Mono-Microbial Diabetic Foot infections: Feasibility for Prompt Treatment

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A B S T R A C T

A detailed analysis of retrospective data on bacterial culture and sensitivity documented in a tertiary care hospital in Puducherry was carried out, with an aim of assessing the feasibility of giving prompt treatment topatients with mono-microbialdiabetic foot ulcer infections. In a patient strength of 40, consisting of 30 males and 10 females,mono-microbial infection rate was 45.0% (18/40),andpoly-microbialinfection was 55.0% (22/40). About 55.5% of patients affected by mono-microbial infection were in the age group of 50 to 69 years, among whom, males formed a majority. Infection by a single pathogen was caused by 5-species of Gram-positive (lone) pathogens(namely, Staphylococcus aureus or MRSA,or CONS, or Enterococcus species, or Streptococcus species), affecting 10 persons; and by 7-species of Gram-negative (lone) pathogens (namely, Pseudomonas species, or Pseudomonas aeruginosa, or Proteus species or Escherichia coli or Klebsiellaoxytoca). This is in contrast to the experiences of Western countries where mono--microbial diabetic foot infections are mostly reported to be caused by Gram-positive pathogens, whereas, Gram-negative pathogens are reported to be associated with mixed-flora of poly-microbial infections. The bacterial sensitivity data indicated that Amikacin was a promising drug for empirical therapy against all Gram-negative organisms involved, in mono-microbial infections, excepting Proteus vulgaris, against which Imipenem, or Levofloxacin was found to be effective. Amikacin was also effective against the three Gram-positive pathogens, namely, Staphylococcus aureus, MRSA and CONS, with the exception of Enterococcus species (against which either Penicillin or Vancomycin or Linezolid was individually effective), and Streptococcus species (against which either Penicillin, or Clindamycin was individually effective).

Introduction

Poorly controlled diabetes in a person can lead to the onset of a diabetic foot ulcer. Different micro-organisms, either alone or in combination, can be the causative agent(s), for the initiation of diabetic foot infections. Empirical therapy must be administered, by choosing appropriate antimicrobial agents, commensurate with the severity of infection, taking care to be guided by the prior knowledge of local data on bacterial epidemiology.

Tobalem and Uckay reported that diabetic foot infections could and would develop rapidly, and hence, would deserve a careful follow-up, after commencing empiric therapy regimen. Subsequently, however, a definitive antibiotic therapy must be decided, based on bacterial

culture and sensitivity test results, in order to correctly target against the microbial causative agents. Generally, local baseline data on bacterial sensitivity can be considered as an effective tool in planning an empirical therapy. Early diagnosis and prompt antimicrobial treatment, when the infection remains mono-microbial, would help obtaining a cure.

Objective of the Study

The purpose of the study was to assess the feasibility of selecting appropriate antimicrobial agents (AMAs) for combating against mono-microbial infections, in respect of each pathogen, in the case of diabetic foot ulcer patients receiving treatment, in a tertiary care hospital, in PuducherryCity (South India), during the period of about ten months, from October 2015 to July 2016.

Scope of the Study

The results of the study can be used for correctly identifying antimicrobial agents (AMAs), in case of mono-microbial infections, in order to promptly arrange for an early treatment, and for preventing further aggravation. This trend is expected to offer some reliable guidance to hospitals located in rural and peripheral-urban settings, where patients belonging to the 'low-income' groups report themselves for treatment, on sensing any initial symptoms,and fearing/suspecting an infection.

Materials and Methods

The data on bacterial culture and antibiotic sensitivity patterns were obtained from the hospital records pertaining to the period of study, in the case of each diabetic foot ulcer patient. The data on antibiotic susceptibility of the various classes of antimicrobial agents was used in the statistical analysis (6)

Results and Discussion

The total number of clinical isolates collected from the 40-diabetic foot ulcer patients was 67. As presented in Figure-1, infection by a single pathogen (mono-microbial infection) was found in 18 patients (45.0%); infection by two pathogens was 42.5% (17/40) and infection by three pathogens was 12.5% (5/40).

