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Association of Staphylococcal Skin and Soft Tissue Infections (SSTIs) among Diabetic Patients

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ABSTRACT

Background: *Skin and soft tissue infection associated with diabetes is one of the major concerns. The early diagnosis of SSTIs is crucial in the patients having diabetes, to prevent deep infection like Osteomyelitis, septicemia, and necrotizing fasciitis.*

Materials and methods: *The clinical sample of SSTI were collected, and screened for staphylococcal infections. The association of Staphylococcal SSTIs was correlated with diabetic patients. All the isolates were further subjected to antimicrobial susceptibility testing as per CLSI guidelines.*

Results: *A total of 134 staphylococcal species isolated from SSTIs infections, Out of 134 isolates, 125(93.3%) were Staphylococcus aureus, 5(3.7%) were S.saprophyticus and 4(3%) were S.epidermidis. The diabetic mellitus (30%) were significantly associated with SSTIs followed by high blood pressure 2(1.5%), however 91 (67.9%) patients don't have any co-morbidity. In our study, Abscesses 40(29.9%) were found to be more frequent SSTI followed by surgical site infection 32(23.9%), diabetic foot ulcer 27(20.1%), boils 20(14.9%), and 15(11.2%) were cellulitis. All the isolates were 100% susceptible to Linezolid, Vancomycin, and ceftaroline.*

Discussion: *The co-morbid conditions are one of the major risk factor associated with skin and soft tissue infections that lead to long term therapy and increasing cost of treatment. In present study the single most co-morbid condition i.e. - diabetes are significantly associated with SSTIs.*

Keywords: *Diabetes, SSTIs, Staphylococci, AST, MLSb phenotypes*

INTRODUCTION

The major risk factor for skin and soft tissue infections (SSTIs) is diabetes and global burden of diabetics with SSTI is one of the major concerns[1]. Bacterial skin and mucous membrane infections were more common in diabetic patients[2]. The diabetic patients are more prone to develop cellulitis as compared to non-diabetic patients[3]. The skin and soft tissue infections range from superficial (impetigo) to deeper and more severe (necrotizing fasciitis). The common examples of SSTIs are abscess, furuncle, carbuncle, cellulitis, diabetic foot ulcer and surgical site infections[4].The skin and soft tissue infections caused by *Staphylococcus aureus* is begin as minor infection (boils, abscesses) which may progress to severe infections involving muscle, bone, which may disseminate to the lungs or heart valves[5].The skin and soft tissue infections are considered to be complicated when deep subcutaneous tissues involves, necrotizing limb threatening infection where the surgery needed in addition to antimicrobial therapy, patients with extensive cellulitis, the patients has severe co-morbidities such as diabetes lead to diabetic foot ulcer or immune-compromised host[6].

The Infectious Diseases Society of America issued the guidelines for the diagnosis and management of SSTIs by framing into five categories. The first one is the superficial uncomplicated infection like impetigo, erysipelas and cellulitis; secondis the necrotizing infection, third is the bites &animal contact associated infections, fourth is the surgical site infections and fifth one is the infections in the immune-compromised host. The given classification of SSTIs will guide the

clinician for clinical management and treatment decisions more efficiently. SSTIs can be associated with serious complications such as osteomyelitis, bacteremia & sepsis and gangrene if not treated in time with proper antimicrobial agents [8].

The Skin and soft tissue infections generally caused by community acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) and the strain is very different from the MRSA strain isolated from hospital source (HA-MRSA).It considered to CA-MRSA when the MRSA culture positivity in OPD patient or first 48 hours of hospitalization and no previous history of hospitalization, Surgery, history of Dialysis, history of MRSA positivity. The Panton valentine leukocidin (PVL), a gamma heamolysin toxin is one of the evident for CA-MRSA and Presence of PVL in MSSA and HA-MRSA is less common. The confirmation of PVL in routine laboratory testing not recommended, as CA-MRSA is sensitive to many oral antibiotics like cotrimoxazole, doxycycline and clindamycin that can be use in most outpatient's setting[9].The proper selection antimicrobial agent is most important while treating PVL producing strains of Staphylococcus including MRSA and MSSA, as the beta-lactam agent that is used against MSSA may trigger and releases toxin leads to pathogenesis. The most of the SSTIs often caused by CA-MRSA and the incidence of PVL toxin production is more in CA-MRSA, hence Clindamycin and Linezolid that are active against MRSA and suppress toxin production can be the good alternative to treat SSTI[10].The appearance of CA-MRSA has changed the scenario of antibiotic management of complicated skin and soft tissue infection, forcing us to choose antibiotics that can suppress toxins, even for MSSA; rather than blindly choosing beta lactam.

