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Incidence, clinical evaluation and antimicrobial susceptibility pattern of bacteria isolated from diabetic patients

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Abstract: Diabetes mellitus (DM) is classified as an endocrinological disorder of metabolism, which is marked by an increased rise in prevalence as well as incidence around the globe. The main aim of the study includes an assessment of the incidence, clinical profile evaluation and susceptibility pattern of bacteria against antimicrobial drugs in diabetic subjects. A total of 280 cases were included in the study of which the patients diagnosed with diabetes were assessed for their biochemical profiles as well as culture and sensitivity assays. 106 patients were diagnosed with diabetes out of 280 and were also associated with certain physiological disorders. Among these 106 patients, 103 patients showed an incidence of microbial infections. Of these patients, 63 were males, and 40 were females. Significant activities were observed against *Klebsiella* by tazobactam (68.8%). Sulzone (cefoperozone + sulbactam) demonstrated the most significant antimicrobial activities against *Staphylococcus aureus* (87.5%). Efficacy of Cefipime against Pseudomonas was quite substantial (66.6%) followed by Sulphamethazole (61.1%). Maximum activities were observed by cefixime against *E. coli* (61.5%) followed by nitrofurantoin (43.5%). Infections caused by Pseudomonas and *Staphylococcus aureus* were present in 18 and 8 patients, respectively.

Keywords: Bacteria, clinical profiles, CLSI, diabetes, HbA1c, susceptibility.

INTRODUCTION

Infections caused due to microbial pathogens, specifically those who demonstrate resistance towards antimicrobial drugs has been posing a potential threat towards their treatment. Mechanism of resistance might be intrinsic or acquired. Several microorganisms have demonstrated severe infections in addition to showing resistance patterns (Bhattacharya, 2013). With the passage of time and changes in the geographic setting, the comparative incidence of pathogens casuing infections might contrast. Apart of these changes in the organisms along with the usage and accessibility of potent antimicrobials, presence of bacteria in bloodstream stands as the most vital marker for prognosis of mortality in children having cancer and febrile neutropenia. Researches state that sepsis has led to a 10-fold rise in the risk of mortality (Basu, Fernandez *et al*., 2005).

Resistance towards antibiotics which may be intrinsic or acquired possesses a substantial effect on managing the treatment of patients. It may demonstrate either phenotypic resistance (a rise in the least concentration of antibiotic essential for inhibiting the growth of the microorganism) or genotypic resistance (particular resistance causing genes are detected via PCR, hybridization and DNA sequencing). Certain cases demonstrate three types of resistance causing mechanisms. Several factors have been held responsible for causing resistance and its spread. These are much clearly shown in an extensive diversity of microbes stretching from viruses (HIV, HBsAg, CMV and Influenza virus), fungi (Amphotericin resistance in *A. terreus*, MDR *Candida haemulonii* and Tuberculosis) to parasites (*Plasmodium falciparum*). Conversely, the threat of multiple drug-resistant pathogens causative of infections are more intensely faced in severe as well as in non-severe settings (Bhattacharya, 2013).

Diabetes mellitus (DM) has been characterized as a disorder of metabolism or endocrinology marked by an increased rise in prevalence as well as incidence around the globe. Most prominent symptoms of DM include raised blood sugar levels. These occur as a result of inadequacy in the secretion of insulin hormone by the pancreas. It might also be due to a deprived insulin-directed mobilization of glucose molecules by target cells. DM is intensified and related to complications of the metabolism, which may gradually be leading towards earlier deaths. DM is a disorder which presents a variety of symptoms. Most frequently characterized by the occurrence of hyperglycemia and glucose intolerance consequence of lack of insulin or defect in insulin or even both the factors (Sicree, Shaw *et al*., 2006). Complexities of these sorts rise because of the imbalances in regulating mechanisms for storing and mobilizing of metabolic fuels comprising catabolism and anabolism of carbohydrates, proteins and lipids originating via defects in secretion of insulin or its action or even both the factors (Piero, Nzaro *et al*., 2015, Shillitoe, 1988).

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DM is accountable for being one of the foremost health problems and has been spreading swiftly around the globe. Studies have predicted that almost 80 million population of India is likely to develop DM. In Asia, the most prevalence of diabetes has been observed in China and India. It has also been observed that Asia comprises more than 60% of the world's population suffering from DM. In countries which are developing, patients develop infections and diabetic foot ulcers which consequently result in amputations, osteomyelitis, mortalities and morbidities. Infectious agent is the most common cause of the foot ulcer. Studies have been conducted regarding the prevalence and susceptibility patterns of *Pseudomonas aeruginosa* in diabetic foot infections against commonly used antimicrobials. Retrospective studies done in South India from Jan-Aug 2013 have indicated the isolation of around 104 bacteria from a total number of 77 patients. The incidence of *P. aeruginosa* infections was found to be highest (37.5%). Resistance patterns were also seen against antimicrobials in case of this bacterium. Most resistance was demonstrated against ciprofloxacin, ofloxacin aztreonam and imipenem. The main problem is the occurrence of resistance towards 3rd and 4th generation cephalosporins. Resistance to carbapenem is also posing a severe threat currently. The rise in the prevalence of MDR *Pseudomonas aeruginosa* is a vital aspect which is to be looked upon (Kamtikar and Mitra, 2014).

