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Research Article

Probiotics in Preoperative Primary Cleft Treatment

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Abstract

The limited availability of tissue for reconstruction in cleft surgery necessitates stringent control of surgical site infections, as any infection can result in detrimental outcomes for the surgical site and fragile tissue. The type, number and antimicrobial resistance of pathogens present preoperatively have increased exponentially in infants with a cleft, adversely affecting treatment outcomes. This study aimed to determine whether the microbial composition and diversity could be altered during the post-obturpaedic period through the application of probiotics.

A prospective study was conducted, including 75 consecutive patients scheduled for primary repair of the cleft in the soft palate. The average age of the patients was 8 months and 15 days, with a gender distribution of 36 males and 39 females. This research was conducted in a private practice specialising in cleft surgery. All procedures were performed by a single surgeon. Written consent from patients or patient's guardian and health clearance from a paediatric physician were prerequisites for inclusion in the study.

Probiotics were administered for 14 days prior to primary palatal surgery. Swabs were collected from the surgical site in all 75 patients before the application of the disinfectant and were analysed for microscopy, culture and sensitivity. The results were compared with three previous studies from the same facility. Pathogenic microorganisms were cultured in 63/75 patients (84%), representing a 13% improvement compared to the study published in 2022 by the same institution. Additionally, the diversity of species decreased from 42 to 23. The most prevelant pathogen, *Klebsiella pneumoniae*, was identified in 30.6% of cases, reflecting a 15% reduction compared to the 2022 study.

The reduction in both the number and diversity of pathogenic microorganisms, as well as in their resistance to antimicrobial preoperative application, is a positive outcome. These findings suggest that probiotics contribute to the prevention of postoperative infections and support improved surgical outcomes.

Keywords: Clefts; Cleft Soft Palate; Probiotics; Infection; Microbial Contamination; Post-Obturpaedics

Abbreviations

AMR: Antimicrobial Resistance; GIT: Gastrointestinal Tract; RCT: Randomized Clinical Trials; C: Cleft; P: Palate; CLP: Cleft-Lip-Palate; sP: soft Palate; CsP: Cleft soft Palate

Introduction

Postoperative infections at surgical sites remain a critical concern for treating professionals, particularly in the context of the different microorganisms with Antimicrobial Resistance (AMR), which is on the rise globally [1]. The report 'Cleft lip and palate repair technique' emphasises that infection is a predominant factor contributing to failure of a surgical procedure [2]. This is especially evident in cases of Cleft soft Palate (CsP) breakdown after surgical reconstruction, where such complications can severely impair a patient's speech development and feeding ability. Aditionally, the loss of soft tissue due to postoperative infection excaserbates morbidity, as the tissue available for subsequent repairs is often limited. These complications, which may result in complete or partial breakdown of the surgical site, have been researched extensively and published - with particular attention given to the formation of oronasal fistulas as a common outcome [3-10].

The prevention of postoperative complications is essential for ensuring favourable outcomes in speech and feeding development, as well as mitigating perturbation of paediatric growth during the orthopaeddontic period [11]. Repeated surgeries pose significant challenges to patients, surgeons, speech pathologists and orthopaeddontist particularly due to the compromised nature of the palatal shelves and palatal-pharyngeal region. Concerns regarding postsurgical infections have intensified as studies have confirmed a steady increase over time in the resistance of pathogenic microorganisms to antimicrobial drugs [12-19]. This resistance represents an escalating global public health threat, affecting all major microbial pathogens and antimicrobial treatments [20].

Optimal surgical outcomes in terms of oro-palatal, oro-pharyngeal function and speech development are achieved through early surgical intervention, typically between 5 or 7 months of age, immediately after the obturpaedic treatment [21,22]. Preventing postoperative infection in this vulnerable patient group is paramount. Current preoperative protocols, including the use of chemical disinfectant solutions such as chlorhexidine, are condsidered standard practice. However, evidence indicated that chlorhexidine is insufficient in eradicating antimicrobial-resistant pathogens [23]. Of greater concern, rescent studies have highlighted the emergence of resistance to chlorhexidine itself, a phenomenon that is developing at an alarming rate [24-29]. Further evidence suggest an increase in microbial contamination and AMR pathogens, as demonstrated by laboratory findings [25,27,29]. In light of these findings, the authors hypothesized that preoperative probiotic application could reduce positively influence oro-pharyngeal microbial diversity, with potentially less severe infections in CsP patients [30].

