Antibiotic Susceptibility of Common Bacterial Pathogens Isolated From Diabetic Pus

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Abstract

To screen various bacterial pathogens present in diabetic pus and to determine their antibiotic sensitivity and resistance pattern against the commonly used standard antibiotics. The common bacterial pathogens isolated from the diabetic pus samples were Gram-positive cocci like *Staphylococcus aureus, S. epidermidis* and ß-*Streptococci*, Gram-negative bacilli like *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli*. The bacterial pathogen showed resistance to most of the antibiotics. The magic bullets, the miraculous drugs, antibiotics can be used to heal the diabetic wounds and thus the amputations, which is the threat of all diabetic patients in the whole world can be minimized to a great extend.

Key words: *Staphylococcus aureus, ß-Streptococci, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli.*

Introduction

Diabetic mellitus is a chronic metabolic disorder with vascular components that is characterized by disturbances in carbohydrate, lipids and protein metabolism. Of the total diabetic population 15-20% will experience a foot ulcer in their lifetime. All diabetic foot ulcers will be superficially colonized by a plethora of microbes (Vinod Kumar and Neelakund, 2004).

An average of 5-6 organisms is often involved in the diabetic foot infections with a mixture of aerobic and anaerobic organisms. Diabetic foot wounds are of the major complications of diabetics resulting in substantial morbidity and mortality (Jeffrey Stone and Paul Cianci, 1997). Antibiotic coverage can then be changed according to the culture and sensitivity results and clinical response (Wagner, 1981). Diabetic wounds represent an increasing burden to health care systems as the age of the population increases (Howell Jones *et al.,* 2005). Diabetic mellitus has been diagnosed in approximately 14 million U.S citizens. It can produce a complex clinical picture due to its involvement in different organ systems (Stephen M Shroeder, 2004).

Mixtures of aerobic and anaerobic organisms are common (Bal, 2000). Anaerobic Gram negative bacilli are common in mixed infections with *Proteus* spp. *Escherichia coli, Klebsiella* spp and *Enterococci* spp, being isolated most often. Antibiotic therapy for infected wounds is composed of good wound care (Kelvin, 1999). The diabetic wounds are mostly infected by pus forming microorganisms like *Enterococci* spp, *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella* spp, *Proteus* spp. (Revathi *et al.,* 1998).

Antibiotic were labeled “Magic bullets” which targeted the microbial pathogens without harming the host. Ofloxacin has been shown to be effective in the treatment of diabetic foot infections in a study conducted. Three weeks of therapy was well tolerated and was effective in treating the diabetic foot infections (Lipsky *et al.,* 1990). In a similar work conducted by Nord and Kager 1983 shown, a combination of oflaxacin 200mg and tinidazole 60mg tablets given twice daily for 14 days was proved to be effective and well tolerated in the treatment of diabetic wounds.

Materials and Methods

The pus samples were collected from Thanjavur Medical College, Tamilnadu in India. The pus samples were examined for its odour, colour, presence of tissue and blood. The initial characterizations of the organisms present in the pus samples were carried out by direct microscopic examination using staining technique. Using selective plate technique did secondary analysis. The usage of selective media suppresses the growth of the unwanted bacteria. Biochemical tests including Indole, Citrate, Oxidase, Coagulase, Catalase, TSI agar, Mannitol motility test were done by confirmation. The antibiotic sensitivity patterns of bacterial pathogens isolated from various pus samples to commonly used antibiotic were measured by the method of Kirby-Bauer.

Results and Discussion

51 samples were collected; some samples showed only pus cells and were found to be sterile after 48 hrs of incubation. However, bacterial pathogens were isolated from 41 samples. The presence of any bacteria in pus is an indication on infection. The patients selected were between the age group of 25-95. They were divided into seven groups and the samples collected from them were inoculated into MacConkey agar and blood agar. The results were noted after 48hrs of incubation and tabulated on Table-1. The percentages of isolates were maximum at the age of 45-54 and minimum at the age group of 85 - 94 and 23- 34.

Table-1: Classification of samples from patients with different age groups

Patient with different age groupso.	Number of samples collected	No. of positive samples collected	No. of samples showed mixed growth	% of organisms isolated
25-34	3	1	Nil	2.17%
35-44	5	3	Nil	6.52%
45-54	15	13	1	30.4%
55-64	12	11	2	28.2%
65-74	10	10	2	26%
75-84	4	2	Nil	4.3%
85-94	2	1	Nil	2.1%

Macroscopic observation

Of the 51 samples analyzed, most of the pus samples (35 nos) were yellow in colour. Pus was mixed with blood in many cases. No characteristic odour was noted.

Microscopic observation

Gram-negative organisms showed highest incidence (71.7%), Gram-positive organisms were also obtained (25.4%). Of the 51 samples analyzed, 10 were found to be sterile (19.6%). Mixed growth was noticed in the cultures obtained from the patients with age group, 45-54 (1 no), 55-64 (2 nos) and 65-74 (2 nos) (Table-1). The G (+) ^{ve} cocci in cluster suggested the presence of *Staphylococci* and G (+) ^{ve} cocci in chains suggested the presence of *Streptococci.* The G (+) ve rods suggested the presence of *Pseudomonas, Klebsiella, Escherichia coli* and other *Enterobacteriacea* members.