Detecting resistant gene in samples is not precisely linked with the presence of pathogen carrying the gene itself. This occurs in case of transferable genes on MGE in which resistant genes are not only extensively spread in microbial agents but also in the healthy flora of patients (Moore, Patel *et al*., 2013). Resistance gene can be harmful for the normal microbiota (Potron, Poirel *et al*., 2011, Shoemaker, Vlamakis *et al*., 2001, Sommer, Church *et al*., 2010). Being focused on detecting MGE-linked antimicrobial resistance might correlate with the problems related to the occurrence of resistant genes in human microbiota as these are known to be highly transferrable to other bacterial pathogens. Adding on, it is to be noted that any previous treatment of the patient with antimicrobial drugs may lead to the persistence of bacteria for a certain period (Costelloe, Metcalfe *et al*., 2010, Jakobsson, Jernberg *et al*., 2010, Nys, Tjhie *et al*., 2005, Penders, Stobberingh *et al*., 2013, Wang, Hang *et al*., 2017).

Phenotypic characterization is a golden criterion for determining resistance mechanisms against antimicrobial drugs. This uses the incompetence of an organism for replicating in the existence of a MIC of an antimicrobial drug. Nevertheless, the de-merit of this technique comprises time delays, sensitivity, as well as the incorporation of methods in comparison with nucleic acid amplification (Maurer, Christner *et al*., 2017). Furthermore, most of the contemporary POC diagnostics depend on nucleic acid amplification techniques (using PCR) (Craw and Balachandran, 2012). Highly transmissible genes could be detected by ease using MGE linked gene detection by DNA/RNA amplification. Though, the resistances occurring because of mutations in genes are not identified with ease by conventional amplification methods. Zankari *et al*., have stated that detecting mutations along with gene amplifying might possess distinguishing merit against amplifying the gene only. The diagnostics might be considered insufficient in providing ample information relevant to the clinical findings (Zankari, Allesøe *et al*., 2017). The objective of the study to includes an assessment of the incidence, clinical profile evaluation and susceptibility pattern of bacteria against antimicrobial drugs in diabetic subjects.

MATERIALS AND METHODS

Materials

Chemicals used in the lab

Constituents of media were attained by the following: E. Merck (Darmstadt, Germany), Difco Labs (Detroit Michigan, USA), Scharlau laboratories (France), Panreac Quimica (Barcelona, Spain) and Sigma-Aldrich Chemicals Co. (St. Louis, USA).

Requirements of laboratory apparatus

The laboratory instruments required for the procedure are as follows: Sterile EDTA tubes, alcohol swabs, gloves, masks, sterile cotton swabs, Petri plates (25ml), Flask (200ml-500ml), Beaker (200ml-500ml), Test tubes (20ml), Glass slides, Sterile syringes, Micropipettes, Sterile tips, Eppendorf tubes, Inoculation platinum wire loop, Bunsen burner, incubator (temp. range 35-37^{o}C), Hot air oven, Water bath, Autoclave (121^{o}C, 15psi), Vortex mixer, Weighing balance, Colony counter and Compound microscope, Biosafety cabinet.

Media and chemicals required

The media and chemical required for the procedure as General purpose media (nutrient agar), Differential media (Cled, Mac Conkey, Blood agar, Mueller Hinton agar), Antibiotics specific media (Muller Hinton Agar), 70% and 95% ethanol, Distilled water, Normal saline, Glycerol, Standard antibiotics.

Antibiotic/drugs used in the study

Antibiotics used in the study are purchased from a pharmaceutical company. Twenty-one antibiotics are selected according to their mechanism of action from 5 diverse groups of antibiotics. These antibiotics are used against the bacteria isolated from different given sources. Following antibiotics are included in the study, Ceftazidime, Fosfomycin, Ciprofloxacin, Imipenem, Chloramphenicol, Tazobactam, Furantoin, Cefipime, Amoxicillin/clavulanate, Cefixime, Gentamycin, Fusidic acid, Ampicillin, Cefuroxime, Meropenem, Vancomycin, Tetracycline, Sulphamethoxazole and Carbenicillin.

Inclusion and exclusion criterion

Inclusion criterion

Both males and females with age group between fifty to seventy years.

Diabetes should be in some range.

Must have certain infections.

Exclusion criterion

Both males and females with age group less than fifty and more than seventy are excluded.

Patients suffering with diseases other than microbial infections.

Collection of samples

280 samples were collected from diabetic patients from the teaching hospital of Lahore University. The collected sample were placed in a sterile container. These collected samples were correctly labelled and transported to the lab safely. Collected samples were transported within two hours after sampling.

Sources of specimen

Blood, pus, Urine, body fluids (ascitic, pleural, synovial), abscesses, wound swabs and serum.

Steps of media preparation

Necessary steps for media preparation as follows: Pour 100ml of distilled water in a conical flask. Weigh 2.3g of nutrient agar and add it in the flask containing distilled water. Mix this solution properly. Wrap the mouth of the flask with aluminium outwits and label it appropriately. Now place this conical flask in the autoclave for 15min at 121^{o}C temp and 15psi Pa. The pH of fresh media is 6.6. After the sterilization is done, allow the media to cool. When it reaches to the suitable temperature or room temperature pour the media in the sterilized petri plates under highly sterilized lab conditions. Let media solidify. After solidification of media plates apply the collected sample dilution carefully under the aseptic condition. Plates must be appropriately labelled with the sample number and date on which culture is applied and Place the media plates in the incubator (35^{o}C-37^{o}C) for 24Hrs for proper bacterial growth.