Table-1 presents the distribution of each pathogen, in the age groups of 30-49 years, 50-69 years, and 70-89 years, in the case of mono-microbial infections. It can be seen that the susceptible group of patients affected with mono-microbial infection among the diabetic foot ulcer patients was found to be maximum in the age-group of 50 to 69, namely, 55.5% affecting 10 patients, followed by 27.75% affecting 5 patients in the age-group of 30-49 years, and 16.65% affecting 3 patients in the age-group of 70-89 years.

The mono-microbial infection pattern, as presented in Table-1, was as follows

5 numbers of Gram-positive organisms were involved in the mono-microbial infection, namely, Staphylococcus aureus, (22.2%, infecting 4 patients), Methicillin-resistant Staphylococcus aureus (11.1%,infecting 2 patients), Coagulase negative Staphylococcus (CONS) species (11.1%,infecting 2 patients), Enterococcus species (5.5%, infecting one person), and Streptococcus species (5.5%, infecting one person).Mono-microbial infection by Gram-positive pathogens corresponded to 55.5%.

7 numbers of Gram-negative organisms were involved in the mono-microbial infections, namely, Pseudomonas species (11.1%, infecting 2 patients), Pseudomonas aeruginosa (5.5%, infecting one person), Escherichia coli (5.5%, infecting one person), Atypical

Escherichia coli (5.5%, infecting one person),Klebsiellaoxytoca (5.5%, infecting one person), Proteus mirabilis (5.5%, infecting one person), and Proteus vulgaris (5.5%,infecting one person).

Mono-microbialinfection by Gram-negative pathogens corresponded to 44.5% (out of which Enterobacteriaceae species corresponded to 27.75%, ie., 62.3% of infection by Gram-negative pathogens), implying that Enterobacteriaceaespecies could play a predominant role in causing mono-microbial infections in diabetic foot ulcers, in contrast to Pseudomonas species (inclusive of Pseudomonas aeruginosa) which cause 16.65% of mono-microbial infections, in the present case

The maximum rate of mono-microbial infection occurred in the age-group of 50 to 69 years (55.5%, infecting 10 persons). Referring to Figure-2, it can be seen that the causative agents for mono-microbial infection were mostly Gram-positive pathogens (55.5%), followed by Gram-negative pathogens (44.5%). It must be noted that the Enterobacteriaceae species correspond to 27.75% (corresponding to 62.3% of the Gram-negative pathogens).

Infection by Pseudomonas species (including P.aeruginosa) was 16.65%. Referring to Figure-3, it can be seen that the distribution of mono-microbial infection was 37.5% among the males, and 7.5% among the females, implying that diabetic foot infection was more prevalent in males than in females, due to the reality that males spend more time outdoors than females. Similarly, infection by two pathogens and three pathogens were lower among the females compared to males.

Microbial Sensitivity Data

In order to evaluate the feasibility of initiating empirical treatment for mono-microbial infections, in respect of each pathogen, the baseline data on microbial sensitivity available at the local centre were used.

The details of choices of appropriate AMAs (Anti Microbial Agents), in the case of each pathogen, are shown in Table-2, according to which the following trends were revealed:

i). Amikacin was found to be effective against Staphylococcus aureus, MRSA, and CONS, among the Gram-positive organisms.

ii). Wherever Amikacin failed, Linezolid, or Penicillin, or Vancomycin was found effective against Enterococcus species, and Penicillin or Clindamycin was effective against Streptococcus species.

iii). Amikacin was effective against all the Gram-negative organisms, excepting Proteus vulgaris against which Imipenem or Levofloxacin was found to be effective.

These choices of AMAs for combating against diabetic foot ulcer infections in the tertiary care hospital at Puducherry (South India) are agreeable to the findings of many other investigators who carried out similar studies in various other locations in South India, as cited in Table-2, column (v).

