



## ORIGINAL RESEARCH

## CHARACTERIZATION OF CARIOGENIC BACTERIA AND THEIR RESISTANCE PROFILES IN AN OBESE IRAQI POPULATION

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## ABSTRACT

**Background:** Obesity is a common yet neglected public health issue worldwide. Oral health, an essential component of overall health and well-being, overlaps with risk factors for obesity, including poor eating habits. This study aimed to investigate the antibiotic susceptibility of bacteria isolated from tooth decay in obese patients.

**Materials and methods:** Sixty specimens were collected randomly from the mouths and teeth of obese patients in the Babylon Governorate, Iraq. The colony size, shape, color, edge, and transparency were examined to determine the morphological and cultural parameters of each bacterial isolate. Antibiotic susceptibility was assessed using 9 types of antibiotics.

**Results:** The results showed that the rate of positive specimens was 37 (56.06%), and the negative rate was 29 (43.93%) for the total of 66 specimens. The number of positive male specimens was 35(58.33%), more than that of the females, 25 (41.66%). The dominant bacterial isolates were *Staphylococcus aureus* (19), *Klebsiella pneumonia* (13), *Candida* spp. (10), *Staphylococcus epidermidis* (8), *Streptococcus* sp., and *Escherichia coli* (5), *Bacillus cereus* (5), *Pseudomonas* sp. (3), *Proteus mirabilis* (2).

**Conclusion:** the study concludes that *S. aureus* was resistant to Clindamycin and Ceftaroline 19(100%), then Erythromycin 15 (78.94%), Gentamycin 14(73.68%), Ofloxacin 9 (47.36%), Rifampin 7(36.84%), Tetracycline 3(15.78%), and Azithromycin and Ciprofloxacin (0 %). *K. pneumoniae* was highly resistant to Penicillin and Ciprofloxacin 13(100%), levofloxacin 9 (53.84%), Gentamycin and Nitrofurantoin 7 (23.7%), Rifampin and Tetracycline 3 (23.07%), and Imipenem and Meropenem (0%).

**Keywords:** Obesity, Patients, Antibiotic Susceptibility, Tooth Decay

## INTRODUCTION

Obesity or overweight is described as an undue or abnormal buildup of fat that poses a concern for well-being, with a body mass index (BMI) greater than 25 is overweight. Globally, over 30% of the population suffers from obesity. The matter has worsened to become a problem of epidemic proportions, as over four million people die each year because of being overweight or obese<sup>1,2</sup>.

Tooth decay is a chronic condition exclusive to humans and one of the most substantial oral health topics currently facing the world. The degradation of dental hard acellular tissue occurs due to acid by-products

produced by the bacterial breakdown of carbohydrates in food, particularly sucrose. Dental caries can affect individuals in multiple ways. Specifically, toothache, dysfunction of the stomatognathic system, or infection may hinder the essential consumption of nutritious diets, thereby impacting growth in both growing children and adults, in addition to their academic achievement, ability to communicate, and recreational pursuits<sup>3,4</sup>. This study aimed to investigate the antibiotic susceptibility of bacteria isolated from tooth decay in obese patients.

## MATERIALS AND METHODS

## Specimen Collection

Sixty specimens were randomly collected from tooth

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obesity patients in Babylon Governorate, Iraq, using a sterile swab. The collected specimens were suspended in ten milliliters broth brain-heart infusion broth and incubated aerobically for 24 h at 37 °C. A loop of the suspension was then transferred to nutrient agar and cultured for 24 h at room temperature. Combined with confirmatory diagnostic procedures, samples with positive cultures were cultured again in mannitol salt, Eosin Methylene Blue, MacConkey, and blood agar in addition to morphological characteristics.

## Identification of Bacterial Isolates

- **Microscopic Examination**

A loopful of water was placed on a tiny slide to initiate the Gram staining reaction. Subsequently, a tiny bacterial colony was moved, combined with water, and spread with a sterile cold loop. After passing through the burner three times, the smear was air-dried and heat-fixed. Next, Gram stain was applied to the smear, which was viewed using an oil immersion lens<sup>5</sup>.

