Microbiological profile of Diabetic Foot Ulcers and its AntimicrobialSusceptibility Pattern at Tertiary Care HospitalValsad. India.

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Introduction: Diabetic foot ulcer and infections are one of the major complications in diabetic patients leadingto frequent hospitalization and increased mortality.

Aim: To evaluate microbiological profile of diabetic foot ulcers and their antibioticsusceptibility pattern.

Method: A total number of 173 patients with Diabetic foot infections were included in this studyfor the period of two year.The samples were processed by using standard microbiological methods. The modified Kirby-Bauer's disc diffusion method was used for antimicrobial susceptibility testing. The isolates of *Enterobacteriaceae family* were initially screened for ESBL production and were further confirmed by double disk synergy test as per Clinical and Laboratory Standards Istitute (CLSI) guidelines. Reference strains of *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC-27853), *S. aureus* (ATCC 25923) and *Klebsiella* 700603 were used as control.

Results: A total of 307organisms, an average 1.26 organisms per lesion were isolated from 244 specimen. Gram negative bacteria (95.77%) were the most frequently isolated pathogen, including *Pseudomonas aeruginosa* (35.83%) followed by*Klebsiella spp*(23.12%), *Proteus spp.*(15.53%)*, E. coli* (12.05%)*, Acinetobacter spp*(5.53%)*,Citrobacter spp*(1.30%), *Morganella morganii* (0.65%). Gram positive accounted for (4.23%) includes*Staphylococcus aureus* (2.60%), *Enterococcusspp*(1.30%)*,* and*Streptococcus spp (0.32%).* Polymyxin B, Meropenem, Imipenem, Piperacillin -Tazobactam and levofloxacin were found to be more susceptible for Gram negative organisms.Linezolid, Vancomycin, Levofloxacin, Chloramphenicol, Amikacin, Gentamicin seems to be more susceptible for Gram positive organisms.50%*Methicillin resistantStaphylococcus aureus*strains wereisolated and 41.91% ESBL production was seen among*Enterobacteriaceae family.*

Conclusion: The study showed a preponderance of gram-negative organisms from thediabetic foot ulcers. It is recommended that antimicrobial sensitivity testing is necessary for initiatingappropriate antibiotic regimen which will help to reduce the drug resistance and minimize the healthcarecosts.

Keywords: Diabetic foot ulcer, antibiotic susceptibility, bacterial isolates

Introduction

We are in the era where more people are dying due to the non-communicable diseases like diabetes, cardiovasculardiseases, stroke, cancer, chronic lung diseases than from the infectious diseases.[1] The global prevalence of diabetes and its complication is continuously growing and becoming the most significant cause of morbidity and mortality. Diabetic foot is the one of the key areas of morbidity associated with diabetes.[2]Approximately one-fourth of people with diabetes will develop an ulcer during their lifetime, and as many as half of these ulcers will become infected[3].If interventionsare not taken at proper time, it can progress to systemic infection,septicaemia, amputation or even death[4].

Hyperglycaemia, neuropathy, peripheral arterial disease,trauma, impaired immunity and infections are the major predisposing factors responsible for diabetic foot ulcer.[2] Increasing incidence of multidrug-resistant organisms from diabetic foot ulcers have created a big health care problem among hospitalised patient[2].The distributions of causative organisms and the antibiotic susceptibility patterns also show variations in diverse geographical regions[5].

Therefore, early diagnosis and prompt initiation of appropriate antimicrobial therapy is essential for controlling the infection and preventing complication and improving the quality of life. The appropriate selection of antibiotics based on the antibiograms of isolates from diabetic foot infections is extremely critical for the proper management of these infections. Therefore, the aim of the present study is to evaluate the microbiological profile of diabetic foot ulcers in order to determine the relative frequencies of microbial isolates cultured from diabetic foot infections and to assess the *in vitro* antimicrobial susceptibility pattern of these isolates.

Materials and Methods

This cross-sectional study was conducted in the Department of Microbiology, GMERS Medical college and Hospital Valsad for the period of two year from May 2017 to April 2019.Ethical clearance was obtained from Institutional Human Ethics Committee. A total number of 173 patients with Diabetic foot infections hospitalised in surgical wards were included in this study. Detailed history of the patients regarding age, sex, site of lesion, occupation, and associated illness were collected on predesigned proforma.

Sample Processing

After admission to the hospital, specimens (Pus swabs, wound swabs and debrided necrotic tissue) were obtained from diabetic patients at the time of admission before starting antibiotic therapy. In those cases when significant improvement was not seen after >7 days of antibiotic treatment another specimen was collected for culture.

Criteria for obtaining the specimens for culture were as follows:

1) 1st Culture- Specimens were collected at the time of admission before starting antibiotic therapy.

2) 2nd Culture- Specimen were collected at the time of debridement (weekly).

3)3rd Culture-Specimen were collected at the time of subsequent debridement. (As and when done).

