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Research Article

Occurrence of Bacterial Flora in Oral infections of Diabetic and Non-diabetic patients, and Antimicrobial Susceptibility Patterns of *the Pathogens*

Mohammad Zakerin Abedin[1], [*]Md. Easin Arfat[2], Md. Oyes Quruni ,[3]Samim Mia[1], Summiya Shamima Prity[1], Md. Babul Aktar[1], Farida Yeasmin[4]and Abdullah Akhtar Ahmed[1]

[1]Department of Microbiology, School of Biomedical Sciences, Khwaja Yunus Ali University, Sirajganj, Bangladesh

[2]Department of Microbiology, University of Chittagong, Chittagong, Bangladesh

[3]Department of Pathology, Shekh Hasina Medical College, Tangail, Bangladesh,

[4]Faculty of Basic Medical and Pharmaceutical Science, University of Science and Technology Chittagong, Chittagong, Bangladesh.

***Correspondence:** zakerin.du2016@gmail.com (Mohammad Zakerin Abedin, Assistant Professor & Head, Dept. of Microbiology, School of Biomedical Science, Khwaja Yunus Ali University, Bangladesh)

Abstract

Oral bacterial infection is one the most common diseases caused by enormously diverse and complex oral cavity microflora consisting of around 1000 bacterial communities. Our study was designed and enrolled to explore the etiological bacterial agents of oral infections and reveal antibiotic susceptibility patterns of pathogens. 56 diabetic and non-diabetic patients of diverse aged, males and females, were involved in the test. Swab samples were collected from the infected area inside the mouth. Pathogens were isolated from the samples growing on culture media and identified by cultural and morphological characteristics. The CLSI guideline analyzed antibiotic susceptibility patterns of the pathogens. Male patients were slightly higher than females and people ranging from 40-60 years of age were more prevalent. The number of male patients was slightly higher than female patients. Klebsiella pneumoniae (39.29%), P. aeruginosa (35.71%), E. coli (14.29%), S. marcescens (8.99%), and C. freundii (1.79%) were isolated from infected sites. Azithromycin and Ciprofloxacin were the most effective (100%) antibiotics, followed by Levofloxacin (98.21%), Imipenem (92.86%), and Erythromycin (78.57%) against the pathogens whereas Ceftazidime (91.07%), Amoxicillin (87.5%), Meropenem (80.36%) and Cefepime (75%) were found most ineffective along with 12.5% intermediate response by Erythromycin and Trimethoprim. These findings are of clinical significance that can provide adequate knowledge to dentists and physicians about the selection of antibiotics to treat oral infections where Azithromycin and Ciprofloxacin should be the preference.

Key Words: Oral Bacterial Infection, Oral Microflora, Periodontitis, Diabetic, Antibiotic Susceptibility Patterns.

1. Introduction

The human oral cavity is the habitat of the most complex and diverse microbiomes in the human body after the colon. Many colonized viruses, bacteria, archaea, fungi, protozoa, etc., possess the oral microbiota of humans. Normal body flora harmonizes with the host through commensalism, except for this oral microbiota, which is responsible for two of the most common chronic oral infections worldwide, including dental caries and periodontal diseases largely influenced by soft and sugar-rich diets (Wade, 2013). Periodontal diseases (gingivitis and periodontitis) are developed by the destruction of tooth support tissues caused by the accumulation and maturation of oral microorganisms (Llambes *et al.*, 2015).

Highly complex bacterial communities with around 1000 species in the mouth are substantially responsible for dental plaque formation, which is considered the primary etiological factor that stimulates dental caries, gingivitis, and periodontitis (Dennison *et al.*, 1996; Dewhirst *et al.*, 2010).

Various common systemic disorders, including diabetes mellitus, preterm birth, heart diseases, pneumonia, etc., are vastly associated with predisposing factors for oral infection (D'Aiuto *et al.*, 2004). Diabetes, one of the leading causes of global morbidity and mortality characterized by chronic hyperglycemia, exhibits a reciprocal relationship with oral infections such as gingivitis and periodontitis, sharing a common pathogenesis of triggering inflammatory responses at both local and systemic levels (Chee *et al.*, 2013). Periodontal diseases and other oral infections are thought to be three-fold more prevalent among diabetic patients due to inappropriate glycemic control (Taylor *et al.*, 1996).

The widespread use of antibiotics for both therapeutic and prophylactic purposes in dental caries, periodontal diseases, and other oral infections is largely responsible for the emergence of antibiotic resistance in low- and middle-income countries due to imprudent, frequent, irrational, and overprescribed antimicrobials by dental surgeons and family practitioners as outpatient care (Haque *et al*., 2019).

Antibiotic resistance is also considered a natural phenomenon attained by microorganisms as they evolve. Therefore, a systematic investigation is necessary to determine the most effective antibiotics to treat oral infections. The current study was designed to unearth the most prevalent etiological agents of oral infections in diabetic and non-diabetic patients of different age groups to uncover antibiotic susceptibility patterns of isolated pathogens associated with different oral infections such as dental caries, gingivitis, periodontal diseases, etc. The study will assist dentists and physicians with the selection of antimicrobials against oral infections.

2. Materials and Methods

2.1 Participants

All diabetic patients were receiving treatment for their diabetes mellitus. The control group of healthy volunteers was matched for age, sex, dental status, and smoking habits. Patients and controls were examined for signs or symptoms of oral infections. Only those who had not been on antibiotics or corticosteroid therapy for the previous four weeks were included in the study. All participants were asked to sign a consent form with the understanding that collected data would be used for non-commercial research purposes and that names would be kept confidential. All participants were also asked to complete a questionnaire addressing their socio-economic status (age, marital status, and occupation), dental status, smoking habits, and duration of diabetes mellitus.

