10.21608/adjc.2024.303956.1599 Advanced Dental Journal
Volume 7 (2025) | Issue 3| Pages 386-396 ©Faculty of Dentistry-Cairo University

Original Article

Screening of Microbial Spectrum of Odontogenic Infections & Their Antibiotic Sensitivity: A Demographic Cross-Sectional Study on Egyptian Patients.

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Submitted: 04-8-2024
Accepted: 20-10-2024

Abstract

Aim: This study aimed to investigate the etiology, microbiology, and antibiotic sensitivity profiles of odontogenic infections among patients presenting to the department of oral and maxillofacial surgery at Cairo University.
Subjects and methods: A total of sixty-five patients with odontogenic infections were enrolled over a 40-month period, following thorough preoperative assessments including clinical, radiographic, and bacteriological examinations. Pus samples were collected via aspiration and subjected to aerobic and anaerobic cultures. Bacterial isolates were identified, and antibiotic susceptibility testing was performed. Treatment protocols consisted of surgical interventions such as incision and drainage, coupled with empirical antibiotic therapy tailored to individual patient profiles.
Results: Predominant bacterial isolates included enterococci, streptococci, and staphylococci, with variable susceptibility to commonly used antibiotics. Notably, enterococci exhibited sensitivity to ampicillin and levofloxacin but demonstrated reduced sensitivity to vancomycin. Streptococci showed sensitivity to cefotaxime and linezolid. Penicillin showed an accepted overall sensitivity rate.
Conclusion: Aerobic bacterial isolates were predominantly found, notably enterococci, differing from some international studies and emphasizing the need for region-specific research in clinical practice. Antibiotic sensitivity varied among isolates, with penicillin group antibiotics showing promise as first-line treatments, but caution advised.

Keywords: odontogenic infections, antibiotic resistance, microbiology, culture & sensitivity

Introduction

In the field of oral and maxillofacial surgery, odontogenic infections rank among the most frequently occurring diseases. (Uppada and Sinha, 2019). The first stage of an odontogenic infection is the inoculation of bacteria into the periapical tissues. This occurs via a carious or non-vital tooth, defective root canal treatment, or deep periodontal pocket. The periapical tissues are the initial sites of bacterial growth inducing the beginning of an immune response in the patient.(Døving, Handal and Galteland, 2020). Occasionally, the immune system alone or together with a therapeutic intervention (i.e. dental treatment combats the infection). But sometimes the infection cannot be restrained and spreads along the path of least resistance to affect the deep maxillofacial spaces. (Kitamura, 2017).

Odontogenic infections are considered polymicrobial. The most commonly isolated microorganisms are facultative anaerobes from *Streptococcus viridans* group.(Robertson and Smith, 2009). These microorganisms are common in the mouth and frequently linked to abscesses and orofacial cellulitis. The strict anaerobes (*Prevotella* and *Porphyromonas*) take over after a few days. Penicillin sensitivity is seen in most facultative *streptococci* that result in odontogenic infections. *Prevotella* and *Porphyromonas* strains are resistant to penicillin in about one-fourth of cases.(Flynn, 2011).

It is crucially important for the clinician to understand the polymicrobial nature of odontogenic infections as well as the principles of appropriate treatment approach, when to prescribe antibiotics, and what type of antibiotics should be prescribed.

Overuse of antibiotics among Egyptian patients,(Elsayed *et al.*, 2021) as well as the lack of national studies on the microbiology of the odontogenic infections in the Egyptian population were the motives behind the design of our study.

Aim of the study

The aim of this study is to screen the current causative organisms of odontogenic infections in Egyptian patients and to test their susceptibility to antibiotics.