To avoid contamination of colonising flora, the wound was cleaned with normal saline thoroughly, after that samples were collected from deeper pockets and immediately

Variables	Isolates per culture	Total

transported to the microbiology laboratory. These specimens were processed for direct microscopy, aerobic/anaerobic culture and sensitivity as per the standard protocol. The samples were inoculated on Nutrient agar (NA), Mac Conkey Agar (MA) and Blood Agar (BA) plates in two sets. One set was incubated aerobically at 37°C for 18-24 hours and another anaerobically. After incubation, identification of different microbes from positive cultures was done with a standard microbiological technique which includes studying the colonial morphology, gram stain and biochemical reactions.[6] The antibiotic sensitivity testing of all isolates was performed by modified KirbyBauer's disc diffusion method on MuellerHinton agar using antibiotics as per CLSI guidelines[7]. Gram-negativebacilli from *Enterobacteriaceae* family were tested for Extended spectrum β lactamase production by using double disk synergy test by using ceftazidime (30 µg) and ceftazidime-clavulanic acid (30 µg/10 µg). Staphylococcus species were tested for methicillin resistance by using 30 µg cefoxitin disk.[7]Reference strains of *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC-27853), *S. aureus* (ATCC 25923) and *Klebsiella* 700603 were tested as control.

Statistical Analysis

Statistical analysis was done in Microsoft Excel 2010.

Result

A total number of 173 patients with Diabetic foot infections were included in this study for the period of 2year. Among these 137 (79.19%)were male and 36 (20.81%) were female. Most common age groups involved in this study was 51-60 years (34.68%) with the mean age of 54.01 years.

Age	Male	Female	Total	Percentage N=173
<30 years	7	1	8	4.62
31-40 year	18	2	20	11.56
41-50	24	11	35	20.23
51-60	50	10	60	34.68
61-70	33	11	44	25.43
>71	5	1	6	3.46
Total	137	36	173	100

Table 1- Age and Sex distribution among patients with Diabetic foot infections

Table:2 Different Variables of this study

|	1st culture	2nd culture	3rd culture	
Specimen	173	56	12	**241**
No growth	23	6	0	**29 (12.03%)**
Growth	150	50	12	**212(87.97%)**
Monomicrobial growth	97	29	4	**130(53.94%)**
Polymicrobial growth	53	21	8	**82(34.03%)**
Organisms	211	74	22	**307 (n)**
GPC	9	4	0	**13 (4.23%)**
GNB	202	70	22	**294 (95.77%)**
Staphylococcus aureus	4	4	0	**8 (2.60%)**
Streptococcus spp.	1	0	0	**1 (0.32%)**
Enterococcus spp.	4	0	0	**4 (1.30%)**
Pseudomonas spp.	74	28	8	**110 (35.83%)**
Acinetobacter spp.	16	1	0	**17 (5.53%)**
Klebsiella spp.	44	20	7	**71 (23.12%)**
Escherichia coli	27	8	2	**37 (12.05%)**
Proteus mirabilis	28	11	4	**43(14%)**
Proteus vulgaris	5	0	0	**5(1.62%)**
Morganella morganii	2	0	0	**2(0.65%)**
Citrobacter spp.	4	0	0	**4(1.30%)**
Providencia spp.	2	2	1	**5(1.62%)**
ESBL- *Klebsiella spp.*	22	6	3	**31(18.56%)**
ESBL- *Escherichia coli*	16	4	2	**22(13.17%)**
ESBL- *Proteus mirabilis*	7	2	2	**11(6.58%)**
ESBL- *Proteus vulgaris*	1	0	0	**1 (0.59%)**
ESBL- *Citrobacter spp.*	2	0	0	**2(1.20%)**
ESBL- *Providencia spp.*	1	1	1	**3 (1.79%)**
(n=167)				**70(41.91%)**
MRSA	2	2	0	**4(50%)**

