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Antibiogram Pattern of Multidrug Resistance of Gram-negative Extended Betalactamase Isolates from Urine of Diabetic Patients

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: The rate of multidrug resistant bacteria is the most worrisome in the health setting because it often associates with nosocomial infection.
Objectives: This present study aim at, evaluating the antibiotic pattern of multidrug resistance of Gram-negative extended betalactamase isolates from urine of diabetic patients.

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Methods: This is a cross sectional study carried out at Nnamdi Azikiwe University Teaching Hospital with a total of 110 diabetic patients and were recruited using convenient sample method. The patients were instructed on how to collect the mid-stream urine samples without contamination. The samples were cultured and characterized the isolates following standard bacteriological methods. The isolated bacteria were subjected to sensitivity test pattern using agar disc diffusion techniques. The multidrug resistant isolates were physically checked for the extended spectrum betalactamase production. Obtained data was subjected to these statistical tools; descriptive statistics, chi-square and pair-test using Statistical Package for the Social Science (SPSS version 26).

Results: Out of 110 urine samples assessed for significant growth of bacteria, it was found that, 55(27%) were the Gram negative bacteria (GNB).The highest prevalence of isolates were *E. coli* 30(54.6%), followed by *Klebsiella pneumonia* 14 (25.5%), *Pseudomonas aeruginosa* 6 (10.9%), and *Proteus* species 5 (9.09%) respectively. The most occurrence of the MDR bacteria were found in Augmentin 46 (83.6%) as well as as Cefuroxime 43 (78.2%) respectively. The significant mean range of Augumentin and Cefixime resistant to ESBL producers were (0.53±1.2; p = 0.000) and (0.600 ± 0.974; p = 000) respectively.

Conclusion: The study detected the high proportion of multi-drug resistant isolates and the most occurrence were found in *E. coli* and *Klebsiella pneumoniae.* Regular monitoring, conducting, supervising, or management of antibiotics and molecular biomarkers for drug resistance are paramount to curtail the rate of drug-resistant pathogens.

Keywords: Extended-spectrum beta-lactamase; multidrug resistance; Escherichia coli; Klebsiella pneumonia; Pseudomonas aeruginosa.

1. INTRODUCTION

There is an increase alarming rate of the occurrence of superbugs globally and common among Gram negative bacteria that cause nosocomial infections and communities-acquired infections in the hospital setting and community at large, due to misused and mismanagement of drugs in the society [1,2].

The great percentage of cases are more prominent in developing countries than developed countries which attribute to antibiotics abuse and multidrug resistance [3].

Currently, the mortality and morbidity rate of antibiotics resistance is worrisome and many people dying on the daily bases due to high rate of substandard drugs over the counters [4]. For instance, they are available drugs for the treatment of bacterial infections caused by Gram negative bacteria and these conventional antibiotics are fluoroquinolones, cephalosporins, and β-lactam/β-lactamase inhibitor combinations [4]. Failure to these drugs have created the fear of the multidrug resistant among the population [5].

The most common Gram-negative bacteria associate with antibiotics resistant may change from one place to another [6,7]. For instance, in the community setting, *Escherichia coli (E. coli)* and *Klebsiella pneumoniae (K. pneumoniae)* cause community acquired infection [8]. While, in the hospital setting, *Acinetobacter* spp., *Pseudomonas aeruginosa*, and carbapenem-resistant *Enterobacteriaceae*. *Enterobacteriaceae* produce extended-spectrum beta-lactamase (ESBL), such as *E. coli* and *K. pneumoniae*, that can cause hospital acquired infections [9].

There is paucity of information on the community and hospital acquired infections reported from researchers [10-13]. In addition, the current study aimed to evaluate the antibiotic pattern of multidrug resistance of Gram-negative betalactamase isolates from urine of diabetic patients.

2. METHODS

2.1 Sample Population

This is a cross sectional study carried out at Nnamdi Azikiwe University Teaching Hospital with a total of 110 diabetic patients and were recruited using convenient sample method. The patients were instructed on how to collect the mid-stream urine samples without contamination.

2.2 Sample Processing and Bacterial Characterization

The early morning clean-catch urine samples were collected with sterile universal containers and cultured on to Cysteine Lactose Electrolyte

Deficient agar (CLED) using a calibrated loop and incubated at 37°C for 24 hours. The culture plates with significant bacteriuria (≥ 10^5 CFU/mL) were considered as pathogenic bacterial growth [14].

