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Studies on the Effect of Antibiotics on Bacteria Isolated from Diabetic Wound Infection

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Abstract: ***The study was to investigate the drug resistance bacteria isolated from diabetic wound infected pus sample. Totally, five bacteria Escherichia coli, Klebsiella pnemoniae, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus pyogens were identified. In antibiotic sensitivity test, twelve antibiotics were used against the pathogens. The E. coli highly sensitive to norfloxacin (30 mm), chloramphenicol and tetracycline (each 24 mm), Klebsiella pnemoniae most sensitive to erythromycin (24 mm), Pseudomonas aeruginosa susceptible to chloramphenicol and rifampicin (each 20 mm), Staphylococcus aureus highly sensitive to tetracycline (24 mm), chloramphenicol (22 mm), rifampicin (20 mm) and Streptococcus pyogens highly sensitive to chloramphenicol (30 mm). All the five isolates were resistance to ampicillin, oflaxacin and penicillin G.***

Keywords: Diabetic wound infection, Bacteria, Antibiotics, Resistance

1. 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). Mostly the diabetic foot infections are mixed bacterial infections (Zubair *et al*., 2010).

According to American Diabetic Association, Diabetes mellitus (DM) is a disease caused by metabolic disorder (www. diabetes.org.br). It is classified in to two basic forms Type I and Type II Diabetes. Type I DM is caused by immunological deficiency in pancreas leading to insufficient insulin production (WHO, 1999). Type II DM occurs when the body becomes insulin resistant or it does not respond to the insulin produced (Uma Makheswari and Sudarsanam, 2012).

The total diabetic population 15-20% will experience a foot ulcers are superficially colonized by a plethora of microbes (Vinod kumar and Veelakund, 2004). An average of 5-6 strains of organisms is often involved in the diabetic foot infections with a mixture of aerobic and anaerobic organisms (Jeffrey stone and Paul cianci, 1997).

The diabetic wound are mostly infected by pus forming microorganisms like *Enterococci* sp. *Staphylococci aureus, Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella* sp. and *Proteus* sp. (Revathi *et al.*, 1998).

Common pathogens isolated from the diabetic pus included Gram positive cocci like (*Staphylococcus aureus* and *Streptococcus pyogenes*) and Gram-negative bacilli like (*Pseudomonas* sp. *Escherichia coli*, *Klebsiella* sp. and *Proteus* sp.). It can be concluded that Gram negative bacteria were present in greater number than Gram positive bacteria in the pus sample. The bacterial pathogens showed resistance to most of the antibiotics (Rajalakshmi and Amsaveni, 2011).

2. Materials and Methods

Sample collection

To isolate and identify the bacteria from diabetic wound infection, samples were collected from 40 diabetic patients affected with wounds at Government Hospital, Perambalur, and Tamilnadu. Among the 40 patients 30 male, 10 female candidates age group between 45-65 years old. The infected diabetic wound pus samples were collected using sterile cotton swabs during February 2017 to March 2017. The swabs were transferred into sterile tubes with 1% peptone broth. The tubes were immediately transported to the microbiology laboratory for further analysis.

Isolation and identification of bacterial pathogen

For isolation of diabetic wound infected bacterial strains, loop full samples were streaked on Mac Conkey agar, Blood agar and Nutrient agar plates (Hi Media, India) and incubated at 37±2ºC for 24 hrs. After incubation, colonies were characterized on the basis of morphological, cultural physiological and biochemical characteristics (Mac Faddin, 2000). A presumptive identification was performed by Gram staining, catalase production, oxidase activity, hydrogen sulfide production, Indole test, Voges-Proskauer test. The bacterial isolates were identified with the help of Bergey's Manual of Systematic Bacteriology (Kreig and Holt, 1984).

Disc diffusion method

The isolated bacterial species were tested for the antibacterial susceptibility test against standard antibiotics. The test was done by disc diffusion method as recommended by CLSI M45 – A2 guidelines on Muller Hinton agar (CLSI, 2015). The commercially available standard antibiotics *viz.* ampicillin, azithromycin, cefotaxime, chloramphenicol, erythromycin, gentamicin, norfloxacin, ofloxacin, penicillin-G, piperacillin tazobactam, rifampicin and tetracycline were used.