Materials and methods

A prospective study of sixty-five patients diagnosed with oral and maxillofacial infections of odontogenic origin were selected from the department of oral and maxillofacial surgery, Cairo university over a period of 40 months. Patients from both genders were recruited with age range from 2 years up to 80 years. Infections of non-odontogenic causes (i.e. post-operative infection, infections of edentulous patients) were excluded from the study. A thorough preoperative assessment of all patients was carried out including case history, physical and radiographic examination. The gathered information was recorded in a diagnostic sheet for each patient that was custom made to address our research. All patients consented to the use of the data in our research, either orally or on the written form.

Case History:

A comprehensive history has been obtained from each patient which included chief complaint, history of present illness, past medical and dental history. History of the swelling and pain. Difficulty in swallowing, breathing, &/or mouth opening. Factors that intensify the symptoms. Presence of fever/ malaise. The data were documented in each patient diagnostic sheet.

Physical Examination:

Extra-oral examination of the patient's head and neck was carefully done with inspection, and palpation to assess areas of erythema, swelling or draining pus. Bimanual palpation of the floor of the mouth, submandibular triangle, and mandibular angle area was done to provide information about the anatomic spaces involved. Palpation of the size of the swelling to detect any fluctuation (which indicates pus formation), tenderness, local temperature and formation of sinus tract or fistula. Diffuse, warm, erythematous, indurated, hard and tender to touch swelling is indication of cellulitis with no true abscess formation. Examination of regional lymph nodes for signs of inflammation (tenderness, or enlargement).

Figure 1 Right buccal space infection spreading to right submandibular space

Intra oral examination was performed to detect the involved teeth pain on percussion, mobility, or extrusion, the site and extent of the swelling, the presence of draining sinus tracts or sulcus obliteration, & the vitality of the related teeth

Bacteriological Examination

Sample Collection and Transport:

Pus samples were acquired through aspiration, with prior disinfection of the overlying skin or oral mucosa using either a 7.5% povidone-iodine solution (Betadine®)[1] or 70% Ethyl Alcohol[2], allowing it to air dry. Sampling was conducted intraorally through intact oral mucosa or extra orally through the skin using a sterile disposable 20 ml plastic syringe mounted with an 18-gauge needle. The needle was inserted into the abscess cavity, and pus was aspirated.

For anaerobic culture, the specimen was placed in a tube containing anaerobic transport media (thioglycolate broth), while the specimen for aerobic culture remained in the aspirating syringe. Subsequently, both specimens were promptly transported to the laboratory.

Isolation and Identification:

Direct smear was prepared from the samples of each case to demonstrate the morphology of the present organism and detecting the pus cells.

The aerobic specimens were inoculated on 5% sheep blood agar, chocolate agar, MacConkey agar and nutrient agar for incubation in temperature of 37 C plus 5% CO2. The anaerobic specimens were inoculated on 5% sheep blood agar, chocolate agar and placed in an anaerobic chamber (anaerobic gas pack jar). Aerobic growth was examined after 24 and 48 hours while anaerobic growth was examined every 3 days for 7 days.

Figure 2 Culture process of bacteria

Identification of Bacterial Isolates: was done by macroscopic examination, microscopic examination, & biochemical reactions including catalase test, coagulase test, optochin sensitivity test & sugar fermentation.

Antibiotic Susceptibility Testing:

Antibiotic susceptibility testing was done by "disk diffusion method" using Muller- Hinton agar and a group of commercially prepared antibiotic discs manufactured by (Bioanalyse). The sensitivity pattern of each bacterial isolate was done. The diameter of the inhibitory zone was measured around each antibiotic disc and interpreted as sensitive, intermediate, or resistant.

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[2] Afak Co. 6th October city Egypt

Selection of antibiotic discs according to the CLSI 2022 document, issued by the department of clinical microbiology/ the central administration of laboratories at the ministry of health.

