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Frequency of Bacterial Infections with Extended-spectrum Beta-lactamases (ESBL) in Diabetic and Non-Diabetic Patients

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Author`s Contribution

[MR] Conception and design, Collection and assembly of data, [SA, MZ] Analysis and interpretation of the data, Statistical expertise, [MA, AS] Final approval and guarantor of the article

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A B S T R A C T

Background: Diabetes is a group of metabolic diseases characterized by high blood sugar levels that persist over long time that may lead to immunocompromised condition. Diabetic patients are vulnerable to multiple infections by multidrug -resistance bacteria and the bacterial resistance is achieved through the production of beta-lactamases against beta-lactam drugs. Extended spectrum beta lactamases (ESBL)-producing pathogens has been the subject of recent pharmacokinetic and pharmacodynamic research.

Objective: This research was aimed to explore the frequency of Extended-spectrum Beta-lactamases (ESBL)-producing bacterial infections in diabetic and non-diabetic patients.

Methodology: A cross-sectional study was conducted and urine sample of Diabetic and non-diabetic Patients with symptoms of stomachaches, diarrhea, pneumonia, and urinary tract infections were used for culture and sensitivity (Kirby-Bauer method). All the data was recorded and analyzed using SPSS version 26. Descriptive statistics were used to present frequency and percentages.

Results: In this study, overall, E. coli and Acinetobacter were found to be most prominent ESBL producing bacteria among them most of the patients (n= 131) were resistant to amikacin.The most frequent pathogen was E. coli, that isolated from 80% in diabetics and 48% in non-diabetics individuals. Klebsiella, Acinetobacter, and Pseudomonas were found Gentamicin-resistant. Meanwhile, most profoundly presented comorbidites were diabetes (n=109) and abdominal pain (n= 119). Uropathogenic bacteria generated ESBL regardless of age, gender, or clinical isolate source. However, mostly patients (n= 131) stayed 2-4 days in hospital that showed significant effect of medication.

Conclusion: These risk variables can identify patients at high risk of ESBL-producing bacteria, enabling more effective empiric antibiotic treatment. Diabetics should get laboratory-assisted antibacterial therapy.

Keywords: Kirby-Bauer method, Resistance, Extended spectrum beta lactamases, Diabetic

Introduction

Diabetes is a group of metabolic diseases characterized by high blood sugar levels that persist over long periods of time.[1] Most people experience an increase in appetite, thirst, and frequency of urinating. If untreated, diabetes can cause a host of complications. Acute consequences of diabetes include hyperosmolar hyperglycemia, diabetic ketoacidosis, and even death.[2] Serious long-term effects include cardiovascular illness, chronic kidney disease, stroke, foot ulcers, nerve damage, cognitive impairment, and eye damage. Both inadequate insulin synthesis by the pancreas and inappropriate insulin utilization by body cells lead to diabetes.[3] Glucose, which comes from the body's digestion of food, must be transported into cells to be used as fuel.[4] The hormone insulin is responsible for this transport .[5]

Multi-resistance to beta-lactam antibiotics such cephalosporins, penicillins, cephamycins, carbapenems, and monobactams is achieved by the production of beta-lactamases by bacteria.[6] As it destroys the structure of antibiotics, beta-lactamase makes them useless. The structural structure of these antibiotics is unified by a beta-lactam ring, a four-atom ring shared by the class.[7] The antibacterial effects of Beta-lactams are destroyed when the enzyme lactamase hydrolyzes the ring of the molecule. There are four main kinds of beta-lactamases according to the Ambler classification (A to D). This categorization system's foundation is based on protein homology (similarity in amino acids), not phenotypic traits.[8]

Serine beta-lactamases fall under classes A, C, and D of the Ambler categorization system. The class B enzymes are metallo-lactamases.[9] According to functional similarity, the Bush-Jacoby-Medeiros categorization system divides -lactamases into groups (substrate and inhibitor profile).[10] This system consists of many subgroups and four primary groupings. This categorization approach takes into account beta-lactamase inhibitors and beta-lactam substrates that are clinically relevant, making it of much more immediate significance in diagnostics.[11]

Penicillins and cephalosporins, the conventional first-line treatment options, are ineffective in vitro against ESBL-producing *E. coli* and K.pneumoniae strains, as well as their excellent resistance to other medications, is the therapeutic arsenal. The most well-established treatment option—and in some cases, the only one—is carbapenems. However, careful use of these drugs is necessary to prevent the growth of pathogens that produce carbapenemase. Another have been desired among frequently used and ignored antibiotics with potential action given the slow development of novel medicines for resistant Gram-negative bacteria. Quinolones, cefepime, aminoglycosides, trimethoprim-sulfamethoxazole, and piperacillin-tazobactam may be effective and considered to be factual therapy. Although aminoglycosides frequently cause coresistance, amikacin may still function.

