Original Research Article

Prevalence of ESBL producing enterobacteriaceae in diabetic foot ulcers

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Abstract

Introduction: Diabetic foot ulcer is one of the dreaded complications among the diabetic patients which are disabling, leading to repeated hospitalizations and even amputation, drastically reducing the quality of life. Hence proper management of diabetic foot infections by choosing appropriate antibiotic is crucial.

The aim of the study: This study was undertaken to know about the prevalence of ESBL producing Enterobacteriaceae in Diabetic foot ulcers and their antibiotic sensitivity pattern to aid in the effective treatment of infection.

Materials and methods: A total of 200 Pus samples were collected from the patients admitted and/ or attending in-patient or out-patient departments of Rajah Muthiah Medical College and Hospital. Only Enterobacteriaceae were isolated and antibiotic sensitivity testing was done according to CLSI guidelines. All the isolates were screened for ESBL and confirmed by phenotypic confirmatory tests.

Results: A total of 60 Enterobacteriaceae were isolated from diabetic foot ulcer patients among which 27 (45%) isolates were ESBL producers. ESBL production is predominantly seen in E.coli (48.1%) followed by Klebsiella (44.4%), P. mirabilis (3.3%) and P. vulgaris (3.3%). ESBL producers were sensitive to Imipenam (88.9%), Amikacin (77.8%) and Gentamicin (55.6%) whereas highly resistant to Ampicillin, Amoxyclav, Cefuroxime and Ceftriaxone.

Conclusion: This study shows the higher prevalence of ESBL producing Enterobacteriaceae in diabetic foot ulcers. Imipenam, Amikacin and Gentamicin can be used for empirical treatment but early identification and treatment according to antibiotic sensitivity pattern helps in preventing the emergence and propagation of multidrug resistance strains.

Key words

ESBL, Diabetic foot ulcers, Enterobacteriaceae, Gram-negative bacteria.

Introduction

The worldwide prevalence of Diabetes Mellitus has risen dramatically over the past two decades, from an estimated 30 million cases in 1985 to 382 million in 2013. In India, about 6.1 million cases of Diabetes had been estimated in 2013. Due to increasing prevalence of diabetes mellitus, infections and complications associated with the condition are also on the rise [1]. Foot ulcers and infections form a major source of morbidity in Diabetic patients. The risk of developing a skin and soft tissue infection ulcer in a diabetic patient during a lifetime is as high as 25% [2]. Also, Diabetes is one of the leading causes of nontraumatic lower extremity amputation. Most of the individuals with Type 2 Diabetes Mellitus develop foot ulcer (great toe or metatarsophalangeal areas are most common), and a significant subset who develop ulceration would undergo amputation (14-24% risk of that ulcer or subsequent ulceration). The increased incidence of foot ulcers in diabetes is due to the interaction of several pathogenic factors like neuropathy, abnormal foot biomechanics, Peripheral arterial disease and poor wound healing. Peripheral arterial disease and poor wound healing impede resolution of minor breaks in the skin, allowing them to enlarge and to become infected [1]. Both aerobic and anaerobic organisms cause diabetic foot ulcers. The infection may exhibit polymicrobial growth [3]. The predominant organisms isolated are *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* species, *Proteus* species, and anaerobic organisms [4]. However, the aetiology of wound infection differs from country to country and from hospital to hospital even within the same region depending mainly on the microbial flora of particular area, metabolic factors, hygiene and the use of antibiotics [5]. In India Gram-negative bacteria are commonly isolated from Diabetic foot infections when compared to western countries where Gram-positive cocci are predominant [6, 8] ESBL production is one of the common mechanism of drug resistance among the Enterobacteriaceae [9] ESBLs are beta-lactamases capable of conferring bacterial resistance to the penicillins, first, second, and third-generation cephalosporins, and aztreonam (but not the cephamycins or carbapenems) by hydrolysis of these antibiotics, and which are inhibited by beta-lactamase inhibitors such as clavulanic acid [10]. Association of multidrug-resistant bacteria in diabetic foot infections pose a great difficulty in treating diabetic foot ulcers [11]. The present study was aimed to know about the prevalence of ESBL producing Enterobacteriaceae in Diabetic foot ulcers and their antibiotic sensitivity pattern to aid in the effective treatment of infection.

Materials and methods

A total of 200 Pus samples were collected from the patients admitted and/ or attending in-patient or out-patient departments of Rajah Muthiah Medical College and Hospital over a period of one year. Patients of both the sexes and age group between 40-80 years with Diabetic foot ulcer yielding Enterobacteriaceae only were included in the study. The samples were processed, and the isolates were identified by standard Microbiological techniques. Out of 200 samples, 60 Enterobacteriaceae were isolated from the patients with a Diabetic foot ulcer. All the Enterobacteriaceae isolates were subjected to antimicrobial susceptibility testing by Kirby Bauer disc diffusion method according to CLSI guidelines [12].

Antibiotics used were Gentamicin (10 µg), Amikacin (30 µg), Ampicillin (10 µg), Cotrimaxazole (25 µg), Cefuroxime (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Imipenam (10 µg), Amoxyclav (20/ 10 µg) and Ciprofloxacin (5 µg).

