Pattern of Bacteriological Culture and Antimicrobial Sensitivity in Diabetic Foot Ulcer: A Cohort Study from District Dera Ismail Khan

Wasim Ahmad[1]
Saqibah Rehman[1]
Amna Khalid[2]
Dastageer Wahid[1]
Saima Bashir[1]

[1]Gomal Medical College, Khyber Medical University, Dera Ismail Khan, Pakistan
[2]Army Medical College, National University of Medical Sciences (NUMS), Islamabad, Pakistan

Abstract. The objective of current study is to identify the bacteria causing infection, drug sensitivity and its effects on the outcome in a diabetic foot ulcer. The article is based on cross sectional descriptive study. This study was carried out at Department of Surgery, District Teaching Hospital Dera Ismail Khan, Pakistan in collaboration with Pathology Department of Gomal Medical College, Dera Ismail Khan, Pakistan, from August 2014 to July 2016. Pus samples were collected from the lesions of diabetic foot and were sent to Pathology laboratory for Culture and Sensitivity analysis. Lesions were categorized according to Wagner's classification. The data was analysed on SPSS version 22. Total 98 patients were included in the study. Male patients were 78 (79.5%) and females were 20 (20.4%). Out of 98 patients, 51 (52%) patients were suffering from diabetes for more than 10 years. A total number of 20 bacteria were included in this study and 84 specimens out of 98 were culture positive. The bacteria isolated in these 84 specimens were further analysed and it was concluded that 46(55%) had single bacterium infection (monomicrobial). While in 36(43.3%) patients two bacteria were isolated from their wounds. However only one patient presented with three bacteria (polymicrobial). The isolated specimens included 98 Gram negative bacteria and 78 Gram positive bacteria. Overall S. aureus (n=35) (41.6%) was the most common bacterium grown from diabetic foot ulcers, it was followed by P. aeruginosa (n=23) (27.3%). While 40 (47.6%) had only gram positive organisms infecting their ulcers. However, specimen from 44 patients (52.3%) grew only gram negative organisms. S. aureus was mostly sensitive to Moxifloxacin, Imipenem/Meropenem, Vancomycin and Linezolid. It showed varying sensitivity to Penicillins and Cephalosporins. The isolated 47.1% of S. aureus were found resistant to Methicillin. Majority of gram negative bacteria were found resistant to Cephalosporins and Moxifloxacin except for P. aeruginosa which showed variable sensitivity to Ceftriaxone, Ceftazidime and Moxifloxacin. Most of the isolates of gram negative rods included in the study were sensitive to Imipenem/Meropenem, Piperacillin-Tazobactam and Ticarcillin-Clavulanate. P. aeruginosa was sensitive to Amikacin in most of the cases while E. colii, Proteus and Klebsiella showed variable sensitivity to Amikacin. The obtained results allow to conclude about early diagnosis of bacterial diabetic foot infections and helps in its prompt treatment and management strategies according to drug sensitivity. It is directly affecting the outcome in diabetic foot and decreasing the amputation rate.

Key words: diabetic foot, culture, drug sensitivity Neuropathy, peripheral arterial disease, Wagner classification.

Introduction

Diabetes Mellitus (DM) is linked with 10–30% decrease in life expectancy. The morbidity and mortality associated with DM is due to its complications (Yazdanpanah et al., 2015: 37). Foot ulceration and infections caused as result of it, are one of the major complications of DM (Noor et al., 2015: 192-199). Damage to the quality of life and perilous outcomes requires prolong hospitalization which is costly for patient and administration. Diabetic foot effects 15% of the diabetic patients while people with diabetes are 15 times more liable to undergo lower limbs amputation (Noor et al., 2015: 192-199). In another study it was approximated that 12–15% of diabetic patients develop foot ulcer in their life time where incidence ranges from 4–10%. It is indicative of life time immobilize and encumbrance (Armstrong et al., 2017: 2367-2375). The prevalence of developing diabetic ulcer is as high as 25% in diabetic patients (Noor et al., 2015: 192-199; Ahmad et al., 2013: 16-18). According to approximate population of 160 million, the incidence of diabetic foot ulcer is about 10% in Pakistan (Ahmad et al., 2013: 16-18). However, the frequency of developing diabetic foot in western world was 2% according to a community based study. While 5–7% of patients have other risk factors such as loss of sensation and foot deformities (Aamir et al., 2011: 58-62). The estimated expenditure for treating a diabetic foot ulcer was 28000 dollars in 1999 (Boulton et al., 2005: 1719-1724). Foot ulceration can result into 85% of non-traumatic lower extremity amputation. These ulcers are repeatedly complicated by infection and this persistence of infection amplify the risk of amputation (Ahmad et al., 2013: 16-18; Aamir et al., 2011: 58-62). Diabetic foot infections are typically polymicrobial and their pathogens are multidrug resistant. Different studies have revealed that samples from these ulcers have grown a variety of bacteria in culture medium. Staphylococcus aureus (S. aureus) was isolated in 44% cases, Proteus in 28%, Pseudomonas aeruginosa (P. aeruginosa) in 25% and Klebsiella in 15% cases according to a study conducted in Malasia on diabetic foot (Amjad et al., 2017: 234-240). The study carried out in Iran has shown a high rate of 65% of antibiotic resistance pathogens. While a study conducted at Mirpur Khas, Pakistan revealed 66% of antibiotic resistant bacteria (Shanmugam and Jeya, 2013: 441). Infections with drug resistant organisms augment surgical intervention, poorer outcomes and elevated healthcare expenses (Boulton et al., 2005: 1719-1724; Amjad et al., 2017: 234-240; Shanmugam and Jeya, 2013: 441).

