Microbial contamination profile change over a 4- year period in nonoperated cleft soft palate

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Abstract

***Aims*:** Surgical site infection is a major concern in cleft soft palate. Knowledge of the type, number and antimicrobial resistance of pathogens present preoperatively contribute to treatment success. The aim of this study is to determine whether or not the microbial contamination (diversity) preoperatively has changed since 2015.

***Methods and Results*:** Swabs were taken from the surgical site in 103 consecutive patients who presented for primary repair of the soft palate cleft. These were sent for microscopy, culture and sensitivity testing. Swabs were taken before disinfecting the site. Results were tabled and compared with two previous studies from the same facility. Out of 103 patients, 100 patients showed positive cultures with 42 different pathogenic micro-organisms identified. Most dominant pathogen was *Klebsiella pneumoniae*, 45.6%, increased by 28% from the previous two studies, with 93.6% of these pathogens resistant to one or more antimicrobials. Most of the other identified pathogens showed an alarming increase in occurrence, with a wide resistance to antimicrobials.

***Conclusions*:** The increase in number and diversity of microbial contamination as well as their resistance to antimicrobials is a real concern. Ways of preventing postoperative infection in a natural way need to be explored.

***Significance*:** Surgeons need to be aware of constant changes in micro-organisms.

KEYWORDS: cleft soft palate, infection, microbial contamination, micro- organisms, resistance

INTRODUCTION

Postoperative infection of a surgical site is always a major concern. As far back as 1937, an article in this regard was published (Kilner, 1937). This is especially true in cleft soft palate repair where the amount of tissue that can be used to restore the defect is already compromised. Any additional loss of soft tissue due to infection will result in significant morbidity and have a detrimental effect on the patient's future. This complication can lead to the breakdown of the surgical site or, in less severe cases, the formation of oro-nasal fistulas (Adesina et al., 2016; Amaratunga, 1988; Deshpande et al., 2014; Murthy, 2014). Repeated surgery in a compromised area to prevent future speech and feeding difficulties and possible growth disturbances poses a significant challenge to both the patient and surgeon.

As the resistance of pathogenic micro-organisms to antimicrobial drugs is growing exponentially so is the concern about postoperative infections. Some opportunistic pathogens like *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Enterobacter cloacae*, *Staphylococcus aureus* and viridans group streptococci (VGS) present with larger resistance to antimicrobial agents (Davin-Regli & Pagès, 2015; Doern & Burnham, 2010; Foster, 2017; Lippmann et al., 2014; Maddi et al., 2017; Mezzatesta et al., 2012; Pang et al., 2018).

Different studies have examined the micro-flora in facial clefts in infants and toddlers with seven of these identifying micro-organisms preoperatively (Arief et al., 2005; Bokhout et al., 1996; Chuo & Timmons, 2005; Cocco et al., 2010; Myburgh & Bütow, 2009; Roode & Bütow, 2018; Roode et al., 2016). A study that aimed to identify possible pathogenic organisms associated with postoperative wound complications cultured 15 different pathogenic organisms preoperatively from 100 babies (Myburgh & Bütow, 2009). Another preoperative evaluation of micro-organisms in cleft soft palate reconstruction by the same institute 7 years later, identified 23 different pathogenic micro-organisms from 115 out of 200 infants and indicated that *K*. *pneumoniae* and *Staph*. *aureus* were the most prevalent pathogenic micro-organisms followed by *Escherichia coli* (Roode et al., 2016). This indicated eight new pathogens associated with the same procedure without changing any clinical settings. A recent review article also indicated the problematic increase of antimicrobial resistance of *E*. *coli* (Poirel et al., 2018). *Streptococcus pneumoniae*, *H*. *influenzae*, *Moraxella catarrhalis*, *Ent*. *cloacae* and *Serratia marcescens* were also indicated as relatively common pathogens in the preoperative evaluation. All these are pathogens that can contribute to postoperative infections. Another study on the resistance of pathogens against chlorhexidine in patients with preoperative cleft soft palate isolated 28 different pathogenic micro-organisms from 50 patients (Roode & Bütow, 2018).

