



Prevalence of Methicillin resistant Staphylococci in diabetic foot infections

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Abstract:

Diabetic foot ulceration & infection is the most common cause of diabetes related admission to hospital & remains one of the major reason for lower limb amputation. Mostly diabetic foot infections are mixed bacterial infections. For proper management of infections in these patients appropriate antibiotic therapy should be started at the earliest based on culture & antimicrobial susceptibility testing. Infection with multidrug resistant organisms is common in diabetic foot infections. So the study was conducted in the Dept. of Microbiology, Dr. D.Y.Patil Medical College, Hospital & Research Institute, Kadamwadi, Kolhapur to find out prevalence of Methicillin Resistant Staphylococci in Diabetic Foot Infections during period December 2009 to August 2011. Total 115 samples from foot ulcer in these patients were studied. Total no. of isolates found were 131. Out of these 56 isolates were of *staphylococci* (42.75%). 39 isolates out of these *Staphylococci* were *Staphylococcus aureus* and 17 isolates were of Coagulase negative *Staphylococci* (CoNS). Antibiotic susceptibility testing of these isolates was done by Kirby – Baur Disk Diffusion method for the antibiotics routinely tested. Results were recorded as per CLSI guidelines. Methicillin resistance was detected by Oxacillin Disk Diffusion & Cefoxitin Disk Diffusion test. Methicillin resistance was found in 30 isolates of *Staphylococcus aureus* (MRSA 76.9%) & 6 isolates of CoNS (MR-CoNS 35.29%). Total methicillin resistance in *Staphylococci* was seen in 36 isolates (64.28%). Minimum Inhibitory Concentration (MIC) for Oxacillin of these resistant isolates was detected by Hi-Comb MIC test. MIC of all these isolates was above 4 µg/ml. All these Methicillin resistant strains of *Staphylococci* were sensitive to Linezolid & Teicoplanin.

Key words: Cefoxitin Disk Diffusion Test; Diabetic foot ulcer (DFU); Methicillin resistant *Staphylococcus aureus* (MRSA); Methicillin resistant Coagulase negative Staphylococci (MR-CoNS); Minimum Inhibitory Concentration (MIC) for oxacillin.

Introduction

Staphylococcus is the commonest cause of community acquired & nosocomial infection. Staphylococcal species have a unique epidemiological pattern & is well known to develop resistance to antibiotics rapidly. The emergence of coagulase negative staphylococci (CoNS) as major pathogens reflects the increased use of implants such as cerebrospinal fluid shunts, intravascular lines & cannulae, cardiac valves, pacemakers, artificial joints, vascular grafts and urinary catheters and increased number of severely debilitated patients in hospitals [1].

Originally penicillin was the drug of choice for treatment of *Staphylococcus aureus* infections. Emergence of resistance to penicillin in *Staphylococcus aureus* was due to acquisition of plasmid born genetic elements coding for β -lactamase production. Semisynthetic penicillin (ie. methicillin & oxacillin) then became drug of choice for treatment of infections due to penicillin resistant *Staphylococcus aureus*. In 1980, resistance to these drugs emerged which was due to altered penicillin binding protein called PBP2a that results from acquisition of chromosomal gene *mecA*. Once the normally present PBP have been inactivated by β -lactam agents PBP2a continues to function & allows the synthesis of stable peptidoglycan structure, thereby allowing the organism to grow & divide. *Staphylococcus aureus* strains expressing *mecA* determinant are termed as MRSA (Methicillin resistant *Staphylococcus aureus*) [2]. The continuously high prevalence of Methicillin resistant Staphylococci (MRS) throughout the world is constant threat to public health, owing to multi resistant characteristics of these bacteria.

Methicillin resistant *Staphylococcus aureus* (MRSA) strains were initially described in 1961 & have emerged, in the last decade, as one of the most important nosocomial pathogen [3].

Foot ulcers are common in diabetic patients with prevalence as high as 25% [4]. Infection is frequent & costly complication of these ulcers represent a major cause of morbidity & mortality. Routine administration of antibiotic therapy to all patients with diabetic foot ulcers (DFU) favors the emergence of antimicrobial resistance, increases financial cost & may cause adverse events [5]. Diabetic patients with foot ulcers often need repeated hospitalization. Indiscriminate use of antibiotics lead to development of multidrug resistant organism pattern among these patients. So the study was

conducted in Dr.D.Y.Patil Medical College, Hospital & Research Institute, Kolhapur to find out Methicillin resistant Staphylococci isolated from diabetic patients with foot ulcer.

