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**Original Research Article**

# A comparative study of Diabetic and Non-diabetic wound infections with special reference to MRSA and ESBL

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Diabetic and Non-diabetic, MRSA and ESBL, *Pseudomonas aeruginosa*, *Escherichia coli*

**A B S T R A C T**

Diabetic patients are more prone to develop infection than their non-diabetic counter parts. The aim of the study was to compare the microbial profile and magnitude of infection in diabetic patients with the non-diabetic patients. A cross sectional study involving 100 diabetic and 100 non-diabetic patients with wound infections was done in a Tertiary care hospital, Salem. Diabetic wounds showed a significant positive culture with a average of 0.9 organisms per case compared with non-diabetic wounds with a average of 0.55 organisms per case. Poor glucose control, i.e. random blood sugar (RBS)>200 mg was found in 64% of the cases. Polymicrobial infections were more common in diabetic wound infections than non-diabetic wound infections. The predominant organism isolated was *Staphylococcus aureus* in both diabetic and non-diabetic wounds followed by Gram negative bacilli. In diabetic wounds the predominant Gram negative bacilli was *Escherichia coli* and in non-diabetic wounds the predominant one was *Pseudomonas aeruginosa*. Cephalosporins, Aminoglycosides, Fluoroquinolones were effective against the sensitive strains of Gram positive and Gram negative organisms. Methicillin resistant *Staphylococcus aureus* (MRSA) strains accounted for about 69.8% in diabetic wounds which were significantly higher compared to non-diabetic cases which accounted for 38.4%. Extended spectrum β-lactamases (ESBL) strains accounted for about 57.4% in diabetic wounds which were significantly higher compared to non-diabetic cases which accounted for 30.4%. Early identification of the infecting organism and appropriate antibiotic therapy are essential to ensure a good outcome.

## Introduction

Diabetes is a prevalent disease worldwide and wound infection is a major complication in diabetic patients. Patients with diabetes have impaired wound healing associated with multitude of factors, including neuropathy, vascular disease, and foot deformities (Jeffcoate and Harding, 2003). Metabolic abnormalities of diabetes lead to impaired leukocyte function, inadequate migration of neutrophils and macrophages to the wound, along with reduced chemotaxis, predispose individuals to an increased risk

of wound infection (Ekta Bansal *et al.*, 2008). Studies have revealed that diabetic wounds showed significantly higher bacterial counts compared with non-diabetic wounds. Natural skin flora itself induced sustained bacterial infections in the wound tissue in diabetic wounds, whereas non-diabetic organisms were able to cope with endogenous bacterial contamination (Tobias Hirsch *et al.*, 2008). It is a fact that diabetic patients are not only more susceptible to infection but that when infection occur they are more severe as the diabetic is a compromised host while certain types of infection do have predilection for the diabetic (Tattersalt *et al.*, 1990).

The predominantly isolated organisms are Staphylococcus aureus, Gram negative bacilli like Escherichia coli, Pseudomonas aeruginosa, Klebsiella species, Proteus species, and anaerobic organisms. The remainders are due to streptococcus species, Candida, etc. The infection may be polymicrobial also and mixed organisms are frequently encountered (Adel Abdulrazak *et al.*, 2005). However, the spectrum of microorganisms depends mainly on microbial flora of particular area, metabolic factors, hygiene and the use of antibiotics. Management of these infections requires isolation and identification of the microbial flora, appropriate antibiotic therapy according to the sensitivity patterns (Chincholikar and Pal, 2002). Emergence of resistance among organisms against the commonly used antibiotics has been clearly outlined in various studies as being largely due to their indiscriminate use (Pathare *et al.*, 1998). Early diagnosis of microbial infections is aimed to institute the appropriate antibacterial therapy and to avoid further complications(Brodsky and Schneilder, 1991) In view of the above facts, a cross-sectional study was done to compare the microbial profile of diabetic wound infections with non-diabetic wound infections, to assess their *in vitro* susceptibility to antibiotics and detection of Methicillin resistant *Staphylococcus aureus* (MRSA) and Extended spectrum β-lactamase (ESBL) producers in Gram negative bacilli.

