Original Research Article

Antibiogram of Multidrug resistance and extended-spectrum beta- lactamase producing Gram-negative bacteria from patients attending Diabetic clinic at Nnamdi Azikiwe University Teaching Hospital, Nigeria.

Abstract

Back ground: Multidrug resistance (MDR), and extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacteria (GNB) is a major public health threat worldwide. Objectives: The aim of the present study was, therefore, to assess Multidrug resistance and extended-spectrum beta- lactamase producing Gram-negative bacteria from patients attending Diabetic clinic at Nnamdi Azikiwe University Teaching Hospital, Nigeria. Methods: A cross-sectional study was conducted from September 2019- April 2020 at Nnamdi Azikiwe University Teaching Hospital, Nigeria. A total of 110 study subjects were recruited using a convenient sampling technique. Urine specimens were aseptically collected. Culturing for identification of bacteria and determination of drug susceptibility testing were done following standard microbiological techniques. Selected MDR isolates were phenotypically assessed for ESBL production. Obtained data was analyzed using Statistical Package for the Social Science (SPSS version 26) and data was computed with descriptive statistics, Chi-square and pair t-test. Results: Of the 110 clinical samples cultured for bacterial growth, 55 (27.5%) were positive for Gram negative bacteria (GNB). The most common GNB identified were *E. coli* 30 (54.6%), *Klebsiella pneumoniae*. 14 (25.5%), *Pseudomonas aeruginosa* 6 (10.9%), and *Proteus* species 5 (9.09%). The highest MDR prevalence of bacteria was found resistant to Augmentin 46 (83.6%) as well as Cefuroxime 43 (78.2%) respectively. Among the total isolates, 36 (65.5%) were resistant to Ceftazidime followed by Cefixime 30 (54.5%). Of which, these ESBL producers had significantly mean resistant to Augumentin (0.53 ± 1.2; $p = 0.000$) and Cefuroxime (0.600 ± 0.974; $p = 000$) respectively. Conclusion: The study detected the high rate of multi-drug resistance and ESBL producing isolates respectively. *E.coli* and *Klebsiella pneumonia* were the most common ESBL producing GNB. Regular monitoring, conducting, supervising, or management of antibiotics and molecular biomarkers for drug resistance are paramount to curtail the rate of drug-resistant pathogens.

Key word: extended-spectrum beta-lactamase, Multidrug resistance, *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa*

Introduction

The continuous spread of multidrug resistance (MDR) Gram-negative bacterial infections in hospitals and community has become one of the global issues, as the result of inadequate availability of alternative effective therapeutic options and high rate of substandard drugs over the counters [1;2]. The problem is more common in developing countries where they reported the high incidence infectious disease burden links to antimicrobial resistance [3]. In addition, the increase in antimicrobial resistance has been proved statistically to cause 700,000 deaths per

annual, the statistics figure is expected to rise up to 10 million deaths per year by 2050, as the result of drug ineffectiveness and high rate of antimicrobial-resistant infections [4]. There are main drugs of choice in used for the management of infectious diseases caused by Gram-negative pathogens, include the fluoroquinolones, cephalosporins, and β-lactam/β-lactamase inhibitor combinations. Resistance to these agents would compromise the efficacy of empiric treatment of suspected Gram-negative infections [5].

Gram-negative bacteria (GNB) are the major cause of drug resistance but they vary from one setting to another [6;7]. In the community setting, *Escherichia coli (E. coli)* and *Klebsiella pneumoniae (K. pneumoniae)* create a critical problem [8]. .Multi-drug-resistant GNBs mainly seen in inpatient wards are *Acinetobacter* spp., *Pseudomonas aeruginosa*, and carbapenem-resistant Enterobacteriaceae. Enterobacteriaceae that can produce extended-spectrum beta-lactamase (ESBL), such as *E. coli* and *K. pneumoniae*, are a major concern in humans, animals, and the environment [9].
However, a few studies have worked on community hospitals [10; 11; 12]. Therefore, the current study aimed to assess the antibiogram of Multidrug resistance and extended- spectrum beta-lactamase producing Gram-negative bacteria from patients attending Diabetic clinic at Nnamdi Azikiwe University Teaching Hospital, Nigeria.

