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RESEARCH ARTICLE

Phenotypic detection of antimicrobial resistance mechanisms in clinically important bacterial isolates from diabetic foot infections in a tertiary care hospital

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Abstract:
Background: Diabetic foot infections are a common complication leading to morbidity and antibiotic resistance. One of the most serious complications of this disease is diabetic foot infection (DFI), which is caused by single or multiple microorganisms. Polymicrobial isolates are mostly observed in chronic wound infections. DFI is caused by multidrug-resistant organisms, such as extended-spectrum ß-lactamase producing Gram-negative rods and Methicillin-resistant *Staphylococcus aureus* (MRSA). This study explored the phenotypic resistance mechanisms in isolates obtained from diabetic foot infections in a tertiary care hospital. **Methods:** A cross-sectional study was conducted in the Department of Microbiology in tertiary care hospital over a period of two years. Clinical samples from 200 patients were collected from patients with diabetic foot infections. The bacterial isolates were identified by standard microbiological procedures and phenotypic tests for ESBL, AmpC, MBL, MRSA were performed.
Results: Among 200 subjects 57% were males, 43% were females. A total of 253 isolates were tested by above phenotypic methods among which 21% and 15% showed ESBL and MBL carbapenamase respectively. MRSA prevalence was 8%. The isolates were stored for further genotypic studies.**Conclusion:** The study highlights high rates of resistance mechanisms in diabetic foot infections, emphasizing the need for early detection and rational antibiotic use.

Keywords: Diabetic foot infection, antimicrobial resistance, polymicrobial, phenotypic

INTRODUCTION

Diabetes mellitus is a common chronic disease, characterized by persistent hyperglycemia and associated skin and soft tissue infection.(1) One of the most serious complications of this disease is diabetic foot infection (DFI), which is caused by single or multiple microorganisms.(2,3) Polymicrobial isolates are mostly observed in chronic wound infections. DFI is caused by multidrug-resistant organisms, such as extended-spectrum β-lactamase producing Gram-negative rods and Methicillin-resistant *Staphylococcus aureus* (MRSA).(4) Phenotypic characters of common bacterial isolates is explored in this study.

MATERIALS AND METHOD

This cross-sectional study was conducted at the Department of Microbiology in a Tertiary care centre after obtaining Institutional Ethical clearance (Ref.no.: 80/SVMCH/IEC-Cert/May 23 Dated 04/05/2023). Clinical samples were collected from patients with diabetic foot infections from September 2023 to December 2024. Wagener classification from grade 1 to grade 5 was used for grading and collection of clinical samples.(5) Non-Diabetic individuals and diabetic cases showing other complications including varicose ulcers, vasculitis, neoplasms were not included in the study.

All patients with diabetic foot infection admitted to Surgery department were enrolled in the study. Clinical samples were collected aseptically from the patients. Briefly, the wounds were cleaned with sterile normal saline to remove debris, sterile swabs were used to collect material from the deep part of the wound bed. Similarly, 1 to 2 ml of pus or aspirate was collected using sterile syringe. Biopsies of infected tendon and tissues were collected aseptically from the base or advancing margin of the wound. All the samples were placed in transport medium immediately. Isolates were identified by standard microbiological procedures.(6) Only aerobes and facultative anaerobes were studied. Clinical isolates were identified, and phenotypic tests for ESBL, AmpC, MBL, MRSA were performed.

Detection of ESBL

Paired discs of Cephotaxime (CTX (30 µg) were each positioned at distances of 20 mm (centre to centre) from Cephotaxime - clavulanate disc (CEC, 30+10 µg). Plates were read after overnight incubation at

37°C.The test is considered positive if the inhibition zone diameter is ≥ 5 mm larger with clavulanic acid than without clavulanic acid.(7)

Detection of MBL

A 10 µg imipenem discs is placed on the surface of the agar plate. One imipenem disk with10 µL EDTA is placed at a distance of 20 mm from the centre of imipenem disk. After overnight incubation, ZOI of imipenem plus EDTA should be equal to and greater than 5 mm that of imipenem. The above isolate is showing the MBL producer.(8)

Detection of Amp-C

A 30-µg cefoxitin disk is placed on inoculated MHA and incubated overnight at 37°CCefoxitin disk shown resistance zone for the isolate. Select the isolates with zone diameters less than 18 mm for confirmation of *Amp*C production.(7)

RESULTS

Among 200 subjects, males were 57% (113), Female were 43% (87). Maximum patients were in the age group more than 45 years as shown in Table 1. Majority of the specimens were pus and wound swab as shown Figure 1. Among 253 isolates 60 *Klebsiella*, 62 *Pseudomonas*, 51 *Staphylococcus aureus* as shown in Figure 2, Among 253 isolates polymicrobial etiology was seen in 48 males and 37 females respectively as shown in Table 2. Majority of the *Klebsiella* and *Pseudomonas* isolates showed ESBL and MBL phenotypically and AmpC was observed in 19 *Staphylococcus aureus* as shown in Table 3.