Taking in account, present study was carried out to find out the association of Staphylococcal SSTIs with diabetes.

Materials And Methods

After the clearance of Research advisory committee and institutional ethical committee, present study carried out in the Department of microbiology at rural based Medical College and Hospital. All the clinical specimens like Pus/Purulent swabs, wound swab, blood, sputum, urine, aspirates and all body fluids obtained from in and out patients having SSTIs were included in the study. Medical case report/Prescription form were used for the record of age, sex, medical history, clinical presentation, co-morbid condition, associated predisposing factors, status of diabetes and prior antibiotic therapy/ antibiotic given.

The isolates first identified as *Staphylococcus aureus* and Coagulase negative staphylococci by standard techniques (gram staining, catalase test, and coagulase test). *Staphylococcus aureus* was differentiated from *Micrococcus* species on the basis of resistance to bacitracin(0.04U).The Coagulase negative strain was further subjected to speciation by using Novobiocin(5µg), polymyxin-B(300U) susceptibility and Urease activity as per standard procedure (Table:1)[11,12].

Table 1: Identification of commonly isolated *Staphylococcus* species[12]

Test	A	B	C	D	E	F	G
Coagulase test	+	-	-	-	-	-	-
Urease test	V	+	+	-	V	+	+
Polymixin-B sensitivity	R	R	S	S	S/R	S	S
Novobiocin sensitivity	S	S	R	S	S	S	S

A- *S.aureus*, **B**- *S.epidermidis*, **C**- *S.saprophyticus*, **D**- *S.Heamolyticus*, **E**- *S.lugdunensis*, **F**- *S.warneri*, **G**- *S.hominis*

All the coagulase negative and positive isolates were subjected to routine antibiotic susceptibility testing by Kirby Bauer's disc diffusion method using different antibiotic disc and E-test strip method for MIC detection (Cefoxitin, Vancomycin, Ceftaroline) as per CLSI guidelines[13].

Antimicrobial Susceptibility Testing

Kirby Bauer disc diffusion method

A well isolated colonies of both the coagulase positive and negative isolates was taken and suspended in peptone water and incubated at 37^0C for 4 hours, the bacterial suspension were compared with 0.5 McFarland turbidity standard, comparison was corrected by using addition of peptone water or further incubation. The bacterial suspension was inoculated on Mueller Hinton agar plates, appropriate antibiotic disc was put and incubated at 37^0C for 24 hours as per CLSI (2015) guidelines[13,14].

E-test strip Method, (MIC)

Ezy-MIC strip procured from Hi-media laboratory Mumbai was used for MIC detection. The E-test strip based on diffusion-dilution principle, concentration gradient of antimicrobial agent range from 0.016 to 256

µg/mlin corporated on nitro-cellulose paper was used and interpreted as follows;

- Ceftaroline (*S.aureus*)-Sensitive: <1µg/ml, Intermediate:2µg/ml, Resistant:>4 µg/ml
- Vancomycin (CoNS)-Sensitive: <4µg/ml, Intermediate: 8-16µg/ml, Resistant:> 32µg/ml
- Vancomycin (*S.aureus*)-Sensitive:<2µg/ml, Intermediate: 4-8µg/ml, Resistant:>16 µg/ml
- Cefoxitin (*S.aureus*)- Sensitive: <4 µg/ml, Resistant: >8 µg/ml

D-test

D-test was performed on erythromycin resistant strains of staphylococcus species to rule out inducible clindamycin resistant strains of staphylococci as per standard guidelines and interpreted as three MLSbphenotypes[13].

Statistical Analysis

Results were analyzed by SPPS 20.0 version software, by using one-sample Chi-square test, one-sample Binomial test and p-value >0.05 were considered as statistically significant.