Preparation of Petri plates

Instruction for preparation of Petri plates: Wash the Petri plates properly with the lukewarm tap water. Let them dry and wrap the plates with the paper. Petri plates are sterilized by dry heat method. Put the wrapped plates in a hot air oven for 30 to 40min at 65 to 70^{o}C temp after the sterilization has done takeout the plates and unwrap them and Pour the autoclaved media in the plates and let them solidify at room temperature (Tosi, Donini *et al*., 1996).

Isolation of bacterial colonies

Procedure for isolation of bacterial colonies as after 24Hrs of incubation takeout the plates from the incubator and observe the growth. Separate different type of colonies and streak them on the new nutrient agar plates. Allow these plates for 24Hrs in incubation. After incubation, at the very next day observe the growth of bacteria on these plates and Record the colony morphology by observing shape, size, margins and elevation.

Method of processing

Following principle was used in methodology as of EDTA blood samples of patients were obtained and tested for HbA1c levels. On confirmation of diabetes, other specimens (blood, pus, urine, wound swab) were requested from them in case if the patient was admitted in the hospital ward. Culturing of these samples was done on respective culture media and plates were incubated at 37°C for 24Hrs. On the second day, gram staining and biochemical tests were put up according to the growth showed on the medium and inoculated biochemical test tubes were incubated at 37°C for 24Hrs.On the third day, results of biochemical tests were noted according to which the organisms were identified and their susceptibility testing was pursued on Mueller Hinton agar. The final day, results of antimicrobial sensitivity testing were read and reported.

Ethical approval

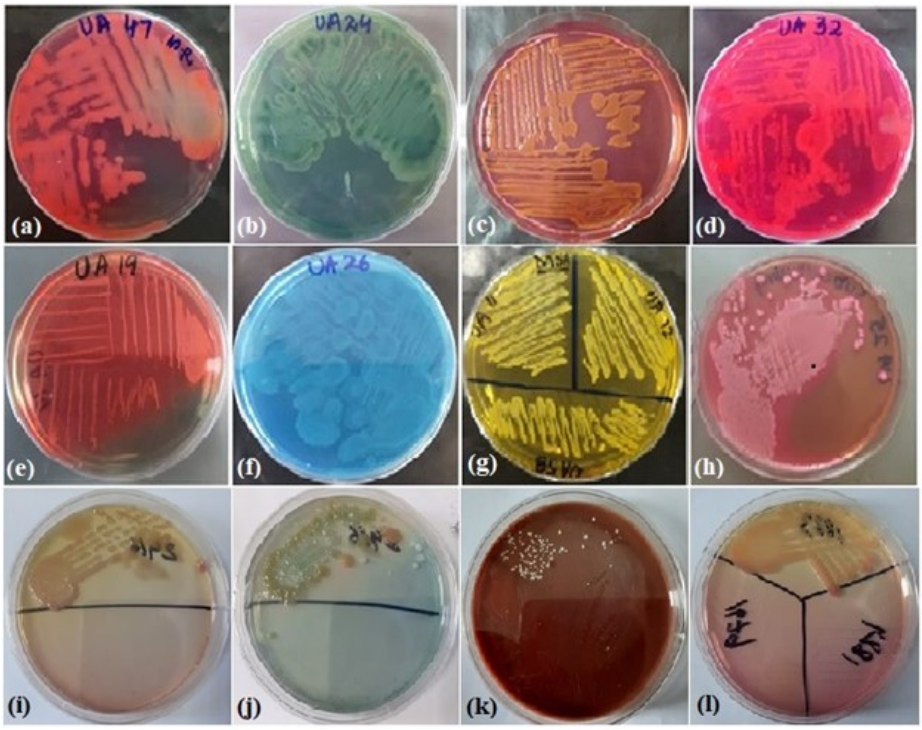
The study was approved by the ethical committee of University of Lahore. The registration number was PMC02173005.

STATISTICAL ANALYSIS

All results were analyzed for statistics using SPSS version 22. We used the sample paired t-test for comparison of groups.

RESULTS

A total number of 280 patients were included in this study. 106 patients were diagnosed with Diabetes mellitus. Of these 106 patients, 103 suffered from severe infections caused by microorganisms. Infected patients were 63 males and 40 females. These patients were assessed for their serum glucose levels, HbA1c levels, CBCs, and various culture and sensitivity specimens. These patient's ratio describe the various pattern among different organism and their different result. Specific research in determining the variations in sensitivity patterns of UTIs among diabetics and non-diabetics. The study included 265 patients, of which 228 were females, and 37 were males. Not all of these patients presented UTIs. Predominantly, *E. coli* was found causative of UTIs among diabetics and was substantially considered lower in case of causing infection in non-diabetics ($P<0.005$) as presented in fig. 3. However, *Klebsiella sp*. was held responsible for causing UTI in diabetics (Chan, Lye *et al*., 1993). In research work fig. 2 show the total population in



