Clinical Research on Foot & Ankle
ISSN: 2329-910X

Jasmine et al., Clin Res Foot Ankle 2013, 1:3
DOI: 10.4172/2329-910X.1000121

Research Article Open Access

Bioburden vs. Antibiogram of Diabetic Foot Infection

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Abstract

Background: Proper management of diabetic foot infection requires appropriate selection of antimicrobials based on culture and antimicrobial susceptibility testing. The aim was to determine the optimal antimicrobial susceptibility to various commonly used antimicrobials for *Gram Positive Cocci* (GPCs) and *Gram Negative Bacilli* (GNBs) in patients with type 2 diabetes and foot infection and also to find out the percentage of MRSA (Methicillin Resistant Staphylococcus aureus) and ESBL (Extended Spectrum of Beta-Lactamase)-producing pathogens and their susceptibility pattern.

Materials and methods: A total of 961 (M: F 697: 264) patients with type 2 diabetes and foot infection were included in this study. After surgical debridement, pus and tissue samples were collected under aseptic conditions in sterile containers and subjected to microbiological analyses. Gram's staining, culture and sensitivity test were done along with quality control procedures.

Results: Among 961 subjects, single pathogens were isolated in 65.3%, poly-microbial organisms were isolated from 14.3% and 20.4% had sterile cultures. A total of 892 pathogens were isolated, of which 41.1% were GPCs, 57.7% were GNBs and 1.1% were *Candida Spp*. Imipenem showed the highest sensitivity of more than 95% and Amikacin above 70% against both GPCs and GNBs. Beta-lactam\beta-lactamase inhibitors showed more than 60% sensitivity to GNBs. GPCs were also >75% susceptible to doxycycline, 99.4% sensitive to vancomycin, 89.1% to linezolid, >55% to clindamycin and erythromycin. 1.35% of MRSA and 3.12% of ESBL were isolated from foot ulcers.

Conclusions: In conclusion, Imipenem was found to be the most potential antimicrobial against both GPCs, and GNBs. Among the combinations, cefipime-tazobactum and cefoperazone-sulbactum was the best choice. Anti-MRSA antimicrobials, linezolid and vancomycin and Anti-ESBLs like Imipenem and meropenem can be given to patients producing MRSA or ESBL.

Keywords: Bioburden; Antibiogram; Foot infection; Type 2 diabetes

Introduction

India, has more people (61.3 million) living with diabetes and issues related to diabetic foot complication represent a significant and often challenging clinical problem [1,2]. It was reported that 25% of diabetic individuals are anticipated to develop severe foot problems at some point in their life time and often end with amputation [3].

Diabetic Foot Infection (DFI) is a leading cause for hospital admission in India [4]. DFI management in India is similar to that practiced in other parts of the world. Nonetheless, the outcome of clinical management is different due to patient related and ulcer related measures.

Diabetic foot problem is characterized by several pathological conditions such as neuropathy, peripheral vascular disease, foot ulceration and infection with or without osteomyelitis, which leads to the development of gangrene and which even necessitates limb amputations [5]. Of all these, infection is the predominant factor that worsens the wound condition. Many organisms, alone or in combinations, can cause DFI which include Gram Positive Cocci (GPCs), and Gram Negative Bacilli (GNBs) [6]. Various microorganisms inevitably colonize the wound leading to tissue damage, followed by a host response accompanied by inflammation. Therefore, it is necessary to routinely assess different microganisms infecting the wound in addition to glycemic control and wound care [7,8].

Majority of the diabetic foot ulcers are initially treated based on empirical antibiotic policy and then tailored as per culture and sensitivity pattern. Proper management of DFI requires, appropriate selection of antimicrobials for all the pathogens based on the culture and antimicrobial susceptibility testing. Several microbiological studies conducted on DFI reported susceptibility pattern and antibiotic therapy, however those studies reported specific species based therapy [9-11]. There is lack of data on antimicrobial therapy for all the species causing DFI and the best choice of antibiotic among the classes of antibiotics for GNBs and GPCs is also unclear. Hence, the aim of this study was to determine the optimal antimicrobial susceptibility to various commonly used antimicrobials for GPCs and GNBs in patients with type 2 diabetes and foot infection and also to find out the percentage of MRSA (Methicillin Resistant Staphylococcus aureus) and ESBL (Extended Spectrum of Beta-Lactamase)-producing pathogens and their susceptibility pattern.