Only one study that indicates surgical antimicrobial prophylaxis usage in hospitals in South Africa is available (Van der Sandt et al., 2019). No study could be found that indicates the frequency of antimicrobial prophylaxis applied by general practitioners in private practice. All of the foregoing seem to indicate that there is an increase in the number of different pathogenic micro-organisms isolated preoperatively, and this study aims to evaluate the current situation and will serve as a follow-up report on publications in 2009 and 2016 from the same institution (Myburgh & Bütow, 2009; Roode et al., 2016).

PATIENTS AND METHODS

A point prevalence study was designed and approved by the Faculty of Health Science Research Ethics Committee (467/2015) of the University of Pretoria. All patients (*N* = 103) who presented for primary repair of the soft palate cleft from November 2015 to November 2019 were included in the study. Other inclusion criteria were as follows: (1) patients from whom written parental consent was received and (2) patients who were cleared as systemically healthy by a paediatrician. The exclusion criterion was the preoperative presence of systemic infections (e.g. flu) and/or any local infections (e.g. tonsillitis) in the patient. History of previous medications prescribed to the patients was not recorded (a possible shortcoming). No data are available on antibiotic prescription rates in paediatric patients, which could indicate whether or not a substantial overall change occurred.

All procedures were executed by the same oral and maxillofacial surgeon. The Copan Transystem Bacteriology Swab Collection system with Amies Agar Gel for aerobic and anaerobic culture was used to collect and transport specimens. A swab was taken preoperatively from the cleft soft palate and adjacent nasopharynx of all patients immediately after they were anaesthetised (general anaesthesia). This was done by removing the swab from the sterile packing, rubbing it gently over the mucosa of the indicated area, re-inserting the swab into the transport tube to seal it and marking it with the patient information. The swab was transported in this format within 1 h to a pathology laboratory for culturing to determine the type, colony size and sensitivity of any possible micro-organisms.

The organisms were isolated using standard microbiological methods: all samples were inoculated onto a nonselective blood agar plate as well as onto a selective and differential MacConkey agar plate. Plates are incubated overnight at 35℃. The next morning, incubated colonies were transferred to a target slide that was introduced into VITEK MS, an automated mass spectrometry microbial identification system for the identification of pathogens. Using the EUCAST disc diffusion method and EUCAST breakpoints, the antimicrobial sensitivity or resistance was determined to indicate which antimicrobial agents would be effective in treating the patient if any infections should develop. Data from the laboratory results were recorded per patient and used for analysis.

RESULTS

Only 3 of the 103 patients included in this study had no preoperative pathogenic micro-organisms cultured. One patient had five different pathogens cultured, and a substantial number of cases hosted four different micro-organisms.

The mean age of the 103 patients is calculated at 7 months and 18 days (standard deviation: 4 months and 22 days). However, three patients were included in the study that was referred to this institution at a much older age for the primary repair of the cleft soft palate than what the normal protocol advises. They were aged 19 months 5 days, 19 months 11 days and 46 months 3 days, respectively. Excluding these three patients, the average age changes to 6 months and 29 days with a much lower standard deviation, namely 2 months

and 8 days. The distribution of sex is close, with 57 male patients and 46 female patients. Race distribution was as follows: Indian (6), Black (29), Coloured (1) and White (67).

Forty-two different pathogenic micro-organisms were identified (Table 1) with *K*. *pneumoniae* the most prevalent, identified in 47 cases. The second most prevalent was *Streptococcus mitis*/*oralis* (in previous studies not considered as pathogenic) in 32 patients, followed by *H*. *influenzae* in 30 patients. The following pathogens were also found in 10 or more patients: *Ent*. *cloacae* (18), *E*. *coli* (16), *Staph*. *aureus* (14) and *Candida albicans* (12). The other micro-organisms occurred in one to eight patients, with 20 of them in only one patient each. A total of 244 pathogens were cultured from the 103 patients (Table 1).

