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RESEARCH ARTICLE

CHARACTERIZATION AND ANTIBIOTIC SENSITIVITY OF BACTERIA IN OROFACIAL ABSCESES OF ODONTOGENIC ORIGIN

Al-Kasem Mohammed Abbas¹, Taghreed Ahmed M Al-Kibsi¹,
 Ameen Abdullah Yahya Al-Akwa², Khaled A AL-Haddad², Hassan Abdulwahab Al-Shamahy³, Mohammed A Al-labani²

¹Department of Maxillo-Facial, Faculty of Dentistry, Sana'a University, Republic of Yemen.

²Orthodontics, Pedodontics and Prevention Department Faculty of Dentistry, Sana'a University, Yemen.

³Department of Basic Sciences, Faculty of Dentistry, Sana'a University, Republic of Yemen.

ABSTRACT

Background: Odontogenic infections vary from minor abscesses to superficial and deep infections that lead to acute infections in the head and neck area that may threaten the patient's life. The objectives of this study are to identify bacteria accountable for orofacial infection of odontogenic origin and patterns of their susceptibility to drugs in order to provide a superior understanding of dental infection management in Yemen.

Methods: The study was conducted on a selected group of patients, regardless of gender and age, who suffer from severe to moderate orofacial infections of odontogenic origin and were admitted to the dental clinic at the Republican University Hospital in Sana'a city. Sample of pus was collected and the bacteria were identified by cultivation in suitable medium and then identified by standard bacteriological techniques. Antimicrobial susceptibility testing was also performed using the Kirby-Bauer disc diffusion method.

Result: A total of 118 cases were positive for bacterial culture, 63 males and 55 females ranged between 5 and 65 years of age, most of them in the age group >45 years (39.8%), 51.7% had dental abscesses and 48.3% had periodontal abscesses. *Staphylococcus aureus*, *Bacteroides* species and *Staphylococcus epidermidis* were isolated from patients with dental abscesses, *Staphylococcus aureus*, *Bacteroides* species, *Staphylococcus epidermidis* and *Streptococcus pyogenes* from perio-Dental abscesses. The most prevalent bacteria were *Staphylococcus aureus* (about 63% of the total isolates), more than 40% of which were resistant to ceftizoxime, clarithromycin, augmentin, tetracyclines, erythromycin and oxacillin. While *Staphylococcus epidermidis* showed less antibiotic resistance than *Staphylococcus aureus*. As for the *Bacteroides* species, it was sensitive to metronidazole and clindamycin (100%), augmentin (98.6%), clarithromycin (94.4%) and finally vancomycin (76.1%).

Conclusion: An elevated occurrence of bacterial isolates was discovered, and *Staphylococcus aureus* was dominant. Most of the isolated bacteria were resistant to diverse classes of antibiotics. Appropriate antibiotics should be administered based on the bacterial isolates, culture sensitivity, and clinical course of disease.

Keywords: Antibiotic susceptibility; Odontogenic infection; Orofacial abscesses, Sana'a, Yemen.

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Address for Correspondence:

Prof. Hassan A. Al-Shamahy, Faculty of Dentistry, Sana'a University, P.O. Box 775 Sana'a, Yemen, Tel- +967-770299847; E-mail: shmahe@yemen.net.ye

INTRODUCTION

The odontogenic infection is the most common infection of the mouth and face area and has overwhelmed the human race for centuries. Oral and facial infections resulting from a purulent cause are the most common odontogenic¹ and vary from minor abscesses to superficial and deep infections of the neck². Usually these infections are due to tooth decay,

gingivitis, crown infection, dental trauma, and to some degree as a result of complications from dental procedures^{3,4}. The successful managing of this infection depends on altering the environment by relieving pressure, removing the causative agent of the disease and choosing an appropriate antibiotic². In the United States of America, the National Center for Disease Control and Prevention estimated that about one third of all prescriptions of outpatient antibiotic are

