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"Identification and Antibiotic Sensitivity of Cariogenic Strains in Caries Lesions: A Study of BECHAR Town Population"

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Abstract:
In this approach, a cariogenic strain is permanently implanted in the host microflora. Once established, the presence of this strain encourages the colonisation or growth of other pathogens.
Objectives: The aim of this study is to isolate and identify the bacteria responsible for these carious infections (cariogenic bacteria) in the human oral cavity from samples taken in private dental practices in Bechar's town.
Methods: Participants completed a questionnaire and were clinically examined according to criteria set by the World Health Organisation. Dental caries samples were collected from 250 subjects with caries lesions. Conventional methods for culturing and identifying aerobic and anaerobic bacteria were used.
Results: This shows that the Streptococcaceae and Lactobacillaceae are the families most represented in our samples. *Streptococcus mutans* is the most cariogenic organism in the oral microflora, due to a number of factors. The analysis of dental plaques of adults and infants with caries revealed the presence of 210 strains belonging to 7 genera of 4 aero-anaerobic (*Streptococcus, Haemophilus, Gamella* and *Staphylococcus*) and 3 anaerobic genera *(Lactobacillus, Peptococcus, Peptostreptococcus*) ($P < 0.05$). Nine (09) bacterial strains were isolated from dental caries, some of them belonging to an aero-anaerobic genus, others to an anaerobic genus. *Streptococcus mutans* (22%), *Streptococcus intermedius* (18%), *Lactobacillus sp* (15%), *Streptococcus mitis* 1 (12%), *Peptococcus sp* (10%), *Haemophilus parainfluenzae* (8%),*Gamella morbillorum* (6%) and *Peptostreptococcus spp*.(5%) and *Staphylococcus aureus* (4%) showed a significantly high prevalence in the group of subjects with carious teeth. Antibiotic sensitivity tests conducted on these microorganisms revealed confirmed resistance in some strains against (Azithromycin, Amoxicillin+ Clavulanic acid, Amoxicillin, Gentamycin, Amikacin, Streptomycin and Spiramycin) and marked sensitivity for other antibiotic (Tobramycin, Oxacillin, Tetracycline, Bacitracin) and as well as having chloramphenicol as an intermediate. *Streptococci* and *lactobacilli* are bacteria that can originate either internally or externally and can cause oral diseases such as dental caries, which is a public health scourge.
Conclusion: The impact of dental pain and infection on quality of life also needs to be considered. Diseases of the oral cavity occur in a complex host-bacterial community interaction that often does not fit a single microbe pathogenesis model. This study corroborates that oral hygiene practices minimize the prevalence of dental caries.

Keywords : Identification, cariogenic strain, oral cavity, caries lesions, *Streptococcus mutans* ,antibiotic sensitivity. BECHAR Town Population

I/Introduction

The mouth, like other surfaces of the body, is colonized from birth by a diverse array of microorganisms (collectively known as the oral microbiota) **[1].** Dental caries is a chronic disease that progresses slowly in most people **[2].** This disease and its sequelae can cause significant pain and are expensive to treat **[3]**. The link between microbial population that form cavity in teeth and oral health are well observed **[4]**.

The important causative organisms of dental caries are *Streptococcus mitis; S. mutans, S. salivarius, S. sanguis and S. sobrinus.* **[5].**More recent studies have shown the bacteria previously referred to as *S.mutans* are now subdivisible into seven distinct species **[6]** .These species are often collectively referred to as the mutans streptococci because they have a number of common properties relevant to caries-inducing. *Lactobacilli* are commonly present in deep carious lesions and are more likely to promote lesion progression than lesion initiation. These bacteria are highly acidogenic **[7]; [8].**

The problem of antibiotic resistance is likely to greatly disrupt patient care. It is therefore up to the dental surgeon to keep abreast of changes in prescribing principles when treating infectious oral pathologies. At the same time, alternatives to conventional antibiotics are being developed in an attempt to circumvent the problem posed by the adaptation of bacterial populations **[9] .**Our study is part of an assessment of microbial biodiversity in healthy teeth and cariogenic bacteria, including in vitro study of the antibiotic resistance of certain oral strains.

