Investigating the resistance of *Streptococcus mutans* bacteria to certain antibiotics in Tikrit city

التحري عن مقاومة بكتريا تسوس الاسنان لبعض المضادات الحيوية

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Abstract

Background: To distinguish *Streptococcus mutans* bacteria from other susceptible streptococcal bacteria, tooth decay is a chronic illness and one of the most prevalent diseases globally. **Aim of the study:** Streptococcus mutans, one of the most prevalent pathogens of tooth decay, is isolated and identified from dental caries lesions. **Materials and methods:** The current study involved collecting 100 samples from individuals with dental caries at the Specialized Dental Center and outpatient clinics of Tikrit Teaching Hospital. The samples were taken from both genders between July 2025 and January 2026 using Gel swabs, which were then cultured on a specific medium. Biochemical and microscopic examinations were performed, and the results showed that 100 isolates were *Streptococcus mutans* on Bacitracin agar salivarius (MSBA). **Result:** of the current study show that the most susceptible to tooth decay are in the 21-30 and 31-40 age groups, representing 32% and 31% respectively. Fifty-seven swabs were collected from males (57%) with dental caries, and 43 swabs were collected from patients with periodontal inflammation (43%). Forty-three swabs were collected from females with dental caries. Antibiotic susceptibility testing was conducted on various commonly used antibiotics. Ampicillin, Azithromicin, Cifotaxime, Cefixime, Erythromycin, Meropenem and Nitroflorantion resistance (100%) was found to be the most common antibiotic. Azithromycin, cifotaxime, cefixime, erythromycin, meropenem, and nitrofloranthin were also found to be resistant. Most bacterial isolates also showed high resistance to more than one antibiotic. The widespread and indiscriminate use of many antibiotics in treating bacterial infections has contributed to an increase in resistance rates. Isolates showed sensitivity to amikacin (80%), imipenem (89%), and augmentin (91%). **Conclusion:** The present study concluded that the bacteria that causes tooth decay is Streptococcus mutans. The bacterial isolates of Strep. mutans can show multidrug resistance to several drugs

Keywords: *Streptococcus mutans*, Antibiotic Sensitivity Test, Tooth decay.

خلاصة

الخلفية: لتمييز بكتيريا العقدية الطافرة عن غيرها من البكتيريا العقدية الحساسة، فإن تسوس الأسنان هو مرض مزمن وواحد من أكثر الأمراض انتشارا على مستوى العالم. **هدف الدراسة**: عزل وتشخيص بكتيريا *Streptococcus mutans* وهي من أكثر مسببات تسوس الأسنان شيوعاً من آفات تسوس الأسنان. **المواد وطرائق العمل**: شملت هذه الدراسة جمع 100 عينة من أفراد مصابين بتسوس الأسنان في مركز طب الأسنان التخصصي والعيادات الخارجية بمستشفى تكريت التعليمي. أُخذت العينات من كلا الجنسين بين يوليو 2025 ويناير 2026 باستخدام مسحات هلامية، ثم زُرعت على وسط غذائي خاص. أُجريت فحوصات بيوكيميائية ومجهرية، وأظهرت النتائج أن جميع العزلات المئة كانت من نوع المكورات العقدية الطافرة *Streptococcus mutans* على وسط باسيتراسين أجار ساليفاريوس .(MSBA) أظهرت نتائج الدراسة أن الفئات العمرية الأكثر عرضة لتسوس الأسنان هي 21-30 و31-40 عامًا، بنسبة 32% و31% على التوالي. جُمعت 57 مسحة من الذكور (57%) المصابين بتسوس الأسنان، و43 مسحة من المرضى المصابين بالتهاب دواعم السن (43%). كما جُمعت 43 مسحة من الإناث المصابات بتسوس الأسنان. أُجري اختبار حساسية المضادات الحيوية على مجموعة متنوعة من المضادات الحيوية شائعة الاستخدام. وُجد أن مقاومة أمبيسيلين، أزيثروميسين، سيفوتاكسيم، سيفيكسيم، إريثروميسين، ميروبينيم، ونيتروفلورانتيون (100%) هي أكثر المضادات الحيوية شيوعًا. كما وُجدت مقاومة للأزيثروميسين والسيفوتاكسيم والسيفيكسيم والإريثروميسين والميروبينيم والنيتروفلورانثين. وأظهرت معظم العزلات البكتيرية مقاومة عالية لأكثر من مضاد حيوي واحد. وقد ساهم الاستخدام الواسع النطاق والعشوائي للعديد من المضادات الحيوية في علاج العدوى البكتيرية في زيادة معدلات المقاومة. وأظهرت العزلات حساسية للأميكاسين (80%) والإيميبينيم (89%) والأوغمنتين (91%). الخلاصة: خلصت هذه الدراسة إلى أن البكتيريا المسببة لتسوس الأسنان هي المكورات العقدية الطافرة .*Streptococcus mutans* تُظهر بكتيريا المكورات العقدية الطافرة مقاومةً متعددةً للعديد من الأدوية.