(a) Klebsiella on MacConkey agar, (b) *Pseudomonas aeruginosa* on Cetrimide agar, (c) Proteus on MacConkey agar, (d) *Staphlococcus epidermidis* on MSA,(e) *E. coli* on MacConkey agar, (f) Entereobacter spp on CLED agar, (g) *Staphlococcus aureus* colonies on MSA, (h) Klebsiella on MacConkey agar, (i) Salmonella spp on MacConkey agar, (j) Klebsiella on CLED agar, (k) *E. coli* on Choclate gar, (l) Klebsiella on MacConkey agar

Fig. 1: a to l. Different types of bacterial growth on different types of media (Representative images)

Table 1: Blood chemistry findings [n =106]

Liver function tests			
Parameter	Results	Normal ranges	Units
A.L.T	52 ± 4	Upto 42	U/L
A.S.T	38 ± 2	Upto 40	U/L
Alkaline phosphatase	95 ± 5	40-129	U/L
Albumin	4.2 ± 0.2	3.5-5.2	g/dl
Total protein	7.6 ± 0.4	6.1-8.6	g/dl
Total Bilirubin	0.4 ± 0.01	0.1-1.2	mg/dl
Direct Bilirubin	0.4 ± 0.01	0-0.3	mg/dl
Gamma G.T	27 ± 3	Less than 60	U/L
Globulin	3.4 ± 0.5	1.8-3.2	g/dl
A/G ratio	1.23 ± 0.1	-	-
Renal function tests			
Urea	28 ± 2	16.6-48.5	mg/dl
Creatinine	0.8 ± 0.05	0.7-1.1	mg/dl
Uric acid	3.6 ± 0.5	3.4-7.0	mg/dl
Serum Vitamin D3	22.46 ± 1.5	30-100	ng/ml
Serum Electrolytes			
Sodium	137 ± 4	136-145	mmol/L
Potassium	4.1 ± 0.3	3.5-5.4	mmol/L
Chloride	104 ± 5	95-110	mmol/L
Blood sugar random	212 ± 30	Upto 160	mg/dl
HbA1c	7.3 ± 0.5	4.5-5.6 > 6.0: poor control	%

Table 2: Hematological findings sample [n =106]

CBC (Complete blood count)			
Parameter	Results	Normal ranges	Units
TLC	8.1 ± 0.4	4-11	$x10^9$/L
RBC	4.41 ± 0.2	4.5-6.0	$x10^{12}$/L
HB	14.4 ± 1.5	M: 14-18, F: 12-16	g/dl
HCT	39 ± 4	40-50	%
MCV	87 ± 6	80-98	Fl
MCH	31 ± 3	27-31	Pg
MCHC	40 ± 5	32-36	g/dl
Platelets	172 ± 8	140-450	$x10^9$/L
Neutrophils	72 ± 4	45-75%	%
Lymphocytes	18 ± 4	15-40%	%
Monocytes	07 ± 2	5-11%	%
Eosinophils	03 ± 1	2-5%	%

Table 3: Susceptibility patterns of antimicrobial drugs against *Klebsiella* from 1 to 15

S. No.	Antibiotics	*Klebsiella*														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Imipenem	S	R	R	R	S	S	R	R	R	R	S	R	S	R	R
2	Ciprofloxacin	R	S	S	S	R	R	R	R	S	I	S	R	S	R	S
3	Cefipime	S	R	R	R	R	R	R	R	I	S	S	R	R	R	R
4	Chloramphenicol	R	R	R	R	R	R	R	I	S	R	R	S	R	R	R
5	Tazobactam	S	S	S	S	S	S	S	S	R	I	S	I	S	S	R
6	Amoxycillin	R	R	I	S	R	R	R	S	I	R	R	R	R	R	S
7	Tetracycline	R	S	S	R	R	R	R	S	S	R	I	S	R	I	R
8	Cefixime	R	R	S	I	S	S	S	R	I	I	S	R	R	R	R
9	Cefuroxime	R	R	I	S	I	S	S	S	S	S	S	R	S	R	R
10	Sulphamethazole/Trimethoprim	R	S	R	R	S	S	S	S	I	R	S	S	R	S	R
11	Fusidic acid	R	R	R	I	S	R	R	R	R	R	I	S	S	R	R
12	Sulzone (Cefoperozone+sulbactam)	R	S	R	R	S	S	I	S	S	S	R	S	I	I	R

Table 4: Susceptibility patterns of antimicrobial drugs against *Klebsiella* from 16 to 30

S. No.	Antibiotics	*Klebsiella*														
		16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	Imipenem	S	R	I	R	S	S	R	R	R	I	S	R	S	R	R
2	Ciprofloxacin	S	S	S	S	R	R	R	R	S	I	S	R	S	R	S
3	Cefipime	R	R	R	R	R	R	R	R	I	S	S	S	S	R	R
4	Chloramphenicol	R	R	R	R	R	R	R	I	S	R	R	S	R	R	R
5	Tazobactam	S	S	S	S	R	S	R	S	R	I	S	I	S	S	R
6	Amoxycillin	S	R	I	R	R	R	R	S	I	R	R	S	R	S	S
7	Tetracycline	S	S	S	R	R	R	R	S	S	R	I	S	R	I	R
8	Cefixime	S	R	R	I	S	S	S	R	I	I	S	S	R	S	R
9	Cefuroxime	S	S	I	S	I	S	S	S	S	S	S	R	S	R	R
10	Sulphamethazole/Trimethoprim	S	S	R	R	S	S	S	S	I	R	S	S	S	S	R
11	Fusidic acid	S	R	R	I	S	R	R	R	R	R	I	S	S	S	R
12	Sulzone (Cefoperozone+sulbactam)	S	S	R	R	S	S	I	S	S	S	R	S	I	I	R