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Received September 28, 2013; **Accepted** November 19, 2013; **Published** November 25, 2013

Citation: Janifer J, Sekkizhar G, Kumpatla S, Viswanathan V (2013) Bioburden vs. Antibiogram of Diabetic Foot Infection. Clin Res Foot Ankle 1: 121. doi: 10.4172/2329-910X.1000121

Materials and Methods

Study subjects

A total of 961 (M: F 697: 264) patients with type 2 diabetes and foot infection who visited a tertiary care centre for diabetes between June 2012 to June 2013 were selected for this study. The selected patients have not received the first dose of antibiotics when they were enrolled in the study. Diagnosis of diabetes was made based on the WHO criteria. Age and duration of diabetes were recorded for all the patients. Wagner's grading was recorded for classification of foot infections [12]. Written informed consent was obtained from all the patients and the Institutional Ethics Committee approved the study.

Specimen collection

Specimens were collected from infected foot ulcers, as advised by current clinical guidelines [13]. Pus from an ulcer is collected at the time the ulcer is incised in the operation theatre. Pus swabs were also collected by standard method based on Levin 1 cm^2 [14]. Tissue scraping were also collected under aseptic conditions and subjected to microbiological analysis.

Microbiological analysis

The specimen was streaked on various media, such as Blood agar and MacConkey agar, (Himedia Laboratories, Mumbai, India) to obtain the bacterial growth and Gram's staining was done before culture and also for the cultured microbes. After overnight incubation at 37°C, the morphology of the pathogens was recorded. Identification of the species of pathogen was done by various bio-chemical and enzamatic tests. Susceptibility tests for the isolated pathogens were performed by disc diffusion method (Kirby-Bauer method) [15]. The pathogens were interpreted as resistant or susceptible on the basis of CLSI (Clinical and Laboratory Standards Institute) guidelines [16]. MRSA and ESBL detection was done as per CLSI guidelines. Quality control procedures were incorporated to assure the quality of stains by gram stained smears (gram positive and gram negative pathogens). Quality control strains like ATCC (American Type Culture Collection) *S. aureus*, *E. coli*, *Pseudomonas aeroginosa* were used to check the quality of both plating and biochemical media. Quality control for antibiotic discs was done by CLSI guidelines.

Pathogens		*(Total=892)*		*p value*
		n	(%)	
Gram Positive Cocci (GPCs)	*Staphylococcus spp*	*312*	*(35)*	*<0.001*
	Streptococcus spp	55	(6.1)	
Gram Negative Bacilli (GNBs)	*Enterobacteriaceae family*	*360*	*(40.4)*	*<0.001*
	Pseudomonas spp	*153*	*(17.2)*	
	Non-fermenters	2	(0.2)	
Yeast like organisms	*Candida spp*	10	(1.1)	

Table 1: Percentage of pathogens isolated from diabetic foot ulcers (DFUs).

Statistical analysis

Data are expressed as percentages. Chi square was used to identify the most prevalent species among GPCs and GNBs and also to determine the most sensitive antibiotic among the classes of antibiotics for GPCs and GNBs. A p value of <0.05 was considered as statistically significant. Statistical analysis was performed using statistical package SPSS version 16.0 (SPSS, Chicago, IL).

Results

The mean age of total study subjects was 57.4 years and the duration of diabetes varied from 1-30 years with a mean duration of 11.9 ± 7.9 years. 502 (52.2%) patients had ulcer in the left foot and 459 (47.8%) in the right foot. 152 (15.8%) patients had Wagners grade 1 ulcer, 463 (48.2%) were present with grade 2 ulcer. 267 (27.8%) had grade 3 ulcer and 79 (8.2%) were with grade 4 ulcer.

Out of 961 patients with DFI, 628 (65.3%) patients had single pathogens, 137 (14.3%) had poly-microbial infection and 196 (20.4%) patients showed sterile cultures.

Table 1 shows the details of pathogens isolated from foot ulcers. A total of 892 pathogens were isolated, of which 367 (41.1%) were GPCs, 515 (57.7%) were GNBs, and 10 (1.1%) were *Candida Spp.* Of the 367 GPCs, *Staphylococcus Spp* were significantly higher compared to *Streptococcus Spp* (p<0.001). Among the GNBs, *Enterobacteriaceae* family were significantly higher in number compared to *Pseudomonas Spp* and Non-fermenters (p<0.001) (Table 1).

MRSA were isolated from 13 patients (1.35%), and ESBLs were found in 30 (3.12%), of which 16 (53%) were *Klebsiella Spp* and 14 (47%) *E. coli* (x^2=6.10, p=0.014).