- **Morphological and Cultural Characteristics**

The colony size, shape, color, edge, and transparency of each bacterial isolate were studied to determine their morphological and cultural traits<sup>5</sup>.

## Antibiotic Susceptibility Test

Antibiotic susceptibility to clindamycin, Ceftaroline, erythromycin, gentamycin, ofloxacin, rifampin, tetracycline, azithromycin, ciprofloxacin, levofloxacin, ciprofloxacin, nitrofurantoin, imipenem, and meropenem. An isolated colony of the tested bacteria was added to two milliliters of Muller-Hinton broth, which was then cultured at 37 °C for 18 h. The McFarland (0.5) turbidity standard, which corresponds to a concentration of  $1.5 \times 10^8$  CFU/ml, was then used to regulate the turbidity of the bacterial suspension. Mueller-Hinton medium was covered with 0.1 ml of bacterial culture, which was then allowed to dry. No more than five antibiotic disks were placed on each plate. The plates were incubated for a full day at 37 °C. A ruler was used to measure the resulting inhibition zones, which were then compared to those found by CLSI<sup>6</sup>.

## RESULTS

### Isolation and Identification:

The results showed the percentage of positive specimens was 37(61.66%) and the negative was 23 (38.33%) from the total 66 specimens Table (1). The result showed the number of positive male specimens 35 (58.33%), more than the female 25(41.66%), Table (2).

**Table 1.** Table 1 summarizes baseline characteristics.

**Table 1. Number and percentage of positive specimens according to age**

Age	Positive samples NO. (%)	Negative samples NO. (%)	Total NO. (%)
11-20	13(21.66)	10(23.33)	27(40.9)
21-30	11(18.33)	5(11.66)	18(27.27)
31-40	5(8.33)	4(6.66)	9(13.63)
41-50	7(11.66)	2(3.33)	9(13.63)
51-60	1(1.66)	2(3.33)	3(4.5)
<b>Total</b>	<b>37 (61.66)</b>	<b>23 (38.33)</b>	<b>60 (100)</b>

**Table 2. Number and percentage of positive specimens according to sex, n=60**

Sex	Positive samples NO. (%)	Negative samples NO. (%)	Total NO. (%)
Male	23(38.33)	12(20)	35(58.33)
Female	14(23.33)	11(18.33)	25(41.66)
<b>Total No (%)</b>	<b>37 (61.66)</b>	<b>23 (38.33)</b>	<b>60(100)</b>

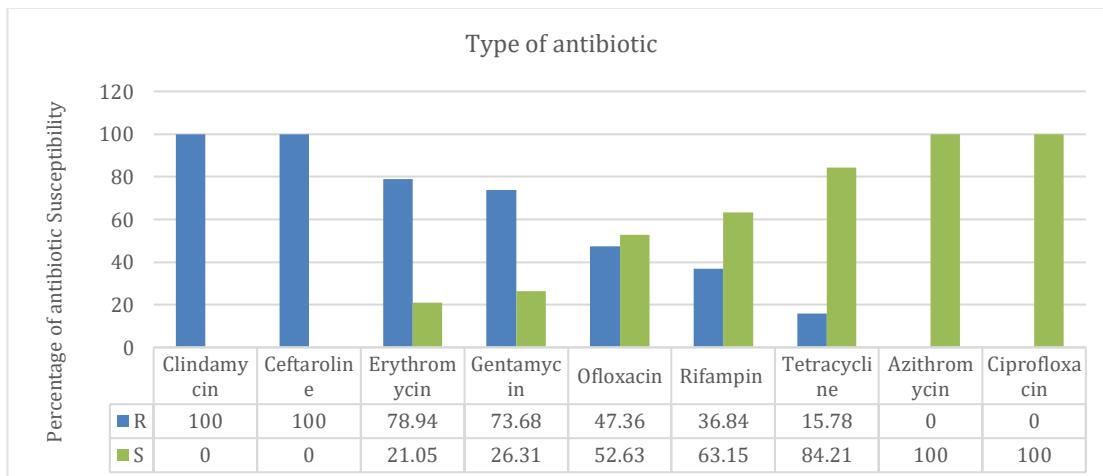
The results showed that the more prevalent type of bacteria isolates was *Staphylococcus aureus* (19) isolates than *Klebsiella pneumonia* (13), *Candida spp.* (10), *Staphylococcus epidermidis* (8), *Streptococcus sp.*, and *Escherichia coli* (5), *Bacillus cereus* (5), *Pseudomonas sp.* (3), *Proteus mirabilis* (2) Table (3).

