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RESEARCH ARTICLE

Bacteriological Evaluation of Diabetic Foot Infections of Patients in Al- Salam Hospital in Mosul City/ Iraq and Antibiotic Sensitivity Pattern

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Abstract

Three hundred thirty three hospitalized and ambulatory patients admitted for treatment of diabetic foot infections in Al-Salam hospital, Mosul/Iraq were enrolled in this study during the period between January/2013 to June/2014 to determine the bacterial spectrum in diabetic foot lesions and analyze the antibiotic susceptibility pattern of the isolated bacteria.

Results revealed that the highest diabetic foot infections were among patients with the age group of 46–55 years, and the lowest infections was among 16-25 years age group, and the male patients' ratio (65.5%) was higher than females (34.5%). 508 bacterial isolates were obtained from 302 positive swab specimens. Gram negative isolates were more predominant (81.2%); in contrast 18.8% of isolates revealed Gram positive isolates. The predominant Gram negative isolate was *Psuedomonas aeruginosa*, with 19.9% of isolates. E. coli were the next most frequently recovered gram-negative bacteria with 18.3%. *Staphylococcus species* was the main Gram positive identified isolates, with MSSA, MRSA, and CoN. Staphylococci presented in 10.1% of the positive specimens. Anaerobes were infrequently isolated and found in 7.5% of bacterial isolates only. The main results of antibiotic susceptibility patterns revealed that, ciprofloxacin, imipenem, and Piperacillin/Tazobactam were the most effective antibiotics against Gram negative isolates, on the other hand; the Gram positive isolates were more susceptible toward Ciprofloxacin and vancomycin followed by imipenem and polymixin B. All *Pseud. aeruginosa* and MRSA were ampicillin resistant and β lactamase producers absolutely.

Introduction

Diabetes mellitus (DM) is a serious health problem that is rapidly expanding worldwide(Shaw et al,2010). One of the more feared diabetic complications is diabetic foot, which results from a complex interaction between a numbers of risk factors**.** Neuropathy (with alterations in motor, sensitive and autonomic functions) has a central role, causing ulcerations because of trauma or excessive pressure on deformed feet that lack protective sensitivity. Once the protective layer of skin is broken, the deep tissues are exposed to bacterial colonization. Infections are facilitated by immunological deficits (especially in neutrophils), which are related to DM, and they rapidly progress to the deep tissues (Richard et al, 2011).

Diabetic patients have a lifetime risk as high as 25% for developing foot ulceration (Singh et al, 2005). Diabetic ulcers have 15–46 times higher risk of limb amputation when compared with foot ulcers due to other causes (Alavi et al, 2007). Every year, more than a million diabetic patients require limb amputation (Khanolkar et al, 2008).

The impaired micro-vascular circulation in patients with a diabetic foot limits the access of phagocytes, thus favoring the development of an infection (Singh et al, 2005). The local injuries and the improper foot wear further compromise the blood supply in the lower extremities (Khanolkar et al, 2008). Infected foot ulcer is a common cause of morbidity in diabetic patients, ultimately leading to dreaded complications such as gangrene and amputations. The lifetime risk to a person with diabetes for

developing a foot ulcer could be as high as 25% (Singh et al, 2005). Infection is most often a consequence of foot ulceration, which typically occurs after trauma to a neuropathic foot (Rao and Lipsky, 2007).

Majority of the diabetic foot lesions are initially treated empirically based on the clinical knowledge of the treating surgeon and on the prevalence of the microbial pattern in the locality and the hospital. It would be prudent if the treatment is directed based on the hierarchy of the organisms most commonly isolated and the most common antibiotic sensitivity pattern of these organisms, at the onset and thus help in a better outcome. Worldwide, several studies have been conducted with respect to the bacteriology and antibiotic sensitivity pattern(Banashankari et al, 2012).