II/Material and methods:

II.1.Study Population and Data Collection

This study was conducted in collaboration with two dental surgeries in the town of Bechar, specifically Dr. LAOUFI and Dr. TIKOUK, and recruited 200 subjects with caries and 50 healthy controls. The study protocol was performed according to the Helsinki declaration and approved by the Tlemcen University Hospital Committee for research on human subjects. Baseline samples were collected from all subjects and for subjects with dental caries, plaque was collected separately from the surfaces of three progressive stages of caries. During the initial visit, we administered a concise written survey to identify the reasons why certain patients did not attend their follow-up appointments. They completed a medical and dental history questionnaire and signed an informed consent document.

II.2. Plaque sampling

Samples were collected in the morning, 12 h after tooth brushing and 2 hours after the last food and/or drink intake. **[10]**

For the healthy subjects dental plaque was sampled from healthy enamel. For the subjects with dental caries plaque was collected separately from the surfaces of each of three progressive stages: 1) intact enamel, 2) white spot lesions, and 3) cavitated lesions, if present. Therefore one sample was collected from each healthy subject, and either two or three samples were collected from each subject with caries, depending on whether cavitated lesions were present.

For subjects with caries, all carious surfaces were scored. Dental plaque was collected by swiping the tooth or lesion surface with a coarse endodontic paper point. Each plaque

sample was obtained by pooling from multiple teeth. Samples were placed in tubes containing 10ml nutrient broth and transported to the laboratory.

The methodology used in this major study is useful because it allows comparison with exogenous and endogenous cariogenic microflora in regards to load, but it is also novel in terms of caries epidemiology.

II.3. Protocol for bacterial isolation in the laboratory

This study examines the cariogenic microflora, the microbial diversity accountable for causing dental caries in both populations, using phenotypic identification methods to classify bacterial strains down to the species level. This was achieved through anoxic cultures, biochemical tests (API miniaturized galleries), and antibiotic sensitivity testing.

The specimens were processed in the laboratory for the cultivation of aerobic and anaerobic bacteria. First, the samples were vortexed for 30s and the suspension was serially diluted (10^{-1}–10^{-4}), with sterile brain heart infusion broth (pH 7.2, Oxoid, Basingstoke, UK), aliquots (100 mL) of each dilution and the undiluted suspension were inoculated onto non-selective and selective media. Enriched blood agar [Columbia agar base (Oxoid, Basingstoke, UK) supplemented with 5% laked blood] was used for the isolation of facultative and anaerobic bacteria. Plates were incubated anaerobically for 3–5 days at 37°C in an environment consisting of 10% CO_2 and aerobically for 24–48 h at 37°C. Selective media such as MacConkey (Oxoid, Basingstoke, UK) used for the isolation of Enterobacteria and Chapman (Oxoid, Basingstoke, UK) for staphylococci were also inoculated and incubated under an aerobic condition for 24–48 h at 37°C.

II.4. Phenotypic identification of isolated bacteria

Bacterial identification was based on the colony morphology and pigmentation, Gram staining and the biochemical reactions (API 20 A, API 20 Strep, API 20 E, API 20 Staph) (Biomerieux, Marcy l'Etoile, France) **[11, 12].** Antibiotics sensitivity test was also used to confirm biochemical test results.

The antibiotic susceptibility of the isolate was determined as per the standard method **[13].** Activated culture of each isolate was swabbed over the surface of Mueller Hinton agar (Biomerieux) agar plates. Twelve different antibiotic discs (Amoxicillin+clavulanic acid (AUG) (AUG, 10 mcg), Azithromycin (AZM, 15 mcg), Streptomycin (STR, 300 mcg), Chloramphenicol (C, 30 mcg), Tobramycin (TOB, 10 mcg), Gentamicin (G, 50 mcg), Amikacin (AK, 30 mcg), Spiramycin (SP, 100 mcg),Bacitracin (B, 10 units), Cefalexin (CN, 30 mcg), Oxacillin (OX, 5 mcg), Tetracycline (T, 10 mcg), (Biomerieux, Marcy l'Etoile, France) were placed over the surface of the inoculated plate. The plates were incubated at 37 °C for 24 h. The diameter of the Zone of inhibition around each disc was measured and expressed in mm. Results were interpreted as sensitive, S (≥ 21 mm); intermediate, I (16-20 mm) or resistant, R (≤ 15 mm) **[14].**