**Table 3. Type and number of bacterial isolates**

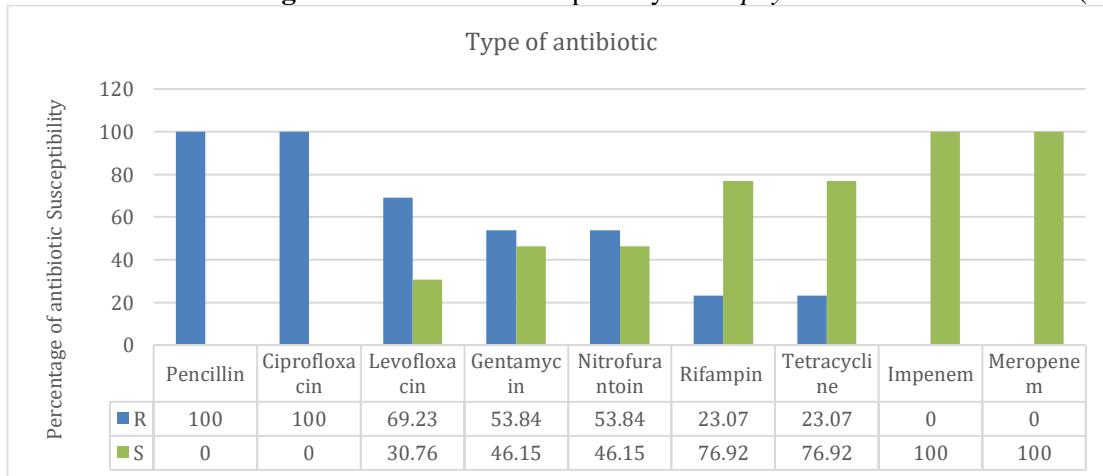
Type of Bacteria	Number
<i>Escherichia coli</i>	5
<i>Staphylococcus aureus</i>	19
<i>Staphylococcus epidermidis</i>	8
<i>Streptococcus sp.</i>	5
<i>Bacillus cereus</i>	4
<i>Klebsiella pneumonia</i>	13
<i>Pseudomonas aeruginosa</i>	3
<i>Proteus mirabilis</i>	2
<i>Candida spp.</i>	10

### Antibiotic susceptibility

Antibiotic susceptibility testing was performed using 14 types of antibiotics mentioned. Measure the diameter of the inhibition zone after cultivating *Staphylococcus aureus* isolates (n=19) and *K. pneumonia* (n=13), according to the standard inhibition zone in CLSI, 2024. The results showed that the higher resistance of *S. aureus* against Clindamycin and Ceftaroline 19(100%) than Erythromycin 15(78.94%), Gentamycin 14(73.68%), Ofloxacin 9(47.36%), Rifampin 7(36.84%), Tetracycline 3(15.78%), and 0% to Azithromycin and Ciprofloxacin. Figure (1). While the high resistance against *K. pneumoniae* was Penicillin and Ciprofloxacin 13(100%), Levofloxacin 9 (53.84%), Gentamycin and Nitrofurantoin 7(23.7%), Rifampin and Tetracycline 3(23.07%), then Imipenem and Meropenem 0% Figure (2).



