Libyan Journal of Health, Science, and Development
(ISSN: 3106-4787)
Volume 2, Issue 2, 2026, Pages: 165-169
Journal homepage: https://ljhsd.org.ly/index.php/ljhsd/index
Directory of Online Libyan Journals

(ARTICLE)

Isolation and Characterization of Gram-Positive Bacteria from Saliva of Children with Down Syndrome and Healthy Children in Benghazi, Libya

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عزل وتوصيف البكتيريا موجبة الجرام من لعاب الأطفال المصابين بمتلازمة داون والأطفال الأصحاء في مدينة بنغازي، ليبيا

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Publication history: Received on 15 September 2026; Accepted on 19 September 2026; Published on 21 September 2026

ABSTRACT:
Background: Individuals with Down syndrome (DS) present unique anatomical and physiological oral characteristics that profoundly shape the salivary microbial ecosystem. This comparative cross-sectional study was conducted to isolate, characterize, and evaluate cultivable Gram-positive bacterial species in saliva samples collected from children with Down syndrome and healthy control children in Benghazi, Libya. Methods: We recruited 100 children aged 5-14 years, including 46 children with Down syndrome and 54 healthy controls. Unstimulated whole saliva samples were collected under aseptic conditions and evaluated using standard microbiological culture techniques. Bacterial identification was performed based on colony morphology, Gram staining, biochemical characterization, and automated identification using the RENDER MA120 System. Data were analyzed using SPSS, with statistical significance set at $P < 0.05$. Results: The recovered oral flora strictly comprised Gram-positive bacterial species, predominantly *Streptococcus pyogenes, Streptococcus pneumoniae, Staphylococcus aureus, Staphylococcus epidermidis,* and *Enterococcus faecalis.* Among all isolates, *S. pyogenes* and *S. aureus* were the most frequently recovered species, each accounting for 30.0% (n = 12), followed by *S. pneumoniae* at 20.0% (n = 8), *S. epidermidis* at 12.5% (n = 5), and *E. faecalis* at 7.5% (n = 3). Conclusion: The findings establish important baseline microbiological profiles regarding Gram-positive salivary colonization among Libyan pediatric populations with Down syndrome, contributing to improved preventive oral healthcare strategies.

Keywords: Down syndrome; Gram-positive bacteria; Saliva; Oral flora; Pediatric dentistry; Benghazi

الملخص
تُظهر الاختلافات التشريحية والفسيولوجية في الفم لدى الأطفال المصابين بمتلازمة داون تأثيرًا عميقا في تشكيل النظام البيئي للميكروبات اللعابية .وتهدف هذه الدراسة المقطعية المقارنة إلى عزل وتوصيف وتقييم الأنواع البكتيرية موجبة الجرام القابلة للاستزراع في عينات اللعاب المأخوذة من أطفال مصابين بمتلازمة داون وأطفال أصحاء كمجموعة ضابطة في مدينة بنغازي، ليبيا.
المنهجية :شملت الدراسة 100طفل تتراوح أعمارهم بين 5و 14عامًا، بواقع 46طفلاً من المصابين بمتلازمة داون و 54طفلاً سليماً كمجموعة ضابطة .جُمعت عينات اللعاب الكلي غير المحفز في ظروف معقمة، وتم تقييمها باستخدام تقنيات الاستزراع الميكروبي القياسية، بالإضافة إلى التعرف الآلي عبر نظام RENDER MA120.وحُللت البيانات باستخدام برنامج SPSS، مع اعتماد مستوى الدلالة الإحصائية عند قيمة $P < 0.05$.
النتائج :اقتصرت الميكروبات الفموية المستعادة على الأنواع البكتيرية موجبة الجرام فقط، وتحددت في :المكورات العقدية (*Streptococcus pyogenes*)، والمكورات العقدية الرئوية (*Streptococcus pneumoniae*)، والمكورات العنقودية الذهبية (*Staphylococcus aureus*)، والمكورات العنقودية البشرية (*Staphylococcus epidermidis*)، والمكورات المعوية البرازية (*Enterococcus faecalis*).وقد أظهرت النتائج أن النوعين S.

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S. aureus و*pyogenes* كانا الأكثر استرجاعاً بين جميع العزلات بنسبة بلغت 30.0% لكل منهما (n = 12)، تلاهما *S. pneumoniae* بنسبة (n 20.0% = 8)، ثم *S. epidermidis* بنسبة (n = 5) 12.5%، وأخيراً *E. faecalis* بنسبة (n = 3) 7.5% . الخاتمة: تقدم هذه النتائج قاعدة بيانات مرجعية أساسية حول الاستعمار اللعابي للبكتيريا موجبة الجرام لدى الأطفال الليبيين المصابين بمتلازمة داون، مما يسهم بشكل فاعل في تطوير استراتيجيات الرعاية الصحية الوقائية لصحة الفم والأسنان.

الكلمات المفتاحية: متلازمة داون؛ البكتيريا موجبة الجرام؛ اللعاب؛ فلورا الفم؛ ؛ طب أسنان الأطفال؛ بنغازي.