## Materials and Methods

This was a cross sectional study involving 100 diabetic and 100 non-diabetic patients with wound infections in a Tertiary care hospital, Salem from June 2012-August 2013. The study group included 1**)**100 diabetic patients which included 68 hospitalized patients and 32 out patients. 2)100 non-diabetic patients which included 57 hospitalized patients and 43 out patients. Both male and female of age group ranging from 20-80 were chosen. Specimens collected were pus samples or wound swabs. Samples were collected in sterile screw-capped containers and hand-delivered immediately to the laboratory**.** In case of wound swabs**,** two swabs per patient with adequate material, taken from the depth of the wound, were collected, one for Gram staining and the other for the culture. Blood was collected for biochemical analysis for estimation of glucose level. The specimens were subjected to Direct Gram staining. Further processing, identification of aerobic bacteria and detection of its antimicrobial susceptibility pattern were done as per standard CLSI guidelines.

The antibiotics used for Gram-positive cocci were Cefoxitin (30mcg), Vancomycin (30mcg), Linezolid (30mcg), Teicoplanin (30mcg), Cefaperazone / Sulbactam (75 / 30 mcg), Cefepime / Tazobactam (30 / 10mcg), Piperacillin/Tazobactam (100/10mcg), Amikacin (30mcg), Gentamycin (10mcg), Levofloxacin (5mcg), Ofloxacin (5mcg), Sparfloxacin (5mcg),

Prulifloxacin (5mcg), Cephataxime (30mcg), Ceftriaxone (30mcg) Cefuroxime (30mcg), Cepodoxime (10mcg), Amoxyclav (20/10)

The antibiotics used for Gram negative bacilli were Imipenam (10mcg) Cefaperazone / Sulbactam (75/30mcg), Cefepime / Tazobactam (30 / 10mcg), Piperacillin/Tazobactam (100/10mcg), Amikacin (30mcg), Gentamycin (10 mcg), Levofloxacin (5mcg), Ofloxacin (5mcg), Sparfloxacin (5mcg), Prulifloxacin (5mcg), Cephataxime (30mcg), Ceftriaxone (30mcg) Cefuroxime (30mcg), Cepodoxime (10mcg), Ceftazidime (30 mcg), cefaperazone (75 µg), Cefixime (5mcg) Amoxyclav (20/10)

**Detection of methicillin resistance**:

The Clinical and Laboratory Standards Institute (CLSI) guidelines (2006) had recommended cefoxitin disc diffusion method for the detection of MRSA. This is performed by using a 30 µg cefoxitin disc and an inhibition zone diameter of ≤19 mm is reported as Methicillin resistant and ≥20 mm is considered as Methicillin sensitive.

**Detection of Extended-spectrum β-Lactamases (ESBL**)

Extended-spectrum β-lactamases (ESBLs) are defined as β-lactamases capable of hydrolyzing oxyimino-cephalosporins and are inhibited by β-lactamase inhibitors**..**Isolates showing a zone of inhibition<22 mm for ceftazidime were tested for ESBL production as per CLSI criteria.

**Combined disc method**

A combined disc method using Cefaperazone (75mcg) and Cefaperazone/Sulbactam (75/30mcg) performed for phenotypic confirmation of ESBL production, as recommended by the latest guidelines of CLSI. Organism was considered as ESBL producer if there was a more than 5 mm increase in zone diameter of Cefaperazone/Sulbactam disc and that of Cefaperazone disc alone.

**Data analysis**

Statistical analysis done using Chi-square test for comparing certain parameters between diabetic and non-diabetic patients. P value less than 0.05 was considered to be statistically significant.

## Result and Discussion

Diabetic patients are quite susceptible to bacterial infections which contribute to excess morbidity. In this study among 100 diabetic wound infection cases, 72% were men, 28% were women and male to female ratio was 2.57%. In 100 non- diabetic wound infection cases, 60% were men, 40% were women and male to female ratio was 1.5%. In this study among diabetic cases majority of patients were in the age group 51-60 and among non-diabetic cases majority of patients were in the age group 21-30. Ekta Bansal *et al.* (2008) had also made similar observation that majority of diabetic wound infection patients (56.31%) were in the age group 51 to 70 years.

