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Prevalence and Antimicrobial Susceptibility Pattern of Extended Spectrum β-Lactamases Producing *Escherichia coli* in Diabetic Foot Infection

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ABSTRACT

To study the prevalence of Extended Spectrum Beta Lactamases producing *E. coli* among diabetic foot ulcer patients attending the tertiary care hospitals and diabetic centers in South India. Thirty four isolates of *E. coli* were obtained from 106 patients. These isolates were subjected to susceptibility testing as per Clinical and Laboratory Standards Institute guidelines. They were further screened for ESBL (Extended Spectrum Beta Lactamases) production by screening test, double disc approximation test and National Committee for Clinical Laboratory Standards Confirmatory test respectively. Of the 34 isolates of *E. coli,* 14 (4%) were found to be ESBL producer. *E. coli* exhibited 100% susceptibility to imipenem and meropenem and resistant to cephalexin, erythromycin, gentamicin and norfloxacin. Intermediate resistance was observed only against cefotaxime (76.5%). High %age of resistance to amikacin (52.9%), gentamicin (64.7%), cloxacillin (70.6%), co-trimoxazole (76.5%) and cephalexin (82.4%) were observed among ESBL producers. These findings suggests that prospective multicenter studies are required to assess the appropriate empirical antibiotic requirements in diabetic foot ulcers taking into consideration the etiology of ulcers.

Key words: *E. coli*, extended spectrum β-lactamase, diabetic foot infections, antibiotic sensitivity test

INTRODUCTION

Diabetic foot infections are common in patients and are associated with high morbidity and risk of lower extremity amputation. Diabetic foot infections are classified as mild, moderate or severe (Boulton *et al*., 2005). Diabetes currently affects more than 194 million people worldwide and is expected to reach 33 million by 2025, with most of the massive burden falling upon developing countries. According to WHO, India has the largest diabetic population (66.58 million in 2004) which is expected to rise to 57 million by 2025 (Krishnan and Rayman, 2005). Worldwide, diabetic foot lesions are a major medical, social and economic problem and are the leading cause of hospitalization for patients with diabetes (Gadepalli *et al*., 2006). The prevalence of diabetic foot ulcers has been estimated to be 3-8% and more than 85% of these amputations are precipitated by a foot ulcer deteriorating to deep infection or gangrene (Apelqvist and Larsson, 2000). The risk

factors identified for the development of foot ulceration among diabetic patients are smoking, presence of ischemic heart disease and hypertension (Chang *et al*., 1996). Foot ulcer grade on admission was a significant factor in determining the surgical intervention. In more superficial infections which is classified according to Wagner (Wagner Grades 1-2), aerobic bacteria are the predominant organisms, in deeper wounds (Wagner Grades 3-5), gram negative bacteria frequently found (Urbancic-Rovan and Gubina, 1997). Grade 0, 1, 2 signifies and classifies patients having high risk foot with no ulceration, superficial ulcer and deep ulcer; grade 3, 4 and 5 classifies patients having osteomyelitis with ulceration, gangrenous patches and gangrene of entire foot (Wagner, 1981; Lavery *et al*., 1996). *Enterobacteria* are an important pathogen group in community and hospital-acquired infections. Patients with diabetes mellitus are at high risk for *Enterobacteria* infection. Unfortunately resistance has become increasingly common among gram-negative bacteria making empirical therapy decisions more difficult. The most serious resistance patterns now emerging among gram-negative organisms include resistance to extended spectrum of cephalosporin and penicillin (Motta *et al*., 2003). Extended Spectrum Beta Lactamases producing strains of Enterobacteriaceae have emerged as a major problem in hospitalized as well as community based patients and Infections due to ESBLs-producers range from uncomplicated UTI to life threatening sepsis (Bhattacharya, 2006). The continuous use of expanded-spectrum cephalosporins in various hospitals for life threatening infections have resulted to the outbreak of resistant organisms that were previously known to be sensitive to these agents and this resistance has spread to *E. coli* and some other gram negative organisms (Iroha *et al*., 2008a). ESBL phenotypes and detection have become more complex due to the diversity of the enzymes produced, emergence of inhibitor resistant ESBL variants plasmid borne resistance genes, concurrent Amp-C production enzyme hyperproduction and porin loss (Sturenburg *et al*., 2004). Mathur *et al*. (2002) from India have reported 68% ESBL positivity rate in their Enterobacteriacae isolates. The increase in ESBL mediated resistance amongst *E. coli* isolates worldwide makes this a major public health threat. In this context, the study was initiated to screen the prevalence of ESBL producing *E. coli* in diabetic foot infection with type II diabetes and assessment of their antibiogram profile.