The AMAs to be tested in future studies against each pathogen are indicated in Table-2, in column (vi), based on the reports of other investigators who conducted studies in various other locations in South India. Information from the available literature suggests that Amikacin can be administered along with a 'matching class' of antibiotic, in order to avert the probable reduction in the efficacy of Amikacin, due to the production of anti-aminoglycoside enzyme (initiated by the long term use of Amikacin by the patient).

Prevalence of mono-microbial infections in various geographical locations in South India havebeen reported in varying percentages. In Kelambakkam near Chennai City (Tamil Nadu State), mono-microbial infection was reported as 50.0%,with 34.8% of Gram-positive pathogens and65.2% of Gram-negative pathogens, out of which 44.0 % was Enterobacteriacea (11). In Chidambaram Town (Tamil Nadu State), it was reported as 23.7%, with 5.8 % of Gram-positive pathogens and 73.5% of Gram-negative pathogens, out of which 58.2% was Enterobacteriacea (20).

In Bengaluru (Kanataka State), it was reported as 100.0%, with 24.4% of Gram-positive pathogens and 75.6% of Gram-negative pathogens, out of which 58.4% was Enterobacteriacea (21). In Thiruvananthapuram (Trivandrum, Kerala State), mono-microbial infection was reported as 41.7% (15).

In Manipal (near Mangalore, Karnataka State), it was reported as 67.0%, with 38.0% of Gram-positive pathogens and 62.0% of Gram-negative pathogens, out of which 37.9% was Enterobacteriaceae (16). In Nellore town (Andhra Pradesh State), mono-microbial infection was reported as 81.2% (18).

In the present study, mono-microbial infection was 45.0%, with 55.5 % of Gram-positive pathogens, and 44.5% of Gram-negative pathogens, out of which 27.75% corresponded to Enterobacteriacae species, mostly agreeing with the results reported for many other locations in South India (11,16, 20, 21).

The presence of each member of the Enterobacteriaceae species (among the Gram-negative pathogens) could imply certain hardships in selecting a mono-drug therapy, as some of the Gram-negative pathogens could correspond to either ESBL-producers, or AmpC beta-lactamase producers, or Carbapenemase-producers, or biofilm-formers, because of which the bacterial resistance to antibiotic treatment would be exhibited in varying degrees.

Khan, et.al (22) reported that significant levels of resistance were exerted against Amikacin and other AMAs, by the Lactose-Fermenting Gram-negative Bacteria (LF-GNB), namely, Escherichia coli, Proteus species, Klebsiella species, Enterobacter species and Citrobacter species (in percentages varying from 16% to 45% of resistance to Amikacin), on par with Non-Lactose-Fermenting Gram-Negative Bacteria (Non-LF-GNB), namely, Pseudomonas aeruginosa and Acinetobacter species (which exerted resistances to Amikacin in percentages varying from 25% to 48%).

The results of the present study, in respect of effective antimicrobial agents, are fairly in agreement with the findings of Rapaka, A.R., et.al (23), according to which Amikacin was effective against the Gram-negative organisms, namely, Escherichia coli, Klebsiella species, and against Gram-positive organisms, namely, Staphylococcus aureusand Coagulase Negative Staphylococcus.

However, Amikacin faced resistance from Proteus species, in the present study. This implies that Gram-negative pathogens need a careful approach in the selection of antimicrobial agents, and that sensitivity of antimicrobial agents could vary from place to place.

Amikacinwas considered to be a better choice for combating against Escherichia coli, Proteus species, and Klebsiella species, 'in case of moderate' grade of foot infections (24).In general, Aminoglycosides, used in treating diabetic foot infections, correspond to 21.46%, whereas, Quinolones correspond to

20.2%, and Cephalosporins correspond to 19.44% (25). Most of the diabetic foot ulcer patients happen to suffer from co-morbid conditions that require analgesic drugs during the treatment for curing diabetic foot ulcers (25, 26). This must be taken into account while choosing thetherapy regimen.

