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Bacteriology of diabetic foot Infection

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ABSTRACT

Objective: To determine the microbiological profile and antibiotic susceptibility pattern of organisms isolated from diabetic foot ulcer in RMMCH Chidambaram

Methods: This is a retrospective study with a review of the bacteriology results of specimens taken from 100 consecutive patients with diabetic foot infection at RMMCH in Chidambaram during the period of 2010 to 2011. The specimens were cultured using optimal aerobic and anaerobic microbiologic technics. Antimicrobial susceptibility testing to different agents was carried out using the diffusion method.

Result: Staphylococcus aureus was the commonest isolate being recovered from 20% cases , including methicillin resistant staphylococcus aureus in 9 of 27(30) patients wounds. The organism isolated were pseudomonas aeruginosa(16%), proteousmirabilis (18%), klebsiella pneumonia(0.7%), Escherichia coli(13%), and klebsiellaoxytoca(0.6%).The antimicrobial suscepteblity testing , showed that vancomycin was the most effective against gram positive and amikoscin and ceftazidime was the most effective against gram-negative organism.

Conclusion: Staphylococcus aureus, pseudomonas aeruginosa, proteous mirabilis, klebsieella pneumonia, Escherichia coli and Klebsiella oxytoca were the most common causes of diabetic foot infection. These wounds require use of combined antimicrobial therapy for initial patient. .

Keywords: Diabetes mellitus, diabetic foot ulcer, microbiological profile.

INTRODUCTION

Diabetic's mellitus (DM) is a serious public health problem and remains an important cause of morbidity and mortality worldwide. Indians with diabetes tend to have more coronary artery disease, ulcer and gangrene as compared to other ethnic groups. Patients with uncontrolled diabetes often develop diabetic complications, some of the most clinically important which are foot ulcers, retinopathy, and neuropathy and macro vascular complications. Foot complications such as foot ulcer constitute a major public health problem and impose a heavy burden on health services.

Diabetes is a growing public health problem and especially in India. The global prevalence of Type 2 diabetes is expected to double in the period between 2002 to 2025, the risk is estimated to increase from 4% in 1995 to 5.4% by the year 2025. In terms of the infecting microorganisms and the like hood of successful treatment with antimicrobial therapy, acute osteomyelitis in people with diabetes is essentially the same as in those without diabetes. Chronic infection in patients with diabetes mellitus is the most difficult infection to cure. Adequate surgical debridement, in addition to antimicrobial therapy, is necessary to cure chronic infections. In order to come over the complication, bacterial isolates were cultured from diabetic foot infections and their in-vitro susceptibility to the commonly used antibacterial agents were assessed, a prospective microbiological study was carried out and results were observed in people residing in and around Chidambaram.

MATERIALS AND METHODS

Subject's hailing in and around Chidambaram attending the out patients as well as in patients services in Rajah Muthiah medical college and hospital were included in the study.

The totals of 100 patients were screened for a period of Jan 2010 to Jan 2011. Discharge from the incised lesions or ulcers were collected with sterile swab. Processing of samples was done in the following way:

1. Direct microscopy.
2. Culture.

1. Direct microscopy

The heat fixed smears were stained by Gram staining technique and examined under oil immersion objective of the light microscope in the Department of Microbiology, M. R. Medical College and Basveshwar Teaching and General Hospital. The smears were examined for the presence of pus cells, gram positive and gram negative organisms. The size, shape, arrangement of bacteria and presence of spores was noted.

2. Culture

The specimens were cultured on nutrient agar, blood agar, Mac Conkey agar & Mannitol salt agar etc .and incubated at 37°c for 24 to 48 hrs. The bacterial isolate were identified by Gram staining, Colony morphology, Oxidase test, Catalase test and by biochemical reaction such as Sugar fermentation, Indole, Methyl red, Voges-proskauer, and Citrate Utilisation test etc, as described in the Practical Microbiology. [Practical Microbiology of Mackie Mac Cartney 14th volume]

All the culture was processes for their antimicrobial susceptibility test by Kirvy-Bauer Disc Diffusion method using Muller Hington agar.

RESULTS

This prospective study was carried out in the Department of Microbiology, Rajah Muthiah Medical College and Research institute from Jan 2010 to Jan 2011. Pus samples were processed from 100 diabetic cases with foot ulcers. These patients sought treatment for ulcer in the Department of Surgery, RMMC&H.