MATERIALS AND METHODS

Patients demographic data: The present study was conducted with the help of the dialectologists of reputed diabetic centers and tertiary care hospitals in and around Erode and Salem, South India. It receives patients from all over the districts comprising both rural and urban. One hundred and six patients with diabetic foot disease presenting from June 2007 to May 2008 were included in the study. Patients with chronic foot disease and previous amputations were also included.

Patients from both outpatient and inpatients admitted in the surgical wards were included. Data was collected by taking a detailed history and clinical examination of foot, its wound or ulcer was recorded using a predesigned pro forma. Age, sex, socioeconomic status, duration of diabetes, types of diabetes, site of ulcer, smoking habits and other associated co-morbid conditions like ischemic heart disease, retinopathy, renal impairment, peripheral disease, polyneuropathy. Wagner's classification (Table 1) and swabs from wound/ulcer were recorded. The patients were evaluated by classifying their disease according to Wagner's classification (Wagner, 1981; Lavery *et al*., 1996) or diabetic foot disease. Data was compiled and frequencies were calculated.

Of the total 106 diabetic patients suffering from foot infections taken for the study 80 were male and 26 were female and the male to female ratio being 3:1. The age of the patients ranged between 41 to 65 years. Among these patients, 10 (9.43%) have undergone amputation. Majority of the

Table 1: Antibiotic discs [conc. (µg)] used for the study

Antibiotics	Conc. (µg)
Amikacin	30
Ampicillin	10
Ciprofloxacin	5
Co-trimoxazole	25
Cephalexin	30
Chloramphenicol	10
Cloxacillin	10
Cefepime	30
Cefotaxime	30
Erythromycin	10
Gentamicin	15
Imipenem	10
Meropenem	10
Norfloxacin	10
Piperacillin	10

diabetic foot ulcer patients were of more than 60 years of age. Males predominated with 75.47%, while females were 24.52%. All the patients included in study had type 2 diabetes and none had type 1 diabetes. Only 18 (16.98%) had positive family history of diabetes.

The number of patients according to Wagner's classification for diabetic foot disease were as follows: 56 patients were classified Majority of the patients presented advanced disease in grade 2 with 52.8%, 37.7% in grade 1, 7.5% in grade 0 and 1.9% in grade 3 and none of the patient's in the current study had grade 4 and 5 disease, respectively.

Collection of samples: A total of 106 pus samples were collected for screening ESBL producer *E. coli* from diabetic foot ulcer patients in the Department of Microbiology, Dr. N.G.P Arts and Science College, Coimbatore, South India. Soft tissue infections includes foot wound and limb threatening infection specimens received during June 2007 to May 2008 were included in the analysis. Specimens were obtained using aseptic techniques to avoid contamination and were promptly transported to the Microbiology laboratory in a sterile swab in an ice-cold condition. All *E. coli* (34) isolates were included in the study. Clinico- demographic data of study patients was noted.

Antibiotic susceptibility testing: The above isolates were tested for antimicrobial susceptibility by disc diffusion technique according to Clinical and Laboratory Standards Institute Guidelines with commercially available discs (Hi Media, Mumbai) on Mueller Hinton agar plates. The antibiotic discs used for the study are shown in Table 1.