The results could be applied toward prevention of antimicrobial resistance, promotion of appropriate antimicrobial use, development of a treatment guideline for drug-resistant infections, or prevention of microbial outbreaks in local community.

Materials and methodology

Sample population

A cross-sectional study was conducted from September 2019- April 2020 at Nnamdi Azikiwe University Teaching Hospital, Nigeria. The urine samples were collected from 110 diabetic patients by using convenient sampling technique. Written consents were obtained from study participants/ assents from parents or guardians. Patient's sociodemographic characteristics were taken urine specimens were aseptically collected.

Sample collection, Processing and bacterial identification

Mid-stream urine samples were collected using a sterile test tube and inoculated on to Cysteine Lactose Electrolyte Deficient agar (CLED) using a calibrated loop (1.3 mm diameter, delivering1 µL) and incubated overnight at 37 ℃. The samples with significant bacteriuria ($\geq 10^5$ CFU/mL) were sub-cultured on to blood agar, MacConkey and Chocolate agar [13].

Identification of Gram-negative bacteria was done using colony characteristics, Gram reaction and different biochemical tests such as, triple sugar iron agar, indole, motility, urease production, hydrogen sulphide production, citrate utilization, and lysine decarboxylase tests [3].

Antibiotic Susceptibility testing

Following identification of bacterial isolates, modified Kirby-Bauer disk diffusion method was done on Muller-Hinton agar according to the Clinical and Laboratory Standard Institute (CLSI) guide line [14]. Up to 4 pure colonies of young culture suspension was prepared in equivalent to 0.5McFarland standards and plated. The plates were allowed to dry for 5 min; antibiotic discs were evenly distributed on the inoculated plate using sterile forceps and incubated at 37 ℃ for 24 hours. The diameter of the zone of inhibition around the antibiotic disc was measured using a ruler. Results were interpreted as Sensitive and Resistance based on CLSI 2017 guide-line. The antibiotic discs used were: ampicillin (AMP, 10 µg), Augmentin (AUG, 20/10 µg), cotrimoxazole (SXT, 25 µg), tetracycline (TET, 30 µg), ciprofloxacin (CIP, 5 µg), chloramphenicol (CHL, 30 µg), gentamycin (GEN, 10 µg), cefepime (FEP, 30 µg), cefixime (CFM, 5 µg), ceftriaxone (CRO, 30 µg), cefoxitin (FOX, 30 µg), and ceftazidime (CAZ, 30 µg) (all from Abtek bio.Ltd UK) and were selected following CLSI guide-line. Multi-drug resistance patterns of the isolates were determined following the criteria set by Magiorakos *et al*. [15].

Detection of extended- spectrum beta-lactamase (ESBL)

Following antimicrobial susceptibility testing bacterial isolates showing zones of inhibition diameters ≤ 22 mm to ceftazidime [30 µg] or ≤ 27 mm to cefotaxime [30 µg] were subjected to ESBL production test. Phenotypic confirmation of ESBL production was done by using the double-disk diffusion method; cefotaxime [30 µg] and cefotaxime-clavulanic acid [30/10 µg] or ceftazidime [30 µg] and ceftazidime clavulanic acid [30/10 µg] as previously described [12].

Data analysis

Data were entered and analyzed using SPSS version 26. Chi-square were applied to see the distribution of sociodemographic variables. Frequency and percentages were computed using descriptive statistics. Pair-T test was set at p-value less than 0.005 at a 95% confidence interval was considered statistically significant.