TABLE 1. Comparison of pathogens identified from soft palate cleft and nasopharynx mucosa pre-operative

Micro-organism *N* (%)	**2020 (103 patients)**	**2016 (200)**	**2009 (100)**
Acinetobacter baumannii	3 (2.9%)	4 (2%)	
Aeromonas hydrophila/caviae/sobria	3 (2.9%)	3 (1.5%)	2 (2%)
Candida albicans	12 (11.7%)	3 (1.5%)	9 (9%)
Candida dublinensis	2 (1.9%)		
Candida famata	1 (1%)		
Candida glabrata	1 (1%)		
Candida kefyr	2 (1.9%)		
Candida krusei	3 (2.9%)		
Candida lusitaniae	1 (1%)		
Candida parapsilosis	4 (3.9%)		
Candida tropicalis	3 (2.9%)		
Chryseobacterium gleum	1 (1%)		
Citrobacter koseri	1 (1%)		
Citrobacter freundii	1 (1%)		
Edwardsiella tarda	1 (1%)		
Enterobacter aerogenes	1 (1%)	2 (1%)	1 (1%)
Enterobacter cloacae	18 (17.5%)	10 (5%)	5 (5%)
Enterobacter gergoviae		1 (0.5%)	
Enterobacter hormaechei	1 (1%)		
Enterobacter kobei	1 (1%)		
Enterococcus faecalis		1 (0.5%)	1 (1%)
Escherichia coli	16 (15.5%)	20 (10%)	9 (9%)
Geobacillus thermoglucosidasius	1 (1%)		
Heamophilus haemolyticus	1 (1%)		
Haemophilus influenzae	30 (29.1%)	13 (6.5%)	
Haemophilus parahaemolyticus	1 (1%)		
Haemophilus parainfluenzae	2 (1.9%)		
Klebsiella oxytoca	8 (7.8%)	3 (1.5%)	1 (1%)
Klebsiella pneumoniae	47 (45.6%)	35 (17.5%)	18 (18%)
Kluyvera cryocrescens		1 (0.5%)	
Moraxella catarrhalis	3 (2.9%)	12 (6%)	6 (6%)
Neisseria subflava	1 (1%)		
Proteus mirabilis	1 (1%)		

Micro-organism *N* (%)	2020 (103 patients)	2016 (200)	2009 (100)
Pseudomonas aeruginosa	4 (3.9%)	4 (2%)	
Pseudomonas putida	1 (1%)		
Saccharomyces cerevisiae	1 (1%)		
Serratia marcescens	4 (3.9%)	8 (4%)	4 (4%)
Staphylococcus aureus	14 (13.6%)	25 (12.5%)	22 (22%)
Staphylococcus epidermidis	1 (1%)	1 (0.5%)	
Stenotrophomonas maltophilia	1 (1%)		
Streptococcus anginosus		1 (0.5%)	
Streptococcus mitis/oralis[a]	32 (31.1%)		
Streptococcus parasanguinis	3 (2.9%)		
Streptococcus pneumoniae	6 (5.8%)	17 (8.5%)	7 (7%)
Streptococcus pseudopneumoniae	1 (1%)		
Streptococcus pyogenes		1 (0.5%)	
Streptococcus salivarius	5 (4.9%)		
Streptococcus viridans		1 (0.5%)	
Total	244	170	85

[a] Not included in the comparison as previously not seen as a pathogen.

Antimicrobial resistance of the pathogens cultured from the swabs was determined, and 159 of the 244 (65.2%) pathogens showed resistance to different antimicrobial agents to which their sensitivity was tested. Table 2 is a summary of the resistance of the seven most prevalent micro-organisms that were isolated in 10 or more cases.

TABLE 2. The antimicrobial resistance profile of the most prevalent pathogens

	MIC:	
	S≤	**R>**
Klebsiella pneumoniae: (*N* = 47)		
Amikacin (1)	16	16
Ampicillin (44)	128	128
Amoxicillin–clavulanic acid (5)	8	32
Cefepime (2)	1	32
Cefotaxime (4)	1	4
Ceftazidime (3)	1	64
Cefuroxime (4)	4	32
Ciprofloxacin (3)	1	4
Cotrimoxazole (6)	40	80
Doripenem (1)	1	4
Ertapenem (1)	0.5	2
Gentamicin (2)	4	16
Imipenem (1)	1	4
Meropenem (1)	1	4
Piperacillin–tazobactum (2)	16	128
Streptococcus mitis/oralis: (*N* = 32)		
Pen-G (18)	0.25	2

