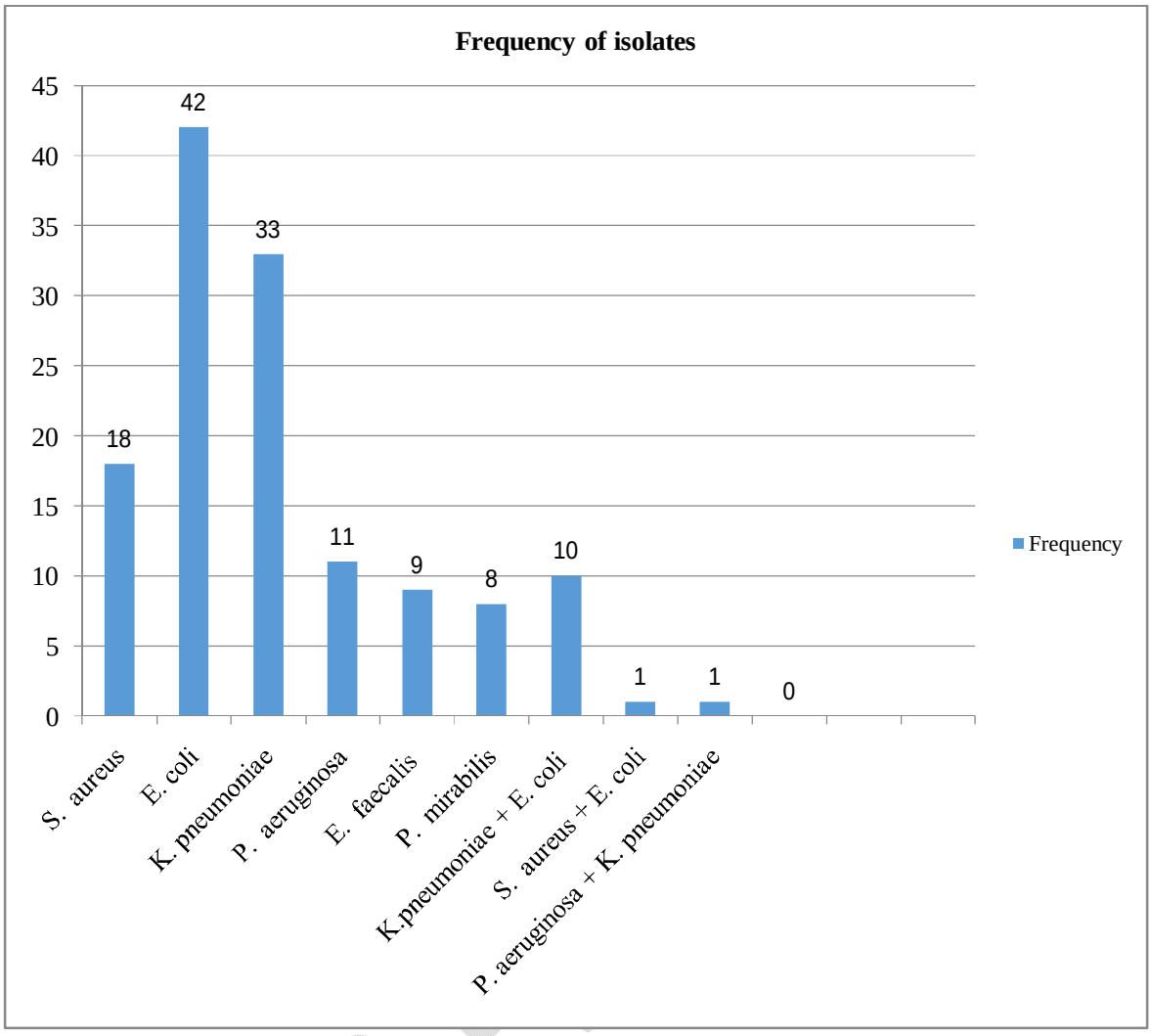

Figure 1: Frequency of Bacteria isolates

Forty nine (49) isolates were ESBL-producing and accounts for 33.8% of the entire isolates while 96(66.2%) were non-ESBL-producing. Figure 2 shows the distribution of ESBL-producing and non-ESBL-producing isolates among participants with UTI. Five (26.3%) of Staphylococcus aureus were resistant to Ceftazidime, Cefotaxime, Cefpodomxime and Cefuroxime but sensitive to Imipenem and Augumentin while 15(28.8%) and 7(87.5%) of K. pneumonia, P. aeruginosa, Enterococcus faecalis and Proteaus mirabilis respectively and exhibited resistance to Ceftazidime, Cetotaxime and Cetpodoxime but sensitive to Cefuroxime Imipenem and Augumentin.