Many studies have reported on the bacteriology of Diabetic Foot Infections (DFIs) over the past 25 years, but the results have been varied and often contradictory. These discrepancies could partly have been due to the differences in the causative organisms, which had occurred over time, geographical variations, or the type and the severity of the infection, as were reported in the studies (Citron et al, 2007). Mostly, the diabetic foot infections are mixed bacterial infections and the proper management of these infections requires an appropriate antibiotic selection, based on the culture and the antimicrobial susceptibility testing results (Zubair et al, 2010).

Numbers of studies have found that *Staphylococcus aureus* and other gram positive aerobes are the main causative pathogen usually isolated in more than 60% of cases (Lipsky et al, 2004). However, many ongoing studies including the two recent prospective studies reported a predominance of gram-negative aerobes (Shankar et al, 2005; Gadepalli et al, 2006). It is of significant importance in health care system because, in the routine practice of management of diabetic foot infections, surgeons / physicians mostly prescribe the antibacterial coverage of gram positive bacteria and the anaerobes. Failure to identify and treat a relatively susceptible organism may result in the development of multidrug resistant strain of gram negative bacteria. The role of anaerobes is particularly unclear, because in many studies specimens were not collected appropriately for anaerobic culture or due to lack of anaerobic culture setup in many institutions. Among those that did use appropriate methods, some report that anaerobes play a minimal role (Armstrong et al, 1995). While others have detected 95% prevalence of anaerobes' in a study with *Bacteroides fragilis* being the predominant anaerobe isolated (Asfar et al, 1993).

More recently, an increase in the incidence of multi-drug resistant (MDR) organisms, namely methicillin-resistant *S. aureus* (MRSA) and extended-spectrum b-lactamase (ESBL)-producing gram-negative bacteria, is threatening the outcome of anti-infectious therapy in the community and in hospitalized patients (Tascini et al, 2011) . Therefore, the current guidelines and expert opinion advise providers to obtain specimens for culture before initiating empiric antibiotic therapy to help with the selection of a definitive therapy (Joseph and Lipsky, 2010).

There are numerous articles on microbiological study of diabetic foot infections along with their susceptibility patterns for antibiotic therapy from different parts of the world. However, in Iraq no such data are available. Therefore, a prospective study was carried out to determine the relative frequency of aerobic and anaerobic bacterial isolates cultured from patients with diabetic foot infections and to assess their in-vitro susceptibility to the commonly used antibiotics. Finally to determine the range of b- lactamase production in ampicillin resistant bacterial isolates.

Materials and Methods

A prospective study was carried out on three hundred thirty three (333) diabetic patients, admitted for treatment of diabetic foot infections in Al-Salam hospital, Mosul/Iraq during the period of January/2013 to June/2014. Patients with foot infections due to any other causes such as non-diabetics - post traumatic, arterial disorder alone, venous disorder alone, non-diabetic peripheral neuropathy and secondary to implant infection were excluded.

The demographics of the patients such as age, sex, complications of diabetes were collected, and identity of microbes, and antimicrobial therapy were extracted from the files of all patients.

The samples were collected after obtaining informed consents from the patients. Specimens were collected from the deeper portion of the ulcers by using 2 sterile swabs which were dipped in sterile glucose broth. The samples were collected by making a firm, rotatory movement with the swabs. One swab was used for Gram staining and a direct Gram stained smear of the specimen was examined, while the other swab was used for inoculation of primary culture media.

The specimens were inoculated onto sheep blood agar, chocolate agar, and MacConkey's agar for isolation of aerobes, while thioglycollate medium was inoculated for isolation of anaerobic bacteria.

The inoculated plates were incubated at 37^0C overnight and the plates were examined for growth. The further processing was done according to the nature of the isolate, as was determined by Gram staining and the colony morphology (Priyadarshini et al, 2013). All isolates were identified by standard methods (Murray, 2003). The Gram-negative colonies were further identified using the API system (Biomerieux, Paris, France).

The antibiotic susceptibility testing was done by the Kirby Bauer disc diffusion method using commercially purchased antibiotic discs and interpreted according to Clinical and Laboratory Standard Institute guidelines (CLSI,2011).

