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STUDY OF ANTIBIOTIC RESISTANCE PATTERN AMONG GRAM POSITIVE ISOLATES OF DIABETIC FOOT ULCER, WITH SPECIAL REFERENCE TO METHICILLIN RESISTANCE AND INDUCIBLE CLINDAMYCIN RESISTANCE

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ABSTRACT One of the important factors contributing to emergence of resistant strains in diabetic foot ulcers (DFUs) is inappropriate and widespread use of antimicrobials, either by patients themselves or primary care providers. So routine testing of antibiotic sensitivity plays a crucial role. Also routine test fails to detect Methicillin resistance (MR) which is mediated by *mec*A, encoding the PBP 2a and inducible Clindamycin resistance (ICR) due to erm genes. Hence, it is advisable to perform MR testing and D test for detection of inducible Clindamycin resistance routinely during the primary antibiotic testing for the knowledge of their prevalence and measures to be taken to control their spread.

The present study included 212 diabetic foot ulcer patients, from which 94 (31.33%) gram positive isolates were obtained, of which 75 (25.0%) were *Staphylococcus aureus,* 9 (3.0%) were Coagulase negative Staphylococci (CoNS) and 10 (3.33%) were Enteroccocus species. Among the Staphylococcal isolates, Methicillin resistance was seen in 25.33% *S. aureus* and 33.33% CoNS species. Inducible Clindamycin resistance was seen in 20.0% *S. aureus* and 33.33% CoNS isolates.

KEYWORDS : Methicillin resistance, Inducible Clindamycin resistance

Introduction

Most of the diabetic foot infections are polymicrobial in nature and mixed organisms are frequently encountered.[1] In recent years, the number of incidents and complications related to diabetic foot infections (DFIs) has drastically increased due to increased incidence of multidrug-resistant organisms (MDROs).[2] The presence of Methicillin resistance *Staphylococcus aureus* (MRSA) strains further worsen the prognosis and increase the risk of amputation.[3] Infection with multidrug-resistant organisms may increase the duration of hospital stay and cost of management and may cause additional morbidity and mortality.[4] Hence there arises the need to evaluate these microorganisms and their antibiotic susceptibility pattern.

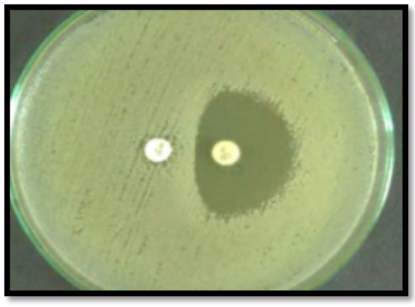
Material and Methods

A total of 212 diabetic foot ulcer patients were included in the study. A detailed history was obtained from each patient**.** Two deep swabs from wound ulcer or pus exudate (when present) were collected from each patient of DFU.[5] Samples collected were immediately processed for isolation of aerobic bacteria as per standard microbiological techniques. Out of the two swabs, one swab was subjected for microscopy and the other used for culture inoculation. Specimens were processed and isolates identified by standard microbiological techniques.[6]

Antibiotic sensitivity testing was done by Kirby Bauer disc diffusion method as per CLSI 2015 guidelines[7] and Methicillin resistance was identified by using Cefoxitin (30 µg) disc and an inhibition zone diameter of ≤ 21 mm (*S. aureus)* and ≤ 24 mm (CoNS) was reported as resistant (figure 1).[7] All Methicillin resistant staphylococcal isolates were subjected to Vancomycin MIC by E strip test. All staphylococcal isolates were tested for ICR by D test on Mueller Hinton agar at $35^0C \pm 2^0C$ for 16-18 hours. Flattening of zone (D shape) of Clindamycin (CD) disk towards side facing Erythromycin (E) disk indicated positive D zone test (figure 2).[7]

Figure 1: Detection of Methicillin resistance

Figure 2: Inducible clindamycin resistance (D zoneTest)



Results

Out of total 300 bacterial isolates obtained, gram positive cocci accounted for 94 (31.33%) isolates comprising 75 (25.0%) *S. aureus,* 9 (3.0%) CoNS and 10 (3.33%) *Enterococcus faecalis.*