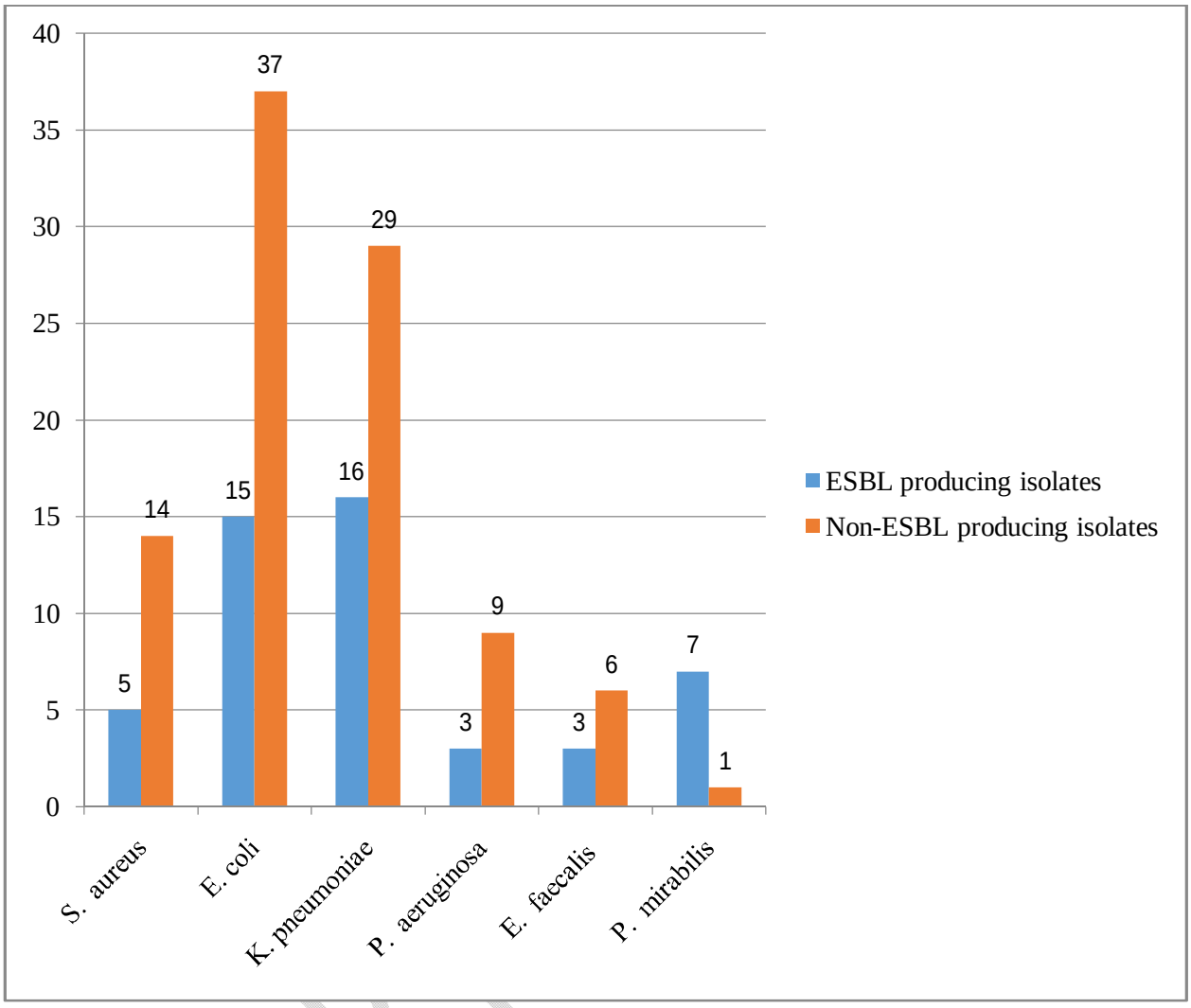

Figure 2: Distribution of ESBL and non-ESBL producing bacterial among the study participants

