Development of bacterial resistance during treatment with topical gentamicin for chronic rhinosinusitis in patients with cystic fibrosis and primary ciliary dyskinesia. Retrospective case series

Authors' Contribution:
A – Study Design
B – Data Collection
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ABSTRACT:

Background: The management of chronic rhinosinusitis (CRS) in patients with cystic fibrosis (CF) and primary ciliary dyskinesia (PCD) is still a challenge. At our institution we have used gentamycin nasal spray, extemporaneously produced, for prophylactic treatment of moderate-to-severe CRS. The aim of this study was to investigate the gentamycin susceptibility of bacteria in sputum samples in CF and PCD patients treated for CRS.

Methodology: Patients with CF and PCD who were prescribed gentamycin nasal spray for CRS and had sputum bacterial cultures taken pre-treatment and followed-up at least once after ≥ 6 months were retrospectively included. Microbiological data were descriptively analysed in terms of bacterial species and resistance to gentamycin.

Results: A case series of 17 CF and 12 PCD patients passed the inclusion criteria. Of those cases, three (18%) CF patients and one (8%) PCD patient developed resistance to gentamycin during treatment with gentamycin nasal spray. In all four cases, the resistant bacterial isolates were *P. aeruginosa*. Additionally, two CF patients already had *P. aeruginosa* isolates resistant to gentamycin in the pre-treatment culture. In further two CF patients, the multi-resistant *Burkholderia cepacia* complex, including gentamycin resistance, was identified. *P. aeruginosa* and *S. aureus* in CF and *P. aeruginosa* and *H. influenza* in PCD were the predominant bacterial species.

Conclusions: The study showed that there was moderate incidence of gentamycin resistance in CF and PCD patients at our institution. However, further prospective studies are needed to confirm the outcomes.

KEYWORDS:

chronic rhinosinusitis, cystic fibrosis, gentamycin nasal spray, gentamycin resistance, primary ciliary dyskinesia

ABBREVIATIONS

BAL – bronchoalveolar lavage
BCC – *Burkholderia cepacia* complex
CF – cystic fibrosis
CRS – chronic rhinosinusitis
ENT – ear, nose and throat
EPOS – European Position Paper on Rhinosinusitis
GAS – group A *Streptococcus*
PCD – primary ciliary dyskinesia

INTRODUCTION

Cystic fibrosis (CF) and primary ciliary dyskinesia (PCD) are two autosomal recessive diseases that disrupt the function of the ciliated

epithelium, either indirectly in CF through mutations in a chloride ion channel or directly in PCD by mutations that result in structural abnormalities of the cilia [1, 2]. The respiratory system in these patients is particularly affected by the accumulation of viscous mucus that interrupts the mucociliary clearance. In the paranasal sinuses, this may lead to bacteria colonization, recurrent infections, inflammation and development of chronic rhinosinusitis (CRS) [3, 4]. CRS is a significant hallmark of CF and affects 70–100% of CF subjects [5]. In PCD the incidence of CRS was recently estimated at 70% [1]. In addition, up to 50% of PCD have Kartagener syndrome characterized by the triad of CRS, situs inversus and bronchiectasis. In comparison, CRS affects over 10% of the general European population [6].

The etiology of CRS has not been clearly defined. However, it is known that bacteria may initiate or amplify the disease. The airways

Tab. I. Case series of 17 CF patients treated for CRS with gentamycin nasal spray. Demographic data and bacteria isolates in pre-treatment and follow-up sputum cultures are presented. ND – not done; N – not found; Y – found; S – sensitive; R – resistant; nm – nonmucoid; m – mucoid; PA – *P. aeruginosa*; SA – *S. aureus*; Sp – *S. pneumoniae*; Mc – *M. catharalis*; Bcc – *Burkholderia cepacia* complex; GAS – group A *Streptococcus*; M – male; F – female; *not sensitivity tested. Patients nos. 1, 7 and 13 (in red) developed resistance to gentamycin during treatment.