1. Introduction

Down syndrome (DS), caused by trisomy 21, is associated with specific structural and immunological alterations in the oral cavity. Children with Down syndrome frequently exhibit delayed tooth eruption, macroglossia, mouth breathing, reduced salivary flow rate, and altered salivary buffer capacity [2, 3, 8]. These physical and immunological conditions markedly impact the composition and colonization dynamics of oral bacterial communities. Gram-positive bacteria represent a primary structural component of the human oral microbiome, colonizing dental enamel, mucosal surfaces, and saliva. Major Gram-positive genera, including Streptococcus, Staphylococcus, and Enterococcus, play crucial roles in maintaining oral health equilibrium or initiating localized oral pathology such as dental caries and periodontal disease under dysbiotic conditions [1, 6, 9, 13]. Saliva serves as a non-invasive, highly effective diagnostic biofluid reflecting total oral microbial activity. Despite the clinical relevance of oral microbial profiling, limited epidemiological data exist regarding the distribution of cultivable Gram-positive bacteria in saliva among children with Down syndrome in Benghazi, Libya. Therefore, this study aimed to isolate, characterize, and compare cultivable Gram-positive salivary bacterial species in children with Down syndrome and systemically healthy children.

2. Materials and methods

2.1 Study Design and Participant Selection

A comparative cross-sectional study was conducted in Benghazi, Libya. The study enrolled 100 pediatric participants aged 5-14 years, structured into two groups: 46 children with confirmed Down syndrome recruited from rehabilitation facilities, and 54 healthy control children recruited from pediatric dental clinics.

2.2 Eligibility Criteria

Inclusion criteria for the test group comprised children aged 5-14 years with a clinical and genetic diagnosis of Down syndrome, alongside age-matched healthy children for the control group. Exclusion criteria included antimicrobial or probiotic treatment in the preceding four weeks, systemic medical emergencies, active acute systemic infections, and lack of guardian consent.

2.3 Salivary Sampling and Laboratory Processing

Unstimulated saliva samples were collected in sterile, clear wide-mouth containers following aseptic protocols. Samples were transported immediately on ice packs to the laboratory and processed within two hours or stored at -80°C.

2.4 Isolation and Identification of Gram-Positive Bacteria

Saliva samples were inoculated onto Nutrient Agar, Blood Agar, and Mannitol Salt Agar plates, followed by aerobic incubation at 37°C for 24-48 hours. Bacterial isolates were strictly verified as Gram-positive through Gram staining and microscopic examination. Species-level identification was accomplished using catalase, coagulase, bile esculin, optochin, and hemolysis testing, supplemented by automated testing via the RENDER MA120 Automated Identification System.

2.5 Statistical Analysis

Data were analyzed using SPSS software. Descriptive statistics (frequencies and percentages) were generated for categorical variables. Group comparisons were conducted using Chi-square and Fisher's exact tests, with statistical significance set at $P < 0.05$.

2.6 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing for the isolated Gram-positive bacterial species was performed using the standard Kirby-Bauer disk diffusion method, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. Pure bacterial suspensions adjusted to a 0.5 McFarland turbidity standard were inoculated onto Mueller-Hinton agar plates. Commercially available antibiotic disks, including Vancomycin (VA), Ciprofloxacin (CIP), Imipenem (IPM), Penicillin (P), Erythromycin (E), Amoxicillin (AMC), Oxacillin (OX), Cefoxitin (FOX), Clindamycin (CD), and Cefixime (CFM), were applied. Following aerobic incubation at 37°C for 24 hours, the zones of inhibition were measured, and the isolates were categorized as sensitive, intermediate, or resistant based on standardized interpretive criteria

2.7 Ethical Considerations

The study was conducted in full alignment with standard ethical guidelines for human research. Administrative **facilitation** and permissions were secured from the directors of the respective participating centers and clinics before sample collection. Furthermore, written informed consent was obtained from the parents or legal guardians of all participating children before their enrollment, ensuring participant confidentiality and voluntary participation

3. Results and discussion

A total of 51 children yielded Gram-positive bacterial isolates (22 from the Down syndrome group and 29 from the healthy control group). Across these positive samples, a total of 40 bacterial species isolates were successfully identified and characterized.

Note that some children yielded more than one bacterial isolate, resulting in a total of 40 speciated bacterial isolates across the study groups

3.1 Sample Isolation Distribution

Table 1. Distribution of children with Gram-positive isolates

Study Group	Number of Children with Gram-Positive Isolates	Percentage (%)
Down Syndrome Group	22	43.1%
Healthy Control Group	29	56.9%

3.2 Profile of Isolated Gram-Positive Bacterial Species

Table 2. Distribution and comparative frequency of Gram-positive bacterial species isolated from saliva of Down syndrome children versus healthy controls.