Table 5: Susceptibility patterns of antimicrobial drugs against *Pseudomonas*

S. No.	Antibiotics	*Pseudomonas*																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	Imipenem	S	I	R	S	R	R	R	R	R	I	R	S	S	R	R	I	R	R
2	Ciprofloxacin	R	S	R	S	R	S	S	R	S	S	R	S	R	S	I	R	R	S
3	Cefipime	S	S	S	S	R	S	I	I	R	S	S	S	R	S	S	S	S	R
4	Chloramphenicol	R	S	R	S	R	S	I	R	S	S	S	I	R	R	R	R	R	R
5	Tazobactam	S	S	R	S	R	I	S	I	R	R	I	S	R	S	S	I	S	R
6	Amoxycillin with Clavulanic acid	R	R	S	R	S	S	R	R	S	R	R	R	S	I	R	S	S	S
7	Tetracycline	R	I	S	R	S	R	R	R	S	S	S	R	S	S	I	R	S	R
8	Cefixime	R	R	S	S	I	R	S	I	S	S	R	S	I	S	S	I	R	I
9	Cefuroxime	R	R	I	R	R	S	I	S	S	R	S	R	S	R	I	S	I	S
10	Sulphamethazole/Trimethoprim	R	R	S	S	S	I	S	I	S	S	S	S	R	R	S	S	S	R
11	Fusidic acid	R	R	S	R	S	S	I	R	S	R	R	R	S	S	S	R	R	S
12	Sulzone (Cefoperozone+sulbactam)	S	I	R	I	R	S	S	S	R	I	R	S	R	R	S	R	R	I
13	Carbenicillin	R	I	R	S	S	R	I	S	R	I	R	I	I	R	I	S	R	R
14	Gentamycin	S	R	S	R	S	S	S	R	S	R	S	R	S	S	S	R	R	S

Table 6: Susceptibility patterns of antimicrobial drugs against *E. coli*

S. No.	Antibiotics	*E. coli*																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	Imipenem	R	R	S	R	R	I	S	S	S	R	S	R	S	R	R	R	R	S	I
2	Ciprofloxacin	R	R	S	S	I	S	R	S	R	S	R	S	R	R	R	R	S	R	R
3	Cefipime	R	S	R	R	R	R	S	S	R	R	S	R	R	I	I	R	R	R	R
4	Chloramphenicol	R	R	R	I	S	R	S	S	R	S	R	S	R	R	R	R	R	R	S
5	Tazobactam	R	S	S	R	S	R	R	S	S	S	S	R	R	S	S	R	I	R	R
6	Amoxycillin with Clavulanic acid	R	S	S	S	R	R	R	S	S	R	I	R	S	S	R	R	R	R	S
7	Tetracycline	S	S	I	S	I	S	R	I	R	S	S	S	R	I	R	S	S	I	S
8	Cefixime	S	S	S	S	R	R	S	R	S	S	S	S	S	S	S	S	I	S	S
9	Cefuroxime	R	S	S	S		R	R	S	S	S	R	S	R	I	I	R	S	R	R
10	Sulphamethazole/Trimethoprim	I	R	S	S	R	S	R	I	S	I	R	S	S	I	I	R	S	S	R
11	Gentamycin	S	S	R	I	S	S	S	S	I	R	S	R	R	R	R	S	R	R	R
12	Carbenicillin	S	S	R	R	S	S	R	S	I	S	S	R	I	S	S	I	S	I	R
13	Fusidic acid	I	I	S	S	S	R	S	R	R	I	R	I	I	S	S	S	R	I	R
14	Nitrofurantoin	I	S	S	R	R	I	R	S	I	S	S	S	R	S	S	S	S	R	R

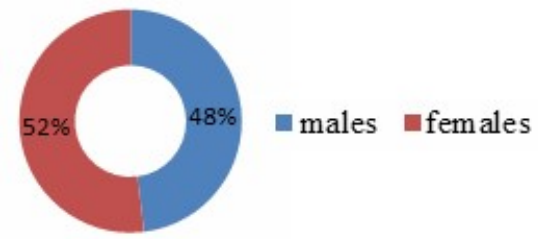


Fig. 2: Graph showing the total population of patients.

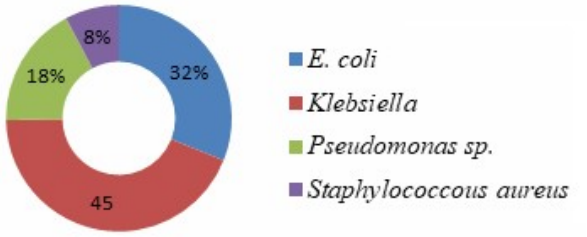


Fig. 3: Distribution of patients affected with the organisms categorized.