The significance of the microbiome begins in utero, as first-pass meconium samples known to harbour microbiomes [31]. Colonization of microflora commences within the first hours of birth and influence various factors, including gestational age, the method of delivery, exposure to the mother, exposure of nearby persons, type of infant-feeding, the infant's environment and possible antibiotic use by the infant [32-35]. Different studies have indicated that the composition of the microflora changes considerably within the first few months after birth [34,36-38]. The difference in composition of intestinal flora in the first days/months of life changes to an adult-like microflora by the age of one year [34]. Notably, a recent study indicated that the composition of microorganisms in the oral and in the nasal cavities of healthy children cleft palate, in the age group 1- to 2-years-old [39]. Potentially explaining the higher incidence of infections in the latter group. The International Scientific Association for Probiotics and Prebiotics (ISAPP) issued a definition of 'probiotics' in 2014, stating that they are 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [40]. Extensive research, including well-designed clinical trials, has been conducted on the health benefits of probiotics, examining how they act, their nutritional mechanism and their clinical effects [41-46]. The beneficial effect of probiotics are primarily associated with the Gastrointestinal Tract (GIT), working against acute gastroenteritis, antibiotic-associated diarrhoea, *Helicobacter pylori* infection, ulcerative colitis, Crohn's disease and functional constipation [47-50]. Additionally, probiotics contributes to a healthy immune system, with one of their mechanisms involving the inhibition of potential pathogenic microbes [40].

A meta-analysis comprising of 84 trails and 74 studies, encompassing a total of 10351 patients, concluded that probiotics are advantageous in the management and prevention of gastrointestinal diseases [51]. Similarly, a review of 12 Randomized Clinical Trials (RCTs) found that probiotics reduced the number of episodes of acute upper respiratory tract infection [52]. A systematic review of 23 trials spanning over 6269 children echo these findings [53]. Furthermore, a meta-analysis of 20 RCTs demonstrated that the use of probiotics reduced the mean acute respiratory tract infection [41].

A prospective, double-blind, randomized, placebo-controlled trial of 2015 indicated that postoperative antibiotic prophylaxis significantly reduces the incidence of surgical-site breakdowns [54]. However, concern about postoperative infections intesifying, as the resistance of both known and emerging pathogenic microorganisms to antimicrobial drugs continues to rise exponentially [30,55-61].

An evaluation of the CsP region revealed a relationship between peri-operative pathogenic organisms and postoperative infections/complications. These complications typically emerge within the week following the initial primary surgical procedure of the CsP and were first documented in 2009 [62]. A wide variety of pathogenic organisms was cultured from swabs taken peri-operatively from the patient's oro-pharyngeal cavity. It was found that some organisms showed resistance to certain antibiotics included in the standard treatment protocol, potentially contributing to the progression of postoperative complications. In the search for alternatives in the fight against multi-drug-resistant bacteria, the following hypothesis was formulated: 'Would preoperative probiotic application reduce pathogenic microbial contamination in the non-operated CsP patients?' This study aimed to compare the results with previous publications from 2009, 2017 and 2022 from the same institution, which documented the presence of pathogenic organisms and their preoperative appearances in cleft palate patients [29,61,62].

Methods

This prospective study was approved by the Faculty of Health Science Research Ethics Committee (467/2015) of the University of Pretoria. Patients (N = 75) who presented for primary repair of the Cleft soft Palate (CsP) were included in the study, conducted between November 2019 and December 2023. The inclusion criteria required (1) written informed consent from the parents or their legal guardian of the patient and (2) paediatric clearance confirming the patient was systemically healthy. The exclusion criterion was any systemic and/or any oro-pharyngeal infections. All surgical procedure(s) were performed by the same surgeon in the same operation theatre to ensure consistency.

Starting two weeks preoperative, a standard probiotic formulation containing *Bifidobacterium lacis*, *Lactobacillus rhamnosus* and *Lactobacillus salivarius* was administered orally. Each patient received four drops of probiotics every morning for 14 consecutive days leading up to the primary CsP surgical procedure. The patients were admitted to the surgical facility on the morning of their surgical intervention.

The Copan Transystem Bacteriology Swab Collection system with *Amies Agar* Gel for aerobic and anaerobic cultures was used to collect and transport the specimens. Preoperative swabs were taken from the CsP and adjacent nasopharynx immediately after the induction of general anaesthesia. The swabbing procedure involved removing the swab from its sterile packaging, gently rubbing it over the mucosa of the indicated area, reinserting the swab in the transport tube and sealing it. Each swab was labelled with the date, time of collection and the patient's information and transported to the pathology laboratory within one hour for further analysis. The purpose of this analysis was to culture the samples in order to identify the type of microorganisms present, assess colony size and determine their sensitivity to microorganisms.