Antimicrobial susceptibility pattern

Figure 1 shows the antibiotic sensitivity pattern of GPCs and GNBs against various commonly used antimicrobial agents. Among the antibiotics tested, Imipenem was highly sensitive (90%) to both

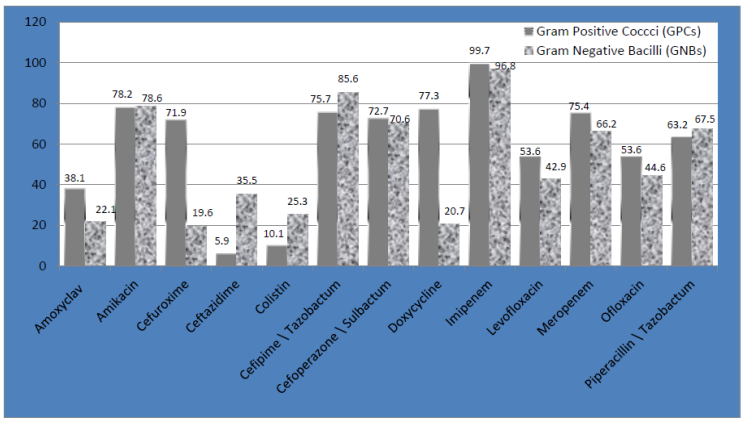

Figure 1: Susceptibility of antimicrobials against Gram positive cocci (GPCs) and Gram negative bacilli (GNBs).

Antibiotics	GPC (n=367)		p value
	n	(%)	
Cephalexin	117	(31.8)	<0.001
Clindamycin	219	(59.6)	
Erythromycin	205	(55.8)	
Linezolid	327	(89.1)	<0.001
Vancomycin	365	(99.4)	

Table 2: Comparison between antibiotics against Gram positive cocci.

Class	Antimicrobials	GNB (n=515) n	(%)	p value
Aminoglycoside	Amikacin	405	(78.6)	
Carbapenems	Imipenem	499	(96.8)	<0.001
	Meropenem	341	(66.2)	
Beta-lactam\ beta-lactamase inhibitors	Cefipime \ Tazobactum	441	(85.6)	<0.001
	Cefoperazone \ Sulbactum	364	(70.6)	
	Piperacillin \ Tazobactum	348	(67.5)	
Quinolones	Levofloxacin	221	(42.9)	0.62
	Ofloxacin	230	(44.6)	
Cephalosporins	Cefuroxime	101	(19.6)	<0.001
	Ceftazidime	183	(35.5)	
	Colistin	130	(25.3)	

Table 3: Comparison between antibiotics against Gram Negative Bacilli

GPCs and GNBs. GPCs and GNBs were also highly susceptible to amikacin, cefipime/tazobactum, cefaperazone/sulbactum, meropenem and piperacillin/tazobactum. GPCs were also highly susceptible to doxycycline and cefuroxime. Levofloxacin and ofloxacin showed >50% sensitivity against GPCs and >40% against GNBs.

Table 2 shows the susceptibility of GPCs against oral antibiotics. It was observed that Clindamycin was significantly more sensitive than Cephalexin and Erythromycin ($p<0.001$). Antibiotic sensitivity of anti-MRSA drugs revealed that vancomycin was significantly highly sensitive compared to Linezolid ($p<0.001$).

Comparison between antibiotics for GNBs was done to identify the optimal antimicrobial therapy for GNB (Table 3). It was observed that GNBs were highly susceptible to imipenem than meropenem ($p<0.001$). Among the beta-lactamase inhibitors cefipime/tazobactum showed the highest sensitivity followed by cefoperazone/sulbactum and piperacillin/tazobactum ($p<0.001$). Aminoglycoside, viz., amikacin also showed high sensitivity against GNBs. Quinolones (levofloxacin and ofloxacin) were equally sensitive (approximately 40%), and among the cephalosporins, ceftazidime showed the highest sensitivity compared to colistin and cefuroxime.

Discussion

Infection is a major cause for the non-healing chronic nature of diabetic foot ulcers. In the present investigation, we assessed the susceptibility testing of commonly used antimicrobials for all GPCs and GNBs to identify the best antimicrobial agent to treat DFI. Among the GPCs isolated, *Staphylococcus spp* were predominant in our study. Of the *Staphyloccus spp*, 56.7% were *Staphylococcus aureus* and 43.3% were Coagulase negative *staphylococci*. *Staphylococus aureus* is the most prevalent isolate in DFI together with other aerobes like *Staphylococcus epidermidis, Streptococcus spp*, *Pseudomonas aeruginosa*, *Enterococcus spp* and *Coliform bacteria* [17,18]. Among the GNBs, *Enterobacteriaceae* group of bacilli were more prevalent (40.4%) than *Pseudomonas* and other species. A similar finding was reported by a recent study by Anjali et al. [19] who showed 37.7% of E. coli, 12.6% of *Klebsiella* and 7.93% of *Proteus spp.* About 17% of *Pseudomonas Spp* were isolated in the present study, which is consistent with the finding of Abdul kadir et al. [20], who reported about 19% of *Pseudomonas Spp* in Brunei.

