Respiratory Medicine 117 (2016) 190–197

Contents lists available at ScienceDirect

Respiratory Medicine

journal homepage: www.elsevier.com/locate/rmed

Review article

The effect of *N*-acetylcysteine on biofilms: Implications for the treatment of respiratory tract infections

Francesco Blasi [a], Clive Page [b], Gian Maria Rossolini [c, d, e, f], Lucia Pallecchi [e], Maria Gabriella Matera [g], Paola Rogliani [h, i], Mario Cazzola [h, *]

[a] Department of Pathophysiology and Transplantation, University of Milan, IRCCS Fondazione Cà Granda Ospedale Maggiore Policlinico, Milan, Italy
[b] The Sackler Institute of Pulmonary Pharmacology, Institute of Pharmaceutical Science, King's College London, United Kingdom
[c] Department of Experimental and Clinical Medicine, University of Florence, Careggi University Hospital, Florence, Italy
[d] Clinical Microbiology and Virology Unit, Careggi University Hospital, Florence, Italy
[e] Department of Medical Biotechnologies, University of Siena, Santa Maria alle Scotte University Hospital, Siena, Italy
[f] Don Carlo Gnocchi Foundation, Florence, Italy
[g] Department of Experimental Medicine, Unit of Pharmacology, Second University of Naples, Naples, Italy
[h] University of Rome Tor Vergata, Department of Systems Medicine, Rome, Italy
[i] University Hospital Tor Vergata, Unit of Respiratory Medicine, Rome, Italy

ARTICLE INFO

Article history:
Received 9 March 2016
Received in revised form
12 June 2016
Accepted 15 June 2016
Available online 16 June 2016

Keywords:
N-acetylcysteine
Biofilm
Airways infections
Topical administration
Inhaled formulation

ABSTRACT

Objectives: In airway infections, biofilm formation has been demonstrated to be responsible for both acute and chronic events, and constitutes a genuine challenge in clinical practice. Difficulty in eradicating biofilms with systemic antibiotics has led clinicians to consider the possible role of non-antibiotic therapy. The aim of this review is to examine current evidence for the use of *N*-acetylcysteine (NAC) in the treatment of biofilm-related respiratory infections.

Methods: Electronic searches of PUBMED up to September 2015 were conducted, searching for 'biofilm', 'respiratory tract infection', '*N*-acetylcysteine', 'cystic fibrosis', 'COPD', 'bronchiectasis', 'otitis', and 'bronchitis' in titles and abstracts. Studies included for review were primarily in English, but a few in Italian were also selected.

Results: Biofilm formation may be involved in many infections, including ventilator-associated pneumonia, cystic fibrosis, bronchiectasis, bronchitis, and upper respiratory airway infections. Many in vitro studies have demonstrated that NAC is effective in inhibiting biofilm formation, disrupting preformed biofilms (both initial and mature), and reducing bacterial viability in biofilms. There are fewer clinical studies on the use of NAC in disruption of biofilm formation, although there is some evidence that NAC alone or in combination with antibiotics can decrease the risk of exacerbations of chronic bronchitis, chronic obstructive pulmonary disease, and rhinosinusitis. However, the usefulness of NAC in the treatment of cystic fibrosis and bronchiectasis is still matter of debate. Most of the studies published to date have used oral or intramuscular NAC formulations.

Conclusions: Evidence from in vitro studies indicates that NAC has good antibacterial properties and the ability to interfere with biofilm formation and disrupt biofilms. Results from clinical studies have provided some encouraging findings that need to be confirmed and expanded using other routes of administration of NAC such as inhalation.

Contents

* Corresponding author.
E-mail address: mario.cazzola@uniroma2.it (M. Cazzola).

http://dx.doi.org/10.1016/j.rmed.2016.06.015
0954-6111/

1. Introduction

Bacteria can exist as single, independent cells (planktonic) or can be organized into sessile aggregates called biofilms. A biofilm is a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface. Acute infections are assumed to involve planktonic bacteria, which are generally treatable with antibiotics, although successful treatment depends on accurate and fast diagnosis, and treatment with an appropriate antibiotic. However, in cases where the bacteria succeed in forming a biofilm within the human host, the infection is often resistant to standard treatment regimes and will therefore develop into a chronic state. Recent advances have demonstrated that biofilms account for most human infections [1,2] and are related to exacerbation or relapse of symptoms. Characteristic features of chronic biofilm-based infections are increased resistance to host defenses and decreased susceptibility to antimicrobial agents. These features make persistent infections difficult or impossible for the immune system to clear and to be eradicated with antibiotics [2,3].

In airway infections, biofilm formation has been demonstrated to be responsible for both acute and chronic events and is a real challenge in clinical practice [1,2]. The observation that systemic antibiotics are not unequivocally effective in eradicating biofilms has led to an increased interest in non-antibiotic therapies. In this review, we discuss the role of biofilms in respiratory infections and current management strategies, focusing on the current evidence regarding the effects of NAC on biofilms.

