AEROBIC BACTERIAL PROFILE OF DIABETIC ULCER IN A TERTIARY CARE CENTRE

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ABSTRACT

BACKGROUND

Diabetes mellitus is one of the most challenging health problem in 21st century and fifth leading cause of death in developed countries.[1] Ulceration of foot is the most common problem affecting approximately 15% of diabetic patients during their lifetime.[2]

The objective of the study was to find the aerobic bacterial profile of diabetic ulcer and to determine their antibiotic sensitivity pattern in a tertiary care centre.

MATERIALS AND METHODS

A total of 147 samples of diabetic ulcers were tested microbiologically using standard techniques and antimicrobial susceptibility testing was performed for the isolated pathogens using Kirby-Bauer disc diffusion method.

RESULTS

The rate of culture positivity was 100% and polymicrobial. Pseudomonas aeruginosa was the most frequent isolated pathogen (50.34%) followed by Escherichia coli (19.72%) and Klebsiella (19.04%). Gram-negative bacilli were highly sensitive to Imipenem and Meropenem. Among Staphylococci (29.92%), 17% were MRSA (Methicillin-Resistant Staphylococcus aureus).

CONCLUSION

Continuous surveillance of antibiotic susceptibility pattern of bacterial pathogen should be done to ensure rational use of antibiotics for empirical and definitive treatment of diabetic ulcer.

KEYWORDS

Diabetic Ulcer, Polymicrobial, Pseudomonas Aeruginosa, Imipenem, Meropenem.

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BACKGROUND

Diabetes Mellitus is a complete metabolic syndrome caused by the lack of insulin resulting in inappropriate high blood glucose level, either because the pancreas does not produce enough insulin or because cells do not respond to the insulin that is produced.[3] The incidence of Diabetes Mellitus throughout the world is increasing strikingly and becoming a public health problem especially in developing countries.

Diabetic foot is the most common complication of diabetes and it is the leading cause of hospitalisation among diabetic patients.[4] The individuals with diabetes have at least a 10-fold greater risk of being hospitalised for soft tissue and bone infections of the foot than individuals without diabetes.[5]

An ulcer is defined as a discontinuity of an epithelial surface and it is characterised by progressive destruction of the surface epithelium and a granulating base. Diabetic ulcers are chronic. A chronic ulcer is defined as an ulcer of more than 6 weeks of duration.

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There are studies from India showing that diabetes, atherosclerosis, tuberculosis, leprosy, etc. are the aetiology of chronic wounds. Chronic diabetic ulcers are usually polymicrobial. Bacterial population of chronic ulcers does not differ significantly and most of them are resistant to usually used and easily available antibiotics. The specific organisms identified in diabetic foot infections can differ not only from patient to patient and hospital to hospital but also from one part of the country to another. India, with approximately 42 million diabetics, is ranked first in the list of the 10 nations most affected with diabetics. Among diabetes mellitus related complications, foot ulcer is the most common, affecting approximately 15% of diabetic patients during their lifetime. The study is to describe the aerobic bacterial profile of diabetic ulcer as well as the susceptibility pattern of the isolates.

MATERIALS AND METHODS

This descriptive study was performed on diabetic ulcer samples sent to Microbiology Department of Government Medical College, Kottayam from July 2014 to June 2015. Samples were taken from patients admitted in the wards of General Surgery Department, with chronic diabetic ulcers of more than 6 weeks, during the study period. A total of 147 double swabs were received during the study period. The bacterial pathogens were isolated and tested for antimicrobial drug sensitivity by standard methods.

Sample Collection

Culture specimens were obtained at the time of admission after local debridement of devitalised tissue, the ulcer wound was scrubbed thoroughly with sterile normal saline to remove the surface colonisers. Sample collection was done using sterile cotton swab. Pus was aspirated with sterile syringe and needle and appropriate two swabs were collected, one for Gram-stain and the other for aerobic culture.

Transport of Specimen

Samples were transported immediately to the laboratory.

Microscopic Examination

The type and relative number of microorganisms and pus cells were identified by direct Gram-stained smear of all samples.

