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## A Identification And Isolation Of Several Bacteria From Oral Cavity Among Human In Soran City

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## Isolation and Identification of Several Bacteria from Oral Cavity among Humans in Soran City

<p><b>Authors Names</b> a. Holem Hashm Balaky b. Alaa Taha Younis Al-Hammadi</p> <p><b>Article History</b> Received on: 1/8/2021 Revised on: 30/8/2021 Accepted on: 9/9/2021</p> <p><b>Keywords:</b> Antibiotics, Bacteria, Oral Cavity, Soran City</p> <p><b>DOI:</b> <a href="https://doi.org/10.29350/jops.2021.26.5.1384">https://doi.org/10.29350/jops.2021.26.5.1384</a></p>	<p><b>ABSTRACT</b></p> <p>This study has aimed to estimate that the number of bacteria in the mouth exceeds the total number of people on the planet. In this context, scientists have discovered more than 700 different types of human oral bacteria. With such a large number of oral bacteria present, many diseases that may threaten health, especially oral health, can emerge. For this reason, this study was employed and conducted on 252 healthy people in the Soran area of the city of Erbil. Both sexes were involved and different ages were selected. Swabs taken from 226 participants gave positive growths on different culture media. The biochemical properties of the isolates were tested according to Bergeys Manual of Systematic Bacteriology and UK Standards for Microbiology Investigation. The results showed that <i>Staphylococcus aureus</i> was the most frequently isolated pathogen (47.83%) followed by <i>Pseudomonas aeruginosa</i> (16.30%), <i>Bacillus subtilis</i> (23.91%), <i>Enterococcus faecalis</i> (5.43%), <i>Enterobacter aerogenes</i> (4.35%), and <i>Klebsiella pneumonia</i> (2.17%) respectively. Antibiotic Susceptibility testing of different species of bacteria was also performed according to Kirby Bauer disc diffusion method on Muller Hinton Agar by using commercial antibiotic discs. It seems that the different species of bacteria showed various sensitivity patterns to several kinds of antibiotics.</p>
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### 1. Introduction

The oral cavity is the only major passage to the internal human body, food passes through the oral cavity to the digestive system, air also passes through the nostrils, and the oral cavity to the respiratory tract. This creates a continuous channel and could cause microorganisms colonizing one area of the oral cavity to have a significant probability of spreading on other neighboring sites. The presence of *Saccharomyces* species and *Candida* has been found to correlate with the severity of hepatitis, pneumonia, cardiovascular diseases, and other systemic diseases (Dolnicar and Chapple 2015).

Also, The oral cavity provides two types of surfaces for bacteria colonization – soft tissue and hard tooth enamel/exposed root surfaces – thus supporting the growth of different bacterial

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communities (Marcotte and Lavoie 1998) (Press *et al.* 2004). Because the microorganisms present in saliva are removed from different surfaces of the mouth, saliva flora can provide an overview of the bacteria in the mouth of an individual (Press *et al.* 2004)

It is known worldwide that the *Staphylococcus* spp. and *Candida* spp., are recognized to be probable pathogens in patients with various systemic debilitations, such as diabetes, neutropenia, agranulocytosis, and AIDS (Cuesta *et al.* 2010). Although *Staphylococci* is assumed to be a transient resident in the oral cavity (Ohara-Nemoto *et al.* 2008), it is suspected of being a pathogen in a variety of oral diseases, including dental caries (Petti *et al.* 2014).

The goal of the study was to determine the most pathogenic bacteria species in the oral cavity of healthy males and females. Mouth rinses containing antimicrobial agents exert beneficial clinical effects when used as adjuncts in treating periodontal disease. Antibacterial activities and the effectiveness in reducing or retarding plaque formation were comprehensively studied (Barkvoll and Attramadal 1989)

## 2. MATERIAL METHODS

### 2.1. Test on the antibiotic activity of Oral rinse technique

#### 2.1.1. Bacteria Oral rinse

The oral cavity in healthy subjects has a well-balanced microbiotic that consists of approximately  $1.0 \times 10^{11}$  microbes/g of dental plaque, and more than 700 species reside on the tongue dorsum, buccal epithelium, hard and soft palates, and other surfaces (Aas *et al.* 2005), some studies have demonstrated that a disturbance of this balance, with an increase of harmful microbes and a decrease of beneficial microbes, causes oral disorders, such as periodontal disease and dental caries (Duran-Pinedo *et al.* 2014).