الكلمات المفتاحية: .*Streptococcus mutans*، اختبار حساسية المضادات الحيوية، تسوس الأسنان.

Introduction

Tooth decay is a chronic disease and one of the most common diseases worldwide. It has occupied many researchers and specialists in the field of dentistry. Tooth decay is considered the leading cause of tooth loss [1]. It not only affects oral health but is also associated with other diseases such as diabetes. Therefore, preventing and treating tooth decay is crucial for mitigating health risks [2]. Neglecting oral and dental health has a significant impact on overall health and is the primary cause in childhood, affecting the crowns and

roots of teeth. The causes of tooth decay include environmental, physical, and biological factors, such as insufficient fluoride exposure [3]. The process of tooth decay begins when acid produced during carbohydrate metabolism breaks down the enamel. The initial stage of decay appears as white spots, and if left untreated, decay forms cavities in the enamel and can reach deeper layers [4]).
Streptococcus mutans A facultative, spherical, Gram-positive anaerobic bacterium that is a cariogenic agent and the primary causative agent of dental caries. It has the ability to produce large quantities of organic acids and is able to outcompete non-cariogenic commensal bacteria under low acid conditions. It is mainly found in biofilms that form on tooth surfaces [5]. *Streptococcus mutans* possesses three key virulence factors that contribute to its cariogenic process: the ability to grow in acidic environments, the ability to produce large quantities of extracellular glucans from sucrose (which help it invade hard surfaces), and the ability to metabolize a wide range of carbohydrates and form organic acids, thus dissolving tooth enamel [6].
S. mutans bacteria are resistant to a number of antibiotics, including bacitracin. Bacitracin is a peptide antibiotic produced by rod-shaped bacteria that targets the bacterial cell wall, specifically by breaking down peptidoglycan in the cell wall and transporting peptidoglycan components across the cell membrane to compensate for cells lacking peptidoglycan in their cell wall. It is effective against both Gram-positive and Gram-negative bacteria and is bactericidal. Some streptococcal bacteria are sensitive to it, while others are resistant. It is added to the growth medium of *S. mutans* bacteria to differentiate them from other susceptible streptococcal bacteria [7]. Optochin is used to differentiate susceptible S. pneumoniae from resistant S. mutans bacteria. It works by inhibiting the enzymes that synthesize adenosine triphosphate (ATP), thus preventing its formation and leading to bacterial cell death. Tetracycline and other antibiotics are also used in this way [8]. Chloramphenicol works in a single way where it inhibits protein synthesis. The tetracycline antibody binds to the 30s ribosomal subunit, which inhibits protein synthesis by preventing the connection of the charged tRNA to the amino acid, thus preventing the amino acids from reaching the peptide chain. Studies indicate that S. mutans is 100% sensitive to amoxicillin, erythromycin, and cephaloth, while sensitivity rates are 95.69% and 69.59%, respectively, to gentamicin and tetracycline [9]. However, a 2009 study by Jain and Pundir found that *S. mutans* isolates were sensitive to amoxicillin, tetracycline, and gentamicin [10]. Erythromycin binds to the 50S subunit and prevents it from forming the 70S complex, thus inhibiting

polypeptide chain synthesis. Streptomycin, an aminoglycoside antibiotic, binds to the p12 protein receptor in the 30S subunit of microbial ribosomes, inhibiting polypeptide chain synthesis, which consists of mRNA + formylmethionine + messenger RNA (mRNA). During the reading of the discriminating region on the rhiosome, the wrong amino acid is added to the polypeptide chain, resulting in a non-functional protein. Finally, the polypeptide chain breaks down into monosomes incapable of protein synthesis, leading to bacterial death. [11]

Aims of study

1. Isolation and identification of *Streptococcus mutans* bacteria from dental caries lesions, as it is one of the most common pathogens of tooth decay.
2. Studying the bacteria's ability to develop antibiotic resistance.