II.5. Ethics Statement

The questionnaire consisted of two parts. The first part consisted of general information such as age, sex and educational qualification. The second part consisted of six questions related to oral health, attitude and practices. We also gathered data on the patient's medical background, fluoride levels, and exposure to cigarette smoke. At each subsequent visit, patients underwent a toothbrush prophylaxis and fluoride varnish application. Furthermore, we offered oral hygiene instructions and advice on how dietary factors can impact the development and advancement of caries.

II.6. Data Management and Statistical Analyses

Chi-squared analysis was used to compare the caries and control groups by gender and race. A *t*-test was used to compare the two groups by age. Levels of each species were calculated as a percent of total bacteria for each sample. Mean relative levels and 95% confidence intervals were determined for the most prevalent species using JMP (JMP, Version 7.0, SAS Institute Inc., Cary, NC). Repeated measures analysis was performed using PROC MIXED in SAS (SAS Institute Inc., SAS 9.2, Cary, NC) using the default structure. The sequential stages of health/caries from which samples were collected were assigned a numeric value and used in the PROC MIXED analysis. Using this scale, healthy control samples were assigned a value of 1, intact enamel samples from subjects with caries 2, white spot lesions 3, and cavitated lesions 4. The p-value represents the probability of obtaining the observed results if the null hypothesis is true. A low p-value (<0.05) suggests a statistically significant association.

III/Results and discussions:

III.1. Results:

Subjects underwent dental examination for caries prevalence by a dentist, who applied the World Health Organization's caries diagnostic criteria to determine the decayed, missing, filled teeth (DMFT) index **[15].** The subjects were divided into two groups: Caries-free (CF) group (DMFT = 0, n = 50) and caries-active (CA) group (20 DMFT 40, n = 200). Exclusion criteria included antibiotic therapy in the previous 3 months, any systemic diseases and having less than 24 permanent teeth.

The questionnaire data revealed that there were 18 males (36%) and 32 females (64%) in the CF group, in which 22 (44%) persons were aged between 30 and 70 years, 16 (32%) between 18–29 years and 12 (24%) between 6 and 17 years age group. Whereas in the second group (CA), there were 96 (48%) males and 104 (52%) females, in which 70 (35%) persons were aged between 30 and 70 years, 86 (43%) between 18–29 years and 44 (22%) between 6 and 17 years age group.

The analysis of dental plaques of adults and infants with caries revealed the presence of 210 strains belonging to 7 genera of 4 aero-anaerobic (*Streptococcus, Haemophilus, Gamella* and *Staphylococcus*) and 3 anaerobic genera *(Lactobacillus, Peptococcus, Peptostreptococcus*) ($P < 0.05$).

Moreover, there was more isolation of Gram-positive bacteria than Gram-negative bacteria for the two groups ($P < 0.05$). There were no significant differences between the isolation rates of various species in examined groups, except for *Streptococcus mutans* (12% vs. 22%) ($P < 0.05$), *Streptococcus intermedius* (10% vs. 18%) ($P < 0.05$), *Lactobacillus sp* (10% vs. 15%) ($P < 0.05$), *Streptococcus mitis* 1 (8% vs. 12%) ($P < 0.05$), *Peptococcus sp* (6% vs. 10%) ($P < 0.05$), *Haemophilus parainfluenzae* (6% vs. 8%) ($P < 0.05$),,*Gamella morbillorum* (4% vs. 6%) ($P < 0.05$) and *Peptostreptococcus spp*. (2% vs. 5%) ($P < 0.05$) and *Staphylococcus aureus.*

Antibiotic sensitivity tests conducted on these microorganisms revealed confirmed resistance in some strains against (Azithromycin, Amoxicillin+ Clavulanic acid, Amoxicillin, Gentamycin, Amikacin, Streptomycin and Spiramycin) and marked sensitivity for other antibiotic (Tobramycin, Oxacillin, Tetracycline, Bacitracin) and as well as having chloramphenicol as an intermediate. *Streptococci* and *lactobacilli* are bacteria that can originate either internally or externally and can cause oral diseases such as dental caries, which is a public health scourge.

