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PREVALENCE OF EXTENDED-SPECTRUM BETA LACTAMASE AND AMPC PRODUCING ENTEROBACTERIACEAE AMONG DIABETIC PATIENTS IN BAUCHI STATE, NIGERIA

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ABSTRACT
Diabetes mellitus (DM) is a diverse group of metabolic disorder which is often associated with a high disease burden in developing countries such as Nigeria. It is one of the dreaded complications leading to repeated hospitalizations and even amputation, drastically reducing the quality of life. Hence proper management of infections among diabetic patients and choosing appropriate antibiotic is crucial. The study aimed to determine the prevalence of Extended Spectrum Betalactamase (ESBL) and Ampicillinases (AmpC) producing Enterobacteriaceae from diabetic patients attending some hospitals in Bauchi state. A total of 196 samples were collected from diabetic patients attending Abubakar Tafawa Balewa University Teaching Hospital Bauchi, Bauchi state. The samples were processed according to standard microbiological techniques, Gram negative; oxidase negative isolates were suspected as Enterobacteriaceae and were confirmed using API20E kit. Antimicrobial susceptibility testing was done using disc diffusion method, ESBL and AmpC production were detected using double disc synergy test and disc approximation assay respectively. A total of 74 (37.8%) Enterobacteriaceae were isolated from study participants, age group 11 to 85 years comprising of 103 male and 93 female, the most frequently isolated enterobacteriaceae was Escherichia coli which has 41(20.9 %,) while the least occurring was Enterobacter spp with 3 (1.5%). Antibiotic screening shows 58 (78.4%) of the isolates were resistant to third generation cephalosporin antibiotics, 54.0% were resistant to cephamycin (cefoxitin). Extended-spectrum Beta lactamase (ESBL) production was noted among 38 (51.3%) of the total isolates, followed by 25 AmpC producing enterobacteriaceae (33.8%), statistically there is significant association between inpatient (hospitalizes diabetes patients) and ESBL producing enterobacteriaceae in the study area (P-value = 0.04).
Keywords: Diabetes mellitus, extended spectrum Betalactamases, AmpC test

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of chronic hyperglycemia characterized by disturbances to carbohydrate, protein, and fat metabolism resulting from absolute insulin deficiency with dysfunction in organ systems (Ogurtsova *et al*., 2017). The prevalence of Diabetes Mellitus has been significantly increase globally from an estimated 30 million cases in 1985 to 382 million in 2013 and recently 463 million in 2019 (Thomas *et al*., 2019; Guariguata *et al*., 2014). In Nigeria , Based on a report by the International Diabetes Federation in 2013, there were 3.9 million cases of diabetes in Nigeria, the highest prevalence rate in Africa (Afolalu *et al*., 2020) with All regions of the country been affected (Balogun *et al*., 2018). Bacterial infections and complications associated with the diabetic mellitus are on the increase due to multiple effects of this disease on the host immune system (Ajayi *et al*., 2019). Common bacterial Infection associated with diabetes mellitus includes; community-acquired and hospital-acquired, sepsis, periodontal disease, urinary tract infection such as cystitis, urethritis, pyelonephritis, Respiratory tract infections such as Pneumonia, bronchopneumonia, and wound infection (Toniolo *et al.,* 2019). Older age is among other factors that enhance the risk of UTI in diabetic patients (Brown *et al*., 2005).

Diabetic patients are more prone to severe infections than non diabetic patients because they have more exposure to antibiotics. Resistance to broad-spectrum betalactam mediated by extended spectrum betalactamases (ESBLs) and types C ampicillinase (AmpC βetalactamases) enzymes is an increasing problem worldwide, hence, determination of their prevalence is essential to formulate an effective antibiotic policy and hospital infection control measures most especially in patients with autoimmune disease conditions such as diabetic patients (Velloso 2013; Grover *et al*., 2013). Infections caused by resistance bacteria may result into prolong hospital stay, disability and greater risk of death due to the delay in administration of effective treatment. Hence, there is a need for surveillance of resistant bacteria among diabetic patients to reduce the risk of major complications. However, knowledge of the proportion of drug-resistant isolates especially extended-spectrum beta lactamase (ESBL)-producing enterobacteriaceae and various risk factors for acquisition is essential. The study aimed at determine the prevalence of ESBL and AmpC producing enterobacteria among diabetic patients attending Abubakar Tafawa Balewa University Teaching Hospital Bauchi, Bauchi State through the following objectives; To screened ESBL and AmpC producing enterobacteriaceae and determine the resistance profile of the isolates phenotypically. To determine the association between demographic characteristics and betalactamases (ESBL and AmpC) producing enterobacteria isolated from the study participants.