Materials and methods

Sample Collection

One hundred gel swabs (43 women and 57 men) were collected from patients with dental caries at the Specialized Dental Center and Tikrit Teaching Hospital in Salah al-Din Governorate. The patients, aged 10-65 years, were collected between July 2025 and January 2026. Patient information, including age, gender, residence, and whether or not they had diabetes, was recorded. The samples were collected using gel swabs and then transferred to the laboratory for culture on appropriate nutrient media and diagnostic testing.

Sample Culture

Samples were cultured immediately upon arrival at the laboratory on Mitis salivarius Bacitracin agar (MSBA) prepared under sterile conditions and incubated anaerobically at 37°C for 48 hours.

Identification of Bacterial Isolates

Cultural Characteristics

Cultural characteristics were studied through the initial identification of bacterial colonies on blood agar and Mitis salivarius agar. The morphological characteristics of the colonies were observed, including color, shape, and texture, as well as their size, height, odor, and border

shape. Their hemolysis capacity was also assessed, as evidenced by the production of hemolysin on sheep blood agar, and their ability to exhibit enthilling [12].

Microscopic Examination of Bacterial Isolates

The microscopic examination of the bacterial isolates under study was performed by taking a portion of the bacterial colony, transferring it to a clean glass slide, and staining it with Gram stain to observe cell morphology and aggregation patterns [12].

Biochemical tests were performed, including: Indole Test, Methyl red test, Voge's-Proskauer (VP) Test, Citrate Utilization Test, Hemolysis test, Catalase Test, Oxidase test, Motility test, and Triple sugar iron agar (TSA) test. Confirmatory methods for diagnosis included using the VITEK 2 Compact System.

Antibiotic Sensitivity Test

The standard Kirby-Baure method was used to test the sensitivity of bacteria to antibiotics, where tablets impregnated with the antibiotic are placed on an agar plate inoculated with bacteria; After incubation, "zones of inhibition" (areas free of growth around the discs) are measured to classify bacteria as "sensitive," "intermediate," or "resistant" to the antibiotic, guiding clinicians in choosing the appropriate treatment. The surface of a Mueller-Hinton agar plate is inoculated with the bacteria to be tested, and small paper discs impregnated with standard concentrations of different antibiotics are placed on top of the agar surface. The plates are incubated, allowing the antibiotic to diffuse from the disc to the agar, creating a concentration gradient. Readings are then determined by measuring the diameter of the bacterial zone of inhibition around each disc in millimeters using a transparent ruler. Bacteria are classified as sensitive (S), resistant (R), or intermediate (I) compared to the standard rates for zone of inhibition diameter listed in international laboratory tables [13].

Statistical Analysis

All results of the current study were subjected to statistical analysis using SPSS version 17. Comparisons were performed using the chi-square test and one-way and multi-way ANOVA based on the least significant

difference (LSD). A p-value <0.05 was considered statistically significant, while a p-value >0.05 was considered non-statistically significant [14].

Result

Isolation and Identification of *streptococcus mutans* according to sex

One hundred samples were collected from individuals with dental caries at the Specialized Dental Center and outpatient clinics of Tikrit Teaching Hospital. These samples consisted of oral swabs taken from the oral cavity (dental caries and periodontal inflammation). Fifty-seven swabs were collected from males (57%) swabs from patients with dental caries and 43 swabs from patients with periodontal inflammation (43%). Forty-three swabs were collected from females with dental caries. Diagnosis was made by studying the morphological characteristics of *Streptococcus mutans* bacteria through culture. The colony was cultured on nutrient agar and then on blood agar. The colony exhibited the ability to decompose blood, producing a transparent alpha-green spore around the colony. It was then cultured on its own medium, Mitis salivarius bacitracin agar (MSBA), and several biochemical and microscopic tests were performed.