	MIC: S≤	R>
Ampicillin (12)	0.5	8
Amoxicillin–clavulanic acid (12)	0.5	2
Clindamycin (3)	0.5	0.5
Haemophilus influenzae: (*N* = 30)		
Cefuroxime (5)	1	2
Cotrimoxazole (15)	20	80
Enterobacter cloacae: (*N* = 18)		
Ampicillin (17)	8	32
Amoxicillin–clavulanic acid (17)	8	32
Cefuroxime (8)	4	32
Escherichia coli: (*N* = 16)		
Ampicillin (10)	8	32
Amoxicillin–clavulanic acid (4)	8	32
Cefuroxime (2)	4	32
Cefotaxime (2)	1	4
Tobramycin (1)	4	16
Ciprofloxacin (2)	1	4
Cotrimoxazole (9)	40	80
Staphylococcus aureus: (*N* = 14)		
Pen-G (10)	0.12	1
Cotrimoxazole (1)	40	80
Tetracycline (1)	1	2
10 were β-lactamase +		
Candida albicans: (N = 12)		
None		

The number of cases showing resistance is given in brackets, indicating the MIC (µg/ml) where S = sensitive and R = resistant (The European Committee on Antimicrobial Susceptibility).

DISCUSSION

The question remains: "Are prophylactic antibiotics necessary before primary cleft soft palate surgery?" A study in the United Kingdom and Ireland found that there is no consensus and that there exists a wide discrepancy in terms of the use of antibiotics between centres performing repair to the cleft palate (Smyth & Knepil, 2008). Another study concluded that patients are at risk of carrying pathogens before primary cleft repair (Chuo & Timmons, 2005), and two publications from India concluded that postoperative antimicrobial prophylaxis decreased the incidence of fistulas after primary cleft palate repair (Aznar et al., 2015; Campbell et al., 2015).

This study found that in 100 of the 103 patients (97%), pathogenic micro-organisms were identified (99/103 patients when *Strep*. *mitis*/*oralis* is excluded as it was not considered pathogenic in previous research). When one compares this study to the study published in 2016 (Roode et al., 2016) where 58% of patients carried pathogens, the above statement that patients are at risk of carrying pathogens before primary cleft repair (Chuo & Timmons,

2005) becomes a real concern. The number of different species also increased from 23 to 42 (Figure 1). The total number of pathogens isolated was 244. One patient had five different pathogens isolated from the surgical site namely *Acinetobacter baumannii*, *Haemophilus parainfluenzae*, *K*. *pneumoniae*, *Staph*. *aureus* and *Strep*. *mitis*/*oralis*. Four different pathogens were identified in each of the 21 patients. The incidence of the isolation of this large number of pathogens preoperatively is cause for great concern as the possibility of postoperative infections is significantly increased.

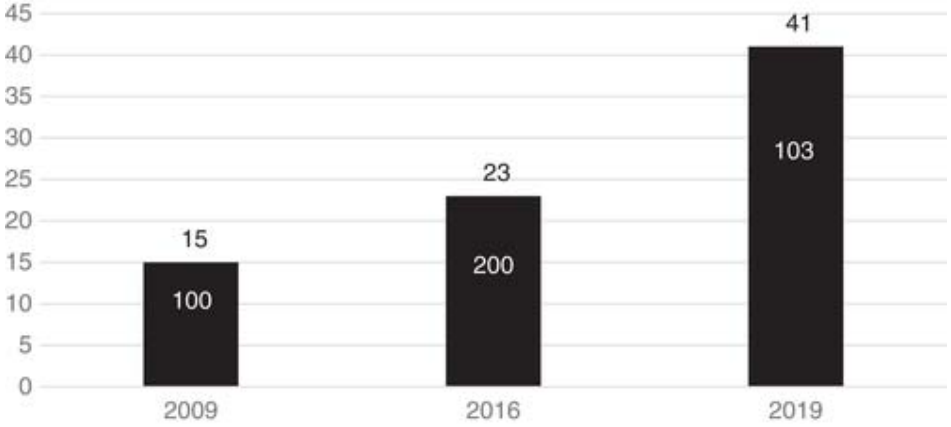

FIGURE 1. Number of different pathogens observed in number of patients presented in graph (excluding *Streptococcus mitis*/*oralis*)