Bacterial Isolation and Identification Procedures[6]

For aerobic culture, the specimens were inoculated on 5% Sheep Blood agar, MacConkey agar and salt agar plates for isolation of aerobic bacteria. Glucose broth was also inoculated as backup broth. The inoculated plates and broth were then incubated at 37°C for 24 hours. The isolates were identified based on colony morphology, Gram-staining, motility, catalase test, oxidase test, coagulase test and biochemical tests. In this study, anaerobic bacteria were not investigated owing to limited laboratory facilities.

Antibiotic Susceptibility Testing

It was done by Kirby-Bauer disc diffusion method as per Clinical and Laboratory Standards Institute Guidelines (CLSI). Muller-Hinton agar plates and commercially available Hi-media discs were used. The antimicrobial discs which were used were those of Ampicillin (20 µg), Gentamicin (10 µg), Amikacin (30 µg), Cefazolin (30 µg), Ceftazidime (30 µg), Cefotaxime (30 µg), Ceftriaxone (30 µg), Cefoperazone/sulbactam (75/10 µg), Piperacillin/tazobactam (100/10 µg), Imipenem (10 µg), Meropenem (10 µg), for Gram-negative bacilli. Penicillin, Ampicillin (10 µg), Cefoxitin (30 µg), Cefotaxime (30 µg), Erythromycin (15 µg), Oxacillin (1 µg), Vancomycin (30 µg), Ciprofloxacin (5 µg), Linezolid (30 µg) were used for Gram-positive organisms. Vancomycin resistance, ESBL and, Amp-C-β Lactamase production were detected as per the CLSI guidelines.

RESULTS

Number of Isolates (%)	Number of Sterile Culture (%)	Total Number of Cases
100	0	147

Table 1. Overview of Culture Done

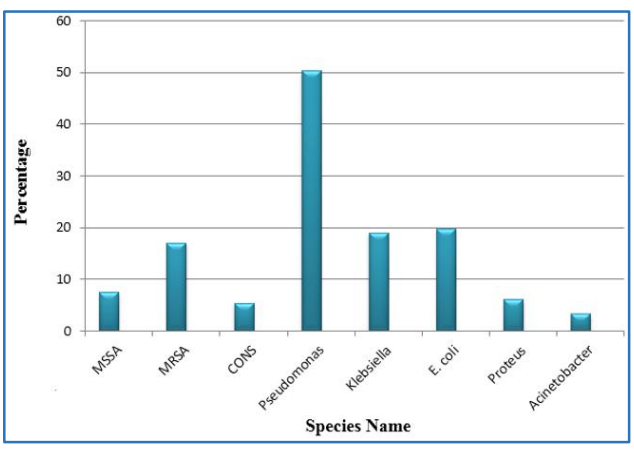

Chart 1. Total Percentage of Bacteria Cultured from Diabetic Patients

Antibiotics	E. coli (%)	Pseudomonas (%)	Klebsiella (%)	Acinetobacter (%)	Proteus (%)
Ampicillin	3.57	-	0	0	25
Gentamicin	28.57	16.40	32.14	20	37.5
1st Generation Cephalosporin	14.22	-	14.28	0	37.5
Amikacin	71.42	56.16	82.14	20	87.5
Cefotaxime	10.71	35.67	21.42	0	50
Ciprofloxacin	32.14	23.28	39.28	0	50
Cefoperazone/Sulbactam	50	50.68	57.14	60	-
Piperacillin/Tazobactam	51.14	61.64	64.28	40	87
Meropenem	85.71	75.34	75	80	100
Imipenem	85.71	75.34	75	80	100

Table 2. Antibiotic Sensitivity Patterns of Gram-negative Bacteria

Antibiotics	MSSA	MRSA	CONS
Penicillin	30	-	33.33
Ampicillin	30	-	33.33
Cefoxitin	100	0	33.33
Gentamicin	90	7.40	33.33
Amikacin	90	66.66	83.33
1st Generation cephalosporin	100	0	0
Vancomycin	100	100	100
Linezolid	100	14	33.33
Erythromycin	40	0	-

Table 3. Antibiotic Sensitivity Patterns of Gram-positive Bacteria

DISCUSSION

A total of 234 pus samples for culture were received in the laboratory during the study period, of which only 147 (62. 8%) were diabetic and 87 (37.2%) were non-diabetic. Pathogenic bacteria were isolated in 147 samples (100%).