MATERIAL AND METHODS

Study design

The study was descriptive cross sectional design as adapted from (Kitchel *et al.,* 2009; Alemayehu *et al.,* 2021).

Sampling Technique

Convenient sampling technique was employed for this study until the sample size was completed (**Mekuanent *et al*., 2020).**

Study population

The study participants comprised of in and out diabetic patients as diagnosed by the attending physician at Abubakar Tafawa Balewa University Teaching Hospitals Bauchi.

Socio-Demographic and Clinical Data Collection

Socio demographic characteristics and clinical data such as age, sex and hospitalization history were collected using patients laboratory request form and interview. The clinical data were collected from the patients' chart and attending physician.

Exclusion criterion

Patients or caregivers/relatives who refused to give consent to participate in the study were excluded from the study.

Ethical considerations

The protocol for this study was submitted to the Health Research Ethics Committee of Abubakar Tafawa balewa University Teaching Hospital Bauchi state, Nigeria, for review and their approval was obtained before the commencement of data collection.

Samples size

A total of 196 samples were calculated using Fisher's formula ($n = Z2pq/d2$) were used for determining minimum sample size for descriptive studies based on standard normal (Z) deviatiation of 1.96 at 95% confidence interval and prevalence rate of 12.8% reported from previous similar study (wanga and, Lemeshow 2018; Aminu, *et al.,* 2021).

Samples Collection

All samples were collected based on the standard operating procedure (SOP) of ATBUTH microbiology laboratory, the samples includes urine 42 (21.4 %), sputum 23 (11.7 %), stool 29 (14.8), wound swab 37 (18.9%) and catheter tips 18 (9.2 %), endocervical swabs 19 (9.7%) and urethal swabs 28 (14.3%) were collected from patients previously diagnosed with hyperglycemia attending Abubakar Tafawa Balewa University Teaching Hospitals Bauchi.

Culture of specimen

The samples were inoculated on nutrient agar, MacConkey agar, and blood agar. Cysteine lactose electrolyte deficient agar was used for inoculation of urine samples. The plates were incubated at 37°C for 18-24hrs.

Identification of Isolates

After overnight incubation, the culture plates were examined for growth. Identification of the isolates was performed microscopically and biochemically using standard microbiological techniques and identification to species level was done using API 20E kit (Badmasti *et al.,* 2021).

Antimicrobial susceptibility Test

All isolates were subjected to antimicrobial sensitivity testing by Kirby–Bauer disk diffusion method on Mueller–Hinton agar (MHA) (Merck, Germany) according to the Clinical and Laboratory Standard Institute. Antibiotics used were Gentamicin (10 µg), Ampicillin (10 µg), Cefotaxime (30 µg), Cefaxitin (30 µg), Imipenam (10 µg), Amoxyclav (20/ 10 µg) and Ciprofloxacin (5 µg) (CLSI, 2020).

Screening for ESBL production

Isolates that showed resistance or intermediate to any two or more of third generation cephalosporins antibiotics with zone size of ≤ 17mm for Cefoxitin and ≤ 25 mm for Cefotaxime were identified as potential ESBL and AmpC producers respectively (CLSI, 2020).

Confirmation of ESBL by double disc synergy test (DDST)

The isolates resistant to two or more betalactam antibiotics were assumed to be potential ESBL producers, and were subjected to phenotypic confirmation by Double Disk Synergy Test (DDST) as described by Iroha *et al.*, (2008) and Iqbal (2017). A suspension of the test isolates were adjusted to 0.5 McFarland turbidity standards, and were aseptically inoculated on Muller-Hinton agar plate using sterile swab sticks. combination disk of amoxicillin-clavulanic acid, AMC (10 µg) was placed at the centre of the plate and cefotaxime (30 µg) were placed each on either sides of the central disk (AMC- 10 µg) at a distance of 15 mm apart. The plates were incubated for 18 to 24 h at 37°C. After incubation, a ≥5 mm increase in zone diameter for either of the cephalosporins (CAZ and CTX) tested in combination with AMC (10 µg) compared to its zone when tested alone confirms ESBL production in the test isolates, *Klebsiella pneumoniae* ATCC 700603 (positive control) and Escherichia *coli* ATCC 25922 (negative control) were used as a control based on criteria described by CLSI, 2020.

