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Comparative Analysis of Culture-Based Salivary Microbiota in Children with Down Syndrome and Healthy Children in Benghazi, Libya

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ABSTRACT

Background: Children with Down syndrome (DS) are at increased risk of oral diseases due to altered immune function, anatomical characteristics, and changes in the oral microbial ecosystem and cultivable salivary microbiota. However, data on the salivary microbiota of Libyan children with Down syndrome remain scarce. **Objective:** This study aimed to compare the cultivable salivary microbiota of children with Down syndrome and healthy controls in Benghazi, Libya, and to assess the antimicrobial susceptibility patterns of the isolated microorganisms. **Methods:** A comparative cross-sectional study was conducted among 100 children aged 5-14 years, including 46 children with Down syndrome and 54 healthy controls. Unstimulated saliva samples were collected under aseptic conditions and cultured using standard microbiological techniques. Bacterial and yeast isolates were identified by colony morphology, Gram staining, biochemical tests, and an automated identification system when required. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method according to CLSI guidelines. Data were analyzed using SPSS, with statistical significance set at $P < 0.05$. **Results:** A total of 110 microbial isolates were recovered from the 100 saliva samples. Gram-positive bacteria constituted 53.6% of the isolates, followed by Gram-negative bacteria (24.6%) and yeasts (21.8%). Staphylococcus aureus and Staphylococcus epidermidis were the most frequently isolated Gram-positive bacteria, while Klebsiella spp. was the predominant Gram-negative isolate. Candida spp. was isolated more frequently from children with Down syndrome. Antimicrobial susceptibility testing showed high susceptibility to vancomycin among Gram-positive isolates and to imipenem among Gram-negative isolates, whereas resistance to amoxicillin, penicillin, erythromycin, and cefotaxime was commonly observed. **Conclusion:** Children with Down syndrome exhibited a distinct cultivable salivary microbiota characterized by increased colonization with opportunistic microorganisms. These findings provide baseline data on the cultivable salivary microbiota of Libyan children with Down syndrome and may contribute to improving preventive oral healthcare strategies and guiding future microbiological research.

1. INTRODUCTION

Down syndrome (DS) is the most common chromosomal disorder in humans and is caused by the presence of an extra copy of chromosome 21 (trisomy 21). It is associated with a wide range of physical, cognitive, immunological, and developmental abnormalities that affect multiple organ systems. The global prevalence of Down syndrome is estimated to be approximately 1 in every 700 live births, although this rate varies according to maternal age, geographic region, and prenatal screening programs. Children with Down syndrome often experience delayed physical growth, intellectual disability, congenital heart defects, endocrine disorders, and immune dysfunction, all of which may contribute to an increased susceptibility to infectious diseases. Oral health is one of the major health concerns among individuals with Down syndrome. Numerous studies have demonstrated that these individuals have a higher prevalence of periodontal disease, oral candidiasis, delayed tooth eruption, malocclusion, mouth breathing, xerostomia, and poor oral hygiene compared with healthy children. These conditions are believed to result from a combination of impaired immune responses, anatomical abnormalities, altered salivary composition, and difficulties in maintaining adequate oral hygiene.