**Figure 1.** Antibiotic susceptibility of *Staphylococcus aureus* isolates (n=19)



**Figure 2.** Antibiotic susceptibility of *Klebsiella pneumoniae* isolates (n=13)

### DISCUSSION

Sixty specimens were collected randomly from the teeth of obese patients aged from 11 to 60 in Babylon Governorate. Isolation and primary identification were performed according to the standard microbiological procedures, including culture characteristics, bacterial cells' arrangements in Gram's stain, biochemical reaction (Catalase, Coagulase, Oxidase, Indole, Methyl red, Citrate, and Voges Proskauer) test <sup>7</sup>. The results showed the percentage of positive specimens was 37(61.66%) and the negative was 23(38.33%) from the total 66 specimens Table (1). The result showed the number of positive male specimens 35 (58.33%), more than the female 25 (41.66%). The results showed that the more prevalent type of bacteria isolates was *Staphylococcus aureus* (19) isolates, then *Klebsiella*

*pneumonia* (13), *Candida spp.*(10), *Staphylococcus epidermidis* (8), *Streptococcus sp.*, and *Escherichia coli* (5), *Bacillus cereus* (5), *Pseudomonas sp.* (3), *Proteus mirabilis* (2). Oral health is unquestionably one of the most significant areas of public health. Since it is among the most crucial elements of individual health, its social relevance must be evaluated. The primary causes of tooth decay, a multiphase illness, are age, social and economic standing, inadequate brushing practices, drinking unhealthy beverages, and unsuitable <sup>8, 9, 10</sup>. The amount of carbohydrates consumed in the daily diet has a significant impact on the disease's onset and course. There are numerous detrimental impacts of obesity on dental health <sup>10, 11</sup>. There is evidence that sugar contributes to tooth decay by reducing pH, which can promote the growth of microbes linked to dental cavities.

It has been demonstrated that those who consume more sugar are much more likely to develop dental decay than those who consume less sugar<sup>12, 13</sup>.

Antibiotic susceptibility testing was performed using 14 types of antibiotics mentioned. Measure the diameter of the inhibition zone after cultivating *Staphylococcus aureus* isolates (n=19) and *K. pneumonia* (n=13), according to the standard inhibition zone in CLSI, 2024. The results showed that the higher resistance of *S. aureus* against Clindamycin and Ceftaroline 19(100%), then Erythromycin 15(78.94%), Gentamycin 14(73.68%), Ofloxacin 9(47.36%), Rifampin 7(36.84%), Tetracycline 3(15.78%), and 0% to Azithromycin and Ciprofloxacin. While the high resistance against *K. pneumoniae* was Penicillin and Ciprofloxacin 13 (100%), Levofloxacin 9 (53.84%), Gentamycin and Nitrofurantoin 7(23.7%), Rifampin and Tetracycline 3(23.07%), then Imipenem and Meropenem 0%. The higher resistant *Staphylococcus aureus* isolated to  $\beta$ -Lactam antibiotic (Penicillin) using in the present study related to the three types of resistant mechanisms including: hydrolyzes the cyclic amide bond of the  $\beta$ -lactam ring by producing  $\beta$ -Lactamase enzymes (decreases bactericidal activity), inhibition the ability of antibiotic to bind to Penicillin Binding Proteins (PBPs) by decreased permeability to the antibiotic through the outer cell membrane of the bacteria. In gram-positive bacteria, the peptidoglycan layer is near the bacteria's surface, and there are few barriers for the drug to reach its target and altered PBPs. This explains the resistance of *Staphylococcus aureus* to most commercially available  $\beta$ -lactams<sup>14</sup>.

Gentamicin. irreversible binding of the aminoglycoside to the bacterial ribosome 30S subunit, which inhibits the production of proteins. Tetracyclines are classified as synthesizing proteins. Broad-spectrum antibiotics are those that inhibit antibiotics. This activity reviews the indications, action, and contraindications for tetracyclines as a valuable agent in treating bacterial infection<sup>15</sup>.

It believes that the spreading of multi-drug resistance to antibiotics is related to the large number and misuse of antibiotics in humans and animals, especially in countries where it is easy to purchase drugs without a prescription, as is the case in most Arab and developing countries. Also, spread in a large number of countries of the world, misuse and frequent use of drugs in animals

## DECLARATION

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This research did not receive any specific grant or

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### Competing Interests

The authors have no competing interests to declare.

### Informed Consent

Not applicable.

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