173 specimens were received as first culture at the time of admission before starting of antibiotic treatment.56 specimens were processed as a 2nd culture and 12 specimens were processed as 3rd Culture. Overall241 samples were received from 173 patients. Among these29(12.08%) samples werehaving no growth and total 212 samples were showing growth. The monomicrobial growth was seen among130 samples(53.94%) and polymicrobial growth was observedamong 82 samples (34.02%).
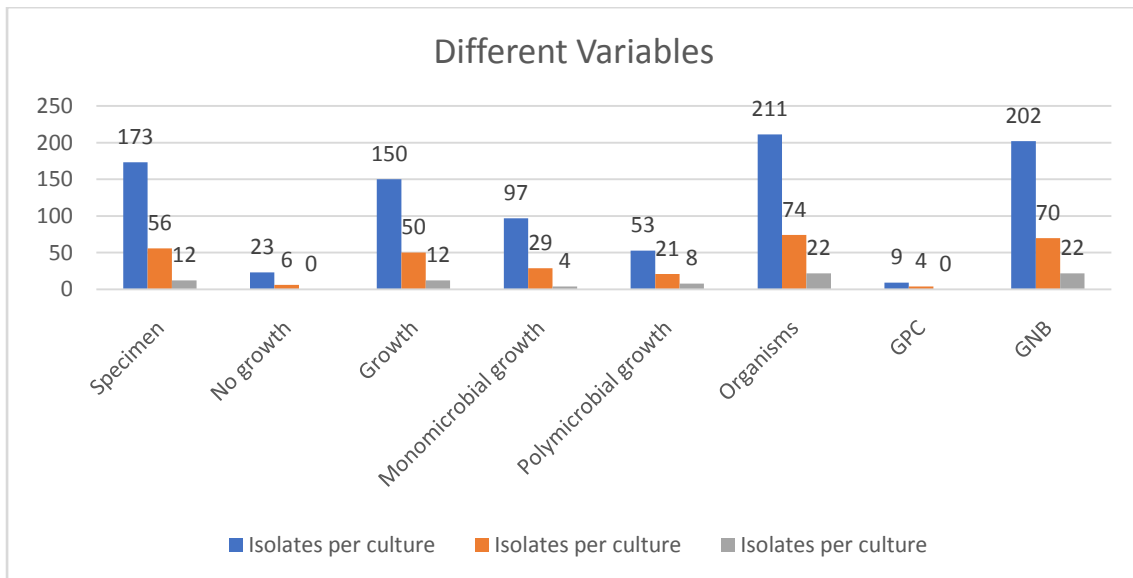
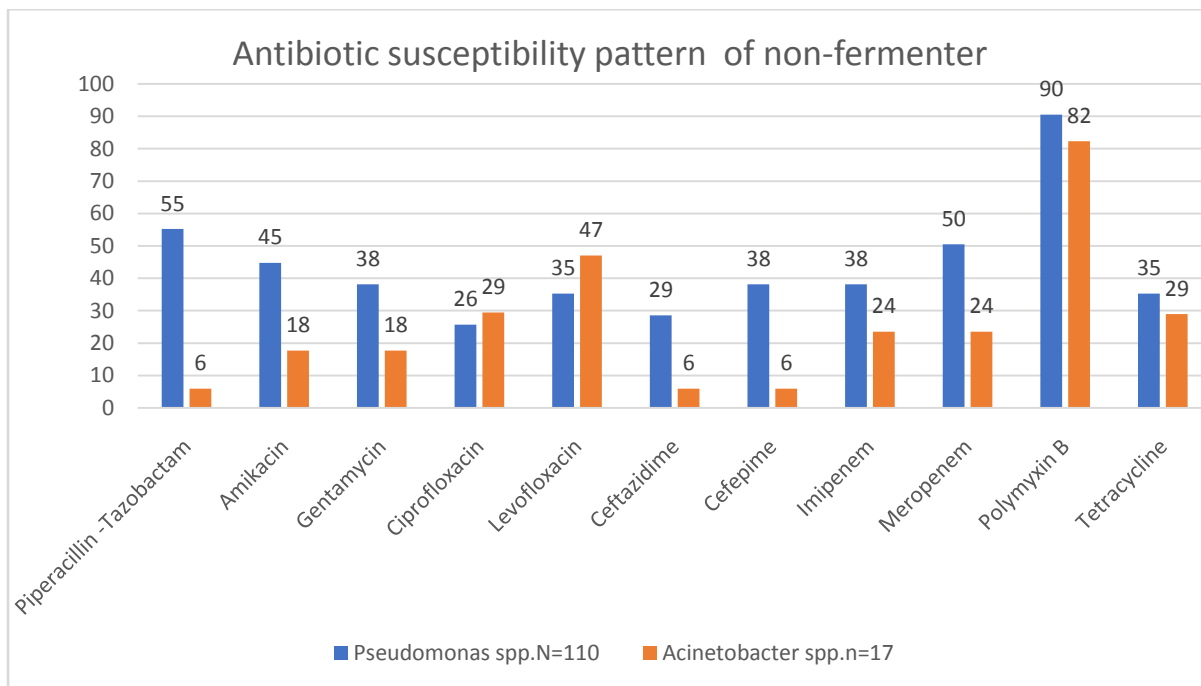Total 307 organisms were isolated. Among them 294 were Gram negative (95.77%), only 13 were Gram positive (4.23%).
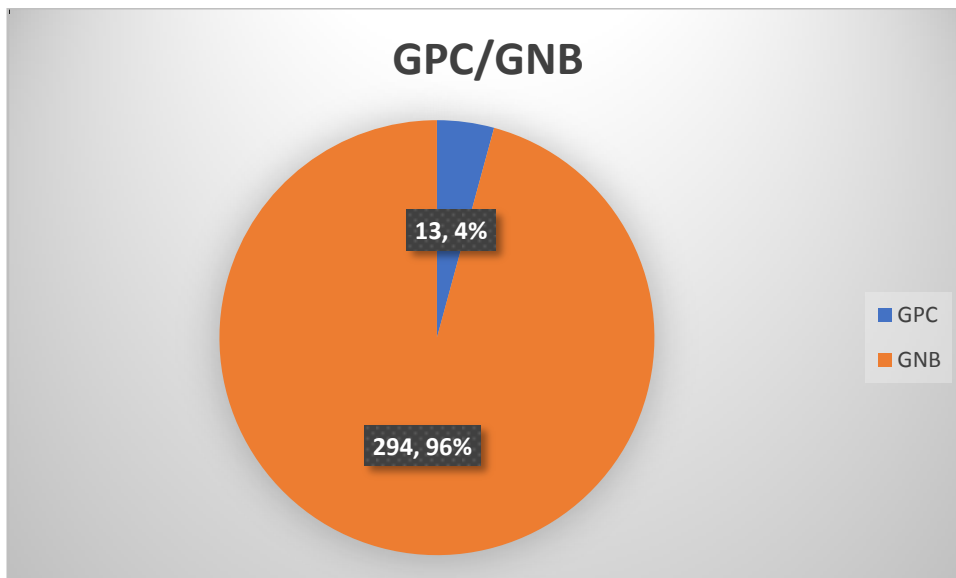
Amongstthe microbes, *Pseudomonas spp.*was the most frequentisolated (35.83%) followed by*Klebsiella spp.*(23.12%), *Proteus spp.* (15.53%), *E. coli* (12.05%), *Acinetobacter spp.*(5.53%),*Staphylococcus aureus* (2.60%),*Providencia spp*(1.62%), *Citrobacter spp.*(1.30%),*Enterococcus spp* (1.30%),*Morganella morganii* (0.65%) and *Streptococcus spp.* (0.32%).