The oral cavity harbors one of the most diverse microbial ecosystems in the human body, containing hundreds of bacterial and fungal species that normally exist in a balanced relationship with the host. This microbial community, collectively known as the oral microbiota, plays a crucial role in maintaining oral health through colonization resistance, immune modulation, and maintenance of ecological stability. However, disruption of this balance, a condition known as microbial dysbiosis, may facilitate colonization by opportunistic microorganisms and contribute to the development of oral and systemic diseases. Saliva represents an ideal biological specimen for investigating the oral microbiota because it reflects microorganisms originating from the teeth, tongue, gingiva, buccal mucosa, palate, and other oral surfaces. Saliva collection is simple, non-invasive, painless, and particularly suitable for pediatric populations and individuals with special healthcare needs. Consequently, salivary microbiological analysis has become an increasingly valuable approach for evaluating oral microbial composition and identifying potential biomarkers associated with disease. Several investigations have reported significant alterations in the salivary microbiota of individuals with Down syndrome. Increased colonization by opportunistic microorganisms, including Candida species, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Enterococcus faecalis, and other Gram-negative bacteria, has been described in comparison with healthy individuals. These microbial changes are believed to be associated with immune dysregulation, altered salivary flow, nutritional habits, oral hygiene practices, and frequent exposure to healthcare settings. Such alterations may increase the risk of dental caries, periodontal disease, oral mucosal infections, and, in some cases, systemic infections. Despite the growing international interest in oral microbiome research, information regarding the salivary microbiota of children with Down syndrome in Libya remains extremely limited. Most published studies have originated from Europe, North America, and East Asia, whereas evidence from North African populations is scarce. Differences in dietary habits, environmental conditions, healthcare accessibility, socioeconomic status, and cultural practices may substantially influence cultivable oral microbial composition, making local epidemiological investigations essential. Therefore, this study was designed to evaluate the cultivable salivary microbiota in Libyan children with Down syndrome and compare it with that of age-matched healthy controls. The study also aimed to identify the predominant bacterial and fungal isolates and evaluate their antimicrobial susceptibility profiles using conventional microbiological techniques supported by an automated identification system. The findings of this study are expected to improve understanding of oral microbial ecology in children with Down syndrome and provide evidence that may contribute to the development of preventive oral healthcare strategies and early microbiological surveillance programs in Libya.

2. METHOD

2.1 Study Design and Setting

A comparative cross-sectional study was conducted to investigate the cultivable salivary microbiota in children with Down syndrome (DS) and compare it with that of age- and sex-matched healthy children in Benghazi, Libya. A total of 100 children aged between 5 and 14 years were enrolled, including 46 children with Down syndrome and 54 healthy controls. Children with Down syndrome were recruited from rehabilitation centers, whereas healthy participants were recruited from dental clinics. Participants in both groups were further classified according to dentition stage into primary dentition and mixed dentition groups.

2.2 Eligibility Criteria

Children aged 5-14 years with a confirmed diagnosis of Down syndrome or healthy age-matched controls were eligible for inclusion. Children with recent antibiotic or probiotic use, active oral infections, severe dental or periodontal disease, systemic disorders other than Down syndrome, recent dental treatment, or conditions affecting saliva composition were excluded from the study to minimize potential confounding factors.

2.3 Saliva Sample Collection

Unstimulated whole saliva samples were collected under standardized conditions using sterile containers. Immediately after collection, samples were transported on ice to the microbiology laboratory within two hours and stored at -80°C until microbiological processing.

2.4 Microbiological Procedures

Saliva samples were initially enriched in nutrient broth and incubated overnight at 37°C. Subsequently, aliquots were cultured on Blood Agar and MacConkey Agar plates. Colony morphology, Gram staining, and conventional biochemical tests were used for preliminary identification of bacterial isolates. Biochemical identification included catalase, coagulase, oxidase, urease, citrate utilization, Triple Sugar Iron (TSI), indole, Mannitol Salt Agar, bacitracin, optochin, and novobiocin susceptibility tests. Isolates requiring further confirmation were identified using the RENDER MA120 Automated Identification and Antimicrobial Susceptibility Testing System according to the manufacturer's instructions.

2.5 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the automated RENDER MA120 ID/AST system. Bacterial suspensions were prepared according to the manufacturer's recommendations, and susceptibility profiles were interpreted automatically following incubation.

2.6 Statistical Analysis

Data was entered into the Statistical Package for the Social Sciences (SPSS) for analysis. Descriptive statistics were used to summarize demographic and microbiological characteristics. Appropriate comparative statistical tests were performed to compare Down syndrome and control groups. Statistical significance was considered at a P-value of less than 0.05.

3. Ethical Approval

Ethical approval was obtained from the directors of the centers where samples were collected before commencement of the study, and all participants who met the participation criteria provided written informed consent permitting the use of their data for research purposes. The source thesis did not state the formal name of the approving research ethics committee or the approval number; both details must be inserted from the original approval document before journal submission**.**

4. RESULT

4.1 Demographic Characteristics

A total of 100 children were enrolled, including 46 children with Down syndrome and 54 healthy controls. No statistically significant differences were observed between the two groups regarding age or sex distribution ($P > 0.05$), indicating that the groups were comparable for subsequent microbiological analyses. Participants were further classified according to dentition stage into primary and mixed dentition groups.