Klebsiella pneumoniae is the most prevalent pathogen, identified in 47/103 cases (45.6%). This is less than in the study from Cocco (Cocco et al., 2010) in which pathogens were identified in 56% of cases, but far more than were identified in previous publications from this institution, that found 18% of 100 cases in 2009 (Myburgh & Bütow, 2009) and 17.5% of 200 cases in 2016 (Roode et al., 2016) (Figure 2). The publication from 2016 included patients treated over a longer time span, including patients treated between January 1992 and March 2015. Percentage-wise, the numbers stayed about the same between the publications of 2009 and 2016, but there is a startling increase of nearly 28% from 2016 to the current year. *Klebsiella pneumoniae* is traditionally seen as an important cause of community-acquired pneumonia, but it can also cause infections in other areas such as the urinary tract and on surgical sites. Paterson and co-workers discussed the *Klebsiella* species in detail, as well as their continuous development of resistance against antimicrobials (Jacoby, 2005; Paterson et al., 2019). A study from India indicates that *Klebsiella oxytoca* is important as an emerging pathogen in causing infections acquired in hospital (Singh et al., 2016). In this study, *K*. *oxytoca* was also identified in eight cases (three identified in 2016, one in 2009) and showed similar antibiotic resistance profiles to *K*. *pneumoniae* with all eight of them resistant to ampicillin. Table 2 indicates the number of *K*. *pneumoniae* cases resistant to different antimicrobials.

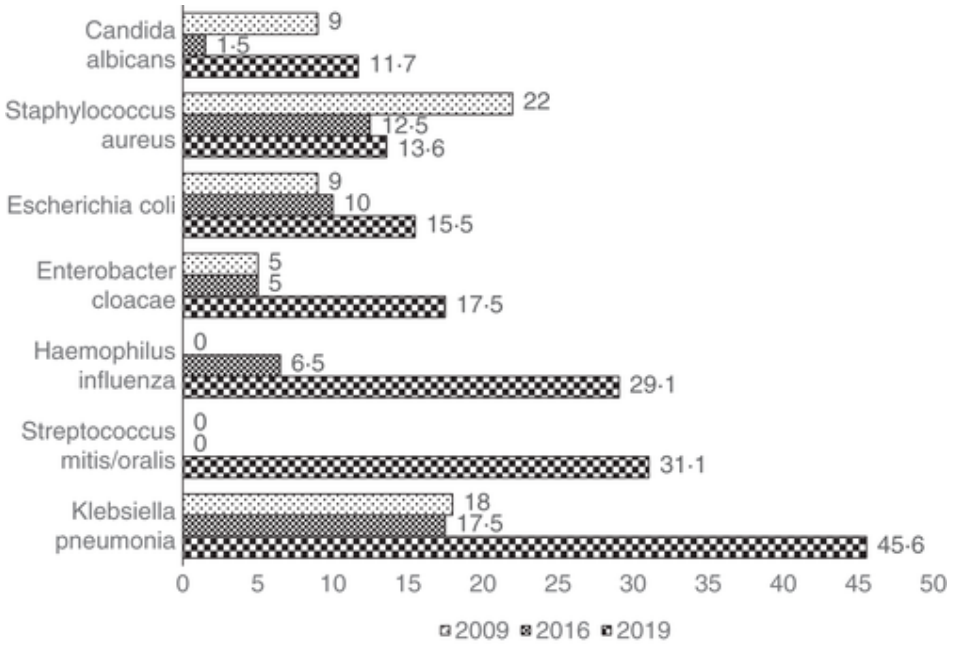

FIGURE 2. Change in percentage of pathogens present in total number of cases. Note: *Streptococcus mitis* was previously not seen as a pathogen

Streptococcus mitis/*oralis* is the second most common pathogen that was found in 32 out of 103 patients (31%) (Figure 2). It is an organism that routinely inhabits the human mouth and was previously not reported as it was seen as normal oral flora, and for this reason, it will not be included in the comparison with the previous studies. Recently, it has been recognised as pathogenic in nature as it causes a wide range of infections in humans, including bacteraemia and infective endocarditis (Arief et al., 2005; Mitchell, 2011; Shelburne et al., 2014; Sitkiewicz, 2018). It has also been shown that *Strep*. *mitis*/*oralis* has brought about epithelial cell and macrophage death (Naji et al., 2018). As part of the VGS, *Streptococcus parasanguinis* (3) and *Streptococcus salivarius* (5) were also identified, and publications on their pathogenic nature exist (Doern & Burnham, 2010; Sadjadi & Ali, 2011; Wilson et al., 2012). Two other *Streptococcus* sspp. isolated were *Strep*. *pneumoniae* (6) and *Streptococcus pseudopneumoniae* (1) (together 6.7%). Incidence of *Strep*. *pneumoniae* came to 7% in each of the previous studies although isolation of *Streptococcus anginosus*, *Streptococcus pyogenes* and *Streptococcus viridans* numbered one each in the 2016 publication.