	PRE-TREATMENT					6-MONTH FOLLOW-UP			12-MONTH FOLLOW-UP			18-MONTH FOLLOW-UP		
	AGE	GENDER	SA	PA	OTHER	SA	PA	OTHER	SA	PA	OTHER	SA	PA	OTHER
1	35	F	N	S	N	N	S m, R nm	N	N	S m	N	N	R m, R nm	N
2	35	F	N	N	Bcc R	ND	ND	ND	N	N	N	N	N	N
3	31	F	N	N	Bcc R	N	N	N	N	N	Bcc R	N	N	Bcc R
4	30	M	Y*	N	N	Y*	N	N	Y*	N	N	S	N	N
5	29	M	N	R nm	N	N	S nm	N	N	R nm	N	N	R m, R nm	N
6	28	F	N	N	N	N	N	N	N	N	N	N	S nm	N
7	40	F	N	N	N	N	N	N	N	N	N	N	R m	N
8	26	M	N	N	N	N	N	N	ND	ND	ND	N	N	N
9	41	M	N	N	Sp	N	N	Sp*	N	N	Sp*	N	N	N
10	24	M	N	N	N	S	N	N	N	N	N	S	N	N
11	18	F	S	N	N	S	N	N	N	N	N	ND	ND	ND
12	15	F	S	N	N	N	N	GAS*	ND	ND	ND	ND	ND	ND
13	42	M	Y*	S nm	N	Y*	R	N	S	R	N	S	R m, R nm	N
14	12	M	S	N	N	N	N	N	S	N	N	S	N	N
15	64	M	S	N	N	Y*	N	N	N	N	N	N	N	N
16	44	M	S	N	N	S	N	N	S	N	N	S	S	N
17	44	M	N	R	N	N	R	N	ND	ND	ND	N	S/R	N

Supplementary Tab. I. The use of antibiotics in patients who developed resistance to gentamycin during treatment with gentamycin nasal spray. The patient's number corresponds to Tab. I. and II.; iv – intravenous treatment; per os – per oral treatment.

PATIENT	INDICATION; ANTIBIOTIC; (IF GIVEN) DOSE/DURATION
CF 13	Pulmonary infection; tobramycin inhalation 300 mg 2 times per day/4 weeks.
CF 1	Chronic lung infection of <i>P. aeruginosa</i> ; multiple courses of tobramycin iv.
CF 7	Several pulmonary infections; several courses of azithromycin per os and sulfamethizol/trimethoprim per os; one course of piperacillin/tazobactam iv, multiple courses of tobramycin inhalation.
PCD 2	Several pulmonary infections; multiple courses of azithromycin per os, ciprofloxacin per os, multiple courses of tobramycin inhalation.

of CF and PCD are commonly colonized by opportunistic bacteria such as gram-negative *P. aeruginosa* and gram-positive *S. aureus* [7]. The management of CRS in CF and PCD patients is generally based on different antibiotic regimens.

In recent years, the topical application of an antimicrobial agent for CRS has become increasingly popular [2]. This form of administration provides direct delivery to the sinonasal cavity without systemic exposure. The high concentration of antibiotics at the site of infection enables effective eradication of bacterial biofilm [8]. Furthermore, it was shown that bacterial isolates found in the sinonasal cavity and the lungs had the same genotype in CF patients, suggesting a direct exchange between sites [9]. This is in accordance with the concept of united airways, which states that upper and lower airway diseases co-occur frequently as they are anatomically and immunologically related [10]. Thus, effectively treated CRS could delay lung disease [11], the main cause of morbidity in CF and PCD patients [1, 2].

Only a few studies have been conducted on topical antibiotics for CRS in the CF and PCD population. They have shown promising results with regards to a reduction in repeat surgery, symptoms,

endoscopic appearance and bacterial burden [2, 12]. Nevertheless, the susceptibility patterns of microbiota in the airways after the administration of topical antibiotics have not been previously investigated. Of particular concern in recent decades is the increasing resistance to antibiotics in CF and PCD [13].

At our ear, nose and throat (ENT) outpatient clinic we have administered empirical treatment with topical gentamycin immediately following isotonic saline nasal irrigation to treat moderate-to-severe CRS in both CF and PCD groups. Gentamycin nasal spray (called 'Wilson's solution') has been produced extemporaneously in accordance with the formula developed in the 1960s at the Mayo Clinic in Rochester, Minnesota [39]. Aminoglycoside agent gentamycin has a broad-spectrum indication for gram-negative bacteria as well as some gram-positive bacteria. Importantly, the nasal administration of gentamycin was proven safe and well tolerable in the previous reports where it was used in a solution for the irrigation of sinuses [14–16]. The aim of this long-term retrospective study was to assess whether treatment using nasal gentamycin spray for CRS contributes to antibiotic resistance in the airways of adolescent and adult CF and PCD patients.