Although ESBL infection should be considered when selecting the initial agent, not every child will necessarily need to take carbapenems. Amikacin and piperacillin-tazobactam are two noncarbapenem agents that could be used as the initial treatment for communicable, superficial infections because they have been shown to be effective at controlling disease without having negative long-term effects in children with pyelonephritis caused by ESBL-producing organisms. [54] These findings are likely explained by the higher drug immersion in urine than those that are properly achieved. The objective of this study was to determine the frequency of ESBL producing bacterial infection in diabetic and non-diabetic patients.

Methodology

A cross-sectional study was conducted from February 2022 to January 2023. Total of 150 samples were taken in total that had been found to have extended-spectrum beta-lactamases. Patients between the ages of 20 and 60 years were included. In this study both males and females were considered. Diabetic and non-diabetic Patients with symptoms of stomachaches, diarrhoea, pneumonia, and urinary tract infections, are included. The diabetic patients which have no infection were excluded.

Urine samples were collected from study participants. Demographic data was collected through questionnaire and urine samples were used for determination of culture and sensitivity through Kirby-Bauer method. Antibacterial sensitivity was observed through measuring the zone of inhibition (mm) of cefotazime (CAZ), ceftazidume (CTX) and augmentin (AUG).

All the data was recorded and analyzed using SPSS version 26. Descriptive statistics were used to present frequency and percentages.

Results

There were 150 participants inclduing 94 males and 56 females and among them, 37 were with primary education, 57 were graduates, 17 were postgraduates, and 39 were uneducated The total of 144 participants were aged 21-40 years, 3 were 41-60 years, and 3 were more than 60 years.

E. Coli was found in 96 patients, *Klebsiella* in 13 patients, *Acinetobacter* in 20 patients, Proteus in 11 patients, and *Pseudomonas* in 10 patients as ESBL producing bacteria (table I).

Table I: ESBL producing bacteria among patients.

Variables	N (%)
E coli	96(64)
Klebsiella	13(8.67)
Acinetobacter	20(13.34)
Proteus	11(7.33)
Pseudomonas	10(6.67)
Total	150(100)

The distribution of the infection caused by the ESBLs-producing bacteria among diabetic and nondiabetic patients is reflected in Table 4, in a total of 150 samples. The samples were equally distributed between both diabetic and nondiabetic ones. *E. coli* was the most predominant bacterium in general, infecting 80% of the diabetic patients and 48% in the nondiabetic. On the other hand, there was a small presence of *Klebsiella* in diabetic patients. It also showed that *Acinetobacter* infections are significantly higher among non-diabetic patients, 21.33%, than in diabetic patients, 5.33%. Whereas Proteus only appears in diabetic patients, 14.67%, and it does not appear at all in non-diabetic patients. *Pseudomonas* shows a higher preponderance in non-diabetic patients, 12%, than in diabetic ones, 1.33%. (Table II).

Table II: Bacterial prevelance in diabetic and nondiabetic patients.

Bacteria	Diabetic Patient	Non- Diabetic Patient
E coli	60 (80%)	36 (48%)
Klebsiella	8 (10.67%)	5 (6.67%)
Acinetobacter	4 (5.33%)	16 (21.33%)
Proteus	11 (14.67%)	0 (0%)
Pseudomonas	1 (1.33%)	9 (12%)

Total of 131 patients were given amikacin and 19 were gentamicin. While all patients were reported with using antibiotics in last 3 months (Table III).

Table III: Antibiotic sensitive pattern of of ESBL producing pathgen.

Antibiotic Sensitive ESBL Positive	**Frequency N (%)**	
	Amikacin	131(87.33)
	Gentamicin	19(12.67)
	Antibiotic use in last 3 months	150(100)

The AST results indicated that both Gentamicin and Amikacin are much clinically active among diabetic individuals. Against *E. coli* , it was highly effective at a 80% rate, compared to the 30% for Gentamicin; also a larger inhibitory area was seen 18.2 ± 2.5 mm vs. 12.5 ± 3. mm). *Klebsiella* was highly susceptible to Amikacin 75%, with the zone of inhibition was 17.5 ±1.5 mm compared to the Gentamicin, to which the percentage susceptibility was 25 %, and had a zone of inhibition 11.0 ± 2 mm. *Acinetobacter* was resistant to Gentamicin at 0%, and was sensitive to 50% of Amikacin, with its inhibition zones