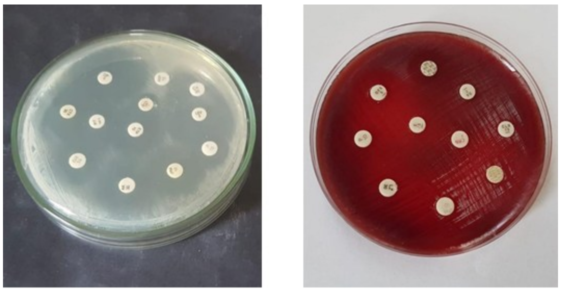

Figure 3 Antibiotic sensitivity using disc diffusion methods

Treatment:

All patients included in the present study were treated by combination of surgical treatment (incision and drainage/ elimination of the cause) and medical treatment.

Statistical methods

Data management and statistical analysis were performed using the Statistical Package for Social Sciences (SPSS) version 24. Numerical data were summarized using means and standard deviations and ranges. Categorical data were summarized as percentages.

Results

Sixty-five patients with maxillofacial infections of odontogenic origin were included in this study. They were selected from the Department of Oral and Maxillofacial Surgery, Faculty of Oral and Dental Medicine, Cairo University. The mean age of patients was 35.13 ±14.7 years and ranged from 6 to 65 years, 37% of them were females and 63% were males. All patients exhibited multiple signs and symptoms. Specifically, each patient presented with both erythema and lymphadenopathy, accounting for 100% of the cases. (65 patients), while 2 patients (3%) presented with fistula and 7 patients (11 %) presented with trismus. The most common site of infection was the submandibular space (48%) followed by the buccal space (46%). Three patients presented with osteomyelitis of the maxilla (6%) & one patient with osteomyelitis of the mandible (2%). 35 % of the patients presented with multiple space infections. Infections arouse more frequently from mandibular teeth 77.5% while it arouses from maxillary teeth in 22.5% of the cases. The most common causative teeth were mandibular third molars (30%) followed by mandibular first molars (28%).

Microbiological Tests Results

A total of 65 pus samples were collected. Bacterial growth was detected in 47 samples (70.8%), fungal growth in 2 samples (3%), and 18 samples (27.7%) showed no growth. Among the 47 samples with bacterial growth, 46 (70.77%) had pure aerobic isolates, 1 (1.54%) had a pure anaerobic isolate, and none had mixed aerobic and anaerobic isolates.

From 65 patients, 66 bacterial strains were isolated, with 63 (95.45%) being aerobic, 1 (1.51%) anaerobic Peptostreptococcus, and 2 (3.03%) Candida. The most frequently isolated bacteria were Enterococci (19 strains, 28.79%), followed by Viridans streptococci (18 strains, 27.27%), and Staphylococcus (15 strains, 22.73%). The least common isolates were E. coli, Peptostreptococcus, Hemophilus, and Methicillin-resistant Staphylococcus aureus (MRSA), with 1 strain each (1.52%).

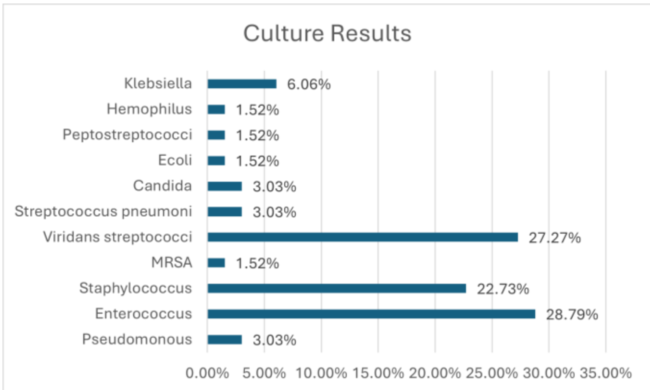

Figure 4 Bar chart showing Culture Results percentile.