MATERIALS AND METHODS

Study design

The study protocol was approved by the Regional Ethical Committee (Dnr2017/142). Data from patients with CF and PCD who were prescribed gentamycin nasal spray to treat CRS between 1996 and 2016 were collected retrospectively at the ENT Clinic, Uppsala University Hospital. The definition of CRS in CF and PCD was similar to the definition used in the European Position Paper on Rhinosinusitis (EPOS) [17]. In brief, CRS is a group of disorders that affects the nose and paranasal sinuses with nasal obstruction, reduced smell or absence of smell, facial pain and nasal discharge lasting for more than 12 weeks. In order to identify the study population, we searched the local drug register for subjects who had been prescribed nasal gentamycin. Following this, we identified via medical records the patients with CF and PCD who had been treated for CRS. Finally, we selected patients with sputum bacterial culture results taken shortly before the treatment (pre-treatment) and at least once after 6 months following treatment induction (follow-up). These sputum samples were collected and analysed via the cystic fibrosis center at the respiratory medicine department at Uppsala University Hospital as part of clinical routine at each 6-month screening. From the patients who met the inclusion criteria we tabulated epidemiological (age and gender), microbiological and clinical data from their medical records. Follow-up sputum samples were divided into three groups taken at 6, 12 and 18 months after gentamycin spray prescription as the patients were prescribed to continuously use the gentamycin spray 6, 12 or 18 months. The treatment compliance could not be evaluated due to the retrospective nature of the study. Major focus was given to the development of resistance to gentamycin. Only the medical records of patients who developed bacterial resistance to gentamycin were searched for other antibiotics (substance, dose and length of treatment) administered during treatment with gentamycin nasal spray. Epidemiological data were presented as medians with lower/upper quartile values in parentheses.

Gentamycin spray

The treatment involved gentamycin spray being delivered twice into each nostril three times per day following isotonic (physiological) saline nasal irrigation. The gentamycin nasal spray contained gentamycin at 80 µg/ml dissolved in saline and produced extemporaneously by APL (Apotek Produktion & Laboratorier AB) in Stockholm, Sweden.

Collection of sputum, culture and susceptibility testing

In brief, CF and PCD patients spat to discard saliva, followed by sputum expectoration into a collection tube. Sputum specimens were then routinely cultured for bacteria on agar plates of various media. Standard microbiological methods and procedures used at the Uppsala University Hospital Laboratory were followed. Gentamycin susceptibility testing for identified bacteria was conducted in accordance with the previously described protocol [18]. This method is internationally accepted, simple, and allows for ease of use when screening a high number of isolates. The distribution of each bacterial species (presumptive phenotype as mucoid and non-mucoid) found, susceptibility pattern and resistance develop-

Fig. 1. The distribution of bacterial species found in pre-treatment and follow-up sputum cultures in patients with CF and PCD treated for CRS with gentamycin nasal spray. *P. aeruginosa* S – sensitive to gentamycin and *P. aeruginosa* R – resistant to gentamycin. *S. aureus* S – sensitive to gentamycin, *S. aureus** – not sensitivity tested; other single isolates such as *P. pneumoniae*, the group A, C and G *Streptococcus*, *M. catarrhalis*, *Burkholderia cepacia* complex.

ment, were analysed separately in the CF and PCD groups, using descriptive statistics. In this study, the eradication rate was calculated (for each bacterial species in CF and PCD) as the number of patients with two subsequent negative cultures (1 year) after one positive (pre-treatment or pre-treatment and 6-month follow up) divided by the number of studied patients.

RESULTS

Patients characteristics

Between 1996 and 2016 a total of 36 patients with CF or PCD were prescribed gentamycin nasal spray for CRS at least once every six months. Seventeen CF and 12 PCD patients met the inclusion criteria ($n = 29/36$; 81%). We therefore presented them as two case series. As the study was retrospective, the most common reason for exclusion was lack of pre-treatment or follow-up sputum sample(s). From the excluded subjects, the majority were treated before 2008 (data not shown). The median age of patients with CF and PCD was 33 years (range 12–44) and 43 years (range 13–71), respectively. Two subjects were under 18 years of age. There were 10 out of 17 (59%) women in the CF group and 6 out of 12 (50%) women in the PCD group. None of the patients had received endoscopic sinus surgery before or during the gentamycin treatment. No side effects such as ototoxicity and nephrotoxicity were reported in the patients' medical records. The characteristics of CF and PCD case series have been summarized in Tab. I. and Tab. II.