ISOLATED ORGANISMS
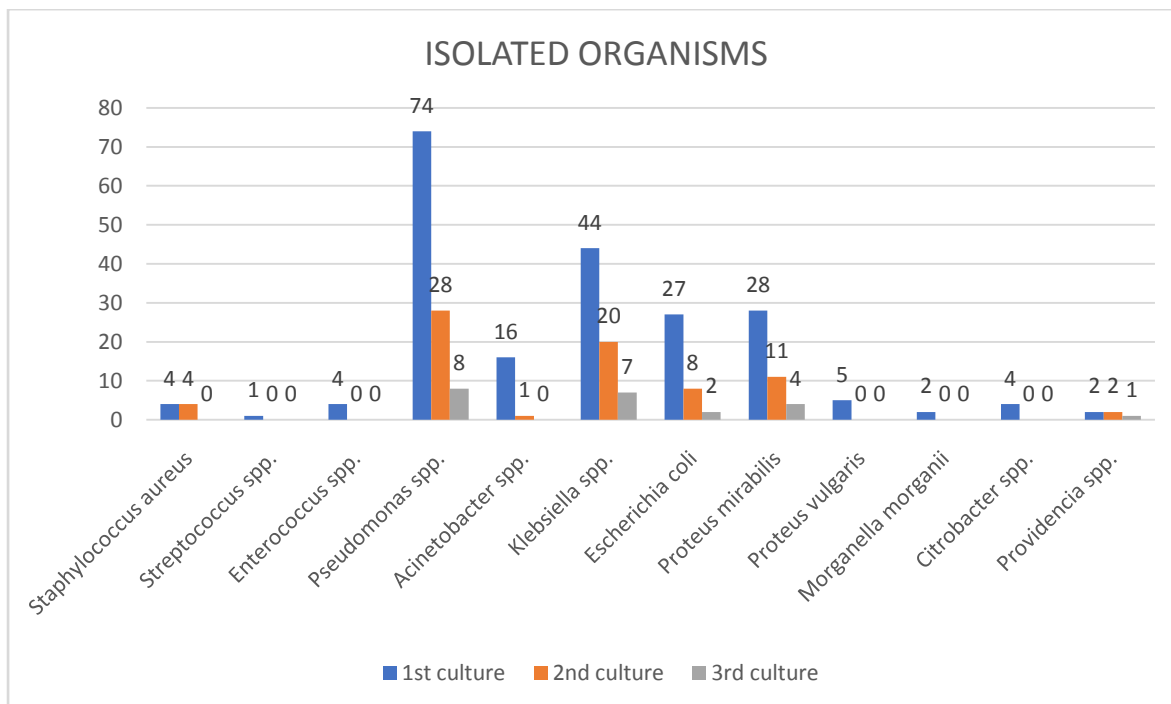
ANTIMICROBIAL SUSCEPTIBILITY PATTERN

Table-3: Antibiotic susceptibility pattern of non-fermenter

	Pseudomonas spp.(n=110) *Sensitivity percentage (%)*	*Acinetobacter spp.(n=17)* *Sensitivity percentage (%)*
Piperacillin -Tazobactam	55	6
Amikacin	45	18
Gentamycin	38	18
Ciprofloxacin	26	29
Levofloxacin	35	47
Ceftazidime	29	6
Cefepime	38	6
Imipenem	38	24
Meropenem	50	24
Polymyxin B	90	82
Tetracycline	35	41

Antibiotic susceptibility pattern of non-fermenter

Looking to the sensitivity pattern, Polymyxin B,Piperacillin -Tazobactam and Meropenem were found to be more susceptible than the other drugs in case of *pseudomonasspp.*, While Polymyxin B, levofloxacin, ciprofloxacin and tetracycline were found to be more susceptible than the other drugs for *Acinetobacter spp*,.9(8.18%) strains of *Pseudomonas aeruginosa* ,4 (23.52%) strains of *Acinetobacter spp.*were resistant to all drugs tested for antimicrobial susceptibility.