Antibiotic Sensitivity of Aerobic Bacterial Isolates:

In this study, various bacterial strains were isolated from pure aerobic cultures and tested for antibiotic sensitivity. Enterococci were the most common isolate (19 instances) showing 100% sensitivity to ampicillin and levofloxacin, 89.4% sensitivity to penicillin and linezolid, and 63.16% sensitivity to vancomycin. Streptococcus viridans, isolated in 18 instances, exhibited 100% sensitivity to cefotaxime and linezolid, 94.4% to levofloxacin, 83.3% to ofloxacin, 72.2% to penicillin, 55.6% to vancomycin, and 50% to clindamycin. Staphylococci were isolated in 15 instances, with 93.3% sensitivity to levofloxacin, 86.6% to linezolid, 80% to cefoxitin, 73.3% to vancomycin, 66.6% to penicillin, and 40% to erythromycin. Klebsiella spp., isolated in 4 instances, showed 100% sensitivity to amoxicillin-clavulanate and levofloxacin, and 50% sensitivity to penicillin and cefoxitin.

Streptococcus pneumoniae, isolated in 2 instances, demonstrated 100% sensitivity to ofloxacin and levofloxacin, 50% sensitivity to penicillin and erythromycin, and 0% sensitivity to clindamycin. Pseudomonas spp. was isolated in 2 instances, showing 100% sensitivity to ceftazidime, imipenem, and levofloxacin, and 50% sensitivity to amikacin. Hemophilus spp. were isolated in 1 instance and were sensitive to ampicillin, imipenem, amoxicillin-clavulanate, ceftazidime, cotrimoxazole, azithromycin, and meropenem. MRSA was isolated in 1 instance, showing sensitivity to erythromycin, linezolid, azithromycin, and levofloxacin, but 0% sensitivity to penicillin, clindamycin, vancomycin, and cefoxitin.

Among the 63 aerobic isolates tested, levofloxacin showed a sensitivity rate of 91.80% (tested on 61 isolates), penicillin 72.88% (tested on 59 isolates), linezolid 92.45% (tested on 53 isolates), vancomycin 62.26% (tested on 53 isolates), ampicillin 100% (tested on 20 isolates), azithromycin 62.50% (tested on 16 isolates), and erythromycin the lowest sensitivity rate at 44.44% (tested on 18 isolates).

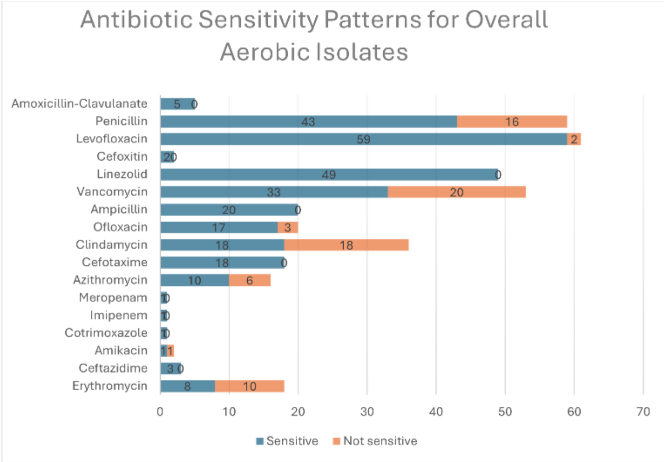

Figure 5 Bar chart representing Antibiotic sensitivity pattern for overall isolated aerobic bacterial strains

Antibiotic Sensitivity of Anaerobic bacterial isolates

Peptostreptococcus:

Peptostreptococcus were isolated in 1 instance, found in pure anaerobic. Levofloxacin, rifampin, linezolid, chloramphenicol & vancomycin had 100 % antibiotic sensitivity rate.

Discussion

Odontogenic infections are one of the most common problems in the daily practice in dentistry. The spread of odontogenic infections can cause serious complications that may eventually escalate to life-threatening situations in rare instances. Understanding the etiology and microbiology of odontogenic infections is of paramount importance to effectively treat them and prevent their spread and associated complications.