Quality control

One hundred and ten questionnaires were distributed to the participants to check for completeness before the commencement of the work. All the research work were conducted following the standard operating procedures. The sterility and the performance of the media were observed daily. The sterility was done by incubation of 5% of the prepared media overnight at 37 ℃ for 24 hour. *E. coli* (ATCC 25922), *K. pneumoniae* (ATCC1705) and *K. pneumoniae* (ATCC1706) were used as quality control for identification and antimicrobial susceptibility testing. The reliability and accuracy of the findings was guaranteed by

implementing quality control measures such as pre-analytical, analytical and post-analytical throughout the whole processes of the laboratory work. Multi-drug resistance was considered as simultaneous resistance to 3 or more antibiotic classes.

Result

A total of 110 patients were enrolled in the present study. Of these 60 (54.54%) were females and 50 (45.45%) were males. Majority of the study participants were age ≤ 80 years, 35(31.82%) followed by age group 61-70 years, 50(45.5%); 51-60 years, 15 (13.0%); 41-50years, 8(7.81%) and the least age groups were age less than 40 years, 2 (1.81%). The occupational status indicated that the major participants were civil servant, 85(77.27%), followed by farmer, 15(13.64%) and the least occupation participated in the study were artisan, 10(9.09%). Fifty-five participants (50.0%) were suffering from urinary tract infection and 55(50.0%) were not associated with urinary tract infection (Table 1).

Majority of Gram-negative bacteria were isolated from urine 55/110 (50.0%). The most common prevalent isolates in urine culture were *E. coli* (53.70%) followed by *Klebsiella pneumoniae* (27.78%), Pseudomonas aeruginosa (11.11%), and proteus species (7.41%) respectively (Figure1).

The overall Cefuroxime resistant prevalence was 43 (86.0%). Among the total isolates, 25 (58.14%) of *E.coli* were resistant followed by *Klebsiella pneumoniae* 10 (23.26%), *Proteus species* 6 (11.63%), *Pseudomonas aeruginosa* 3(6.97%) respectively (figure 2).

The overall Ceftazidime resistant prevalence was 38 (78.0%). Among the total isolates, 22 (57.89%) of *E.coli* were resistant followed by *Klebsiella pneumonia* 8 (21.05%), *Proteus species* 1 (2.63%), *Pseudomonas aeruginosa* kD6(15.79%) respectively (figure 3).

The overall Cefixime resistant prevalence was 31 (62.0%). Among the total isolates, 20 (64.52%) of *E.coli* were resistant followed by *Klebsiella pneumoniae* 9 (29.03%), *Proteus species* 1 (3.23%), *Pseudomonas aeruginosa* 1(3.23%) respectively (figure 4).

The overall Augumentin resistant prevalence was 42 (84.0%). Among the total isolates, 9 (21.43%) of *E.coli* were resistant followed by *Klebsiella pneumonia* 9 (21.43%), *Proteus species* 4(9.52%), *Pseudomonas aeruginosa* 4(9.52%) respectively (figure 5).

Table 1: Demographic data of Type-2 diabetes individuals with Urinary Tract Infection

Variables	Type-2 diabetes(n=110)	Non-T2diabetes(n=10)	X2	p-value
Age (in years)	20-80	20-80		
30 - 40	2(1.81%)	3		
41-50	8(7.27%)	2		
51 - 60	15(13.64%)	3		
61 - 70	50(45.5%)	1		
71 - 80	35(31.82%)	1		
Chi-square Test			25.057a	0
Gender				
Male	50(45.45%)	5(50.00%)		
Female	60(54.54%)	5(50.00%)		
Chi-square Test			0.076a	0.782
Educational Levels				
Primary	10(9.09%)	0		
Secondary	40(36.36%)	1(10.00%)		
Teritary	60(54.55%)	9(90.00%)		
Chi-square Test			4.778a	0.092
Occupation				
Farmer	15(13.64%)	0		
Civil servant	85(77.27%)	6(60.00%)		
Artisan	10(9.09%)	4(40.00%)		
Chi-square Test			9.231a	0.01
Asymptomatic infection				
UTI subject	55(50.00%)	3(30%)		
NON-UTI subject	55(50.00%)	7(70%)		
Chi-square Test			0.052a	0.819