Characterization of the Gram-negative bacteria was carried out using colony morphology, Gram reaction and various biochemical tests like urease production, triple sugar iron agar, indole, motility, hydrogen sulphide production, citrate utilization, and lysine decarboxylase tests [3].

2.3 Antibiotic Sensitivity Testing

The modified Kirby-Bauer disk diffusion method was carried out on Muller-Honton agar following the Clinical and Laboratory Standard Institute (CLSI) guide line [15]. Up to 4 pure colonies of young culture suspension was prepared in equivalent to 0.5McFarland standards and plated. The plates left to dry for 5 minutes and antibiotic discs were evenly distributed on the inoculated plate using sterile forceps and incubated at 37°C for 24 hours. The diameter of the zone of inhibition around the antibiotic disc was observed using a meter ruler. Obtained Results were interpreted as Sensitive and Resistance based on CLSI 2017 guide-line. These antibiotic discs were made available: Ciprofloxacin (CIPR, 5 µg), Cefuroxime (CRX, 30 µg), Cefazidime (CAZ, 30 µg), Cefixime (CXM, 5µg), Augmentin (AUG, 20/10 µg), Gentamycin (GEN, 10 µg),Nitrofuratoin (NI, 30 µg), and Ofloxacin (OFL,5 µg) respectively (all from Abtek bio.Ltd UK) and were chosen according to CLSI guide-line. Multi-drug resistance patterns of the isolates were evaluated following the criteria set by Magiorakos et al. [14].

2.4 Identification of Extended- Spectrum Beta-Lactamase (ESBL)

The antibiotic sensitivity testing of the bacterial isolates were measured the zones of inhibition diameters based on its susceptibility and resistance patterns as follows Ciprofloxacin ≤ 20mm, Cefuroxime≤ 14mm, Ceftazidime ≤ 17mm, Cefixime ≤ 15mm, Augmentin ≤ 16 were considered as resistance while, Ciprofloxacin ≤ 30mm, Cefuroxime≤ 18mm, Ceftazidime ≤ 21mm, Cefixime ≤ 19mm, Augmentin ≤ 18 were considered as susceptibility as well. Phenotypic confirmation of ESBL production was done 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].

2.5 Data Analysis

Obtained data was subjected to these statistical tools; descriptive statistics, chi-square and pair-test using Statistical Package for the Social Science (SPSS version 26) and, set at p-value less than 0.005 at a 95% confidence interval was considered statistically significant.

2.6 Quality Control

One hundred and ten questionnaires were distributed to the participants and also ascertained their willingness to participate in the study. The sensitivity and specificity test of the media were done regularly. This is carried out by incubation of 5% of the prepared media overnight at 37°C for 24 hour. *E. coli* (ATCC 25922), *K. pneumoniae* (ATCC1705) and *K. pneumoniae* (ATCC1706) were used as quality control for the performance of antibiotic susceptibility testing. Multi-drug resistance was considered as simultaneous resistance to 3 or more antibiotic classes.

3. RESULTS

The present study recruited 110 diabetic patients and 60 (54.54%) of them, were female whereas 50 (45.45%) were males. it was found that the highest age range participated in this research work were age ≤ 80 years, 35(31.82%) followed by age bracket 61-70 years, 50(45.5%); 51-60 years, 15 (13.0%); 41-50years, 8(7.81%) and minority of the 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).

The great proportion of bacteria isolated from urine specimens were Gram negative bacteria 55/110 (50.0%) and more prominent isolates were *E. coli* (53.70%) followed by *Klebsiella pneumoniae* (27.78%), *Pseudomonas aeruginosa* (11.11%), and *proteus* species (7.41%) respectively (Fig. 1).

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 (Fig. 2).

