Original Research Article

Isolation and identification of bacterial pathogens from wounds of diabetic patients

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A B S T R A C T

Keywords

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Diabetes Mellitus is a serious health problem worldwide and India has the highest number of people with diabetics. In the present study, out of 105 samples collected from wounds of diabetic patients 95 showed bacterial infections. Among males 93.82% and among females 79.16% showed positive results. In the age wise distribution of positive cases there is not much difference among the age groups. In the same way there is not much variation in the positive cases of inpatient and outpatient categories. *Staphylococcus* sp was the primary pathogen in most of the wound infections in the study. The frequently reported bacterial pathogens of wound infections such as *Klebsiella sp.* and *Pseudomonas sp.* were not so common in the present study. Almost all isolates showed resistance against most of the broad spectrum and narrow spectrum antibiotics tested. Thus, multidrug resistance among the pathogens is a problem in the present study. Proper management of diabetic wound infection with appropriate antibiotic must be implemented, keeping in mind the high incidence of drug resistance among the bacteria isolated from diabetic cases.

Introduction

Diabetes mellitus (DM) is a serious public health problem worldwide (Ozer *et al*., 2010). Among the 191 WHO members states, India has the highest number of people with diabetes (Chellan *et al*., 2010). Worldwide, the prevalence of DM has been estimated to be 2.8% in 2000, and projected to be 4.4% in the year of 2030. Foot ulcers are a serious complication of diabetes with recent studies suggesting that life time risk of developing a foot ulcer in diabetic patients may be as high as

25% (Hayat *et al*., 2011). They are now the most common proximate and non-traumatic causes of leg amputation (Hayat *et al*., 2011).Various microorganisms colonize the wound and in some patients one or more species of organisms proliferate in the wound, which may lead to tissue damage, host response accompanied by inflammation, that is, clinical infection (Lipsky *et al.,* 2012). Mostly the diabetic foot infections are mixed bacterial infection (Zubair *et al.,*

2010). Among the bacterial pathogens isolated, Gram positive organisms such as *Staphylococcus aureus* and *Staphylococcus epidermidis* are the most common in wound infection, while the Gram negative organisms such as *Pseudomonas aeruginosa*, *Escherichia coli, Proteus* species and *Klebsiella* species are rare.

Materials and Methods

Pus samples from wound of diabetic patients were collected from a private hospital and a Government Hospital in Pondicherry. Oral informed consent was obtained from the patients before sample collection. Patient's personal details, antibiotic regime and other relevant information were collected using a questionnaire.

A total of 105 samples were collected from both inpatients and outpatients. Pus samples were collected by using sterile cotton swabs which are moistened with sterile saline to prevent drying. For each specimen two swabs were used. The swabs were brought to the laboratory in a sterile container within one hour after the collection and processed immediately. One swab was used for Gram staining and the other one was inoculated on Mac-conkey agar and blood agar plates for isolating the pathogens. The inoculated plates were incubated at 37˚C overnight.

After incubation, the plates were observed for growth and the isolated colonies were identified by morphological and biochemical characteristics.

The tests performed include Gram staining, Motility, Catalase, Oxidase, Indole, Methyl Red, Voges Proskauer, Citrate Utilization production, Urease production, Triple Sugar Iron, Mannitol and Phenylalanine.

Antibiotic susceptibility pattern of the isolates was studied by Kirby Bauer's disc diffusion method (Bauer *et al*., 1966). Both broad spectrum and narrow spectrum antibiotics were used. The antibiotics tested were Penicillin (10 units), Amoxycillin (10 mcg), Ciprofloxacin (5 mcg), Vancomycin (30 mcg), Gentamicin (30 mcg), Ampicillin (10 mcg), Tetracycline (30 mcg), Erythromycin (15 mcg), Co-Trimoxazole (25 mcg) and Cephalothin (30 mcg).

Result and Discussion

In the present study, a total of 105 samples, 16 from Hospital I and 89 from Hospital II, were collected (Table: 1).Of the 105 samples, bacteria were isolated from 95 (90.47%) samples and were considered positive for infection.