Disk Approximation Techniques for detection AmpC.

For the detection of inducible AmpC activity, 10-µg imipenem, 30-µg cefoxitin, and 20/10-µg amoxicillin-clavulanate disks were used as the inducing substrates as the reporter substrate. Disks were applied at a distance of 20 mm, and any obvious blunting or flattening of the zone of inhibition between the ceftazidime disk and the inducing substrates was interpreted as a positive result for AmpC (Tan *et al.*, 2009; Aruhomukama, 2018).

Data Processing and Analysis

Data were analyzed using SPSS statistical software version 21 and presented in table and 95% confidence level and p-value <0.05 was used for statistical significance.

RESULTS

Table 1 shows a total of 196 samples collected from study participants in which 187 (95.4%) of the respondents were adults, the median age was 52 years, with a range of 11-89 years and 103 (52.5%) were males. A total of 129 (65.8%) participants were outpatients, while 67 (34.2%) were hospitalized (inpatients).

Table 2 shows that 74 out of 196 samples were isolated with the following distributions; *E. coli* 41 (55.4%), *K. pneumoniae* 23 (31.0%), *Proteus mirabilis* 5 (6.7%), *Enterobacter aerogenes* 3 (4.0%) and *Serratia mercescens* 2 (2.7%). However, the highest frequency of enterobacteriaceae was observed in urine samples 40 (54.1%) followed by wound swabs which has 13 (17. 6 %), Endocervical swabs recorded the lowest frequency 2 (2.7%) of enterobacteriaceae.

Table 3 shows resistance profile of enterobacteriaceae tested against different antibiotics, 60 isolates equivalent to 81.1% were found to be resistant to ampicillin, and amoxiclav each, 78.4% isolates were resistant to cefotaxidine and 54.0% Cefoxitin. Nonetheless, lower resistance rate to carbapenem antibiotics was also observed from *E. coli* which has 10.5% resistance rate to imipenem antibiotic, while the highest resistance rate to imipenem was observed among *Klebsiella pneumoniae* with percentage frequency of 6.7 %.

Table 4 Distribution of ESBL producers from 58 (78.3%) suspected enterobacteriaceae, shows that 38 (65.5%) isolates were found to be confirmed ESBL producers based on NCCLS breakpoint Test. Highest ESBL production was observed in *E. coli* 25 (65.7%), followed by *K. pneumoniae* 9 (23.7%), *Proteus mirabilis* 3 (7.9 %) and *E. aerogenes* 1 (2.6 %), in addition, zero prevalence of ESBL was observed among *Serratia marcescens* (0.0%). The prevalence of AmpC producers as 25 (33.8%), with *E. coli* and *K. pneumoniae* as the only AmpC producers among the isolated enterobacteriaceae with percentage frequency of 22 (57.9) and 3 (20.0%) respectively and it was showed in Table 5.

Table 6 shows the resistance profile of ESBL and AmpC producers and it was observed that all the enterobacteriaceae screened were resistance to ampicillin, amoxiclavulanic acid and ceftaxidime with 100% resistance rate each. However, resistance rate of imipenem antibiotics was observed to be 15.7% among family enterobacteriaceae that produce ESBL and AmpC, while the highest resistance rate to imipenem was observed among *K. pneumoniae* with percentage frequency of 33.3%.

Factors associated with developing ESBL and AmpC infections (*n*=76) are presented in table 7. The result shows that out of 103 male participants 21 (20.4%) developed ESBL producing enteric bacterial infection while female's participants had the lowest frequency of 17 (18.3%). In addition highest prevalence of ESBL producing enteric bacteria 27 (40.3%), was observed among inpatients participants when compare with outpatients participants 11 (8.5%), statistically there is significant association between ESBL and hospitalization in the study area (P-value = 0.04).