Table (1): Percentage of tooth decay, number of S. mutans bacterial isolates according to sex

Sex	NO.	%	Cases of	NO.	%	P value	X2
Male	57	57	Tooth decay	57	57	0.3	0.82
Female	43	43	Tooth decay	43	43		
Total	%100	%100	Total	100	%100		

Percentage of tooth decay, number of *S. mutans* bacterial isolates according to age group

The samples were taken from individuals aged 10 to 65 years of age, of both sexes, between July 2025 and January 2026. The results of the current study show that the most susceptible to tooth decay in 21-30 and 31-40 age group, representing a percentage of 32% and 31% respectively . While the least susceptible to tooth decay in **41-50**, **51-60** and **>65** age group, representing a percentage of 9%, 8% and 5% respectively as in Table (2). Statistical analysis showed **P value** 0.01 a highly significant difference between age groups in the incidence of tooth decay, specifically regarding the relationship between the number of isolates and age groups.

Table (2): Percentage of tooth decay and number of *S. mutans* isolates by age group

Age group	NO.	%
10-20	15	15
21-30	32	32
31-40	31	31
41-50	9	9
51-60	8	8
>65	5	5
Total	100	100
X2	14.6	
P value	0.01	

Percentage of tooth decay, number of *S. mutans* bacterial isolates according to smokers and oral and dental hygiene

The results of the current study show the distribution number of *S. mutans* of dental caries cases among smokers. The highest incidence of the disease was among smokers (62%), while the lowest incidence was among non-smokers (38%). As well as according to oral and dental hygiene recorded that (35%),

while not interested in oral and dental hygiene recorded that (65%) as shown in Table 3. Statistical analysis showed P value 0.001 a highly significant difference between age groups in the incidence of tooth decay.

Table (3): Percentage of tooth decay and number of *S. mutans* isolates according to smokers and oral and dental hygiene

Cases of tooth decay	Smokers	Son-smokers	Oral and dental hygiene	not interested in oral and dental hygiene
	Total=100		Total=100	
NO.	62	38	35	65
%	62	38	35	65
X2	52.41		36.78	
P value	0.001		0.001	

Antibiotic Sensitivity of *S. mutans*

The test results showed a clear variation in the pattern of sensitivity and resistance to antibiotics. *S. mutans* total number of samples: 45 isolates of *S. mutans* exhibited complete resistance (100%) to Ampicillin, Azithromicin, Cifotaxime, Cefixime, Erythromycin, Meropenem and Nitroflorantion. Most bacterial isolates also showed high resistance to more than one antibiotic [15]. The widespread and indiscriminate use of many antibiotics in treating bacterial infections has contributed to an increase in resistance rates. Isolates showed sensitivity to Amikacin (80%), Imipenem (89%), and Augmentin (91%). Resistance to Amoxicillin (91%) and Gentamicin (89%), Ciprofloxacin (76%), and Streptomycin (96%)was moderate. (Table 4).

Table (4): Susceptibility of (45) *S. mutans* isolates to antibiotics

Antibiotics	**45 isolates of *S. mutans***			
	Number of isolates Resistance	٪	**Number of isolates Sensitive**	٪

Amikacin	9	٪20	36	80٪
Amoxicillin	41	٪91	4	9٪
Ampicillin	45	٪100	0	٪0
Azithromicin	45	٪100	0	٪0
Cifotaxime	45	%100	0	٪0
Cefixime	45	%100	0	٪0
Erythromycin	45	٪100	0	٪0
Gentamicin	40	٪89	5	٪11
Ciprofloxacin	34	٪76	11	٪24
Imipenem	5	٪11	40	٪89
Meropenem	45	٪100	0	٪0
Nitroflorantion	45	٪100	0	٪0
Augmentin	4	9٪	41	٪91
Streptomycin	43	96	2	٪4