In the inpatient category, 74 out of 79 (93.67%) samples were positive and in the outpatient category, 21 out of 26 (80.76%) samples were positive. Age wise positive cases among diabetic patients are shown in Figure: 1.

Based on the biochemical tests the isolates were identified as *Staphylococcus* sp., *E. coli*, *Proteus* sp., *Pseudomonas* sp. and *Klebisella* sp. Three of the isolates could not be identified by the routine biochemical tests. The distribution of the bacterial isolates among the inpatients and outpatients are listed out in Table: 2. A total of 49 Staphylococcal isolates were obtained in the present study of which 43 were from inpatients. Samples collected from hospital I yielded only *Staphylococcus* sp. and *E. coli*, whereas samples collected from hospital II yielded

Table.1 Hospital wise details of samples

Sample Collection Area	In patient	Out patient	Grand Total
Hospital I	11	5	16
Hospital II	68	21	89
Total	79	26	105

Table.2 Distribution of the isolates between in-patients and out-patients

Organisms	In-Patient	Out-Patient	Total
Staphylococcus **sp.**	43	6	49
Escherichia coli	11	7	18
Proteus **sp.**	8	1	9
Pseudomonas **sp.**	3	2	5
Klebsiella **sp.**	6	4	10
Unidentified	3	1	4
Total	74	21	95

Table.3 Antibiotic susceptibility patterns of the isolates (Hospital I)

S.No.	Antibiotics	*Staphylococcus* sp.			*Escherichia coli*		
		R(%)	I(%)	S(%)	R(%)	I(%)	S(%)
1	Penicillin	100	-	-	100	-	-
2	Amoxycillin	100	-	-	100	-	-
3	Ciprofloxacin	88	12	-	80	20	-
4	Vancomycin	100	-	-	100	-	-
5	Gentamycin	63	25	12	60	20	20
6	Ampicillin	100	-	-	100	-	-
7	Tetracycline	63	25	12	100	-	-
8	Erythromycin	50	50	-	100	-	-
9	Co-trimoxazole	100	-	-	100	-	-
10	Cephalothin	100	-	-	100	-	-

R- Resistant; I – Intermediate; S- Sensitive.

Proteus sp., *Pseudomonas* sp. and *Klebsiella* sp. along with Staphylococci and *E. coli*.

All the isolates were tested for their antibiotic susceptibility (95). The antibiotic susceptibility patterns of the isolates are listed in Table: 3 and 4. Of the ten antibiotics tested in the present study, *Staphylococcus* sp. isolated from hospital I

samples were sensitive to Gentamicin and tetracycline and *E. coli* were sensitive only to Gentamicin

The most serious complications affecting diabetic patients are the foot ulceration. Suresh *et al.,* (2011) has reported that 15% of all diabetics develop a foot ulcer at some time in their lives as they are highly susceptible to an infection that spreads

quite rapidly leading to tissue destruction and subsequent amputation.

Microorganisms are known to aggravate the problem of wound infection and the same has been proved in the present study by the observation that 90.47% of cases showing positive results, that is almost each and every case showed a bacterial infection. Among males 93.82% and among females 79.16% showed positive results. Thus, wound infections in the diabetic were common among the males than females as the present study represents 79% of the positive cases were in male against 21% of cases in female.

In the age wise distribution of positive cases there is not much difference in different age groups. More positive cases were found in the age group 41-70 years and it reflects the fact that the complication of diabetics is high among aged people. In the same way there is not much difference in the number of positive cases between inpatients and outpatients. In the present study Staphylococci are the most common pathogen isolated (49/95). This results is in agreement with Goldstein *et al.,* (1996), Hayat *et al.,* (2011) and Oates *et al.,* (2012) who also reported a high frequency of *S. aureus* being isolated from diabetic foot infection.

Next to Staphylococci, the predominant bacterium in wound infection is *Escherichia coli* which is similar to the finding made by Banashankari *et al.,* (2012). The frequently reported organisms such as *Klebsiella* species and *Pseudomonas* species were not so common in the present study. None of the samples resulted in more than one or two bacterial pathogens indicating the absence of mixed infections and this is in contrast to the findings of several authors (Zubair *et al.,* 2010; Lipsky *et al.,* 2012).