2. Literature search methodology

Literature searches, conducted in the period August–September 2015, were performed using the PubMed database (with no date limitations), searching with the terms 'biofilm', 'respiratory tract infection', '*N*-acetylcysteine', 'cystic fibrosis', 'COPD', 'bronchiectasis', 'otitis', and 'bronchitis' in titles and abstracts, and restricting the results primarily to articles written in English. A few publications in Italian were also included. The authors examined the resulting lists of abstracts and excluded those that did not fit within the scope of the present review.

2.1. Biofilms in respiratory tract infections

2.1.1. Device-related infections

In ventilator-associated pneumonia (VAP), biofilms are responsible for microbial persistence and impaired response to treatment. Biofilm formation within the first 24 h after intubation has been demonstrated in 95% of endotracheal tubes. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are the most frequent bacteria that colonize the devices [4–6].

2.1.2. Tissue-related infections

2.1.2.1. Cystic fibrosis (CF). In CF, the incidence of bacterial lung infections is high since the mucoid polysaccharidic material that accumulates on the respiratory epithelium due to impaired mucociliary clearance in the bronchi of such patients favors biofilm formation. *P. aeruginosa* is the most common bacterial species involved in respiratory tract infection in CF patients and can be found in about half of all cases and in up to 70% of adults (Cystic Fibrosis Foundation Patient Registry. Annual data report 2013 Cystic Fibrosis Foundation, Bethesda, MD). The ability of *P. aeruginosa* to form biofilms is thought to be the primary reason for its survival in the CF lung, despite an exuberant inflammatory response and intensive antibiotic treatment [7,8]. Other pathogens such as *Burkholderia cepacia* complex, *Staphylococcus aureus*, *Achromobacter xylosoxidans*, and *Stenotrophomonas maltophilia* have also been identified in CF and are related to biofilm formation [9].

2.1.2.2. Chronic obstructive pulmonary disease (COPD). A role of biofilms in patients with COPD has not been directly demonstrated but has been hypothesized considering the evidence indicating that the airways of these patients are frequently colonized by pathogens such as *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pneumoniae*. COPD is characterized by frequent exacerbations and resistance to antibiotics. Even if direct evidence of biofilms *in vivo* is lacking, biofilms may reasonably be considered to be involved in the vicious cycle of infection/inflammation leading to disease progression in patients with COPD [10–12]. However, the role of biofilms in acute exacerbations needs to be further investigated (i.e. acute episodes caused by new strains or species compared to those accounting for chronic colonization).

2.1.2.3. Non-cystic fibrosis bronchiectasis. In bronchiectasis not due to CF, infections cause a change in the muscular and elastic components of the bronchial wall, which become distorted and enlarged. Airways slowly become unable to clear mucus, leading to serious lung infections that in turn cause more damage to bronchi. Biofilm formation has recently been demonstrated *in vivo* and is assumed to play a relevant role in the pathophysiological cascade of this disease [13–15]. Bacterial biofilm formation by *P. aeruginosa* or *Klebsiella pneumoniae* is common in bronchiectasis and could be an important factor that makes infections in bronchiectasis intractable. Other pathogens such as *Veilonella* sp., *Prevotella* sp. and *Neisseria* sp. have also been recently identified in patients with bronchiectasis [16,17].

2.1.2.4. Bronchitis. Protracted bacterial bronchitis may be caused by chronic infections of the airways. Especially in children, the condition appears to be secondary to impaired mucociliary clearance that creates an environment favorable for bacteria to become established, often in the form of biofilms [18]. The most commonly involved bacteria include *H. influenzae* (30–70%), *S. pneumonia*, and *M. catarrhalis*.

2.1.3. Upper respiratory infections

In otitis media, infections are due to both respiratory viruses and bacteria such as *S. pneumonia* (25–40%), non-capsulated *H. influenzae* (25–40%), *M. catarrhalis* (20%), *Streptococcus pyogenes*, and *S. aureus* (<10%), causing the appearance of polymicrobial biofilms [19–21]. Biofilms were identified in the sinus tissues of 72% of patients affected by chronic rhinosinusitis; the cultured organisms identified included *S. aureus* (50%), *H. influenzae* (28%), *P. aeruginosa* (22%), and fungi (22%). The presence of bacterial biofilms was strongly associated with persistent mucosal inflammation after endoscopic sinus surgery [22].