not necessary and this rate is higher in developing countries such as Yemen, where most prescriptions are unnecessary⁵. Failure to prescribe appropriate antibiotics may be linked with the expansion of resistance and adverse side effects⁶. Regardless of all the developments in diagnostic testing and the accessibility to modern antibiotic treatment, such infections persist to cause important morbidity and mortality rates, particularly once there is no early treatment^{7,8}. The significant problems linked with antibiotic use have encouraged studies looking at antibiotic prescribing practices for dentists⁹⁻¹³. Furthermore, the option of antibiotic to manage a dental infection preferably depends on the appropriate culture and susceptibility report. Patients with this infection are normally prescribed antibiotics on an experimental basis without knowledge of the exact pathogen. This antibiotic treatment may or may not lead to positive results due to a variety of factors such as microbial specificity and drug resistance. In patients of immunocompromised, this treatment schedule directs to a faster decline in health situation. These limits are compounded by a need of advanced amenities to quickly and precisely detect pathogenic microorganisms. Dentists must understand pathobiology and proper management of this infection as it is so important. In addition, differences in geography, prevalence of resistant bacterial strains and local antimicrobial prescribing practices lead to variation in the antibiotic properties of the bacteria between populations in different regions of the world¹⁴. Updated information on the pattern of microbial resistance at the national and local levels must inform the rational use of offered antimicrobial drugs¹⁵. It is known that early finding of pathogens and enhanced diagnostic methods are necessary to improve the healthcare system. Because microorganisms differ from region to region in addition to their sensitivity, it is vitally important to conduct such studies that will help monitor the constant development of the susceptibility of bacteria to regularly utilized drugs. Several studies have found that there are changes that have occurred in the organisms that cause maxillofacial infection in the world in general¹⁶. Therefore, this clinical and microbiological study was planned to verify the legitimacy of these allegations. The aim of this study was to detect the bacteria responsible for orofacial abscesses infections and to find out the pattern of their sensitivity to antimicrobials against the usually prescribed antibiotics in Yemen.

SUBJECTS AND METHODS

Patients

This study integrated 118 patients suffering from moderate and severe oral infections of odontogenic origin (abscesses), who were admitted to the dental clinic at the Republican University Hospital in Sana'a, in a period of nearly one year, which started in December 2019 and ended in November 2020, of whom 63 were males and 55 were females. Their ages varied from 5 to 65 years by an average of 36.2 years.

Study design

This study hospital base cross sectional study. The dental clinic at the Republican University Hospital was chosen as a search site. All dental clinics in government hospitals in Sana'a have been asked to refer cases of odontogenic origin (abscesses) for examination and to collect pus specimens and culture bacteria.

Data collection and processing

The patient's personal and clinical data were collected on a questionnaire for each patient. This included age, gender, occupation and relevant clinical information regarding bacterial oral infections. Upon initial hospital registration, cultures were acquired from pus collected by surgeons in order to isolate various bacterial etiological agents.

Microbiological methods

Patients who had incision and drainage and/or had undergone antibiotic treatment were excluded. Pus specimens were collected by aspiration of the abscess from inside the mouth after further oral sterilization, using a disposable syringe (5 ml) with a disposable needle of 18/22 gauges. Each sample was rigorously examined for manifestation with respect to color and uniformity. The collected pus was inoculated immediately in trypticase Soy broth and/or thioglycollate broth then cultured into proper solid media in aerobic and anaerobic conditions at 37°C for 18-48 h. Each separate morphological colony species was counted using a digital colony counter after incubation. If no growth is observed, the culture is reported as "no growth". All identical individual colonies were also handled for Gram staining, and pure cultures were obtained and also used for identification. All specimens were operated according to the procedures of Clinical Microbiology Laboratory Standard Operating Procedures¹⁷.

Antibiotic sensitivity tests

Antimicrobial susceptibility testing was carried out using the Kirby-Bauer disk diffusion method on Muller-Hinton agar according to CLSI guidelines. Antimicrobial susceptibility have been determined using commercial antimicrobial discs (Oxoid, UK). We selected ten antibiotics with a wide range of mechanisms of action, including drugs that target the cell wall, DNA, and protein (Tables 5, 6, 7). After incubation, the antimicrobial effectiveness was determined by measuring the diameter of the inhibition zones. The bacterial strains were classified as Sensitive (S), Intermediate (I), or Resistant (R) according to the diameter of the inhibition zone¹⁸.

Data analysis

The clinical and personal data in addition to the results of culture of the specimens were entered into a questionnaire and analyzed by the Epi Info, Version 6. The significance of difference in proportion was analyzed by Pearson Chi-square (χ^2) which is Greater than 3.84 and probability value (p) is less than 0.05 was considered statistically significant.