Test for ESBL production:

- Screening test (CLSI, 2005)
- Double Disc Approximation Test (Jarlier *et al.*, 1988)
- NCCLS Confirmatory test (NCCLS, 2000)

Statistical analysis: Statistical analysis was performed by one way ANOVA SPSS statistical software package version 11. Statistical significance was assumed at the $p<0.05$ level.

RESULTS

The socio-demographic and clinical characteristics of the study population are shown in Table 2. It was found from Table 3, that the duration of diabetes for 20 patients relate to the category of less than 1 year, 74 patients relate to the category of 1-5 years, 8 patients relate to the category of 5-10 years and 4 patients were related to the category of more than 10 years. In the present study, more number of patients had duration of diabetes about 1-5 years. The mean duration of diabetes was 8.83±10.77 years and only 3.7% of the patients had the condition for more than 10 years. The localization of wounds was mostly on toes with 41.50% on fore foot, 35.85% on hind foot and 22.64% on mid foot. It was noted from table 2 that among diabetic patients with foot ulcers, 37.73 (n = 40) were smokers. With increasing severity of diabetes based on the presence of one or more complications, more number of patients with foot infections was observed. The associated co-morbid conditions recorded from 106 patients, 38 (35.84%) patients were found to have diabetic polyneuropathy, 8 (7.54%) patients showed diabetic retinopathy, 6 (5.66%) patients

Table 2: Socio demographic and clinical features of 106 diabetic patients with infected foot ulcer

Variables	Total (n = 106)	Percentage
Sex		
Male	80	75.47
Female	26	24.52
Age/years		
41-50	18	15.00
51-60	40	39.62
>60	48	45.28
Types of diabetes		
Type1	-	-
Type 2	106	100.00
Smoking habits		
Yes	40	37.73
No	66	62.265
Site of ulcer		
Fore foot	44	41.50
Hind foot	38	35.84
Mid foot	24	22.64
Family history		
Present	18	16.98
Absent	88	83.01
Socioeconomic status		
Lower	76	71.69
Middle	18	16.98
Upper class	-	-
Not-mentioned	12	11.32

Table 3: Duration of diabetes

Duration of diabetes (Years)	No. of patients (%)
Less than 1	20 (18.9)
1-5	74 (69.8)
5-10	8 (7.5)
More than 10	4 (3.7)

Table 4: Associated co-morbid conditions in 106 patients with diabetic foot infections

Co-morbid condition	No. of patients (%)
Diabetic polyneuropathy	38 (35.84)
Diabetic retinopathy	8 (7.54)
Diabetic nephropathy	6 (5.66)
Ischemic heart disease	2 (1.88)
Others	52 (49.05)

Table 5: The overall status of cultural specimens (pus) of diabetic subjects with soft tissue infections

Status	Values
No. of pus specimen processed	106.00
No. of positive specimen	82(77.35%)
No. of negative specimen	24 (22.64%)
Number of confirmed *E. coli*	34 (41.46%)
Others	48 (58.53%)

showed diabetic neuropathy, 2 (1.88%) patients showed ischemic heart disease and the rest 52 (49.05%) patients were diagnosed with some other disorders (Table 4). Of the 106 specimens of diabetic patients with foot infection, 82 (77.35%) specimens showed culture positive and the rest 24 (22.64%) were negative. Among the positive isolates, aerobic gram negative *E. coli* was the most frequent pathogen found in 34 (41.46%) samples and other organisms was found in 48 (58.53%) samples (Table 5).

Extended spectrum β-lactamases in *E. coli* isolates of diabetic foot infection: The increased prevalence of Enterobacteriaceae producing ESBL creates a great need for testing methods that until accurately identifies the presence of enzymes in pus samples. The detection of these ESBL strains is of vital importance because they are responsible for spread of resistance gene in a hospital setting. From 34 *E. coli* isolates ESBL production was observed in 41.17% of *E. coli* by NCCLS confirmatory test. The double disk approximation test failed to detect ESBLs in six isolates of *E. coli*. These ESBL positive isolates were obtained from 80 male and 26 female with a male female ratio of 3:1 (Table 6).