Bacterial resistance has emerged as a significant challenge in treating odontogenic infections in recent years. This resistance is increasing, primarily due to factors like patient self-medication and bacterial mutations.(Cuevas-Gonzalez *et al.*, 2022)**.** Self-medication is a widespread problem within the Egyptian patients (Elsayed *et al.*, 2021). While international literature extensively covers microbial spectrum and antibiotic sensitivity in odontogenic infections, there is a notable absence of studies conducted in Egypt on this subject. This study examined the microbial spectrum and antibiotic sensitivity of odontogenic infection in 65 patients in Cairo Government.

Out of 65 patients diagnosed with odontogenic infection, samples were obtained via aspiration and sent to the laboratory for microbiological analysis. Culturing was performed using blood agar and nutrient agar to facilitate the growth of both fastidious and nonfastidious bacteria, while chocolate agar was employed as a selective medium for gram-negative bacteria. Bacterial growth was observed in 47 cases. However, 18 samples (27.69%) showed no growth, which may be attributed to factors such as errors during sample collection, contamination of specimens during pus collection, and most importantly, preoperative self-medication by patients before their hospital visit.

The current investigation showed a predominance of males (63%), aligning with findings from many studies in the literature.(Katoumas *et al.*, 2019),(Heim *et al.*, 2017, 2020),(Wang, Huang and Long, 2022), (Adamson *et al.*, 2019). This could be attributed to potentially better oral hygiene practices among females compared to males, resulting in fewer instances of dental caries in females.

The submandibular space emerged as the most frequently affected space (48%), consistent with findings reported in most of the literature. Similarly, mandibular molars were identified as the primary causative teeth.

Pure aerobic bacterial isolates were found in 70.77% of the cases. This result was considerably high compared to ***Habib et al., (2017), Abdel Fatah (2020) & Zeyad (2021)*** who reported 36%, 31% & 38% of the cases respectively. In Poland, *Zawiślak and Nowak* found pure aerobic bacterial isolates in 36.5% of the patients(Zawiślak and Nowak, 2021).

Pure anaerobic bacterial isolates were found in 1.54% of the cases. ***Zeyad (2021)*** reported pure anaerobic isolates in 23.3% of the cases, which is consistent with ***Abdel Fatah. (2020)*** who reported 22% of the cases, while ***Habib et al., (2017)*** reported only 5%. In this study significantly lower values of anaerobic isolates were encountered which may be due to errors in sample collection/ transfer or delayed start of the culture in the laboratory which may affect the outcome significantly. Further assessment of the cause of low levels of anaerobes in these results must be assessed in the following research.

In recent studies, bacteriological assessment of organisms causing odontogenic infections shows a significant shift towards anaerobes. 65.3% anaerobes in (López-González *et al.*, 2019), & 87.5% (Katoumas *et al.*, 2019).(Böttger *et al.*, 2021) stated, using experimental 16S rRNA gene next-generation sequencing that 98% of pus samples were anaerobic bacteria. Microbiome analysis identifies a considerably higher number of bacteria compared to traditional culture-based methods, providing results even when conventional cultures yield negative results.

In their review, (Robertson and Smith, 2009) mentioned that a mix of aerobes and anaerobes accounts for most of odontogenic infections with a range of 59-75%. This contradicts the currently conducted study as mixed infections were absent. ***Zeyad (2021)*** encountered (26.6%) mixed infections while ***Habib et al., (2017) and Abdel Fatah. (2020)*** reported 53%, 46% respectively.

In most international studies, Streptococcus viridans was typically identified as the most common bacterial isolate. However, the present study revealed enterococci (28.79%) as the predominant bacterial isolate, with

Streptococcus (27.74%) closely following. This finding closely aligns with the results of ***Zeyad (2021)***, where Streptococcus viridans was the predominant aerobic bacteria (28.5%), followed by enterococci (17%). Notably, ***Habib et al., (2017) and Abdel Fatah (2020)*** did not report the presence of enterococci in their studies. (Mochalov *et al.*, 2023) reported an enterococci prevalence of 23.01%, albeit with a small sample size (13 patients), similarly (Weise *et al.*, 2019) reported enterococcus (35%) with sample size of 16 patients. The prevalence of enterococcus in odontogenic infections among Egyptian patients warrants further investigation and research.