In the present study 49 isolates of *Staphylococcus* sp. were obtained and all of them were resistant to Vancomycin. A similar observation was made by Citron *et al.* (2007) in a study on diabetic foot infection. Vancomycin resistance among *Staphylococcus aureus* is a frequent phenomenon and infact, the first two isolates of Vancomycin resistant *S. aureus* were isolated from diabetic patients with foot lesion (Chang *et al.,* 2003). Similarly all the isolates of *Pseudomonas* sp., and *E. coli* in the present study also showed resistance to Vancomycin. Only Ciprofloxacin and Gentamicin were found to be effective against the two major pathogens viz. *S. aureus* and *E. coli* isolated in the present study.

Figure.1 Age wise distribution of positive cases
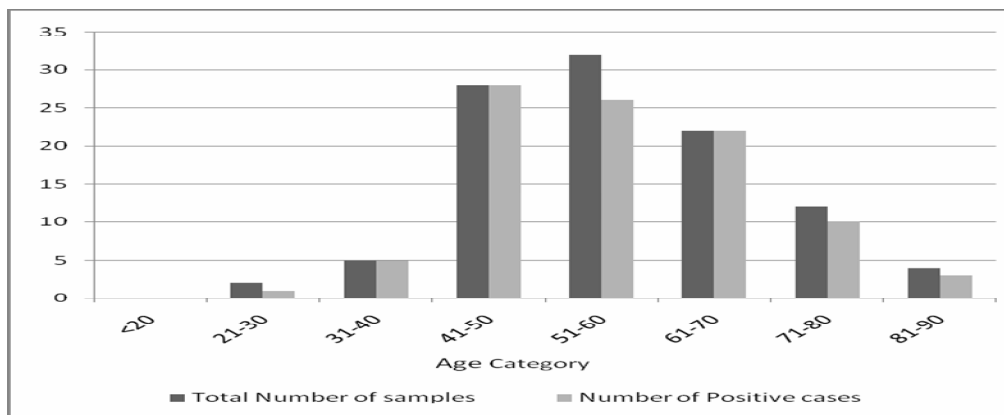
Table: 4 Antibiotic susceptibility patterns of the isolates (Hospital II)

S. No.	Antibiotics	*Staphylococcus* sp.			*Escherichia coli*			*Proteus* sp.			*Pseudomonas* sp.			*Klebsiella* sp.			Unidentified		
		R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
1	Penicillin	100	-	-	85	-	15	100	-	-	100	-	-	100	-	-	100	-	-
2	Amoxicillin	100	-	-	100	-	-	100	-	-	100	-	-	100	-	-	100	-	-
3	Ciprofloxacin	88	12	-	92	8	-	77	11	12	60	20	20	30	20	30	75	-	25
4	Vancomycin	100	-	-	92	-	8	89	-	11	100	-	-	60	30	10	100	-	-
5	Gentamycin	63	35	2	69	8	23	89	-	11	80	20	-	70	-	30	75	25	-
6	Ampicillin	100	-	-	100	-	-	100	-	-	100	-	-	100	-	-	100	-	-
7	Tetracycline	56	15	29	69	15	16	100	-	-	100	-	-	50	40	10	100	-	-
8	Erythromycin	50	50	-	85	8	7	88	-	12	80	20	-	60	20	10	100	-	-
9	Co-trimoxazole	100	-	-	77	8	15	88	-	12	100	-	-	70	10	20	75	-	25
10	Cephalothin	100	-	-	77	8	15	88	-	12	100	-	-	60	10	30	100	-	-

R - Resistant, I - Intermediate, S - Sensitive

The possibility of nosocomial infection is insignificant among the cases sampled in the study, as most of the patients had been admitted in the hospital for the treatment of wound infections. However, the role of Staphylococci in causing severe infection leading to hospitalization is a point to be noted seriously.

In conclusion, proper management of foot infection with appropriate antibiotic must be implented keeping in mind the incidence of drug resistance among the pathogens isolated from diabetic population.

Acknowledgement

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