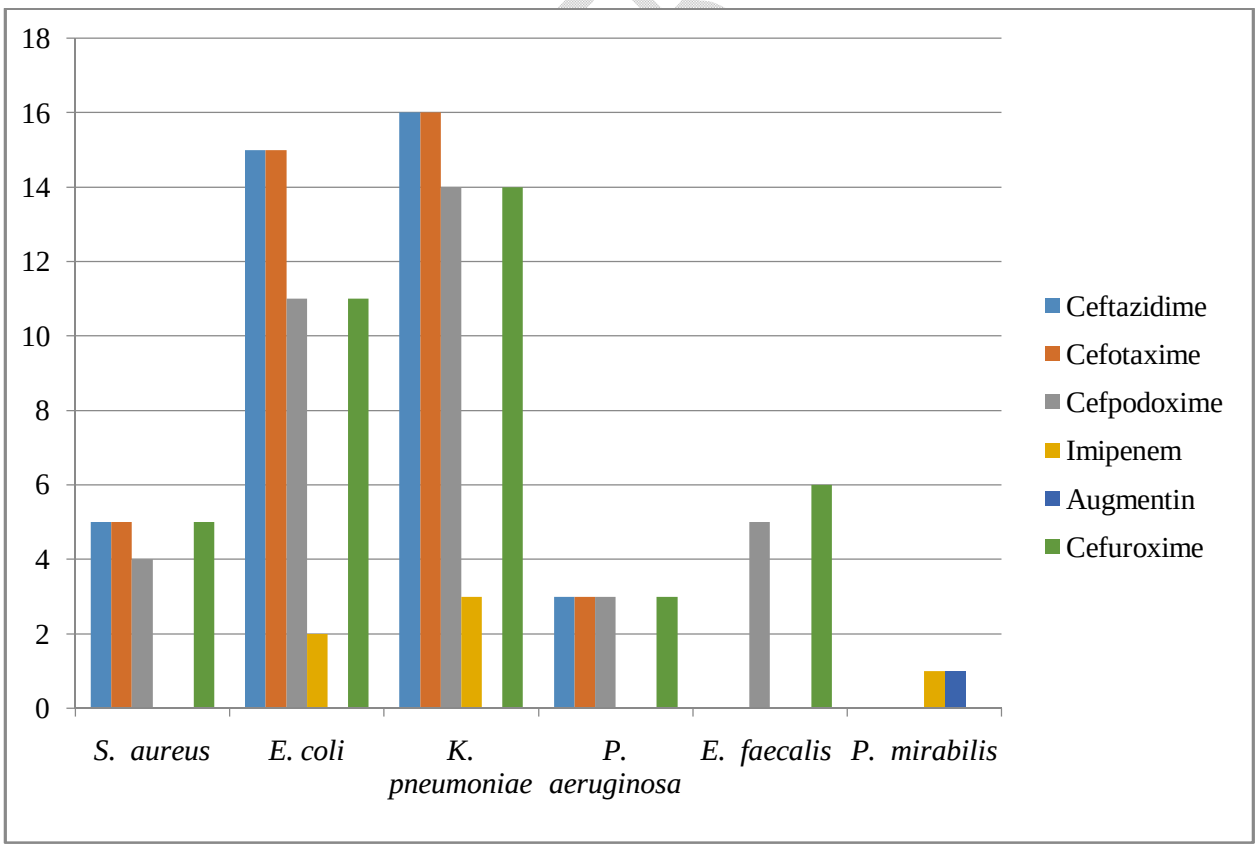

Figure 3: Antibiotics Resistance Pattern of ESBL producing Bacteria

Discussion

Against the previous report that ESBL is most common in Klebsiella and Escherichia species, we isolated six (6) different species of bacteria (gram negatives and gram positives). They includes.S. aureus, E. coli, K. pneumonia, P. aeruginosa, E .faecalis and P. mirabilis.This is consistent with the report of Mitu et al(15) who isolated S. aureus, E. coli, K. pneumonia, P. aeruginosa and E. faecalis among patients with UTI and the reports of Chukwujim et al(16) who reported that Klebsiella and Escherichia species are not the only ESBL producing bacteria implicated in UTI. A total of 52(35.9%) of E. coli, 45(31.0%) of K. pneumonia, 19(13%) of S. aureus, 12(8.3%) of P. auroginosa, 9(6.2%) of E. faecalis and 8(5.5%) of P. mirabilis were isolated. Thus, E. coli was the leading cause of UTI in the present study, followed by Klebsiella pneumonia, S. aureus and others. This is consistent with other studies which implicated E. coli as the major cause of UTI (17).Contrary to this finding, Klebsiella pneumonia was the highest occurring urinary tract pathogen followed by E. coli (18).This shows that the preponderance of the agents of UTI may differ from one location to another, hence further investigations should be carried out to ascertain the role of location in the preponderance of uropathogens in UTI.The prevalence of UTI in this study was 34.6%.A total of hundred and thirty-three (133) out of 384 participants had positive urine culture implying UTI. This prevalence is similar to 32.2% prevalence reported in Uganda (19) but varies with 5.8% reported among HIV patients in Nigeria (20).The reason for the lower prevalence could be attributed to the use of antibiotics in the management of opportunistic infections in HIV/AIDS which may help reduce the rate of infection. A higher