Aerobic Bacterial Profile of Diabetic foot and its Antibiogram in RIMS, Ranchi - a Tertiary Care Hospital

Ankur Kumar[1], A. K. Agrawal[2], Manoj Kumar[2], Ashok Kumar Sharma[3], Pinki Kumari[1]

ABSTRACT

Introduction: Diabetic foot is the most serious complications of diabetes. Hyperglycemia impairs body defense mechanism, leads to increase infection rate. Thus for proper management it is necessary to know about most effective drug acting against isolated organism. Study aimed to know the different aerobic bacteria associated with Diabetic foot and their antimicrobial sensitivity pattern.

Material and Method: In the present study 100 specimens were collected from the out and in patients, departments of Surgery and Medicine in RIMS, Ranchi. The samples were collected from June 2014 to August 2015. Specimens were cultured using standard microbiological procedure and antibiotic susceptibility testing was performed through the Kirby Bauer's disc diffusion method recommended by "Clinical and Laboratory Standards guideline.

Result: 134 pathogens were isolated from 100 patients, an average of 1.34 organisms per lesion. The most frequently isolated pathogens were Gram-negative bacteria (56.7%), including *Pseudomonas aeruginosa* (22.4%), *Escherichia coli* (17.9%), *Klebsiella pneumonia* (15%) and Proteus sp. (1.5%). Gram-positive bacteria accounted for40.3% of all bacterial isolates. *Staphylococcus aureus* was predominant (32.8%) among Gram-positive bacteria, followed by streptococci (4.5%) and Coagulase Negative Staphylococcus (2.9%).The antimicrobial susceptibility testing, showed that vancomycin and linezolid were the most effective drugs against gram positive organisms and imipenem was the most effective drug against gram negative organisms.

Conclusion: Most of specimens were poly microbial infection and predominant bacteria were *S. aureus* and Pseudomonas sp. These wounds may require use of combined antimicrobial therapy for initial management.

Keywords: Diabetic foot, Kirby Bauer's Disc Diffusion, S. Aures.

INTRODUCTION

Diabetic foot problems are among the most serious and costly complications of diabetes. The principal pathogenetic mechanisms in a diabetic foot disease are neuropathy, infection, micro vascular dysfunction and ischemia. Infection is most often as a consequence of foot ulceration, which typically follows trauma to a neuropathic foot. Poorly controlled diabetes is prone to skin infections because elevated blood sugar reduces the effectiveness of bacteria fighting cells due to reduced resistance and immunocompromised situation.[1,2] Most of these infections are polymicrobial in nature and mixed organisms are frequently encountered. However, the spectrum of microorganisms depends mainly on microbial flora of lower limb, metabolic factors, foot hygiene and the use of antibiotics. Antibiotic resistant is also a major problem for diabetic foot patients. Multidrug resistant organisms is a potential risk factor in management of diabetic foot infections which may lead to devastating complications like systemic toxicity, gangrene formation and may herald amputation of lower extremity.[3,4] These multidrug resistant organisms are frequently resistant to many classes of antibiotics so it is necessary for the clinician to be completely aware of the prevalence rate of multidrug resistant organisms and their management strategies. So this study will help the clinicians to choose appropriate antibiotic or combination of antibiotics for the treatment of Diabetic Foot Ulcer. For better outcome of diabetic foot patients it is necessary to know their bacteriological profile and most effective drugs act on these isolated bacteria. Thereafter appropriate suitable antibiotic in full doses for full course should be instituted for the treatment of infection to prevent the development of antibiotic resistance. Study aimed to know the different aerobic bacteria associated with Diabetic foot and their antimicrobial sensitivity pattern.

MATERIAL AND METHODS

In the present study 100 specimens (Pus, debrided ulcer material or aspirate of material from infected wound) were collected from the different cases of Diabetic Foot. The cases were randomly taken from the out and in patients, departments of Surgery and Medicine in Rajendra Institute of Medical Sciences, Ranchi. The samples were collected from June 2014 to August 2015. This study was reviewed by ethics committee, RIMS, Ranchi. (Memo no. - 69 IEC/IAEC RIMS Ranchi). Sample was taken randomly of 100 cases with following criteria –

Inclusion criteria

Patients should be diabetics with diabetic foot infection presented with Wagner grade 1-5 ulcers and include both who are not taking and/or taking anti-diabetic treatment with/without supporting other treatment.

Exclusion criteria

Patients not fulfilling the inclusion criteria, those who do not give consent and improper, Inadequate collected sample.