MRSA, was detected according to the CLSI guidelines (CLSI,2011). Briefly, the phenotypic test for the detection of MRSA was done by using a cefoxitin (30 µg) disc. A zone of inhibition which was equal to or more than 22 mm was considered as susceptible to Cefoxitin and the organism was reported as Methicillin Sensitive *Staphylococcus aureus*. Those isolates which produced a zone of inhibition which was less than or equal to 21 mm were considered as Methicillin Resistant *Staphylococcus aureus* (MRSA).

All the ampicillin resistant isolate isolates were subjected to test for β- lactamase activity by Iodometric method (Catlin, 1975).

Statistical analysis used in present study to test the variables was done by using the Chi-square test and the p value of <0.05 was considered to be statistically significant.

Results

The present study included 333 patients, of which the male patients' ratio (65.5%) was higher than females (34.5%). The age ranged from 16 to 70 years, and the mean age was 43 years. The highest diabetic foot infections were among the age group of 46–55 years, followed by the 36–45 years age group, on the other hand the lowest infections was among 16-25 years age group. Table (1).

Table (1): Distribution of patients according to sex and age groups.

	Age group (years)						Total	
Sex	16-25	26-35	36-45	46-55	56-65	≥66	No.	%
Male	17	23	41	68	49	20	218	65.5
Female	10	16	15	34	18	22	115	34.5
Total	27	39	56	102	67	42	333	100

We cultured a total of 333 specimens; one specimen was taken from each patient, and isolated 508 microorganisms. Gram negative isolates were 81.2%; in contrast 18.8% of isolates revealed Gram positive isolates. Of the 302 positive culture specimens, 22.5% had growth of a single isolate, while therest were polybacterial isolates, with 30.2% yielding three or more organisms. Of the total positive specimens192 had given aerobic bacterial isolates solely, with percentage of 63.6, while4.3% of positive specimens were anaerobic isolate only. The rest ratio comprised of mixed (aerobic and anaerobic cultures). Table (2).

Table (2): Characteristics of diabetic foot ulcer specimens taken prior to antimicrobial therapy of 333 patients.

Characteristic	Value	
	No.	%
Specimens	333	100
Positive cultures	302	90.7
Negative cultures	31	9.3
Aerobes only	192	63.6
Anaerobes only	13	4.3
Mixed (aerobes & anaerobes)	97	32.1
Positive cultures with:		
One isolate	68	22.5
Two isolates	59	19.5
Three isolates	54	17.9
four isolates	25	8.3
Five isolates	12	4.0
Gram negative isolates	403	81.2
Gram positive isolates	105	18.8
Total isolates	508	100

The data in Table (3)explain the profile of the pathogenic isolated bacteria. A total of 508 bacterial pathogens were identified with an average of 1.53 organisms per patient.

Aerobes were presented in92.5% of bacterial isolates, with Gram negative aerobes comprised 75.2% of the isolated organisms. The predominant Gram negative isolate was *Psuedomonas aeruginosa*, with 19.9% of isolates. *E. coli* were the next most frequently recovered gram-negative bacteria with 18.3%,followed by *Proteus species* and *Klebsiella species*. The other most frequently encountered Gram negative organisms were *Acinetobacter species* and *Morganella morganii*.

Gram positive bacteria comprised17.3%of the total number of isolates. Staphylococcus species was the main Gram positive identified isolates, with MSSA, MRSA, and CoN. Staphylococci presented in 10.1% of the positive specimens. The second frequently isolated Gram positive genus was *Streptococcus species* with 4.8% of isolates. Other Gram positive genera were infrequently.

Anaerobes were infrequently isolated and found in 7.5% of bacterial isolates only, with *Bacteroides fragilis* accounted for 2.1% of all isolates, followed by the *Peptostreptococcus species*, which accounted for1.4% of isolates.

The antibiotic susceptibility patterns of the predominant Gram negative and Gram positive bacterial isolates have been tabulated in tables (4,5, and 6).The main results revealed that, ciprofloxacin, imipenem, and Piperacillin/Tazobactamwere the most effective antibiotics against Gram negative isolates, on the other hand; the Gram positive isolates were more susceptible toward Ciprofloxacin and vancomycin followed by imipenem and polymixin B. Other antibiotics showed different effectiveness on different tested bacterial isolates.