Table 1. Demographic data of type-2 diabetes individuals with urinary tract infection

Variables	Type-2 diabetes (n=110)	Non-T2 diabetes (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

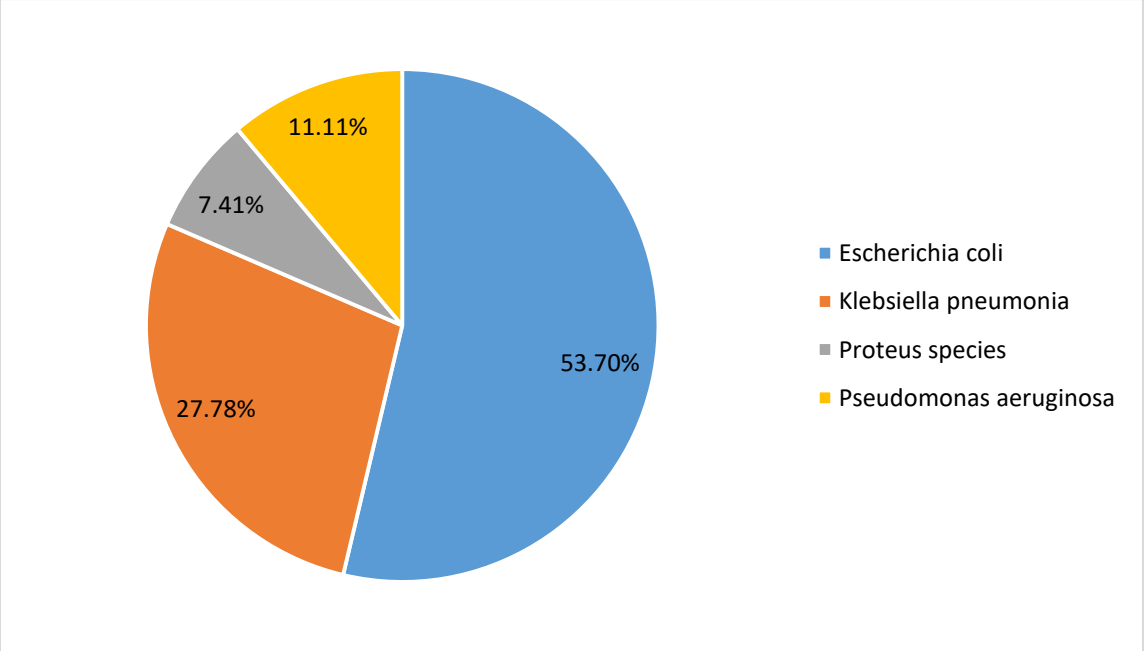

Fig. 1. Prevalence of urinary tract infection among T2D patients

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 (Fig. 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 (Fig. 4).