Of the 12 antibiotics tested only four antibiotics namely Tobramycin, Oxacillin, Tetracycline and Bacitracin were found to inhibit some strains (*Peptococcus sp*. *Haemophilus parainfluenzae and Gamella morbillorum, Lactobacillus sp and Streptococcus mitis* 1).

Mueller Hinton agars showing the zone of inhibition of different antibiotics to the tested isolates are shown in **Figure 1**. In general antibiotics elicit their action by inhibiting synthesis of bacterial cell wall, protein or by inhibiting the action of DNA gyrase.

This analysis aims to determine if there is a statistically significant association between bacterial species and antibiotic resistance patterns.

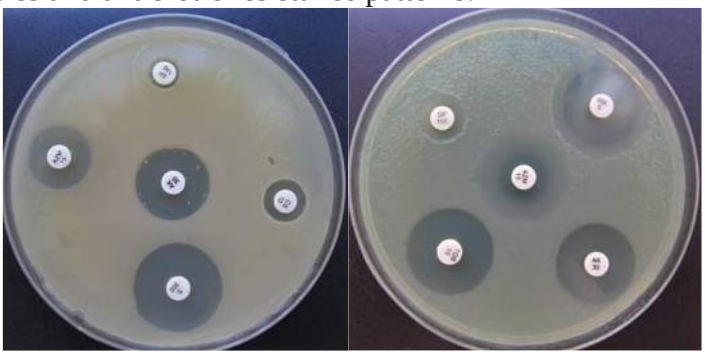

Figure1: Antibiogram of somes strains (*Streptococcus mutans "left"* and *Gamella morbillorum "right"*)

We treated bacterial species; *Streptococcus mutans, Streptococcus intermedius, Lactobacillus sp., Streptococcus mitis 1, Peptococcus sp., Haemophilus parainfluenzae, Gamella morbillorum, Peptostreptococcus sp., Staphylococcus aureus* and antibiotic resistance as categorical variables (Azithromycin, Amoxicillin+clavulanic acid, Streptomycin, Amikacin, Tobramycin, Chloramphenicol, Spiramycin, Gentamicin, Oxacillin, Tetracycline, Bacitracin. There is a significant association between bacterial species and antibiotic resistance (table 1).

Table 1: Susceptibility and resistance profiles of each bacteria species to the listed antibiotics.

Species	Antibiotic	Susceptibility
Streptococcus mutans	Azithromycin	Resistant
	Amikacin	Sensitive
	Streptomycin	Sensitive
	Spiramycin	Sensitive
	Gentamicin	Sensitive
Streptococcus intermedius	Amoxicillin + Clavulanic Acid	Resistant
	Streptomycin	Sensitive
	Amikacin	Sensitive
	Tobramycin	Sensitive
	Chloramphenicol	Sensitive
	Azithromycin	Sensitive

	Spiramycin	Sensitive
Lactobacillus sp.	Azithromycin	Sensitive
	Chloramphenicol	Sensitive
	Oxacillin	Sensitive
	Tetracycline	Sensitive
	Amoxicillin	Resistant
	Gentamicin	Resistant
	Amikacin	Resistant
	Streptomycin	Resistant
Streptococcus mitis 1	Streptomycin	Sensitive
	Chloramphenicol	Sensitive
	Gentamicin	Sensitive
	Bacitracin	Sensitive
	Spiramycin	Resistant
Peptococcus sp.	Streptomycin	Sensitive
	Amikacin	Sensitive
	Tobramycin	Sensitive
	Amoxicillin + Clavulanic Acid	Resistant
Haemophilus parainfluenzae	Streptomycin	Sensitive
	Amikacin	Sensitive
	Bacitracin	Sensitive
	Tobramycin	Sensitive
	Spiramycin	Resistant
	Chloramphenicol	Resistant
	Gentamicin	Sensitive
	Tobramycin	Sensitive
	Amikacin	Sensitive