#### 2.1.2. Sample Collection and study area

In this study, 226 samples gave a positive result for growth out of a total of 352 collected from Hospital by toothpicks 0.1 ml of suspension was cultivated by spread plate method on each blood agar, mannitol agar, McConkey agar. Then, the dishes were incubated in aerobic conditions at 37 °C for 24-48 hours. After incubation, the colonies were counted and the developing bacteria were diagnosed using Macfaddin (2000) (Medicine 1984) (Patricia M.Tille 2015).

#### 2.1.3. Effect of antibiotics on bacterial isolates

Antibiotic disk diffusion test depends on measuring the growth inhibition zone around antibiotic disks and comparing it with these reported by Clinical and Laboratory Standards Institution (CLSI) guidelines [12,13]. Rely on the standard (Kirby - Bauer method) as mentioned in Mueller-Hinton agar medium was inoculated with the young suspension of bacteria using a sterile cotton swab. Then the following antibiotic tablets were distributed Penicillin G: P10 $\mu$ g, Ampicillin AMP10 $\mu$ g, Tetracycline: TET 10 $\mu$ g, Chloramphenicol: C 30 $\mu$ g, Cefotaxime: CTX 30 $\mu$ g, Erythromycin: Eyr 10 $\mu$ g, (CF) 5 $\mu$ g: Ciprofloxacin, and, Gentamycin: CN10 $\mu$ g. The interpretative criteria for all antimicrobials are those recommended by the National Committee on Infectious Diseases and Diseases (Turnbull *et al.* 2004).

## 3. RESULTS AND DISCUSSION

This *in vitro* study to assess the antibacterial activities was carried out using oral cavity assays. this study included (252) samples were collected from 226 participants who gave positive growths on different culture media. by plaque and swab samples from the oral cavity without any patients, such as bad smell, no smoking, regular teeth brushing, and prevent of soda pop consumption. The total of 252

samples divided to gender ranges from male (150) sample with gender ranges from female 102 samples with the positive and negative culture of results.

However, a total of (252) Thus, the positive culture from male was 88%, and female was 91% with the negative culture from male 13%, and female 8% respectively shown Table 1, and Figure 1.

**Table 1:** Distribution of human pathogen with an oral cavity in human

Culture results	Male		Female		Average (Male and Female)	
	No.	%	No.	%	No	%
Positive Culture	126	88%	91	91%	217	89.5
Negative Culture	24	13%	11	8%	35	10.5
Total	150	65%	102	35%	252	100