The third most abundant pathogen found was *H*. *influenzae*, in 30 of the 103 patients (29.1%). The study from 2009 identified only 8 cases (8%) and the study from 2016 included 13 cases (6.5%) (Figure 2). This indicates an alarming increase over the period November 2015 to current. Although *H*. *influenzae* is seen as an opportunistic pathogen, its presence in contact with a surgical site can be detrimental to the success of the surgical procedure. Multiple studies have looked into the multidrug resistance of this pathogen (Aguirre-Quinonero et al., 2018; Atkinson et al., 2017; Chen et al., 2018; Jacoby, 2005; Maddi et al., 2017; Sakata et al., 2019). The resistance profile as found in this study (Table 2) is in line with some of those findings. Another *Haemophilus* ssp. identified in this study was *Haemophilus haemolyticus* (1), which is difficult to differentiate from *H*. *influenzae*, and was found in 2016 to show resistance to different antimicrobials (Le Floch et al., 2013; Marti et

al., 2015). The *H*. *haemolyticus* cultured in this study showed no resistance. Other subspecies cultured was *Haemophilus parahaemolyticus* (one case, with resistance to levofloxacin and moxifloxacin), which should also be reckoned as an opportunistic pathogen (Marti et al., 2015), and *H*. *parainfluenzae* (two, both resistant to cotrimoxazole). These three subspecies were not identified in the previous studies.

The fourth most common micro-organism identified was *Ent*. *cloacae* in 18 of the 103 cases (17.5%). Compared with the 2009 and 2016 studies (where both showed an occurrence of 5%) (Figure 2), an alarming rise is again noted. It is also a common nosocomial pathogen that is capable of generating a wide variety of infections (Annavajhala et al., 2019). Broad-spectrum antibiotic resistance is reported, including the recent development of resistance to last-resort carbapenems (Breurec et al., 2016; Mezzatesta et al., 2012; Pang et al., 2018). Resistance found in this study is listed in Table 2. One each of three other *Enterobacter* sspp. were identified in this study (numbers for 2016; 2009 and current resistance is given in brackets) namely *Enterobacter aerogenes* (2; 1 resistant to ampicillin and amoxicillin–clavulanic acid), *Enterobacter hormaechei* (0; 0 ampicillin, amoxicillin–clavulanic acid and cotrimoxazole) and *Enterobacter kobei* (0; 0 ampicillin and amoxicillin–clavulanic acid). The latter two are part of the *Ent*. *cloacae* complex group. None of the *Enterobacter* sspp. isolated in this study were carbapenem resistant. Known formerly as *Enterobacter agglomerans*, *Pantoea agglomerans* was not isolated in this study, although four cases were reported in the 2016 and three in the 2009 publications.

The occurrence of *E*. *coli* was quantitatively similar to *Ent*. *cloacae*, being cultured from 16 of the 103 patients (15.5%). There was also a rise in numbers from the 2009 study that isolated 9% of total cases and the 2016 publication, indicating 10% (Figure 2). *Escherichia coli* is mainly a noninvasive bacterium of the gastro-intestinal tract, although some strains are associated with food poisoning, causing diarrhoea and vomiting. Reports on its cause in a wide spectrum of other infections including surgical site infections have been published (Petkovsek et al., 2009; Tourmousoglou et al., 2008). *Escherichia coli* in this study showed similar resistance to ampicillin and cotrimoxazole.

Staphylococcus aureus is perceived as the most dangerous of the *Staphylococcus* sspp. (Bush, 2018; Tong et al., 2015). It was the most common pathogen in the 2009 study (22%) and the second most common in the 2016 publication, decreasing to 12.5% (Figure 2). In the current study, a similar number of cases were noted—14/103 (13.6%). A declining incidence of pathogens is favourable and reassuring, especially when this is compared with the study by Chuo who included 121 cleft palate patients over a time span of nearly 15 years and identified *Staph*. *aureus* in 38% of cases (Chuo & Timmons, 2005). One other species of *Staphylococcus* that could play a role in surgical site infections was identified, namely *Staphylococcus epidermidis*, (Namvar et al., 2014). This pathogen was not present in either previous study. No resistance to antimicrobials was found.