Table (3): Profile of bacterial pathogens isolated from infected foot ulcers in diabetic patients' specimens.

Isolated microorganisms	Frequency	
	No.	%
Aerobic isolates	**470**	**92.5**
Gram negative	**382**	**75.2**
Pseudomonas aeruginosa	101	19.9
E.coli	93	18.3
Klebsiella species	47	9.3
Proteus mirabilis	38	7.5
Proteus vulgaris	33	6.5
Acinetobacter species	33	6.5
Morganella morganii	20	3.9
Citrobacter species	8	1.5
Enterobacter species	6	1.2
Providencia species	3	0.6
Gram positive	**88**	**17.3**
Staph. aureus	19	3.7
MRSA	19	3.7
Coagulase negativeStaphlococci	14	2.7
Streptococcus pyogenes	12	2.4
Streptococcus pneumoniae	12	2.4
Enterococcus species	6	1.9
Micrococcus species	6	1.9
Anaerobic isolates	**38**	**7.5**
Gram negative	**21**	**4.1**
Bacteroides species	11	2.1
Veilonella species	6	1.2
Fusobacterium species	4	0.8
Gram positive	**17**	**3.4**
Peptostreptococcus species	7	1.4
Clostridium species	6	1.2
Eubacterium species	2	0.4
Other gram^{+ve}, non-spore-forming rods	2	0.4
Total	**508**	**100**

Table (4): The frequency of resistance to used antibiotics among *Pseudomonas aeruginosa, E.coli,* and *Klebsiella species* isolates.

Antibiotics	Bacterial Isolates					
	Pseudomonas aeruginosa		*E. coli*		*Klebsiella species*	
	No.	%	No.	%	No.	%
Ampicillin	101	**100**	50	**53.8**	40	**85.1**
Carbencillin	101	**100**	56	**60.2**	43	**91.5**
Piperacillin/Tazobactam	22	**21.8**	3	**3.2**	11	**23.4**
Cefozolin	66	**65.3**	55	**59.1**	36	**76.6**
Ceforuxime	78	**77.2**	45	**48.4**	30	**63.8**
Cefotaxime	77	**76.2**	42	**45.2**	31	**66.0**
Cefaperazone/Sulbactam	64	**63.4**	2	**2.2**	3	**6.4**
Cefepime	77	**76.2**	33	**35.5**	16	**34.0**
Tobramycin	68	**67.3**	53	**57.0**	30	**63.8**
Nitlimicin	44	**43.6**	22	**23.7**	9	**19.1**
Amikacin	90	**89.1**	9	**9.7**	4	**8.5**
Aztreonam	79	**78.2**	34	**36.6**	14	**29.8**
Imipenem	20	**19.8**	4	**4.3**	0	**0.0**
Livofloxacin	76	**75.2**	76	**81.7**	31	**66.0**
Ciprofloxacin	3	**3.0**	1	**1.1**	0	**0.0**
Nitrofurantion	44	**43.6**	23	**24.7**	3	**6.4**
Nalidexic acid	90	**89.1**	66	**71.0**	20	**42.6**
Cotramoxazole	96	**95.1**	69	**74.2**	37	**78.7**
Chloramphenicol	100	**99.0**	71	**76.3**	40	**85.1**
Polymixin B	98	**97.0**	33	**35.5**	11	**23.4**

Table (5): The frequency of resistance to used antibiotics among *Proteus species, Acinetobacter species,* and *Morganella morganii* isolates.