Specimens were inoculated onto a non-selective blood agar plate as well as onto a selective and differential MacConkey agar plate. These plates were incubated overnight at 35˚C. The following morning, colonies from these incubated plates were transferred to a target slide and processed using the VITEK MS automated mass spectrometry microbial identification system to identify the pathogens. The organisms were isolated using standard microbiological methods. Kirby-Bauer antibiotic testing was utilized to determine antimicrobial sensitivity or resistance. This analysis aided in identifying the effective antimicrobial agent for potential treatment if infections developed. Labaratory data received were recorded for each patient in a Microsoft Office Excel Database. These records were analysed and compared with previous studies conducted at the same institution [30,62,63].

Results

The mean age of the 75 patients is calculated at 8 months 15 days (standard deviation: 6 months 9 days). However, five patients included in the study were referred to this institution were of older age (between 18 and 40 months) than the standard normal institutional protocol for primary repair of the CsP. Excluding these five patients, the average age changed to 7 months 15 days, with a narrower standard deviation of 2 months 9 days. The sex distribution, 36 male and 39 female patients was quite close. In twelve patients (12/75) no preoperative pathogenic microorganisms were cultured. A single microorganism and two different ones were found in the majority of patients (52/75). In 8/75 patients presented with three different pathogens and in 3/75 patients four different pathogens were cultured. Twenty-three (23) different pathogenic microorganisms were identified (Table 1). The *Klebsiella pneumonia* was the most prevalent one and identified in 23 patients. The second most prevalent was *Streptococcus mitis/oralis* in 19 patients, followed by *Staphylococcus aureus* in 11 patients and the *Enterobacter cloacae* and *Escherichia coli* both isolated in 10 patients. *Candida albicans* presented in 9 patients and *Haemophilus influenza* in 8 patients. A total of 122 pathogens were cultured from the 75 patients (Table 1).

Microorganism **N (%)**	**2024** (75 patients)	**2022** (103 patients)	**2016** (200 patients)	**2009** (100 patients)
Acinetobacter baumannii	1 (1.3%)	3 (2.9%)	4 (2%)	0
Aeromonas hydrophila/caviae/sobria	0	3 (2.9%)	3 (1.5%)	2 (2%)
Candida albicans	9 (12%)	12 (11.7%)	3 (1.5%)	9 (9%)
Candida dublinensis	0	2 (1.9%)	0	0
Candida famata	0	1 (1%)	0	0
Candida glabrata	2 (2.7%)	1 (1%)	0	0
Candida kefyr	0	2 (1.9%)	0	0
Candida krusei	1 (1.3%)	3 (2.9%)	0	0
Candida lusitaniae	0	1 (1%)	0	0
Candida parapsilosis	5 (6.7%)	4 (3.9%)	0	0
Candida tropicalis	1 (1.3%)	3 (2.9%)	0	0
Chryseobacterium gleum	0	1 (1%)	0	0
Citrobacter freundii	1 (1.3%)	1 (1%)	0	0
Citrobacter koseri	0	1 (1%)	0	0
Edwardsiella tarda	0	1 (1%)	0	0
Enterobacter aerogenes	0	1 (1%)	2 (1%)	1 (1%)
Enterobacter cloacae	10 (13.3%)	18 (17.5%)	10 (5%)	5 (5%)
Enterobacter gergoviae	0	0	1 (0.5%)	0
Enterobacter hormaechei	1 (1.3%)	1 (1%)	0	0
Enterobacter kobei	0	1 (1%)	0	0
Enterococcus faecalis	0	0	1 (0.5%)	1 (1%)
Escherichia coli	10 (13.3%)	16 (15.5%)	20 (10%)	9 (9%)
Geobacillus thermoglucosidasius	0	1 (1%)	0	0
Heamophilus haemolyticus	1 (1.3%)	1 (1%)	0	0
Haemophilus influenzae	8 (10.6%)	30 (29.1%)	13 (6.5%)	
Haemophilus parahaemolyticus	0	1 (1%)	0	0
Haemophilus parainfluenzae	0	2 (1.9%)	0	0
Klebsiella oxytoca	7 (9.3%)	8 (7.8%)	3 (1.5%)	1 (1%)