It is claimed that *C*. *albicans* is the best studied of the human fungal pathogens that commonly live in our bodies (Berman, 2012). It is also considered the third or fourth most common cause of infections acquired in the hospital and as the most prevalent basis of fungal infections in humans, more specifically in facial cleft patients (Rawashdeh et al., 2011; Seladi-Schulman, 2018). In this study, 12 of the 103 cases (11.7%) were cultured. The

2009 study reported 9%, and the 2016 publication reported 1.5% (Figure 2). Eight other species of *Candida* were also cultured in this study totalling 17 cases (Table 1) with *Candida parapsilosis* presenting 4 of them. *Candida parapsilosis* was reported in 2008 as becoming a major threat for the future (Singh & Parija, 2012; Trofa et al., 2008). *Candida krusei* and *Candida tropicalis*, which contributed three cases each in this study, were also reported to add to the increase of *Candida* infections in India (Singh & Parija, 2012). A recent update on *C. tropicalis* (Zuza-Alves et al., 2017) discussed the development of resistance to azoles and amphotericin B in this species. Other studies point out that *C. krusei* may be less susceptible to the treatment with amphotericin B and fluconazole (Abbas et al., 2000; Pfaller et al., 2008). The three isolates of each of these pathogens in this study did not show any resistance. *Candida dublinensis* and *Candida kefyr* were present in two cases each. *Candida dublinensis* is closely related to *C. albicans* (Sullivan & Coleman, 1998; Sullivan et al., 2005) but is not virulent (Moran et al., 2012). In one study in patients with cleft, *C. kefyr* constituted of 7.9% of *Candida* infections (Rawashdeh et al., 2011). A similar result (6.9%) was found in this study. The three species *Candida famata*, *Candida glabrata* and *Candida lusitaniae* were isolated in one case each. *C. famata* and *C. glabrata* are not common yeasts that contribute to infections but are reported to display reduced susceptibility to antifungal treatment (Beyda et al., 2012; Karapetsa et al., 2019; Schulman, 2019). *Candida lusitaniae* is referred to being resistant to amphotericin B (Hawkins & Baddour, 2003). All these species can contribute to postoperative infections, but in our study they did not show any resistance to antimicrobials.

Four isolates each of *Pseudomonas aeruginosa* and *Ser. marcescens* (3.9% of total) were identified. A lower incidence (2%) of cases of *P. aeruginosa* was described in the 2016 publication (0% in 2009). It is reported to be “the most common pathogen isolated from patients who have been hospitalized longer than 1 week” (Friedrich et al., 2018). It is also a regular cause of infections acquired in hospital and is resistant to antimicrobials (Fernández et al., 2015). In this study, these four organisms showed no antimicrobial resistance. One other species of the same genus was found namely *Pseudomonas putida*, which did not manifest in the 2016 study. The number of *Ser. marcescens* compares similarly to the 2009 and 2016 studies where it contributed 4% of cases. From an antimicrobial perspective, all these organisms demonstrated resistance to ampicillin, amoxicillin–clavulanic acid and tobramycin.

Three other micro-organisms were present in three cases (2.9%) each. *Acinetobacter baumannii* increased marginally from 2% in 2016 (0% in 2009) and is reported to “specifically target(s) moist tissues such as mucous membranes” (Howard et al., 2012). None of these cases showed any resistance profile.

Aeromonas hydrophila/*caviae*/*sobria* was present in 1.5% and 2% of cases in 2016 and 2009, respectively. Although this pathogen mainly causes diarrhoea, it is indicated that it can cause mild to severe wound infections (Morris & Horneman, 2019). Two cases in this study manifested no resistance, but the third showed resistance to imipenem and meropenem.

Incidence of *M. catarrhalis* is down by half compared with the 6% in 2016 and 2009. It is a common cause of otitis media (Goldstein et al., 2009) with reported resistance to antimicrobial agents. The three isolated cases were β-lactamase positive with resistance to

ampicillin and cefuroxime, and one was also resistant to azithromycin, clarithromycin, cotrimoxazole and telithromycin.