2.2. Collection of Biological Samples

2.2.1. Collection and transport of oral swab

A good light inside the mouth affected area was swabbed using a sterile cotton wool swab. Carefully without contaminating the swab with saliva, it was returned to its sterile container—the swab with a completed questionnaire form was delivered to the laboratory within two hours of collection.

2.2.2. Collection of blood samples for glucose estimation

Fasting blood glucose was taken from all of the participants. Blood glucose was measured by Accu-Check, Roche Diabetes Care, Inc. (USA).

2.3. Sample preparation and Bacterial species analysis

An imprint culture technique was used to determine bacterial species' frequency of isolation and density at up to nine intraoral sites (Murray *et al.*, 2003). Aseptically collected oral samples were inoculated on Chromogenic agar, MacConkey agar, and Nutrient agar (Biomaxima, Poland) and then incubated at 37° C for 24 hours.

2.4. Isolation, Identification and Characterization of Bacterial species

2.4.1. Cultural characterizations

The colonies of bacteria were observed and categorized based on their color, texture, outline, opacity, pigmentation, etc., and different types of colonies appearing on all the media were counted.

2.4.2. Morphological characterizations

The colonies were picked and processed for the Gram staining technique to differentiate between Gram-positive and Gram-negative bacteria, shape, and arrangement of cells.

2.4.3. Chromogenic agar medium

It is recommended for the rapid isolation and identification of bacterial species from mixed cultures in clinical samples. Chromogenic differential agar is a selective and differential medium, which facilitates rapid isolation of bacteria from mixed cultures and allows differentiation of *bacterial* species, namely *K. pneumonie, E. faecallis, Proteus mirabilis, P. aeruginosa, S. aureus,* and *E. coli*. Still, sometimes *S. saprophyticus* is based on coloration and colony morphology. On this medium, results are obtained within 24 hours, which is helpful for the rapid and presumptive identification of common bacteria in the Mycology and Clinical Microbiology Laboratory.

2.5. In vitro Antibacterial Susceptibility Testing by Disc Diffusion method

2.5.1. Inoculums preparation

The colonies were mixed in 0.85% sterile normal saline (5 ml volume) and adjusted to a turbidity of 0.5 McFarland standards. Meuller-Hinton agar with 2% glucose and 0.5 µg/ml of methylene blue was used.

2.5.2. Antibacterial Susceptibility Testing

A sterile swab was used to inoculate the plate by making a lawn culture by rotating the plate 180 degree in three directions. The antibacterial discs such as Amoxicillin (30µg), Azithromycin (15µg), Cefixime (5µg), Ceftazidime (30µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Meropenem (10µg), Trimethoprim (25µg), Nalidixic acid (30µg), Cefepime (30µg), Imipenem (10µg), Erythromycin (15 µg), Cefotaxime (30µg), Ceftriaxone (30µg), Cotrimoxazole (25 µg) were placed on the plate and incubated at 37°C for 18-24 hours. The zone sizes were interpreted as resistant, intermediate and sensitive, standardizing with Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI, 2020).

2.6. Statistical Analysis of Experimental Data

Data obtained were analyzed by SPSS version 20 and Excel 2019.

3. Results

In this study, 56 individuals with various types of oral infections were included, where 26 samples were collected from diabetic patients and 30 samples from healthy non-diabetic individuals, taking similar socioeconomic status (age, marital status, and occupation), dental status, smoking habits, and duration of diabetes mellitus into account. All the collected samples were growth-positive, including people of all ages, with the highest prevalence in the 40–60 year age group was 51% of total population, followed by 29% of> 60 years and 20% of <40 years age groups respectively (**Fig. 1**).

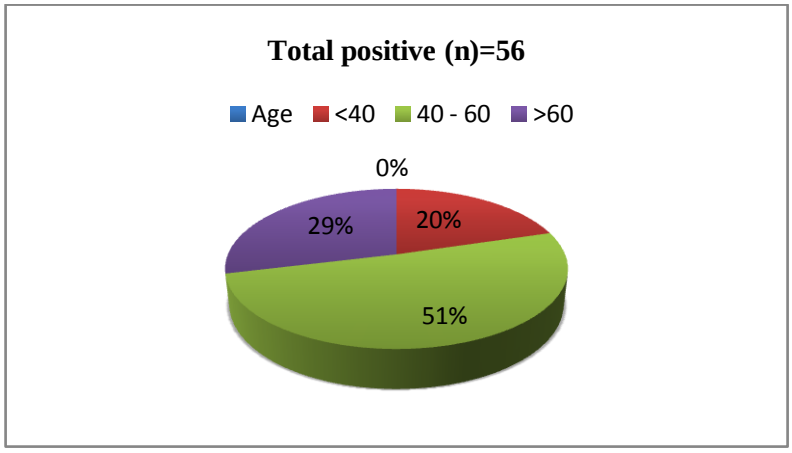

Fig. 1: Distribution of individuals based on their age

Among those 56 positive samples, 29 (51.79%) were male participants, comprising 15 people with diabetes and 14 non-diabetic patients, and 27 (48.21%) were female, with 11 people with diabetes and 16 non-diabetic patients. Total diabetic patients were 26 (46.43%), whereas a total of 30 (53.57%) non-diabetic infected controls were assigned for the investigation (**Fig. 2**).