In this study, in diabetic patients, 81% of the total specimens yielded significant bacterial growth (83.8% from hospitalized patients and 75% from outpatient cases). In non-diabetic patients 52% of the total specimens yielded significant bacterial growth, 56.1% from hospitalized patients and 46.5% from outpatient cases. In this study Diabetic wounds showed a significant positive culture compared to non-diabetic wounds

with P<0.05 (Table 1). Tobias Hirsch *et al.*, (2008) in his study showed that diabetic wounds were significantly more susceptible to wound infections by endogenous bacterial challenge as well as external contamination than non-diabetic wounds. In this study total organisms isolated in 100 diabetic wounds were about 90 giving an average of 0.9 organisms per case and total organisms isolated in 100 non-diabetic wounds were about 55 giving an average of 0.55 organisms per case (Table 2).

Poor glycemic control (Random blood sugar [RBS]>200mg) was present in 64% of the patients (Table 3). This correlated with the study done by Ekta Bansal *et al.* (2008) where poor glycemic control, i.e., random blood sugar (RBS) >200 mg/dL, was found in 69 (67%) patients.

In these study polymicrobial infections among diabetic wounds were slightly higher (11.1%) as compared to non-diabetic wounds (5.8%) and monomicrobial infections among diabetic and non-diabetic wounds were 88.9% and 94.2% respectively (Table 4). Raja (2007) and studies from various literature have documented the polymicrobial nature of diabetic wound infections.

In this study among bacterial isolates in diabetic wounds, Gram positive organisms accounted for 53.9% and Gram negative organisms accounted for 46.1%. Among bacterial isolates in non-diabetic wounds, Gram positive organisms accounted for 57.4% and Gram negative organisms accounted for 42.6% (Table 5). Lipsky *et al.* (1987) had a similar observation in their study that aerobic Gram-positive cocci were isolated as the sole pathogen in 42% of cases, while anaerobes and aerobic Gram-negative bacilli were infrequently recovered.

In this study among diabetic wound infection cases, the predominant organism isolated was *Staphylococcus aureus followed by Escherichia coli, Pseudomonas aeruginosa, Klebsiella oxytoca, Klebsiella pneumoniae, Streptococcus species, Proteus vulgaris, Proteus mirabilis, Citrobacter species, Coagulase negative Staphylococcus,* and *Candida species.* Among non-diabetic wound infection cases, the predominant organism isolated was *Staphylococcus aureus followed by Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Streptococcus species, Coagulase negative Staphylococcus, Klebsiella oxytoca, Proteus vulgaris, Proteus mirabilis, Acinetobacter species*, and *Candida species.* Similar results were obtained by Chincholikar and Pal (2002) as their study reported highest positivity of *S. aureus* (31%) followed by *P. aeruginosa* (19%), *K. pneumoniae* (18%), *E. coli* (15%) and *Proteus sp* . (9.3%) (Table 6).

In this study Gram positive cocci isolated from diabetic wounds showed 100% sensitivity to Vancomycin, Linezolid, Teicoplanin, more than 95% sensitivity to Cefaperazone /Sulbactam, Cefepime /Tazobactam, Piperacillin/ Tazobactam, Amikacin & Gentamycin, 80% to Fluoroquinolones, 65% to Cephalosporins and only 30% to Cefoxitin. In non-diabetic wounds the same pattern was observed for all antibiotics except for about 61% sensitivity to Cefoxitin.

In this study Gram negative bacilli from diabetic wounds showed absolute sensitivity to Imipenam, Cefaperazone/ Sulbactam, Cefepime/ Tazobactam, Piperacillin/ Tazobactam, moderate sensitivity to Aminoglycosides& Quinolones and poor sensitivity to Cephalosporins, and Amoxyclav.