Pus samples from the infected foot lesions were collected aseptically by using sterile cotton swab. These sterile cotton swab sticks were moistened with sterile normal saline before collecting the specimens. The swab sticks were extended deeply into the depth of the lesion avoiding touching of surrounding skin area around the wound. The collected samples were properly labeled and transported without any delay, to the laboratory of Microbiology Department, RIMS Ranchi.

[1]Junior Resident Academic, [2]Associate Professor, [3]Assistant Professor, Department of Microbiology, RIMS, Ranchi, Ranchi, India

Corresponding author: Dr. Ankur Kumar, JRA, Department of Microbiology, RIMS, Bariatu, Ranchi- 834009, India

How to cite this article: Ankur Kumar, A. K. Agrawal, Manoj Kumar, Ashok Kumar Sharma, Pinki Kumari. Aerobic bacterial profile of diabetic foot and its antibiogram in RIMS, Ranchi - a tertiary care hospital. International Journal of Contemporary Medical Research 2017;4(1):251-253.

Mixed type of organisms isolated	Number of patients	Single organism isolated	Number of patients
Staphylococcus aureus + Pseudomonas sp.	6	Staphylococcus aureus	24
Staphylococcus aureus + Escherichia coli	6	Coagulase Negative Staphylococcus	4
Staphylococcus aureus +Klebsiella	4	Streptococci sp.	6
Staphylococcus aureus + Candida	2	Pseudomonas sp.	16
Pseudomonas sp. + Escherichia coli	4	Escherichia coli	6
Pseudomonas sp. +Klebsiella sp.	4	Klebsiella sp.	4
Escherichia coli +Klebsiella sp.	6	Proteus sp.	2
Staphylococcus aureus + Escherichia coli. +Klebsiella sp.	2	Candida sp.	2

Table–1: Pattern of Isolation of microorganisms (n =100)

Microbial isolate		Number(Percentage)	Total	Percentage
Gram positive aerobes	Staphylococcus aureus	44 (32.8%)	54	40.3
	Coagulase Negative Staphylococcus (CoNS)	4 (2.9%)		
	Streptococci	6 (4.5%)		
Gram negative aerobes	Pseudomonas	30 (22.4%)	76	56.7
	Escherichia coli	24 (17.9%)		
	Klebsiella	20 (14.9%)		
	Proteus	2 (1.5%)		
Candida		4 (2.9%)	4	2.9

Table–2: Pathogenic microbes isolated from diabetic foot infection (n = 134)

Isoletes	Present Study	Chincholikar	Ekta
Avg. organism/patient	1.34	1.3	1.52
Predominant isolate	GNB (56.7%)	GPC	GNB
S. aureus	35.7%	31%	19%
Pseudomonas	22.4%	19%	22%
Klebsiella spp.	14.9%	18%	17%
E coli	17.9%	15%	18%
Proteus spp.	1.5%	9.3%	11%

Table-3: Comparison of studies on isolates in diabetic foot

Identification of bacterial isolates was performed by standard microbiological procedure (Macroscopic evaluation, Microscopic examination, Culture, motility and biochemical test) and antibiotic susceptibility testing was performed through the Kirby Bauer's disc diffusion method (recommended by "Clinical and Laboratory Standards Institute" guidelines[1]).

STATISTICAL ANALYSIS

Quantitative variables, Continuous demographic variables (age, sex, and others) were expressed as number and means±SD while qualitative variables were expressed as percentages.

RESULT

Out of 100 specimen 134 pathogens were isolated in consistent of 8 different types of microorganisms either single in number or mixed. Monomicrobial etiology was 65% and polymicrobial 35% (Table-1), in which Gram negative aerobes (56.7%) are predominant than Gram positive aerobes (40.3%). But predominant aerobic bacteria isolated from these infections were *S. aureus* (32.8%) followed by *Pseudomonas* sp.(22.4%), *Escherichia coli* (17.9%), Klebsiella sp.(14.9%), *Streptococci* sp.(4.5%), Coagulase Negative *Staphylococcus* (2.9%) (Table-2). The antimicrobial susceptibility testing showed that vancomycin and linezolid were the most effective drugs against gram-positive organisms and imipenem was the most effective drug against gram-negative organisms (Table-4).