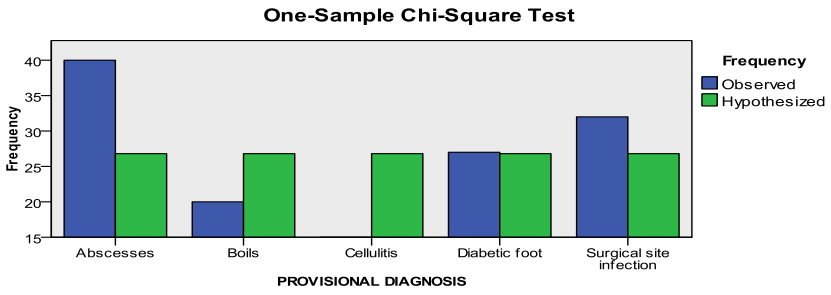
Observations And Results

Table 2.*Staphylococcus species isolated from SSTIs*

Isolates	Frequency	Percent	Cumulative Percent
Staphylococcus aureus	125	93.3	93.3
Staphylococcus epidermidis	4	3.0	96.3
Staphylococcus saprophyticus	5	3.7	100.0
Total	134	100.0	

Table 3.*Demographic data pertaining SSTIs*

Source		Frequency	Percent	Cumulative Percent
Gender	Female	83	61.9	61.9
	Male	51	38.1	100.0
OPD/IPD	IPD	115	85.8	85.8
	OPD	19	14.2	100.0
Clinical Departments	OBGY	5	3.7	3.7
	Orthopedic	17	12.7	16.4
	Surgery	112	83.6	100.0
History of prior antimicrobial therapy	Amoxy-clav	52	38.8	38.8
	Cefotaxime	7	5.2	44.0
	Cefuroxime	8	6.0	50.0
	Levofloxacin	2	1.5	51.5
	Ofloxacin	8	6.0	57.5
	Piperacillin-Tazobactum	2	1.5	69.0
	Not given	55	41.0	100.0
Distribution of SSTIs	Abscesses	40	29.9	29.9
	Boils	20	14.9	44.8
	Cellulitis	15	11.2	56.0
	Diabetic foot ulcer	27	20.1	76.1
	Surgical site infection	32	23.9	100.0



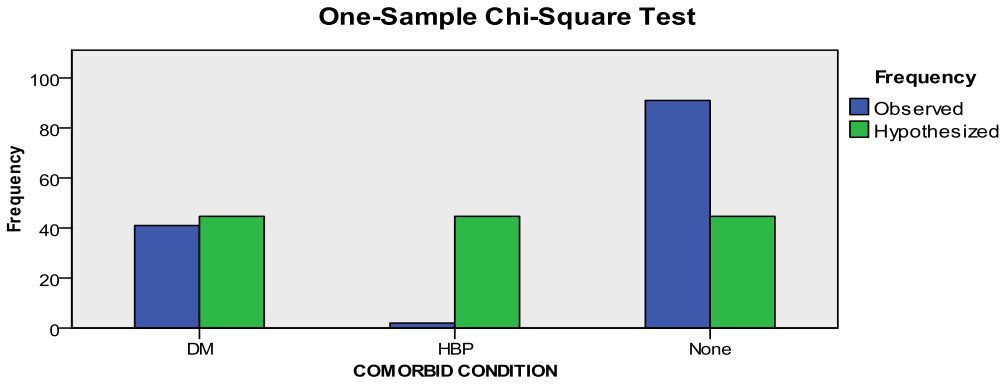
Total N	134
Test Statistic	14.433
Degrees of Freedom	4
Asymptotic Sig. (2-sided test)	.006

1. There are 0 cells (0%) with expected values less than 5. The minimum expected value is 26.800.

Graph-1(*Table 3):The categories of SSTIs occur with equal probabilities by One-Sample Chi-square Test (p-value .006). Hence null hypothesis rejected.*

Table 4. *Co-morbid condition associated with SSTIs*

Co-morbidity	Criteria	Status
Diabetes- 41(30.6%)	Controlled (<140 mg/dl since last 3 months)	09(22%)
	Uncontrolled(> 200 mg/dl since last 3 months)	32(78%)
Blood pressure- 2(1.5%)	High blood pressure	02(1.5%)
	Low blood pressure	00
None- 91(67.9%)	No co-morbidity	



Total N	134
Test Statistic	89.119
Degrees of Freedom	2
Asymptotic Sig. (2-sided test)	.000