Table 4- Antibiotic susceptibility pattern of *Enterobacteriaceae*

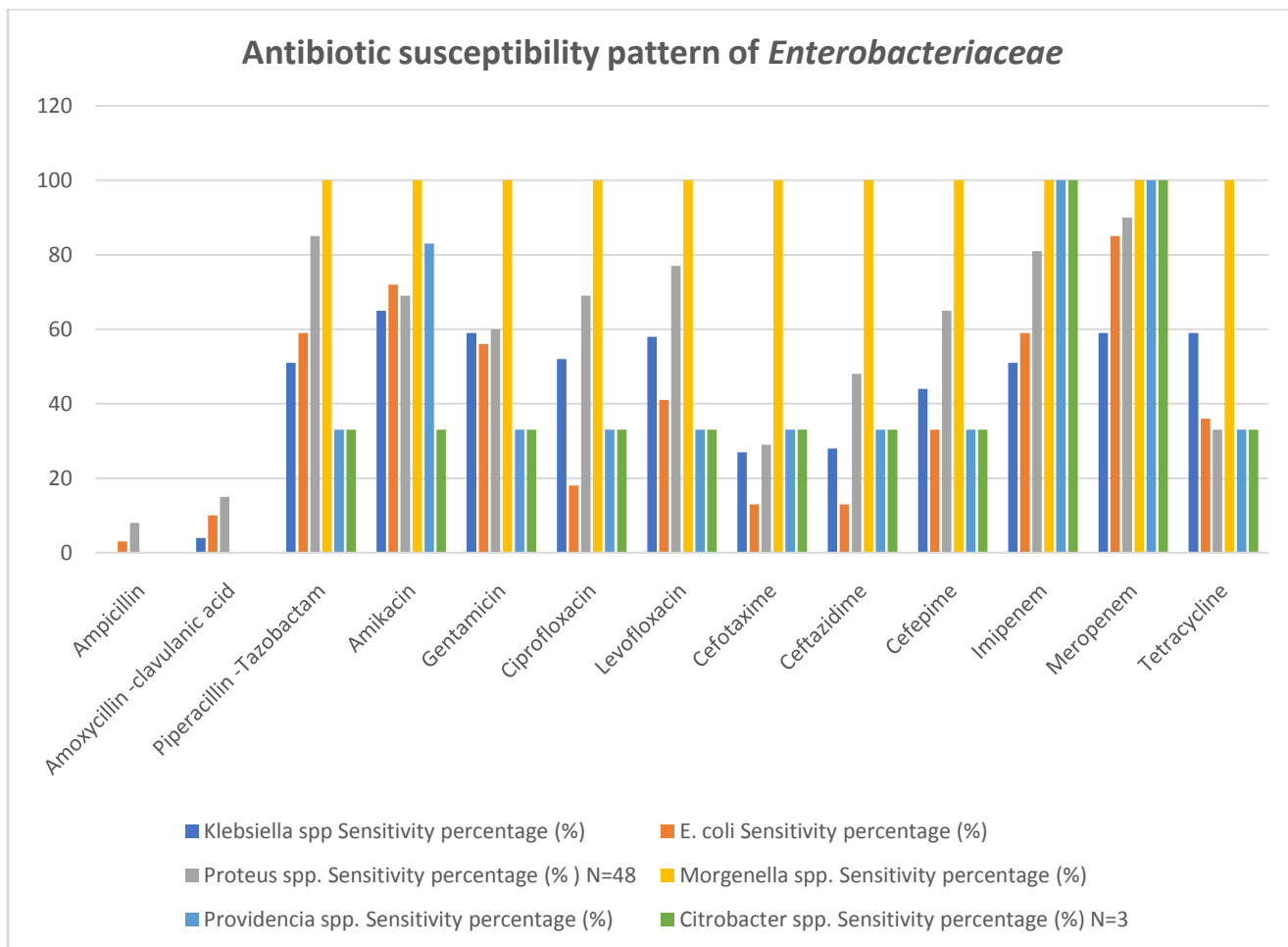|	*Klebsiella spp Sensitivity percentage (%) N=71*	*E. coli Sensitivity percentage (%) N=39*	*Proteus spp. Sensitivity percentage (%) N=48*	*Morgenella spp. Sensitivity percentage (%) N=2*	*Providencia spp. Sensitivity percentage (%) N=6*	*Citrobacter spp. Sensitivity percentage (%) N=3*
Ampicillin	--	3	8	0	0	0
Amoxycillin -clavulanic acid	4	10	15	0	0	0
Piperacillin -Tazobactam	51	59	85	100	33	33
Amikacin	65	72	69	100	83	33
Gentamicin	59	56	60	100	33	33
Ciprofloxacin	52	18	69	100	33	33
Levofloxacin	58	41	77	100	33	33
Cefotaxime	27	13	29	100	33	33
Ceftazidime	28	13	48	100	33	33
Cefepime	44	33	65	100	33	33
Imipenem	51	59	81	100	100	100
Meropenem	59	85	90	100	100	100
Tetracycline	59	36	33	100	33	33