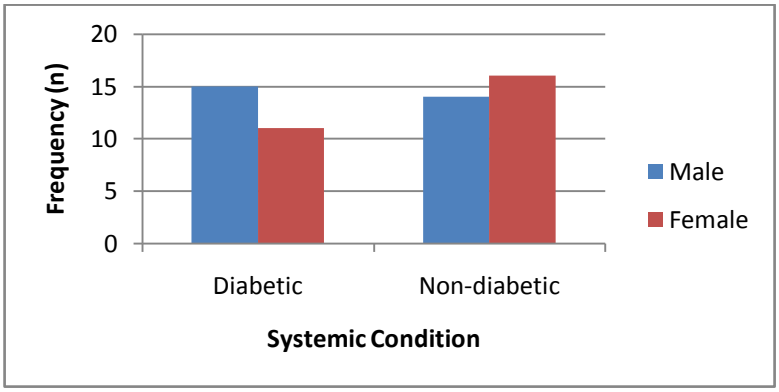

Fig. 2: Distribution of patients based on gender and systemic condition (Diabetic/ non-diabetic)

Various bacterial isolates from the patients were identified where *K. pneumoniae* (39.29%) was the most prevalent, followed by *P. aeruginosa* (35.71%), *E. coli* (14.29%), *S. marcescens* (8.99%) and *C. freundii* (1.79%) (**Fig.3**). *K. pneumoniae*, *P. aeruginosa*, *E. coli*, and *S. marcescens* are common in diabetic and non-diabetic individuals, except for *C. freundii*, which is apparent only in diabetic patients (**Fig. 4**).

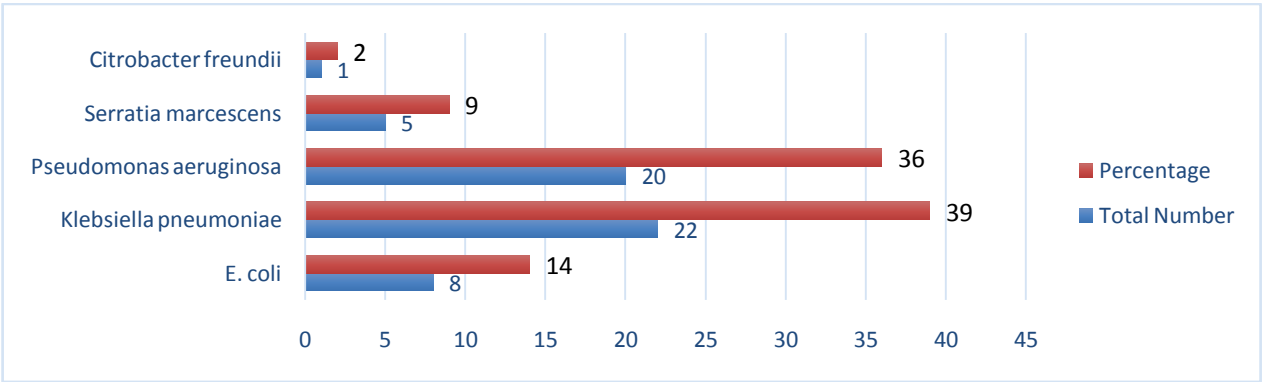

Fig. 3: Prevalence of bacterial pathogens in oral infections

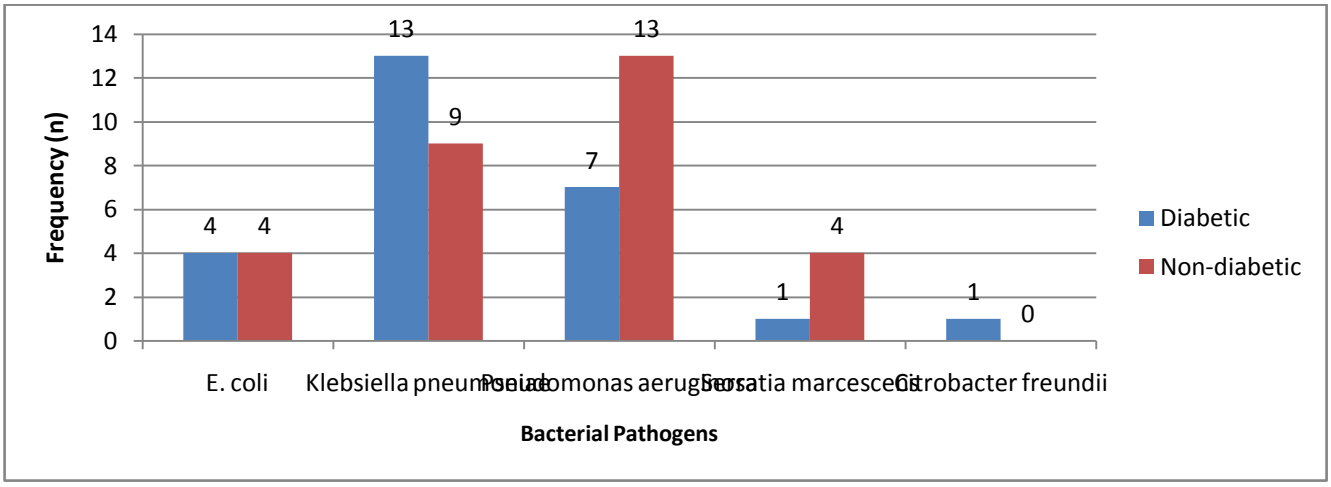

Fig. 4: Prevalence of bacterial pathogens in diabetic and non-diabetic patients

Fifteen different antibiotics such as Amoxicillin, Azithromycin, Cefixime, Ceftazidime, Ciprofloxacin, Levofloxacin, Meropenem, Trimethoprim, Nalidixic acid, Cefepime, Imipenem, Erythromycin, Cefotaxime, Ceftriaxone, and Cotrimoxazole were used to understand the antibiotic susceptibility patterns of the isolated pathogens. Our current study found Azithromycin, Ciprofloxacin, Imipenem, and Levofloxacin most effective (100%) against *K. pneumoniae*. In contrast, the highest resistance (86.36%) was found against Amoxicillin and Ceftazidime, followed by Meropenem (81.82%) and Cefepime (63.64%). The highest intermediate response (13.64%) was found in the Erythromycin and Trimethoprim groups (**Fig. 5**).