Ethical approval

The ethical approval was obtained from the Medical Research and Ethics Committee at the Faculty of Medicine and Health Sciences at Sana'a University with Document No. 311 dated September 1, 2019. All

data, including patient identification, have been kept confidential.

RESULTS

A total of 118 cases were positive for bacterial culture, 63 males and 55 females ranged in age from 5 to 65 years, most of them in the age group >45 years

(39.8%), 51.7% had dental abscesses and 48.3% had perio-dental abscesses (Table 1 and Table 2). *Staphylococcus aureus*, *Bacteroides* species and *Staphylococcus epidermidis* were isolated from patients with dental abscesses, *Staphylococcus aureus*, *Bacteroides* species, *Staphylococcus epidermidis* and *Streptococcus pyogenes* were isolated from perio-dental abscesses (Table 3 and Table 4).

Table 1: The number and percentage of clinical diagnosis of different bacterial oral/facial abscesses infections.

Age in years	Total Number (%)	Dental abscess n=61		Perio-dental abscess n=57	
		No.	%	No.	%
< 16	7 (5.9%)	5	8.2	2	3.5
16-25	18 (15.3%)	9	14.8	9	15.8
26-35	23 (19.5%)	13	21.3	11	19.3
36-45	22 (18.6%)	11	18	11	19.3
>45	47 (39.8%)	23	37.7	24	42.1
Total	118 (100%)	61	51.7	57	48.3

Table 2: The number and percentage of clinical diagnosis of different bacterial oral/facial infections among different sexes.

Type of infections	Total n=118		Male n=63		Female n=55		χ^2	p
	No.	%	No.	%	No.	%		
Dental abscesses	61	51.7	32	50.8	29	52.7	0.02	0.89
Periodontal abscesses	57	48.3	31	49.2	26	47.3	0.21	0.61

χ^2 =Chi-square \geq 3.84 (significant), p=Probability value= $<$ 0.05 (significant)

The most prevalent bacterium was *Staphylococcus aureus* (about 63% of the total isolates, and more than 40% of them were resistant to ceftizoxime, calrithromycin, augmentin, tetracycline, erythromycin, and oxacillin (Table 5), while *S. epidermidis* showed

less resistance to tested antibiotics than *Staphylococcus aureus* (Table 6). For *Bacteroides* species the paternal sensitivity is: metronidazole and clindamycin (100%), augmentin (98.6%), clarithromycin (94.4%) and finally vancomycin (76.1%) (Table 7).

Table 3: The number and percentage of the isolated microorganisms from the 61 patients suffering from dental abscesses in respect to gender.

Bacterial isolates	Total n=61		Male n=32		Female n=29		χ^2	P
	No.	%	No.	%	No.	%		
<i>S. aureus</i>	37	60.7	20	62.5	17	58.6	0.10	0.75
<i>S. epidermidis</i>	6	9.8	4	12.5	2	6.9	0.54	0.46
Total Gram positive	43	70.5	24	75	19	65.5	-	-
<i>Bacteroides</i> spp	10	16.4	5	15.6	5	17.2	0.03	0.86
Mixed growth	8	13.1	3	9.4	5	17.2	1.2	0.27

χ^2 =Chi-square \geq 3.84 (significant), p=Probability value = $<$ 0.05 (significant)

Table 4: The number and percentage of isolated microorganisms from the 57 patients suffering from periodontal abscesses in respect to gender.

Bacterial isolates	Total n=57		Male n=31		Female n=26		χ^2	P
	No.	%	No.	%	No.	%		
<i>S. aureus</i>	36	63.2	19	61.3	17	65.4	1.33	0.24
<i>S. epidermidis</i>	10	17.5	7	22.6	3	11.5	1.19	0.27
<i>Bacteroides</i> spp	7	12.3	2	6.5	5	19.2	2.14	0.14
<i>S. pyogenes</i>	2	3.5	2	6.5	0	0	1.74	0.18
Mixed growth	2	3.5	1	3.2	1	3.8	0.02	0.87

χ^2 =Chi-square \geq 3.84 (significant), p=Probability value = $<$ 0.05 (significant)