p-value <0.05 was considered as significant

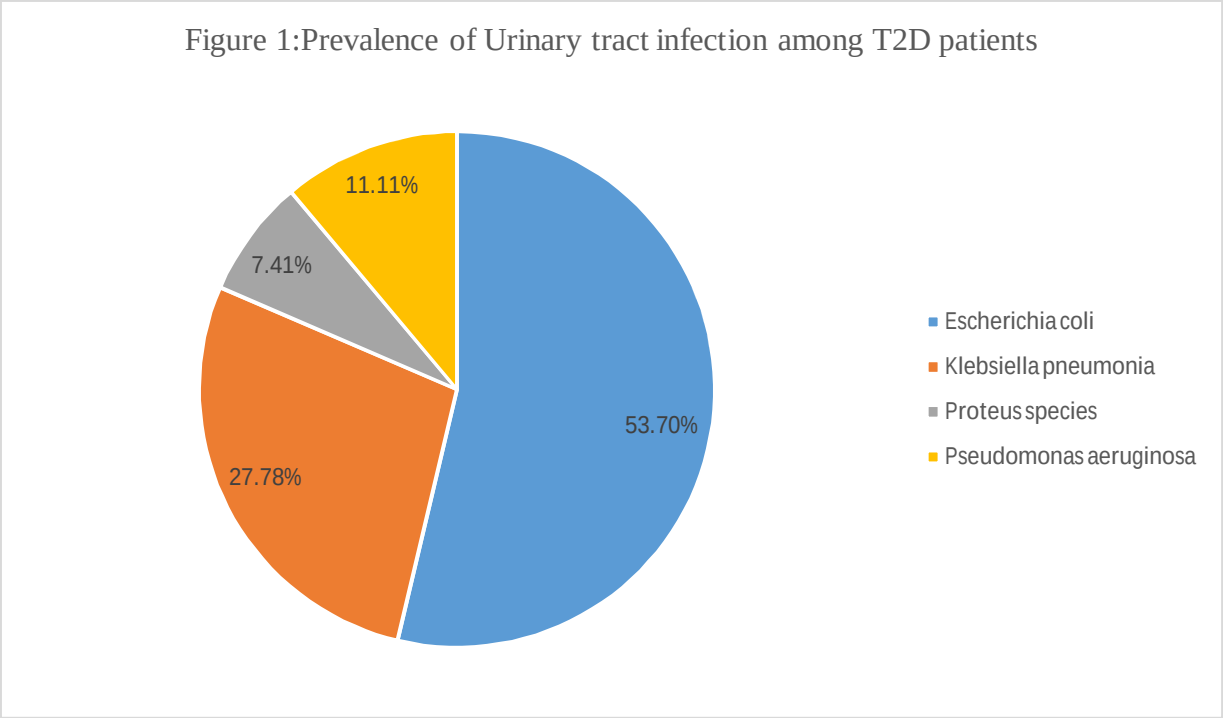

Figure 1:Prevalence of Urinary tract infection among T2D patients

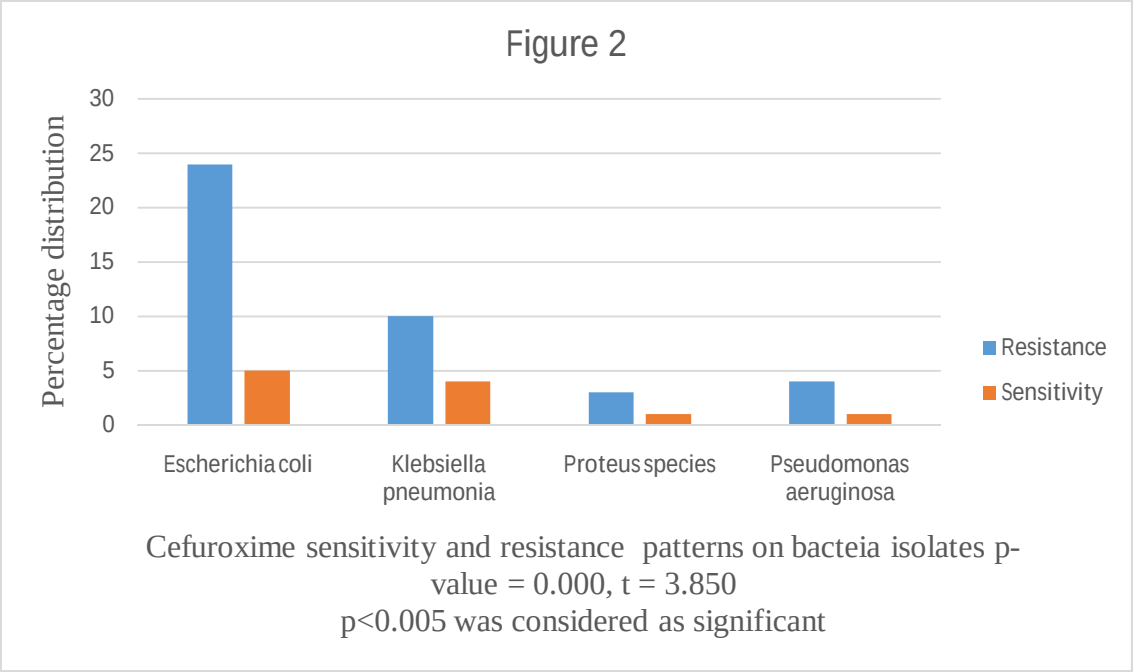

Figure 2: Cefuroxime sensitivity and resistance patterns on bacteia isolates p-value = 0.000, t = 3.850

