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Original Research Article

Increasing the Effectiveness of Some Antibiotics Using Zinc Oxide Nanoparticles against Bacteria Isolated from the Oral Cavity

Fanar Dawas Mahmood[1]*
[1]Directorate General of Education of Nineveh., Iraq

***Corresponding Author:** Fanar Dawas Mahmood
Directorate General of Education of Nineveh., Iraq

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Abstract: This study focused on isolating and identifying bacteria associated with oral cavity infections and exploring the potential of zinc oxide nanoparticles as a supportive antibacterial agent. A total of 62 oral swab samples were collected from patients with oral conditions, including inflammation and ulcers. The samples were cultured on selective media, and the bacterial isolates were identified using morphological characteristics, biochemical tests, and the VITEK 2 system. The results showed that most samples (96.77%) exhibited bacterial growth, and all isolates were Gram-positive. Four main bacterial species were identified, with *Streptococcus mutans* being the most prevalent, followed by *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Staphylococcus haemolyticus*. These bacteria are commonly associated with oral infections and dental problems. The study also examined antibiotic susceptibility and the potential of zinc oxide nanoparticles to enhance antibacterial activity. The findings suggest that Gram-positive bacteria largely dominate oral infections and highlight the importance of exploring alternative strategies, such as nanomaterials, to improve the effectiveness of antibiotics and help reduce bacterial resistance.

Keywords: Oral Bacteria, *Streptococcus Mutans*, Zinc Oxide Nanoparticles, Antibiotic Resistance, Antibacterial Activity.

1. INTRODUCTION

More than 390 types of bacteria live in the human mouth, which are found associated with the teeth, gums, tongue surface and tonsils, including positive or negative bacteria such as *Staph aureus, Staph epidermis, Staph hemolyticus, Strep muntas, Pseudomonas, Klebsiella baumannii and E. coli* (Doran *et al.,* 2004).

Tooth decay is one of the most common chronic infections, along with periodontal and dental inflammation, gingivitis, and mouth ulcers. Studies indicate that 60% of patients aged 45-55 suffer from these periodontal and dental inflammations, and 80% suffer from gingivitis and mouth ulcers when they are over 60 years old. The oral cavity environment supports the growth and survival of a wide range of microorganisms, including bacteria, viruses, and yeasts, due to the presence of water, nutrients, and heat (Ranganatha *et al.,* 2019).

Because of the excessive use of classic antibiotics, which many people resort to eliminate germs causing oral diseases, the decreasing sensitivity of pathogenic bacteria to antibiotics and their increasing resistance have raised concerns and challenges in the healthcare sector. To avoid antibiotic resistance, it has become essential to find alternatives and implement various medical interventions (Laxmerarian, 2013).

Therefore, finding alternative antibacterial systems is a significant achievement in bacteriology. This achievement has been facilitated by the emergence of new antibacterial agents, both novel and complementary. These agents fill gaps that existing active agents cannot address due to their physical and chemical properties. Different agents may employ various mechanisms, such as the specificity of certain ions against bacteria. Reports have indicated specificity against different types of microbes, such as zinc oxide (ZnO), magnesium oxide (MgO), gold (Au), and silver (Ag), which are effective against drug-resistant bacterial, viral, and fungal strains (Seil *et al*., 2012; Beyth, 2015)

2. MATERIALS AND METHODS

2.1Sample Collection

A study of 62 oral swabs (melancholia, inflammation, and oral ulcers) from patients of both sexes attending some dental outpatient clinics from mid-July to October 2025 showed that the swabs were placed in sterile tubes containing a transport medium and then transferred directly to the laboratory, where they were incubated at 37ºC for 24 hours.

2.2Culture Specimens

The samples were streaked onto nutrient agar using the streaking method and incubated at 37°C for 24 hours. Then, several differential media suitable for bacterial growth (Manitol, Blood agar, MSBA) were used to confirm their suitability. They were then recultured for purification under the same conditions and stored at 4ºC until diagnostic and biochemical tests were performed (Leboff *et al*., 2011).

2.3 Morphological and Microscopic Diagnosis

This is done by studying the morphological and microscopic properties of isolated bacteria grown on multiple differential media, based on the colony's color, size, texture, height, edge shape, and hemolysis of different types, after fixing and staining them with Gram stain by transferring a part of the bacterial culture to the slide using a loop carrier. They were examined under an electron microscope under a 100X oil lens (Wanger *et al*., 2017).

2.4 Biochemical Tests

These tests were performed according to the method adopted by Laber (2016).

The physiological characteristics of bacteria isolated from the oral cavity were studied using several tests, including IMVIC, oxidase, catalase, urease, motility, coagulase, menthol fermentation, lactose fermentation and hemolysin.