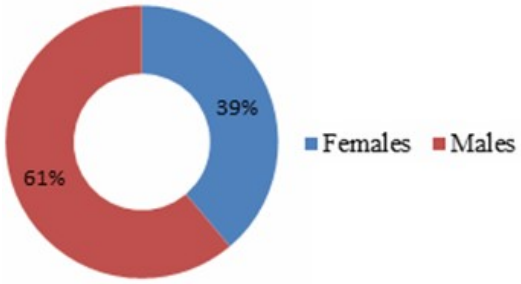


Fig. 4: Graph showing the percentage of males and females affected.

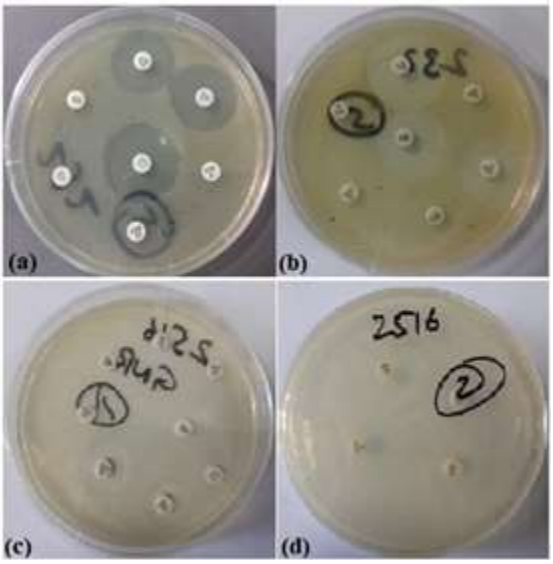


Fig. 5: (a to d) Pattern of sensitivity observed in *Pseudomonas aeruginosa* using different antibiotics (Representative images)

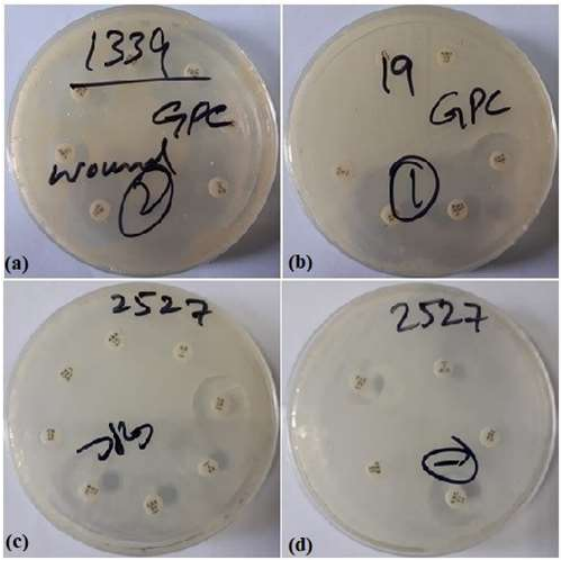


Fig. 6: (a to d) Pattern of sensitivity observed in *Staphylococcus aureus* using different antibiotics (Representative images)

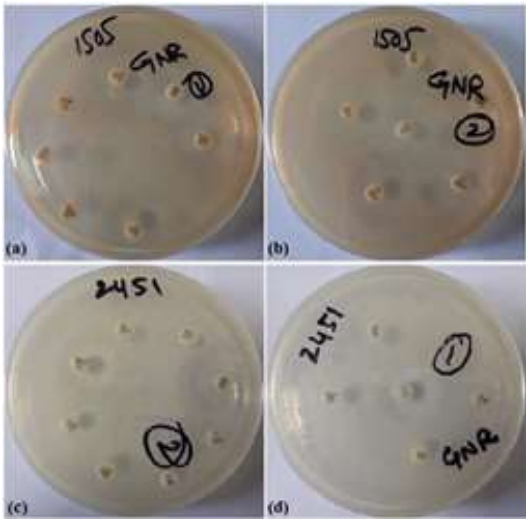


Fig. 7: (a to d) Pattern of sensitivity observed in *E. coli* using different antibiotics (Representative images)

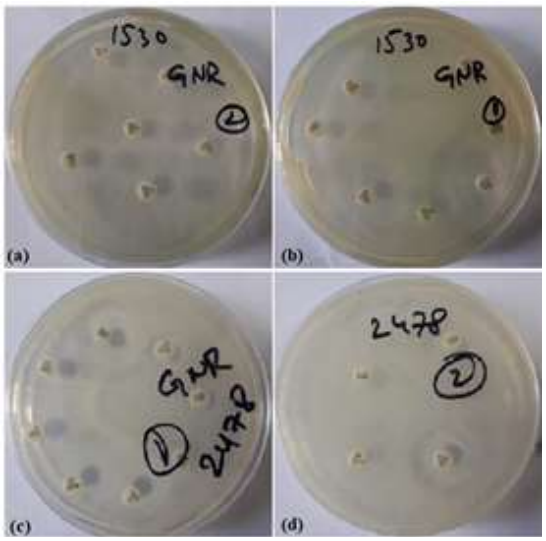


Fig. 8: (a to d) Pattern of sensitivity observed in *Klebsiella* using different antibiotics (Representative images)

the form of graphical representation as compare to the results of the study conducted by (Chan, Lye *et al*., 1993) which were considerably similar results to our study. In our study, infections caused by *Klebsiella sp*. in diabetics were enumerated as 30 in males and 15 in females. According to the results of (Chan, Lye *et al*., 1993) a resistance pattern was observed by the organism against 3rd generation cephalosporin group. The research concluded that the incidence of nosocomial UTI was more than community-acquired UTI in diabetics. Fig. 2 showed the 106 patients were diagnosed with diabetes out of 280 and were also associated with specific physiological disorders. Among these 106 patients, an incidence of microbial infections was observed in around 103 patients who include infections of the urinary tract, wounds and skin. Infections were most commonly caused by *E. coli*, *Klebsiella sp*., *Pseudomonas sp*., and *Staphylococcus aureus*.

