PREVALENCE OF *STAPHYLOCOCCUS AUREUS* IN DENTAL INFECTIONS AND THE OCCURRENCE OF MRSA IN ISOLATES

ABSTRACT

Background and aims: *Staphylococcus aureus* is an opportunist that causes dental infection and systemic infections in the human body. This organism increases its resistance to many types of antibiotics every day and becomes more resistant, and this led to a growing feeling of concern in this era. Given this fact, the aims of this study were to determine the frequency of *Staph.aureus* in oral infections and to determine the prevalence of MRSA strains and the sensitivity of isolated *Staph.aureus* to antibiotics, in patients who attended dental clinics in major public hospitals and private clinics in the city of Sana'a - Yemen.

Subjects and methods: The study was conducted for a year, starting in December 2018 and ending in November 2019. The study included 296 patients, 153 male and 143 female, ages 5 to 65, with an average age of 36.2 years. Clinical and demographic data were collected in the standard questionnaire, then oral swabs or pus were collected from patients, cultivated, isolated and identified using standard laboratory techniques. MRSA was determined by means of the disc diffusion method to 5 micrograms of methycillin disc and 1 micrograms of oxicillin disc; an antimicrobial sensitivity test was performed by means of disc diffusion method of selected antibiotics.The oral infections include dental abscesses, periodontal abscesses, gingivitis, periodentitis, dental caries, pulpitis and oral thrush.

Results: Of a total of 296 cultured swabs, only 217 swabs produced a positive culture (73.3%). Gram-positive bacteria formed 67.4% of the total isolates where *Staph. aureus* was the predominant pathogen (43.1%). The prevalence of MRSA was 23.5%. There was a higher rate of antibiotic resistance tested in MRSA isolates compared to a lower rate of resistance in MSSA as well as 22.2% of MRSA isolates were vancomycin resistant, while only 11.4% of MSSA were vancomycin resistant.

Conclusion: It can be concluded, *Staph.aureus* was the most common bacterial isolate in dental infections, high rate of MRSA, the emergence of *Staph.aureus* isolates resistant to vancomycin and other wide range of antibiotics have raised MRSA in oral infections into a multi-drug-resistant, making it more and more dangerous in oral infections. Regular surveillance of oral associated infections and monitoring antibiotic sensitivity pattern and strict drug policy for antibiotics are recommend.

Key words: Aantibiotic resistance, Dental infection, MRSA, *Staphylococcus aureus*, Sana'a, Yemen

INTRODUCTION

The human oral cavity acts as a growth medium for pathogenic microorganisms due to its moisture, temperature, and nutritional content such as fats, carbohydrates, and protein[1]. There are several types of dental infections that occur in the patient's oral cavity such as periodontal disease, tooth decay, dental ache, dental plaque, dental abscess, dentin hypersensitivity, dental calculus, hyperdontia, malocclusion, acid erosion, ulcerative gingivitis, acute necrotizing, dental fluorosis, tooth impaction, etc. *Staph.aureus* is a presumed pathogen for many oral diseases, such as oral mucositis, periodontitis, peri-implantitis, endodontic infections and even dental caries [2-5].

Staph.aureus is a Gram-positive, non-spore forming, non-motile, grape like clusters and the most important coagulase positive pathogen from *staphylococci* due to mixture of invasiveness, toxic mediated virulence and antibiotic resistance [6]. Some *Staph.aureus* strains have developed drug-resistant[7]. Methicillin-resistant *Staph.aureus* (MRSA)[8] are the strains of *Staph.aureus* that have been resistant to beta-lactam antibiotics, which include penicillins, amoxicillin, ampicillin, methicillin, oxacillin, cephalosporins, etc.[9]. The tendency of *Staph.aureus* to acquire antibiotic resistance led to a global dissemination of clone expressing various antimicrobial resistances. Many bacterial diseases occur in hospitals and in community due to MRSA strains and sometimes lead to death [10-12].

Staph.aureus infection, including MRSA strains, has long been common in Yemen[13]. Because the indiscriminate use of antibiotics is a typical practice, hospital environments are not clean enough and crowding of patients and health workers supports the spread of infectious germs including *Staph.aureus* [13]. The potential presence of *Staph.aureus* is especially important in dental infections due to its increased resistance[13,14]. Therefore it is very logical to check the status of the microbial resistance against the commonly used antibiotics for the treatment of dental infections that occur by *Staph.aureus*. Considering this , the aims of this study were to determine the frequency of of *Staph.aureus* in oral infections and to identify the prevalence of MRSA strains and antibiotic sensitivity of isolated *Staph.aureus*, in patients attended the dental clinics at the main general hospitals and private clinics in Sana'a city-Yemen.

PATIENTS, MATERIALS AND METHODS

Patients: The study was conducted for a year, beginning in December 2018 and ending in November 2019. The

study included 296 patients, 153 male and 143 female, ages 5 to 65, with an average age of 36.2 years. Clinical and demographic data were collected in the standard questionnaire, then oral swabs or pus were collected from patients, cultivated, isolated and identified using standard laboratory techniques. The oral infections include dental abscesses, periodontal abscesses, gingivitis, periodentitis, dental caries, pulpitis and oral thrush.