All gram positive cocci were found sensitive to Linezolid. Among *S. aureus* isolates, 70.67% were reisistant to Ciprofloxacin, 64% to Gentamicin, 33.33% to Amikacin and Clindamycin, 44.0% to Erythromycin and 48.0% to Chloramphenicol. CoNS isolates showed least resistance to Amikacin (44.44%) followed by 55.56% resistance to Chloramphenicol and Clindamycin, whereas resistance to Ciprofloxacin and Gentamicin was seen in 77.78% and 66.67% respectively. All staphylococcal isolates were resistant to Penicillin G. Majority of *Enterococcus faecalis* isolates (90%) showed resistance to Penicillin, Ampicillin. Resistance to both HLG and HLS were observed in 40.0% isolates followed by Erythromycin in 60% isolates (figure 3).

Figure 3: Antimicrobial resistance amongst Gram positive cocci in DFU (n = 94)

Drugs	*Staphylococcus aureus* n = 75 (25.0%)	Coagulase negative Staphylococci n = 9 (3.0%)	*Enterococcus faecalis* n = 10 (3.33%)
Penicillin	75 (100.0)	9 (100.0)	9 (90.0)
Ampicillin	-	-	9 (90.0)
Cefoxitin	19 (25.33)	3 (33.33)	-*
Gentamicin	48 (64.0)	6 (66.67)	-*
Amikacin	25 (33.33)	4 (44.44)	-*
High level Gentamicin (HLG)	_	_	4 (40.0)
High level Streptomycin (HLS)	_	_	4 (40.0)

Erythromycin (E)	33 (44)	8 (88.89)	6 (60.0)
Clindamycin(CD)	25 (33.33)	5 (55.56)	-*
Tetracycline	45 (60.0)	6 (66.67)	_
Ciprofloxacin	53 (70.67)	7 (77.78)	_
Chloramphenicol	36 (48)	5 (55.56)	5 (50.0)
Linezolid	0 (0)	0 (0)	0 (0)
Vancomycin	0 (0) #	0 (0) #	0 (0)

Vancomycin sensitivity by E-test was performed in only MRSA and MRCoNS isolates (n = 21)

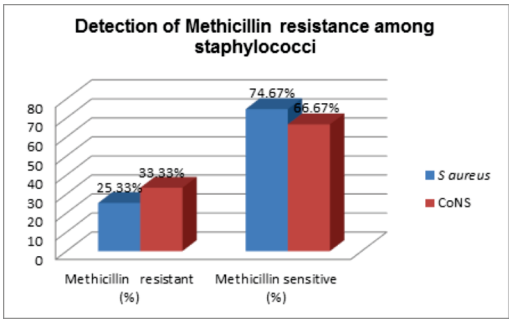
* Intrinsic resistance

Among gram positive isolates of DFU, CoNS were found to be most MDRO with 77.80% isolates resistant to three or more class of drugs, followed by 64.0% *S. aureus* and 50.0% *E. faecalis* isolates.

Out of 75 *S. aureus* isolates, 19 (25.33%) were MRSA and 56 (74.67%) were Methicillin sensitive *Staphylococcus aureus* (MSSA). Out of the total 9 CoNS, 3 (33.33%) isolates were MRCoNS (Methicillin resistant CoNS) and 6 (66.67%) were MSCoNS (Methicillin sensitive CoNS) (Figure4).

All methicillin resistant isolates that were subjected to MIC testing were found to be susceptible to Vancomycin with MICs of ≤ 4µg/ml.

Figure 4: Detection of Methicillin resistance among staphylococi



Out of total 75 *S. aureus* isolates, 42 (56.0%) were sensitive and remaining 33 (44.0%) were resistant to to Erythromycin (E). Of these 33 Erythromycin resistant isolates, 10 showed resistance to and 23 showed susceptibility to Clindamycin (CD) on disc diffusion testing. These 23 Erythromycin resistant and Clindamycin sensitive isolates were then subjected to D- test, of which 15 showed inducible Clindamycin resistance (ICR). The remaining 8 isolates (E resistant and CD sensitive) which were D- test negative were referred to as MS phenotype. Total 10 (13.33%) isolates which were resistant to both Clindamycin and Erythromycin are the constitutive Clindamycin resistant *S. aureus* isolates. ICR were found in 15 (20.0%) *S. aureus* isolates (figure 5).