Fig. 3 showed the distribution of patients affected with the organisms categorized as *E. coli* 32%, *Klebsiella* 45%, *Pseudomonas* species 18% and *Staphylococcus aureus* with 8%. The number of patients varies from a given type of bacteria. Fig. 4 showed the percentage of males and females affected. Of the 43 female diabetic patients, 15 suffered from infections of *Klebsiella*. 4 cases of *Staph aureus* infections were also observed. *Pseudomonas* and *E. coli* infections were observed in 8 and 13 cases,

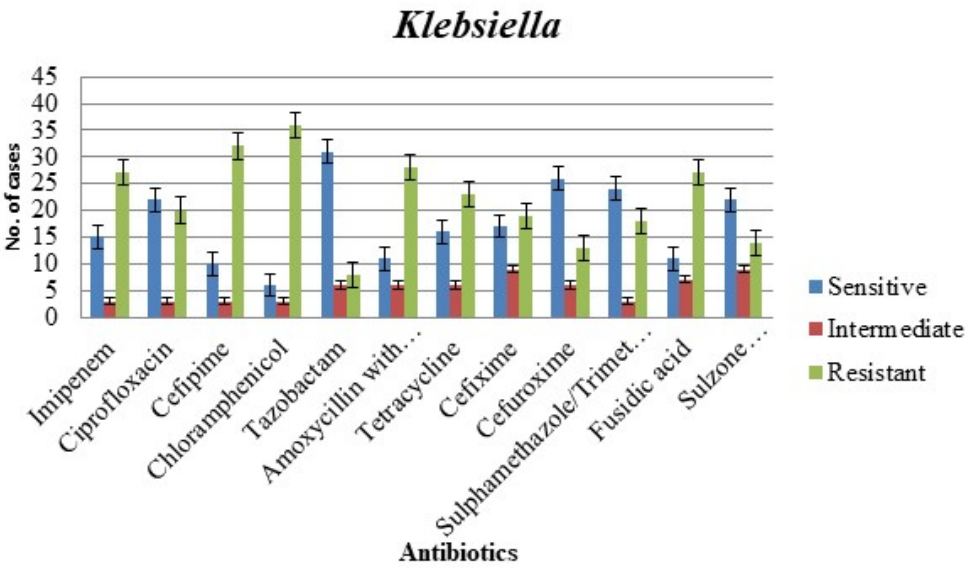


Fig. 9: Graph showing the susceptibility pattern of antimicrobial drugs against *Klebsiella*.

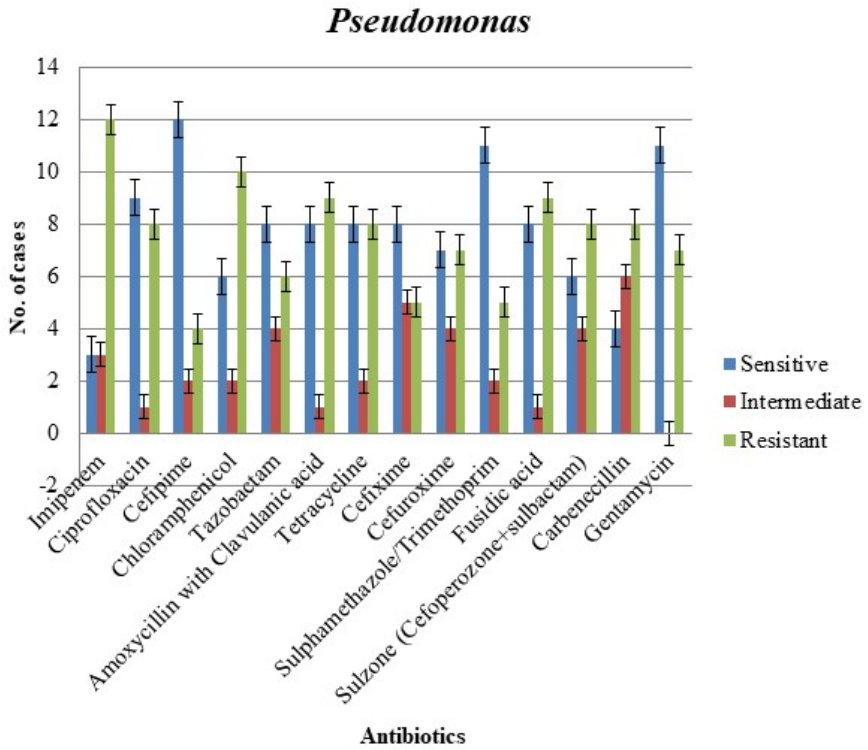


Fig. 10: Graph showing the susceptibility pattern of antimicrobial drugs against *Pseudomonas*