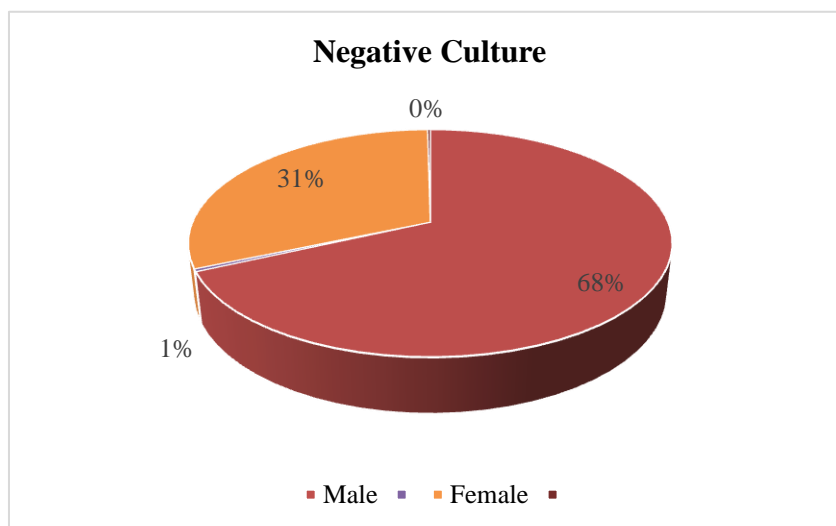


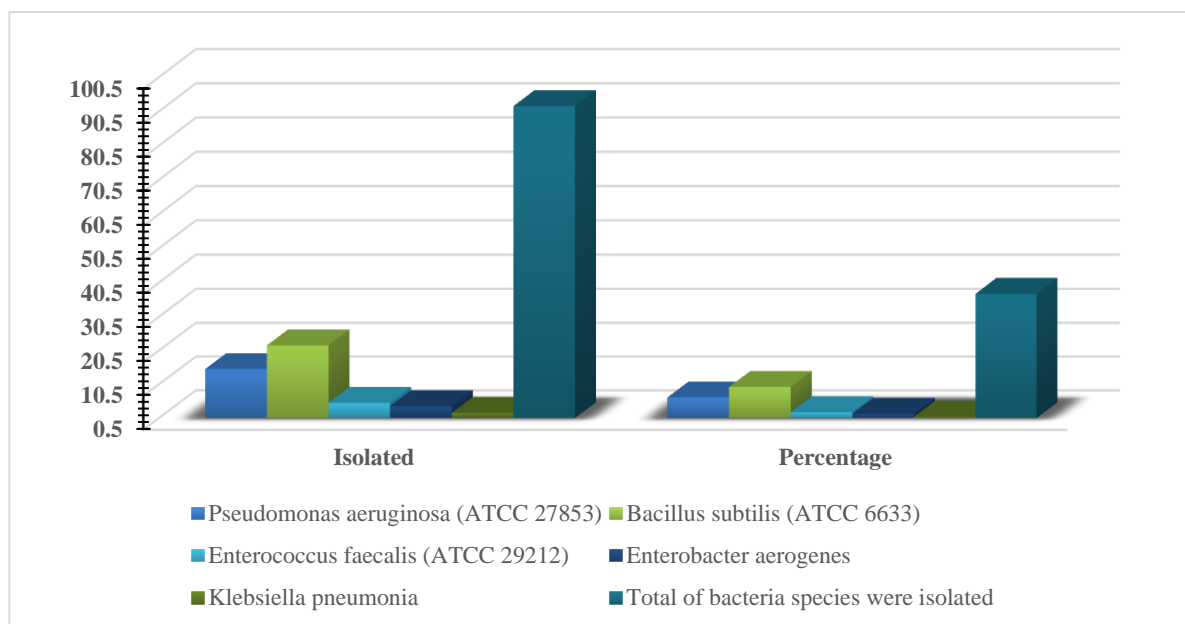
Figure 1: Distribution of human pathogen with an oral cavity in Male and Female

The present study compares favorably with investigations by the antibiotic. six oral mucosal bacterial species were identified from the oral cavity of 252 people, in decreasing order of dominance; *Staphylococcus aureus* (47.83%), *Pseudomonas aeruginosa* (23.91%), *Bacillus subtilis* (5.43%), *Enterococcus faecalis* (4.34%), *Enterobacter aerogenes* (2.17%), and *Klebsiella pneumonia* (0.85%), but all total of bacteria species were isolated 92 shown Table 2, and Figure 2. Therefore, the results were shown that the gram-positive bacterial isolates were the most abundant (71.74%). the *Staphylococcus aureus* more common isolates from male and female as value (47.83%), followed by *Bacillus subtilis* (23.91 %), while the Gram-negative bacterial isolates recorded (28.24%) as shown in Table 2. his group of the (Hua Xie *et al.* 2000), and (Y Li, Y Ge 2007), confirmed that Gram-positive bacteria are prevalent in adults and children and that *Staphylococcus* bacteria is the most common, and this is spent with (Al-Abdul AA 2017) (Lateef and Ibrahim 2017). Because it has surface antigens and degrading enzymes that help it penetrate different body tissues. However, the bacteria negative-gram

was lower than the gram-positive bacteria. According to several studies, most gram-negative bacteria originate from inflammation of the respiratory tract and the gastrointestinal tract.