Only one in each of the following pathogens (in alphabetical order) was isolated in this study, with the numbers for 2016 and 2009 indicated in brackets:

- *Chryseobacterium gleum* (0; 0) very rarely causes infections in humans and shows varying susceptibility to antibiotics (Lin et al., 2019). There was no resistance identified from the one case isolated in this study.
- *Citrobacter freundii* and *Citrobacter koseri* (0; 0) are well-known causes of urinary tract, diarrheal and surgical site infections, which are showing an increase in multidrug resistance (Liu et al., 2018; Samonis et al., 2009). These organisms were resistant to ampicillin and amoxicillin–clavulanic acid.
- *Edwardsiella tarda* (0; 0) is a pathogen that very rarely causes wound infections (Hirai et al., 2015). Resistance to ampicillin was noted in this case.
- *Geobacillus thermoglucosidasius* (0; 0). No report could be found on infections related to this micro-organism. The one isolated case was resistant to ampicillin, amoxicillin–clavulanic acid and cotrimoxazole.
- *Neisseria subflava* (0; 0) is part of the nasal and oropharyngeal flora but has sometimes been reported to cause disease in immune-compromised patients (Liu et al., 2015). This isolate was resistant to Pen-G.

The main clinical manifestations of *Proteus mirabilis* (0; 0) are urinary tract infections (Jamil et al., 2019). No antimicrobial resistance was noted in this case.

Saccharomyces cerevisiae (0; 0) is a yeast commonly used as a probiotic as part of the treatment of diarrhoea caused by the use of antibiotics but can lead to *S. cerevisiae* fungemia. No antimicrobial resistance was noted.

Stenotrophomonas maltophilia (0; 0) is reported to be associated with wound infections and to exhibit multidrug resistance (Adegoke et al., 2017). The identified pathogen in this study was not resistant to any antimicrobials.

The following pathogens were present in the previous studies, but none of them were identified for the current one: *E. agglomerans* (2% in 2016), *Enterobacter gergoviae* (0.5% in 2016), *Enterococcus faecalis* (0.5% in 2016; 1% in 2009), *Kluyvera cryocrescens* (0.5% in 2016), *S. anginosus* (0.5% in 2016), *Strep. pyogenes* (0.5% in 2016) and *S. viridans* (0.5% in 2016).

The treatment of patients with cleft soft palate deformity is particularly challenging because of the scarcity of tissue that can be used for reconstructive purposes. The risk of tissue compromise due to infections is a critical concern. The results from studies from within a consistent setting over three separate periods of time indicate a major alarm that should be noted. This concern is intensified as in the latest study pathogens were cultured preoperatively in 99/103 patients, with 22 patients (21.4%) harbouring four or more pathogens each. In addition, the multiplication in number of different pathogens, that has risen from 15 in 2009 and 23 in 2016 to 41 (Figure 1) in the current study, is alarming. The

increase in total number of pathogens isolated is also disturbing: 85/100 patients in 2009, 170/200 patients in 2016 and an astonishing 212 pathogens occurring in 103 patients in the current study. *Streptococcus mitis*/*oralis* has added 32 additional pathogens as its status has changed from 'normal' to 'pathogenic'. There was, however, only one case where it was isolated as the one and only pathogen present. The other concern is the rise in antimicrobial resistance. It is, therefore, recommended that alternative methods such as the use of diluted Manuka honey or Anolyte be researched to control the presence of microbial contamination in the oral and naso-oropharyngeal region preoperatively.

TRANSPARENCY DECLARATION

Gieljam J Roode (corresponding author), Kurt-Wilhelm Bütow and Sharan Naidoo did not receive any payment or services from a third party for any aspect of the submitted work 'Microbial contamination profile change over a 4-year period in nonoperated cleft soft palate'. There is no relevant conflict of interest, and there are no patents planned, pending or issued relevant to this work. No other relationships or activities have influenced what is written in this work. We have nothing to disclose. The study was self-funded by the private practice of K-W Bütow, who is also the guarantor of the data.

CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

AUTHOR CONTRIBUTIONS

GJ Roode collected and analysed the data and has reported the findings. K-W Bütow assisted in collecting the data, reviewed and approved the manuscript. S Naidoo reviewed and approved the manuscript.

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