2.2. Biofilm development and functioning

Five stages have been identified in biofilm development (Fig. 1). Early attachment is the first reversible stage: planktonic microbial cells adhere to the surface through weak, reversible van der Waals forces. If the process progresses, the early attachment is followed by irreversible late attachment where bacteria firmly attach to the surface through fimbrial and nonfimbrial adhesins and begin producing extracellular polymeric substances (EPS). Next, early-stage biofilms (third stage: maturation stage I) take form that consist of microcolonies immersed in EPS. When the biofilm matures (maturation stage II), it is characterized by microcolonies separated by open water channels that act as a primitive circulatory system. The mature biofilm begins to release planktonic cells and bacterial aggregates (septic emboli) in the dispersion stage.

This complex process relies on the ability of bacteria to function cooperatively through a cell-cell communication process called quorum sensing. Bacterial gene expression is regulated by bacterial density leading to either an enhancement or a decrease of their virulence factors [2].

Due to their nature, biofilms are more resistant than planktonic cells to host defenses and antibiotics. Resistance to host defenses (phagocytes, complement and antibodies) is related to the presence of the EPS, which protect bacteria growing in the biofilm from phagocytes and humoral effectors [2,3,20]. Resistance to antibiotics is due to several factors including: i) a reduced penetration of drugs across the EPS matrix (demonstrated for some antibiotics that may actually be trapped by the EPS matrix, such as glycopeptides) [2,3,20]; ii) the physiological state of vegetative cells growing in the biofilm (slow growth, anaerobic environment) that may render them less susceptible to some antibiotics (e.g. beta-lactams, aminoglycosides) [2,3,20]; iii) the presence of persister cells that, due to their state, are highly resistant to antibiotics and can subsequently regenerate vegetative cells within the biofilm [2,3,20].

Anti-biofilm strategies may act by preventing bacterial adhesion (e.g. modifying roughness and physicochemical properties of biomaterials), impairing survival of the attached biofilm (e.g. using surfaces covered with Cu/Ag nanoparticles, antibiotics, or other antimicrobial agents), inhibiting the quorum-sensing response that is essential to biofilm formation, or disrupting the formed biofilm (using enzymes that degrade the matrix such as dispersin, DNase I) [2] (Fig. 2). A very promising perspective, although still at an early stage of development, is the use of substances that are active against persister cells or that sensitize these cells to antimicrobial agents [2,23].

In respiratory tract infections many strategies have been developed. Antibiotics that penetrate the biofilm matrix and have a bactericidal rather than bacteriostatic mode of action can be useful. Combined antibiotic therapies seem to be better than monotherapy, and high dosages appear to be necessary to disrupt biofilms.

However, antibiotics alone seem unable to resolve the problem of biofilm infections, not only because of biofilm resistance, but also because of dispersion limitations posed by the biofilm extracellular matrix [2,24,25]. Apart from antimicrobials, several different compounds have been investigated in vitro for their potential to reduce biofilm formation. For example, non-steroidal anti-inflammatory drugs (NSAIDs) and mucolytics have been shown to have inhibitory effects on biofilm production [26–28].

2.3. The role of N-acetylcysteine against biofilms

2.3.1. In vitro studies

In vitro studies have indicated a potential role of NAC as an anti-

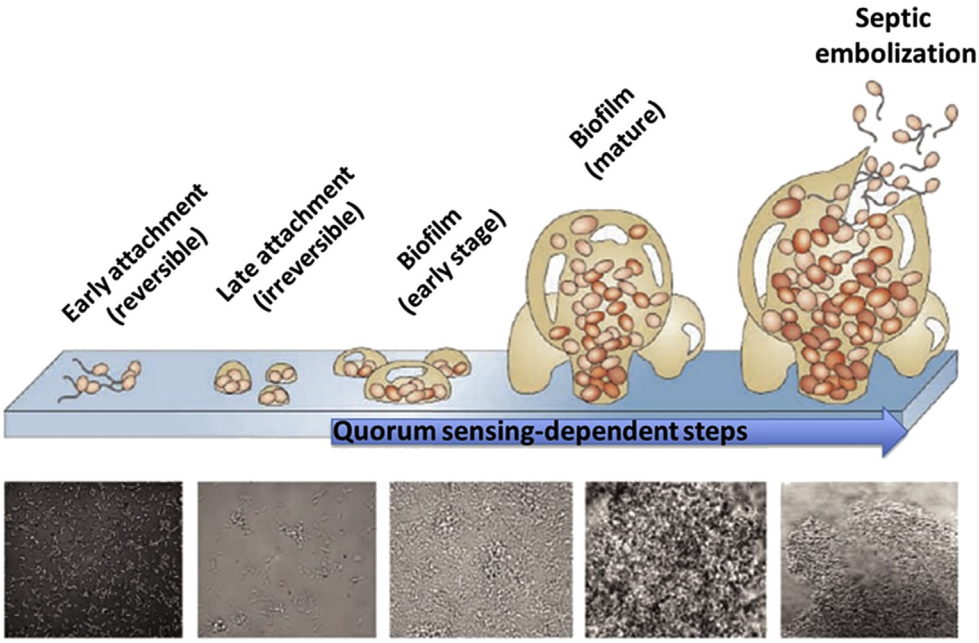

Fig. 1. Stages of biofilm development. Each stage in the diagram has been paired with a photomicrograph of a developing *Pseudomonas aeruginosa* biofilm. Adapted from Davies [3]