There was a general agreement between the bacterial species prevalent in the mono-microbial flora observed in the present study and the findings of Priyadarshini, S., et.al (11), in the sense that many Gram-negative species were individually present in the mono-microbial diabetic foot infections, whereas many investigators in foreign countries indicated that mono-microbial diabetic foot infections were mostly caused by Gram-positive pathogens, namely, Staphylococcus aureus, Streptococcus species, etc (27,27b,28).

The management of diabetic foot ulcers cannot fully rely upon antimicrobial treatment alone. It must be combined with concurrent debridement procedures and wound-dressing arrangements (29, 8, 30). The role of a microbiologist or an expert on infectious diseases must be made available at several stages of the treatment period, to enable the clinician to take judicious decisions,in the choice of antibiotic therapy, at his/her exclusive discretion.

However the baseline data on the culture-cum-sensitivity available at the local centre would be of great value, as a reference document. Trials of appropriate dosages of Ceftriaxone plus Ciprofloxacin versus Amikacin plus Ceftazidime, have been suggested by some investigators (31, 32).Peter, N., et.al (33) indicated Amikacin plus Cefotaxime as a dual therapy, and Amikacin plus Metronidazole plus Amoxicillin/clavulanic acid by way of triple therapy, probably, in the case of poly-microbial diabetic foot infections. By way of mono-therapy, Ciprofloxacin and Metronidazole were indicated.

Ramirez and Tolmasky (34) reported that Amikacin, as an aminoglycoside, could deserve the status of an essential component of armamentarium against serious infections caused by Gram-negative and Gram-positive bacterial species, and that Amikacin could be used always along with another class of antibiotic (in such a way that it would not involve any antagonistic side effect).

The efficacy of Amikacin could get reduced due to the production of aminoglycoside-modifying enzymes, particularly, AAC(6')-1b, and this is the reason thatAmicacin is administered always in combination with one more antibiotic of another 'matching' class. It was reported that un-modified therapy by Imipenem was as effective as a combination therapy by Ceftazidime/Amikacin in clinically and 'bacteriologically documented' infections (35).

A combination therapy with Amikacin and Piperacillin/tazobactam, or Imipenem and Vancomycin was reported as successful, in the treatment for diabetic foot infections (36). Ali & Al-Shabaki (37) reported that Amikacin was susceptible to all the bacterial species, namely, Staphylococcusaureus, Pseudomonas species, Escherichia coli, Klebsiella species, etc.,in percentages higher than 66.7%, excepting Pseuodomonasaeruginosa and Proteus mirabilis. This is in agreement with the findings of the present study..

The treatment approach would include procedures such as wound-cleaning, debridement, wound-dressings, antibiotic-impregnated dressings, and antibiotic treatment, based on clinical trial results (38, 39, 40, 4).

Table.1 Age Group-wise Distribution of Mono-microbial Infection

S,no.	Pathogen	30-49 Yrs.	50-69 Yrs.	70-89 Yrs.	Total
1.	*S.aureus*	…	16.65(3)*	5.55(1)*	22.20
2.	**MRSA	5.55(1)	5.55(1)	…	11.10
3.	+CONS	…	5.55(1)	5.55(1)	11.10
4.	*Enterococcus spp.*	5.55(1)	…	…	5.55
5.	*Streptococcus spp.*	…	…	5.55(1)	5.55
6.	Beta-hemolytic *Streptococcus* spp.	….	…	…	…
7.	*Pseudomonas spp.*	5.55(1)	5.55(1)	…	11.10
8.	*P.aeruginosa*	5.55(1)	…	…	5.55
9.	*Enterobacter spp.*	…	…	…	…
10.	*E.coli*	5.55(1)	…	…	5.55
11.	Atypical *E.coli*	…	5.55(1)	…	5.55
12.	ESBL-producing *E.coli*	…	…	…	…
13.	*K.pneumoniae*	…	…	…	…
14.	*K.oxytoca*	…	5.55(1)	…	5.55
15.	*Klebsiella spp.*	…	…	…	…
16.	*P.mirabilis*	…	5.55(1)	…	5.55
17.	*P.vulgaris*	…	5.55(1)	…	5.55
18.	*Proteus spp.*	…	…	…	…
19.	*Citrobacter spp.*	…	…	…	…
	Total	27.75(5)	55.50(10)	16.65(3)	~100.0