Aim:

To find out prevalence of methicillin resistance in Staphylococci isolated from wounds in diabetic patients with foot infection.

Objectives

- 1) To study the prevalence of methicillin resistant Staphylococci isolated from wounds in diabetic patients with foot infection.
- 2) To study antimicrobial susceptibility pattern of Staphylococcal isolates obtained from wounds in diabetic patients with foot infection.

Materials & Methods

The study was done in Dr.D.Y.Patil Medical College, Hospital & Research Institute, Kolhapur during period December 2009 to August 2011. Permission from ethics committee was obtained. Informed consent was obtained from all the patients.

Collection of sample:

Clinical samples from wound were obtained from 115 patients of type II diabetes presented with foot ulcer. Clinical samples from the wounds of these patients such as pus, exudates, scrapings from the base of ulcer & debrided material were studied.

Processing of samples:

Preliminary gram staining was done to determine likely organisms present. The samples were inoculated on Nutrient agar, Blood agar, MacConkey agar which were incubated at 37°C aerobically. Staphylococcal colonies were identified by colony morphology, pigment production & smear. Gram positive cocci isolated from primary culture were subjected to biochemical tests. Identification of Staphylococci was done by Catalase test, Mannitol fermentation, slide & tube coagulase test to differentiate between coagulase positive & coagulase negative strains.

All Staphylococcal isolates were tested for antibiotics routinely used by Kirby – Bauer Disk Diffusion method. Results were recorded as per CLSI norms [6]. Antibiotics used were Penicillin (10 μ g), Amoxy-clav (30 μ g), Clindamycin (2 μ g), Oxacillin (1 μ g), Erythromycin (15 μ g), Cefoxitin (30 μ g), Ciprofloxacin (5 μ g), Vancomycin (30 μ g),

Linezolid (30 µg), Gentamycin (10 µg), Teicoplanin (30 µg).

Detection of Methicillin resistance in Staphylococci

Methicillin resistance was detected by -1) Oxacillin Disk Diffusion Test 2) Cefoxitin Disk Diffusion Test 3) MIC by Hi-Comb Test

1) **Oxacillin Disk Diffusion Test** - A bacterial suspension adjusted to 0.5 Mc Farland standard was inoculated on Mueller Hinton Agar plate supplemented with 2% NaCl. Oxacillin disk (1 µg) was placed on plate. Plates were incubated for 24 hrs at 35°C. Results were interpreted according to CLSI guideline [6]. Zone of inhibition less than 10mm or any growth within zone of inhibition was indicative of methicillin resistance.

2) **Cefoxitin Disk Diffusion Test** – Standard disk diffusion test was performed on Mueller Hinton Agar using Cefoxitin disk (30 µg). Plate was incubated for 24 hrs at 37°C. Cefoxitin zone less than or equal to 21 mm was reported as resistant as per CLSI guidelines [6]. (*Staphylococcus aureus* 25923 was used as standard control strain)

3) **Hi-Comb MIC Test** – Minimum Inhibitory Concentration was detected using Hi-Comb MIC strips of Oxacillin. Mueller Hinton Agar plates supplemented with 2% NaCl were inoculated by suspension of Methicillin resistant Staphylococcal isolate adjusted to 0.5 Mc- Farland Std. Hi-Comb MIC strips containing Oxacillin were applied on the surface of agar. After incubation of plate at 37°C for 24 hrs in air, MIC was read directly from intersection of zone of inhibition with the test strip. CLSI breakpoints were used for interpretation. (MIC < or equal 2 µg/lit. as susceptible & MIC > or equal to 4 µg/lit as resistant) [7]. (*Staphylococcus aureus* 25923 was used as standard control strain)

Data collection was done from Dr.D.Y.Patil Medical College, Hospital & Research Institute, Kolhapur. Data analysis is done by using MS – Excel computer language. (Data analysis tool park option) Antibiotic disks & MIC strips manufactured by Hi-Media, Mumbai were used.