p<0.005 was considered as significant

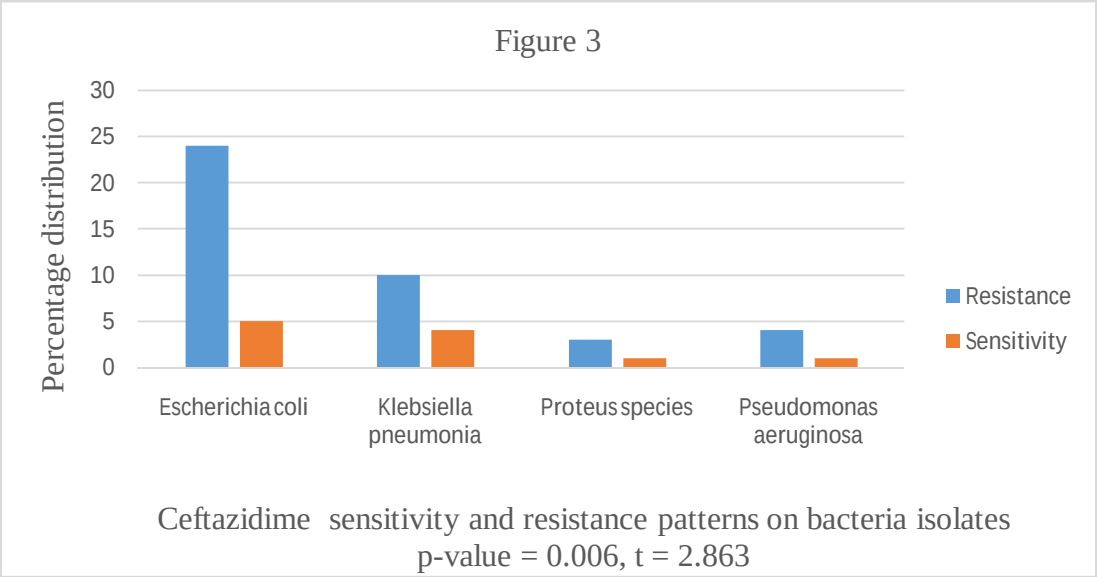

Figure 3: Ceftazidime sensitivity and resistance patterns on bacteria isolates

p-value = 0.006, t = 2.863

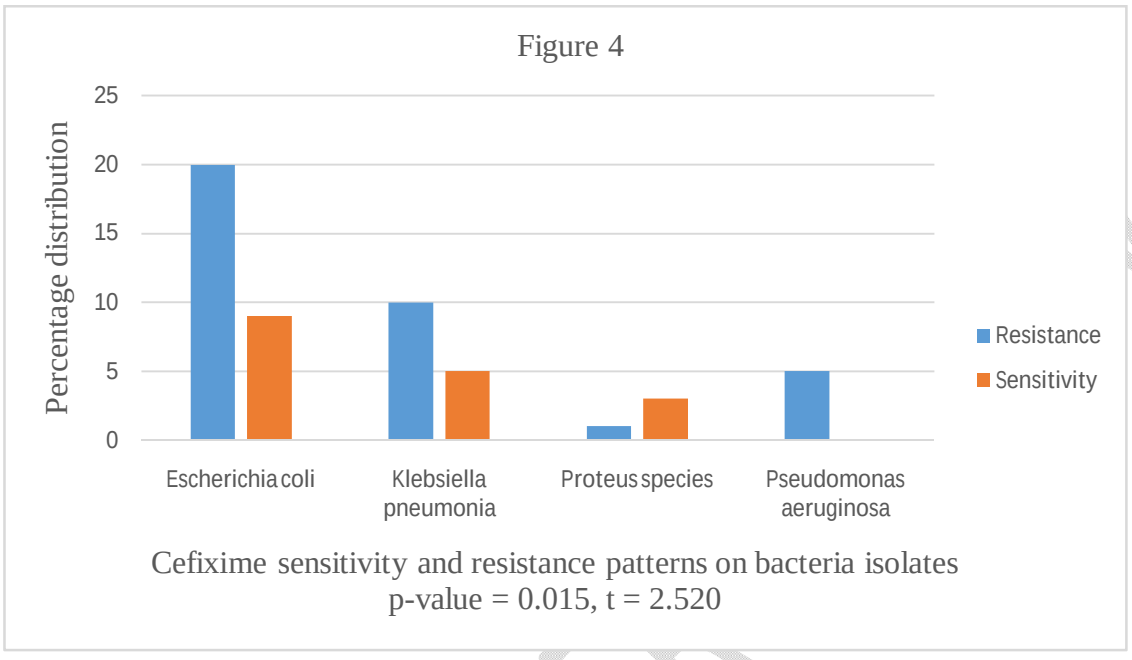

Figure 4: Cefixime sensitivity and resistance patterns on bacteria isolates

p-value = 0.015, t = 2.520

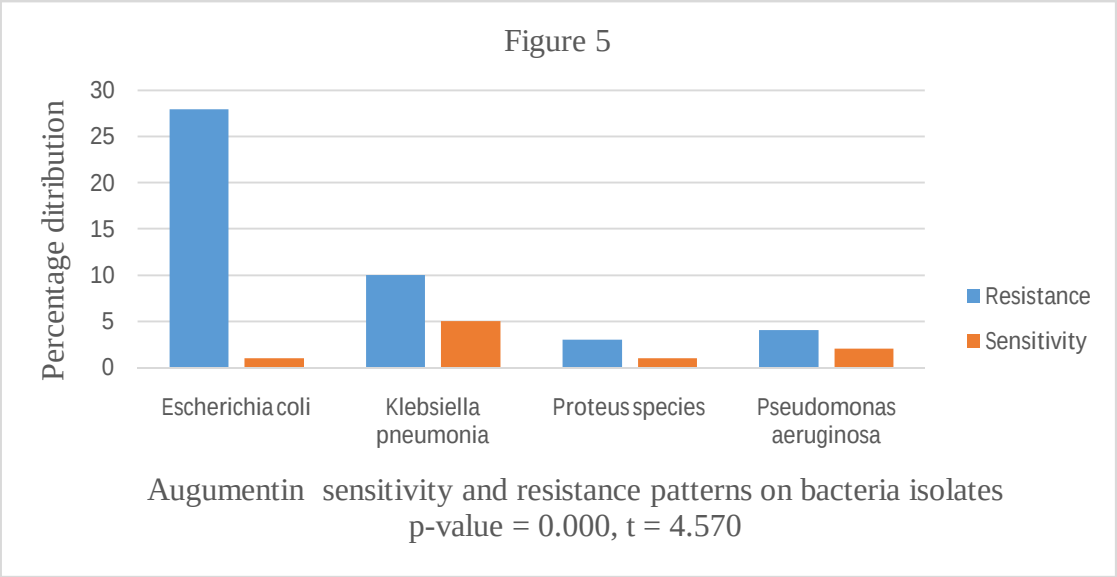

Figure 5: Augumentin sensitivity and resistance patterns on bacteria isolates

p-value = 0.000, t = 4.570

Discussions

The overall prevalence of Gram-negative bacteria isolated from urine sample of type-2 diabetic patients attending diabetic clinic was 50%. However, it is higher than a study conducted from other Africa countries (10; 18; 19). The most common isolates in this study were *E. coli*, 53.70% and *Klebsiella pneumoniae*, 27.78%. Similarly, a Saudi Arabian report showed that the prevalence of gram negative bacteria isolates same to results in which, *E. coli*, 69.8% and K. *pneumoniae*, 17.2% were the most frequently isolated Gram-negative bacteria [16]. The sources and numbers of the urine sample collected, patients with comorbidity, severity of diabetic condition, patient on/without antibiotic medications, at which the samples obtained, and geographical differences used in each study may explain the observed variations among the overall prevalence and occurrences of Gram-negative bacteria.