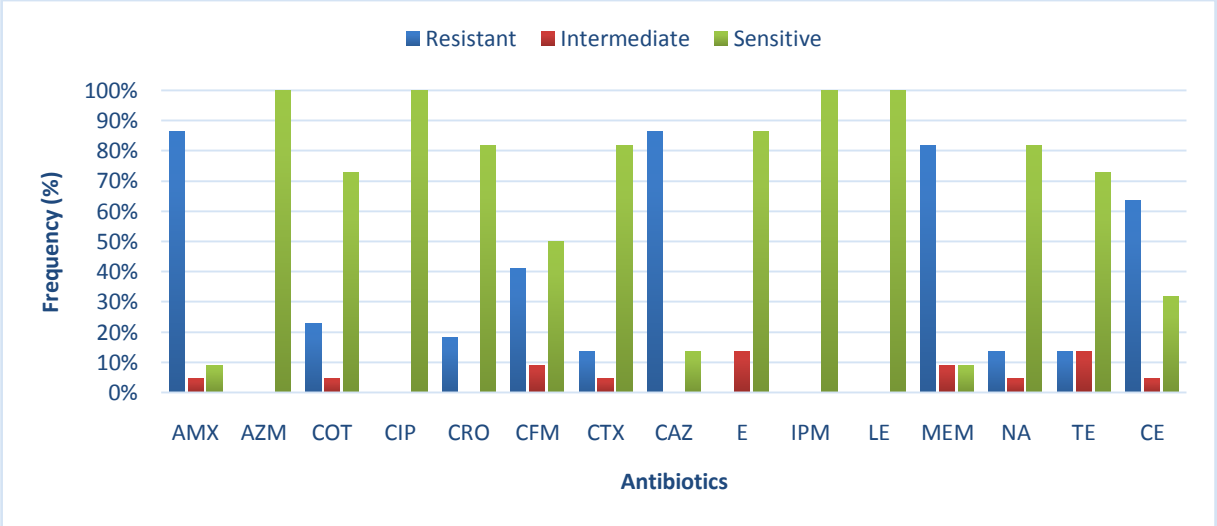

Fig. 5: Overall antibiotics susceptibility pattern of *K. pneumoniae*

Note: AMX=Amoxicillin, AZM=Azithromycin, COT=Cotrimoxazole, CIP=Ciprofloxacin, CFM=Cefixime, CAZ=Ceftazidime, LE=Levofloxacin, MEM=Meropenem, TE=Trimethoprim, NA=Nalidixic acid, CE=Cefepime, IPM=Imipenem,E= Erythromycin, CTX=Cefotaxime, CRO=Ceftriaxone

Diabetic *K. pneumoniae* was found to be resistant (30.77%) against Ceftriaxone which was found 100% effective against non-diabetic *K. pneumoniae*. Moreover, non-diabetic *K. pneumoniae* was 55.56% resistant against Meropenem, reaching 100% in diabetic *K. pneumoniae*. The highest intermediate response (23.08%) was also found in diabetic *K. pneumoniae*, which was against Trimethoprim (**Fig. 6 & 7**).