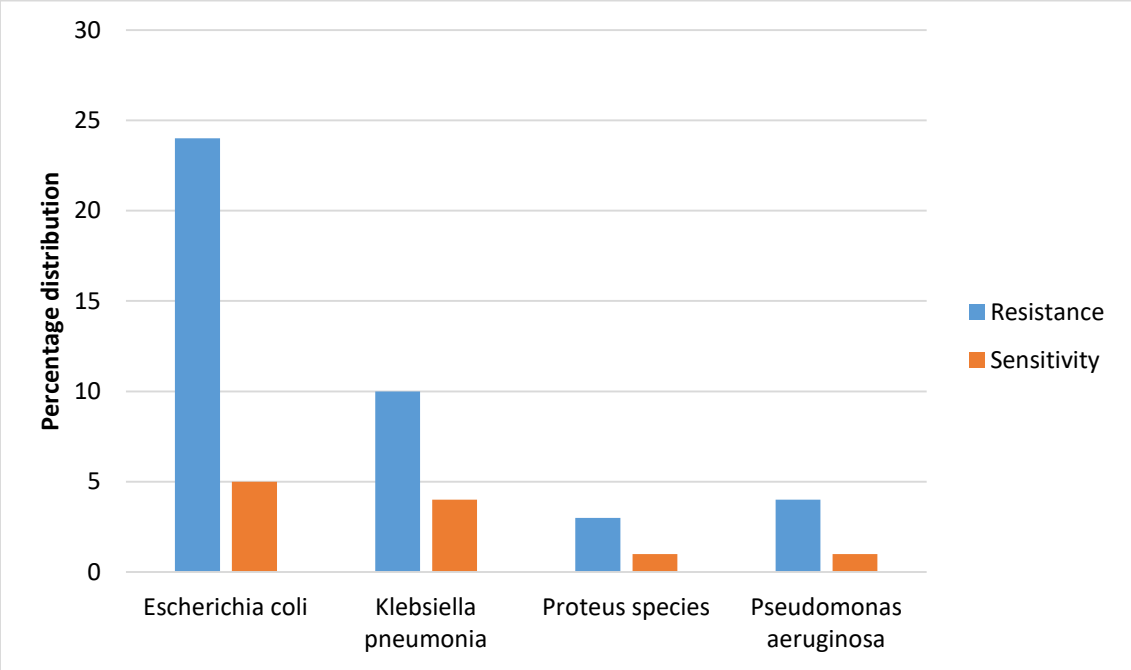

Fig. 2. Cefuroxime sensitivity and resistance patterns on bacteia isolates p-value = 0.000, t = 3.850

p<0.005 was considered as significant

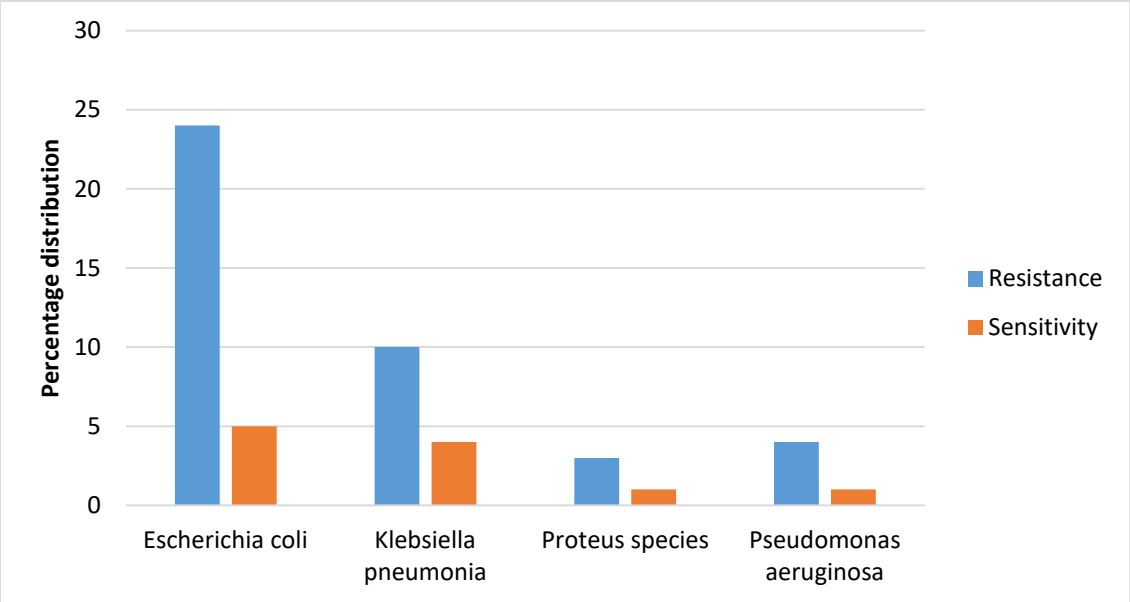

Fig. 3. Ceftazidime sensitivity and resistance patterns on bacteria isolates p-value = 0.006, t = 2.863

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 (Fig. 5).

4. DISCUSSION

The great proportion of bacteria isolated from urine specimens were Gram negative bacteria and more prominent isolates were *E. coli* followed by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *proteus* species respectively. However, it is higher than a study conducted from other Africa countries (10;18;19). Similarly, this study is in agreement with previous findings which showed the most proportion of gram negative bacteria isolated were *E. coli,* and K. *pneumoniae,* respectively [16]. Although, there are various underline factors that might affect the data generated from this research work and included the followings; sample size of the population, participant's commitment to the study, patients with comorbidity, severity of diabetic condition, patient on/without antibiotic medications.