Antibiotic susceptibility pattern of ESBL producer *E. coli* from infected foot ulcers of diabetic patients: Antibiotic susceptibility pattern in ESBL producer *E. coli* isolated from diabetic foot ulcer patients were found to be as follows: the high percentage of susceptibility to cefotaxime (88.2%), chloramphenicol (88.2%), piperacillin (82.4%), cefepime (76.5%), norfloxacin (64.7%), ampicillin (58.8%) and erythromycin (58.8%) and high percentage of resistance to cefpodoxime (82.35%), co-trimoxazole (76.47%), cloxacillin (70.58%), imipenem (70.05%), ampicillin (52.94%) and meropenem (52.94%) were observed among ESBL producers. The antibiotic susceptibility results of ESBL producers are shown in Fig. 1.

Antibiotic resistance pattern in ESBL and non ESBL producers: The antibiotic resistance pattern in ESBL producers (14) and non-ESBL producers (20) are presented in Table 7. High percentage of resistance to amikacin (52.94%), gentamicin (64.7%), cloxacillin (70.6%), co-trimoxazole (76.5%), cephalexin (82.4%) were observed among ESBL producers. Resistance to the above drugs was found to be less among non-ESBL producers (0.2-37.9%).

Table 6: ESBL detection by three methods

		ESBL producer *E. coli*	
Specimen	Methods	No. of strains	%
Pus	Screening test	22	64.70
	DDAT test	8	23.52
	Phenotypic confirmatory test	14	41.17

Table 7: Antibiotic resistance pattern in ESBL and non ESBL producers

Antibiotics	ESBL producers resistance (%)	Non ESBL producer resistance (%)	p-value
Cephalexin	82.35	0.2	<0.001
Co-trimoxazole	76.47	37.9	<0.001
Cloxacillin	70.58	27.9	<0.001
Gentamicin	64.70	11.4	<0.001
Amikacin	52.94	3.1	<0.001

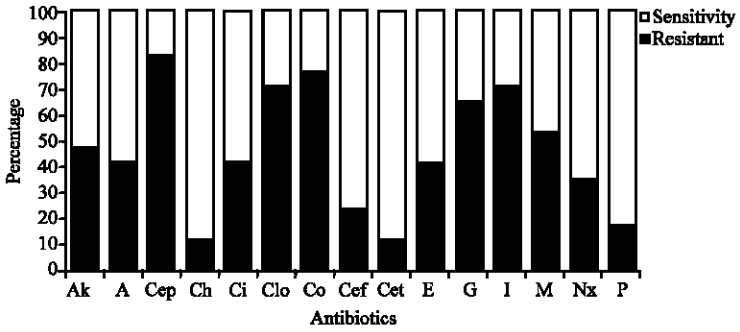

Fig. 1: Antimicrobial susceptibility pattern of ESBL producer *E. coli*. Ak: Amikacin, A: Ampicillin, Cep: Cephalexin, Ch: Chloramphenicol, Ci: Ciprofloxacin, Clo: Cloxacillin, Co: Cotrimoxazole, Cef: Cefepime, Cet: Cefotaxime, E: Erythromycin, G: Gentamicin, I: Imipenem, M: Meropenem, Nx: Norfloxacin, P: Piperacillin

DISCUSSION

Foot infection and the subsequent amputation of a lower extremity are the most common causes of hospitalization among diabetic patients (Yonem *et al*., 2001). It is more common in males which formed 75.47% of the study patients with diabetic foot infection. In another study 66.6% were males and 33.3% were females (Zaffar, 2001). Dhanasekaran *et al*. (2003) reported 14.80% of *E. coli* in Chennai, Shankar *et al*. (2005) reported 22.0% in Chennai and Gadepalli *et al*. (2006) had reported only 12.0% in New Delhi. These findings indicate that *E. coli* is becoming predominant in diabetic foot infection patients. Alavi *et al*. (2007) reported that *E. coli* (23.8%) was the most predominant gram negative organisms.