Another study from South India showed only the antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* from diabetic foot ulcer [21]. 1.4% of DFI was with *candida spp* of total isolates [22]. We have isolated 10 cases of *Candida spp* with the percentage of 1.1% in our study.

Prevalence of MRSA in DFIs ranged from 5% to 30% and there is an alarming trend for increase in many countries [23]. An increase in the incidence of multi-drug resistant (MDR) organisms, namely MRSA and ESBL-producing gram negative bacteria, is threatening the outcome of anti-infectious therapy in the community and in hospitalized patients [24]. 1.35% of MRSA were isolated in our study. In recent years, there has been an increase in the incidence and prevalence of ESBLs also. Currently there was paucity of data on ESBL-producing organisms from DFI especially in this part of world. Our study from South India found 3.12% of ESBL-producers.

It was reported that literature regarding antibiotic therapy is inadequate to determine the best antimicrobial agent [25]. In the current study, it was observed that Imipenem was the best choice for both GPCs and GNBs with sensitivity of 99.7% and 96.8% respectively and thus can be used to treat severe foot infection and it can also be used as a best choice for ESBL producers. Another recent study by Banashankari et al. [10] also reported 100% susceptibility to imipenem when tested for *Enterobacteriaceae* family. Other antimicrobials such as amikacin, cefipime-tazobactum, cefaperazone-sulbactum, meropenem and piperacillin-tazobactum also showed considerable sensitivities against both GPCs and GNBs in our study. Similar findings have been reported in another study from Africa where amikacin was 77.5% sensitive for *Pseudomonas spp* and 58.3% sensitive for *E. coli* [26]. A recent study from North India showed that pipercillin-tazobactum showed the highest sensitivity for polymicrobial nature of foot infection [27].

Amikacin can be a better choice for *E. coli, Proteus* and *Klebsiella spp* which can be used for severe and moderate grade of foot infections as noted in our study.

Cefipime-tazobactum combination, showed more than 80% sensitivity against *Enterobacteriaceae* family [28]. Cefipime-tazobactum combination showed 75.7% susceptiblity to GPCs and 85.6% susceptiblity for GNBs in our study. An important finding in the present study was that cefuroxime, which was commonly used only against GNBs, was more than 70% sensitive against GPCs, as well. This implies that the clinicians can incorporate cefuroxime in their panel of antibiotics against both GPCs and GNBs. Doxycycline was more than 75% sensitive against GPCs, which indicates its potential use against GPCs, including infections caused by MRSA. The present study showed that GPCs were more than 50% susceptible to the quinolones (levofloxacin) than GNBs.

Among the oral forms of antimicrobials tested for GPCs in our study, Clindamycin was found to be highly sensitive than erythromycin and cephalexin. Among the intravenous (IV) anti-MRSA antimicrobials, linezolid and vancomycin showed higher sensitivities against GPCs, with the latter showing significantly higher potential. This finding indicates that patients with known MRSA infection can be directly treated with the IV drugs instead of starting with the oral forms, since MRSA is known to have contact transmission. The most reliable predictor for MRSA as a cause of DFI is a previous history of

MRSA infection [29] but one of the limitation of the current study was nonavailablity of data on previous history of MRSA.

In vivo (response) changes that happen whenever an antimicrobial drug is administered is still unclear. Therefore, in vitro studies are necessary to derive at an appropriate decision on the use of antimicrobials in the treatment of DFIs.

In conclusion, among the most potential antimicrobials, Imipenem was found to be the best drug of choice against both GPCs and GNBs. Among the combinations, cefipime-tazobactum was the best, among quinolones: ofloxacin was a better choice, and among the cephalosporins: ceftazidime can be used for mild infections. Appropriate usage of antibiotics based on local antibiogram pattern can certainly help the clinician in reducing the bioburden of DFI, which could ultimately reduce the rate of amputations.

Acknowledgement

We acknowledge the help rendered by Mr. Dhamodharan Umapathy in the preparation of the manuscript. Also, we would like to acknowledge the patients with DFIs who participated in this research.

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