Table 1: Sociodemographic characteristics of the study participants (*n*=196)

Factor	Frequency (*n*=196), *n* (%)
Gender	
Male	103 (52.5)
Female	93 (47.4)
Age	
≤30	16 (8.7)
≥31	180 (91.8)
Ward	
In patients	67 (34.2)
Out patients	129 (65.8)

Table 2: Frequency of occurrence of Enterobacteriaceae isolated from diabetic patients N=74

Enterobacteriaceae	Urine (%)	Spu (%)	Ws (%)	Ct (%)	Es(%)	Us (%)	Total
E. coli	19 (25.0)	6 (8.1)	8 (10.8)	4 (5.4)	2 (2.7)	2 (2.7)	41
K.. pneumoniae	14 (18.9)	3 (4.0)	5 (6.7)	1 (1.3)	0 (0.0)	0 (0.0)	23
P. mirabilis	3 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.7)	5
E. aerogenes	3 (2.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3
S. marcescens	1 (2.7)	0 (0.0)	0 (0.0)	1 (8.1)	0 (0.0)	0 (0.0)	2
Total	40 (54.1)	9 (12.1)	13 (17.6)	6 (8.1)	2 (2.7)	4 0 (0.0)	74

Key: Spu = Sputum, Ws = Wound swab, Ct = Catheter tips, Es = Endocervical swab, Us = Urethral swab

Table 3: Susceptibility pattern of *Enterobacteriaceae* isolates resistant to the tested antimicrobial agents

Enterobacteriaceae (N=74)	Ampiciline (10µg)	Amoxy-clav (30µg)	Cefoxitin (30µg)	Ciproflaxacin (5µg)	Gentamicin (10µg)	Ceftaxidine (30µg)	Ceftaxidime (30µg)	Imipenem (10µg)
	R %	R %	R %	R %	R %	R %	R %	R %
E. coli (41)	40 (54.0)	40 (54.0)	32 (43.2)	26 (35.1)	25 (33.8)	35 (47.3)	38 (51.3)	4 (5.4)
K. Pneumoniae (23)	15 (20.3)	13 (17.0)	8 (10.8)	9 (12.2)	9 (12.2)	8 (10.8)	9 (12.2)	5 (6.7)
E. aerogenes (3)	2 (2.7)	1 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.4)	1 (1.4)	1 (1.4)
P. mirabilis (5)	4 (5.4)	4 (5.4)	0 (0.0)	2 (2.7)	1 (1.4)	3(4.0)	3 (4.1)	0 (0.0)
S. marcesens (2)	2 (2.7)	2 (2.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	60 (81.1)	60 (81.1)	40 (54.0)	37 (50.0)	35 (51.4)	36 (48.6)	51 (68.9)	10 (13.5)

Table 4: Screening and Confirmation of ESBLs production among Enterobacterial isolates based on Double Disk Synergy Test

Isolates	No. screened	No. of suspected ESBL producers (%)	No. of confirmed ESBL producers (%)
E. coli	41	38 (92.7)	25 (65.8)
Klebsiella spp	23	13 (56.5)	9 (69.2)
Proteus spp	5	4 (80.0)	3 (75.0)
Sarraia mercescens	2	2 ((100)	0 (0.0)
Enterobacter spp	3	1(33.3)	1 (33.3)
Total	74	58 (78)	38(65.5)

Table 5: Screening and Confirmation of AmpC production among Enterobacterial isolates based on Disk Approximation Techniques

Isolates	No. screened	No. of suspected AmpC producers (%)	No. of confirmed AmpC producers (%)
Escherichia coli	41	38 (92.7)	22 (57.8)
Klebsiella pneumoniae	23	13 (56.5)	3 (23.0)
Proteus spp	5	1(25.0)	0 (0.0)
Sarraia mercescens	2	1 (50.0)	0 (0.0)
Enterobacter spp	3	1(33.3)	0(0.0)
Total	74	54 (72.9)	25 (33.8)

R= Resistance, ESBL= Extended spectrum betalactamases, AmpC= type C Ampicillinases.