**Table.1** Data regarding bacterial growth

| Diabetes | Positive culture | Negative culture |
|---|---|---|
| IP (68) | 57 (83.8%) | 11 (16.2%) |
| OP (32) | 24 (75%) | 8 (25 %) |
| Total (100) | 81 (81%) | 19 (19%) |

| Non-diabetes | Positive culture | Negative culture |
|---|---|---|
| IP (57) | 32 (56.1%) | 25 (43.9%) |
| OP (43) | 20 (46.5%) | 23 (53.5%) |
| Total (100) | 52 (52%) | 48 (48%) |

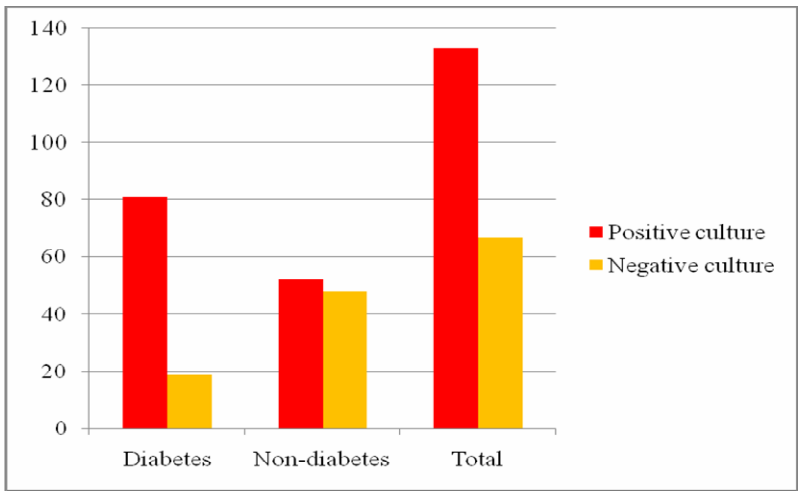



**Table.2** Total organisms isolated

| | Diabetes | Non-diabetes |
|---|---|---|
| IP | 61 | 34 |
| OP | 29 | 21 |
| Total | 90 | 55 |
| Average organism/case | 0.9 | 0.5 |

**Table.3** Correlation of glycemic control with diabetic wound infections

| Total diabetics | RBS>200mg | RBS<200mg |
|---|---|---|
| 100 | 64 | 26 |

**Table.4** Polymicrobial and monomicrobial infections

| | Diabetes | | Non-diabetes | |
|---|---|---|---|---|
| | Polymicrobial | Monomicrobial | Polymicrobial | Monomicrobial |
| IP | 4 | 53 | 2 | 30 |
| OP | 5 | 19 | 1 | 19 |
| Total | 9(11.1%) | 72(88.9%) | 3(5.8%) | 49(94.2%) |

**Table.5** Gram positive and Gram negative infections

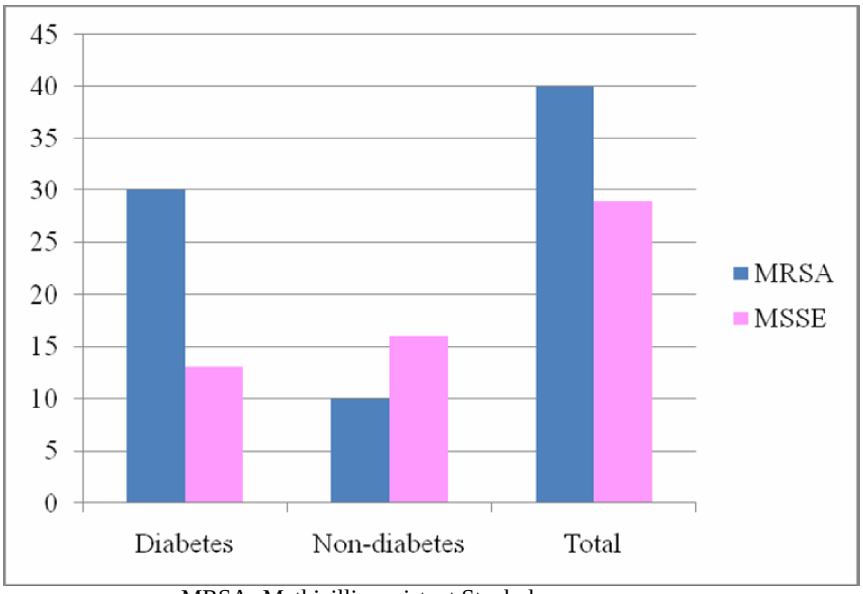
| Total Organisms | Diabetes | | Non-diabetes | |
|---|---|---|---|---|
| | No of GPC | No of GNB | No of GPC | No of GNB |
| IP | 29(48.3%) | 31(51.7%) | 20(60.6%) | 13(39.4%) |
| OP | 19 (65.5%) | 10(34.5%) | 11(52.4% | 10(47.6%) |
| Total | 48(53.9%) | 41(46.1%) | 31(57.4%) | 23(42.6%) |