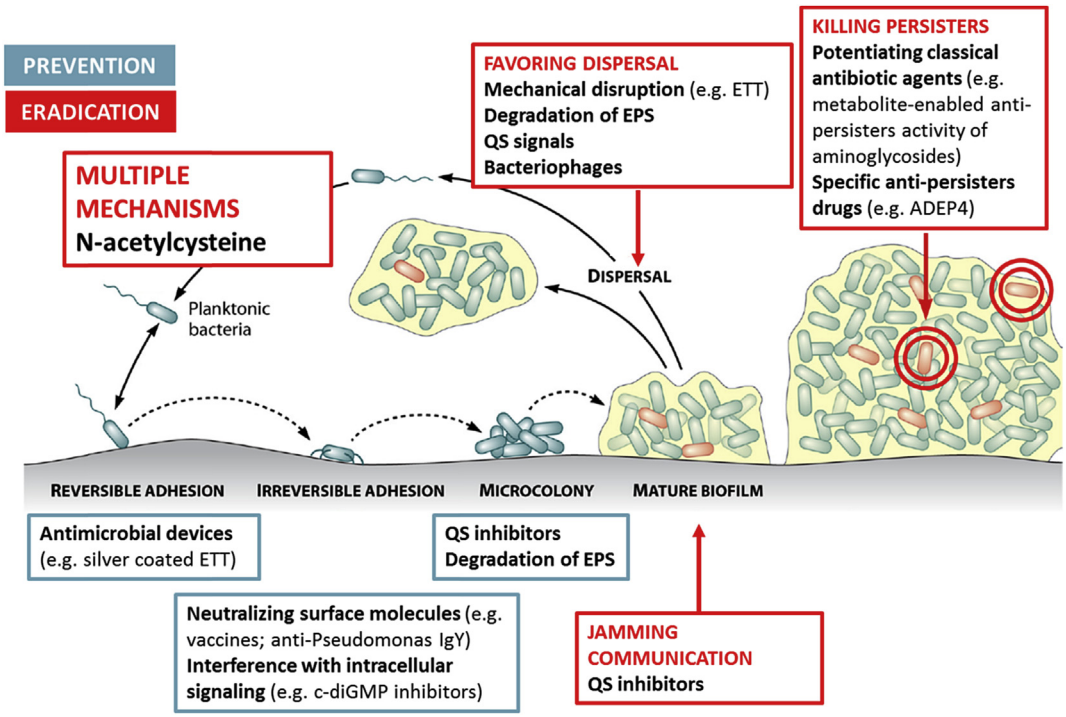

Fig. 2. Anti-biofilm strategies. EPS = extracellular polymeric substances; ETT = endotracheal tubes; QS = quorum sensing. Adapted from Lebeaux et al. [2]. Copyright © 2014, American Society for Microbiology. All Rights Reserved.

biofilm agent. In fact, NAC has been reported to have antimicrobial activity against different microorganisms, and has been suggested to play a role in the various steps of biofilm formation: adhesion to inert and living surfaces, matrix production and organization, and dispersal of preformed biofilms (see below).

The ability of NAC to interfere with biofilm formation was first demonstrated by Pérez-Giraldo and colleagues in 1997 [29]. In that investigation, the authors evaluated the effects of different NAC concentrations on bacterial growth and biofilm formation in cultures of *Staphylococcus epidermidis*. This study reported a concentration-related decrease in biofilm formation (at concentrations >0.25 mg/ml); furthermore, the inhibitory effect of 2 mg/ml of NAC on matrix formation was demonstrated by electron microscopy.

Since then, many other studies have demonstrated the efficacy of NAC in reducing biofilm formation induced by a variety of microorganisms (including Gram-negative and Gram-positive bacteria, and yeasts), and shown its ability to impair matrix architecture and promote disruption of biofilm. Table 1 reports a selection of publications on these topics.

One of these studies investigated the effect of NAC on biofilm formation and dispersal with a collection of clinical isolates of *P. aeruginosa* [38], which are known to be among the most important opportunistic pathogens that are responsible for biofilm-associated chronic respiratory colonization in patients with cystic fibrosis, COPD, and bronchiectasis. The results showed that NAC had some antimicrobial activity against planktonic cultures (minimum inhibitory concentrations [MIC] for the majority of isolates were ≤40 mg/ml). Mature biofilms of *P. aeruginosa* PAO-1 expressing a green fluorescent protein could be detached from glass cover slips at NAC concentrations as low as 0.5 mg/ml, as shown by confocal laser scanning microscopy. Using the dimethylthiazol diphenyltetrazolium bromide assay for determining viability of biofilm cells, the authors observed a dose-dependent dispersal of mature biofilms formed by clinical isolates, despite the low concentrations of NAC tested (i.e. 0.5–2.5 mg/ml), and a synergistic interaction with ciprofloxacin. In addition, EPS production by *P. aeruginosa* was found to decrease by 27% and 44% at NAC concentrations of 0.5 mg/ml and 1 mg/ml, respectively. Recently, NAC was also demonstrated to significantly potentiate the efficacy of photodynamic therapy against *S. aureus* biofilms [48].