prevalence of 56% was obtained in another study among pregnant women (1). This was attributed to hormonal changes in pregnancy, sexual intercourse and douching among others(1).The prevalence of ESBL producing bacteria has been investigated in other countries – 30% in the Korea, 36% in Pakistan, 68% in India, 20% in Algeria, 29% in Spain, 28.4% in Taiwan and 44% in the USA (10).The prevalence of ESBL producing bacteria recorded in the present study is 33.8%.This is consistent with the ESBL prevalence in Korea and Pakistan but lower than that obtained in India and higher than the 20% in Algeria, 29% in Spain and 28.4% in Taiwan. The reason for low and high prevalence in different 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 other countries like Nigeria. Regarding the antibiogram, the isolates showed a high susceptibility to Imipenem and Amoxicillin-Clavulanic than the other antibiotics. The organism, P. Mirabilis exhibited a high rate of resistance to Amoxicillin-Clavulanic and Imipenem which has been the most successful antibiotic against ESBL-producing bacteria because of their beta-lactamase stability.These findings suggests the emergence of new resistance mechanisms which could be due to the abuse or under-dosing of these antibiotics in the Enugu community.

Conclusion

The overall prevalence of UTI in Enugu within the period of this study was 34.6%. This study revealed that ESBL producing bacteria were implicated in UTI with a prevalence rate of 33.8%. This is alarming and a major concern to both clinicians and patients. Hence, there is an urgent need to establish an evidence-based protocol and guideline for the implementation of surveillance programs such as Study for Monitoring Antimicrobial Resistance Trends (SMART)in Enugu. This will help limit the spread of resistant strains and reduce antibiotic treatment failures.

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