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Molecular Identification Of Different Gram Positive And Gram Negative Bacteria From Diabetic Foot Patients In Khyber Teaching Hospital (KTH) Peshawar KP Pakistan

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Abstract

The World Health Organization defines the diabetic foot as a "situation of infection, ulceration or also destruction of tissues depths of the feet, associated with abnormalities neurological and varying degrees of vascular disease peripheral in the lower limbs of patients with Diabetes Mellitus. The objective of the work was identify the prevalence of microorganisms that caused infections in diagnosed patients with diabetic foot treated at Khyber Teaching Hospital (KTH) Peshawar during the year 2020.Descriptive, cross-sectional and retrospective study. The sampling was non-probabilistic, for convenience, and 117 samples corresponding to 96 patients. Of the 117 patients with diabetic foot, 55% were mens. 28% of infections occurred in patients aged 51 to 60 years. 23 different microorganisms were isolated in the 117 samples. 82% (76) were monomicrobial, and 22% (41) polymicrobial. Among the Gram positives, the most frequently isolated microorganism was the Staphylococcus aureus 21% (23)

and Enterococcus spp. 6% (6) and among the Gram negative were the Klebsiella pneumoniae15% (17) and Acinetobacter spp. 14% (15). The results of sensitivity tests antimicrobial agents showed that 100% of the S. aureus strains were resistant to Oxacillin and high resistance of K. pneumoniae strains to Cephalosporins. Acinetobacter Strains spp. were 100% resistant to cephalosporins and Piperacillin. Isolated microorganisms and antimicrobial resistance profile as the same present coincide with the bibliography, and it is very important to implement prevention programs this pathology in order to avoid amputations in this type of patients.

Keywords: Bacteria; Foot; Diabetic.

INTRODUCTION:

Diabetes Mellitus (DM) is one of the major public health problems due to its high prevalence, morbidity, mortality and high healthcare costs that represent (1). Condition where the body is unable to control blood sugar, which can be defined as "A chronic condition that is triggered when the body loses its ability to make enough insulin or to use it effectively (2). A diabetic does not absorb glucose properly, so this remains circulating in the blood (hyperglycemia), damaging tissues over time (3). East deterioration causes health complications that they can be potentially lethal (4). It is classified into 3 main types; DM type 1 (DM1),type 2 (DM2) and gestational, which present with hyperglycemia, causing acute complications and severe, chronic, macro vascular, microvascular, can cause myocardial infarction, accident vascular brain, kidney failure, blindness, injury peripheral nerves (diabetic neuropathy) and amputations 2.Amputations and foot ulcers are frequent complications in diabetics, where the risk of lower limb amputation is approximately 40 times greater than in the population general. Mortality related to amputation immediate is estimated at 21% and survival is 67%in three years and 41% in five years. This complication is known as "diabetic foot", occupying one of the first places among the main problems health, and it is estimated that by the year 2025 the total of affected with this disease will amount to 300 millions of people around the world (5). The World Health Organization (WHO) defines the diabetic foot as a "situation of infection, ulceration or also destruction of tissues depths of the feet, associated with abnormalities neurological and varying degrees of vascular disease peripheral in the lower limbs of patients with DM (6). Diabetic foot infections are most frequently due to microorganisms from the genus Staphylococcus spp. and to a lesser extent by Streptococcus spp. Most infections are polymicrobial, and more than 50% of ulcers infected contain Gram negative rods aerobic and anaerobic, promoting the development of a rapid and progressive wet gangrene that does not prompt treatment can be fatal(7). Path gnomonic sign of fulminant infection may be subcutaneous emphysema, although this also can occur in diabetics with infections caused by less virulent microorganisms, such as Escherichia coli and other coli forms (7).The disease is mainly concentrated in the segment of the elderly, although currently there are also young people and even children who suffer, which is due to the little lifestyle healthy that are maintained, in which sedentary lifestyle and poor diet (8). In the kids and adolescents is more common type 1 that has a incidence of 1.8 per 100,000 inhabitants, which represents between 28 and 30 new cases each year (9).This indicates that there is a high probability that these patients could develop diabetic foot, such as consequence of DM.