Pre-treatment culture characteristics

Twenty out of 29 (69%) patients [CF 12/17 (71%); PCD 8/12 (67%)] demonstrated bacterial growth in the pre-treatment culture. The most frequent bacteria in CF patients were *S. aureus*, which were identified in 7 out of 17 (41%) CF patients. Five out of seven *S. aureus* isolates were tested for susceptibility to gentamycin and all were found to be sensitive, whereas the remaining two isolates were not tested for susceptibility. The second most common bacteria in CF patients were *P. aeruginosa*, which were identified in

Tab. II. The case series of 12 PCD patients treated for CRS with the gentamycin nasal spray. Demographic data and bacteria isolates in pre-treatment and follow-up sputum cultures are presented. ND – not done; N – not found; Y – found; S – sensitive; R – resistant, nm – nonmucoid; PA – *P. aeruginosa*; SA – *S. aureus*; Hi – *H. influenza*; Mc – *M. catarrhalis*; G strep – group G *Streptococcus*; C strep – group C *Streptococcus*; M – male; F – female; *not sensitivity tested. Patients nr 2 developed resistance for gentamycin under treatment.

	PRE-TREATMENT					6-MONTH FOLLOW-UP			12-MONTH FOLLOW-UP			18-MONTH FOLLOW-UP		
	AGE	GENDER	SA	PA	OTHER	SA	PA	OTHER	SA	PA	OTHER	SA	PA	OTHER
1	71	F	N	S nm	N	N	S nm	N	N	S nm	N	N	S nm	N
2	68	F	N	S	N	S	S	N	N	R nm	Hi*	N	N	Hi*
3	13	M	N	N	N	N	N	N	N	N	N	S	N	N
4	63	M	N	N	N	N	N	N	N	N	Hi*	N	N	Hi*
5	53	F	Y*	N	N	N	S	N	ND	ND	ND	N	S	N
6	48	M	N	N	N	N	N	N	N	N	N	N	N	N
7	25	M	N	N	N	N	N	N	N	N	N	N	N	Hi*
8	27	F	N	N	N	N	N	N	N	S	N	N	N	Hi*
9	26	F	N	N	Hi*	N	N	N	N	N	N	S	N	N
10	39	M	N	N	Hi*	N	N	Hi*	Y*	N	Mc* G strep*	N	N	Hi*
11	38	F	N	N	Hi*	N	N	N	N	N	N	N	S	N
12	35	F	N	S	N	N	N	N	S	N	C strep*	S	N	N

4 out of 17 (24%) individuals with CF. Two of these patients had isolates sensitive to gentamycin and an additional two were resistant to gentamycin. *Burkholderia cepacia* complex (Bcc) was found in two other CF patients (n = 2/17, 12%) and both isolates were multi-resistant, including showing resistance to gentamycin. *S. pneumoniae* was found in one patient (n = 1/17, 6%). In PCD patients, the most common bacteria were *P. aeruginosa* (n = 3/12, 25% of PCD) and *H. influenza* (n = 3/12, 25% of PCD). This was followed by *S. aureus* presented in 8% (n = 1/12) of PCD patients. Each patient's pre-treatment culture is shown in Tab. I and Tab. II. and a summary of bacterial distribution is presented in Fig. 1.

Follow-up culture characteristics

The follow-up sputum cultures in CF and PCD patients were divided into three groups taken at 6, 12 and 18 months following gentamycin spray induction. Taken together, in the follow-up samples the most common bacterial strain in CF patients was *P. aeruginosa* (n = 19/47, 40%), followed by *S. aureus* (n = 15/47, 32%). In PCD patients the most common bacterial strain was also *P. aeruginosa* (n = 9/35, 26%), followed by *H. influenza* (n = 8/35, 23%) and *S. aureus* (n = 6/35, 17%). From other bacteria were *S. pneumoniae* and group A *Streptococcus* (GAS) in CF, as well as *S. pneumoniae*, group C and G *Streptococcus*, *M. catarrhalis* in PCD.