DISCUSSION

In the present study, 134 organisms (monomicrobial etiology was 65% and polymicrobial 35%) were isolated from 100 patients (Table-1). Among the aerobic bacteria isolates, gram negative comprised of 56.7% and gram positive accounted for 40.3%(Table-2). The observations are similar with Ekta et al[2] while differ significantly from Chincholikar[3] in which the major organism are Gram Positive Cocci (GPC) which may be due to the role of geographical variations in microbial etiology (Table-3). In present study (Table-2) Staphylococcus species (35.7%) was most common isolate followed by Pseudomonas (22.4%), Escherichia coli (17.9%) and Klebsiella (14.9%) which is in concordance with other studies carried out in India (Bansal et al., 2008 and Viswanathan et al., 2002[4]) and outside India (Nadeem Sajjad Raja[5], DR. Naomi Kemunto Ratemo et al[6], Maryam Amini et ai[7], Sharma VK[8], Meghna Dharod[9] and Tuttolomondo et al[10]). Among the Gram negative aerobes the most commonly encountered were P. aeruginosa, K. pneumoniae and E. coli in agreement with the other studies carried out in India (Gadepalli et al., 2006[11]).

Gram positive isolates were most susceptible to vancomycin, linezolid and piperacillin/tazobactam (Table-4). These findings are similar to those reported by Kaup*et ai.,* 2014 that gram positive isolates were highly sensitive to vancomycin, teicoplanin, linezolid and chloramphenicol (Kaupet al[12].,2014). *S.aureus* in this study is most sensitive to linezolid (100%), vancomycin (95.4%) and piperacillin/tazobactam (86.4%). Similar findings by Kaup et al, 2014 reported that *S.aureus* was sensitive to vancomycin (100%), and linezolid (100%). Shriyan et al[13]., 2010 also reported *S.aureus* to be sensitive to vancomycin (100%) and linezolid (100%) (Shriyan et al., 2010). However Daniel et al[14], 2013 reported 100% vancomycin resistant *S.aureus*.

Gram negative isolates were most sensitive to colistin, imipenem and piperacillin/tazobactam (Table-4). This is in agreement with the study Rao et al., 2014 that gram negative isolates were most

Antibiotics	Number of sensitive strains (Percentage)						
	S. aureus (n = 44)	CoNS (n = 4)	Streptococci (n = 6)	Pseudomonas (n = 30)	E. coli (n =24)	Klebsiella (n = 20)	Proteus (n = 2)
Amoxicillin- clavulanic acid	28 (63.6%)	2 (50%)	4 (66.6%)	8 (26.6%)	8 (33.3%)	12 (60%)	2 (100%)
Cefotaxime	34 (77.3%)	2 (50%)	6 (100%)	12 (40%)	10 (41.6%)	2 (10%)	0 (0%)
Ciprofloxacin	32 (72.7%)		2 (33.3%)	26 (86.6%)	12 (50%)	10 (50%)	0 (0%)
Colistin	-	-	-	30 (100%)	24 (100%)	20 (100%)	2 (100%)
Erythromycin	32 (72.7%)	2 (50%)	0 (0%)	-	-	-	-
Gentamycin	28 (63.6%)	2 (50%)	2 (33.3%)	16 (53.3%)	14 (58.3%)	10 (50%)	0 (0%)
Imipenem	-	-	-	28 (93.3%)	24 (100%)	20 (100%)	2 (100%)
Linezolid	44 (100%)	4 (100%)	6 (100%)	-	-	-	-
Piperacillin-Tazobactam	38 (86.4%)	4 (100%)	4 (66.6%)	24 (80%)	18 (75%)	16 (80%)	2 (100%)
Vancomycin	42 (95.4%)	4 (100%)	6 (100%)	-	-	-	-

Table-4: Number of sensitive strains

susceptible to imipenem, amikacin and piperacillin/tazobactam (Rao et al[15]., 2014). *E.coli* showed high sensitivity to imipenem (100%), piperacillin/tazobactam (75%). This findings are also similar with a study by Kaup et al.,2014 that showed *E.coli* was most sensitive to piperacillin/tazobactam (100%), imipenem (100%). Mahmood 2000[16] also reported *E.coli* was most sensitive to piperacillin/tazobactam (100%), imipenem (100%) and meropenem (100%). *Klebsiella* showed more than 80% sensitivity only to imipenem and piperacillin/tazobactam. This is similar to those by Rao et al., 2014 that showed *Klebsiella* to be most sensitive to imipenem (76.92%), levofloxacin (76.92%) and amikacin (76.92%).

CONCLUSION

Infection was of mixed spectrum with Staphylococcus aureus being predominant single most isolate. Imipenem, colistin, piperacillin/ tazobactam were the most effective agents against Gram negative organisms while vancomycin, linezolid were the most effective agents against Gram-positives organisms.

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Source of Support: Nil; **Conflict of Interest:** None

Submitted: 01-01-2017; **Published online**: 10-02-2017