2.5 Diagnosis by the Vitek-2 System

The studied isolates were tested using the VITEK 2 system with the VITEK 2 GP CARD diagnostic kit dedicated to Gram-positive isolates to enhance confirmation to the species level. In this system, the bacterial suspension must be from the cultured sample (Sanders, 2019).

2.6 Antibiotic Sensitivity Testing Against the Bacteria under Study

Biochemical tests were performed on the study isolates using the disc diffusion method, as described in Perez *et al*., (2019). To determine the absence of the antibiotics used in the study (ciprofloxacin, Amikacin, and Azithromycin), 3–4 colonies growing on nutrient agar were transferred using ring tubes containing 5 mL of nine physiological salts. The tubes were shaken to remove the solution, which was then compared to a standard McFarland solution. The solution was spread onto Mueller-Hinton agar using a cotton swab and allowed to dry at room temperature for 15 minutes. The antibiotics were then placed onto the bacterial-saturated dish using sterile forceps and kept separate to prevent interference from inhibitors. It was stored at 37°C for 24 hours. The results were recorded by measuring inhibition and comparing them with international measurements reported by the CLSI Institute (2020).

2.7 Preparation of Solution Concentrations of the Nanomaterial Used on Bacterial Isolates

The nanomaterial was prepared to measure its effect on the bacterial isolates under study at different concentrations (100%, 75%, 50%, 25%) as follows: The first concentration (100%) was prepared by dissolving 10,000 µg/ml of the nanomaterial and diluting the volume to 1 ml with distilled water. The remaining concentrations (75%, 50%, and 25%) were prepared by using a specific ratio of the first concentration mentioned above, and each concentration was diluted to 1 ml with distilled water.

2.8 Measuring the Inhibitory Effect of the Nanomaterial on the Bacterial Isolates under Study

To measure the effectiveness of the nanomaterial on bacterial isolates, Mueller-Hinton culture medium (0.1) was inoculated with a bacterial suspension of the studied isolates prepared in heart and brain infusion medium equivalent to McFarland's solution. Holes were then drilled in the dish using a cork drill to add the nanomaterial at the previously prepared concentrations for each of the studied bacteria. It was incubated in an incubator at 37 degrees Celsius for 24 hours, after which the inhibition diameters were measured to determine the extent of its effect and its efficacy.

2.9 Synergistic Effect of Antibiotics with Nanobiops:

First, dissolve the antibiotics at a concentration of 50 µg/mL.
Second – Dissolve nanobodies at a concentration of 50 µg /ml.

Third – Prepare a 50:50 mixture of antibiotics and nanobodies.
Fourth – Measure the inhibitory effect on the studied bacteria using the same method as above (pitting) and incubate for 24 hours at 37°C.

3. RESULT AND DISCUSSION

3.1 Isolation and Diagnosis

Sixty-two different oral swabs were isolated. Upon culture, the results showed that (60) oral swabs exhibited positive growth (96.77%), while (2) swabs showed no growth (3.22%), meaning they were negative. The results also showed that all isolates were Gram-positive (100%). These results were very close to those reported by Saud (2016). The study concluded that all bacterial isolates were Gram-positive (60) out of a total of 62 oral swabs, representing the highest isolation rate of (96.7%). This percentage is very close to that reported by Al-Lahibi (2020), which was 94.17%. The distribution was as follows:

Strep mutans (24) isolates (40%), Staph. Staph. epidermidis (21) isolates (35 %), Staph. aureus (10) isolates (16.6.33%), Staph. haemolyticus (5) (8. 3%), This study is consistent with (Borty *et al*., 2015), who did not record any Gram-negative bacteria

Table 2: Bacterial isolates, their types, numbers and proportions

No	Type of bacteria	Number	Lineage %
1	*Strep.mutans*	24	40
2	*Staph. epidermidis*	21	35
3	*Staph.aureus*	10	16.6
4	*Staph. haemolyticus*	5	8.3
Total		60	100

The results showed the identification of (4) Gram-positive bacterial species, with the highest percentage belonging to Streptococcus mutans at (40%). This aligns with the findings of Al-Dulaimi (2014), who also achieved a 40% isolation rate, and is consistent with (Ayoub *et al*.,2020) and (Yadav *et al*., 2015), where Streptococcus mutans was the dominant isolating bacterium, exhibiting the highest isolation rate. However, the percentages differ from those of (Anejo-okopi *et al.,* 2015), who obtained an isolation rate of (18.0%), and from the study by (Kaur *et al*., 2015), who did not obtain any isolation rate for this bacterium. High levels of Strep. mutans bacteria are a direct risk factor for developing tooth decay in the present and future, and are the most common type associated with oral cavities (Nomura *et al*., 2020).