Screening for ESBL production

All the Enterobacteriaceae isolates were screened for ESBL production. The isolates which showed resistance to any one of the antibiotics with zone size of < 22 mm for Ceftazidime, < 27 mm for Cefotaxime or <25 mm for Ceftriaxone were identified as potential ESBL producers and they were tested further.

The double disc synergy test (DDST)

The isolates which were identified as potential ESBL producers by the screening test were picked up and emulsified in saline to a 0.5 McFarland's turbidity. Discs of ceftazidime (30 µg), cefotaxime (30 µg) and amoxiclav (20 µg amoxycillin and 10µg clavulanic acid) were placed at a distance of 20 mm from center to center in a straight line, with the amoxyclav disc in the centre on a plate of Mueller Hinton Agar (MHA) inoculated with the test strain. The plates were incubated at 37°C for 18-24hrs. Isolates which showed an enhancement of the zone of inhibition towards the disc with clavulanic acid as compared to that which was seen on the side without amoxyclav, were confirmed as ESBL producers [13].

The Phenotypic Confirmatory Disc Diffusion Test (PCDDT)

All the strains which were screened for ESBL production were also subjected to confirmation by using the PCDDT, as recommended by the CLSI [12]. The ceftazidime (30 µg) discs alone and in combination with clavulanic acid (ceftazidime + clavulanic acid, 30/10 µg discs) were applied onto a plate of Mueller Hinton Agar (MHA) which was inoculated with the test strain incubated at 37°C for 18-24hrs. An increase of ≥ 5mm in the zone of inhibition of the combination discs in comparison to the ceftazidime disc alone was considered to be a marker for ESBL production.

E-test for ESBL

Combination of disc diffusion and Minimum Inhibitory Concentration (MIC) were studied using the E-test strips. The E-test strip contains Ceftazidime gradient at one end and Ceftazidime plus Clavulanate gradient on the opposite end. E-test strips were placed on the Muller Hinton agar inoculated with the test strain and incubated at 37°C for 18-24hrs. MIC was the point of intersection of the inhibition ellipse with the E-test strip edge. The ratio of ceftazidime MIC and Ceftazidime Clavulanic acid MIC ≥ 8 indicated the presence of ESBL.

Results

In the present study, the predominant Enterobacteriaceae isolated from Diabetic foot ulcer is *E.coli*(43.3%) and *Klebsiella* (43.3%) followed by *Proteus mirabilis* (5%), *Proteus vulgaris* (3%), *Citrobacter* (1.7%) and *Enterobacter* (3%) as shown in **Figure - 1**. Patients with diabetic foot ulcers were predominantly between the age group of 50-70 years and males (68.3%) were more commonly affected than females (31.7%). Out of 60 isolates, ESBL production was observed in 27 isolates (45%). Distribution of ESBL producing isolates was shown in **Table - 1**. ESBL production is predominantly seen in *E.coli* (48.1%) followed by Klebsiella (44.4%). The antibiotic sensitivity pattern of the ESBL producing strains was shown in **Table - 2**.

Discussion

Diabetic foot ulcer is one of the dreaded complications of diabetes and is the leading cause of the increase in morbidity among the diabetic patients. Diabetes mellitus is a metabolic disorder that impedes the normal wound healing process leading to severe infections. Many organisms causing diabetic wound infections are developing resistance to commonly used antibiotics due to indiscriminate use. ESBLs forming a major group of drug resistance mechanisms are common among the Enterobacteriaceae, which are the commonly encountered organisms in diabetic foot ulcers [14]. In this study, patients with diabetic foot, the ulcer is common between the age group of 50-70 years which could attribute to the fact that diabetic ulcers are common among the elderly

age group with chronic diabetes due to neuropathy and peripheral arterial disease.

Among the Enterobacteriaceae, *E.coli* (43.3%) and *Klebsiella* (43.3%) are the predominant organisms isolated followed by *Proteus mirabilis* and *P.vulgaris* respectively in this study. This finding correlates with a study by Saroj Golia, et al. [15] which showed *E.coli* (40.8%) and *Klebsiella* (38.8%). Kavitha, et al. [16] and Gadepalli, et al. [17] had reported 46.7% and 44.7% of ESBL production in diabetic foot ulcers respectively which correlated with this study showing 45% the isolates from the diabetic foot ulcer were ESBL producers. ESBL production was observed in 48.1% of *E.coli* and 44.4% of *Klebsiella* in this study which correlates with Varaiya, et al. [18] where 46.5% *E.coli* and 44.4% *Klebsiella* were ESBL producers, Gadepalli, et al. [17] has reported 54.5% of ESBL production in *E.coli* isolates whereas Umashankari, et al. [19] has reported higher percentage of ESBL production in *Klebsiella* (59.5%) than *E.coli* (40%) in diabetic foot ulcers.Antibiotic sensitivity pattern of ESBL producing Enterobacteriaceae shows that all strains were resistant to Ampicillin followed by 96.3% resistance to Amoxyclav, Cefuroxime, and Ceftriaxone each. The rate of sensitivity is high to Imipenam (88.9%) followed by Amikacin (77.8%) and Gentamicin (55.6%). Varaiya, et al. [18] had shown similar antibiotic sensitivity patterns in which *E.coli* and *Klebsiella* are 100% resistant to Ampicillin and sensitivity to Amikacin and Gentamicin are 66.2% and 31.3 % for *E.coli* and 63.5% and 35.6% for *Klebsiella* respectively. Imipenam is 100% sensitive for all ESBL strains of Enterobacteriaceae as reported by Varaiya, et al. [18]; Kavitha, et al. [16] and Priyadharshini, et al. [20] when compared with this study which showed 88.9% sensitivity to Imipenam.