1. There are 0 cells (0%) with expected values less than 5. The minimum expected value is 44.667.

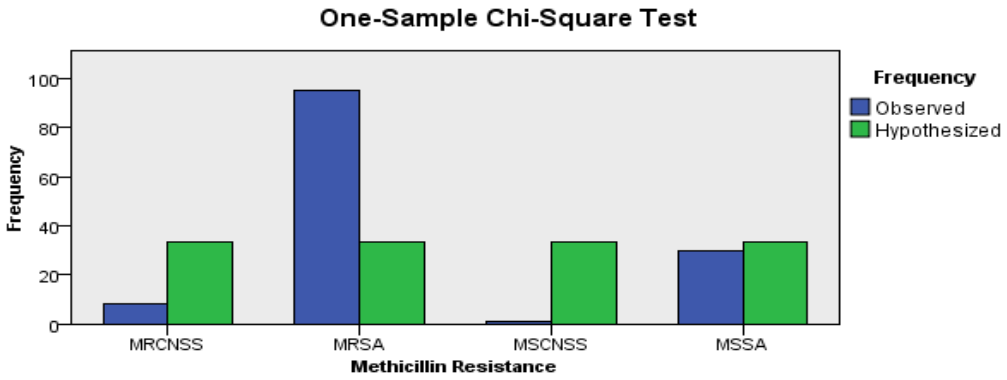
Graph-2. (*Table-4*)*:The categories of Comorbidities occur with equal probabilities by One-Sample Chi-square Test (p-value .000). Hence null hypothesis rejected.*

Table 5. *Antimicrobial susceptibility of Staphylococcus species exhibiting SSTIs*

Antimicrobial agents	Sensitive		Resistant	
	Frequency	Percent	Frequency	Percent
Penicillin	0	0	134	100
Cefoxitin(MIC)	31	23.1	103	76.9
Erythromycin	5	3.7	129	96.3
Clindamycin	47	35.1	87	64.9
Trimethoprim/Sulfamethoxazole	54	40.3	80	59.7
Tetracycline	27	20.1	107	79.9
Chloramphenicol	26	19.4	108	80.6
Ofloxacin	37	27.6	97	72.4
Gentamycin	26	19.4	108	80.6
Rifampin	133	99.3	1	0.7
Linezolid	134	100	0	0
Vancomycin(MIC)	134	100	0	0
Ceftaroline(MIC)	134	100	0	0

Table 6. *Methicillin resistant Staphylococcus species among SSTIs*

Methicillin Resistance	Frequency	Percent	Cumulative Percent
MRSA	95	70.9	70.9
MSSA	30	22.4	93.3
MR-CoNS	8	6.0	99.9
MS-CoNS	1	0.7	100.0
Total	134	100.0	



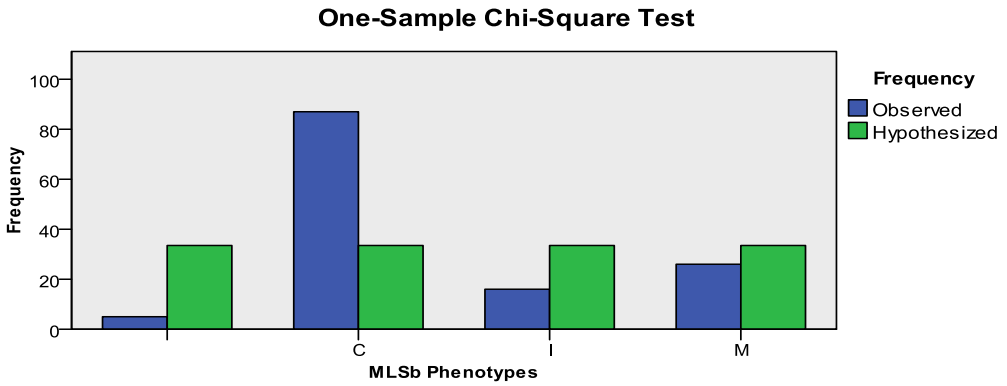
Total N	134
Test Statistic	164.209
Degrees of Freedom	3
Asymptotic Sig. (2-sided test)	.000

1. There are 0 cells (0%) with expected values less than 5. The minimum expected value is 33.500.

Graph-3. *(Table 5):The categories of methicillin resistant strains occur with equal probabilities by One-Sample Chi-square Test (p-value .000). Hence null hypothesis rejected.*

Table 7. *MLSb phenotypes strains of Staphylococcus aureus among SSTIs*

Type of resistant genes	Frequency	Percent	Cumulative Percent
Erythromycin sensitive	5	3.7	3.7
cMLSb phenotype	87	64.9	68.7
iMLSb phenotype	16	11.9	80.6
MSb phenotype	26	19.4	100.0
Total	134	100.0	



Total N	134
Test Statistic	120.507
Degrees of Freedom	3
Asymptotic Sig. (2-sided test)	.000

1. There are 0 cells (0%) with expected values less than 5. The minimum expected value is 33.500.

Graph-4. *(Table 7):The categories of MLSb phenotypes occur with equal probabilities by One-Sample Chi-square Test (p-value .000). Hence null hypothesis rejected.*