respectively. Total of 63 male patients was diagnosed with diabetes. Out of these 63 patients, 30 were reported to be infected with *Klebsiella* infections, 4 with *S. aureus*, 10 suffered from infections caused by *Pseudomonas sp*. and 19 were infected with *E. coli.* Significant activities were observed against *Klebsiella* by tazobactam (68.8%). Sulzone (cefoperozone+sulbactam) demonstrated the most significant antimicrobial activities against *Staphylococcus aureus* (87.5%). Efficacy of Cefipime against *Pseudomonas* was quite substantial (66.6%) followed by Sulphamethazole (61.1%). Maximum activities were observed by cefixime against *E. coli* (61.5%) followed by nitrofurantoin (43.5%). 45 patients were affected with *Klebsiella* while 32 patients suffered from infections caused by *E. coli*. Infections caused by *Pseudomonas* and *Staphylococcus aureus* were present in 18 and 8 patients, respectively.

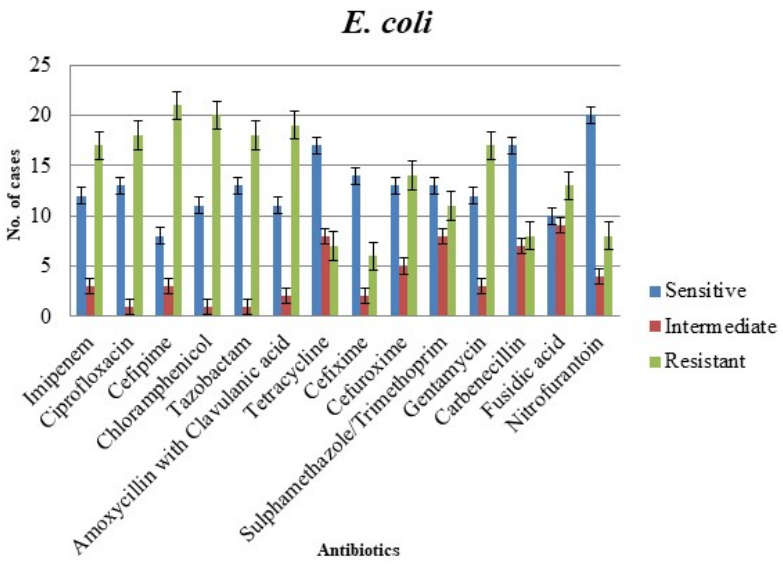


Fig. 11: Graph showing Susceptibility pattern of antimicrobial drugs against *E. coli*

DISCUSSION

Percentage frequency of male and female affected, and this comparison with the other research work and concluded that susceptibility patterns of UTIs in diabetics observed by (Sewify, Nair *et al*., 2016), their study involved Gram +ve as well as Gram –ve organisms. According to their results, resistance patterns were observed in 47% cases, whereas our study has reported 53.3% cases of sensitivity and in 40% cases, the organism was observed showing resistance. In 48% of cases, sulbactam was sensitive to the pathogen while in 31% of cases, resistance patterns were observed. Compared with the study done by (Sewify, Nair *et al*., 2016) resistance towards sulbactam was observed in 42% cases. In 46% of cases, sensitive results were seen in the case of ciprofloxacin while 44% of cases displayed resistance patterns in our study. Patterns of resistance were recorded as 34% in case of ciprofloxacin in the study of (Sewify, Nair *et al*., 2016) 62% cases of resistance were observed in our study concerning amoxicillin with clavulanic acid and these results differ from those of (Sewify, Nair *et al*., 2016) in which resistance pattern was observed as 24% against the antibiotic.

Susceptibility pattern of antimicrobial drugs against various pathogen and correlate the effect of this study with the incidence of MRSA is quite high in diabetics. McKinnell *et al*., have conducted studies involving analyses for estimation of MRSA among diabetics. It was concluded that among 11577 diabetic patients, the incidence of MRSA was calculated to be 9.2% (McKinnell, Miller *et al*., 2013). Incidence of DFIs was 16.7%. Infections of foot, skin and soft tissues were observed in 18% of the cases. Yamasaki *et al*., have reported the incidence of MRSA infections as 13.4%. Estimates have stated that the incidence of MRSA infections is around 2.6% in Japan (Yamasaki, Takeuchi *et al*., 2018). Studies were done by Lin *et al*., have also stated that around 3.9% of the healthy young age group has also been affected by it (Lin, Peng *et al*., 2016). Den Heijer *et al*., have estimated the incidence to be around 2.6% among 9 various countries of Europe. Health Protection Scotland, 2010 has revealed the incidence of MRSA infections around 3.8% which might be progressing to about 20% in the patients with renal diseases, older subjects as well as those undergoing vascular surgeries. Correctly, a higher incidence has been observed in diabetics (den Heijer, van Bijnen *et al*., 2013).

CONCLUSION

In this study Incidence, clinical evaluation and antimicrobial susceptibility pattern of bacteria isolated from diabetic patients were investigated experimentally. The conclusion can be summarized as follows. 103 diabetic patients were affected by bacterial infections. Of these patients, 63 were males, and 40 were females. The subjects were assessed for their blood chemistry tests and hematological findings.

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