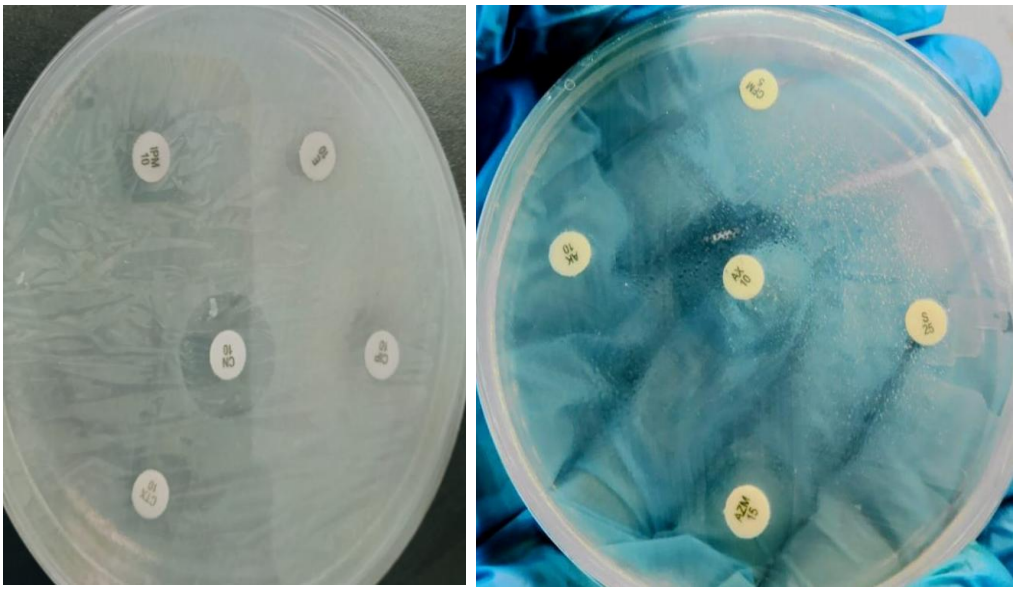

Figure (1): Antibiotic sensitivity test

Bacterial Diagnosis of *S.mutans* bacteria

Isolates grown on solid (MSA) medium after anaerobic incubation at 37°C for 48 hours showed small, elevated, irregular, and tightly packed colonies, while isolates grown on (MSBA) medium were characterized by being 1 mm in diameter, resembling beads or drops, and fermenting sugars, as shown in Figure (1).

Cultivation characters

Figure (2): *S.mutans* bacteria on mitis salivarus bactracin agar

Figure (3) of bacteria on mannitol salt agar medium to assess their activity in mannitol fermentation and the result is positive.

Figure (3): *S.mutans* bacteria on mannitol salt agar medium

Microscopic Examination

The results of staining dry smears prepared from growing bacterial colonies showed Gram-positive spherical or oval-shaped, chain-like structures arranged

in pairs or chains (Figure 4). Bacteria stained with Gram stain. Bacteria are Gram-positive [16].

Figure (4): *S. mutans* bacteria under a microscope (100X)

Biochemical examinations

All bacterial isolates gave negative results for oxidase, catalase, citrate, while positive results were obtained for indole, and Voges-Proskauer (VP), methyl red (MR), fermentable sugars (alanine, sorbitol, sucrose, and manthol), and trisaccharide (TSI), consistent with Salh et al., [17]. The citrate test result on Simmons citrate agar medium. Result: Negative. Bacteria fermenting sucrose, glucose, and fructose. A/A (Acid/Acid) no H2s no gas