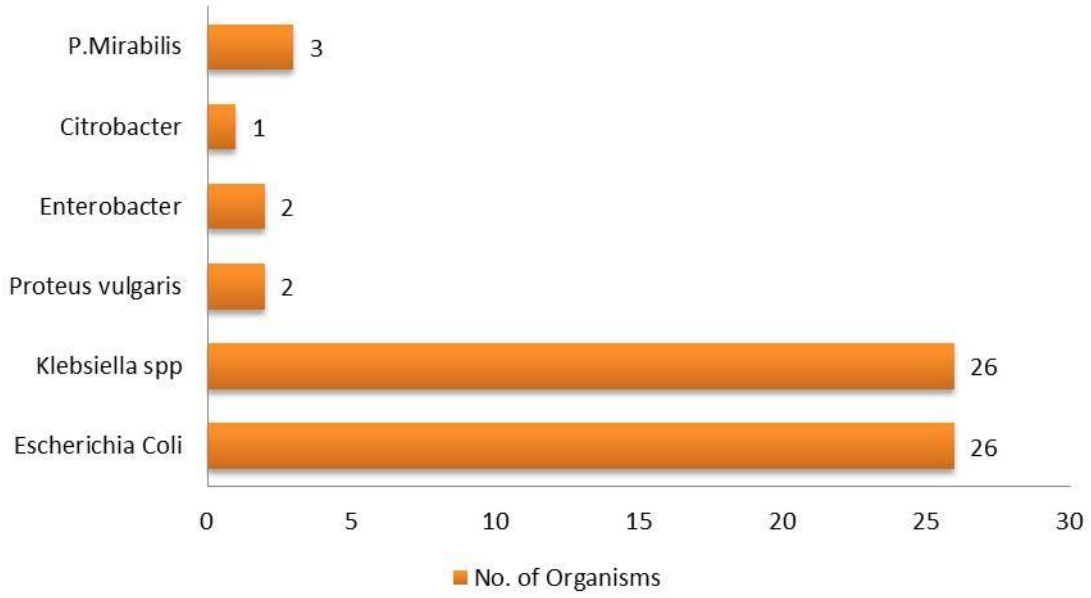
Table – 1: Distribution of ESBL producing enterobacteriaceae in diabetic foot ulcer.

Organism	**Total isolates**	**ESBL**	
		Positive	**Percentage**
Escherichia coli	26	13	48.1
Klebsiella spp	26	12	44.5
Proteus vulgaris	2	1	3.7
Enterobacter	2	0	0
Citrobacter	1	0	0
Proteus mirabilis	3	1	3.7
Total	**60**	**27 (45%)**	**100.0%**

Table – 2: antibiotic sensitivity pattern of ESBL producing enterobacteriaceae.

Antibiotics	**Resistance**		**Sensitive**	
	No of isolates	**Percentage**	**No of isolates**	**Percentage**
Ampicillin	27	100.0%	0	0%
Gentamicin	12	44.4%	15	55.6%
Amikacin	6	22.2%	21	77.8%
Cotrimoxazole	20	74.1%	7	25.9%
Ciprofloxacin	16	59.3%	11	40.7%
Amoxyclav	26	96.3%	1	3.7%
Cefuroxime	26	96.3%	1	3.7%
Ceftriaxone	26	96.3%	1	3.7%
Ceftazidime	25	92.6%	2	7.4%
Imipenam	3	11.1%	24	88.9%

Figure - 1: prevalence of enterobacteriaceae in diabetic foot ulcers.



Limitations of this study

This study has some limitations such as the only Enterobacteriaceae were included in the study for ESBL detection not other important Gram-negative bacilli like Pseudomonas. ESBLs were confirmed by phenotypic tests whereas few strains of ESBL cannot be detected by Phenotypic methods. Nuesch and Hachler stated that genotypic techniques are more reliable than phenotypic methods. But genotypic methods are expensive, time-consuming, the requirement of special apparatus and expertise limits its application in routine usage. [21]

Conclusion

This study shows that prevalence of ESBL producing Enterobacteriaceae is high in the diabetic foot ulcers. This constitutes a serious threat of treatment failure by Beta-lactam group of antibiotics which are commonly used to treat infections. The sensitivity of ESBL producing strains for Imipenam, Amikacin and Gentamicin was good when compared to other antibiotics. Thus regular monitoring of the drug-resistant pattern helps the clinician in starting the empirical therapy. There is an urgent need to rationalize the use of antibiotics to prevent and control the increasing emergence of Multidrug-resistant strains.

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