Despite the efficacy of NAC in association with antibiotics in some infections (i.e. urinary tract infections, device related infections) [33], few studies to date have been focused on biofilm-associated respiratory tract infections. In particular, Lea and colleagues [36] evaluated the effects of ciprofloxacin alone, ciprofloxacin + dexamethasone, NAC alone, and NAC + ciprofloxacin on 15 strains of *P. aeruginosa* isolated from patients with suppurative otitis media. While *P. aeruginosa* strains grew in the presence of ciprofloxacin + dexamethasone and ciprofloxacin alone, no growth was found in the sessile or planktonic state among all 15 strains when NAC (≥5 mg/ml) was used either alone or in combination with ciprofloxacin. Another study [49] assessed the ability of 11 pneumococcal strains (serotypes 3, 6B, 9V, 19F, and 23F) to form biofilms on polystyrene plates. Human serum albumin at 25,000 μg/ml and ibuprofen at 128 μg/ml both significantly reduced biofilm formation in 7 and 5 strains, respectively. Amoxicillin, erythromycin, and levofloxacin at concentrations above the MIC were very active against planktonic cells of 3 strains, but less or no active against biofilms. NAC alone had little activity against planktonic and sessile cultures, but when combined with the 3 antibiotics, a slightly enhanced activity against biofilms was observed in some strains.

Some *in vitro* studies have also demonstrated the ability of NAC to affect adherence to respiratory epithelial cells of relevant respiratory pathogens [50,51]. Riise and colleagues [50] studied the effects of four compounds (NAC, lidocaine, hydrocortisone, and terbutaline) on bacterial adherence of oropharyngeal epithelial cells after short-term exposure and long-term incubation. *S. pneumoniae* and *H. influenzae* were the target bacteria. Following

Table 1
In vitro studies demonstrating anti-biofilm activity of NAC against bacterial and fungal pathogens.

Pathogens examined	Reference	NAC concentrations tested (mg/ml)
Gram-negative bacteria		
Escherichia coli	El Feki et al., 2009 [30]	2 and 4
	Marchese et al., 2003 [31]	range 0.007–8
Klebsiella pneumoniae	Mohsen et al., 2015 [32]	2.5
	Aslam and Darouiche, 2011 [33]	80
	El Feki et al., 2009 [30]	2 and 4
	Aslam et al., 2007 [34]	80
	Olofsson et al., 2003 [35]	range 0.25–2
Enterobacter cloacae	Aslam and Darouiche, 2011 [33]	80
	Olofsson et al., 2003 [35]	range 0.25–2
Proteus spp.	Mohsen et al., 2015 [32]	2.5
	El Feki et al., 2009 [30]	2 and 4
Pseudomonas aeruginosa	Mohsen et al., 2015 [32]	2.5
	Lea et al., 2014 [36]	12.5
	Drago et al., 2013 [37]	range 3–24
	Aslam and Darouiche, 2011 [33]	80
	Zhao et al., 2010 [38]	range 0.5–10
	El Feki et al., 2009 [30]	2 and 4
Pseudomonas mendocina	Olofsson et al., 2003 [35]	range 0.25–2
Acinetobacter baumannii	Olofsson et al., 2003 [35]	range 0.25–2
Prevotella intermedia	Moon et al., 2015 [39]	range 0.375–3
Gram-positive bacteria		
Staphylococcus aureus	Mohsen et al., 2015 [32]	20
	Drago et al., 2013 [37]	range 6–24
	Aslam and Darouiche, 2011 [33]	80
	El Feki et al., 2009 [30]	2 and 4
	Aslam et al., 2007 [34]	80
	Roveta et al., 2004 [40]	8
	Bozzolasco et al., 2002 [41]	range 0.007–8
Staphylococcus epidermidis	Leite et al., 2013 [42]	4 and 40
	Kirmusaoğlu et al., 2012 [43]	0.03, 0.12, 0.5, and 2
	Gomes et al., 2012 [44]	4 and 40
	Aslam and Darouiche, 2011 [33]	80
	El Feki et al., 2009 [30]	2 and 4
	Venkatesh et al., 2009 [45]	range 0.5–32
	Aslam et al., 2007 [34]	80
	Perez-Giraldo et al., 1997 [29]	range 0.003–8
Enterococcus faecalis	Quah et al., 2012 [46]	range 12.5–50
Yeast		
Candida albicans	El-Baky et al., 2014 [47];	range 0.312–40
	Venkatesh et al., 2009 [45]	range 0.5–32