Results

Total 115 pus samples from wounds of diabetic patients with foot infection were studied. 6

samples were culture negative. Number of isolates obtained were 131. Out of these isolates 56 were

Table 1: Resistance pattern of MRSA isolated from pus samples obtained from wounds in Diabetic foot infections (n=30)

Antibiotic	No. of resistant isolates	Percentage of resistant isolates
Penicillin	27	90%
Amoxy-clav	26	86.66%
Clindamycin	10	33.33%
Oxacillin	30	100%
Cefoxitin	30	100%
Erythromycin	24	80%
Gentamycin	22	73.33%
Ciprofloxacin	25	83.33%
Vancomycin	2	6.66%
Linezolid	0	00
Teicoplanin	0	00

Table 2: Resistance pattern of MR-CoNS isolated from pus samples obtained from wounds in Diabetic foot infections (n=6)

Antibiotic	No. of resistant isolates	Percentage of resistant isolates
Penicillin	5	83.33%
Amoxy-clav	5	83.33%
Clindamycin	2	33.33%
Oxacillin	6	100%
Cefoxitin	6	100%
Erythromycin	4	66.66%
Gentamycin	3	50%
Ciprofloxacin	5	83.33%
Vancomycin	0	00
Linezolid	0	00
Teicoplanin	0	00

Staphylococci (42.75%). Remaining 75 isolates were gram negative bacilli (57.25%), 39 isolates out of 56 were Coagulase Positive Staphylococci

(*Staphylococcus aureus* (69.64%) & 17 were Coagulase Negative Staphylococci (CoNS) (30.35%) Methicillin resistance was detected in 30 isolates of *Staphylococcus aureus* (76.92%). 6 isolates of CoNS were Methicillin resistant (35.29%). It was found that 36 isolates out of 56 strains Staphylococci were Methicillin resistant (MRS – 64.28%).

Table 3: Minimum Inhibitory Concentration (MIC) values of oxacillin for MRSA Isolates (n=30)

MIC values (µg/ml)	No. of isolates	Percentage of isolates
0.016 - 2	0	00
4	2	6.66%
8	14	46.66%
16	5	16.66%
32	3	10%
64	2	6.66%
128	2	6.66%
256	2	6.66%

Table 4: Minimum Inhibitory Concentration (MIC) values of Oxacillin for MR-CoNS Isolates (n=6)

MIC values (µg/ml)	No. of isolates	Percentage of isolates
0.016 - 2	0	00
4	1	16.66%
8	3	50%
16	1	16.66%
32	1	16.66%
64	0	00
128	0	00
256	0	00

All MRSA & MR-CoNS isolates were further confirmed by Hi-Comb MIC Test for oxacillin. MIC value of all isolates was above 4µg/ml.

Resistance pattern of MRSA was as follows – Penicillin (90%), Amoxy-clav (86.66%), Clindamycin (33.33%), Oxacillin (100%), Cefoxitin (100%), Erythromycin (80%), Ciprofloxacin (83.33%), Gentamycin (73.33%), Vancomycin (6.66%), Linezolid & Teicoplanin (0%).

Resistance pattern of MR-CoNS was as follows – Penicillin (83.33%), Amoxy-clav (83.33%), Clindamycin (33.33%), Oxacillin & Cefoxitin (100%), Erythromycin (66.66%),

Ciprofloxacin (83.33%), Gentamycin (50%), Vancomycin, Linezolid & Teicoplanin (0%). All Methicillin resistant Staphylococci were sensitive to Linezolid & Teicoplanin.

Discussion

Increase in both prevalence & incidence of Type II diabetes has occurred globally. It is estimated that 20% current global type II diabetic population resides in South-East Asia region [8]. Diabetic foot lesion is one of the most common cause of hospitalization & caused by number of socio-cultural practices in India like bare foot walking, inadequate facilities for diabetic care, low level of education & poor socio-economic conditions [9].

In present study, 115 patients of type II diabetes mellitus with foot infections were studied. 103 patients were from In Patient Dept. (IPD) & 12 patients were from OPD. 82 patients were male & 33 were female. Age ranged from 36 – 76 yrs. (mean age 50 yrs) Bacteriological culture yielded negative in 6 patients (5.21%).