Table 5-Antibiotic susceptibility pattern of Gram-Positive Organisms

	Staphylococcus aureus Sensitivity percentage (%) (n=8)	*Streptococcus spp.* Sensitivity percentage (%) (n=1)	*Enterococcus spp.* Sensitivity percentage (%) (n=4)
Penicillin	0	100	25
Amoxycillin-Clavulanic acid	25	-	-
Vancomycin	100	100	100
*Gentamycin High Level Gentamycin tested for *Enterococcus spp.*	25	-	75
Azithromycin	25	-	-
Erythromycin	25	-	-

Clindamycin	25	100	-
Tetracycline	100	-	-
Ciprofloxacin	50	-	-
Levofloxacin	50	100	-
Linezolid	100	100	100
Chloramphenicol	100	100	100
Ampicillin	-	100	25
Imipenem	-	100	-
Cotrimoxazole	75	100	-
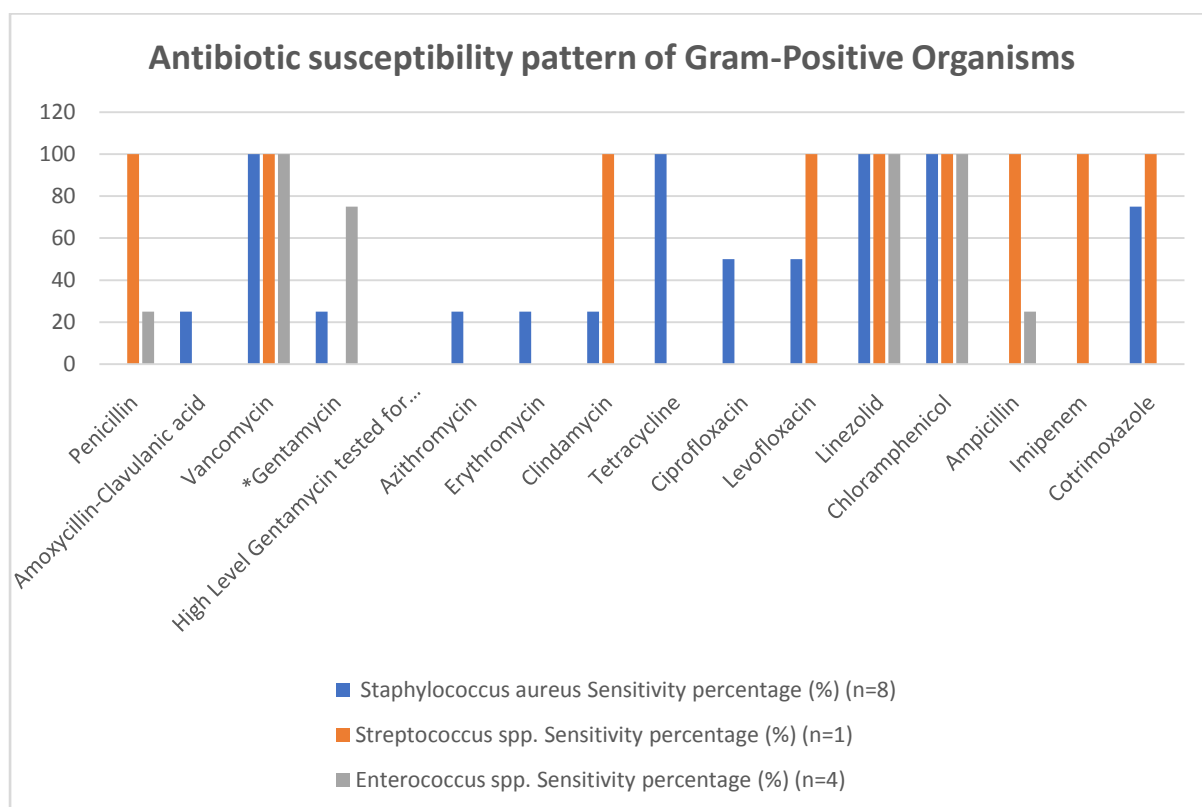
Among 167 total *Enterobacteriaceae* 70 were ESBL producer (41.91%). ESBL production was seen among 22 strains of *E. coli* (13.71%),31 strains of *Klebsiella pneumoniae*(18.56%) and 12 strains of *Proteus spp.*(7.17%).

Gram-positive organismswere found to be 100% susceptible to Linezolid, Vancomycin, and Chloramphenicol.50 % MRSA strains were isolated in this study.