The eradication rate for *S. aureus* was 37% (n = 3/8) for CF. There were no cases of *S. aureus* eradication in PCD and no *P. aeruginosa* eradication in CF and PCD patients. There was a 40% (n = 2/5) eradication rate of *H. influenza* in PCD patients (Tab. I. and II.). The pathogen characteristics in each patient and each sputum sample are shown in Tab. I. and Tab. II. In addition, the distribution of bacterial species each time bacterial samples were collected are summarized in Fig. 1.

Resistance to gentamycin

Three patients with CF (18%) and one patient with PCD (8%) developed bacterial strains that were resistant to gentamycin dur-

ing treatment with gentamycin spray. All resistant strains were *P. aeruginosa* of both phenotypes mucoid and non-mucoid (Tab. I. and II.). We also found that all four patients had been administered multiple courses of other antibiotics primarily for pulmonary infections during treatment with gentamycin spray (the indication, antibiotic(s), dose and length of treatment are summarized in the Supplementary Table). Additionally, a further two CF patients had a multi-resistant Bcc already isolated in the pre-treatment culture.

DISCUSSION

Patient characteristics

In CF and PCD patients, resistance to antibiotics can lead to a significant increase in morbidity and mortality [1, 2]. Nevertheless, the potential impact of longitudinal low dose local antibiotic exposure on respiratory tract bacteria in CF and PCD patients has not been previously studied. To address this knowledge gap, we retrospectively identified patients with CF or PCD who had been prescribed gentamycin nasal spray for CRS. The therapy was empirical and continued for at least 6 months. We assessed the distribution of each bacterial species found in pre-treatment and follow-up cultures at 6, 12 and 18 months and placed great emphasis on pathogen susceptibility to gentamycin.

Resistance development

Our most important finding was that 18% (n = 3/17) of CF patients and 8% (n = 1/12) of PCD patients developed bacterial strains resistant to gentamycin during treatment with gentamycin nasal spray. In all four cases the resistant bacteria were *P. aeruginosa* isolates, a gram-negative opportunistic pathogen. In fact, all these patients had also been treated with different antibiotics for pulmonary infections during the time that gentamycin nasal spray was being administered which leaves numerous questions unanswered regarding the mechanisms behind the resistance. However, this treatment

is not surprising as, in accordance with international guidelines, PCD and CF patients positive for pathogenic bacteria isolated from the lower airways should be treated with systemic antibiotics for two weeks, even in the absence of clinical symptoms [19]. The only PCD patient who developed resistance to gentamycin had a resistant *P. aeruginosa* isolate at the 12-month follow-up but no bacterial growth 6 months later. Instead, in all three CF patients, *P. aeruginosa*-resistant isolates remained in the sputum culture up to the 18-month follow-up. A further two CF patients already had *P. aeruginosa* resistant isolates in the pre-treatment culture and they were also present in the follow-up. Thus, this confirms that in the majority of CF cases, once bacteria have established a chronic infection they can hardly ever be eradicated [20].

In addition, two other CF patients had multi-resistant gram-negative Bcc including resistance to gentamycin in the pre-treatment culture. One of the two CF patients had no bacterial growth in the subsequent three follow-up sputum samples. Such successful eradication of Bcc is uncommon. Instead, infections with multiple antibiotic-resistant bacteria have been associated with accelerated progression of CF [21, 22]. Previous reports have shown that the prevalence of Bcc is rather low in CF patients. However, it may lead to necrotizing pneumonia and sepsis with a high mortality rate [13]. According to a recent systematic review, Bcc have never been isolated from PCD patients [13]. We did not find either Bcc in any of the PCD patients in our case series.

Pathogen distribution

In our study, the most common bacteria in pre-treatment sputum were *S. aureus* (41%) followed by *P. aeruginosa* (24%) in CF patients and *P. aeruginosa* (25%) and *H. influenza* (25%) followed by *S. aureus* (8%) in PCD patients. In the follow-up, *P. aeruginosa* (39%) followed by *S. aureus* (32%) were the most frequently identified bacteria in CF patients while *P. aeruginosa* (26%) followed by *H. influenza* (23%) and *S. aureus* (17%) were the most frequently identified bacteria in PCD patients. Other bacteria comprised only single strains of *S. pneumonia*, *M. catarrhalis* and staphylococci in both CF and PCD patients and Bcc in CF patients. Similar findings in the bacterial distribution in adults have been reported in previous studies [1, 13]. The risk of *P. aeruginosa* and *S. aureus* infections increases with disease progression and the age of patients [13]. Importantly, *S. aureus* can be a predecessor of later infections by *P. aeruginosa* in CF patients [23], which we also observed in our case series. It is known that *S. aureus* employs adhesion protein and a wide array of factors that weaken the host defence, including the production of penicillin-binding protein that is responsible for methicillin resistance and MRSA phenotype. Although recent epidemiological reports showed an increase of *S. aureus* MRSA in CF patients in particular [24], we did not observe any MRSA in our patients (data not shown).