Material and Methods

This cross- sectional descriptive study was conducted at the Department of Surgery, District Headquarter teaching Hospital Dera Ismail Khan in collaboration of Pathology Department of Gomel Medical College from August 2014 to August 2016. Ethical committee of Gomal Medical College approved the study protocol. The sample size was calculated by WHO calculator. A total number of 98 patients with Diabetes Mellitus (type 1 or 2) who presented with chronic foot lesions were included in this study after taking informed written consent on predesigned questionnaire. Patients on anti-bacterial treatment were excluded.

A sterile swab was used to collect pus for culture and sensitivity. The samples were inoculated on Blood Agar and Mac Conkey Agar; the disc diffusion method was used for testing antibiotic sensitivity. All the Data was analyzed by SPSS-Version 22.

Results

Out of 98, there were 78 (79.5%) males and female were 20 (20.4%) with mean age of 56.9±11 years.

The cases positive for culture were found to be 84 out of 98. While 14 cases were negative for any bacteriological growth. Amongst the positive cases, Staphylococcus aureus 35 (41.6%) was the most common bacterium isolated from Diabetic Foot ulcers, followed by Pseudomonas aeruginosa 23 (27.3%), Escherichia coli 18(21.2%), Staphylococcus epidermidis 5 (5.9%), Proteus vulgaris 2 (2.3%) and Klebsiella 1(1.1%). The Table 1 shows, S. aureus was most often sensitive to Moxifloxacin, Imipenem/Meropenem, Vancomycin and Linezolid. However, it showed varying sensitivity to Penicillins and Cephalosporins. While 47.1% isolates of S. aureus were found resistant to Methicillin and hence considered as Methicillin Resistant Staphylococcus aureus (MRSA). Similarly, majority of the isolates of S. epidermidis were found resistant to Penicillins and Cephalosporins except Cefepime and Cefuroxime. However, most of S. epidermidis isolates were sensitive to Moxifloxacin, Imipenem/Meropenem, Vancomycin and Linezoild. None of the gram negative bacteria were sensitive to Ampicillin-Cloxacillin and Cephradine. Majority of gram negative bacteria were found resistant to Cephalosporin and Moxifloxacin except P. aeruginosa which showed variable sensitivity to Ceftriaxone, Ceftazidime and Moxifloxacin. The Table 2 shows that most of the isolates of gram negative rods included in the study were sensitive to Imipenem/Meropenem, Piperacillin-Tazobactam and Ticarcillin-Clavulanate. P. aeruginosa was found to be sensitive to Amikacin in most of the cases while E. colii, Proteus and Klebsiella showed variable sensitivity to Amikacin. Multiple organisms were identified but Staphylococcus aureus was the most common organism isolated from foot wounds. While 67 (68.4%) patients had healed and 9 (9.2%) had unhealed ulcers. Amputation was done in 21(20.9%) patients while 2 (1.5%) patients expired during study.