Among 147 cases, 31.97% cultures were polymicrobial. The most common isolate was Pseudomonas aeruginosa (50.34 %) followed by Staphylococci (29.92 %) of which 17% were MRSA. The microbial profiles from diabetic ulcer vary widely among studies from different parts of country, but all of them show a Gram-negative preponderance. In a study of Parve et al from North India, Gram-negative organisms were

isolated in 66.2% patients and Enterobacteriaceae family accounted for almost 50% of bacterial isolates in diabetic ulcers. This Gram-negative preponderance has been shown to be similar over a span of two decades by Ramakant et al. Pseudomonas aeruginosa has been consistently shown to be the most common organism isolated from diabetic ulcer in our country. However, others have shown Escherichia coli as the most common organism followed by Staphylococcus aureus.

In a study by Mrs Smita Watwe, Dr Sadhana Chate, Dr Charan K Dardi and Dr Aruna Khare, Dept. of Microbiology, MIMER Medical College, Talegaon-Dabhade, Maharashtra, out of total 86 samples, growth was obtained in 74 samples i.e. 86% cases.[7] Polymicrobial growth was seen in 35 cases (41%). Polymicrobial infections were predominant in diabetic group. From non-diabetic group, 25% cases yielded polymicrobial growth. Overall Staphylococcus aureus was the single most common isolate, from 34 of 86 cases (40%). Non-sporing anaerobic Gram-negative bacilli were isolated on 12 occasions. In a study by Marek Kucharzewski, Jolanta Misztal-Knyra, Edward Błaszczak and Andrzej Franek, analysis of the flora of venous and diabetic ulcerations showed Staphylococcus aureus as the most frequently isolated organism. In two studies conducted respectively by Jain Manisha, Patel Mitesh,[8] et al, Department of Microbiology, GMERS Medical College, Sola, Ahmedabad and in B. J. Medical College, Ahmedabad, a tertiary care hospital from February 2009 to April 2010, Gram-negative organisms accounted for 82.80% and Gram-positive organisms accounted for 17.20% isolates. Pseudomonas aeruginosa was the most common isolate, accounting for 30.57%, followed by Klebsiella species, Escherichia coli and Staphylococcus aureus, comprising 22.29%, 16.56% and 12.74% respectively. Antibiotic sensitivity pattern of Staphylococcus aureus showed that oxacillin resistance i.e., the prevalence of Methicillin-resistant Staphylococcus aureus (MRSA) was 55%. In a recent study by Lpsky et al, the most common pathogen isolated was Staphylococcus aureus (44%). Nearly 80% of them were methicillin sensitive. Other common isolates were Peptostreptococcus spp., Prevotella, etc. The study noted that hospitalisation, surgical procedures and broad spectrum antibiotic therapy predispose the patients to infections with MRSA or vancomycin-resistant strains.

Pseudomonas

Pseudomonas aeruginosa was the most common pathogen associated with foot ulcers in patients with Diabetes mellitus. It is aerobic Gram-negative motile bacilli, which grows well on ordinary media producing large opaque irregular colonies with musky or earthy smell. The metabolism is oxidative and non-fermentative. Indole, Methyl Red-Voges Proskauer and H_2S tests are negative. Catalase, oxidase and arginine dihydrolase tests are positive. In this study, Pseudomonas aeruginosa was the most common isolate (50.34%). It has been reported that Imipenem is the most effective antibiotic against Gram-negative organisms, including Pseudomonas aeruginosa. In our study, 75.34% of the Pseudomonas aeruginosa isolates were sensitive to Imipenem and Meropenem. Additionally, we found that only 16.40% of Pseudomonas aeruginosa isolates were sensitive to gentamicin. Differences in the results obtained in many studies shows that the patterns of microbial infection are not consistent in patients with diabetic foot infections; therefore, repeated evaluation of microbial characteristics and the antibiotic sensitivity is necessary for the selection of appropriate antibiotics. Pseudomonas aeruginosa showed 75.34% sensitivity to Imipenem and meropenem, 61.64% sensitivity to Piperacillin and tazobactam. Only 23.28% is sensitive to Ciprofloxacin.