Cases definition: All patients enrolled in this study, who had a major complaint of various oral infections and entered dental clinics in the city of Sana'a.

Data collection and processing: A questionnaire was filled out for each patient with the patient's personal and clinical data. This included age, gender, profession and relevant clinical information regarding bacterial and fungal oral infections. Upon initial hospitalization, cultures were obtained from the oral infection sites in order to isolate the causative agents of various bacteria and fungi.

Antimicrobial susceptibility test: Antibiotic resistance phenotypes (Methicillin / Oxacillin sensitivity test): All isolates of *Staph.aureus* were tested for the susceptibility to 5 µg Methicillin disc and 1 µg Oxacillin disc provided by Difco using the disk diffusion method as described by NCCLS. The resistance breakpoints were ≥14 mm to ≤ 10 mm for 5 µg Methicillin, and ≥ 12 mm to ≤ 10 mm for 1 µg Oxacillin. The ability of other antibiotic disc to inhibit MRSA or MSSA were estimated according to the guidelines provided by NCCLS using commercially available discs which include: Augmenitin (AC 30 µg), tetracycline (T,30 µg), erythromycin(E,15 µg), ceftizoxime (CEF 20 µg), ciprofloxacin(Ci 5 µg), clindamycin(CC, 2 µg), clarithromycin (Cl 15 µg) and vancomycin(V, 30 µg). The zone of inhibition produced by *Staph. aureus* against each antibiotic was measured and interpreted as resistant and susceptible according to standards of Clinical Laboratory and Standards Institute[15].

RESULTS

The positive culture rate was 73.3% and 26.7% of the specimens were negative (table 1). A hundred and eighty 180 (67.4%) were Gram positive bacteria, 71 (26.6%) were Gram negative bacteria and 16 (6.0%) were *C.albicans*. The most frequent microorganism isolated was *Staph.aureus* (115 isolates), followed by *Bacteroides* spp (71 isolates) and *Strept. pyogens* (38 isolates) with percentages of 43.1%, 26.6% and 14.2% respectively. Table 3shows the susceptibility patterns of *Staph.aureus* isolates towards the different commonly used antibiotics. The resistant results for MRSA of antibiotics represented in number and percentages are shown in the following order: vancomycin (22.2%), clindamycin (26%), ciprofloxacin (29.7%), ceftizoxime (40.7%), calrithromycin (37%), augmentin (55.6%), tetracycline (74%), and erythromycin (23.3%). The resistant results for MSSA of antibiotics represented in number and percentages are shown in the following order: vancomycin (11.4%), clindamycin (30.3%), ciprofloxacin (22.7%), ceftizoxime (30.3%), calrithromycin (26.3%), augmentin (30.7%), tetracycline (72.7%), and erythromycin (60.2%).

DISCUSSION

Dental patients typically take antibiotics primarily to treat postoperative and secondary infections. In the current study all 115 coagulase positive isolates of *Staph.aureus* were subjected to disc diffusion method to 5 µg Methicillin disc and 1 µg Oxacillin disc to determine MRSA; the test results discovered that 23.5% of isolated *Staph.aureus* were MRSA strain. The current rate of 23.5% of MRSA in all isolates of *Staph.aureus* is lower than the rate reported from Yemen in previous reports in which MRSA was isolated from 55% of health workers in Taiz, Yemen[16], also it is very lower than that reported by al-Baidani and others, [17] among health care workers in Al Hodeida City, Yemen where the MRSA rate was 86%. On the other hand, it was almost similar to that mentioned by Al-Safani *et al.* [13] (19.3%) among patients attending Military Hospital, Sana'a City; and Alyahawi, and others among patients of some private hospitals in Sana'a City (17.6%),[18].

HA-MRSA occurred at a higher rate than CA-MRSA in the world, but in Yemen the rates were similar for the HA-MRSA and CA-MRSA (19.4% and 17%, respectively), as mentioned by Al-Safani *et al.*[13] and Alyahawi *et al.* [18]. This result can be explained by long hospitalization, random use of antibiotics, lack of awareness, and receiving antibiotics before coming to hospital, which are some of the potential predisposing factors for the appearance of MRSA in the hospital and community. Our result differs from that reported in the United States of America where a high incidence of MRSA occurred in a hospital-acquired *Staph.aureus* infection (HA-MRSA) (59%), compared to a community-acquired infection of *Staph.aureus* (17%) [19]. This difference can be explained by the CA-MRSA biology appearing to be different from the HA-MRSA and the MSSA, which may allow CA-MRSA to cause diseases other than those expected from MSSA[20,21].

With the advent of HA-MRSA, it is likely that it not only replaced HA-MSSA, but also led to a comprehensive increase in *Staph.aureus* infection in healthcare settings [22,23]. In addition, almost all researchers say the same thing that inpatients and outpatients suffer from *Staph.aureus* / MRSA infection higher than *Staph.aureus* / MSSA due to the widespread prevalence of MRSA in a community environment and hospitals [23-25]. When comparing our MRSA rate with the MRSA rate in *Staph.aureus* dental infections, our result (23.5%) was almost lower than the 30% MRSA reported by Das Manisha *et al.* [26]. Also ,the prevalence of MRSA (23.5%) was

higher than the results of Ayepola *et al.* [27] who reported 2.4%, as well as Smith *et al.* [28] 6% of MRSA positive isolates were reported in oral infection. Another study by Renvert *et al.* [29] in Sweden, observed similar results associated with periodontitis patients.