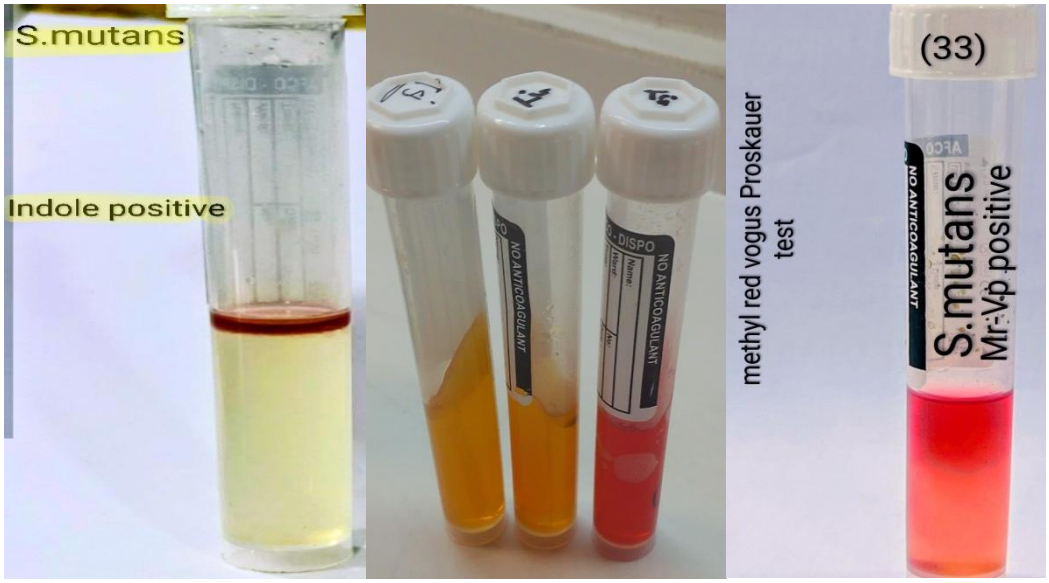

Fig. 5: Biochemical test examinations

Discussion

The results contradicted those of Al-Dulaimi [18], who explained in his study that the number of affected females was generally higher than that of affected males, which may be explained by their more frequent visits to hospitals and clinics. The results also agreed with those of Khamise [1] in Najaf Governorate, who found that the infection rate among males was 25.2% in permanent teeth, and higher than that among females (31.2%). However, the percentage in primary teeth was higher among males than females, at 31.8% for males and 20.4% for females. The results also agree with those of Al-Mosawi [20] in Baghdad, where the infection rate among males was 87% (64%), higher than the infection rate among females, which was 34% (36%). They also agree with the results of Ketabi *et al.* [21]. It showed that the level of oral health is better in females than in males.

The current results show that the 20-40 age groups is the most susceptible to the disease. This group represents the most frequent segment of society and is the least able to protect itself from pollutants and external factors, due to its high consumption of sugary foods and drinks. These results differ from those of Al-Sultani *et al.* [22] in Babylon Governorate, where they found the highest incidence among children aged 5-7 years, at 94%. Abbas [23] also found that the 7-9 age group is the most affected, with rates of 80-90% for primary teeth and 60% for permanent teeth in this age group.

The over-50 age group had the lowest incidence of tooth decay at 5.4%, likely due to their higher prevalence of diabetes and heart disease, and their lack of referral to the specialized dental center in Baquba. These results were similar

to those of the study by JIANG *et al.* [24], who found that adults had a lower incidence of tooth decay. However, a study by Noor [25] in Baghdad showed that the prevalence of the disease was higher among the older age group (53.9%) than among the younger age group (14.8%).

The study's findings were similar to those of Efunwole [26], whose *S. mutans* isolates showed sensitivity to the antibiotics Augmentin and Imipenem.

It also partially agreed with the results of Shamrin [27], where the isolates showed sensitivity to the antibiotic Amikacin. These results were similar to those found by AL-Kaziragy [28], where *S. mutans* exhibited high resistance to Azithromycin, Cifotaxime, and Cefixime, and moderate resistance to Gentamicin (89%) and Ciprofloxacin. A group of researchers [29] also found complete resistance of the bacteria to the antibiotics Meropenem and Nitroflorantion due to indiscriminate use of antibiotics in Iraq, particularly in Tikrit province.

study highlights a strong link between smoking, poor oral hygiene, and increased dental caries, showing smokers (62%) and those neglecting hygiene (65%) have higher rates, compared to non-smokers (38%) and those with good habits (35%), aligning with research showing smoking increases cariogenic bacteria like *S. mutans* and harms oral health.

Smoking is associated with a significantly higher incidence of tooth decay compared to non-smokers [30]. Poor oral hygiene is strongly associated with higher rates of decay compared to good oral hygiene. *Streptococcus mutans* bacteria play a major role in exacerbating decay and are more prevalent among smokers. The findings support a link between smoking and increased decay activity through its impact on oral bacteria and the oral environment. Good oral hygiene may reduce the risk of decay even in the presence of other risk factors. Related studies indicate that smoking impairs oral integrity and affects tooth and periodontal structures. There is evidence from various sources confirming that smoking increases the factors that aggravate the oral environment and promote decay [31].

Conclusion

1. The study showed that the highest incidence of tooth decay in males was in the age group of 21-30 and 31-40 years, and the lowest in the age group over 50 years.
2. *Streptococcus mutans* bacteria is one of the causes of tooth decay.
3. The Strep.mutans bacterial isolates have the ability to exhibit multidrug resistance to a number of antibiotics.

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