Table 1. Percentage of Antibiotic sensitivity pattern of Gram Positive Bacteria

Drugs	Staphlococcus aureus (n-35)	Staph- epidermidis (n-5)
Ampicillin/cloxacillin	26(38.8%)	60%
Amoxicillin/clavulanate	58.8%	50%
Cephradine	45.6%	40%
Cefuroxime	60.3%	70%
Ceftriaxone	54.4%	50%
Ceftizidime	50%	60%
Cefixime	33.8%	50%
Cefipime	64.7%	80%
Moxifloxacin	69.1%	70%
Imipenum/meropenum	80.9%	80%
Flucloxacillin	48.5%	40%
Methicillin	51.5%	70%
Vancomycin	73.5%	80%
Fusidic acid	57.4%	60%
Linezolid	69.1%	80%

Table 2. Percentage of Antibiotic sensitivity pattern of Gram Negative Bacteria

Drugs	Pseudomonas aeruginosa (n=23)	E-coli (n=18)	Proteus (n=2)	Klebsiella (n=1)
Ampicillin/cloxacillin	0%	0%	0%	0%
Amoxicillin/clavulanate	6.25%	29.7%	25%	20%

Cephradine	0%	0%	0%	0%
Cefuroxime	16.7%	16.2%	12.5%	20%
Ceftriaxone	56.3%	16.2%	12.5%	20%
Ceftizidime	50%	32.4%	25%	0%
Cefixime	4.2%	5.4%	0%	20%
Cefipime	37.5%	21.6%	37.5%	40%
Moxifloxacin	56.3%	46%	50%	20%
Imepenam/Meropenam	88.3%	83.8%	75%	80%
Piperacillin-Tozobactam	72.9%	83.8%	75%	80%
Ticarcillin-Clavulanate	85.4%	78.4%	62.5%	80%
Amikacin	75%	62.2%	37.5%	40%

Discussion

Diabetes is being on the increase across the world. It is expressed as a global epidemic of the 21st century. Diabetic foot is a foremost health problem as result of this disease which can damage the quality of life, extended stay in hospital is required which is expensive for the patient (Noor et al., 2015: 192-199; Ahmad et al., 2013: 16-18). Diabetes influences 15% of the diabetic foot related problems while the patients with uncontrolled diabetes are 15 times more liable to amputation (Yazdanpanah et al., 2015: 37; Ahmad et al., 2013: 16-18). Diabetic foot bacterial infections spread speedily, resulting into irreversible tissue damage. It may lead to lower extremity amputations if not treated judiciously and appropriately. In diabetic foot infections, the patterns of microbial infection are not steady (Rahim et al., 2016: 528-533). Consequently, continual assessment of causative organisms and their antibiotic susceptibility is needed for the choice of appropriate empirical therapy (Bessa et al., 2015: 47-52). The judicious use of antibiotics in diabetes may result into progression of drug resistant organisms. Therefore, it is better to treat only the clinically infected wounds and use narrowest-spectrum antimicrobial agent potentially. Failure to treat diabetic foot duly can lead to unfortunate outcomes as sepsis or limb amputation (Rahim et al., 2016: 528-533). The clinician should settle on the empirical therapy on the foundation of regional data available presenting prevalence of causative organisms and also think about the local antibiotic resistance patterns, in particularly in view of MRSA (Rahim et al., 2016: 528-533). This study illustrated that both gram-positive and gram-negative species were isolated from diabetics with moderate to severe diabetic foot infections who did not receive antimicrobial therapy. The mean age was 56.9 years while the mean duration of diabetes was 11.4 years. It was found to be same as the study conducted in Pakistan by Ahmad et al. (2013: 16-18). In our study 79.5% were males and 20.4% were females. It was demonstrated that large number of males presented with diabetic foot as compared to females. Similar pattern was also observed in different national studies (Ahmad et al., 2013: 16-18; Boulton et al., 2005: 1719-1724; Rahim et al., 2016: 528-533). It is most likely due to more exposure of male to external environment and increased risk of trauma to foot. Amputation was associated with foot ulceration, ankle brachial index of less than 0.9, raised HbA1C levels and peripheral neuropathy. In this study incidence of sensory neuropathy was 40.1 %. Out of 98 specimens 85.7% were found positive for bacterial growth. A total of 45% of culture positive diabetic foot ulcers had polymicrobial infection which is consistent with the findings of studies conducted by Alavi et al. (2007: 681-684) and others who have accounted for polymicrobial growth in the range of 42-52.4%. However, Anandi et al. have reported a much higher rate of polymicrobial infection (Anandi et al., 2004: 175). This