TABLE – 1. NUMBERS AND PERCENTAGE OF AEROBES ISOLATED

GRAM POSITIVE	NO. OF AEROBES (n=132)*	PERCENTAGE (%)
Staphylococcus aureus	27	20.45
Enterococcus faecalis	12	09.09
Coagulase negative Staphylococcus (CONS)	11	08.33
Group A Streptococci	02	01.51

*Multiple aerobes are present, hence n>100

Among Gram positive aerobes, *Staphylococcus aureus* (20.45%), *Entrococcus faecalis* (9.09),Coagulase negative *Staphylococcus*(8.33) and Group A Streptococci (01.51%).

FIGURE – 1. NUMBER AND PERCENTAGE OF AEROBES ISOLATED

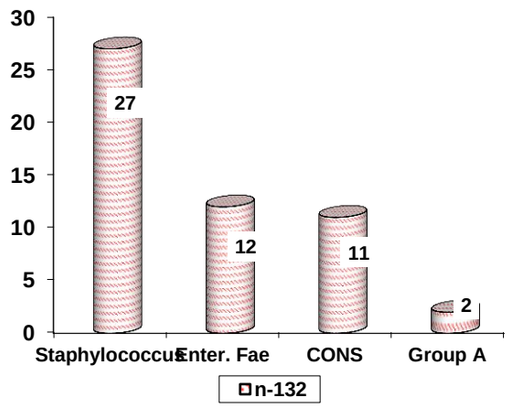

TABLE – 2 NUMBER AND PERCENTAGE OF AEROBES ISOLATED

GRAM NEGATIVES	NO. OF AEROBES (n=132)*	PERCENTAGE (%)
Proteus mirabilis	13	09.84
Pseudomonas aeruginosa	22	16.66
Klebsiella pneumoniae	10	07.57
Escherichia coli	18	13.63
Proteus vulgaris	09	06.81
Klebsiella oxytoca	08	06.06

*Multiple aerobes are present, hence n>100

Among Gram negative aerobes, Pseudomonas aeruginosa(16.66%), Eschericha coli (13.63%), Proteus mirabilis (09.84%), Klebsiella pneumonia (7.57%), Proteus vulgaris (6.81%)and Klebsiella oxytoca (6.06%). Staphylococcus aureus showed 81.4% sensitivity to cefotaxime, 74% sensitivity to oxacillin and it is 62.9%,96.2% resistant to erythromycin and ampicillin respectively. Enterococcus feacalis showed 66.6% sensitivity to ampicillin. Coagulase negative Staphylococcus

showed 90.9% sensitivity to amikacin and ciprofloxacin with 45.5% sensitivity to penicillin and ampicillin. All these isolates showed 100% sensitivity to vancomycin.

Sensitivity to amikacin in Proteus mirabilis was 107.6%, 100%, 92.3% to ceftriaxone and ceftazidime, 93.02% to cefotaxime. 84.6%, 53.8% were resistant to ampicillin and gentamicin respectively. 90.9% of Pseudomonas aeruginosa were sensitive to ceftazidime. All other isolates showed 100% sensitivity to amikacin except Klebsiella oxytoca which was 75% sensitive to amikacin. Staphylococcus aureus showed 81.4% sensitivity to cefotaxime, 74% sensitivity to oxacillin and it is 62.9%, 96.2% resistant to erythromycin and ampicillin respectively. Enterococcus feacalis showed 66.6% sensitivity to ampicillin. Coagulase negative Staphylococcus showed 90.9% sensitivity to amikacin and ciprofloxacin with 45.5% sensitivity to penicillin and ampicillin. All these isolates showed 100% sensitivity to vancomycin. Sensitivity to amikacin in Proteus mirabilis was 107.6%, 100%, 92.3% to ceftriaxone and ceftazidime, 93.02% to cefotaxime. 84.6%, 53.8% were reistant to ampicillin and gentamicin respectively. 90.9% of Pseudomonas aeruginosa were sensitive to ceftazidime. All other isolates showed 100% sensitivity to amikacin except Klebsiella oxytoca which was 75% sensitive to amika

DISCUSSION

Diabetic foot Infections are a major and increasing problem word wide. In our country 25% of the more than 18 million diabetic patients develop foot ulcerations during their lifetimes, and over half of these become infected. In the present study, Staphylococcus aureus was the predominant aerobic isolate (27%). Our study is in correlation with Wheat et al, Ramani et al Dipali AC et al and Vijaya et al, Tahaway et al 17 and Unachukwu et al. 18 who isolated Staphylococcus aureus as their major aerobe.

Often dismissed as a contaminant or colonizer, s. epidermidis is increasingly being recognized as a true pathogen. In the mean study Staphylococcus epidermidis comprised nearly (8.33%) which is especially true not only in nosocomial infections involving catheters and prosthetic devices but also in various other types of wound infections. (Refsahl, K et al., and B. M. Andersen et al. 1992., Yao etal, Y., D. E. Sturdevant et al, and M. Otto et al. 2005).