Total 131 organisms were isolated amongst which 56 were Staphylococci (42.75%) & 75 were GNB (57.25%). Ravisekhar Gadepalli, Arti Kapil et al, (2006), from AIIMS, New Delhi, India [10], reported in his Clinico-Microbiological study of DFU, Gram positive aerobes 33.3% & GNB were 51.4%. Azizul Hasan Amir et al, (2011), in his study [11], reported commonest pathogen isolated was *Staphylococcus aureus* (46%). Sayed Alavi et al, (2007), from Razvi Medical School hospital, Iran [12], reported gram positive bacteria from DFU accounted for 42.9%. In the present study, out of these 56 isolates of Staphylococci, 39 were *Staphylococcus aureus* (69.64%) & Coagulase negative Staphylococci (CoNS) were 17 ie. 30.35%. 30 out of 39 isolates of *Staph. aureus* were MRSA (76.92%) & six isolates out of 17 CoNS were Methicillin resistant (35.29%). In India incidence of MRSA shows variation from 6.9% to 81% [13]. Arumugam Suresh et al from Chennai, (2011), in his study of diabetic foot ulcer found MRSA (70.45%) & MR-CoNS (62.5%) [14]. Shazia Parveen, Jothsna K. from Andhra Pradesh, India in 2011, reported MRSA 48% [15]. Mohammad Zubair et al (2010), from Aligarh, India reported MRSA in DFU as 57.1% [16]. Azizul Hasan Amir et al, (2011), from Pakistan, reported MRSA in diabetic foot infections 9% [11]. A study from Australia shows MRSA prevalence from Multidisciplinary diabetic foot clinic in Melbourne 23% [17].

In the present study, all MRSA isolated were 100% resistant to Oxacillin & Cefoxitin. MIC values of Methicillin Resistant Staphylococci for Oxacillin ranged between 4µg/ml to 256µg/ml. S.Vidhani, P L Mehndiratta et al, (2001), reported high MIC values of Oxacillin for MRSA which ranged in between 4µg/ml to 512µg/ml [18]. Resistance showed by MRSA to other antibiotics was Amoxy-clav (86.66%), Penicillin (90%), Ciprofloxacin (83.33%), Erythromycin (80%), Clindamycin (33.33%). S.Vidhani, P L Mehndiratta et al, (2001), reported resistance of MRSA to Penicillin & amoxicillin (100%), Augmentin (87.4%) [18]. Arumugam Suresh et al, (2011) reported sensitivity of all strains of MRS to Linezolid & Vancomycin [14]. Only two isolates of MRSA showed resistance to Vancomycin (6.66%) Sasirekha B et al reported Vancomycin resistance in three isolates of *Staph.aureus* [19]. All MR-CoNS were also 100% resistant to oxacillin & cefoxitin. Resistance rate of MR-CoNS –Penicillin (83.33%), Amoxy-clav & Ciprofloxacin (83.33%), Erythromycin (66.6%), Gentamycin (50%), Clindamycin (33.33%). All MRSA & MR-CoNS isolates were sensitive to Linezolid & Teicoplanin. Mohammad Aghazadeh et al, (2009), reported 100% sensitivity of MRSA to Linezolid, Teicoplanin & Tigecyclin [20].

Conclusion

Foot ulcers remain one of the most distressing complication of diabetic patients. Diabetic foot infections are mixed bacterial infections for which the patient needs repeated hospitalization. MRSA is commonest nosocomial pathogen causing morbidity & mortality worldwide. Prevalence of Methicillin Resistant *Staphylococcus aureus* in Type II Diabetic foot infections was 76.9% & MR-CoNS was 35.29%. Total Methicillin Resistant Staphylococci in Diabetic foot infections detected were 64.28%. Present study showed high rate of resistance to commonly used antibiotics. All resistant Staphylococci were sensitive to Linezolid & Teicoplanin. Successful treatment of diabetic foot infection often requires correctly targeted antimicrobial therapy. Reporting Methicillin Resistant Staphylococci (MRS) in DFU will be helpful in selecting proper antibiotic at the earliest to reduce complications & medical as well as surgical care cost of the patients.

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