As for S. epidermidis, we obtained an isolation rate of 35%. This study is similar to what Al-Ani (2021) achieved with an isolation rate of (37.54%). As for S. aureus bacteria, the isolation rate was (16.6%), and the approach of (Anejo-okopi *et al.,* 2015) came as the isolation rate reached (14.4%), which is not in agreement with(Al-Husseini *et al*.,2012), as the isolation rate reached (29.2%). However, S. hemolyticus bacteria had the lowest isolation rate among bacteria isolated from oral cavities, at (8.3%) of the total number of isolated bacteria. This is inconsistent with previous studies.

3.2 Morphological and Microscopic Diagnosis

The bacterial isolates isolated from the oral cavity were diagnosed after being cultured on culture media based on morphological and microscopic characteristics as a primary diagnosis, then cultured on differential media to distinguish them from the rest of the isolates from these media: Mitis Salivarius Bacitracin Agar (MSB) for S. muntas bacteria, Manitol Salt Agar (MSA) for S. aureus bacteria such as Staphylococcus aureus, and blood agar medium to test for hemolysis of all isolates according to the paragraphs of the diagnostic methods mentioned in the Materials and Methods of Work, in Table nomber (2). The results showed that Staph aureus bacteria produced growth on MSA agar medium in the form of golden colonies fermenting mannitol, and also produced growth on blood agar medium in the form of yellow colonies and produced complete β-hemolysis, It was also found that Staphylococcus aureus bacteria are positive for the coagulase test acharacteristic that distinguishes them from other staphylococci such as S. epidermis and S. hemolyticus.

As for Strep. mutans bacteria produced growth on MBS medium, forming small colonies with a blue center, and on blood agar medium, hemolyzing type α as for S. hemolyticus, Staph. Candida epidermis bacteria produced growth on blood agar medium, with white colonies that did not hemolyse. Also, after microscopic examination, some bacterial species appear as ordered clusters or chains, and their positive Gram staining indicates that they belong to the genus Staphylococcus. As for Strep. mutans bacteria, they were in the form of chains of varying lengths, as in paragraphs 2 and 3:(Karen *et al*., 2016).

Table 2: Morphological and culture characteristics of bacteria isolated from the oral cavity

Morphological and implantation characteristics		Types of Bacteria			
		Strep.mutans	*Staph. aureus*	*Staph haemolyticus*	*Staph epidermis*
Appearance characteristics		Chains of similar length	clusters or pairs	clusters or double groups	cluster-shaped groups
Gram stain		+	+	+	+
Growth in the media	Mitis Salivarius BacitracinAgar(MSB)	Small colonies with a blue center.	-	-	-
	MSA	-	Menthol fermentation colonies	-	-
	Biood agar	α-type hemolytic colonies	ß-Hemolytic yellow colonies-	Y- hemolytic white colonies	Y- hemolytic white colonies

Positive Test + negative Test

Biochemical tests diagnosed the isolated bacteria under study to confirm the diagnosis, which include several tests mentioned in paragraph (4) of Materials and Methods above, as shown in Table 3.

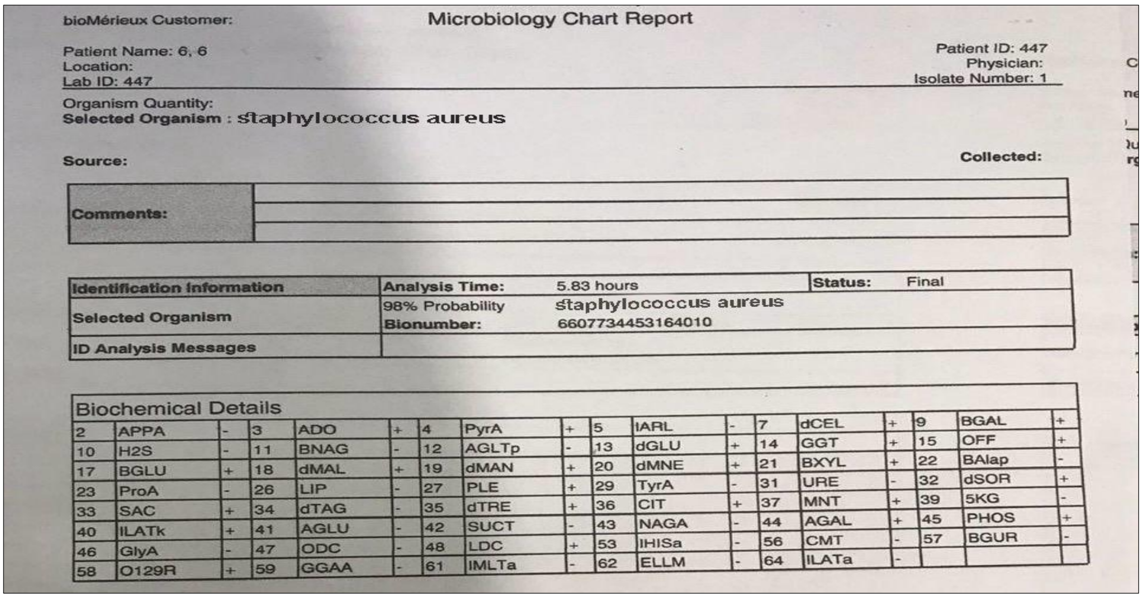
Table 3: The biochemical tests for the isolated bacterial species