Gamella morbillorum	Streptomycin	Sensitive
	Chloramphenicol	Intermediate
	Azithromycin	Resistant
	Spiramycin	Resistant
	Amoxicillin + Clavulanic Acid	Resistant
Peptostreptococcus sp.	Spiramycin	Resistant
	Streptomycin	Sensitive
	Chloramphenicol	Sensitive
	Gentamicin	Sensitive
Staphylococcus aureus	Chloramphenicol	Sensitive
	Amikacin	Resistant
	Spiramycin	Resistant

This analysis does not identify specific associations between each species and each antibiotic. Further analysis, such as calculating odds ratios or performing post-hoc tests, would be needed to explore specific relationships. This analysis only considers categorical data (resistant/sensitive) and does not consider the degree of resistance.

The Chi-Squared test can provide insight into whether a statistically significant association exists between bacterial species and antibiotic resistance patterns. However, this analysis is not exhaustive and further investigation is needed to fully understand the specific relationships between species and antibiotic sensitivities.

The questionnaire data revealed that there were 18 males (36%) and 32 females (64%) in the CF group, in which 22 (44%) persons were aged between 30 and 70 years, 16 (32%) between 18–29 years and 12 (24%) between 6 and 17 years age group. Whereas in the second group (CA), there were 96 (48%) males and 104 (52%) females, in which **70** (35%) persons were aged between 30 and 70 years, **86** (43%) between 18–29 years and **44** (22%) between 6 and 17 years age group.

Table 2: Distribution of free and active caries according to gender (male and female) and age.

Age	**Carries free (CF)**		**Carries active (CA)**	
	male	**female**	**male**	**female**
6-17	04	08	19	25
18–29	07	09	41	45
30-70	07	15	36	34

Total	**18**	**32**	**96**	**104**
Effective	50		200	

III.2. Discussion:

The present study offers the first description of the microflora associated with supragingival plaque in CF and CA Algerian adults based on culture methods. A wide diversity of bacterial species was observed. The relationship between cultural level, socioeconomic status, the food intake, oral hygiene practices, tooth pain, and oral malodor with dental caries was revealed by the questionnaires data.

Women had higher prevalence and severity of caries compared to men, which was consistent with the findings of Lukacs [**16**],Doyal and Naidoo[**17**], and Ferraro and Vieira [**18**]. This higher caries prevalence in females may be due to different salivary composition and flow rate, hormonal fluctuations during pregnancy, dietary habits, and particular social roles of women among their family (caretaker, meal preparation, etc.) [**18**]. Other studies have proposed that the differential effects of genes influencing dental caries may partly explain the observed differences in the two sexes [**19**].

Eighty-three studies found at least one measure of caries to be significantly higher in low socioeconomic position compared with high socioeconomic position individuals, while only three studies found the opposite [**20**]

In the present study, there were no association between socioeconomic status and caries prevalence in Algeria ($P < 0.05$) due to the free medical services that allow access to dental care services to all socioeconomic levels.

The frequency of sugar intake was most important in CA adults (80%) than that of in CF adults (30%). Previous studies have observed a linear relationship between sugar consumption and caries [**21**].

In this study, mutant streptococci and lactobacilli were more frequently isolated from supragingival plaques of CA subjects (20% and 22% respectively). Several studies in humans are largely based on the mathematical relationship between various streptococci, lactobacilli and dental caries [**22**].