DISCUSSION

Microbial and anatomical factors and the devastation of host resistance, as well as a delay in getting appropriate treatment in the initial stages, can lead to the development of a dental local infection³. Deep space

infection can carry a high rate of morbidity and mortality¹⁹. The presentation of the patient's condition is determined by the complex microflora, the anatomical methods of spread and the teeth involved²⁰. An understanding of these microorganisms engaged in the infection, and the sensitivity profile will aid improve the treatment regimen, while incision and

drainage is definitely the primary treatment²¹. For the above motives, understanding the nature and dynamics of oral flora is significant in orofacial infections²². Most of the patients in the current study were adults in the >45 years age group as reported in other previous studies²³⁻²⁵. A possible cause of adults at higher risk is the high rate of systemic diseases that compromise immunity and negligence of oral hygiene²⁶. The male to female ratio presented in our study is 1.2: 1, which is consistent with the majority of the earlier reported studies²⁶⁻²⁸. This possibility may be due to the reason why women tend to improve oral health and more frequently search for oral health care²². In this study,

Gram-positive bacteria were isolated more repeatedly than Gram-negative bacteria as the origin of dental abscess by 75% versus 10% for Gram-negative bacteria (Table 3). This result is in agreement with a study conducted in Spain of 68% and 30% for Gram-positive and Gram-negative bacteria, respectively²⁹. In addition, this result was lower than that recognized in Brazil, which indicates that Gram-positive bacteria account for 96.6% of patients³⁰. Additionally, *Staphylococcus aureus* is the dominant bacteria isolated in our study which account for 60.7% of the total microorganisms isolated (Table 3).

Table 5: The susceptibility patterns of *S. aureus* isolates (73) towards the different commonly used antibiotics.

Antibiotics	Susceptibility test					
	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Augmentin (30µg)	41	56.2	-	-	32	43.8
Oxacillin (1µg)	17	23.5	12	16.4	44	60.3
Tetracycline (30µg)	21	28.8	6	8.2	46	63
Erythromycin (15µg)	19	26	10	13.7	44	60.3
Ceftizoxime (30µg)	43	59	7	9.6	23	31.5
Ciprofloxacin (5µg)	52	71.2	4	6.1		22.6
Clindamycin (2µg)	56	76.7	2	2.7	15	20.5
Clarithromycin (15µg)	42	57.5	4	5.5	27	37
Vancomycin (30µg)	67	91.8	-	-	6	8.2

Table 6: The susceptibility patterns of *S. epidermidis* isolates (16) towards the different commonly used antibiotics.

Antibiotics	Susceptibility test					
	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Augmentin (30µg)	10	62.5	1	6.3	5	31.2
Oxacillin (1µg)	9	56.3	0	0	7	43.7
Tetracycline (30µg)	13	81.2	1	6.3	2	12.5
Erythromycin (15µg)	8	50.0	5	31.2	3	18.8
Ceftizoxime (30µg)	11	68.8	3	18.8	2	12.5
Ciprofloxacin (5µg)	12	75	2	12.5	2	12.5
Clindamycin (2µg)	14	87.5	0	0	2	12.5
Clarithromycin (15µg)	10	62.5	2	12.5	4	25
Vancomycin (30µg)	15	93.7	0	0	1	6.3

High occurrence of *Staphylococcus aureus* in oral infections can be justified by the fact that *Staphylococcus aureus* often colonizes nasal mucosa, where it can cause internal oral cavity infections³¹ and *S. aureus* virulence by generating various enzymes

such as coagulase that coagulate the plasma and coat the bacterial cell to likely prevent phagocytosis; Hyaluronidase breaks down hyaluronic acid and aids the spread of *S. aureus*³². What is more, the prevalence of *Bacteroides* spp isolates was 16.4%. This result was

lower than that documented in Brazil (26.7%)³⁰. The results of this study indicate that the microbiotics of root canals with periapical abscesses have multimicrobial organisms and are predominantly Gram-positive cocci. Van Winkelhof has previously described the difficulties in isolating these bacterial species through the use of culture methods³³. Furthermore, Dymock et al.,³⁴ and Baumgartner et al.,³⁵ they are reported that different populations have different configurations of microbes or these species may consist of both cultured and non-cultivable biotypes³⁶. In this study the antibiotic results indicated that the antibiotics that remained hypersensitive to *Staphylococcus aureus* were vancomycin (91.8%), followed by clindamycin (76.7%). In contrast, the antibiotics that were highly resistant to *Staphylococcus aureus* were tetracycline (63%), followed by erythromycin and oxacillin (60%). The results of the study agreed with those of the Rams et al., they observed that some strains of *Staphylococcus aureus* isolated from periodontal abscesses were resistant to tetracycline and erythromycin³⁷. The antibiotic results in the current study also revealed that metronidazole was 100% effective against *Bacteroides* spp. This result was consistent with that reported in Germany (100%)³⁸. These results are also consistent with the results of Roche et al., which found anaerobes very sensitive to metronidazole³⁹. Metronidazole is only active when it is reduced to form an unstable medium that binds to microbial DNA and results in damage that prevents reproduction and transcription⁴⁰. In addition, strict anaerobic isolates (*Bacteroides* spp.) also showed to be hypersensitive to 100% clindamycin, and this result was higher than that documented in Spain