In the present study Coagulase Negative Staphylococcus was 11% where as by Bessman. et al, by 9% in 1984, Fass, R. J et al., by 8% in 1986, Refsahl, K.,et al by 5% in 1992. A. N., Mamatha P.Samaga et al by 11% in 2006.

In its ability to cause serious infections that are mediated in part by the production of virulence factors, such as clumping factor, a thermostable DNase, esterase, lipase, protease,
and a fatty acid-modifying enzyme.

Among Gram positive aerobes, Staphylococcus aureus was the predominant isolate (20.45%). Group A Streptococci was least isolated (1.51%). Whereas, by McMillan et al, 1% in 2007, Nagamune, H et al., by 3% in 2000, Mamatha P.Samaga et al by 2% in 2006.

Enterococci are considered commensals with low virulence except in compromised patients, such as diabetics, in whom they can act as opportunistic pathogens. We recovered Enterococcus species from 12 patients 9%, including pure culture from two patients. (Diane M el al., Citron et al, Ellie J et .al. Mamatha P. Samaga et al by 12% in 2006.)

In our study twelve patients with ulcer revealed three types of mixed bacterial infection; (a) four patients had Pseudomonas spp., E.coli and S.aureus; (b) six patients had Proteus spp. and S.aureus and (c) two patients had Enterobacter spp., Proteus spp. and S.aureus.

No anaerobes were isolated from the patients with ulcers. The role of anaerobes is particularly unclear, because in many studies specimens were not collected or cultured properly to recover these organisms. Mamatha P.Samaga et al Staphylococcus aureus was the major gram positive isolate accounting for (22.01%) of the aerobes, Methicillin resistant strains in (16.7%) All the isolates of MRSA showed 100% susceptibility to vancomycin. Staphylococcus aureus isolates showed 81.2% susceptibility to cefotaxime, 68.7% susceptibility to amikacin and 62.5% to cotrimoxazole. Enterococci showed 66.6% susceptibility to ampicillin with 100% susceptibility to vancomycin. CONS accounted for 5.04% of the aerobes with 100% susceptibility to vancomycin and oxacillin, 10(90.9%) to amikacin and ciprofloxacin. (Mamatha P.Samaga et al)

Mamatha P.Samaga et al Proteus mirabilis (19.7%) was the major gram negative aerobe isolated which showed 100% sensitivity to amikacin with 95.34 % sensitivity to ceftriaxone and ceftazidime each. It showed lowest sensitivity to ampicillin (27.9%). Pseudomonas aeruginosa showed highest sensitivity to ceftazidime (86.3%) followed by amikacin (81.8%). Klebsiella pneumoniae showed highest sensitivity to amikacin and ceftriaxone (85.71%). Klebsiella oxytoca showed highest sensitivity to ceftriaxone (87.5%). All other gram negative isolates were 100% sensitive to amikacin. This is close to the findings of the above authors who have noted highest susceptibility to amikacin. In our study, Proteus mirabilis (9.84%) was the major gram negative aerobe isolated which showed 69.2% sensitivity to amikacin with 107.6 % sensitivity to ceftriaxone and 100% sensitivity to ceftazidime. It showed lowest sensitivity to Gentamycin (53.8%).Pseudomonas aeruginosa showed highest sensitivity to Ampicillin (100%) followed by Gentamycin(72.7%). Klebsiella pneumoniae showed highest sensitivity to Ampicillin and ceftazidime(110%). Klebsiella oxytoca showed highest sensitivity to ceftriaxone (87.5%). This is close to the findings of the above authors who have noted highest susceptibility to amikacin.

Our study demonstrates the large number and variety of organisms that can be isolated from properly obtained specimens that are optimally processed. While many factors must be considered, good glycemic control including antibiotic therapy, knowledge of the usual causative organisms in these infections and their antibiotic susceptibilities will allow clinicians to make informed choices.

CONCLUSION

We encourage clinicians to obtain proper, post debridement specimens of culture and urge clinical microbiology laboratories to report all organisms, at least to the genus level, recovered from such specimens. Reports of "normal cutaneous flora" or "no S. aureus isolated" are not helpful for properly collected specimens. Also, if necrotic tissue swabs are submitted, laboratories should not be expected to waste time and resources working up organisms of questionable etiologic importance. However, susceptibility testing should be performed routinely for staphylococci and gram-negative rods with unpredictable resistance. Other organisms may be tested selectively.

Hence, awareness of the causative organisms in diabetic foot infections and their antimicrobial susceptibility pattern is essential for the institution of appropriate antimicrobial therapy.

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