The prevalence of Staphylococci in this investigation is significant, reaching 22.73%, despite meticulous disinfection of the skin and mucosa and solely employing pus aspiration without any swabbing. Staphylococci are common skin contaminants, potentially leading to sample contamination during aspiration from extraoral abscesses through the skin. In this study, staphylococci were detected in 15 specimens collected from extraoral abscesses, with 10 of these specimens showing co-isolation with other bacteria. This finding suggests that staphylococci may not be the primary pathogenic bacteria in these cases. (Heim *et al.*, 2017) observed a prevalence of 13.5% Staphylococci, ranking as the third most common isolate in their study. Their pus sampling method involved using a swab, which could potentially lead to cross-contamination with normal skin pathogens. In a retrospective study by (Zawiślak and Nowak, 2021), staphylococci spp. constituted 30% of their bacterial isolates, making it the second most common species. However, details regarding the pus sampling method were not provided in their study.

Peptostreptococcus was found to be isolated only once in the current study, contrasting with the findings of ***Habib et al., (2019), Zeyad (2021)***, and ***Abdel Fatah (2020)***, where it was isolated at almost equivalent percentages (17.5%). As previously mentioned, this study did not successfully isolate anaerobic bacteria. The hypothesis for this discrepancy is summarized as potential sampling errors, transfer issues, or laboratory delays in cultivation.

Regarding the antibiotics tested in this investigation, the CLSI 2022 guidelines were followed. Enterococci in this study was sensitive to ampicillin and levofloxacin, with the lowest sensitivity rate to vancomycin (63.16 %). Enterococci exhibit intrinsic resistance to numerous antibiotics such as clindamycin, certain penicillin, cephalosporins, and erythromycin. Furthermore, it's essential to recognize that while enterococci possess intrinsic resistance to these antibiotics, they have the potential to acquire resistance to a broad spectrum of antibiotics through genetic mutations or by acquiring resistance genes from other bacteria. (Said, Tirthani and Lesho, 2024). This highlights the significance of evaluating their prevalence in odontogenic infections among the Egyptian population.

Viridans streptococci exhibited sensitivity to cefotaxime and linezolid, while levofloxacin demonstrated a sensitivity rate of 94.4%. Penicillin showed a sensitivity rate of 72.2%, while vancomycin exhibited a sensitivity rate of 55.6%. However, clindamycin displayed the lowest sensitivity rate at 50%.
In contrast, ***Zeyad (2021)*** reported sensitivity rates for amoxicillin (88.4%), amoxicillin/clavulanic acid, clindamycin, and ciprofloxacin (96.1%), erythromycin (84.6%), cefotaxime, and ceftriaxone (92.3%). These findings were consistent with those of ***Habib et al., (2017)*** except for amoxicillin, which showed a much lower sensitivity rate (15.7%).
Additionally, ***Abdel Fatah (2020)*** reported lower rates for ciprofloxacin and amoxicillin/clavulanic acid (33.3% and 16.7%, respectively).

As noted earlier, peptostreptococci was observed only once, and it displayed sensitivity to all tested antibiotics. However, due to the limited sample size, it is not possible to determine the significance of the relationship between peptostreptococci and their sensitivity to the tested antibiotics. (Zeyad, 2021) discovered that the sensitivity rates of the isolated peptostreptococci to various antibiotics were as follows: amoxicillin (31.2%), amoxicillin/clavulanic acid (93.7%), ciprofloxacin (93.7%), cefotaxime (78.1%), ceftriaxone (78.1%), clindamycin (96.8%), and metronidazole (100%). These findings aligned with those of ***Habib et al., (2017)***, except for amoxicillin, where all strains were resistant. Additionally, ***Abdel Fatah (2020)*** reported a similar rate for metronidazole sensitivity but a substantially lower rate for amoxicillin/clavulanic acid and ciprofloxacin (20%).