Antibiotics	Bacterial Isolates							
	Proteus mirabilis		*Proteus vulgaris*		*Acinetobacter species*		*Morganella morganii*	
	No.	%	No.	%	No.	%	No.	%
Ampicillin	29	**76.3**	26	**78.8**	22	**66.7**	15	**75.0**
Carbencillin	34	**89.5**	26	**78.8**	23	**69.7**	18	**90.0**
Piperacillin/Tazobactam	4	**10.5**	2	**6.1**	0	**0.0**	2	**10.0**
Cefozolin	17	**44.7**	18	**54.5**	18	**54.5**	5	**25.0**
Ceforuxime	16	**42.1**	13	**39.4**	10	**30.3**	7	**35.0**
Cefotaxime	16	**42.1**	16	**48.5**	14	**42.4**	7	**35.0**
Cefaperazone/Sulbactam	3	**7.9**	2	**6.1**	1	**3.0**	0	**0.0**
Cefepime	11	**28.9**	12	**36.4**	13	**39.4**	3	**15.0**
Tobramycin	30	**78.9**	12	**36.4**	10	**30.3**	7	**35.0**
Nitlimicin	4	**10.5**	3	**9.1**	0	**0.0**	1	**5.0**
Amikacin	3	**7.9**	2	**6.1**	1	**3.0**	0	**0.0**
Aztreonam	13	**34.2**	10	**30.3**	8	**24.2**	5	**25.0**
Imipenem	1	**2.6**	0	**0.0**	0	**0.0**	0	**0.0**
Livofloxacin	25	**65.8**	17	**51.5**	15	**45.5**	6	**30.0**
Ciprofloxacin	0	**0.0**	0	**0.0**	0	**0.0**	0	**0.0**
Nitrofurantion	6	**15.8**	4	**12.1**	6	**18.2**	5	**25.0**
Nalidexic acid	17	**44.7**	17	**51.5**	19	**57.6**	7	**35.0**
Cotramoxazole	26	**68.4**	22	**66.7**	20	**60.6**	8	**40.0**
Chloramphenicol	33	**86.8**	30	**90.9**	28	**84.8**	3	**15.0**
Polymixin B	32	**84.2**	21	**63.6**	23	**69.7**	5	**25.0**

Table (6): The frequency of resistance to used antibiotics among the most predominant aerobic Gram positive isolates.

Antibiotics	Bacterial Isolates									
	Staph. aureus		MRSA		CoN.Staphlococci		*Strept. pyogenes*		*Strept. pneumoniae*	
	No.	%	No.	%	No.	%	No.	%	No.	%
Ampicillin	15	**78.9**	19	**100**	12	**85.7**	7	**58.3**	6	**50.0**
Piperacillin/Tazobactam	13	**68.4**	19	**100**	11	**78.6**	5	**41.7**	4	**33.3**
Cefozolin	16	**84.2**	19	**100**	9	**64.3**	6	**50.0**	7	**58.3**
Ceforuxime	15	**78.9**	19	**100**	9	**64.3**	7	**58.3**	6	**50.0**
Cefotaxime	15	**78.9**	19	**100**	8	**57.1**	7	**58.3**	6	**50.0**
Cefaperazone/Sulbactam	10	**52.6**	17	**89.5**	3	**21.4**	4	**33.3**	5	**41.7**
Cefepime	13	**68.4**	11	**57.9**	4	**28.6**	6	**50.0**	4	**33.3**
Daptomycin	11	**57.9**	8	**42.1**	5	**35.7**	5	**41.7**	4	**33.3**
Doxycycline	10	**52.6**	6	**31.6**	7	**50.0**	3	**25.0**	4	**33.3**
Tetracycline	11	**57.9**	7	**36.8**	6	**42.9**	2	**16.7**	3	**25.0**
Tobramycin	12	**63.1**	5	**26.3**	8	**57.1**	2	**16.7**	4	**33.3**
Amikacin	2	**10.5**	4	**21.1**	0	**0.0**	0	**0.0**	2	**16.7**
Vancomycin	2	**10.5**	0	**0.0**	0	**0.0**	0	**0.0**	0	**0.0**
Livofloxacin	10	**52.6**	3	**15.8**	4	**28.6**	4	**33.3**	2	**16.7**
Ciprofloxacin	0	**0.0**	2	**10.5**	0	**0.0**	0	**0.0**	0	**0.0**
Imipenem	3	**15.8**	4	**21.1**	5	**35.7**	5	**41.7**	3	**25.0**
Nalidexic acid	6	**31.6**	8	**42.1**	5	**35.7**	6	**50.0**	6	**50.0**
Cotramoxazole	9	**47.4**	4	**21.1**	4	**28.6**	5	**41.7**	5	**41.7**
Chloramphenicol	9	**47.4**	4	**21.1**	8	**57.1**	5	**41.7**	4	**33.3**
Polymixin B	4	**21.1**	4	**21.1**	4	**28.6**	4	**33.3**	4	**33.3**