Table 6: Resistance profiles of ESBL and AmpC producing Enterobacteriaceae isolated from diabetic patients

Enterobacteriaceae (N=38)	Ampiciline (10µg)	Amoxy-clav (30µg)	Cefoxitin (30µg)	Ciproflaxacin (5µg)	Gentamicin (10µg)	Cefotaxime (30µg)	Ceftaxidime (30µg)	Imipenem (10µg)
	R %	R %	R %	R %	R %	R %	R %	R %
E. coli (25)	25 (100)	25 (100)	32 (43.2)	22 (88.0)	22 (88.0)	25 (100)	25 (100)	2 (8.0)
K. Pneumoniae (9)	9 (100)	9 (100)	3 (33.3)	3 (33.3)	3 (33.3)	8 (88.0)	9 (100)	3 (33.3)
E. aerogenes (1)	1 (100)	1 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)	1 (100)	0 (0.0)
P. mirabilis (3)	3 (100)	3 (5.4)	0 (0.0)	1 (33.3)	1 (33.3)	3(100)	3 (100)	1(33.3)
Total	38 (100)	38(100)	35(92.1)	26 (68.4)	26 (68.4)	37 (97.4)	38 (100)	6 (15.7)

Table 7: Socio demographic factors associated with ESBL among study participants

Factor	Total	ESBL & AmpC positive	ESBL Negative	X^2	df	P-value
Gender						
Male	103	21	82	0.139	1	.70 9
Female	93	17	76			
Age						
≤30	16	5	11	1.568	1	.210
≥31	180	33	147			
Ward						
In patients	67	27	40	3.920		.04*
Out patients	129	11	118			

DISCUSSION

This study presents a microbiological profile of infected diabetic patients in ATBUTH Bauchi. The prevalence of Enterobacteriaceae were 37.8% from various samples with urine samples having the highest frequency (54.1%) and the most predominant Enterobacteriaceae was *E. coli* (25.0%). The hyperglycemic condition of the diabetic patients may predispose the patients to bacteria causing urinary tract infection as reported by Datta *et al*., 2019. And this finding correlates with the finding in Imo State, Southeastern Nigeria, in 2021 (Nwanko *et al*., 2021; Shashikala *et al*., 2016). The study contradict the finding of Okwume *et al.,* 2021 from Enugu, Nigeria who reported P*roteus spp* as the most frequent species from only urine samples of 51 diabetic patients, this variance could be associated with the differences in sample size and type. Furthermore, study from Mohammad *et al*., 2020, reported *Klebsiella pneumoniae* as the most predominant enterobacterial specie isolated from diabetic patients which is also in disagreement with this study.

This study agreed with the findings of Datta *et al*., 2019 on Evaluation of various risk factors associated with multidrug-resistant organisms from diabetic foot ulcer patients in which low susceptibility to antibiotics was observed from Ampicillin (81.1%), Amoxicillin (81.1%) and Ceftaxidime (68.9%), the reason of high degree of antibiotic resistance found in our isolates, may be due to the fact that the study area is a tertiary care hospital with widespread usage of broad spectrum antibiotics leading to selective survival advantage of pathogen. The antimicrobial resistance pattern was similar to the studies done in India (Shankar *et al*., 2005; Raja, 2007; Marquez-Algaba, 2021).

In addition *Enterobacteriaceae* isolated from this study were sensitive to imipenem which agrees with the finding of Zubair *et al*., 2010.

The prevalence ESBL and AmpC producing *Enterobacteriaceae* were 63.3% and 41.0% respectively and it is in contrast with the finding of Rawat, 2013 who reported the prevalence of ESBL and AmpC as 18.7%, 20.8% respectively. These differences may be due to the time factor and differences in Geographical location. This study also agreed with the finding of Datta, *et al*., 2020, where they reported a high prevalence of ESBL production among enterobacteriaceae with *E. coli* as the most frequent species. The current study shows that there was no significant association between gender and ESBL producing enterobacteriaceae with P- value > 0.05 (P =.70 9). Age group 31 years above had the highest prevalence of 35 (18.2%) ESBL producing enterobacteriaceae when compare to age group 30 years below which has 5 (16.7%). Therefore, there is no association between age of participants and ESBL producing enterobacteria as P= 0.210. This agrees with the work of Liu *et al*., 2018, Lilian *et al.,* 2015; Mohanasoundram, 2012; Nwachukwu *et al.,* 2009..