Table 1: Age and distribution of study participants

Age group	Down syndrome (N = 46)	Non-down syndrome (N = 54)
5-6 Y	4 (8.7%)	4 (7.4%)
7-8 Y	12 (26.1%)	16 (29.6%)
9-10 Y	16 (34.8%)	8 (14.8%)
11-12 Y	10 (21.7%)	13 (24.1%)
13-14 Y	4 (8.7%)	13 (24.1%)

4.2 Distribution of Microbial Isolates

Culture-based microbiological analysis recovered 110 microbial isolates from saliva samples collected from both study groups. Gram-positive bacteria represented the predominant isolates (53.6%), followed by Gram-negative bacteria (24.6%) and yeasts (21.8%).

Although microorganisms were isolated from both groups, opportunistic organisms, particularly Candida spp. and several Gram-negative bacteria, were more frequently recovered from children with Down syndrome (Table 2).

Table 2: Overall distribution of microbial isolates

Isolated microbial species	Count	Percentage
E. faecalis	10	9.09%
S. aureus	18	16.36%
S. epidermidis	18	16.36%
S. pneumoniae	1	0.91%
S. pyogenes	7	6.37%
S. viridans	5	4.54%
E. coli	5	4.54%
E. hormaechei	1	0.91%
K. pneumoniae	4	3.64%
Klebsiella spp.	14	12.73%
Pseudomonas spp.	3	2.73%
Candida spp.	24	21.82%
Total	110	100%

4.3 Microbial Distribution According to Study Group and Gender

Comparative analysis demonstrated differences in the salivary microbial profiles between children with Down syndrome and healthy controls. Opportunistic microorganisms, particularly Candida species and several Gram-negative bacteria, were recovered more frequently from children with Down syndrome. In contrast, healthy controls exhibited a microbial profile predominantly composed of commensal oral microorganisms. The distribution of microbial isolates according to study group and sex is summarized in Table 3.

Table 3: Microbial distribution according to study group and gender

Isolates	DS Male	DS Female	DS Total	Control Male	Control Female	Control Total
E. faecalis	3 (5.45%)	1 (1.82%)	4 (7.3%)	6 (10.9%)	0	6 (10.9%)
S. aureus	3 (5.45%)	5 (9.09%)	8 (14.5%)	6 (10.9%)	4 (7.3%)	10 (18.28%)
S. epidermidis	6 (10.9%)	3 (5.45%)	9 (16.3%)	6 (10.9%)	3 (5.45%)	9 (16.3%)
S. pneumoniae	1 (1.82%)	0	1 (1.82%)	0	0	0
S. pyogenes	3 (5.45%)	1 (1.82%)	4 (7.3%)	1 (1.82%)	2 (3.6%)	3 (5.45%)
S. viridans	2 (3.6%)	1 (1.82%)	3 (5.45%)	2 (3.6%)	0	2 (3.6%)
E. coli	1 (1.82%)	1 (1.82%)	2 (3.6%)	2 (3.6%)	1 (1.82%)	3 (5.45%)
E. hormaechei	1 (1.82%)	0	1 (1.82%)	0	0	0
K. pneumoniae	0	1 (1.82%)	1 (1.82%)	2 (3.6%)	1 (1.82%)	3 (5.45%)
Klebsiella spp.	3 (5.5%)	2 (3.6%)	5 (9.09%)	4 (7.3%)	5 (9.09%)	9 (16.3%)
Pseudomonas spp.	2 (3.6%)	1 (1.82%)	3 (5.45%)	0	0	0
C. albicans	1 (1.82%)	0	1 (1.82%)	0	0	0
C. guilliermondii	0	1 (1.82%)	1 (1.82%)	0	0	0
Candida spp.	8 (14.5%)	4 (7.3%)	12 (21.8%)	7 (12.7%)	3 (5.45%)	10 (18.28%)
Total	34 (61.82%)	21 (38.18%)	55 (50%)	36 (65.45%)	19 (34.55%)	55 (50%)

5. DISCUSSION

The present study provides important insights into the cultivable salivary microbiota of children with Down syndrome (DS) compared with healthy controls in Benghazi, Libya. The findings demonstrated distinct differences in microbial composition between the two groups, with children with DS showing increased colonization by opportunistic bacterial and fungal microorganisms. These results support the concept that Down syndrome is associated with alterations in the oral microbial ecosystem and increased susceptibility to microbial imbalance (dysbiosis).