Figure (1) and table (7) explain the frequency of beta-lactamase producers among ampicillin resistant bacterial isolates. All Pseud. aeruginosa and MRSA were ampicillin resistant and β lactamase producers absolutely. The most other β lactamase producer isolates were Staph. aureus, Proteus vulgaris,E. coli,and Proteus mirabilis.

Figure (1): The frequency of beta-lactamase producers among ampicillin resistant bacterial isolates.

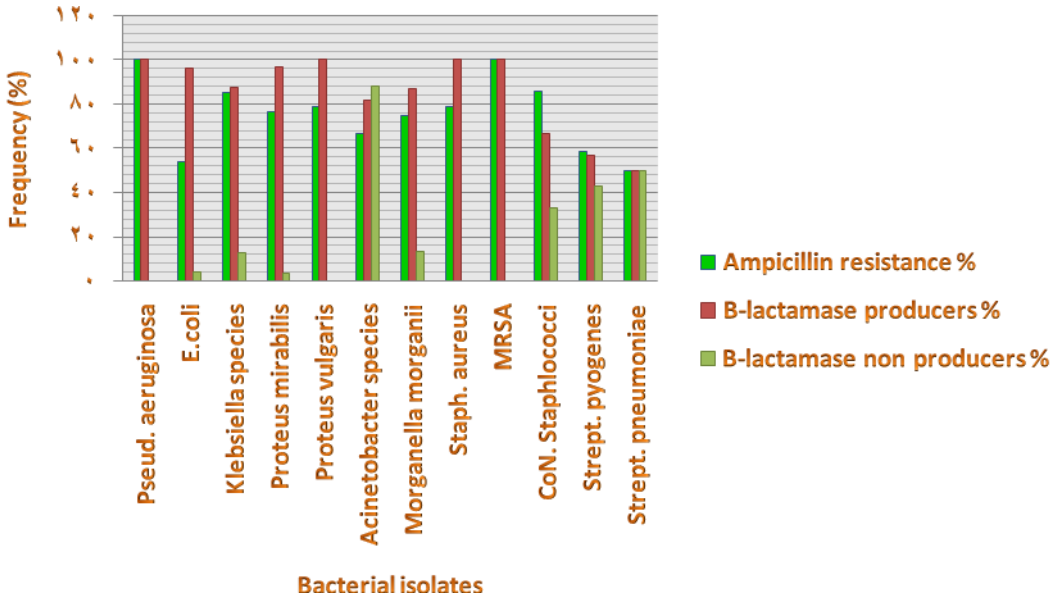

Table (7): The frequency of beta-lactamase producers among ampicillin resistant bacterial isolates.

Bacterial Isolates	Ampicillin resistance		B-lactamase producers		B-lactamase non producers	
	No.	%	No.	%	No.	%
Pseud. aeruginosa	101	**100**	101	**100**	0.0	**0.0**
E.coli	50	**53.8**	48	**96.0**	2.0	**4.0**
Klebsiella species	40	**85.1**	35	**87.5**	5	**12.5**
Proteus mirabilis	29	**76.3**	28	**96.6**	2	**3.4**
Proteus vulgaris	26	**78.8**	26	**100**	0.0	**0.0**
Acinetobacter species	22	**66.7**	18	**81.8**	4	**88.2**
Morganella morganii	15	**75.0**	13	**86.7**	2	**13.3**
Staph. aureus	15	**78.9**	15	**100**	0.0	**0.0**
MRSA	19	**100**	19	**100**	0.0	**0.0**
CoN. Staphlococci	12	**85.7**	8	**66.7**	4	**33.3**
Strept. pyogenes	7	**58.3**	4	**57.1**	3	**42.9**
Strept. pneumoniae	6	**50.0**	3	**50.0**	3	**50.0**