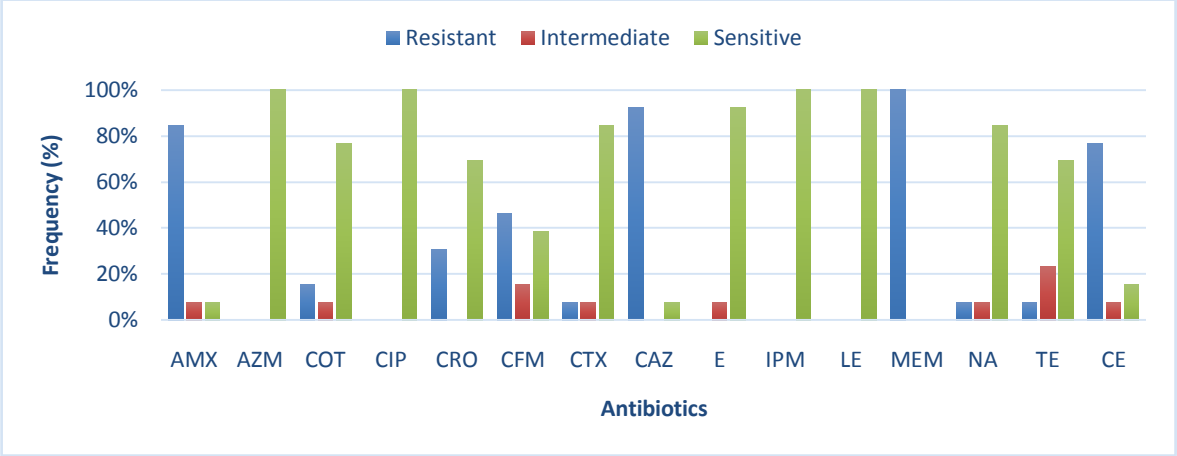

Fig. 6: Antibiotic susceptibility pattern of diabetic *K. pneumoniae*

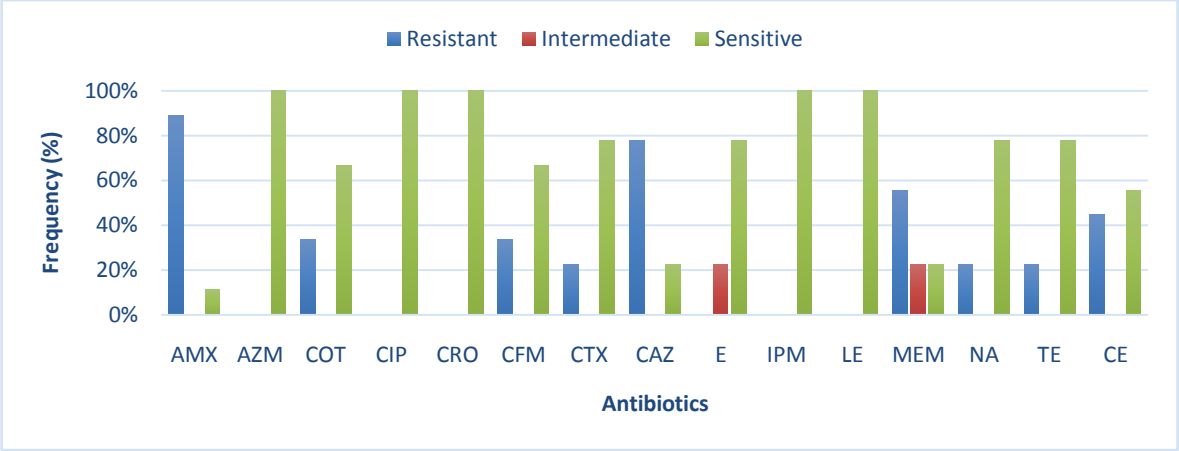

Fig. 7: Antibiotic susceptibility pattern of non-diabetic *K. pneumoniae*

Azithromycin, Ciprofloxacin, and Levofloxacin were the most effective (100%) antibiotics, followed by Imipenem (90%), Ceftriaxone (85%), Trimethoprim (85%), and Erythromycin (75%) against *P. aeruginosa*. In contrast, the highest resistance (95%) was shown by *P. aeruginosa* against Amoxicillin and Cefepime, followed by Ceftazidime (90%), Cefixime (85%), Meropenem (75%), and cefotaxime (65%). Nalidixic acid had the highest intermediate activity (25%) against *P.* aeruginosa (**Fig. 8**).

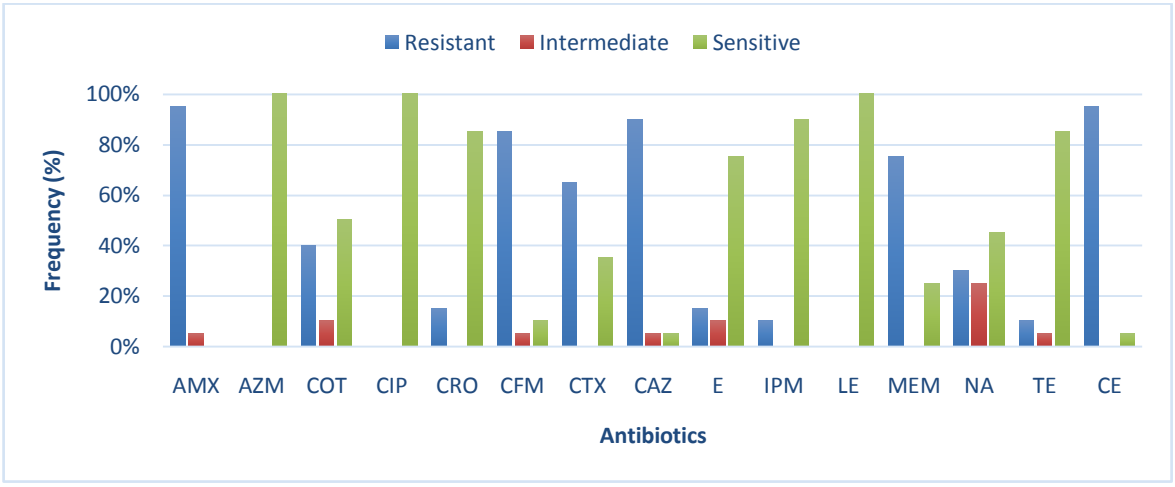

Fig. 8: Overall antibiotic susceptibility pattern of *P. aeruginosa*

Non-diabetic *P. aeruginosa* showed 84.62% resistance against Cefixime, whereas 100% resistance was exhibited by diabetic *P. aeruginosa*. No sensitivity by diabetic *P.aeruginosa* was observed against Cefepime, where mild sensitivity (7.79%) was shown by non-diabetic *P. aeruginosa* (**Fig. 9 & 10**).