Table IV: Antimicrobial sensitivity pattern of isolates from diabetic patients

Bacteria	Antibiotic	Sensitive	Intermediate	Resistant	Zone of inhibition (Mean ± SD)
E. coli	Gentamicin	18 (30%)	12 (20%)	30 (50%)	12.5 ± 3mm
E. coli	Amikacin	48 (80%)	9 (15%)	3 (5%)	18.2 ± 2.5mm
Klebsiella	Gentamicin	2 (25%)	1 (12.5%)	5 (62.5%)	11 ± 2mm
Klebsiella	Amikacin	6 (75%)	1 (12.5%)	1 (12.5%)	17.5 ± 1.5mm
Acinetobacter	Gentamicin	0 (0%)	1 (25%)	3 (75%)	10 ± 1.8mm
Acinetobacter	Amikacin	2 (50%)	1 (25%)	1 (25%)	16 ± 2mm
Proteus	Gentamicin	4 (36.36%)	2 (18.18%)	5 (45.45%)	14 ± 2.5mm
Proteus	Amikacin	8 (72.72%)	2 (18.18%)	1 (9.09%)	18 ± 1.5mm
Pseudomonas	Gentamicin	0 (0%)	0 (0%)	1 (100%)	9.5 ± 1mm
Pseudomonas	Amikacin	1 (100%)	0 (0%)	0 (0%)	18 ± 1mm

measuring 10.0± 1.8 mm and 16. 0±2 mm respectively. Proteus are more sensitive to Amikacin, 72.72%, than to Gentamicin, 36.36%; the zones of inhibition are also bigger in the case of Amikacin, 18.0 ± 1.5 mm versus 14.0 ± 2.5 mm. *Pseudomonas* shows complete resistance to Gentamicin and complete sensitivity to Amikacin; the average zone of inhibition was 9.5 ± 1 mm for the former and 18.0 ± 1 mm for the latter. In general, with most bacteria, amikacin would be rather more effective than gentamicin, indicating high sensitivity and large areas of inhibition (Table IV).

AST results showing the sensitivity of both antibiotics, Gentamicin and Amikacin, to the bacteria varied within the non-diabetic category. Very high susceptibility of 86.1% was demonstrated by Amikacin against *E. coli* with a mean zone of inhibition at 19.0 ± 1.5 mm compared to sensitivity by Gentamicin which presented 55.5% and a zone of inhibition at 14.8 ± 2 mm. *Klebsiella* is also more sensitive to Amikacin, 80%, compared to Gentamicin, 40%; the zones of inhibition are 18.2 ± 1 and 12.0 ± 1.2 mm, respectively. *Acinetobacter* is highly resistant to Gentamicin, 68.75%, but relatively sensitive to Amikacin, 62.5%, with mean zones of inhibition of 10.5 ± 2.2 and 17.0 ± 2 mm, respectively. On the other hand, Proteus species showed complete inhibition against Gentamicin and perfect sensitivity to Amikacin, with an inhibition zone of 13.0 ± 1.5 mm and 18.5 ± 1.2 mm, respectively. *Pseudomonas* was most resistant; 88.9% of the isolates showed resistance to this antibiotic, namely, Gentamicin, with a very high sensitivity of 66.7% to Amikacin, whose inhibition zones were 10.2 ± 1.8 and 17.5 ± 1.2 mm, respectively. As a whole, Amikacin has a broader sensitivity rate and inhibits a larger inhibition zone in most bacteria than Gentamicin (Table V).

Total of 131 patients stayed 2-4 days in hospital, 13 patients stayed 5-7 days in hospital, and 6 patients stayed 8-10 days in hospital. There were 130 participants with diabetes, 109 with fever, 119 with abdominal pain, and 97 with flank pain.

Discussion

In this study, *E-Coli* was found in 96 patients, *Klebsiella* in 13 patients, *Acinetobacter* in 20 patients, *Candida* in 11 patients, and *Pseudomonas* in 10 patients as ESBL producing bacteria. It has been stated that 68 percent of Indians are ESBL producers, according to recent research. Forty percent of K. pneumoniae isolates and 41 percent of *E. coli* isolates were ESBL producers in the research cohort by another study [12]. A further investigation found that ESBL-producing *E. coli* occurred in 58% of cases. Based on these findings, another study[13] estimate that between 19.2% and 21.2% of *E. coli* and *K. pneumoniae* isolates, respectively, generate ESBLs.[14] The prevalence of ESBLs in diabetic foot infection is not well-characterized at present. This condition was shown to be uncommon among *E. coli* isolates in a research done in Brazil, with a frequency of just 6%. Some 54.5% of *E. coli*