The current study represents one of the limited investigations assessing salivary microbiota among children with DS in North Africa and provides baseline microbiological data from the Libyan population.

In the present study, Gram-positive bacteria represented the predominant group of recovered microorganisms in both DS and control children. This finding agrees with the general characteristics of the oral microbiota, where Gram-positive cocci constitute a major component of the normal oral microbial community. However, despite the presence of common oral commensals, children with DS exhibited a higher recovery of microorganisms considered opportunistic or potentially pathogenic, suggesting a shift from microbial equilibrium toward dysbiosis.

The increased detection of Candida species among children with DS was one of the important findings of this study. Candida spp. represented a considerable proportion of the recovered isolates and were more frequently observed in children with DS compared with healthy controls. Previous studies have reported increased Candida colonization in individuals with DS, particularly Candida albicans and other Candida species. This increased susceptibility may be explained by several factors associated with DS, including immune dysregulation, impaired neutrophil function, altered salivary antimicrobial activity, and changes in the oral environment. In addition, difficulties in maintaining optimal oral hygiene due to developmental and motor limitations may further promote fungal colonization.

The presence of higher levels of Gram-negative bacteria among children with DS also indicates a possible alteration in the oral microbial environment. Although some Gram-negative organisms may exist transiently in the oral cavity of healthy individuals, their increased frequency in individuals with DS may reflect reduced ecological stability of the oral microbiota. Previous investigations have suggested that immune alterations, frequent exposure to healthcare environments, dietary factors, and differences in oral hygiene practices may contribute to increased colonization by opportunistic microorganisms in this population.

The findings of the current study are consistent with previous microbiome investigations demonstrating altered microbial profiles among individuals with DS. Molecular studies have reported differences in bacterial diversity and increased abundance of microorganisms associated with periodontal disease in DS populations. Although the current study was based on culture-dependent methods rather than sequencing approaches, the identification of clinically relevant cultivable microorganisms provides valuable information regarding organisms that may have direct implications for oral infection risk and antimicrobial management.

Antimicrobial susceptibility analysis demonstrated variations among bacterial isolates recovered from saliva samples. Although many isolates remained susceptible to commonly used antimicrobial agents, differences in susceptibility patterns were observed among certain organisms. These findings highlight the importance of continuous monitoring of antimicrobial resistance among oral microorganisms, particularly in populations with increased vulnerability to infections and frequent healthcare exposure. Appropriate antimicrobial stewardship is essential to prevent the development and spread of resistant oral pathogens.

Several mechanisms may explain the observed microbial differences between children with DS and healthy controls. The presence of an additional chromosome 21 in DS is associated with complex genetic and immunological changes that can influence host–microbial interactions. Altered immune responses, including changes in innate and adaptive immunity, may reduce the ability of the host to maintain microbial balance. Furthermore, anatomical characteristics, reduced salivary flow, oral motor difficulties, and increased prevalence of periodontal problems may collectively contribute to changes in microbial colonization patterns.

An important contribution of this study is its focus on Libyan children with DS, as most previous studies have been conducted in high-income countries. Geographic location, dietary habits, environmental exposures, healthcare accessibility, and cultural practices may influence oral microbial composition. Therefore, establishing local microbiological data is essential for developing appropriate preventive strategies and improving oral healthcare programs for children with special healthcare needs.

6. CONCLUSION

Children with Down syndrome in Benghazi exhibited a distinct cultivable salivary microbiota characterized by increased colonization with opportunistic microorganisms. These findings provide baseline data on the cultivable salivary microbiota of Libyan children with Down syndrome and may contribute to improving preventive oral healthcare strategies and guiding future microbiological research.

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