MATERIALS AND METHODS

Descriptive, cross-sectional and retrospective study from 1st January to 31st December of the year 2020. Non- probability sampling, for convenience.117 samples were included corresponding to 94 diabetic patients, reported as carriers standing diabetic. The samples were grown on Mac Conkey agar, 5% Sheep Blood Agar and Chocolate Agar, the sowing method used was by depletion, Identification of genus and species was carried out by conventional biochemical tests. The bacteria Gram negatives were identified using the Lysine, SIM, Urea Oxidase, TSI, Citrate, Orinithine, and Phenylalanine and Gram positives were identified from tests for coagulase, bileesculin, catalase, specific latex for Staphylococcus aureus, Orinithine and Polymyxin B. For the susceptibility tests, the technique was used Kirby-Bauer. The antibiogram for Gram germs positive was performed using antibiotics Ciprofloxacin, Cefoxitin, Rifampicin, Clindamycin, Erythromycin, and Vancomycin. In the group of Gram negatives, the antibiotics used were Amoxicillin/ Ac.clavulanate, Cefotaxime, Ampicillin, Ceftazidime, Cefepime, Ciprofloxacin, Imipenem, Meropenem, Piperacillin and Colistin.

RESULTS

117 samples were analyzed from 94 diabetic patients.25% (23) of the patients were aged 51 to60 years, being the most common age group, and of the 94 patients with DM, 52% (49) were men and 48% (45)they were women. In both sexes, the highest frequency of bacterial infections occurred in the group ofage 51 to 60 years. (Table 1).

Ages	Men		Women		Total	
	n	%	n	%	n	%
21-30	4	8.2	4	8.9	8	9.0
31-40	4	8.2	2	4.4	6	6.0
41-50	9	18.4	11	24.4	20	21.0
51-60	12	24.5	11	42.4	23	25.0
61-70	11	22.4	4	8.9	15	15.0
71-80	3	6.1	7	15.5	10	11.0
81-90	6	12.2	4	8.9	10	11.0
> 91	-	-	2	4.4	2	2.0
Total	49	100	45	100	94	100

In the 117 samples analyzed, 21 types were isolated of different microorganisms. It was infections monomicrobial 80% (75) and polymicrobial the 20% (40). Of the polymicrobial cultures, 17% (16) presented 2 concomitant microorganisms and a3% (3) presented 3 infecting microorganisms.58% (12) of the microorganisms were classified as Gram negative bacteria (GNB), and 42% (9) asGram positive bacteria (BGP). The microorganism most frequently isolated was S. aureus 19% (22) followed by Klebsiella pneumoniae 13% (19) (Table 2).

Table2. Percentage distribution of isolated microorganisms of bacterial infections in

Patients with diabetic foot.

Microorganism	Frequency Per Sample	Percentage (%)
Staphylococcus aureus	22	30.1
Klebsiella pneumoniae	16	21.9
Acinetobacter spp.	14	19.1
Pseudomonas aeruginosa	11	15.0
Enterococcus spp.	6	8.2
Group A Streptococcus	4	5.5
Total	73	100

Among the CGPs, the three most common microorganisms frequently isolated were Staphylococcus aureus,19% (22), Enterococcus spp. 6% (6) and Streptococcus of group A 4% (4). Gram negative rods most frequently isolated were Klebsiella pneumoniae13% (16), Acinetobacter spp. 12% (14) and Pseudomonas aeruginosa 11% (13). Table 3.

Table3. Percentage distribution of Gram bacteria positive and Gram negative isolates From patients with diabetic foot.

Gram Bacils	Positive Frequency Per Sample	Percentage
Staphylococcus aureus	22	48,9
Enterococcus spp.	6	13,3
Staphylococcus coagulase (-)	4	8,9
Streptococcus group "A"	4	8,9
Streptococcus viridians	3	6,7
Enterococcus faecalis	2	4,4
Streptococcus spp.	2	4,4
Staphylococcus epidermidis	1	2,2
Streptococcus agalactiae	1	2,2
Total	45	100
GRAM BACILSNEGATIVES		
Klebsiella pneumoniae	16	23,2