**Table.6** Isolation of pathogens from diabetic and non-diabetic wounds

| | Total Sample | Positive culture | *S.aureus* | *E.coli* | *P.aeruginosa* | *K.oxytoca* | *K.pneumoniae* | *P.vulgaris* | *P.mirabilis* | *Streptococcus. sp* | *Citrobacter .sp* | *Acinetobacter.sp* | *CONS* | *Candida* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DM IP | 68 | 57 (83.8%) | 24 (42.1%) | 12 (21.1%) | 8 (14%) | 3 (5.3%) | 2 (3.5%) | 2 (3.5%) | 2 (3.5%) | 4 (7%) | 2 (3.5%) | - | 1 (1.8%) | 1 (1.8%) |
| DM OP | 32 | 24 (75%) | 19 (79.2%) | 3 (12.5%) | 4 (16.7%) | 1 (4.2%) | 1 (4.2%) | 1 (4.2%) | - | - | - | - | - | - |
| DM Total | 100 | 81 (81%) | 43 (53.1%) | 15 (18.5%) | 12 (14.8%) | 4 (4.9%) | 3 (3.7%) | 3 (3.7%) | 2 (2.5%) | 4 (4.9%) | 2 (2.5%) | - | 1 (1.2%) | 1 (1.2%) |
| ND IP | 57 | 32 (56.1%) | 18 (56.3%) | 3 (9.4%) | 5 (15.6%) | 1 (3.1%) | 2 (6.3%) | 1 (3.1%) | - | 2 (6.3%) | - | 1 (3.1%) | - | 1 (3.1%) |
| NDM OP | 43 | 20 (46.5%) | 8 (40%) | 3 (15%) | 3 (15%) | - | 3 (15%) | - | 1 (5%) | 1 (5%) | - | - | 2 (10%) | - |
| NDM Total | 100 | 52 (52%) | 26 (50%) | 6 (11.5%) | 8 (15.4%) | 1 (1.9%) | 5 (9.6%) | 1 (1.9%) | 1 (1.9%) | 3 (5.8%) | - | 1 (1.9%) | 2 (3.8%) | 1 (1.9%) |

**Table.7** Detection of Methicillin resistant *Staphylococcus aureus* (MRSA)

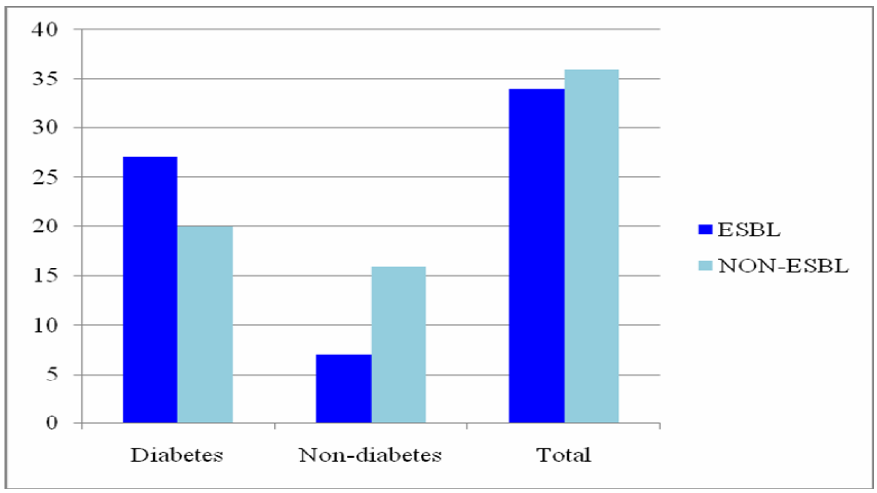
| Total Organisms | Diabetes | | Non-diabetes | |
|---|---|---|---|---|
| | *Staphylococcus aureus* | MRSA | *Staphylococcus aureus* | MRSA |
| IP | 24 | 15(62.5%) | 18 | 7(38.9%) |
| OP | 19 | 15(78.9%) | 8 | 3(37.5%) |
| Total | 43 | 30(69.8%) | 26 | 10(38.4%) |