(79.1%)⁴¹. But in accordance with that documented in Germany (100%)³⁸. Clindamycin is a useful dental antibiotic, able to penetrate into bone and prevent biofilms from forming⁴². In addition, sensitivity to augmentin by *Bacteroides* spp in the current study was 94.1%, and this result was higher than that documented in Spain (76.7%)⁴¹. The remaining antibiotics from *Bacteroides* spp. was clarithromycin (94.1%) (macrolide and the erythromycin analog). The most resistant antibiotic used in this study was vancomycin (23.5%) for the same bacteria. The indiscriminate use of antibiotics to supplement dental treatments should be avoided as they may lead to allergic reactions, the development of super-infection with induction of resistant bacterial species and unnecessary exposure to patients with both toxicity and other drug side effects⁴³. Antibiotic prescription should be an aid to appropriate clinical treatment. The antibiotic regimen choice should be based on knowledge of the effectiveness of the antibiotic for the bacteria. It must be considered that infections of dental caries are ecosystems of bacteria where the producers of one type of bacteria can be nutrients for other types of bacteria²³. Studies of antibiotic resistance have generally relied on isolating bacteria on antibiotic-free plates and post-testing of sensitivity to a group of antibiotics. The majority of the isolated antibiotic-resistant bacteria were members of the normal oral microflora; some pathogenic and opportunistic bacteria were also isolated. Antibiotic-resistant bacteria have been isolated, which are important factors for otitis media, pneumonia, sinusitis, meningitis, endocarditis, deep abscesses, and dental diseases including caries and gum disease.

Table 7: The susceptibility patterns of *Bacteroides* spp isolates (17) towards the different commonly used antibiotics.

Antibiotics	Susceptibility test					
	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Augmentin (30µg)	16	94.1	0	0.00	1	5.9
Metronidazole (50µg)	17	100	0	0.00	0	0.00
Clindamycin (2µg)	17	100	0	0.00	0	0.00
Clarithromycin (15µg)	16	94.1	1	5.9	00	0.00
Vancomycin (30µg)	13	76.5	0	0.00	4	23.5

These opportunistic pathogens carriage is of clinical importance since infection caused by antibiotic-resistant bacteria can lead to failure treatment and lead to chronic infection. All subjects had antibiotic-resistant bacteria, which indicates the ability of the oral cavity to function as a reservoir for antibiotic-resistant organisms. A small number of people had very high levels of oral bacteria that are resistant to certain antibiotics. The high percentage of oral bacteria that are resistant to a particular antibiotic indicates the existence of a subgroup that can act as a reservoir for antibiotic-resistant bacteria²⁹⁻³¹.

CONCLUSION

Species of *staphylococcus* are the most common pathogens of orofacial infections of odontogenic origin. Most of the bacteria were resistant to diverse classes of antibiotics. Suitable antibiotics must begin to correlate with clinical emergence devoid of not remember the importance of early surgical intervention to decrease morbidity and complications. Furthermore, administered antibiotics should be based on the bacterial isolates, culture sensitivity, and clinical course of disease. This study also indicates the effect of iceberg and that the potential public health significance

of periodontal disease is perhaps underestimated in Yemen. The pattern of illnesses presented in a hospital is influenced by many confounding factors of choices that operate from the patient's home to the point at which their condition is diagnosed and treated in the hospital. Regarding the patient, his act is based on his awareness that he is sick and his knowledge that relief is available in a particular healthcare facility.

AUTHOR'S CONTRIBUTION

This study was under the supervision of Prof. Dr. Al-Kasem M Abbas, Professor of Dental Surgery, Faculty of Dentistry, Sana'a University. Other authors analyzed the data and wrote the manuscript, and reviewed it.

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CONFLICT OF INTEREST

No conflict of interest associated with this work.

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