Human oral microbiota is gradually developing into the oral cavity with age, and more than 600 species are usually seen in the adult population as a whole [**23**]**.**

Dental caries results from interactions over time between bacteria that produce acid, a substrate that the bacteria can metabolise, and many host factors that include teeth and saliva. Dental caries results from an ecological imbalance in the physiological equilibrium between tooth minerals and oral microbial biofilms. [**24,25**]

The mechanisms of the caries process are similar for all types of caries. Endogenous bacteria (largely mutans streptococci [*Streptococcus mutans* and *Streptococcus sobrinus*] and *Lactobacillus* spp) in the biofilm produce weak organic acids as a by-product of metabolism of fermentable carbohydrates. [**26,27**]**.**

Moreover, diet, mouth hygiene, health status, genetics, and lifestyle are biological and cultural factors that strongly affect oral microbiota diversity [**28**].

Colonisation by mutans streptococci, and other cariogenic bacteria at a young age could be a key risk factor for caries development. [**12,29**]**.** Early studies of caries lesions found higher proportions and incidence of *Streptococcus mutans* and *Streptococcus sobrinus* than sound enamel; lactobacilli were isolated from advanced lesions [**30**]. These observations led to the proposal that caries is only caused by a limited subset of

the many species found in dental biofilms (the 'specific plaque hypothesis') [**31**].

Dental caries is still a neglected topic, despite the acknowledgment of the WHO that is still a major health problem in most industrialized countries, in which 60–90% of children and the vast majority of adults are affected by dental caries[**32**].Although caries has been considered a childhood disease, in reality, it continues into adulthood [**33**]. Health inequalities exist in the burden of dental caries in both children and adults [**34**].

Many studies, such as those by Huang et *al.* [**35**]. and Araujo et *al.*[**36**]., were conducted in vitro, limiting the generalizability of their findings to clinical settings. Additionally, there is a notable lack of research directly addressing the antibiotic resistance patterns of *S. mutans*, an area crucial for developing effective treatment strategies.

IV/Conclusion:

Dental caries is a considerable public fitness issue global, and know-how the prevalence and variety of cariogenic microorganisms is essential for growing powerful prevention and treatment strategies. This study aimed to isolate and discover cariogenic strains from the BECHAR town population, focusing on the cariogenic ability of oral microorganisms. The take a look at employed a mixture of morphological and antibiogram techniques to perceive the isolated traces and compare their sensitivity.

The sensitivity of the isolated strains was evaluated using a disk diffusion test and a method described previously with some modification. Nine (09) bacterial strains were isolated from dental caries, as potentially cariogenic, including *Streptococcus mutans* (22%), *Streptococcus intermedius* (18%), *Lactobacillus sp* (15%), *Streptococcus mitis* 1 (12%), *Peptococcus sp* (10%), *Haemophilus parainfluenzae* (8%),*Gamella morbillorum* (6%) and *Peptostreptococcus spp*.(5%) and *Staphylococcus aureus* (4%) .

The antibiotic sensitivity tests conducted on these strains revealed confirmed resistance in some strains against (Azithromycin, Amoxicillin+ Clavulanic acid, Amoxicillin, Gentamycin, Amikacin, Streptomycin and Spiramycin) and marked sensitivity for other antibiotic (Tobramycin, Oxacillin, Tetracycline, Bacitracin) and as well as having chloramphenicol as an intermediate.

This study demonstrates the importance of isolating and identifying cariogenic strains from the BECHAR town population. The results provide useful information on the role of lactic acid bacteria from fermented foods and oral commensal streptococci in dental caries. Future studies should focus on the in vivo cariogenicity of these strains and their potential as probiotics for treating bad breath and regulating intestinal flora. Implement oral health education programs to raise awareness about the importance of oral hygiene and the role of cariogenic microorganisms in dental caries. Develop targeted dental care strategies that incorporate the identification and management of cariogenic microorganisms in the BECHAR town population.

Highlights

The literature on the characterization of oral cariogenic strains and their antibiotic susceptibility highlights several research gaps, particularly concerning the clinical implications of antibiotic resistance in *Streptocossus mutans* and other strains. This highlights the real challenges facing epidemiological studies on caries, whereby apparently comparable results from Bechar town have, in fact, been collected at different time-points with very variable levels of training and calibration and, thus, record caries.

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