DISCUSSION

The staphylococcal skin and soft tissue infection is a global burden, especially MRSA infection which leads to severe form of deep infection. A total of 421staphylococci isolated from various clinical samples. Out of which 134 isolates were associated with skin and soft tissue infections (SSTIs).Out of 134 isolates, 125(93.3%) were *Staphylococcus aureus*, 5(3.7%) were *S.saprophyticus* and 4(3%) were *S.epidermidis*.The distribution of SSTIs among different gender shows that females 83(61.9%) were found to be predominant than males 51(38.1%) and the majority of the pathogens were isolated from hospitalized patient 115(85.8%), while 19(14.2%) were from OPD. Benjamin A Lipsky et.al[15]also stated that SSTIs infections are frequent among hospitalized patients.

It was also observed that clinical specimen exhibiting skin and soft tissue infection were received from department of surgery 112(83.6%) followed by 17(12.7%) from department orthopedic and 5(3.7%) were from received from department of medicine.

Skin and soft tissues infections is an broad range of infection and categorized on the basis of site of infection, underlying condition and severity of the infections. In our study, Abscesses 40(29.9%) were found to be more frequent SSTI followed by surgical site infection 32(23.9%), diabetic foot ulcer 27(20.1%), boils 20(14.9%), and 15(11.2%) were cellulitis which is similar to the study conducted by Zarrin Afroz et al[16].

The co-morbid conditions are one of the major risk factor associated with skin and soft tissue infections that lead to long term therapy and increasing cost of treatment. In present study the single most co-morbid condition i.e.- diabetic mellitus (30%) were significantly associated with skin and soft tissue infection followed by high blood pressure 2(1.5%), however 91 (67.9%) patients don't have any co-morbidity. In a study conducted by Shah et al[17] who observed that the individuals suffering from diabetic mellitus have 1.21 risk ratios (RR) for all infectious diseases as compared to non-diabetic individuals.

The patients suffering from SSTIs had history previous antimicrobial therapy were also evaluated and found that majority of the patients had taken Amoxycillin-clavulanic acid 52(38.8%), followed by Cefuroxime & Ofloxacin 8(6% each), Cefotaxime 7(5.2%) and 2(1.5%) had taken Levofloxacin & Piperacillin-Tazobactum. However, 55(41%) patients don't have history of any medication. In present scenario, prior antimicrobial therapy was not appropriate which may lead to antimicrobial resistance, hence evaluation of infection; microbiological findings are necessary for judicial use of the drugs and proper institution of the therapy.

Antimicrobial susceptibility testing revealed that the all the isolates were resistant to penicillin (100%) followed by Erythromycin (96.3%), Gentamycin & Chloramphenicol (80.6% each), Tetracycline (79.9%), Cefoxitin (76.9%) indicating MRSA strains, Ofloxacin (72.4%), Clindamycin (64.9%) which is further evaluated for inducible clindamycin resistant strains of Staphylococci to rule out true susceptibility. However Linezolid, Vancomycin, Ceftaroline were found to be 100% susceptible followed by Rifampin (99.33%).

Methicillin resistant strains of Staphylococci were found out by Cefoxitin disc which a surrogate marker and universally accepted method and as per CLSI guidelines. Among the *Staphylococcus aureus*, 95(70.9%) isolates were MRSA and 30(22.4%) strains were MSSA. Among the CoNS, 8(6%) isolates were MR-CoNS and 1(0.7%) isolates were MS-CoNS. Methicillin resistance among staphylococcus species were noted in number of studies carried out in India and abroad as well, and very high prevalence of methicillin resistance is making the condition worsen day by day [18,19,20].

Out of 134 isolates of Staphylococci, 129(96.3%) isolates studied for MLSb phenotypes. Out of which 16(11.9%) were D-test positive indicating inducible clindamycin resistant, 26(19%) were D-test negative indicating true susceptibility to clindamycin and 87(64.9%) were resistant to both erythromycin and clindamycin (constitutive MLSb phenotypes). Similar studies carried out in India and vary from different geographical area[21,22,23].

CONCLUSION

The majority of SSTIs were diagnosed in inpatient settings for patients with diabetes compared to patients without diabetes. The abscess was most commonly diagnosed infection among diabetic patient as compare to non-diabetic patients. The higher incidence of methicillin resistant strains of staphylococci causing SSTIs and its association diabetes is one

of the major concerns. Present study conclude that before dealing with staphylococcal SSTIs, control of diabetes is necessary for better outcome of the therapy and hence to reduce risk of severe and complicated infections.

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