Considering antimicrobial resistance rate of gram negative bacteria, a high resistance rate was noticed to the commonly prescribed cephalosporins, with exception of ceftazidime and

cefixime to which the isolates exhibited below 40% resistant while, a significant resistance rate of cefuroxime and Augmentin were ($p=0.00$, $t= 3.850$; $p = 0.00$, $t = 4.570$) respectively. This agrees with results of a Mexican that reveals the isolated GNB exhibited a high resistance rate for ampicillin (95.85%), cefuroxime (84.17%), piperacillin (82.93%), cefotaxime (78.07%), ceftriaxone (77.41%), aztreonam (75.23%), cefazolin (75.00%), and ceftazidime (73.19%) [17]. This study is also disagreed with a study from Nepal, *E. coli* was found to be most sensitive to cephalosporins and tetracycline and most resistant to quinolones, fluroquinolones and sulphonamides [18]. These high rates of antimicrobial resistance observed in our setting, alarms the stakeholders to have more surveillance and control of the use of antimicrobials to combat infections. Other reasons for inappropriate use of antibiotics may include a wrong indication, wrong duration, improper route of administration, use of leftover antibiotics from a family member, and immature discontinuation of antibiotics.

Conculsion

The study detected the high rate of multi-drug resistance of cefuroxime and Augmentin respectively. In this hospital setting, the clinician should recommend the patients on cefixime and ceftazidime for urinary tract infection. *E. coli* and *Klebsiella pneumonia* were the most common ESBL producing GNB. Regular monitoring, conducting, supervising, or management of antibiotics and molecular biomarkers for drug resistance are paramount to curtail the rate of drug-resistant pathogens.

References

1.Shaikh S, Fatima J, Shakil S, Rizvi SMD, Kamal MA (2015). Prevalence of multidrug resistant and extended spectrum beta-lactamase producing Pseudomonas aeruginosa in a tertiary care hospital. Saudi J Biol Sci. 22(1):62–64

2. Negvekar V, Sawant S, Amey S (2020). Prevalence of multidrug-resistant Gram-negative bacteria cases at admission in multispecialty hospital. Journal Global Antimicrobial Resistance 9(22):457–61

3.Ayukekbong JA, Ntemgwa M, Atabe AN (2017). The threat of antimicrobial resistance in developing countries: causes and control strategies. Antimicrob Resist Infect Control. 6(1):47.