Klebsiella pneumonia	23 (30.6%)	47 (45.6%)	35 (17.5%)	18 (18%)
Kluyvera cryocrescens	0	0	1 (0.5%)	0
Moraxella catarrhalis	3 (4%)	3 (2.9%)	12 (6%)	6 (6%)
Neisseria subflava	0	1 (1%)	0	0
Pluralibacter gergoviae	1 (1.3%)	0	0	0
Proteus mirabilis	0	1 (1%)	0	0
Pseudomonas aeruginosa	0	4 (3.9%)	4 (2%)	0
Pseudomonas putida	0	1 (1%)	0	0
Raoultella ornithinolytica	1 (1.3%)	0	0	0
Saccharomyces cerevisiae	0	1 (1%)	0	0
Serratia marcescens	0	4 (3.9%)	8 (4%)	4 (4%)
Staphylococcus aureus	11 (14.6%)	14 (13.6%)	25 (12.5%)	22 (22%)
Staphylococcus epidermidis	0	1 (1%)	1 (0.5%)	0
Stenotrophomonas maltophilia	0	1 (1%)	0	0
Streptococcus agalactiae	1 (1.3%)	0	0	0
Streptococcus anginosus	0	0	1 (0.5%)	0
Streptococcus mitis/oralis	19 (25.3%)	32 (31.1%)	0	0
Streptococcus parasanguinis	1 (1.3%)	3 (2.9%)	0	0
Streptococcus pneumoniae	4 (5.3%)	6 (5.8%)	17 (8.5%)	7 (7%)
Streptococcus pseudopneumoniae	0	1 (1%)	0	0
Streptococcus pyogenes	0	0	1 (0.5%)	0
Streptococcus salivarius	1 (1.3%)	5 (4.9%)	0	0
Streptococcus viridans	0	0	1 (0.5%)	0
Total number of pathogens	122	244	170	85
Pathogens/patient	1.6	2.36	0.85	0.85

Table 1: Preoperative comparison of number of pathogens identified from Cleft of soft Palate (CsP) and nasopharynx mucosa.

Antimicrobial resistance of the pathogens cultured from the swabs was determined. Resistance to different antimicrobial agents, against which their sensitivity was tested, was shown by 77/122 (63.1%) number of pathogens. Table 2 is depicting a summary of the antimicrobial resistance of the five most prevalent microorganisms that were isolated in ten or more patients.

Klebsiella pneumonia (N = 23):		MIC: S ≤	R > 16
Amikacin	(1)	16	128
Ampicillin	(23)	128	32
Amoxicillin-Clavulanic Acid	(4)	8	32
Cefepime	(3)	1	64
Ceftazidime Cefuroxime	(3)	1	32
Ciprofloxacin Cotrimoxazole	(3)	4	4
Gentamicin Tobramycin	(2)	1	80
	(3)	40	16
	(1)	4	128
	(2)	64	
Streptococcus mitis/oralis (N = 19): Pen-G		MIC: S ≤	R >
Ampicillin	(7)	0.25	2
Amoxicillin-Clavulanic Acid	(7)	0.5	8
Ceftriaxone	(7)	0.5	2
	(1)	1	4
Staphylococcus aureus (N = 11):		MIC: S ≤	R >
Azithromycin Clarithromycin	(1)	1	8
Cotrimoxazole	(1)	0.25	8
	(1)	2	8
Enterobacter cloacae (N = 10): Ampicillin		MIC: S ≤	R > 32
Amoxicillin-Clavulanic Acid	(10)	8	32
Cefuroxime	(10)	8	16
Cotrimoxazole	(4)	4	16
	(1)	2	
Escherichia coli (N = 10): Ampicillin		MIC: S ≤	R > 32
Amoxicillin-Clavulanic Acid	(4)	8	32
Cefuroxime	(3)	8	32
Cefotaxime	(1)	4	4
Tobramycin	(1)	1	16
Ciprofloxacin	(1)	4	4
Cotrimoxazole	(2)	1	80
	(4)	40	
The number of cases showing resistance is given in brackets, indicating the Minimal Inhibitory Concentration (MIC) (μg/ml) where S = sensitive and R = resistant (According to: 'The European Committee on Antimicrobial Susceptibility').			

Table 2: The antimicrobial resistance profile of the most prevalent pathogens.

Discussion

Since 2009, this CLP unit has been continuously investigating the role of microbial infection in complications arising from the repair of facial Cleft Deformities (CLP). The growing prevelanvce of AMR raises and the question is: What alternatives are at our disposal in anti-infectious therapeutics to combat multi-drug-resistant bacteria? [64]. The principal hypothesis of the current study aimed to determine whether the prevention of post-operative infection following CsP repair could be supported by pre-operative administration of probiotics.