**Table 2:** incidence of all bacteria isolated in male and female culture from the oral cavity

Bacteria code.	Bacteria species	Isolated	Percentage (%)
B 1	<i>Staphylococcus aureus</i>	44	47.83%
B2	<i>Pseudomonas aeruginosa</i>	15	16.30%
B3	<i>Bacillus subtilis</i>	22	23.91%
B4	<i>Enterococcus faecalis</i>	5	5.43%
B5	<i>Enterobacter aerogenes</i>	4	4.34%
B6	<i>Klebsiella pneumonia</i>	2	2.17%
<b>Total</b>	Total of bacteria species were isolated	92	



**Figure 2:** incidence of all bacteria isolated from the oral cavity

Many studies were conducted to Identification and Isolation bacteria from the oral cavity, that the amounts and types of microorganisms found in the oral cavity may vary among individuals with different males and females. Also, there are many bacteria inhibited by some antibacterial agents. Furthermore, the inhibition zone significantly was seen in (Table 3).and (Figure 4), the antibacterial activities of the treatment of oral cavity by synthetic antibacterial discs used positive control as P10, AMP10, TE10, (Ch)C30, (Ce)CTX30, Eyr10, (Ci)CPR5, and (Ge)CN10. Furthermore. the topmost antibacterial values ranged between in (Ch)C30 (13.70 -28.49), and (Ce)CTX30 (26.80-29.53) respectively, as significant by the diameters of inhibition zones of most the activities against the organism's test, whereas the inhibition zone ranged from 7.80 to 29.53mm against all bacteria tests. Also, the highest antibacterial activity was seen against the *Staphylococcus aureus* and *Klebsiella pneumonia* at all diameter zone of antibacterial activity respectively. Therefore, among all bacteria, just five artificial antibacterial were significantly affected against the growth of microorganisms.

On the other hand, all the studied microorganisms seem to be resistant to Cefotaxime, Tetracycline, cephalosporin, and Chloramphenicol, respectively. This resistance might be attributed to the beta-lactamase production by these microorganisms which destroys the beta-lactam ring of penicillin's and 7- amino cephalosporin acid ring of cephalosporin respectively. Since these rings are the site of antimicrobial activity of these drugs, therefore destruction of these rings renders microorganism's resistant to these antibiotics. These results are expected regarding all six bacteria since all these microorganisms are known to produce beta-lactamase enzymes as indicated in the literature (Ibrahim *et al.* 2014), (Alaa 2016).

Table 3: Results of Diameter of Antibiotic zone against the oral cavity in micro dilution assay

Bacteria	Antibiotic (Antimicrobial Agent) / Susceptible							
	P10	AMP10	TE10	(Ch)C30	(Ce)CTX30	Eyr10	(Ci)CPR5	(Ge)CN10
B.1	12.94	12.83	21.73	24.70	29.53	11.60	22.76	16.90
B.2	11.09	10.59	21.66	20.72	26.80	16.45	18.58	10.19
B.3	14.30	No	20.08	24.91	28.25	11.37	21.85	11.10
B.4	8.27	7.80	22.23	13.70	26.36	13.14	21.50	10.71
B.5	10.02	11.42	22.37	26.13	27.48	8.80	21.29	17.66
B.6	10.61	12.64	21.54	28.49	28.41	12.98	19.42	14.21

I.Z.mm: inhibition zone of Concentration; Bacteria: B.1=*Staphylococcus aureus* (ATCC 29213), B.2= *Pseudomonas aeruginosa* (ATCC 27853), B.3= *Bacillus subtilis* (ATCC 6633), B.4=*Enterococcus faecalis* (ATCC 29212), B.5=*Enterobacter aerogenes*, B.6=*Klebsiella pneumonia* (CCM 2318); Antibiotic (Antimicrobial Agent) / Susceptible: Penicillin G: P10 µg, Ampicillin (other) : AMP10 µg, Tetracycline : TET 10 µg, Chloramphenicol : C 30 µg, Cefotaxime : CTX 30 µg, Erthromycin : Eyr 10 µg , (CF) 5 µg : Ciprofloxacin, and, Gentamycin : CN10 µg; N.D: Not Detected.