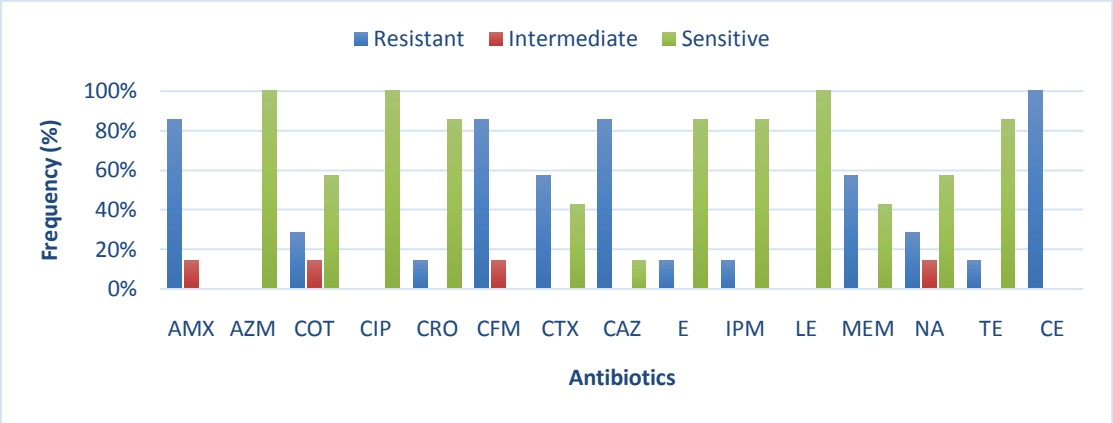

Fig. 9: Antibiotic susceptibility pattern of diabetic *P. aeruginosa*

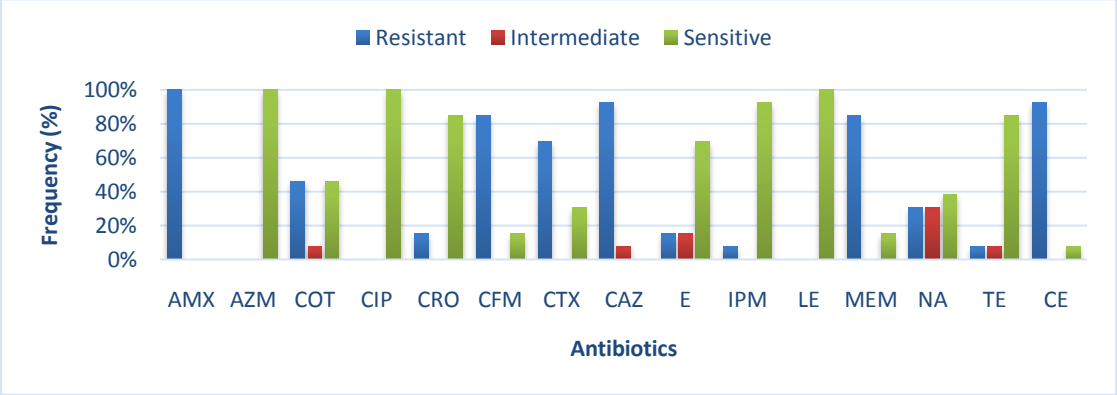

Fig. 10: Antibiotic susceptibility pattern of non-diabetic *P. aeruginosa*

E. coli showed the highest sensitivity (100%) against Azithromycin, Ciprofloxacin, Imipenem, and Levofloxacin, followed by Nalidixic acid (87.5%), Cotrimoxazole (75%), Cefotaxime (75%), and Erythromycin (62.5%). In contrast, the most resistance (100%) was shown against Amoxicillin and Ceftazidime, followed by Cefixime (87.5%), Meropenem (87.5%), and Cefepime (50%). Besides that, the highest intermediate activity (25%) was shown against Erythromycin, Trimethoprim and Cefepime by *E. coli* (**Fig. 11**).

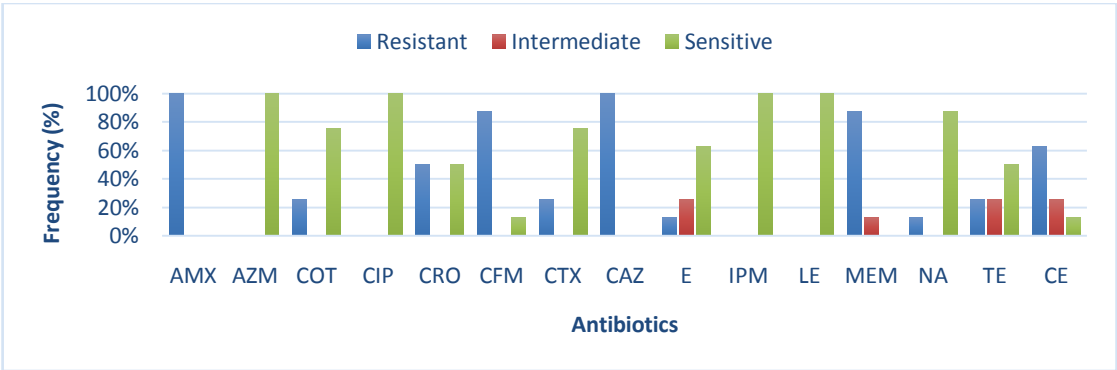

Fig. 11: Overall antibiotic susceptibility pattern of *E. coli*

S. marcescens was susceptible (100%) against Azithromycin, Ciprofloxacin, Erythromycin, Levofloxacin, Nalidixic acid, and Trimethoprim, followed by Cotrimoxazole, Cefotaxime, and Imipenem with an efficiency of 80%. In contrast, it exhibited the highest resistance against Ceftazidime (100%), followed by Cefixime (80%),Meropenem (80%), Ceftriaxone (60%), and Cefepime (60%). Amoxicillin was the antibiotic with the highest intermediate response (40%) against *S. marcescens* (**Fig. 12**).

C. freundii was a single pathogen isolated from an oral infection. Patients with diabetes showed susceptibility to azithromycin, ciprofloxacin, ceftriaxone, and nalidixic acid. In contrast, it possessed resistance against Amoxicillin, Cotrimoxazole, Cefixime, Cefotaxime, Ceftazidime, Erythromycin, Imipenem, Levofloxacin, Meropenem, and Cefepime, and an intermediate response against Trimethoprim (**Fig. 13**).