4. Piddock LJ (2016). Reflecting on the final report of the O'Neill Review on Antimicrobial Resistance. Lancet Infectious Disease. (7):767–768

5. Izadpanah M, Khalili H (2015). Antibiotic regimens for treatment of infetions due to multidrug-resistant Gram-negative pathogens: An evidence-based literature review. J Res Pharm Practice. 4(3):105–14

6. Cassini, A.; Högberg, L.D.; Plachouras, D.; Quattrocchi, A.; Hoxha, A.; Simonsen, G.S.; Colomb-Cotinat, M.; Kretzschmar, M.E.; Devleesschauwer, B.; Cecchini, M (2019). Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: A population-level modelling analysis. *Lancet Infectious Diseaase.* 19:56–66.

7. Morris, S.; Cerceo, E (2020). Trends, epidemiology, and management of multi-drug resistant gram-negative bacterial infections in the hospitalized setting. *Antibiotics*. *9*:196.

8. Sumpradit, N.; Wongkongkathep, S.; Malathum, K.; Janejai, N.; Paveenkittiporn, W.; Yingyong, T.; Chuxnum, T.; Vijitleela, A.; Boonyarit, P.; Akaleephan, C (2021). Thailand's national strategic plan on antimicrobial resistance: Progress and challenges. *Bull. World Health Organ.* 99: 661–673.

9. National Antimicrobial Resistance Surveillance Thailand. Global and National Antimicrobial Resistance Situation 2019. Available online: **http://narst.dmsc.moph.go.th/** (accessed on 31 May 2021).

10. Baramee, J.; Unahalekhaka, A.; Klunklin, P (2021). Implementation and barriers in prevention of multidrug-resistant organisms transmission among community hospitals. *Nursing Journal Chiang Mai University. 48*: 95–106.

11. Alali, W.Q.; AlFouzan, W.; Dhar, R (2021). Prevalence of antimicrobial resistance in Gram-negative clinical isolates from a major secondary hospital in Kuwait: A retrospective descriptive study. *Germs.* 11:498–511.

12. Andrés Lasheras, S.; Martín Burriel, I.; Aspiroz, C.; Mainar Jaime, R.C.; Robres, P.; Sevilla, E.; Kuijper, E.; Chirino Trejo, M.; Bolea, R (2019). Incidence and characterization of Clostridium difficile in a secondary care hospital in Spain. *Rev. Esp. Enferm. Dig. 111*: 338–344.

13 Chesbrough M. District laboratory practice in tropical countries. New York: Cambridge university press, 2006

14. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; twenty-second informational supplement. 27th ed. CLSI Supplement M100 Wayne, PA: CLSI; 2017

15. Magiorakos AP, Srinivasan A, Carey R, Carmeli Y, Falagas M, Giske C, et al. Multidrug-resistant, extensively drug-resistant and pan drug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology infection. 2012:268-281.

16. Jaffar A, Rabaan A, Justin VS, Ali MB(2 . Antimicrobial resistance of gram-negative bacteria: A six-year longitudinal study in a hospital in Saudi Arabia. J Infect Public Health. 2020;13(5):737–745.

17. Uc-Cachón AH, Gracida-Osorno C, Luna-Chi IG, Jiménez-Guillermo JG, Molina-Salinas GM (2019). High prevalence of antimicrobial resistance among gram-negative isolated bacilli in intensive care units at a tertiary-care hospital in Yucatán Mexico. Medicina (Kaunas). 55(9):588.

18. Lamichhane B (2014). Antibiotic resistance patterns of Gram-negative isolates in a tertiary care hospital of Nepal. *Asian Journal Pharm Clinical Resources.*7(3):30-33