Discussion:

Studies on Microbiological profile of diabetic foot infection is widely done all over the world and also differ in different region of the world.In the present study 79.19% males were affected which was similar to the most of the studies done from different parts of world.Male population is involved in hard work with greater risk of trauma is the major reason behind this. More common age groups involved in this study was 50-60 years which was also similar to so many studies where elder patients are more commonly affected due to the burden of life and exercise habits. Table6 and 7 shows the comparison of the present study with recent studies from India as well as from the different part of the world.

In this study monomicrobial growth was observed in 53.94% specimens which is similar to other studies done by Shareef J[8], Wu M[9]. But contrary to study by Belefquih B[10], and Saseedharan S.[11]in their studiespolymicrobial infection was predominates.

In microbiological evaluation of this study showedpreponderance of Gram‐ negative organisms 95.77% overGram‐ positive organisms 4.23% which is similar to recent studies done in India by Shareef J[8], Saseedharan S[11], Khare J[12], Jain S[13]and also similar to study done by Wu M[9] at China. But contrary to Indian study done by Malepati S[14] and Korean study done by Son T[15].

In Present study, Amongst the microbes, *Pseudomonas spp.*was the most frequentisolated (35.83%)organismfollowed by*Klebsiella spp.* (23.12%), *Proteus mirabilis* (14%)*, E. coli* (12.05%).*Pseudomonas aeruginosa* was the most common isolated organism in Shareef J[8], Malepati S[14] but*Staphylococcus aureus* was the most common organism the other studies done bySaseedharan S[11], Jain S[13], Wu M[9], Belefquih B[10],, Son T[15].Table 8 shows the comparison of different microorganisms isolated from diabetic foot infections from recent studies done in India as well as studies done in China and Korea. We can find the huge diversity of organisms. *P. aeruginosa* is more common in developingcountries especially in Asia and Africa.While in Western developed countries *S.aureus* is more common. Thereasons for this are not clear but the environmental factors, footwear, personal hygiene, antimicrobialpre-treatment, or other factors may be related to this.

Multi-drug resistant (MDR) Gram-negative microorganisms,including extended-spectrum beta-lactamase (ESBL) or carbapenemase-producing Enterobacteriaceae and MDR non-fermenters,are becoming a serious concern in tertiary referral hospitalsin developing countries.Looking to the sensitivity pattern of the non -fermenter, among all isolates, 19 (27.14%) strains of *Pseudomonas aeruginosa* ,2 (18.18%) strains of *Acinetobacter baumanii* were resistant to all drugs tested for antimicrobial susceptibility.Observing the susceptibility pattern of non -fermenter, Polymyxin B, Meropenem, Imipenem, Piperacillin -Tazobactam and levofloxacin were found to be more susceptible than the other drugswere the better choice as an empirical treatment for these organisms.

In the present study from 167 total *Enterobacteriaceae* 70 were ESBL producer (41.91%). ESBL production was seen among 22 strains of *E. coli* (13.71%),31 strains of *Klebsiella pneumoniae*(18.56%) and 12 strains of *Proteus spp.* (7.17%).Which was higher than the

study of Wu M[9] reported 10.6% ESBL in China. While in Indian study there was a much higher incidence of ESBL were noted by Jain S[13] (77.67%). With the emergence of ESBL‑ producing bacteria, the woundcondition deteriorates and treatment becomes difficult resultingin a poor outcome.Meropenem, Imipenem, Piperacillin -Tazobactam Amikacin, Gentamicin and levofloxacin, Cefepime were found to be more susceptible than the other drugs among the*Enterobacteriaceae*familywhich will bethe choice of drugs according to susceptibility pattern.

In present study two strains of MRSA was isolated that was from 2nd Culture Most probably due long duration of wound or due to antibiotic treatment. Previous study suggests that Methicillin-resistant *S. aureus* (MRSA) is more often isolatedfrom patients who have recently received antibiotic therapy,have been previously hospitalized, have nasal carriage of MRSAor osteomyelitis, or have a long-wound duration (≥4 weeks).Looking to the sensitivity pattern Gram positive organisms are found to be 100% susceptible to Linezolid, Vancomycin, Teicoplanine, Levofloxacin, Chloramphenicol, Amikacin, Gentamycin, Rifampin and Cotrimoxazole even MRSA Strain was also susceptible to these drugs. So, these drugs seem to be effective as empirical treatment for Gram positive organisms.