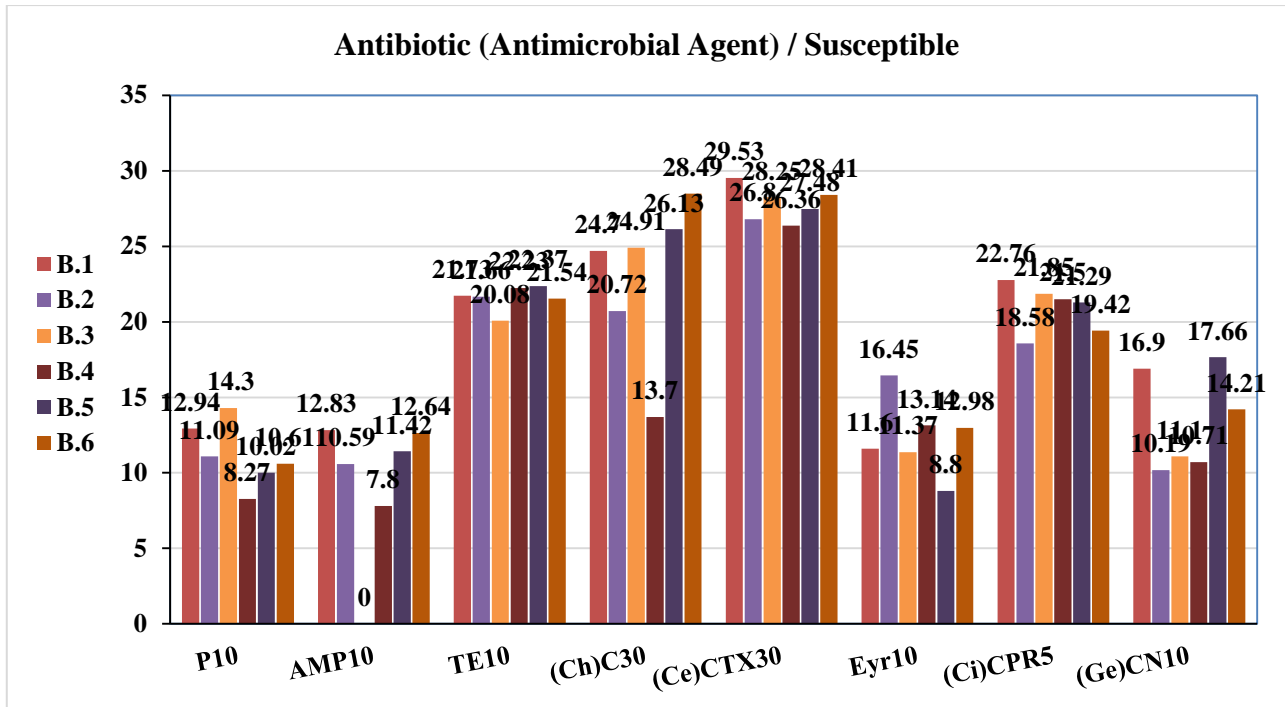


Fig 4: Prevalence of oral bacteria species with antibiotics in human

#### 4. CONCLUSION

The positive culture from males was 88%, and females were 91% with the negative culture from males 13% and females 8% respectively. However, the results were showed that the gram-positive bacterial isolates were the most abundant (71.74%). the *Staphylococcus aureus* is a more common isolate from males and females as value (47.83%). On the other hand, the highest antibacterial activity was seen against the *Staphylococcus aureus* and *Klebsiella pneumonia* at all diameter zone of antibacterial activity respectively. Therefore, among all bacteria, just five artificial antibacterial were significantly affected against the growth of microorganisms. that the different species of bacteria showed various sensitivity patterns to several kinds of antibiotics.

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