Escherichia coli

This is aerobic and facultatively anaerobic Gram-negative bacilli which grows in ordinary media. Colonies are large, thick, greyish white, moist, smooth, opaque or partially translucent. It is haemolytic in blood agar. On MacConkey medium, colonies are bright pink due to lactose fermentation. Indole and MR positive, VP and citrate utilisation tests are negative. Escherichia coli showed maximum sensitivity to Meropenem and Imipenem with 85.71%, Amikacin - 7.4%, Piperacillin/tazobactam - 51.17%, Ciprofloxacin - 32.14%. All the isolates showed resistance to Ampicillin.

Klebsiella Pneumoniae

This is Gram-negative non-motile, non-sporing, encapsulated coccobacilli. Produces large dome-shaped mucoid colonies and pink colonies on MacConkey agar. Is catalase positive, oxidase negative. Indole not produced**,** MR negative**,** VP positive**,** Citrate utilised, Urea hydrolysed, decarboxylate lysine, but not arginine and ornithine. Klebsiella isolates showed maximum sensitivity to Amikacin 82.14%, followed by Meropenem and Imipenem (75%). Gentamicin sensitivity is only 32%.

Acinetobacter

This is Gram-negative non-motile coccobacilli. Forms non–haemolytic colonies on blood agar and pale, non-lactose fermenting colonies on MacConkey agar. Catalase positive, oxidase negative, nitrate not reduced to nitrite, Indole not produced, does not ferment sugars, utilises 10% lactose. Acinetobacter showed maximum sensitivity to Meropenem and Imipenem (80%).

Proteus

Gram negative, pleomorphic motile bacilli. MR positive and VP negative. Proteus spp. isolated were 100% sensitive to Imipenem and Meropenem.

The most frequently isolated organisms like Pseudomonas, Escherichia coli, Klebsiella and, Acinetobacter showed resistance to commonly used antibiotics.

Staphylococci

Staphylococci were tested for methicillin resistance using oxacillin and cefoxitin discs as recommended by the guidelines of Clinical Laboratory Standards Institute (CLSI). Novobiocin discs were used to distinguish Staphylococcus saprophyticus, which is resistant to Novobiocin in culture, from other coagulase-negative Staphylococci (CoNS). Of all the Staphylococcus aureus isolates, 17% were resistant to Cefoxitin i.e. MRSA (Methicillin-Resistant Staphylococcus Aureus). All MRSA isolates were found to be sensitive to Vancomycin (100%). Methicillin-Resistant Staphylococcus Aureus (MRSA) is resistant to many antibiotics. In medical facilities MRSA causes life threatening blood stream

infections. These bacteria are typically resistant to most of beta lactam and non-beta lactam antibiotics.

Coagulase-Negative Staphylococci

Identified by Gram-positive cocci with white opaque colonies in salt agar. It is catalase positive, phosphatase test positive, slide and tube coagulase negative.

Streptococcus Pyogenes

Were confirmed with blood agar culture and bacitracin (0.04 U) disc sensitivity test, which is used in the presumptive identification of group A, beta-haemolytic Streptococci.

Streptococcal isolates showed 100% sensitivity to Penicillin, Ampicillin.

Limitation of the Study

This study includes the use of FBS (Fasting blood sugar) to determine glycaemic control, the use of patients' records and interviews to determine the duration of diabetes and the fact that anaerobic organisms were not isolated due to our inability to use anaerobic methods in the culturing of the wound specimen is a limitation.

CONCLUSION

Studies from India suggest that the third generation cephalosporins and piperacillin are appropriate empirical antibiotic choices until definitive culture reports are available, because of predominant Gram-negative bacterial isolates. But continuous surveillance of antibiotic susceptibility pattern of bacterial pathogen should be done to ensure rational use of antibiotics for empirical and definitive treatment of diabetic ulcer. However, it confirmed the high prevalence of multidrug-resistant pathogens in diabetic foot ulcers. Diabetic foot infections were predominantly due to Pseudomonas aeruginosa, Staphylococcus or were polymicrobial infections. Many studies on the bacteriology of diabetic foot infections have reported results that vary and are often contradictory. Therefore, it is necessary to evaluate the different microorganisms infecting the wound on a routine basis and to know the antibiotic susceptibility patterns of the isolates from the infected wound in patients with diabetic foot lesions. This knowledge is crucial for planning the treatment of these patients with the appropriate antibiotics, reducing resistance patterns, and minimising healthcare costs.

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