difference is due to small number of isolate per case, which was owing to the duration of ulcer and non-inclusion of anaerobic bacteria in the study. The alliance between polymicrobial infection and deep ulcers was statistically significant (p=0.006). Gram negative aerobes were the widespread (55.7%) isolates among all the bacteria cultured. This finding is consistent with studies conducted by Umadevi S et al and others (Umadevi et al., 2011). It was documented that gram positive bacteria were predominant organisms in 47% and gram negative in 57 % cases. Therefore, a changing trend in the organisms causing diabetic foot infections with gram-negative bacteria replacing gram-positive bacteria as the commonest agents was observed. Overall, S. aureus was the most common bacterium isolated (41.6%). Alavi et al. (2011: 681-684) have concluded S. aureus to be mainly common organism in diabetic foot ulcers in both regionally and internationally conducted studies (Ahmad et al., 2013: 16-18; Rahim et al., 2016: 528-533; Anandi et al., 2004: 175).

Amongst the isolated bacterium gram negative bacteria, P. aeruginosa was the most recurrent (27.3%). This finding was consistent with Rahim et al and others (Rahim et al., 2016: 528-533; Alavi et al. 2007: 681-684; Anandi et al., 2004: 175). Majority of S. aureus isolates were found sensitive to Vancomycin (73.5%), and Linezolid (69%). Amjad et al. also reported similar pattern of drug sensitivity in a national study (Amjad et al., 2017: 234-240). S. Aureus showed variable sensitivity to Penicillins and Cephalosporins, and was found resistant to most members of Penicillin and Cephalosporin group of antibiotics used in the study. This verdict was similar to observations of Rahim et al where all S. aureus isolates were resistant to the Penicillin group (Rahim et al., 2016: 528-533). A total of 47% S. aureus isolates were found resistant to Methicillin, and these were considered MRSA. This was startling situation in our setup which needs emergency measures to control MRSA. The MRSA rate in the study conducted by Reveles et al and others has shown about 17 % MRSA (Reveles et al., 2016: e0161658). This wide range of MRSA rates in these ulcers may be accounted for a number of reasons including the differences in the use of empirical antibiotics for these ulcers before presenting to the center of study, duration and grades of ulcers and the degree of contamination of wounds by the hands of hospital personnel.

Most of the gram negative bacteria were sensitive to Imipenem/Meropenem, Piperacillin- Tazobactam and Ticarcillin-Clavulanate. Imipenem was revealed as the most efficient antibiotic against gram negative bacteria by Spichler et al and others (Spichler et al., 2015: 2). Similarly, the sensitivity pattern of P. aeruginosa which was the most common gram negative bacteria isolated, to Imipenem/Meropenem, Piperacillin-Tazobactam, Ticarcillin-Clavulanate and Amikacin in this study was found to in consistent with the findings of Amjad et al. (2013) and others (Rahim et al., 2016: 528-533, Anandi et al., 2004: 175). Majority of gram negative bacteria were found resistant to oftenly used penicillins and cephalosporins. Similar sensitivity pattern was observed by Sekhar et al (Sekhar et al., 2014: 742-745) This also take account of E. coli which was isolated with a higher frequency (21%) and was in consistent with Alavi et al. (2007: 681-684). E. coli has never been isolated this frequently in any other studies on the subject. The high frequency of resistant E. coli among the gram negative isolates cannot be explained effectively. But an alarming level of antibiotic resistance was witnessed among common organisms like S. aureus, P. aeruginosa and E. Coli.

Conclusion

Bacteriological study of diabetic foot ulcer and drug sensitivity is vital in managing diabetic foot ulcer. The treatment of infection is directly related to increase in healing and

low amputation rate. This study offered an eye opening message to our setup where emerging resistance of different broad spectrum antibiotics and presence of MRSA is soaring. It is a genuine epidemic and requires efficient course of action. Pragmatic treatment of all wounds should stop instantly. Culture should be performed first in line of therapy and antibiotics should strictly be given according to their reports. MRSA should be eradicated and eliminated in the patients and in all healthcare professions.

Limitations

The study should be conducted on large sample size and on provincial or national level to avoid any bias

References

Aamir, A.H., Nasir, A., Jadoon, M.Z., Mehmood, K., Ali, S.S. (2011). Diabetic foot infections and their management in a tertiary care hospital. Journal of Ayub Medical College Abbottabad, 23(1), 58-62. Available at: http://applications.emro.who.int/imemrf/J_Ayub_Med_Coll_Abbotabad_Pak/J_Ayub_Med_Coll_Abbotabad_Pak_2011_23_1_58_62.pdf

Ahmad, W., Khan, I.A., Ghaffar, S., Al-Swailmi, F.K., Khan, I. (2013). Risk factors for diabetic foot ulcer. Journal of Ayub Medical College Abbottabad, 25(1-2), 16-18. Available at: https://jamc.ayubmed.edu.pk/index.php/jamc/article/view/1775