MRSA- Methicillin resistant Staphylococcus aureus
MSSA- Methicillin sensitive Staphlococcus aureus

**Table.8** Detection of Extended spectrum β-actamases (ESBLS)

| Organism | Diabetes | ESBL | Non-diabetes | ESBL |
|---|---|---|---|---|
| *Escherichia coli* | 15 | 12(80%) | 6 | 2(33.3%) |
| *Pseudomonas aeruginosa* | 12 | 7(58.3%) | 8 | 3(37.5%) |
| *Klebsiella oxytoca* | 4 | 2(50%) | 1 | 0 |
| *Klebsiella pneumoniae* | 3 | 19(33.3%) | 5 | 1(20%) |
| *Proteus vulgaris* | 3 | 1(33.3%) | 1 | 0 |
| *Proteus mirabilis* | 2 | 2(100%) | 1 | 0 |
| *Citrobacter species* | 2 | 2(100%) | - | - |
| *Acinetobacter species* | - | - | 1 | 1(100%) |
| Total | 47 | 27(57.4%) | 23 | 7(30.4%) |



In non-diabetic wounds, they showed absolute sensitivity to Imipenam, Cefaperazone/ Sulbactam, Cefepime/ Tazobactam, moderate sensitivity to Aminoglycosides, Quinolones & Cephalosporins and poor sensitivity to Amoxyclav. Raja (2007) antimicrobial susceptibility results showed that Gram-negative bacterial isolates were sensitive to Imipenam and Amikacin while Vancomycin showed good activity against Gram-positive bacteria.

In this study 69.8% of the *Staphylococcus aureus* isolates were MRSA in diabetic wounds which was higher compared to non-diabetics where only 38.4% of the isolates were MRSA. MRSA strains in diabetic wounds showed a significant difference with non-diabetic wounds with $P<0.05$ (Table 7). This study finding was in concordance with findings by Gadepalli *et al.* (2006) in which MRSA was seen in 56% of the cases of *S. aureus;* whereas in the study by Tentolouris *et al.* (1999) MRSA was present in 40% of the cases. Vancomycin, linezolid and Teicoplanin were most effective drug against MRSA.

In this study most of the isolates i.e.57.4% among diabetic wounds was found to be ESBL producers which is higher compared with from non-diabetic wounds where only 30.4% of the isolates were ESBL producers. ESBL strains in diabetic wounds showed a significant difference with non-diabetic wounds with $P<0.05$ (Table 8). Varaiya *et al.* (2008) had reported 51.61% of *K. pneumoniae* isolates and 48.38% of *E. coli* isolates to be ESBL producers, Kapil *et al.* (2006) have reported 54.5% *E. coli* isolates to be ESBL producers, which have caused diabetic foot infections. All the ESBL-producing isolates were found to be 100% sensitive to carbapenems (Imipenam and Meropenem) which correlated with this study. Imipenam. Cefaperazone /Sulbactam, Cefepime/Tazobactam and Piperacilllin /Tazobactam were the most effective drugs against ESBL producing Gram negative bacilli.

The prevalence of ESBLs among members of *Enterobacteriaceae* constitutes a serious threat to the current beta-lactam therapy, leading to treatment failure. There is an urgent need to emphasize rational use of drugs to minimize the misuse of available antimicrobials. In conclusion, Pus culture and sensitivity testing played an important role in the treatment of wound infection in patients. Surveillance of MRSA and ESBL are essential to formulate new therapeutic strategies.

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