Acinetobacter spp.	14	20,2
Pseudomonas aaeruginosa	13	18,8
Pseudomona spp.	7	10,1
Escherichia coli	5	7,2
Proteus mirabilis	4	5,8
Proteus vulgaris	4	5,8
Enterobactercloacoe	2	2,9
Klebsiella oxytoca	2	2,9
Enterobacter spp.	1	1,4
Providenciaalcalifaciens	1	1,4
Total	69	100

The results of sensitivity tests antimicrobial agents showed that 100% of the S. aureus strains were resistant to Oxacillin. The genus Enterococcus spp. was sensitive to Ampicillin, Ciprofloxacin and Vancomycin. Streptococcus group. A were sensitive to Ampicillin, Ciprofloxacin and Clindamycin, but resistant to Erythromycin. The antimicrobial susceptibility profile for K. pneumonia proved to be sensitive to quinolones and carbapenems, but resistant to the others. The Acinetobacter spp. turned out be sensitive to carbapenems and resistant to others antibiotics. The Pseudomonas spp. they were sensitive to fourth generation cephalosporins, quinolones and carbapenems. Table 4.

Table 4. Antimicrobial sensitivity of Gram bacteria positives and negatives isolated From patients with foot diabetic.

Antibiotics	Staphylococcus aureus				Enterococcus spp.				Streptococcus Group A			
	S		R		S		R		S		R	
	N	%	N	%	N	%	N	%	N	%	N	%
Ampicicline	-	-	-	-	6	100	0	0	4	100	0	0
Ciprofloxacin	20	90,8	2	9,1	4	66,6	2	33,3	4	100	0	0
Clindamycin	17	77,2	5	22,7	-	-	-	-	4	100	0	0
Erythromycin	18	81,8	4	18,1	-	-	-	-	0	0	4	100
Levofloxacin	34	100	0	0	-	-	-	-	-	-	-	-
Oxacycline	0	0	22	100	-	-	-	-	-	-	-	-
Rifampicin	22	100	0	0	-	-	-	-	-	-	-	-
Vancomycin	22	100	0	0	6	100	-	0	-	-	-	-
Antibiotics	Klebsiellapneumoniae				Acinetobacter spp.				Pseudomona aeruginosa			

	S		R		S		R		S		R	
	N	%	N	%	N	%	N	%	N	%	N	%
Ampicicline	0	0	16	100	0	0	14	100	-	-	-	-
Cefotaxime	5,8	36,3	10,1	63,3	0	0	14	100	-	-	-	-
Cefepime	5	31,2	11	68,7	4	28,5	10	71,4	7,4	57,1	5,5	42,8
Ciprofloxacin	9,8	61,5	6,1	38,4	3	21,4	11	78,6	9,2	71,4	3,7	28,5
Imipenem	16	100	0	0	8	57,1	6	42,8	8,3	64,2	4,6	35,7
Meropenem	16	100	0	0	10	71,4	4	28,5	13	100	0	0
Piperacycline	3	18,7	13	81,2	0	0	14	100	9,2	71,4	3,7	28,5
Piperazilin / Tazo	5	31,2	11	68,7	3	21,4	11	78,5	6,5	50	6,5	50

Regarding the distribution of microorganisms by sex, the highest number of infections by Pseudomonas spp. and S. aureus occurred in the sex female, while in males, the majority of the infectious ones were produced by S. aureus, K.pneumoniae and Pseudomonas spp. Table 5.

Table5. Percentage distribution of microorganisms by sex isolated from Patients with diabetic foot.

WOMEN			MAN		
GRAM NEGATIVE BACTERIA	FREQUENCY	%	GRAM NEGATIVE BACTERIA	FREQUENCY	%
Pseudomonas spp.	11	22	Klebsiella spp.	10	16,4
Acinetobacter spp.	9	18	Pseudomonas spp.	10	16,4
Klebsiella spp.	7	14	Proteus spp.	6	9,8
Proteus spp.	2	4	Acinetobacter spp.	5	8,2
Providencia spp.	1	2	Enterobacter spp.	2	3,3
E. coli	1	2	E. coli	2	3,3
			Citrobacter spp.	1	1,6
GRAM BACTERIA POSITIVE			GRAM BACTERIA POSITIVE		
Staphylococcus spp.	10	20	Staphylococcus spp.	16	26,2
Streptococcus spp.	4	8	Streptococcus spp.	5	8,2
Enterococcus spp.	4	8	Enterococcus spp.	4	6,6