Biochemical Test	*Strep. mutan*	*Staph. epidermidis*	*Staph.aureus*	*Staph. haemolyticus*
Catalase	-	+	+	+
Oxidase	-	-	-	-
Lactose	+	+	+	-
Methyl red	+	-	+	+
Indol	-	+	+	-
Motility	-	-	-	-
Ureaus	-	+	+	-
Simon citrate	-	-	+	+
Voges proskaur	+	+	+	+
Coagulase	+	-	-	-
Optochin	+	-	-	-
Manitol	+	-	+	-

Test positive+ Test negative –

3.3 Diagnosis by the Vitek$_2$ system

All studied isolates were identified using the Vitik 2 system, which consists of 64 biochemical tests. The results were consistent with the biochemical test results shown in the images below.


bioMérieux Customer:
Microbiology Chart Report

Patient Name: 6, 6
Location:
Lab ID: 447
Patient ID: 447
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : staphylococcus aureus

Source:
Collected:

Comments:

Identification Information	Analysis Time: 5.83 hours	Status: Final
Selected Organism	98% Probability staphylococcus aureus Bionumber: 6607734453164010	
ID Analysis Messages		

Biochemical Details

2	APPA	-	3	ADO	+	4	PyrA	+	5	IARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	-	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	+	53	IHISa	-	56	CMT	-	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

Al-Mansur Laboratory

Microbiology Chart Report

bioMérieux Customer:

Patient Name: 6, 6
Location:
Lab ID: 447

Patient ID: 447
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : staph epidermis

Source:

Collected:

Comments:	

Identification Information	**Analysis Time:** 5.83 hours	**Status:** Final
Selected Organism	98% Probability staph epidermis **Bionumber:** 6607734453164010	
ID Analysis Messages		

Biochemical Details

2	APPA	-	3	ADO	+	4	PyrA	+	5	IARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	-	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	+	53	IHISa	-	56	CMT	-	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	-			

bioMérieux Customer:

Microbiology Chart Report

Printed June 17, 2023 13:11:50 PM CST

Patient Name: 4 .
Location:
Lab ID: 187

Patient ID: ds4
Physician:
Isolate Number: 4

Organism Quantity:
Selected Organism : Streptococcus mutans

Source:

Collected:

Comments:	

Identification Information	**Analysis Time:** 4.83 hours	**Status:** Final
Selected Organism	98% Probability **Streptococcus mutans** **Bionumber:** 190011564753531	
ID Analysis Messages		

Biochemical Details

2	AMY	+	4	PIPLC	-	5	dXYL	-	8	ADH1	-	9	BGAL	-	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	+	23	ProA	-	24	BGURr	-	25	AGAL	+	26	PyrA	-	27	BGUR	-
28	AlaA	+	29	TyrA	-	30	dSOR	+	31	URE	-	32	POLYB	+	37	dGAL	+
38	dRIB	-	39	ILATk	-	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	+	50	NC6.5	-	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	+	58	O129R	-	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Al-Mansur Laboratory

bioMérieux Customer:

Microbiology Chart Report

Patient Name: 4, 4
Location:
Lab ID: 451

Patient ID: 451
Physician:
Isolate Number: 5

Organism Quantity:
Selected Organism : Staphylococcus haemolyticus

Source:

Collected:

Comments:	

Identification Information	**Analysis Time:** 4.82 hours	**Status:** Final
Selected Organism	96% Probability **Staphylococcus haemolyticus** **Bionumber:** 010002101320231	
ID Analysis Messages		

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	-
13	APPA	(-)	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	+	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	-	37	dGAL	-
38	dRIB	+	39	ILATk	-	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	-
47	NOVO	-	50	NC6.5	+	52	dMAN	-	53	dMNE	-	54	MBdG	-	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Staph. epiderm Strep. mutan Staph. haemolyticus, Staph. aureus

4. CONCLUSION

The present study showed that oral cavity infections are mainly associated with Gram-positive bacteria, with *Streptococcus mutans* being the most prevalent species, followed by *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Staphylococcus haemolyticus*.

The applied diagnostic methods, including biochemical tests and the VITEK 2 system, provided accurate identification of the isolates.

The study also highlights the potential role of zinc oxide nanoparticles as an effective adjunct to antibiotics, potentially improving antibacterial activity and reducing microbial resistance.

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