The resistance profile of ESBL producing *Enterobacteriaceae* shows that all strains were 100% resistant to Ampicillin followed by Amoxiclavulanic acid and Cefeftaxidime, 97.4% isolates were resistance to Cefotaxime and 68.4% were resistant to Ciproflaxacin and Gentamicin each. High sensitivity rate was observed with Imipenam (84.3%) followed by Cefoxitin (92.1%). This study agreed with the finding of Datta, *et al*., 2020; Anitha and Natarajan 2017, in another study Varaiya, *et al*., 2008, reported Imipenam as 100% sensitive for all ESBL strains of Enterobacteriaceae from *Escherichia coli* and *Klebsiella pneumonia*e in diabetic foot infections.

CONCLUSION

The current study established the prevalence of ESBL and Ampc producing Enterobacteriaceae among diabetic patients from Bauchi state.Therefore, Early identification and adequate management of the risk factors incriminated in ESBL and AmpC should be a priority for physicians in order to limit the dissemination of the ESBL producing strains and thus to improve the outcome of these patients

REFERANCES

Afolalu, T. D., Wada, O. Z., Olawade, D. B., and Suntai, A. D. (2020). Prevalence of diabetes mellitus among adult residents of Tinda rural community, Nigeria. *Journal of Biosciences and Medicines*, 8(11), 107-116.

Anitha S., Natarajan, V. (2017). Prevalence of ESBL producing Enterobacteriaceae in diabetic foot ulcers. *International Archives of Integrated Medicine* 4(11): 122- 127.

Ajayi, E. I. O., Modo, E. U., Kiakubu, O. T., & Molehin, O. R. (2019). Diabetes Care and Wound Healing Using Nauclea latifolia, Manihot esculenta, and Other Natural Products. In *Bioactive Food as Dietary Interventions for Diabetes* (pp. 545-558). Academic Press.

Alemayehu, T., Asnake, S., Tadesse, B., Azerefegn, E., Mitiku, E., Agegnehu, A., & Nigussie, N. (2021). Phenotypic detection of carbapenem-resistant gram-negative Bacilli from a clinical specimen in Sidama, Ethiopia: A cross-sectional study. *Infection and Drug Resistance*, *14*, 369.

Badmasti, F., Azizi, O., Mohaghegh, M. A., & Solgi, H. (2021). Data on the prevalence and distribution of carbapenemase genes in Enterobacterales species isolated from clinical specimens in the center of Irans. *Data in Brief*, *38*, 107368.

Balogun, W. O., Uloko, A. E., Ipadeola, A., Enang, O., Adamu, A. N., Mubi, B. M., Okafor, C.I, Odeniyi, I., Lawal, I.U, Adeleye, J.O. and Fasanmade, O. A. (2018). Prevalence of obesity and ethno-geographic variation in body sizes of Nigerians with type 2 diabetes mellitus-A multi-centre study. *Nigerian Journal of Medicine*, 27(1), 69-77.

Brown, J. S., Wessells, H., Chancellor, M. B., Howards, S. S., Stamm, W. E., Stapleton, A. E., Steers, W. D., Van Den Eeden, S. K., & McVary, K. T. (2005). Urologic complications of diabetes. *Diabetes care*, *28*(1), 177–185.

Weinstein, M. P., & Lewis, J. S. (2020). The clinical and laboratory standards institute subcommittee on antimicrobial susceptibility testing: background, organization, functions, and processes. *Journal of clinical microbiology*, *58*(3), e01864-19.

Datta, P., Chander, J., Gupta, V., Mohi, G. K., & Attri, A. K. (2019). Evaluation of various risk factors associated with multidrug-resistant organisms isolated from diabetic foot ulcer patients. *Journal of Laboratory Physicians*, *11*(01), 058-062.

Grover, N., Sahni, A. K., & Retd, S. B. (2013). Therapeutic challenges of ESBLS and

AmpC beta-lactamase producers in a tertiary care center. *Medical Journal Armed Forces India*, *69*(1), 4-10.

Guariguata, L., Whiting, D. R., Hambleton, I., Beagley, J., Linnenkamp, U., & Shaw, J. E. (2014). Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes research and clinical practice*, *103*(2), 137-149.