short-term exposure, NAC had an inhibitory effect on *H. influenzae* adhesion and was seen to be effective in inhibiting adherence even after long-term incubation. Both NAC and hydrocortisone lowered adherence of both strains in a concentration-dependent manner. NAC was also effective at inhibiting bacterial adhesion in the majority of *H. influenzae* (3 of 4) and all *S. pneumoniae* (n = 3) strains. Zengh and colleagues demonstrated a significant reduction in the attachment to human pharyngeal epithelial cells by *M. catarrhalis* after exposure to mucoregulating drugs, including NAC [51]. In this study, three strains of *M. catarrhalis* isolated from sputum of patients with respiratory infections were treated with NAC or S-carboxymethylcysteine and their ability to attach to pharyngeal epithelial cells was measured thereafter. A statistically significant reduction in attachment for both drugs was seen that was concentration-dependent.

Taken together, in vitro studies suggest that NAC has a promising anti-biofilm activity. The mechanisms accounting for the antimicrobial and anti-biofilm activity of NAC, however, are still largely unknown and deserve further investigation to fully understand the potential for NAC in the management of biofilm-related infections. It has been suggested that the antimicrobial activity of NAC could be related to: i) competitive inhibition of cysteine utilization; ii) reaction of the NAC sulfhydryl group with bacterial proteins; and iii) perturbation of the intracellular redox equilibrium with potential indirect effects on cell metabolism and intracellular signal transduction pathways [35,38]. The perturbation of microbial physiology induced by NAC might, in turn, represent the key factor accounting for NAC-mediated inhibition of biofilm formation, since the processes leading to the switch from planktonic to sessile mode of growth are known to be controlled by complex regulatory networks [2]. The reported activity of NAC in promoting dispersal of preformed biofilms could be related either to perturbation of microbial physiology or to a direct effect of NAC in affecting biofilm matrix architecture (e.g. by chelation of calcium and magnesium or interaction with crucial components in the matrix) [35,38].

The multifactorial activity of NAC against microbial biofilms that has been hypothesized represents a strength for its potential use as an anti-biofilm agent. In particular, if further studies reinforce the available data, NAC may indeed be a promising candidate for prevention of biofilm formation and for potentiating conventional anti-biofilm treatments (including antimicrobial drugs and photodynamic therapy approaches). In addition, the non-antibiotic nature of NAC and the relevance of biofilms in many technical systems (e.g. paper mills) have raised a multidisciplinary interest for this molecule [35,52]. In this perspective, further in vitro studies on this molecule are warranted in order to overcome important knowledge gaps and try to understand some apparent inconsistences in the available data, which are possibly related to the complex and still unclear mechanisms of NAC activity and to the difficulties and lack of standardization of in vitro biofilm models.

2.3.2. Clinical studies

Most studies have been conducted using oral or intramuscular NAC formulations.

2.3.2.1. Cystic fibrosis. The role of NAC in CF is still debated: a recent review [53] on the use of thiol derivates such as NAC concluded that there was not enough evidence to support the use of these compounds in clinical practice, but further studies were encouraged. Recently, Skov and colleagues [54] evaluated the effect of 4 weeks of treatment with oral NAC (2400 mg/day divided into two doses) on biochemical parameters of oxidative stress in an open-label, controlled, randomized trial on 21 patients (11 patients in the NAC group and 10 in the control group). Significantly decreased levels of oxidized vitamin C and increased vitamin C levels were seen in the NAC group; this group also had an improvement, though not significant, in lung function.

In another study [55], 70 CF subjects received NAC or placebo orally three times daily for 24 weeks. Oral NAC (900 mg × 3) maintained stable or slightly increased lung function in the treated group, while the control group showed a reduction in spirometric parameters. However, no change was observed in selected biomarkers of neutrophilic inflammation. These promising preliminary results suggest that further studies are required to better understand the role of NAC in treating patients with CF.

2.3.2.2. COPD and chronic bronchitis. The role of NAC in preventing exacerbations of patients with COPD and chronic bronchitis has been the basis of a recent meta-analysis by Cazzola and colleagues [56]. From the data of 13 studies (of 48 eligible full text articles), the records of 4155 COPD patients (1933 treated with NAC and 2222 placebo or control) were analyzed. It was seen that patients treated with NAC had a decreased risk of exacerbations of chronic bronchitis or COPD, but the effect was higher in patients with an absence of airway obstruction. NAC was well tolerated and the risk of adverse effects was not significantly higher at the higher dose. Furthermore, the data showed that in the case of airway obstruction, higher doses (≥1200 mg per day) are needed to prevent exacerbations [51,57], while regular doses (600 mg per day) are sufficient in patients with chronic bronchitis [58,59].