By performing the routine biochemical tests and other specific tests the confirmation of these isolated organisms (Table 2 and 3). A standard antibiotic multi-disc containing 12 antibiotics was used for Antibiotic sensitivity test. *Staphylococcus aureus* showed highest degree of sensitivity to tetracycline, cefotaxime, ampicillin and gentamycine (Table-4). The antibiotic sensitivity pattern of *Staphylococcus epidermidis* is produced (Table-5). Another G (+) ^{ve} cocci isolated was ß *Streptococci*, which showed highest sensitivity to ampicillin (Table- 6).

The G (-) ^{ve} organisms isolated were *K. pneumoniae*, *E. coli* and *P. aeruginosa.* Separate multidiscs were used for G (+) ve and G (-) ve

Table-3: Special identification tests for the confirmation of the isolated organisms

Sl.No.	Organisms	Catalase	Coagulase	Oxidase
1	*Staphylococcus aerues*	+	+	NC
2	*Staphylococcus epidermidis*	+	-	NC
3	*ß-Streptococci*	+	NC	NC
4	*Escherichia coli*	+	-	NC
5	*Klebsiella pneumoniae*	+	+	NC
6	*Pseudomonas aeruginosa*	+	+	+
NC = Not Carried Out.				

Table-4: Antibiotic Sensitivity for *Staphylococcus aureus*

Antibiotics	S/R	ZOI	S	MS
Ampicillin	S	19	17	14 - 16
Cotrimoxazole	R	-	16	11 15
Ciprofloxacin	S	21	21	16 20
Pefloxacin	R	14	23	15 22
Cefotaxime	S	25	23	15 22
Roxythromycin	R	17	21	18 19
Cephalexin	MS	17	18	15 17
Ofloxacin	S	17	17	14 16
Tetracycline	S	21	19	15 18
Gentamycin	S	17	15	13 14
Lincomycin	MS	18	19	16 - 18

S/R = Sensitive or Resistance; ZOI = Zone of Inhibition
S = Standard Sensitivity; MS = Moderate Sensitivity

Table-2: Biochemical characterization and Colony Morphology of the isolated cultures

S.No	Organisms	Colony Morphology	Gram's staining	Indole	Citrate	Mannitol	Motility	TSI
1	*Staphylococcus aerues*	Golden, yellow colonies, haemolysis on blood agar	G(+)ve cocci in clusters.	NC	NC	NC	NC	NC
2	*Staphylococcus epidermidis*	White colored colonies	G(+)ve cocci in clusters.	NC	NC	NC	NC	NC
3	*ß-Streptococci*	Shiny, small, semi transparent colonies, haemolysis on blood agar.	G(+)ve cocci in chains.	NC	NC	NC	NC	NC
4	*Escherichia coli*	Foul smelling, Lactose fermenting colonies.	G(-)ve rods	+	-	+	-	Yellow slant & Yellow butt with gas.
5	*Klebsiella pneumoniae*	Highly mucoid, large dome shaped, lactose-fermenting colonies.	G(-)ve stout rods	-	+	+	-	-DO-
6	*Pseudomonas aeruginosa*	Greenish, non-lactose fermenting colonies.	G(-)ve slender rods	-	-	-	-	-
NC = Not Carried Out.								

Table-5: Antibiotic Sensitivity for *Staphylococcus* epidermidis

Antibiotics	S/R	ZOI	S	MS
Ampicillin	S	20	17	14 - 16
Cotrimoxazole	R	14	16	11 15
Cephalexin	S	19	18	15 17
Tetracycline	R	-	19	15 18
Cefotaxime	S	25	23	15 22
Ciprofloxacin	S	24	21	16 20
Pefloxacin	S	19	18	16 17
Ofloxacin	S	17	16	13 15
Gentamycin	S	17	15	13 14
Cloxacillin	R	-	14	12 13
Roxythromycin	R	-	19	16 18
Lincomycin	R	-	18	16 - 17

S/R = Sensitive or Resistance; ZOI = Zone of Inhibition
S = Standard Sensitivity; MS = Moderate Sensitivity

Table-6: Antibiotic Sensitivity for *ß-Streptococci*

Antibiotics	S/R	ZOI	S	MS
Ampicillin	S	20	17	14 - 16
Cotrimoxazole	R	-	16	11 15
Cephalexin	S	19	18	15 17
Tetracycline	S	20	19	15 18
Cefotaxime	S	23	23	15 22
Ciprofloxacin	S	23	21	16 20
Pefloxacin	S	20	18	16 17
Ofloxacin	S	16	16	13 15
Cloxacillin	S	16	14	12 13
Gentamycin	S	17	15	13 14
Lincomycin	R	-	18	16 17
Roxythromycin	R	-	19	16 18

S/R = Sensitive or Resistance; ZOI = Zone of Inhibition,
S = Standard Sensitivity; MS = Moderate Sensitivity