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Pseudomonas	Gentamicin	0 (0%)	0 (0%)	1 (100%)	9.5 ± 1mm
Pseudomonas	Amikacin	1 (100%)	0 (0%)	0 (0%)	18 ± 1mm

isolates, according to another study[15] are ESBL producers and can lead to infections in people with diabetes affecting the feet. The double disc diffusion test, a quick and easy procedure suggested by CLSI for confirming phenotypes. [16]

In more than 95% of cases, a single bacterial species is responsible for the infection, and Escherichia coli is by far the most common infecting organism in cases of acute illness.[17] This study's range of uropathogens isolated from urine samples is not wildly dissimilar from what has been described elsewhere. *Escherichia coli* was the most prevalent type of bacteria found in this research, followed by *Klebsiella*, Enterobacter, and Proteus. Similar findings were found by in another study, who examined Indian urine isolates, where Escherichia coli was the most prevalent isolate, followed by Enterobacter species, Proteus species, and *Klebsiella* species among the Enterobacteriaceae in the in-patients group.[18] For diabetic individuals, Escherichia coli was shown to be the most common cause of UTIs.[19] This data suggests that the incidence of Escherichia coli is increasing, as opposed to that seen in previous investigations.

In this study, total of 131 patients were given amikacin and 19 were gentamicin. While all patients were reported with using antibiotics in last 3 months. Despite the fact that liver disease has been linked to on-admission ESBL carriage elsewhere[20], connective tissue disease and diabetes mellitus have not been found to have a similar role. Infections with ESBL-E in individuals with severe liver disease are linked with worse outcomes.[21] More research is needed to determine the underlying processes of this correlation. One suggestion is that chronic liver illness may be serving as a surrogate marker for prophylactic fluoroquinolone treatment against spontaneous bacterial peritonitis as a recognised risk factor for ESBL-E acquisition. [22] Extensive time in the hospital and the usage of gentamicin were both factors in our study's discovery of an association between ESBL-E carriage and the development of an infection. These characteristics have previously been identified to be linked with nosocomial infection with ESBL. [23]

Total 131 patients stayed 2-4 days in hospital, 13 patients stayed 5-7 days in hospital, and 6 patients stayed 8-10 days in hospital. Prevalence of ESBL generating strains of Enterobacteriaceae varies from nation to country and from species to species throughout Asia. 2 In a major research carried out in India, another study showed the overall prevalence of 68.78% for ESBL generating organisms which is virtually close to our data which reveals the overall prevalence of 65.7%.[24] The significant incidence of ESBLs among Enterobacteriaceae has also been found by two additional investigations conducted in Pakistan. According to a study done by Zaman et al.[7,8] the total prevalence of ESBL generating Enterobacteriaceae was 35%. 7 While Jabeen et al. found the ESBL prevalence of 40% among Enterobacteriaceae.[25] Another study observed a frequency of 35.5% among gramme negative bacilli in clinical isolates.[26] This reveals that the prevalence of ESBL producing Enterobacteriaceae differs from hospital to hospital and this prevalence of ESBL producing Enterobacteriaceae is growing. This is because the frequency with which ESBL-producing organisms are found varies greatly, both between different regions and over time within a single region. Because ESBL generating strains typically develop in focused outbreaks, regional and local estimates are probably more relevant than are larger global evaluations in clinical decision making and for infection control purpose.

There were 130 participants with diabetes, 109 with fever, 119 with abdominal pain, and 97 with flank pain. *Klebsiella* species appeared as the top most ESBL producing organism, another study indicated a high frequency of ESBL producing organisms in Southeast Asian contexts.[27] Urinary isolates from hospitalised patients showed that 38.5% were

Klebsiella species and 24.7% were Escherichia coli, according to research by Khurana et al. In contrast, Mathur et al. found that *Klebsiella* species accounted for 80% of all ESBL-producing bacteria.[27] *Klebsiella* species were also recognised as the most prevalent ESBL producers by the SENTRY surveillance initiative in Asia Pacific and South Africa.[28] This research showed that ESBL-producing microbes were widespread across the age spectrum. Differences in age groups did not reach statistical significance. Patients younger than 5 and older than 60 were found to have considerably higher rates of ESBL generating organisms, as reported by another study.[29] With this data, we can see that the frequency of ESBL-producing organisms is rising across all demographics, which calls attention to the general rise in the load of ESBL-producing organisms. Other surveillance studies from the Asia-Pacific area and South Africa corroborate this idea by revealing a similarly concerning increase in ESBL positive.[30]

Prior hospitalisation during the past 3 months was revealed as the strongest independent risk factor, supporting previous reports that hospitalisation is a major cause of the development of infection by ESBL-producing bacteria. We found that ESBL-positive group had not been hospitalised in the previous three months, suggesting that infection with ESBL-producing strains can occur in the community as well as in hospitals. In our analysis, we found that hospital stay duration did not affect outcomes. Another study (Kumar et al) found that the only independent risk factor for colonisation with ESBL-producing Escherichia coli or *Klebsiella* spp. was length of hospital stay. However, their analysis was based on a limited sample size, and 62% of patients in the research were transfers from other hospitals.