Iqbal, R., Ikram, N., Shoaib, M., Muhammad, J. A., Raja, T. M., Abid, A. N. and Faiza, N. (2017). Phenotypic confirmatory disc diffusion test (PCDDT), double disc synergy test (DDST), E-test OS diagnostic tool for detection of extended spectrum beta lactamase (ESBL) producing Uropathogens. *Journal of Applied Biotechnol Bioeng*, 3(3), 344-349.

Kitchel, B., Rasheed, J. K., Patel, J. B., Srinivasan, A., Navon-Venezia, S., Carmeli, Y., Brolund, A. & Giske, C. G. (2009). Molecular epidemiology of KPC-producing Klebsiella pneumoniae isolates in the United States: clonal expansion of multilocus sequence type 258. *Antimicrobial agents and chemotherapy*, *53*(8), 3365-3370.

Liu, C., Shi, J., & Guo, J. (2018). High prevalence of hypervirulent *Klebsiella pneumoniae* infection in the genetic background of elderly patients in two teaching hospitals in China. Infection and drug resistance, 11, 1031.

Lwanga, S., K., Lemeshow S. (2018). Sample Size Determination In Health Studies A Practical Manual

Marquez-Algaba, E., Pigrau, C., Bosch-Nicolau, P., Viñado, B., Serra-Pladevall, J., Almirante, B., and Burgos, J. (2021). Risk Factors for Relapse in Acute Bacterial Prostatitis: the Impact of Antibiotic Regimens. *Microbiology Spectrum*, 9 (2), e00534-21.

Mohamed, E. S., Khairy, R. M., and Abdelrahim, S. S. (2020). Prevalence and molecular characteristics of ESBL and AmpC β-lactamase producing Enterobacteriaceae strains isolated from Urinary tract infections in Egypt *Antimicrobial Resistance & Infection Control*, 9(1), 1-9.

Nwankwo, E. O., Nwagbara, E. E., & Onusiriuka, K. N. (2021). Antibiotic Sensitivity Pattern and Plasmid Profile of Bacteria Isolated from Diabetic Ulcers in Mbano Metropolis, Imo State, South eastern Nigeria. *Umaru Musa Yar'adua University Journal* 6 (1), 38 – 46.

Ogurtsova, K., da Rocha Fernandes, J. D., Huang, Y., Linnenkamp, U., Guariguata, L., Cho, N. H., Cho, Cavan, D. Shaw, J.E., Makaroff, L. E. (2017). IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes research and clinical practice*, *128*, 40-50.

Okwume, C. C., Onyemelukwe, N. F., Abdullahi, I. N., Okoyeocha, O. E., & Asamota, S. D. (2021). Prevalence of symptomatic urinary tract infection and bacterial spectrum of diabetic and non-diabetic patients at the two teaching hospitals in Enugu, Nigeria. *African Journal of Clinical and Experimental Microbiology*, *22*(4), 480-488.

Umasankari, J., Jeya, M., & Sekar, C. (2012). Microbiological study of diabetic foot ulcers and analysis of ESBL activity of the bacterial isolates. *Journal of Pharm. Res*, *5*, 2329-2332.

Shashikala,V., Farman, A., Narendranath, L. and Joseph, M. (2016). Diabetic foot ulcer and biofilm formation. *International Journal of Biomedical and Advance Research*, **7**(9):428-433.

Thomas, R. L., Halim, S., Gurudas, S., Sivaprasad, S., and Owens, D. R. (2019). IDF Diabetes Atlas: A review of studies utilising retinal photography on the global prevalence of diabetes related retinopathy between 2015 and 2018. *Diabetes research and clinical practice*, *157*, 107840.

Toniolo, A., Cassani, G., Puggioni, A., Rossi, A., Colombo, A., Onodera, T., and Ferrannini, E. (2019). The diabetes pandemic and associated infections: suggestions for clinical microbiology. *Reviews in Medical Microbiology*, 30(1): 1.

Velloso, L. A., Eizirik, D. L., & Cnop, M. (2013). Type 2 diabetes mellitus an autoimmune disease? *Nature Reviews Endocrinology*, *9*(12), 750-755.

Varaiya, A. Y., Dogra, J. D., Kulkarni, M. H., Bhalekar, P. N. (2008). *Escherichia coli* and *Klebsiella pneumonia* in diabetic foot infections. *Nature Reviews Endocrinology* 51: 370–372.