A multicenter double blind study [60] on 180 patients with acute bronchitis, tracheo-bronchitis, or acute exacerbations of chronic bronchitis compared the effects of thiamphenicol glycinate acetylcysteinate (TGA; n = 92) and thiamphenicol glycinate (TG; n = 88), both administered by aerosol. Both groups received the equivalent of 500 mg of thiamphenicol. Symptoms improved in both groups in terms of reduced frequency and cough severity and difficulties in expectoration. Furthermore, TGA was significantly more effective in eliminating cough within 6 days of treatment (82% versus 65%). Treatment efficacy was judged as "very good" (the maximum rating) by physicians in 37% of TGA-treated patients and in 28% of TG-treated patients. Both treatments were well tolerated.

2.3.2.3. Bronchiectasis. In bronchiectasis, intervention should ideally target bacterial colonization, airway inflammation, and impaired mucociliary clearance at the same time. NAC seems to be useful in this latter process, but the evidence to date is not sufficiently supported by clinical studies [15,61].

2.3.2.4. Other airway infections. A large study by Serra and colleagues [62] enrolled 398 patients (age 18–75 years) with recurrent infections of the upper airways (rhinosinusitis, pharyngotonsillitis, and acute otitis media), and assessed the effect of TGA in 149 patients versus other oral antibiotics. TGA was administered by aerosol (500 mg ½ ampoule daily for 6–10 days); antibiotics used in other groups (amoxicillin/clavulanate, cefixime, cefaclor, clarithromycin, levofloxacin, moxifloxacin, or telithromycin) were administered orally in accordance with the standards of the trial center. The etiological agents isolated included *Streptococcus pyogenens* (up to 75% in pharyngotonsillitis), *S. pneumoniae* (up to 50% in otitis), *H. influenzae* (up to 35% in rhinosinusitis), and *M. catarrhalis* (up to 20% in rhinosinusitis). The clinical results showed symptom disappearance in 88% of patients with pharyngotonsillitis, 91.7% in otitis media, and 87% of rhinosinusitis in patients treated with inhaled TGA. In patients treated with oral antibiotics, percentages of symptom resolution were generally lower, although the differences were not statistically significant. In patients with rhinosinusitis, topical NAC (nasal douche) associated with flunisolide has been demonstrated to be more effective than ambroxol plus flunisolide in terms of symptom improvement and number of exacerbations at 3 and 6 months. Moreover, the time to first exacerbation was significantly increased with NAC compared with ambroxol [63]. The results of this study confirm that NAC added to standard flunisolide treatment via atomized nasal douche is an effective strategy to break the vicious circle of recurrent acute rhinosinusitis and improve patients' conditions for up to 6 months following the end of treatment (Fig. 3).

Further evidence for the efficacy of NAC in rhinosinusitis comes from the review by Smith and colleagues [64] where TGA was shown to be effective in treating chronic rhinosinusitis and eradicating bacterial biofilms.

3. Discussion

In the respiratory infection field, the available data indicate that NAC has good antibacterial properties and suggest that this drug has the ability to interfere with biofilm formation and to disrupt biofilms. *In vitro* studies strongly support this assumption, although more clinical evidence is required.

NAC is usually given orally, with several formulations and dosage forms available for both short- and long-term treatment of respiratory diseases, but an inhalation route might also be considered a practical option. In particular, topical NAC causes a clear mucolytic effect by passing into the mucus and changing its physiochemical properties. The use of topical drugs has the advantage to reach the right anatomical target, at high concentrations, thus avoiding that the drug is metabolized by liver and intestines.

Therefore, the use of topical NAC in respiratory airway diseases may help in clinical practice, not only because of its efficacy [60,62], but also because it can reach the anatomical target thus paving the way for enhanced antibiotic action within the lung. Furthermore,

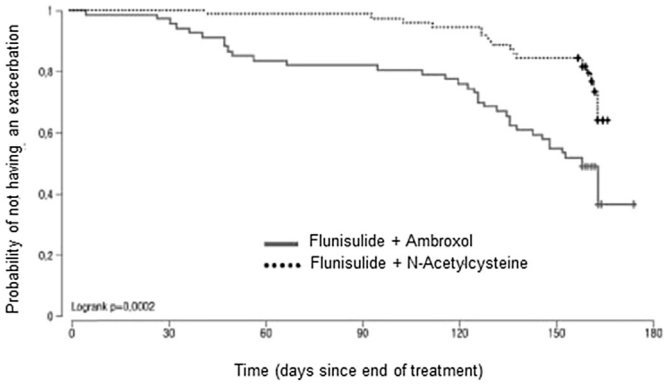

Fig. 3. Results of Kaplan-Meier analysis showing time to first exacerbation after stopping treatments in the study by Macchi et al. [60].

inhaled formulations of NAC have been demonstrated to be effective when used in association with antibiotics, possibly because of the ability of NAC to inhibit biofilm formation and cause biofilm disruption [28–30,43,50]. The use of inhaled NAC may be limited by the individual susceptibility to bronchoconstriction because of its acidic properties. Consequently, we do not believe that this is true for all patients and the use of NAC must always be based on the characteristics of the individual subject to be treated. Furthermore, NAC may help antibiotics to penetrate biofilms, allowing improved accessibility to bacteria.