The present study reports the higher incidence of *E. coli* isolates among diabetic foot ulcer patients in South India. There is a paucity of Indian data on the prevalence of ESBL producing pathogen in diabetic foot infection. In a study conducted in 2001, the prevalence was only 6% amongst *E. coli* isolates (Motta *et al*., 2003). Gadepalli *et al*. (2006) reported 54.5% *E. coli* isolates to be ESBL producers in a tertiary care hospital in New Delhi. The present study reports 41.17% of *E. coli* isolates to be ESBL producers. Mathai *et al*. (2002) reported unstable pattern of antimicrobial resistance and wide variation in the reported rates of resistance from diverse geographical location within India. Mansouri and Ramazanzadeh (2009) reported the prevalence

of ESBL-producing *E. coli* as 16.8% in Iran and prevalence of ESBL was found to be 25.2% in Tiruchirapalli, South India (Selvakumar and Jasmine, 2007) and 31.86% prevalence of ESBL producing E. coli was observed in Turkey (Arabaci *et al*., 2009).

Oteo *et al*. (2002) also reported resistance to non β-lactam antibiotics among ESBL producers. Resistance to co-trimoxazole, cephalexin and cloxacillin was more prevalent in ESBL producing strains than non-ESBL strains. ESBLs are encoded by plasmids, which may also carry resistance genes for other antibiotics like aminoglycosides, tetracycline and trimethoprim sulphamethoxazole which results in strains that are multi drug resistant (Jacoby and Sutton, 1991). The difference in resistance between ESBLs and non-ESBLs was found to be statistically significant from Table 6. Villa *et al*. (2000) reported similar observations of multi drug resistance in ESBL strains. In a study conducted by Iroha *et al*. (2008b), they have used combinational drug therapy combining gentamicin and fluroquinolones which showed enhanced activity against ESBL producers.

This study was conducted mainly in the age group of 40-60 years and only with type 2 diabetes. It remains to be determined whether this result would apply to younger subjects or persons with type 1 diabetes. One of the limitations of this study is that it has confined only to *E. coli* and hence cannot comment on other aerobic gram positive, gram negative and anaerobic infections. The future attempts must be targeted at understanding the role of bacterial pathogens in diabetic foot ulcers and initial therapy should be directed at both aerobic anaerobic bacteria. The validation of a simple, cost effective algorithm for the diagnosis of diabetic foot infections shall be of per amount use. However, as suggested by Lipsky *et al*. (2004) detection of neuropathy and its complications is the best way to prevent diabetic foot infections. Patients infected with *E. coli* strains cannot be treated with β-lactam antibiotics and monobactams. Such resistance to non β-lactam antibiotics like norfloxacin, cotrimoxazole, gentamicin, cloxacillin was observed. Amikacin, piperacillin, cefotaxime and cefepime are found to be alternative and may be used for treating diabetic foot infection patients at low cost.

CONCLUSION

These findings suggest that prospective multicenter studies are required to assess the appropriate empirical antibiotic requirements in diabetic foot ulcers taking into consideration the etiology of ulcers. Proper management of antibiotics must be implemented to decreases the incidence of drug resistant in this population. Proper education regarding foot wear and foot care is strongly recommended in diabetic foot patients. The management of ESBL requires a multi-disciplinary approach. Coordinated participation of microbiologist, clinical, nursing personnel and hospital infection control team is essential. Therapeutic decision-making requires a sound appreciation of clinical perspective. Potential for screening exists but it must be tailored to the institutional need and patient profile.

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