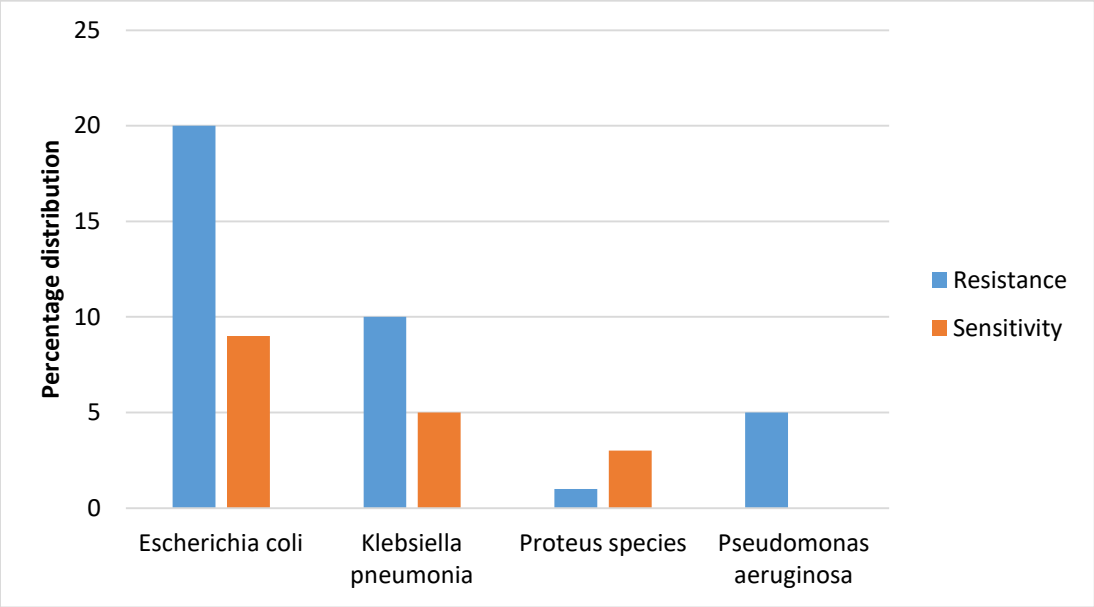

Fig. 4. Cefixime sensitivity and resistance patterns on bacteria isolates p-value = 0.015, t = 2.520

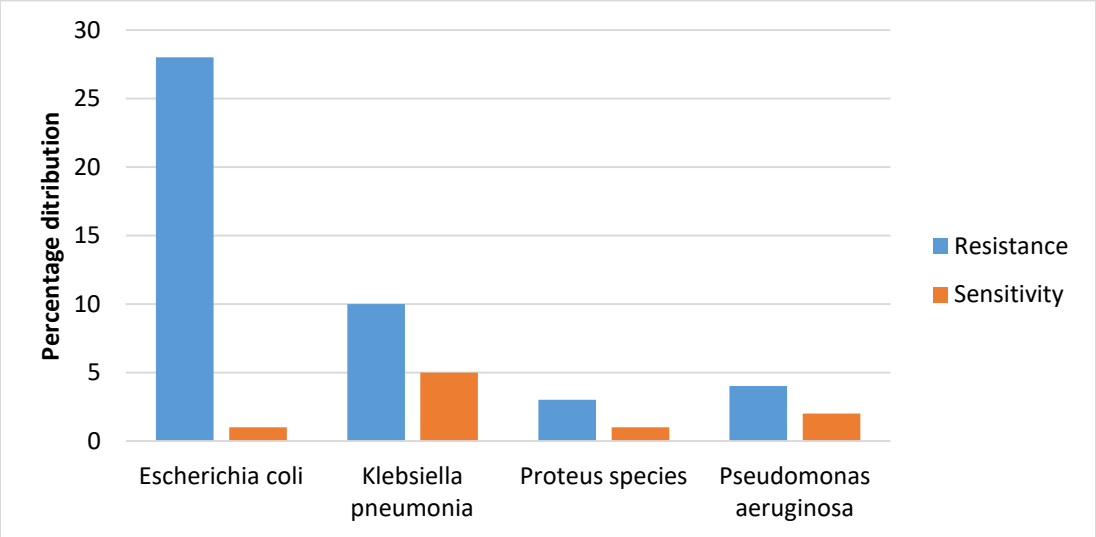

Fig. 5. Augumentin sensitivity and resistance patterns on bacteria isolates p-value = 0.000, t = 4.570

Considering the way by which the people acquired antibiotic resistance, it is noted that the clinician empirically prescribed cephalosporin group as drugs of choice to their patients. Following the resistance of cephalosporins in this study, only the ceftazidime and cefixime produce 40% resistant, whereas, a significant resistance rate were found in Cefuroxime ($p=0.00$, $t= 3.850$), and Augmentin ($p = 0.00$, $t = 4.570$). The present study is agreed with other findings which reveal the high percentage of cephalosporin groups were resistant to Gram negative bacteria [17]. The study is also disagreed with other previous studies which demonstrated the cephalosporins group and other antibiotics as the most susceptible to Gram negative bacteria [18]. However, the variation of the research data might attribute to the attitude of the patient towards antibiotics, self-medication, drug abuse and misused of antibiotics in this locality.