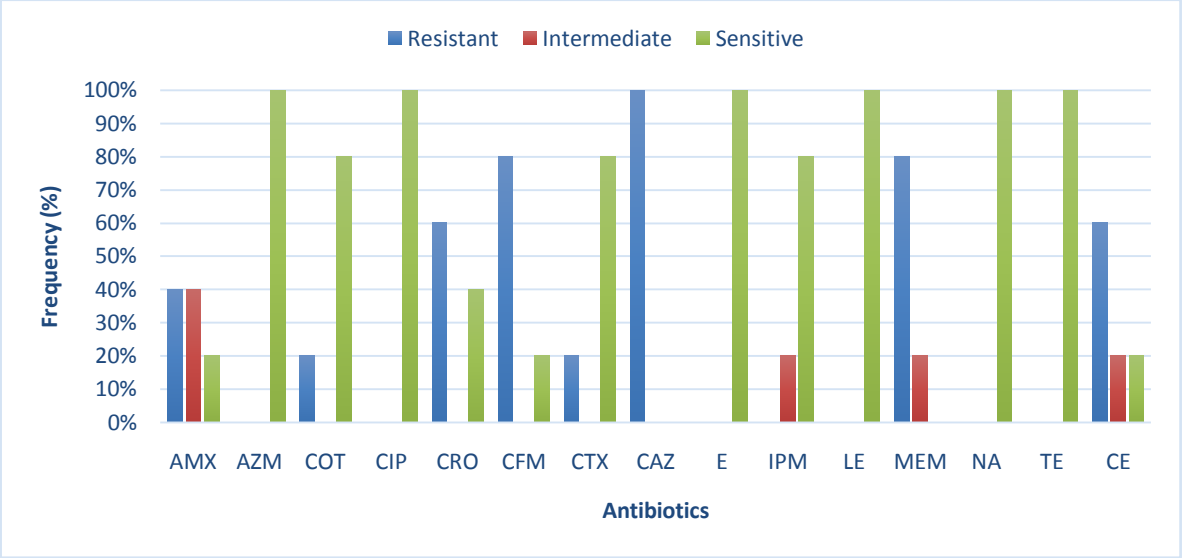

Fig. 12: Overall antibiotic susceptibility pattern of *S. marcescens*

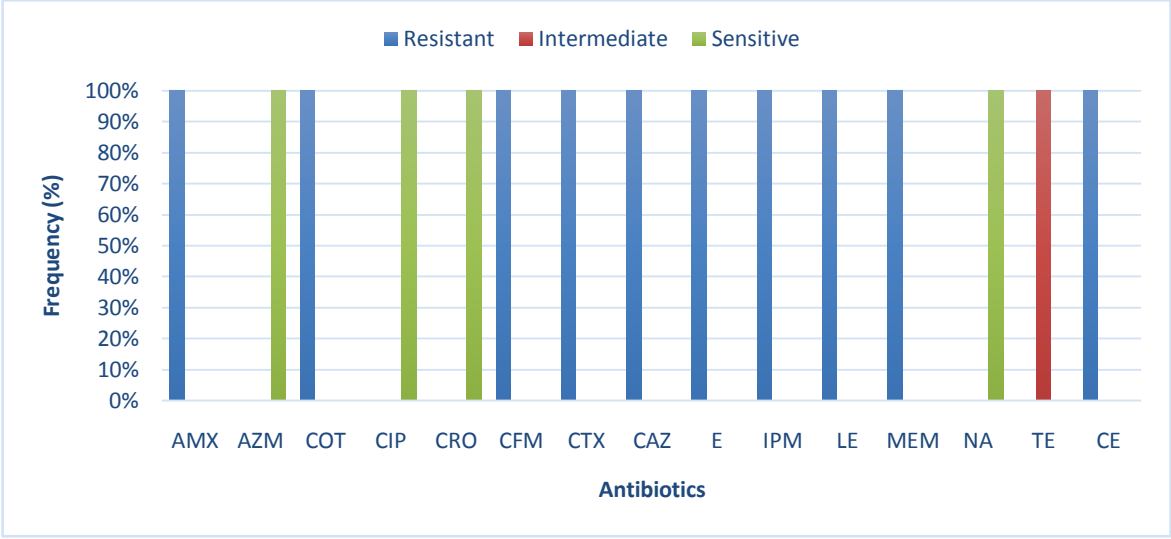

Fig. 13: Antibiotic susceptibility pattern of *C.freundii* isolated from diabetic patient

Overall, the antibiotic susceptibility pattern in our study revealed that *K. pneumoniae*, *P. aeruginosa*, *E. coli*, *S. marcescens*, and *C. freundii* were found relatively (100%) sensitive against Azithromycin and

Ciprofloxacin, followed by Levofloxacin (98.21%), Imipenem (92.86%), and Erythromycin (78.57%). On the other hand, the highest resistance was exhibited against Ceftazidime (91.07%), followed by Amoxicillin (87.5%), Meropenem (80.36%), and Cefepime (75%) by the isolated pathogens. Besides that, the highest intermediate response (12.5%) was shown against Erythromycin and Trimethoprim (**Fig. 14**).

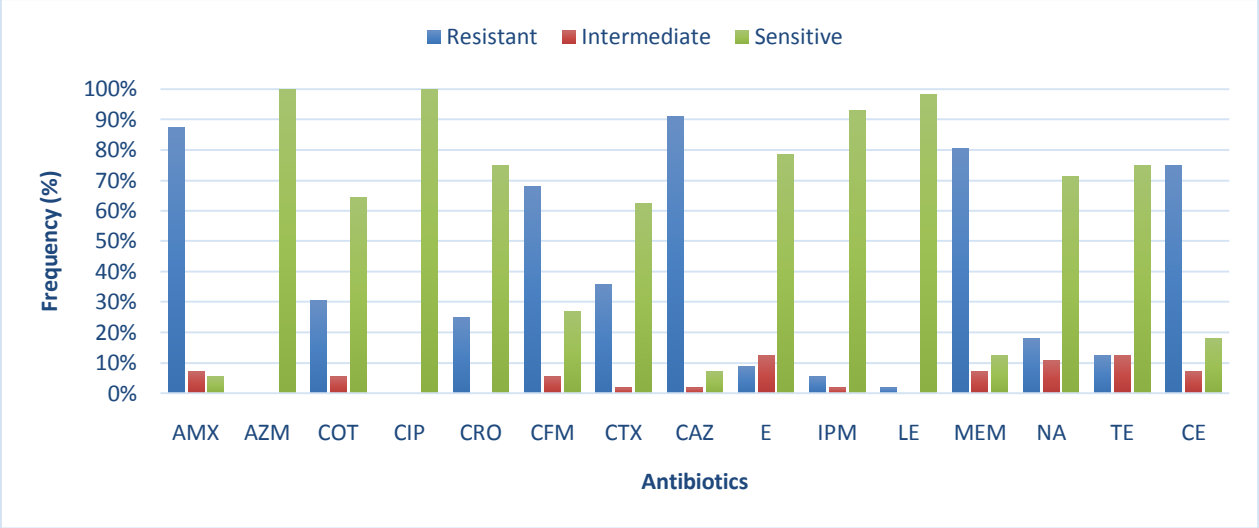

Fig. 14: Overall Antibiotic susceptibility pattern of bacterial pathogens of oral infections