Table-7: Antibiotic Sensitivity for *Klebsiella* pneumoniae

Antibiotics	S/R	ZOI	S	MS
Ampicillin	S	20	17	14 - 16
Cotrimoxazole	R	-	16	11 15
Cephalexin	S	19	18	15 17
Tetracycline	S	20	19	15 18
Cefotaxime	S	23	23	15 22
Ciprofloxacin	S	23	21	16 20
Pefloxacin	S	20	18	16 17
Ofloxacin	S	16	16	13 15
Cloxacillin	S	16	14	12 13
Gentamycin	S	17	15	13 14
Lincomycin	R	-	18	16 17
Roxythromycin	R	-	19	16 18

S/R = Sensitive or Resistance; ZOI = Zone of Inhibition,
S = Standard Sensitivity; MS = Moderate Sensitivity

Table-8: Antibiotic Sensitivity for *Escherichia coli*

Antibiotics	S/R	ZOI	S	MS
Ampicillin	S	19	17	14 16
Cephalexin	S	19	18	15 17
Cotrimoxazole	R	-	16	11 15
Cefotaxime	S	25	23	15 22
Cholramphenical	R	-	18	13 17
Ciprofloxacin	R	-	21	16 20
Tetracycline	R	-	19	15 18
Amikacin	S	26	17	13 16
Pefloxacin	R	-	23	15 22
Ofloxacin	S	17	17	14 16
Gentamycin	S	18	15	13 14

S/R = Sensitive or Resistance; ZOI = Zone of Inhibition,
S = Standard Sensitivity; MS = Moderate Sensitivity

Table-9: Antibiotic Sensitivity for *Pseudomonas aeruginosa*

Antibiotics	S/R	ZOI	S	MS
Ampicillin	R	-	17	14 16
Cotrimoxazole	R	-	16	11 15
Cefotaxime	S	23	23	15 22
Cephalexin	R	-	18	15 17
Cholramphenical	R	-	18	13 17
Ciprofloxacin	S	21	21	16 20
Tetracycline	R	-	19	15 18
Ofloxacin	MS	16	17	14 16
Gentamycin	S	18	15	13 14
Pefloxacin	R	-	23	15 22
Amikacin	S	21	17	13 16

S/R = Sensitive or Resistance; ZOI = Zone of Inhibition,
S = Standard Sensitivity; MS = Moderate Sensitivity

Table-10: Prevalence of Microbes in Collected Specimens

Total No. of organisms isolated	Types of organisms isolated	No. of organisms present	% of the organism present
46	*Klebsiella pneumoniae*	15	32.6%
	Escherichia coli	7	15.2%
	Pseudomonas aeruginosa	11	23.9%
	Staphylococcus aureus	7	15.2%
	Staphylococcus epidermidis	3	6.52%
	ß-Streptococci	3	6.52%

Table-11: Prevalence of Microbes in collected specimens

Total No. of organisms isolated	Types of organisms isolated	No. of organisms present
46	*Klebsiella pneumoniae (5 nos)* *Escherichia coli (1 no)* *Pseudomonas aeruginosa (4 nos)*	5

organisms. Usually, *Klebsiella* and *E. coli* were found to be resistant to most of the antibiotics. *Klebsiella* showed sensitivity to ampicillin, gentamycin, amikacin and chloramphenicol and of this ampicillin showed highest zone of inhibition (table- 7). The antibiotic sensitivity pattern of Escherichia coli and P. aeruginosa was produced Table-8 and 9 respectively. 18 pus swabs and 33-bottle pus were found to be sterile after the period of incubation. Of the total isolates *Klebsiella* showed highest incidence (32.6%), which was followed by *P. aeruginosa* (23.9%), *E. coli* (15.2%), *S. aureus* (15.2%), *S. epidermidis* (6.52%) ß-Streptococci (6.52%) respectively (Table-10). Mixed growth of *Klebsiella, E. coli* and *P. aeruginosa* were also noted (Table-11).

An average of 5 or 6 organisms is often involved in diabetic pus (Birrer *et al.,* 1996). The polymicrobial nature of most infections of the diabetic foot is well known, with an average of 5-6 organisms involved. Mixtures of aerobic and anaerobic organisms are common (Bal, 2000). The above two observations were found to be identical to that of the present study. In this work, Gram-negative organisms showed highest incidence (71.7%). Gram-positive organisms were also present (25.4%). A similar study by Kamal *et al.*, 1996, reflected that the diabetic wounds are superficially colonized by a plethora of microbes.

The present study evaluates the necessity of screening the bacterial pathogen in time and to determine the antibiotic susceptibility of the suspected organisms, which was found to be very useful in the case of empirical therapy and by the usage of antibiotic multidisc, we can find out the appropriate antibiotic which inhibits the growth of a particular species. Thus, it promotes the treatment against the original pathogen and reduces the consumption of wide spectrum of antibiotic which in turn reduces a wide spectrum of side effects and thus save the diabetic victims from the extremity amputations in time.

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