Table 1: Age-wise Distribution of subjects

S.N.	Age (years)	Male (n = 113)	Female (n =87)
1	< 35	7	2
2	36–45	9	7
3	46–55	22	21
4	56–65	41	32
5	> 66	34	25

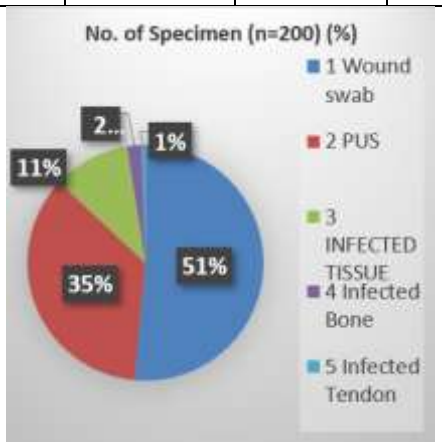

Figure 1: Specimen types

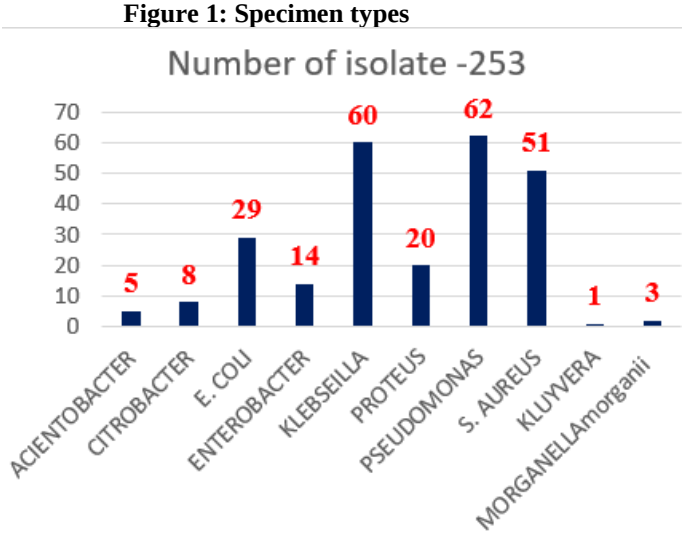

Figure 2: Bacterial Isolates

Table 2: Number of Polymicrobial isolates

Subjects	Total Isolates (n = 253)	Polymicrobial Isolates	Single Isolates
Male (n = 113)	157	48 (2 organisms) 41 (>2 organisms)	109
Female (n = 87)	96	31 (2 organisms) 29 (>2 organisms)	65

Table 3: Phenotypic characteristics of bacterial isolates

S. N.	Isolates (n = 253)	ESBL Positive (n = 53)	MBL Positive (n = 39)	ESBL & MBL Positive (n = 29)	AmpC Positive (n = 19)
1	*E. coli* (29)	9	1	1	–
2	*Klebsiella* (60)	21	6	19	–
3	*Enterobacter* (14)	2	0	0	–
4	*Acinetobacter* (5)	1	2	1	–
5	*Proteus* (20)	6	1	1	–
6	*Pseudomonas* (62)	13	29	7	–
7	*Citrobacter* (8)	1	0	0	–
8	*Kluyvera* (1)	0	0	0	–
9	*Morganella* (3)	0	0	0	–
10	*Staphylococcus aureus* (51)	–	–	–	19

DISCUSSION

Among 113 males, wound swab was collected 70, Pus from 48. Among 200 patients 5 had deep infections affecting infected bone and tendon all were males. Maximum no of cases were in age group 56-65. Similar report was given by Aiswariya et al., and

Malepati et al (6,9). Among the 253 isolates 62 were *Pseudomonas*, 60 were *Klebsiella*. Similar report was presented by Sanjith SS et al.(10) Among 113 males 48 had polymicrobial isolates and 7 had more the 2 numbers of species. Similar report of isolation of Polymicrobial species was reported by Aiswariya et al. and Sanjith et al.(6,10) Among 87 females 31 had polymicrobial isolates and 2 had more than 2 numbers of species.

Maximum numbers of combination was noted in *Klebsiella* and *Pseudomonas*. *Pseudomonas* shows maximum numbers of MBL positives. Vinodkumar et al., reported higher percentage of MBL in *Pseudomonas* isolates.(11) *Klebsiella* shows maximum numbers of ESBL positives. *Klebsiella* showed maximum combinations of ESBL and MBL positive. Khan et al., has discussed similar findings for *Klebsiella* species.(12) A total of 253 isolates were tested by above phenotypic methods among which 21% and 15% showed ESBL and MBL carbapenamase respectively. *Staphylococccus aureus* had 19 isolates which had AmpC positive. MRSA prevalence was 8%. Similar resistance was reported in earlier studies by Gadepalli et al.(13) All the isolates were stored for future genotypic studies. This study did not check anaerobic and non-cultivable organism in DFI.

CONCLUSION

Polymicrobial infection was noted in diabetic foot infection patients. The study highlights high rates of resistance mechanisms in diabetic foot infections, emphasizing the need for early detection and rational antibiotic use. *Pseudomonas* was commonly isolated. *Klebsiella* showed maximum resistance. Future genotypic studies will throw light on the resistance mechanisms.

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