5. CONCLUSION

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. Regular monitoring, conducting, supervising, or management of antibiotics and molecular biomarkers for drug resistance are paramount to curtail the occurrence of this superbug.

CONSENT

A written consents were taken from the participants before the commencement of the urine collection. Also, sociodemographic data were obtained from the diabetic patients.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Shaikh S, Fatima J, Shakil S, Rizvi SMD, Kamal MA. Prevalence of multidrug resistant and extended spectrum beta-lactamase producing *Pseudomonas aeruginosa* in a tertiary care hospital. Saudi J Biol Sci. 2015;22(1):62–64.
2. Negvekar V, Sawant S, Amey S. Prevalence of multidrug-resistant gram-negative bacteria cases at admission in multispecialty hospital. Journal Global Antimicrobial Resistance 2020;9(22):457–61.
3. Ayukekbong JA, Ntemgwa M, Atabe AN. The threat of antimicrobial resistance in developing countries: Causes and control strategies. Antimicrob Resist Infect Control. 2017;6(1):47.
4. Piddock LJ. Reflecting on the final report of the O'Neill review on antimicrobial resistance. Lancet Infectious Disease. 2016;(7):767–768.
5. Izadpanah M, Khalili H. Antibiotic regimens for treatment of infetions due to multidrug-resistant Gram-negative pathogens: An evidence-based literature review. J Res Pharm Practice. 2015;4(3):105–14.
6. Cassini A, Högberg LD, Plachouras D, Quattrocchi A, Hoxha A, Simonsen GS, Colomb-Cotinat M, Kretzschmar ME, Devleesschauwer B, Cecchini M. 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. 2019;19:56–66.
7. Morris S, Cerceo E. Trends, epidemiology, and management of multi-drug resistant gram-negative bacterial infections in the hospitalized
setting. Antibiotics. 2020;9:196.
8. Sumpradit N, Wongkongkathep S, Malathum K, Janejai N, Paveenkittiporn W, Yingyong T, Chuxnum T, Vijitleela A, Boonyarit P, Akaleephan C. Thailand's national strategic plan on antimicrobial resistance: Progress and challenges. Bull. World Health Organ. 2021;99:661-673.
9. National Antimicrobial Resistance Surveillance Thailand. Global and National Antimicrobial Resistance Situation; 2019. Available:http://narst.dmsc.moph.go.th/ Access on 31 May 2021
10. Baramee J, Unahalekhaka A, Klunklin P. Implementation and barriers in prevention of multidrug-resistant organisms transmission among community hospitals. Nursing Journal Chiang Mai University. 2021;48:95–106.
11. Alali WQ, AlFouzan W, Dhar R. Prevalence of antimicrobial resistance in gram-negative clinical isolates from a major secondary hospital in Kuwait: A retrospective descriptive study. Germs. 2021;11:498–511.
12. Andrés Lasheras S, Martín Burriel I, Aspiroz C, Mainar Jaime RC, Robres P,

Sevilla E, Kuijper E, Chirino Trejo M, Bolea R. Incidence and characterization of *Clostridium difficile* in a secondary care hospital in Spain. Rev. Esp. Enferm. Dig. 2019;111:338–344.
13. Chesbrough M. District laboratory practice in tropical countries. New York: Cambridge University Press; 2006.
14. 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.
15. 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.
16. Jaffar A, Rabaan A, Justin VS, Ali MB. 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. High prevalence of antimicrobial resistance among gram-negative isolated bacilli in intensive care units at a tertiary-care hospital in Yucatán Mexico. Medicina (Kaunas). 2019;55(9): 588.
18. Lamichhane B. Antibiotic resistance patterns of gram-negative isolates in a tertiary care hospital of Nepal. Asian Journal Pharm Clinical Resources. 2014; 7(3):30-33.

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