Discussion

Diabetic foot ulcer is one of the most common complications requiring hospitalization among diabetic patients. A diabetic foot infection is common, complex, and costly and defined as any inframalleolar infection in a diabetic that include paronychia, cellulitis, myositis, abscesses, necrotizing fasciitis, septic arthritis, tendinitis, and osteomyelitis. The most common and classical lesion, however, is the infected diabetic "mal-perforans" foot ulcer.(Shaw et al, 2010; Lipsky et al, 2004) .

The present study showed that males ratio presented with diabetic foot infection was more than females one, which is agreed with findings of number of other studies(Zubair et al, 2011; Taher, 2011). This may be due to higher level of outdoor activity among males compared to females (Zubair et al, 2011).

To our knowledge, this is the first big published study; regarding with number of patients, that is reported the infectious microbiota of diabetic foot infections inpatients located in Mosul/ Iraq.

Wound cultures revealed more than three quarter (77.5%) positive culture patients had polybacterial isolates(both Gram negative and positive, aerobic and anaerobic bacteria),while 22.5% had monobacterial infection (Table 3.In most studies, polybacterial isolates were most common(Citron et al, 2007; Tascini et al, 2011). In contrast more less studies showed that monobacterial isolates were most common like(Martínez-Gómez et al, 2009). This kind of inconsistency could be because of

geographical variations, or the types and severity of infection included in the studies.(Shankar et al, 2005; Gadepalli et al, 2006).

In present study, a total of 508bacterial species were isolated from 333patients with an average of 1.5 microorganisms per case. Studies by (Vishwanathan et al, 2002; Banashankari et al,2012)yielded results similar to present study.

As mentioned in table (3) Gram negative bacteria predominated in the study population. Gram negative isolates constituted 403(81.2%), followed by105 (18.8%) Gram- positive ones, while the anaerobic bacteria have were isolated infrequently. In several studies, gram-positive bacteria were most common (Citron et al, 2007; Martínez-Gómez et al, 2009; Lipsky et al,2004) but recent investigations reported a predominance of gram-negative aerobes (Shankar et al, 2005; Gadepalli et al, 2006).

The commonest isolate was *Pseudomonas aeruginosa*, followed by *E. coli*. The other predominant bacteria were Proteus *species* and *Staphylococcus species*. These findings correlated in many ways with those of Pappu K et al, 2011 who reported that 76% of the organisms which were isolated were gram negative bacilli, Pseudomonas *aeruginosa* being the predominant pathogen (23%), followed by *Staphylococcus aureus* (21%). Zubair et al, 2010 reported *E. coli* (26.6%) and *Pseudomonas aeruginosa* (10.6 %) as the predominant gram negative isolates. In the study of Benwan et al, 2012 which was done in Kuwait, they reported that more gram-negative pathogens (51.2%) were isolated than gram-positive pathogens (32.3%) or anaerobes (15.3%).In contrast, Zubair et al, 2010; Citron et al, 2007; and Alavi et al, 2007 reported *Staphylococcus aureus* as the predominant pathogen, which comprised 28%, 57.2%, and 26.2% of their isolates respectively. *Streptococcus pyogenes* was isolated in 10.6 % of the diabetic foot ulcers. Citron et al,2007; Zubair et al, 2010; and Ozer et al,2010 reported incidences of 15.5%, 6.6% and 6.8% of *Streptococcus pyogenes* respectively.