**Note : Numbers within parentheses indicate the number of patients infected with a single, specific pathogen
** MRSA = Methicillin Resistant Staphylococcus aureus); + CONS =(Coagulase Negative Staphylococcus

Table.2 AMAs selected against mono-microbial infections

S.No	Pathogen	No. of Cases	AMAs Sensitive	AMAs Of choice (references)	++ Other AMAs To be Tested
(i).	(ii)	(iii)	(iv)	(v)	(vi)
G(+) 1.	*S.aureus*	3M,1F	Ak,Cd,, V,Lin	Ak(7,8,9,10)	Amc,Cip,At,E,Dox, Cpm,E,G,Cfxtn, Tet,Dox,Oxa,Tmp/Smx,Cd, Cfzln
2.	*MRSA*	2M	Ak,V	Ak(8,11)	At,Cd, Lin,Dox Tmp/Smx
3.	*CONS*	2M	Ak,Cd.	Ak(7,12,13)	P,E,Lin, Ctr,Tet
4.	*Enterococcus*	1M	*P,V,*	Lin(14,15,13)	Amp,Amc,Clrm, Dox,G,E, I,Tet,Cip
5.	*Streptococcus sp.*	1F	P,Cd,	P(9,11)	Amp,At,E,Ctr,V, Cot,G,Teic,Rif, Amox
G(-)					
6.	*Pseudomonas sp.*	2M	Ak,Pit	Ak(7,11,13)	G,Cpm,Caz
7.	*P.aeruginosa*	1M	Ak, Pit,	Ak(8,16)	At,G,Cip,Cfs,Caz,I,Cpm,Cfxtm,Cfrxm,Ntlm cn,Pi,Tob, Tet,Tcc,Cot,Lin
8.	*E.coli*	1M	Ak, I	Ak(7,8,11,13,17,18)	G,Cfs,Pit,Tcc
9.	*Atypical*	1M	Ak,	Ak (not isolated	G,I,Cfs, Pit,Tcc
10.	*K.oxytoca*	1F	Ak	Ak(9,11,13)	Cot,Clrm,Cftxm,G, Pit,I,Tet
11.	*P.mirabilis*	1F	Ak,Pit	Ak (12,13,18)	G,I,Caz,Tet,Cip,Ctr,Cpm,Cftxm,
12.	*P.vulgaris*	1M	I, Lev	I (11,12,13,17,18,19)	,G,Pit,Cip Cpm,Cftxm,Tet, Caz.Ctr,Ntlmcn,
	Total	18			

++ Note: Additional AMAs to be included in testing the susceptibility; as per clues indicated by other investigators in South India.