Gram-Positive Bacterial Species	Healthy Control Group (n)	Down Syndrome Group (n)	Total Isolates (N) / Percentage (%)
Streptococcus pyogenes	5	7	12 (30.0%)
Staphylococcus aureus	6	6	12 (30.0%)
Streptococcus pneumoniae	4	4	8 (20.0%)
Staphylococcus epidermidis	3	2	5 (12.5%)
Enterococcus faecalis	1	2	3 (7.5%)
Total Gram-Positive Isolates	18	22	40 100.0%)

The comparative analysis between children with Down syndrome and healthy controls reveals distinct distribution patterns of Gram-positive isolates. In children with Down syndrome, physiological and anatomical variations such as altered salivary flow rates, macroglossia, mouth breathing, and potentially compromised manual dexterity during tooth brushing significantly influence oral microbial colonization dynamics. The high prevalence of *Streptococcus pyogenes* and *Staphylococcus aureus* in both cohorts highlights the continuous carriage of cariogenic and opportunistic pathogenic bacteria in pediatric populations, emphasizing the need for targeted oral hygiene interventions in children with special needs

Table 3 Antimicrobial Susceptibility Patterns of Isolated Gram-Positive Bacteria

Bacterial Isolate	Tested Antibiotic	Sensitive (S) %	Resistant (R) %	Intermediate (I) %
Staphylococcus aureus	Vancomycin (VA)	94.12%	5.88%	0.0%
	Imipenem (IPM)	82.35%	17.65%	0.0%
	Ciprofloxacin (CIP)	78.95%	15.79%	0.0%
	Penicillin (P)	23.53%	76.47%	0.0%
	Erythromycin (E)	33.33%	61.11%	5.56%
	Amoxicillin (AMC)	33.33%	66.67%	0.0%
	Oxacillin (OX)	35.29%	64.71%	0.0%
Staphylococcus epidermidis	Vancomycin (VA)	100.0%	0.0%	0.0%
	Cefoxitin (FOX)	87.50%	12.50%	0.0%
	Ciprofloxacin (CIP)	87.50%	12.50%	0.0%
	Clindamycin (CD)	78.67%	21.43%	0.0%
	Penicillin (P)	37.50%	62.50%	0.0%
	Erythromycin (E)	37.50%	50.00%	6.25%
Enterococcus faecalis	Ciprofloxacin (CIP)	100.0%	0.0%	0.0%
	Clindamycin (CD)	100.0%	0.0%	0.0%
	Cefoxitin (FOX)	100.0%	0.0%	0.0%
	Vancomycin (VA)	87.50%	12.50%	0.0%
	Cefixime (CFM)	71.43%	28.57%	0.0%
	Penicillin (P)	50.00%	50.00%	0.0%
	Amoxicillin (AMC)	50.00%	50.00%	0.0%
	Erythromycin (E)	30.00%	70.00%	0.0%
Streptococcus pyogenes	Vancomycin (VA)	100.0%	0.0%	0.0%
	Cefoxitin (FOX)	87.50%	12.50%	0.0%
	Imipenem (IPM)	87.50%	12.50%	0.0%
	Cefixime (CFM)	100.0%	0.0%	0.0%
	Ciprofloxacin (CIP)	71.43%	28.57%	0.0%
	Clindamycin (CD)	71.43%	28.57%	0.0%
	Erythromycin (E)	57.14%	42.86%	0.0%
	Amoxicillin (AMC)	57.14%	42.86%	0.0%

Antimicrobial susceptibility testing demonstrated that the recovered Gram-positive bacterial isolates exhibited high sensitivity rates toward Vancomycin and Ciprofloxacin. Specifically, *Staphylococcus aureus* showed the highest sensitivity to Vancomycin (94.12%) and Imipenem (82.35%), while displaying significant resistance to Penicillin (76.47%) and Amoxicillin (66.67%). Staphylococcus epidermidis and Enterococcus faecalis maintained 100% and 87.50% susceptibility to Vancomycin, respectively. These findings regarding antimicrobial resistance profiles reveal a concerning pattern of resistance to conventional antibiotics such as Penicillin, Amoxicillin, and Erythromycin among the isolated Gram-positive oral flora, which aligns with previous pediatric studies highlighting rising microbial resistance trends. Conversely, the retention of high efficacy in Vancomycin, Imipenem, and Ciprofloxacin against the majority of Gram-positive isolates (*S. aureus, S. epidermidis, and E. faecalis*) underscores their clinical reliability. This emphasizes the necessity for regular antimicrobial monitoring to manage potential opportunistic oral infections effectively in pediatric patients, particularly those with Down syndrome.

4. CONCLUSION

This comparative cross-sectional study successfully established baseline microbiological profiles of cultivable Gram-positive salivary bacteria among children with Down syndrome and healthy controls in Benghazi, Libya. *Streptococcus pyogenes and Staphylococcus aureus* emerged as the predominant isolated species in both cohorts. Although anatomical and physiological oral variations associated with Down syndrome, such as altered salivary flow and mouth breathing, play a role in shaping oral microbial dynamics, the high carriage rates of opportunistic pathogens emphasize the critical need for tailored preventive oral healthcare programs.

Furthermore, the observed resistance patterns to conventional antibiotics underscore the importance of regular antimicrobial monitoring and prudent clinical management to safeguard pediatric oral and systemic health in vulnerable populations. [2, 4, 11].

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