In the current research, Clindamycin demonstrated a decreased sensitivity rate of 50%, largely attributable to the predominance of aerobic isolates in this study. Yet consistent with recent literature findings showing increased resistance to clindamycin as observed in studies by (Kang and Kim, 2019),(Sebastian *et al.*, 2019), (López-González *et al.*, 2019), (Heim *et al.*, 2017, 2020)& (Katoumas *et al.*, 2019). Clindamycin is associated with well-known adverse effects such as antibiotic-associated colitis due to Clostridium difficile, gastrointestinal intolerance, metallic taste, and hypersensitivity reactions, as documented by (Flynn, 2019). Despite these side effects, Clindamycin is frequently prescribed as a primary antibiotic for patients with confirmed penicillin allergies in many countries. This highlights the necessity for further research and investigations into the escalating trend of bacterial resistance to Clindamycin.

Erythromycin exhibited the lowest sensitivity rates in this study (44.4%), coinciding with recent publications stating that it should no longer be used in dentistry.(Flynn, 2019).

Levofloxacin and linezolid presented the greatest sensitivity rates among all bacterial isolates. Nevertheless, it's crucial to highlight that these antibiotics should not be used as first-line treatments for odontogenic infections because of their potential adverse effects. Instead, they should be reserved for managing severe infections induced by multidrug-resistant bacteria. Side effects of linezolid include peripheral and ocular neuropathy, anemia, thrombocytopenia, hyperlactatemia, hypoglycemia & reticulocytopenia. (Hashemian, Farhadi and Ganjparvar, 2018). On the other hand, levofloxacin's side effects include photosensitivity, tendonitis, or tendon rupture, and rare yet serious side effects such as nervous system effects such as nervous system effects, including seizures or hallucinations, and cardiac arrhythmias. (Rusu *et al.*, 2023)

Members of the penicillin family still show accepted sensitivity rates in the present study; Penicillin 72,88%, ampicillin 100%, amoxicillin-clavulanic 100% & oxacillin 85%. That coincides with our colleagues ***Zeyad (2021), Abdel Fatah (2020), Habib et al., (2017)*** in their previous studies and with international studies as well. (Heim *et al.*, 2017),(Flynn, 2019), (Farmahan *et al.*, 2014).

Based on the results of the current study penicillin group antibiotics are recommended to be the first-choice antibiotic for treatment of odontogenic infection as it has a good efficacy against bacterial isolates in this study, also it has fewer side effects when compared to linezolid and levofloxacin.

Conclusion

In conclusion, the present study sheds light on the microbial spectrum and antibiotic sensitivity of odontogenic infections among patients in Cairo Government, Egypt. This investigation revealed a predominance of pure aerobic bacterial isolates, notably with enterococci emerging as the predominant bacterial isolate which differs from some international studies but underscores the importance of region-specific research in guiding clinical practice. Additionally, the relatively low prevalence of anaerobic isolates prompts further inquiry into potential sampling errors and laboratory procedures.

Antibiotic sensitivity testing demonstrated varying responses among different bacterial isolates, with penicillin group antibiotics showing promising efficacy as first-line treatment options. However, caution is warranted in prescribing antibiotics like clindamycin and erythromycin due to rising resistance rates and lower sensitivity.

In summary, this study underscores the significance of region-specific research in understanding odontogenic infections and highlights the importance of evidence-based antibiotic prescribing practices in dental care. By aligning clinical decisions with microbiological insights, we can enhance patient care and combat the challenge of antibiotic resistance effectively. Further research is warranted to delve deeper into the factors influencing microbial trends and antibiotic resistance in odontogenic infections, ultimately improving treatment strategies and patient outcomes.

Conflict of interest: No conflict of interest.

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors

Ethics: This study protocol was approved by the ethical committee of the faculty of dentistry- Cairo university on: 13/7/2016.

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