3. Results and Discussion

A total of 40 diabetic wound infected samples were analysed for isolation of predominant bacterial pathogens. Out of which most of the samples showed prominent bacterial count. Few of the samples showed very low bacterial count. The demographic characterization of the patients showed that, the significant proportions were males (75%), in the age group of 45 to 65 years, 27 (67.5%) people were having normal wound and 13 (32.5%) people having diabetic wound infection, 30% people capable of read and write, up to SSLC grade (20%) and HSC level 17.5% (Table 1). Five bacteria were isolated from 40 diabetic wound infected pus samples. The isolates were characterized and identified by studying different properties as mentioned in materials and methods. The identification characteristics were confirmed with standard manual (Krieg and Holt, 1984). The biochemical characteristics revealed that, these isolates belonging to 5 genera (Table 2). Of these *Escherichia coli, Klebsiella pnemoniae, Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus pyogens* were identified (Table 3). Similarly, Joseph *et al.* (2013) reported that the frequently reported bacterial pathogen of wound infection such as *Klebsiella* sp and *Pseudomonas* sp. and also Anuradha *et al.* (2008) reported that the most common isolates was *Pseudomonas aeruginosa* (55.0%), followed by *Staphylococcus aureus* (19.29%), *Klebsiella* sp. (11.43%), *Acinetobacter* sp. (7.14%), *Proteus* sp. (4.29%), *Escherichia coli* (2.85%). Swab samples were collected from diabetic patient's foot ulcers from hospitals in and around Chennai. Out of 22 isolates, 4 strains showed high multiple Antibiotic Resistant (MAR) index. 16S rRNA gene was amplified using PCR technique in all the 4 strains and they were characterized as *Staphylococcus aureus, Morganella morganii, Acinetobacter baumannii* and *Acinetobacter* sp. Phylogenetic tree for each of the isolate was constructed to analyze its evolutionary relationship with closely related species (Mathangi *et al.*, 2013).

In antibiotic sensitivity test *E. coli* most sensitive to norfloxacin (30 mm), chloramphenicol (24 mm), tetracycline (24 mm) and Rifampicin (22 mm), moderate sensitive to gentamicin (20 mm) Resistance to other antibiotics. *Klebsiella pnemoniae* sensitive to erythromycin (24 mm) rifampicin (19 mm), azithromycin (18 mm) moderate sensitive or resistance to other antibiotics. Similarly, Rajalakshmi and Amsaveni, (2011) reported that the bacterial pathogens showed resistance to most of the antibiotics. *Pseudomonas aeruginosa* sensitive to chloramphenicol and rifampicin (each 20 mm) resistance to other antibiotics. Similarly, Shailesh kumar *et al.* (2011) reported that the members of *Enterobacteriaceae* as well as *Pseudomonas* sp. and *Acinetobacter* sp. were found to be susceptible mainly to amikacin, piperacillin-tazobactam and imipenem. The *Staphylococcus aureus* highly sensitive to Tetracycline (24 mm), chloramphenicol (22 mm), rifampicin (20 mm) moderate sensitive to azithromycin, gentamicin and piperacillin resistance to other antibiotics. The *Streptococcus pyogens* most sensitive to chloramphenicol (30 mm), piperacillin (20 mm) moderate sensitive to rifampicin and resistance to other antibiotics. In the present study correlated with Suzan *et al.* (2016) antimicrobial susceptibility test against wound bacterial isolates, the imipenem and ciprofloxacin were found to be the most effective drugs against most of the isolates, followed by amikacin. doxycycline, tetracycline and azithromycin were less sensitive to some isolates, while gentamycin and oxacillin were the weakest antibiotics.

Table 1: Characteristics of diabetic wound culture positive patients

Variables	Number	Percentage
Age	40-65	
Sex		
Male	30	75
Female	10	25
Diabetic wound		
Normal	27	67.5
Post operative	13	32.5
Education level		
Write and read only	12	30
SSLC	8	20
HSC	7	17.5
University level	13	32.5

Table 2: Biochemical characteristic testing of bacterial isolates from diabetic wound infected sample

Bacterial Strains	Gram Strains	Motility	Shape	Indole	MR	VP	Citrate	TSI	H_2S	Urease	Catalase	Oxidase
1	-	+	Rod	+	+	-	-	-	-	-	+	-
2	+	-	Club bacilli	-	-	+	+	+	+	+	+	-
3	-	+	Rod	-	-	-	+	+	-	-	+	+
4	+	-	Cocci	-	+	+	+	+	-	+	+	-
5	+	-	Cocci arranged chain	-	-	+	-	-	-	-	-	-

Table 3: Bacteria isolated from the diabetic wound infected sample

Bacterial strains	Name of the organism
1	*Escherichia Coli*
2	*Klebsiella pnemoniae*
3	*Pseudomonas aeruginosa*
4	*Staphylococcus aureus*
5	*Streptococcus pyogens*

Table 4: The effect of antibacterial susceptibility testing of isolated diabetic wound infected bacterial pathogens

S. No.	Organisms	Ampicillin	Azithromycin	Cefotaxime	Chloramphenicol	Erythromycin	Gentamicin	Norfloxacin	Ofloxacin	Penicillin-G	Piperacillin Tazobactam	Rifampicin	Tetracycline
		Zone of inhibition in mm											
1	*Escherichia Coli*	10	7	12	24	9	20	30	7	7	15	22	24
2	*Klebsiella pnemoniae*	10	18	10	8	24	8	11	10	7	15	19	6
3	*Pseudomonas aeruginosa*	8	9	13	20	7	9	7	7	4	12	20	4
4	*Staphylococcus aureus*	10	18	14	22	9	15	6	6	6	14	20	24
5	*Streptococcus pyogens*	10	9	10	30	10	8	4	7	6	20	17	10

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