According to Kurita *et al.* [30] dental patients are not the only ones responsible for spreading MRSA bacteria, but a health professional may transfer this pathogen through their tools, so there are consistent guidelines for controlling MRSA as the CDC some standard precautions may be recommended which may help reduce the prevalence of MRSA among dental patients [31].

The reason for conducting the current study was to know the prevalence of MRSA and the current antimicrobial profile of *Staph.aureus* in order to choose the appropriate empirical treatment for these oral infections. In our study, vancomycin resistance (VRSA) was 22.2% in isolated MRSA. This result differs from that reported in Asian countries where the vancomycin resistance rate was no more than 10% [32]. The occurrence of VRSA in Asian countries has also been documented by Kaleem *et al.* [33] in Pakistan to be 3.3%, 6% in India, by Sonavane and Mathur,[34], 7.5% in Iran by Mehdinejad *et al*. [35] and 9% in Jordan are from Al-Zoubi and others[36]. The current study results revealed that 73% dental *Staph.aureus* isolates were found resistant to tetercycline followed by 53.9% to erythromycin, 46.5% to augmanten, and 35.6% to Cefotaxime where low rates of resistant occurred for ciprofloxacin (24.3%), Clarithromycin (28.7%), and Clindamycin (29.6%) (Table 3). Kim and Lee[37] and Das Manisha *et al.* [26] reported more sensitive strains of *Staph.aureus* isolated from the periodontal patients showed sensitivity 95% to ciprofloxacin (vs 75.7%) and 90% to tetracycline (vs 31%), 90% to erythromycin (vs 46.1%), and to 3rd generation cephalosporins 95% (vs 62.4%) that is comparatively higher than the current study. Similar antimicrobial susceptibility results were reported by previous authors [8,38-40]. The higher resistant rates in Yemen to commonly used antibiotics indicates indiscriminate or haphazard use that may have effect on treatment cost, poor prognosis as well as enhance the bacterial infection and growth virulent pathogens.

CONCLUSION

Prevalence of *Staph.aureus* in dental patients is quite high and showed resistance to commonly used antibiotics as well as carried high rate of MRSA. Despite these results, the sample size of this study is not sufficient and study period was too short to uncover actual picture of MRSA involved in dental infection in Sana'a city, Yemen. Large scale studies could be done both in hospitalized patients and in community to identify prevalence of MRSA, genome analysis, identification of toxin gene and other antibiotic resistant gene. Regular brushing of teeth, keeping up oral cleanliness and consulting with dental doctors to check up the teeth once in a month should be taken to maintain a distance from dental infections.

AUTHOR'S CONTRIBUTION

This research work is part of a research work under the supervision of Hassan Al-Shamahy. The field, clinical and laboratory works of the research was done by the corresponding author, the fifth author, the sixth author and the eighth author. The first, second, fourth, and seventh author supervised the work and edited the manuscript.

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CONFLICT OF INTEREST

"There is no conflict of interest related to this work."

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Table 1: Cultural results of the 296 patients with bacterial and fungal oral infections.

Results	**No.**	**%**
Positive cultures	217	**73.3**
Negative cultures	79	**26.7**
Total	**296**	**100**

Table 2: Distribution of the 217 positive culture isolates according to their group and genus.

Isolates	No.	%
Gram positive bacteria	**180**	**67.4**
Staph. aureus	115	43.1
Strept. pyogenes	38	14.2
Staph. epidermidis	16	6.0
Strept. mutans	11	4.1
Gram negative bacteria	**71**	**26.6**
Bacteroides spp	71	26.6
Yeasts	**16**	**6.0**
Candida albicans	16	6.0
Total	**267**	**100.0**

Table 3: The antibiotic sensitivity for 115 isolated MRSA and MSSA for tested antibiotics.

Antibiotics	MRSA n=27 (23.5%)		MSSA n=88 (76.5%)	
	Sensitive	Resistant	Sensitive	Resistant
Augmentin	12 (44.4%)	15 (55.6%)	61 (69.3%)	27 (30.7%)
Cefotaxime	11 (40.7%)	16 (59.3%)	61(69.3%)	27 (30.3%)
Ciprofloxacin	19 (70.3%)	8 (29.7%)	68 (77.3%)	20 (22.7%)
Clarithromycin	17 (63%)	10 (37%)	65 (73.7%)	23 (26.3%)
Clindamycin	20 (74%)	7 (26%)	61(69.3%)	27 (30.3%)
Erythromycin	18 (66.7%)	9 (23.3%)	35 (39.8%)	53 (60.2%)
Tetracycline	7 (26%)	20 (74%)	24 (27.3%)	64 (72.7%)
Vancomycin	21(77.8%)	6 (22.2%)	37 (42%)	51 (58%)