The results of this study on the use of probiotics preoperatively indicated that: 1) only in 63/75 (84% of patients) pathogenic microorganisms were cultured; 2) that there is a 13% improvement on the study published in 2022 by the same institution [30]. Preoperative application of probiotics reflects a positive outcome; however, it is 26% more severe than the outcome of the research of 2017; 3) the variety of species decreased from 42 to 23 different species, equating to the 2017 study; 4) there was one patient in the 2022 publication who was carrying five different pathogens and 21 patients with four different pathogens each and this is presently a major positive result in this study as the number of different pathogens per patient was maximum four and isolated only in three unconnected patients [63].

The most prevalent pathogen identified was *Klebsiella pneumonia*, detected in 23/75 (30.6%) cases. This represents a 15% reduction compared to the 2022 findings and is slightly over half the percentage reported by Cocco, et al., who observed a prevalence of 56% [65]. This reduction is significant, as the *Klebsiella pneumonia* is a known cause of community-acquired pneumonia, urinary tract infections and postoperative infections at a surgical site. Moreover, *Klebsiella species* are continuously studied for their development of antimicrobial resistance [66,67].

The second most prevalent pathogen, consistent with the 2022 study from this institution, was *Streptococcus mitis/oralis*. This micro-organism was not identified before 2018 as a pathogen type. The preoperative application of probiotics contributed to a reduction in its prevalence, from 31% (32/103 patients in 2022) to 25.3% (19/75 patients) in this study. This pathogen is significant due to its role in postoperative complications through macrophage and epithelial cell deaths [68].

Haemophilus influenza was the third most abundant pathogen in the 2022 study, with a prevalence of 29.1% (30/103 patients). As an opportunistic pathogen, *Haemophilus influenza* can adversely affect the outcomes of reconstructive surgical procedures [62]. Multiple studies have been conducted about the multi-drug resistance of this pathogen [60,67,69-72]. In the study, the application of probiotics reduced its prevalence to 10.6% (8/75 patients).

However, the application of probiotics had no effect on the following pathogens and presented, with some showing a slight increase, compared to the 2022 study [30]. *Candida albicans* (+0.3%); *Candida glabrata* (+1.7%); *Candida parapsilosis* (+2.8%); Klebsiella *oxytoca* (+1.5%); *Moraxella catarrhalis* (+1.1%); *Staphylococcus aureus* (+1.0%). As Probiotics contains three non-pathogen microorganisms that suppresses pathogenic microorganisms, the slight increase may be attributed to insignificant fungal overgrowth.

The following microorganisms (in alphabetical order) were also identified in single patients: *Acinetobacter baumannii, Candida krusei, Candida tropicalis, Citrobacter freundii, Enterobacter hormaechei, Heamophilus haemolyticus* and *Streptococcus salivarius*. However, in this research three new single pathogens were cultured in three separate patients: *Pluralibacter gergoviae, Raoultella ornithinolytica* and *Streptococcus agalactiae*.

Conclusion

Palatal reconstruction in facial cleft CLP deformity faces a significant challenge due to the limited availability of tissue adjacent to the CsP during reconstruction. The risk of tissue loss caused by infections is a major underlying concern. The results from previous studies conducted within a consistent setting over three separate time frames have raised serious concerns. In 100/103 patients (2009/2022), pathogenic microorganisms were cultured preoperatively, with 22 (21.4%) of these patients harbouring four or more pathogens each. Furthermore, there has been a notable proliferation in both the diversity and the total number of pathogens: 15 species identified in 2009; 23 in 2016; 42 in 2022, with pathogen prevelance decreasing from 85% in 2009 and 2016 to 23.7% in 2022. This research explored an alternative approach involving preoperative application of probiotics for 14 days preoperatively prior to primary CsP surgical intervention.The findings indicated a substandial reduction in both the total number and the diversity of pathogens identified presurgically. Preoperative probiotic use was effective in decreasing peri-operative infections, reducing the diversity of pathogenic microorganisms and mitagating AMR through a more natural method. Additionally, probiotics supports a healthy immune system, providing a dual benefit that creates a ‘win-win situation’ for both infection prevention and immune health.

Ethical Approval

A point prevalence study was designed, amended and approved by the Faculty of Health Science Research Ethics Committee (467/2015) of the University of Pretoria.

Data Availability

Additional data available on request.

Conflict of Interest

The authors have no conflict of interest to declare.

Language Evaluation
AC Wolmarans.

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