To further understand the frequency and characteristics of ESBL-producing Enterobacteriaceae in community-acquired bacteremia, another study[31] recently undertook a research in southern Israel. Significant risk factors for ESBL production were identified in their investigation, including advanced age, ICU admission, urinary catheterization, and bedridden circumstances.[32]Drawing any firm findings is rather hard because to the tiny number of ESBL-positive individuals. It is unclear whether or not combination therapy has been included in the evaluation of antibiotic usage in prior research.[33] Multiple studies have found that having been hospitalised within the past three months for any reason, including renal problems like UTIs and genitourinary system operations, as well as systemic infections, indirect hyperbilirubinemia, prematurity, and operations on other systems, is an independent risk factor for developing ESRD. [34]

Antibiotic usage over the previous three months was also found to be a significant independent risk factor to hospitalisation. Analysis of antibiotic classes used in pretreatment revealed no statistically significant risk factors, including trimethoprim-sulfamethoxazole, macrolides, aminoglycosides, and nitrofurantoin. While another study (Wang et al., 2020) reported that trimethoprim-sulfamethoxazole usage was an independent risk factor, we found the opposite to be true. Since the first three medications are often used in everyday life, these results are promising. Further, we have previously demonstrated that nitrofurantoin is the medicine of choice for treating community-acquired UTI, and resistance rates are still rather low in our region.[21]

Since amikacin/gentamicin is still the medicine of choice for treating streptococcal pharyngitis, the association observed in this study between its usage and the emergence of ESBL-producing strains is a serious issue.[35] The use of quinolones in outpatient settings is also common. An higher likelihood of infection with ESBL-producing strains was seen in patients with a greater variety of underlying conditions, including diabetes, cardiovascular disease, genitourinary disease, neurological disease,

recurrent UTI, and malignancies.[20] Study of all underlying disorders showed that diabetes was the sole independent risk factor for infection with ESBL-producing bacteria. It may also not be economically viable for some facilities that serve areas with low ESBL prevalence to do routine testing for this resistance mechanism.[36] For these reasons, it is crucial to identify community-level risk factors for the emergence of ESBL-producing bacteria.

Conclusion

This study identified *E. coli* in 96 patients, *Klebsiella* in 13 patients, *Acinetobacter* in 20 patients, *Candida* in 11 patients, *Pseudomonas* in 10 patients as ESBL producing bacteria. Total of 131 patients were given amikacin and 19 were gentamicin. While all patients were reported with using antibiotics in last 3 months. Total of 130 participants with diabetes, 109 with fever, 119 with abdominal pain, and 97 with flank pain. Total of 131 patients stayed 2-4 days in hospital, 13 patients stayed 5-7 days in hospital, and 6 patients stayed 8-10 days in hospital. The ESBL generation by the isolated uropathogenic bacteria did not differ substantially by age, gender, or source of clinical isolates.

Recommendations: Empiric antibiotic treatment can be administered with greater success by using these risk variables to identify patients who are at high risk of harbouring ESBL-producing bacteria. By completing many complimentary investigations and assessing the efficacy of phages, it is believed that they will be employed therapeutically as an effective therapeutic agent against K. pneumoniae that produces ESBL in patients. Diabetes patients should adhere to an appropriate and efficient antimicrobial treatment plan, with laboratory support as necessary. In order to reduce the risk of MDR acquisition, persons with underlying conditions who carry MDR bacteria need to be better understood.

Limitations: There are a few limitations with this study. First of all, while being the largest study to precisely examine the variables impacting the recurrence of ESBL-induced infections, the sample size was limited. Second, because this was a retrospective analysis, we were unable to consistently track the drugs that patients were taking after being discharged. All potential ESBL genes from those E. coli isolates were not tested due to the restricted molecular resources and funding. We were unable to conduct sequencing for further verification of those genes, which was another limitation of this investigation. Diabetes recurrence may be impacted by therapeutic medications used to treat some chronic conditions; aspirin, for instance, has been demonstrated in trials to lower the likelihood of diabetes recurrence. Additionally, the patients in this research were all from the same region, and geographic considerations may have an impact on the epidemiology of ESBL.

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