Alavi, S.M., Khosravi, A.D., Sarami, A., Dashtebozorg, A., Montazeri, E.A. (2007). Bacteriologic study of diabetic foot ulcer. Foot, 23(5), 681-684. https://doi.org/10.1016/j.ijid.2008.05.519

Amjad, S.S., Zafar, J., Shams, N. (2017). Bacteriology of Diabetic Foot in Tertiary Care Hospital; Frequency, Antibiotic Susceptibility and Risk Factors. Journal of Ayub Medical College Abbottabad, 29(2), 234-240. Available at: https://pdfs.semanticscholar.org/26c4/9f1ac79cd6a2d35fc54b95045eee8c393650.pdf?_ga=2.264091215.2116049015.1580152576-318957245.1568298533

Anandi, C., Alaguraja, D., Natarajan, V., Ramanathan, M., Subramaniam, C., Thulasiram, M. (2004). Bacteriology of diabetic foot lesions. Indian journal of medical microbiology, 22(3), 175. Available at: http://www.ijmm.org/text.asp?2004/22/3/175/11213

Armstrong, D.G., Boulton, A.J., Bus, S.A. (2017). Diabetic foot ulcers and their recurrence. New England Journal of Medicine, 376(24), 2367-2375. https://doi.org/10.1056/NEJMra1615439

Bessa, L.J., Fazii, P., Di Giulio, M., Cellini, L. (2015). Bacterial isolates from infected wounds and their antibiotic susceptibility pattern: some remarks about wound infection. International wound journal, 12(1), 47-52. https://doi.org/10.1111/iwj.12049

Boulton, A.J., Vileikyte, L., Ragnarson-Tennvall, G., Apelqvist, J. (2005). The global burden of diabetic foot disease. The Lancet, 366(9498), 1719-1724. https://doi.org/10.1016/S0140-6736(05)67698-2

Noor, S., Zubair, M., Ahmad, J. (2015). Diabetic foot ulcer – a review on pathophysiology, classification and microbial etiology. Diabetes & Metabolic Syndrome: Clinical Research & Reviews., 9(3), 192-199. https://doi.org/10.1016/j.dsx.2015.04.007

Rahim, F., Ullah, F., Ishfaq, M., Afridi, A.K., ur Rahman, S., Rahman, H. (2016). Frequency of common bacteria and their antibiotic sensitivity pattern in diabetics presenting with foot ulcer. Journal of Ayub Medical College Abbottabad, 28(3), 528-533. Available at: https://jamc.ayubmed.edu.pk/index.php/jamc/article/view/1416/408

Reveles, K.R., Duhon, B.M., Moore, R.J., Hand, E.O., Howell, C.K. (2016). Epidemiology of methicillin-resistant Staphylococcus aureus diabetic foot infections in a large academic hospital: implications for antimicrobial stewardship. PLoS One, 11(8), e0161658. https://doi.org/10.1371/journal.pone.0161658

Sekhar, S.M., Vyas, N., Unnikrishnan, M., Rodrigues, G., Mukhopadhyay, C. (2014). Antimicrobial susceptibility pattern in diabetic foot ulcer: A pilot study. Annals of medical and health sciences research, 4(5), 742-745. https://doi.org/10.4103/2141-9248.141541

Shanmugam, P., Jeya, M. (2013). The bacteriology of diabetic foot ulcers, with a special reference to multidrug resistant strains. Journal of clinical and diagnostic research: JCDR, 7(3), 441. https://doi.org/10.7860/JCDR/2013/5091.2794

Spichler, A., Hurwitz, B.L., Armstrong, D.G., Lipsky, B.A. (2015). Microbiology of diabetic foot infections: from Louis Pasteur to 'crime scene investigation'. BMC medicine, 13(1), 2. https://doi.org/10.1186/s12916-014-0232-0

Umadevi, S., Kumar, S., Joseph, N.M., Easow, J.M., Kandhakumari, G., Srirangaraj, S. (2011). Microbiological study of diabetic foot infections. Indian journal of medical specialities, 2(1). https://doi.org/10.7713/ijms.2011.0004

Yazdanpanah, L., Nasiri, M., Adarvishi, S. (2015). Literature review on the management of diabetic foot ulcer. World journal of diabetes., 6(1), 37. https://dx.doi.org/10.4239%2Fwjd.v6.i1.37