Banashankari et al,2012revealed that *Proteus species*, gram negative (19%) and *S.aureus*, gram positive (18%) were the predominantly isolated pathogens. In similar distribution *E.coli* (16%), *Pseud. aeruginosa* (13%) was isolated. A study by Ramani et al, 1991 also made similar observation and found *Proteus* (20.73%), *Klebsiella* (12.35%), *Pseudomonas* (11.73%) as the most common pathogens. Prabhakar et al, 1981 also showed a predominant gram negative (*Proteus, E.coli*) growth. Hefni et al, 2013reported that *Pseudomonas aeruginosa* (19.4%), *Klebsiella pneumoniae* (15.3%) and *Acinetobacter spp*. (10.2%) were the majority of the causative Gram-negative microorganisms, while among the Gram-positive microorganisms, *Staph. aureus* (10.2%), *Str. pyogenes* (7.1%), and MRSA (7.1%) were more predominantly isolated.

The results of antibiotic susceptibility patterns of the predominant Gram negative and Gram positive bacterial isolates in the present study revealed that ciprofloxacin, imipenem, and Piperacillin/Tazobactam, vancomycin, and polymixin B appeared to be the best antibiotics for therapy against tested isolates. Other antibiotics showed different effectiveness on different tested bacterial isolates.

Studies included antibiotic susceptibility patterns revealed partly similarities and differences in their results with present study in, where Sivanmaliappan and Sevanan, 2011, in their study which was done in 2011, reported that the *Pseudomonas aeruginosa* strains showed 100% resistance to Ampicillin, and Norfloxacin, 83.3% resistance to piperacillin, ticarcillin and tetracycline, 66.6% resistance to ceftazidime, imipenem, gentamicin, amikacin, tobramycin, and cotrimoxazole and 50.0% resistance to cefoperazone. 83.3% of the *Pseudomonas aeruginosa* strains were susceptible to cefotaxime. These findings correlate partly with our study findings. Shanker et al, 2005 has reported that 44% of the Pseudomonas isolates were multi drug resistant. Gadepalli et al 2006 documented that E.coli was the second highest ESBL producer in their study. An increased resistance to cefuroxime and ceftriaxone was noted among the Escherichia coli which were isolated in their study.(Sivaraman et al 2011).

Priyadarshini et al, 2013 in their study reported that among the 5 isolates of *Klebsiella pneumoniae*, 40% were ESBL producers and 20% were Carbapenamase producers. *Proteus mirabilis* showed 100% resistance to Ampicillin, Cotrimoxazole, Gentamicin and Cefazolin. More than an 80% resistance was noted, to the 3rd and 4th generation cephalosporins, quinolones and amikacin. It was susceptible to chloramphenicol, piperacillin tazobactam, imipenem, meropenem, aztreonam and cefaperazone/sulbactam. 80% of the *Proteus mirabilis* isolates were ESBL producers and 40% were Carbapenemase producers. They also revealed that all the strains of Staphylococci isolates were resistant to Penicillin and they were susceptible to Vancomycin, Teicoplanin and Linezolid. Alavil et al, 2007 reported that the *Staphylococcus aureus* isolates were resistant to all the tested antibiotics, except Ciprofloxacin and Amikacin, of which the sensitivity rates were 91% and 80% respectively. Girish et al, 2010 reported that 15% of the MRSA strains were resistant to Ampicillin, Cephalosporins and Gentamicin and that they were sensitive to Amikacin, Vancomycin, Teicoplanin and Linezolid. Raja, 2007 also report that vancomycin was effective against Gram positive cocci.

The study of beta-lactamase producers among ampicillin resistant bacterial isolates showed that all *Pseud. aeruginosa* and MRSA were ampicillin resistant and β lactamase producers absolutely. The most other β lactamase producer isolates were *Staph. aureus, Proteus vulgaris, E. coli,*and *Proteus mirabilis*. Amita et al, 2003 reported that 63.6% of *E. coli* and 86.6% of *Klebsiella species* were β lactamase producers, while all the isolates of *Pseudomonas species* were none β lactamase producers.

Resistance to β lactam antibiotics in gram positive cocci is mediated by production either of a β lactamase that hydrolyzes and in activates drug or of an altered target or targets. β lactamase as a clinically important resistance mechanism is almost exclusively found in staphylococci.(Henry, 1999).

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