Out of total 9 CoNS, only one isolate (11.11%) was sensitive and remaining 8 (88.89%) were resistant to Erythromycin. Of these eight Erythromycin resistant isolates, 2 (22.22%) isolates showed constitutive resistance to CD, 3 (33.33%) showed ICR and 3 (33.33%) were of MS phenotype (figure 5).

Figure 5: Phenotypic pattern of Clindamycin resistance among Staphylococcal isolates of DFU

Susceptibility pattern (Phenotype)	*S. aureus* (%)	CoNS	Total (%)
Inducible resistance	15 (20.0%)	3 (33.33%)	18 (21.43%)
Constitutive resistance	10 (13.33%)	2 (22.22%)	12 (14.29%)
MS phenotype	8 (10.67%)	3 (33.33%)	11 (13.10%)
Susceptible to E and CD	42 (56.0%)	1 (11.11%)	43 (51.19%)
Total	75	9	84

Discussion

In present study, 31.33% isolates were gram positive cocci, with 25.0% being *S. aureus*. In the study by Bansal et al[1], gram-positive accounted for 24% of which 18.88% were *S. aureus*.

All gram positive isolates were sensitive to Vancomycin and Linezolid. In study by Gadepalli et al[4], *S. aureus* exhibited a high frequency (56.0%) of resistance to the antibiotics tested. High levels of resistance to Erythromycin, Tetracycline and Ciprofloxacin (40%) were found in *Enterococcus* species. All isolates were uniformly susceptible to Vancomycin and Linezolid. Banashankari et al[8] reported that *S. aureus* exhibited a high frequency of resistance to the antibiotics tested including Methicillin (47%), Erythromycin (34%). Resistance to Erythromycin was found in 23% of Enterococcus species.

Of the *S. aureus* isolates studied, 25.33% were MRSA and 74.67% were MSSA. Among CoNS isolates, 33.33% were MRCoNS and 66.67% were MSCoNS. Bansal et al[1] showed 55.50% MRSA. In the study by Yerat and Rangasamy[9], 23.08% of Staphylococci were MRSA. The study of Juyal et al[10] and Roopa et al[11] reported 28.35% and 33% of MRCoNS isolates respectively.

Importance of MRSA lies in the fact that it becomes resistant to other β –lactam agents i.e. Penicillins, β -lactam and β lactamase inhibitor combinations, Cephems (with the exceptions of the Cephalosporins with anti MRSA activity) and Carbapenems. In most staphylococcal isolates, it is axiomatic that the sooner an MRSA infection is diagnosed and the susceptibility to antimicrobial agents established, the sooner appropriate therapy and control measures can be initiated. Laboratory diagnosis and susceptibility testing are crucial steps in treating, controlling and preventing MRSA infections.

MRSA strains usually require treatment with intravenous Vancomycin, which has disadvantages such as longer hospitalization, increased costs and increased risk of complications.

ICR was seen in 20.0% and constitutive Clindamycin resistance in 13.33% *S. aureus* isolates. Among CoNS, 33.33% isolates showed ICR, 22.22% showed constitutive Clindamycin resistance and 33.33% was referred as MS phenotype. In study by Regeer et al[12], prevalence of *S. aureus* isolated in a DFU was 59%, of which half were Clindamycin resistant *S. aureus*. Banashankari et al[8] found Clindamycin sensitivity to be 33% in *S. aureus*.

Isolates with inducible resistance are resistant in vivo, but appear to be susceptible in vitro to Clindamycin on routine disc diffusion test, resulting in treatment failure.[13] Hence, double disc diffusion (D zone) test is recommended by CLSI guidelines for detection of ICR.[7]

CONCLUSION

There is an increasing rate of multidrug resistant organisms in the diabetic foot patients because of indiscriminate use of broad spectrum antibiotics. Therefore, a detailed knowledge of susceptibility to antimicrobial agents is necessary to facilitate the development of effective strategies to combat the growing problem of resistance especially the Methicillin resistance and inducible Clindamycin resistance.

To conclude, judicious use of antibiotics based on local antibiotic susceptibility pattern can certainly help the clinician in reducing the burden of DFI, which could translate into reduced rate of amputations and improve the overall antibiotic utilization and reduce the emergence of multidrug resistant organisms.

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