We found that *P. aeruginosa* was the predominant bacterial species in pre-treatment sputum samples in PCD and in follow-up sputum samples in both CF and PCD. The presence of *P. aeruginosa* in particular emerges due to its ability to adapt to the hypoxic condition of the CF/PCD airways, including slow growth, loss of motility, quorum sensing and overproduction of alginate [25]. This leads to phenotypic changes [26] from a non-mucoid form to a more aggressive mucoid form that produces biofilm associated with

reduced bioavailability and greater resistance to antibiotics [27]. A mucoid phenotype was shown in four CF patients. Three of the patients were those who developed resistance to gentamycin during treatment with topical gentamycin and one patient was already resistant to gentamycin isolate in the pre-treatment sputum sample. Since mucoid forms indicate a chronic *P. aeruginosa* infection, we suspected that this was the case in the four CF patients. Similarly to other studies [28], in PCD patients we found only a nonmucoid phenotype. As suggested by other studies, the lack of a mucoid form in PCD means that bacteria may have a longer adaptive process in PCD patients compared to CF patients [28].

Gentamycin nasal spray characteristics

Gentamycin nasal spray, called the “Wilson solution” [39], has been used at our institution for precisely 20 years as an empirical long-term treatment, in addition to isotonic saline nasal irrigation, for moderate-to-severe CRS in CF and PCD patients. Gentamycin is a wide-spectrum aminoglycoside antibiotic that is primarily effective for gram-negative *P. aeruginosa* but also certain gram-positive bacteria such as *S. aureus*, the major bacterial pathogens found in CF and PCD patients. The use of isotonic saline nasal irrigation prior to gentamycin aims to remove inflammatory cells and mucopurulent secretion and expose the mucosal membrane to subsequently administered local medication in order to optimize its pharmacological efficacy [29]. The intranasal administration of gentamycin will be delivered at a high concentration directly at the site of infection and could avoid systemic exposure. The benefits include a reduced risk of the dose and duration limiting the systemic toxicities of gentamycin, particularly in hearing loss and nephrotoxicity [30]. Importantly, none of the patients included in our study reported any adverse effects. Thus, we assumed that gentamycin spray treatment at 80 µg/ml used three times daily twice in each nostril had been uneventful. In the previous study when patients were administered 2.5 mg gentamycin twice daily as sinus irrigation, the serum gentamycin level elevation measured after 3 weeks was 0.42 µg/ml [14]. This is low in comparison with 4 µg/ml, which is recommended in addition to systemic administration [40]. Even one high dose of gentamycin (60 mg) during endoscopic sinus surgery showed only a slight serum gentamycin elevation [16]. There was no sign of hearing loss in either of the two studies [14, 16].

Previous studies on local antibiotics for CRS in CF and PCD

Based on the sinus-lung transfer theory, microbiota first adapts to the sinuses and then migrates to the lungs, causing pulmonary infections [7]. Thus, early treatment of CRS may not only improve the patient's quality of life but also have a long-term benefit on the degree of bacterial transmission to the lungs. The intensive eradication of bacteria is a crucial part of the management of CF and PCD patients who are highly susceptible to bacterial respiratory infections. Recent evidence-based studies showed that inhaled antibiotics (the most often-used tobramycin) significantly reduce pulmonary colonization in CF [24] and PCD patients [31]. It is still unclear whether the use of gentamycin spray for CRS in CF and PCD patients is beneficial. No systematic review has been published thus far. Previous studies on CF where topical antibiotics were administered as adjuvant therapy after endoscopic sinus surgery have been promis-

ing in terms of reduced symptoms, endoscopic appearance and the need for repeat surgeries [2, 32]. Nevertheless, in non-operated patients, the outcomes of topical antibiotics were less beneficial in CF compared to non-CF [33]. In the present study none of the patients were treated with endoscopic sinus surgery before treatment with gentamicin spray. It has been suggested that surgery might reduce penetration of antibiotics to non-operated sinuses [33]. Interestingly, in a recent study that evaluated the distribution of local antibiotics with radioactive marked saline administered into the sinuses of CF patients, no difference in fluid deposition was noted prior to or after surgery. It was proposed that sinonasal mucosa could be pre-operatively covered in mucus and swell after surgery, obstructing penetration of an antibiotic agent [34].