4. Discussion

Oral diseases, including dental caries, gingivitis, and periodontal diseases, are the most widespread forms of oral bacterial infections worldwide. Periodontal diseases affect 47.1% of people in the United States under the age of 30, rising to 70.1% as people reach the age of 65 (Eke *et al*., 2012). In our current study, the age group ranging from 40 to 60 years was found to be more exposed to oral infections, covering 51% of total samples, followed by > 60 years (29%) and < 40 years (20%) age groups.

In our study, oral infections were slightly more frequent in males (51.79%) than in females (48.21%). Resembling findings were mentioned by Eke *et al*. (2012), revealing a statistic that 56.4% of males exhibited worse periodontal conditions, whereas 38.4% of females corroborated our findings. However, several studies have also elucidated the prevalence of oral infection in females more than in males (Shillitoeet al., 2012; Petrovic *et al.*, 2015). Besides age and gender, poor personal oral hygiene, limited education, and cigarette smoking are risk factors for increased periodontal progression (Eke *et al.*, 2012). Although-diabetic infected controls (53.57%) were higher than diabetic patients (46.43%) in our study participants, Taylor *et al*. (1996), Mealey and Rose (2008), Chee et al. (2013) and many other studies commissioned about the higher prevalence of oral infections even with greater severity in diabetic patients concerning poor control of glycemic status.

K. pneumoniae was the most prevalent (39.29%) pathogen, followed by *P. aeruginosa* (35.71%), *E. coli* (14.29%), *S. marcescens* (8.99%) and *C. freundii* (1.79%) in our sample. The results were consistent with Yacoubi (2013) and Saeb *et al*. (2019), who reported the appearance of diverse microorganisms in addition to our isolated bacterial pathogens, such as *Streptococcus viridans*, *S. mutans, Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia, Staphylococcus aureus*, and so on.

The maximum resistance exhibited by isolated pathogens in our current study was against Ceftazidime (91.07%), followed by Amoxicillin (87.5%), Meropenem (80.36%), and Cefepime (75%). Like in our study, in recent studies, Amoxicillin was found ineffective or less effective in the treatment of

oral infections by Ardila and Bedoya-Garcia (2020) and Bhat *et al.* (2021). However, it was found effective previously by Minguezet al. (2018) and Bhat *et al*. (2019). Unlike our study, due to the lower dose concentration applied in our investigation, Meropenem was found to be one of the most effective antibiotics. All the isolated pathogens showed extreme sensitivity of 100% to Azithromycin and Ciprofloxacin, followed by Levofloxacin (98.21%), Imipenem (92.86%), and Erythromycin (78.57%). Unlike in our study, Azithromycin was found less effective in various studies, with 28% and 40.5% resistance by Minguez*et al*. (2018) and Ardila and Bedoya-Garcia (2020), respectively. Erythromycin and Trimethoprim showed our study's highest intermediate response of 12.5%. Moreover, 90–100% of isolates of *K. pneumoniae*, *P. aeruginosa*, and *E. coli* showed resistance to multiple antibiotics, one of the most alarming findings of the study.

5. Conclusion

The present study was designed to investigate the etiological pathogens of oral infections in diabetic patients compared with non-diabetic patient controls and to observe the antibiotic susceptibility patterns of isolated pathogens to commonly prescribed antibiotics. For the investigation, 56 diabetic and non-diabetic patients, both male and female, of diverse ages were employed. Males (51.79%) were more than females (48.21%), whereas the 40- to 60-year age group was more prevalent (51%). *K. pneumoniae* was the most prevalent (39.29%) pathogen, along with *P. aeruginosa* (35.71%), *E.coli* (14.29%), *S. marcescens* (8.99%) and *C.freundii* (1.79%). Azithromycin and Ciprofloxacin were the most effective (100%) antibiotics, followed by Levofloxacin (98.21%), Imipenem (92.86%), and Erythromycin (78.57%). The maximum resistance exhibited by the isolates was against Ceftazidime (91.07%), followed by Amoxicillin (87.5%), Meropenem (80.36%) and Cefepime (75%), whereas the highest intermediate activity of 12.5% was shown by erythromycin and Trimethoprim.

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7 Authors Contributions

Research concept- Mohammad Zakerin Abedin, Research design- Mohammad Zakerin Abedin & Md. Oyes Quruni, Supervision-Mohammad Zakerin Abedin, Materials-Md. Easin Arfat & Farida Yeasmin, Data collection-Samim Mia & Summiya Shamima Prity, Data analysis and Interpretation- Md. Easin Arfat, Literature search- Md. Easin Arfat & Farida Yeasmin, Writing article-Md. Easin Arfat, Critical review-Md. Babul Aktar & Mohammad Zakerin Abedin, Article editing-Abdullah Akhtar Ahmed & Md. Babul Aktar, Final approval- All authors

8. Funding

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9. Conflict of Interests

The authors declare no conflicts of interests.

10. Ethical Statement

Institutional ethical clearance was taken from the ethical committee of KYAU.

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