Ak= Amikacin; Amox= Amoxicillin; Amc= Amoxicillin/clavulanic acid; Amp=Ampicillin; At=Aztreonam; Cd=Clindamycin; Cip : Ciprofloxacin; Clrm=Chloramphenicol; Caz= Ceftazidime; Cftxm=Cefotaxime; Cfrxm= Cefuroxime; Cftxn= Cefoxitin; Cfzln= Cefazolin; Cflxn= Cefalexin; Cfs= Cefaperazone/sulbactam; Cpm= Cefepime; Cot= Cotrimoxazole; Ctr=Ceftriaxone; Dox :Doxicycline; E=Erythromycin; G : Gentamicin; I=Imipenem; Lin=Linezolid; Lev = Levofloxacin; Ntlmcn= Netilmycin; Oxa= Oxacillin; P : Penicillin; Pi= Piperacillin; Pit :Piperacillin/tazobactam; Rif=Rifampicin; Teic= Teicoplanin; Tet=Tetracycline;Tcc= Tetracycline/clavulanicacid; Tob= Tobramycin; Tmp/smx= Trimethoprim/sulfamethoxazole; V= Vancomycin.
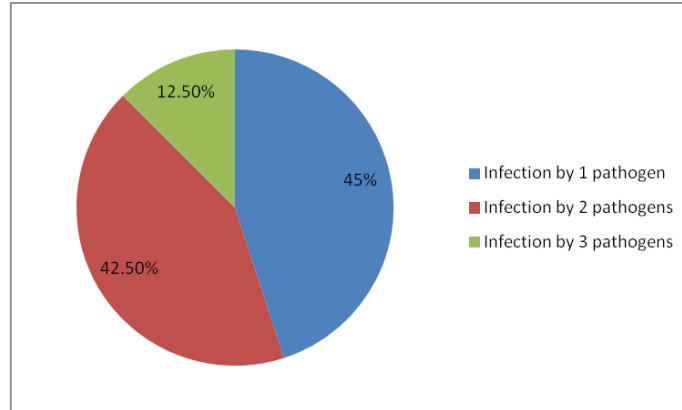
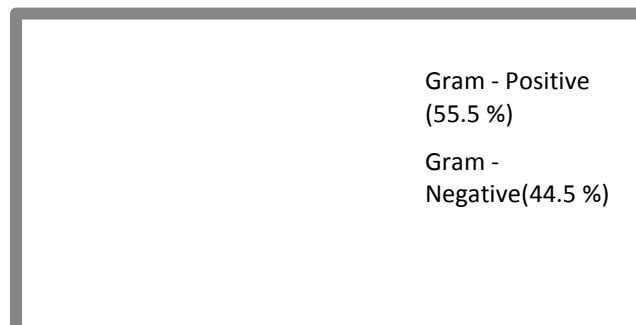Fig.1 Infection pattern in a single patient

Fig.2 Distribution of Bacterial Pathogens in Mono-microbial Infection
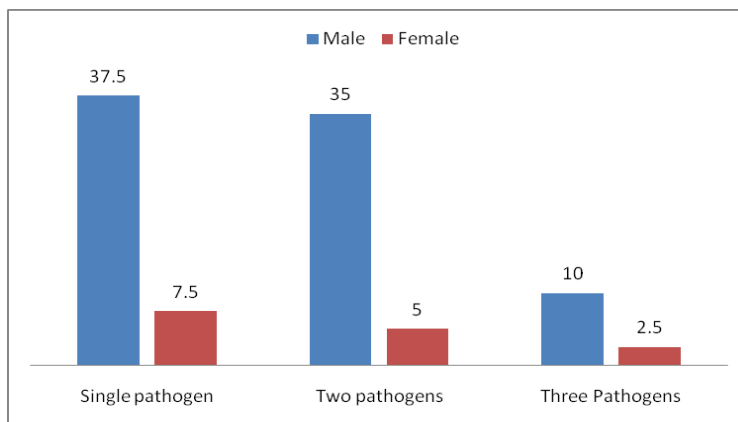

Fig.3 Infection rate among males versus females

In case of mono-microbial infections, Amikacin was found to be a promising antimicrobial agent, in treating diabetic foot infections, in the case of mono-microbial infections, against some Gram-positive pathogens such as Staphylococcus aureus, MRSA, and CONS. Amikacin faced resistance from Enterococcus species and Streptococcus species.

Amikacin was also found to be effective against some Gram-negative organisms such as Pseudomonas species, Pseudomonas aeruginosa, Escherichia coli, Atypical Escherichia coli, and Klebsiellaoxytoca.

Amikacin faced resistance from Proteus vulgaris. The best approach in treating mono-microbial infection of diabetic foot ulcers will be to select the empiric therapy based on the local baseline data, and arrange for early identification of the causative pathogen, and to select the confirmatory therapy as guided by bacterial sensitivity results.

Additional AMAs identified in Table-2 (column vi) will be helpful for future studies toward strengthening the data-bank on bacterial sensitivity at the local centre,

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