No previous reports have investigated resistance development during the administration of local antibiotics. The single study on the use of the Wilson solution is a report on 38 paediatric non-CF/PCD patients with CRS. Notably, the Wilson solution was administered as nasal irrigation twice daily (2 mg per nostril/dose) for a minimum of 3 months and demonstrated a reduction of symptoms and an increase in patient satisfaction compared to those patients treated with saline irrigation only [39].

Strengths and limitation of the study

The strength of our study is that it investigates the long-term follow-up of resistance development after the use of topical antibiotics for CRS in CF and PCD patients. Choosing patients with CF and PCD as the treatment group is not a coincidence as they are the group of patients most susceptible to respiratory tract infections. Also, the use of topical antibiotics for CRS has been poorly investigated, particularly in PCD. Our study is also unique as it investigates both PCD patients and CF patients simultaneously.

The main limitation of our study is that it is a retrospective case series with no control group. The follow-up cultures were only taken at 6-month intervals whereas recent protocols recommended at least every 3 months in order to use the Leeds criteria. According to the Leeds criteria, patients can be divided into groups based on airway culture results over the course of one year [35]. More than 50% of positive airway cultures for a specific pathogen is a definition of a chronic infection. In contrast, only subsequently repeated positive cultures are classified as intermittent infections. In this study we disposed of only two bacterial cultures per year and in certain patients one or two follow-ups were missed. This could have resulted in intermittent infections being omitted. Further, for cases in which one (or two) of three followed-up bacterial culture were missing, treatment was continued without verification of the bacterial species, which is against current recommendations. However, as suggested by other studies in the case of local and long-term therapy, susceptibility could be of less significance as a resistant bacterium may still be susceptible due to the high

concentration of antibiotics applied directly to the site [36]. Even routine microbiological testing and susceptibility to antibiotics may not necessarily correlate with the clinical outcome [28].

Further, we based our study on sputum samples that represented lower airway microbiota. However, the sputum could be contaminated by oropharyngeal flora. The current gold standard for evaluation of microbiota in the upper and lower airways is sinus aspiration [17] and bronchoalveolar lavage (BAL), respectively. These sampling procedures are unfortunately painful, time-consuming and expensive and, like us, many institutions have replaced these procedures with non-invasive alternatives such as sputum sampling [37].

Another significant weakness of this study is that not all of the *S. aureus* strains and none of the *H. influenza* strains – two out of the three most frequent bacteria in our patients' sputum samples – were sensitivity tested for gentamicin. Other gram-positive bacteria such as *S. pneumoniae* and *Streptococcus* were either susceptibility tested for gentamicin in accordance with our laboratory routine or were tested only when ordered. Furthermore, we did not study any alternative to the gentamicin spray such as nasal irrigation with antibiotics. Moreover, the interactions between the gentamicin spray and the use of systemic antibiotics in those patients who developed resistance to gentamicin were not taken into account. This could be significant because systemic antibiotics may amplify as well as reduce local antimicrobial activity [38].

CONCLUSIONS

The current study is a retrospective case series that offers a glimpse into topical gentamicin for CRS in CF and PCD patients. Antibiotic resistance in CF and PCD patients is a major concern because of the multiple courses of antibiotic treatment that such patients often receive. Our study could be the first of its kind to focus on the long-term resistance development of nasal topical antibiotics used as maintenance therapy and prophylaxis against worsening of the CRS. Despite all study weaknesses, we clearly demonstrated that there was moderate incidence of gentamicin resistance in CF and PCD patients during the use of gentamicin nasal spray at our institution. However, there is still little evidence to support the use of topical antibiotics for the management of CF and PCD and a prospective cohort study is necessary in order to fully evaluate the therapy. To date there are two ongoing clinical trials on topical gentamicin for CRS: on adults, retrospective and case-based study and on a paediatric non-operated population (Phase 4) [41].

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