Enterobacter spp.	1	2			
TOTAL	50	100	TOTAL	61	100

DISCUSSION

The Current study show the prevalence rate of bacterial infection in Diabetic Foot Patients is 52% in men with a 25 % distribution in the age group 61 to 60 years. Other study show different result from our study as other study show the frequency rate of infection in men is 26.9%. (10) In our study, 20% of the infections were polymicrobial. These results are to be expected, as that in a review conducted in Wales by Howell-Jones et al. in 2005, he mentions that the microflora of diabetic foot ulcers are almost always polymicrobial, presenting from 2 to 4Concomitant bacteria in infection (11).Studies using molecular techniques emphasized the complex ecology of these wounds and using technique use conventional the mean number of bacteria per ulcer has a range of 1.6 to 4.4, observing that ulcers that do not show signs of infection contain more than one bacterial species. In an investigation Staphylococcus epidermidis was isolated in 20.6%of diabetic foot ulcers, Pseudomona aeruginosa in a range of 7 to 33%, other species isolated were E. coli, Enterobacter cloacae, Klebsiella spp., Streptococcus spp., Enterococcus spp. and Proteus spp. The most frequent anaerobic bacteria were Bacteroides spp. in 12% and Peptos treptococcus spp. On 8% (9). The most frequently isolated microorganism was the S. aureus, which is part of the normal microbiota of the human body, which can cause diseases opportunists. Although the mucous membranes of the skin favor adherence to S. aureus, offer a mechanical barrier very effective against tissue invasion. When is barrier is disrupted, microorganisms gain access to the underlying tissue creating a lesion with characteristic local obsessive, as occurs in the foot diabetic. S. aureus is believed to be responsible for more than 80% of suppurative diseases, since they constitute 80% of clinical isolates (12,13). On the General University Hospital "José María Morales Meseguer", in Spain, 55% of germs isolated with more frequent were Gram microorganisms positive and of these, S. aureus was the most common (33%). Pseudomonas frequently followed aeruginosa (12%) and Enterococcus spp. (9%), which coincide with the results presented in this research (14,15). In another study by the National Toxicology Center of Cuba, 63 samples, 33 were confirmed positive for S. aureus, 29 of these being Methicillin Resistant (MRSA) (16,17). Regarding the antimicrobial susceptibility profile, most of the BGP were sensitive to Ciprofloxacin, generally used to treatment of urinary tract infections, diarrhea bacterial and prostate infections, however, these same bacteria were for the most part resistant to Oxacillin and Erythromycin. Oxacillin belongs to the group of penicillins resistant to beta-lactamase, and resistance is increasingly common from Staphylococcus to these antibiotics. In these cases, combined treatment between two antibiotics of different classes that are sensitive according to the antibiogram (15). In the study by Macias AE et al, eight strains were isolated of S. aureus, of which three (38%) were MRSA (18,19). Currently In our study the analysis of gram-negative bacteria show that the majority of them were sensitive to carbapenems, and some bacteria are presenting resistance to this group of antibiotics, making more treatment of these infections is difficult (20,21). The resistance of the strains to beta-lactams is known as strains. Extended Spectrum Betalactamases (ESBL), and are microorganisms capable of producing beta-lactamase and hydrolyze the betalactam ring of penicillins and cephalosporins. In the Spanish study cited previously, E. coli presented almost 30% of resistance to the combination of Amoxicillin with Clavulanic Acid and Ciprofloxacin. In other research, of the 68 Gram negative rods isolates, 24 were resistant to Ciprofloxacin

(35%),and in the 55 isolated Enterobacteriaceae and 4 (7%) were ESBL (22).

Conclusion

In conclusion, most foot infections diabetic were monomicrobial, being S. aureus,K. pneumaniae and Acinetobacter spp. with a profile very high antimicrobial resistance. It would be very important to implement prevention programs this pathology in order to avoid amputations in this type of patients.

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