Table 6: Comparisonof present study with otherIndian studies

	Present study	Shareef j[8]	Saseedharan S[11]	Jain SK[13]	Malepati[14]
Study Period	May 2017-April 2019	Aug 2016-March 2017	Jan-June 2014	Feb 2015-Jan 2016	Jan-Dec 2015
Country	India	India	India	India	India
State	Gujarat	Karnataka	Maharashtra	Assam	Andhra Pradesh
Total Cases	173	71	261	150	346
M: F Ratio	3.80:1	1.86:1	1.48:1	-	2.53:1
Commonest Age group involved	51-60 years (34.68%)	60-69 years (39.43%)	58 years	60-65 Years	46-55 years (42.8%)
Specimen	241	71	216	150	346
No growth	29 (12.03%)	0	38 (17.6%)	11 (7.3%)	0
Growth	212 (87.97%)	71 (100%)	178 (82.4%)	139 (92.67)	346 (100%)
Monomicrobial growth	130 (53.94%)	38 (53.5%)	79 (44.3%)	96 (64%)	286 (82.7%)
Polymicrobial growth	82 (34.03%)	33 (46.47%)	99 (55.7%)	43 (28.6%)	60 (17.3%)
Organisms	307 (n)	122	289	185	438
GPC	13 (4.23%)	43 (35.24%)	117 (41.5%)	73 (39%)	224 (51.1%)
GNB	294 (95.77%)	79 (64.79%)	165 (58.5%)	112 (61%)	214 (48.9%)
Commonest organism isolated	*Pseudomonas aeruginosa* (35.83%)	*Pseudomonas aeruginosa* (18.03%)	*Staphylococcus aureus* (26.9%)	*Staphylococcus aureus* (24.86%)	*Pseudomonas aeruginosa, MRSA*

					(19.2%)

Table 7: Comparison of present study with otherstudies done in different countries.

	Present study	Belefquih B[10]	Wu M[9]	Son T[15]
Study Period	**May 2017-April 2019**	Jan 2009-June 2014	Jan 2014-June2017	Jan 2011-Dec 2015
Country	**India**	Morocco	China	Korea
Total Cases	**173**	157	428	745
M: F Ratio	**3.80:1**	4.06:1	1.56:1	2.63:1
Commonest Age group involved	**51-60 years (34.68%)**	>50 years (80%)	>70 years (39.8%)	-
Specimen	**241**	199	428	745
No growth	**29 (12.03%)**	23 (11.55%)	74 (17.28%)	132 17.72%
Growth	**212 (87.97%)**	176 (88.44%)	354 (82.71%)	613 (82.28%)
Monomicrobial growth	**130 (53.94%)**	69 (34.67%)	201 (56.8%)	-
Polymicrobial growth	**82 (34.03%)**	107 (53.76%)	153 (43.2%)	-
Organisms	**307 (n)**	307	555	
GPC	**13 (4.23%)**	138 (45%)	205 (36.9%)	478 (57.75%)
GNB	**294 (95.77%)**	150 (48.8%)	283 (51.0%)	333 (40%)
GPB	-	19 (6.2%)	-	-
Fungi	-	-	67 (12.1%)	9 (1.1)
ANAEROBES	**-**	-	-	12 (1.4%)
Commonest organism isolated	*Pseudomonas aeruginosa (35.83%)*	*Staphylococcus aureus (12.6%)*	*Staphylococcus aureus (41.5%)*	*Methicillin Resistant Staphylococcus aureus (13.7%)*

Table 8: Comparison of microorganisms with other studies

	Present study	Shareef j[8]	Jain SK[13]	Wu M[9]	Son T[15]
Country	India	India	India	China	Korea
Staphylococcus aureus	8 (2.60%)	15 (12.29%)	46 (24.86%)	85 (41.5%)	218 (26.2%)
Streptococcus spp.	1 (0.32%)	-	-	-	-

Enterococcus spp.	**4 (1.30%)**	13 (10.65%)	27 (14.59%)	36 (17.6%)	105 (12.6%)
Pseudomonas spp.	**110 (35.83%)**	22 (18.03%)	22 (11.89%)	39 (13.8%)	78 (9.4%)
Acinetobacter spp	**17 (5.53%)**	10 (8.19%)	7 (9.78%)	15 (5.3%)	13 (1.6%)
Klebsiella spp.	**71 (23.12%)**	18 (14.75%)	22 (11.89%)	35 (12.4%)	27 (3.2%)
Escherichia coli	**37 (12.05%)**	12 (9.83%)	37 (20%)	33 (11.7%)	60 (7.2%)
Proteus spp.	**48 (15.53%)**	12 (9.83%)	9 (4.86%)	32 (11.3%)	13 (2.0%)
Morganella morganii	**2 (0.65%)**	3 (2.45%)	4 (2.16%)	26 (9.2%)	12 (1.4%)
Citrobacter spp.	**4 (1.30%)**	2 (1.63%)	1(0.54%)	12 (4.2%)	13 (1.6%)
Providencia spp.	**5 (1.62%)**	-	3(1.62%)	-	54 (6.5%)
ESBL production	**41.91%**	-	61.44%	47.1%	10.6%
MRSA	**50%**	-	48.14%	40%	13.7%

Conclusion:

This study showed the predominance of gram-negativeorganisms over gram-positiveorganisms with the majorityof the infections to be monomicrobial in nature. It isnecessary to evaluate the culture sensitivity test fromthe infected wound and the knowledge on theantibioticsensitivity pattern of the isolates helps in planning treatmentwith the appropriate antibiotic regimen. This, in turn, helpsto prevent the emergence of drug-resistant organisms andminimizing healthcare costs.

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