Since NAC has been demonstrated to reduce bacterial attachment [51], it could also be considered as a prophylactic agent in respiratory infections where topical administration of the drug to the upper respiratory tract may be a choice even for patients in whom prevention of respiratory infections, rather than expectoration of sputum, is the primary reason for treatment.

References

[1] T. Bjarnsholt, The role of bacterial biofilms in chronic infections, APMIS 121 (Suppl. 136) (2013) 1–54.
[2] D. Lebeaux, J.M. Ghigo, C. Beloin, Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics, Microbiol. Mol. Biol. Rev. 78 (3) (2014) 510–543.
[3] D. Davies, Understanding biofilm resistance to antibacterial agents, Nat. Rev. Drug Discov. 2 (2) (2003) 114–122.
[4] S. Gil-Perotin, P. Ramirez, V. Marti, J.M. Sahuquillo, E. Gonzalez, I. Calleja, R. Menendez, J. Bonastre, Implications of endotracheal tube biofilm in ventilator-associated pneumonia response: a state of concept, Crit. Care (2012), 23;16(3):R93.
[5] C. Mietto, R. Pinciroli, N. Patel, L. Berra, Ventilator associated pneumonia: evolving definitions and preventive strategies, Respir. Care 58 (6) (2013) 990–1007.
[6] N. Safdar, C.J. Crnich, D.G. Maki, The pathogenesis of ventilator-associated pneumonia: its relevance to developing effective strategies for prevention, Respir. Care 50 (6) (2005), 725–39; discussion 739–741.
[7] C. Koch, N. Hoiby, Pathogenesis of cystic fibrosis, Lancet 341 (1993) 1065–1069.
[8] J.W. Costerton, P.S. Stewart, E.P. Greenberg, Bacterial biofilms: a common cause of persistent infections, Science 284 (1999) 1318–1322.
[9] O. Ciofu, T. Tolker-Nielsen, P.Ø. Jensen, H. Wang, N. Høiby, Antimicrobial resistance, respiratory tract infections and role of biofilms in lung infections in cystic fibrosis patients, Adv. Drug Deliv. Rev. 85 (2015) 7–23.
[10] D.J. Hassett, M.T. Borchers, R.J. Panos, Chronic obstructive pulmonary disease (COPD): evaluation from clinical, immunological and bacterial pathogenesis perspectives, J. Microbiol. 52 (3) (2014) 211–226.
[11] N. Eldika, S. Sethi, Role of nontypeable Haemophilus influenzae in exacerbations and progression of chronic obstructive pulmonary disease, Curr. Opin. Pulm. Med. 12 (2) (2006) 118–124.
[12] L. Martínez-Solano, M.D. Macia, A. Fajardo, A. Oliver, J.L. Martinez, Chronic Pseudomonas aeruginosa infection in chronic obstructive pulmonary disease, Clin. Infect. Dis. (2008), 15;47(12):1526–1533.
[13] J.D. Chalmers, A.T. Hill, Mechanisms of immune dysfunction and bacterial persistence in non-cystic fibrosis bronchiectasis, Mol. Immunol. 55 (1) (2013) 27–34.
[14] R.L. Marsh, R.B. Thornton, H.C. Smith-Vaughan, P. Richmond, S.J. Pizzutto, A.B. Chang, Detection of biofilm in bronchoalveolar lavage from children with non-cystic fibrosis bronchiectasis, Pediatr. Pulmonol. (2014 Mar 18), http://dx.doi.org/10.1002/ppul.23031.
[15] J.D. Chalmers, S. Aliberti, F. Blasi, Management of bronchiectasis in adults, Eur. Respir. J. 45 (5) (2015) 1446–1462.
[16] M.M. Tunney, G.G. Einarsson, L. Wei, et al., Lung microbiota and bacterial abundance in patients with bronchiectasis when clinically stable and during exacerbation, Am. J. Respir. Crit. Care Med. 187 (2013) 1118–1126.
[17] G.B. Rogers, C.J. van der Gast, D.J. Serisier, Predominant pathogen comptetition and core microbiota divergence in chronic airway infection, ISME J. 9 (2014) 217–225.
[18] K.N. Priftis, D. Litt, S. Manglani, M.B. Anthracopoulos, K. Thickett, G. Tzanakaki, P. Fenton, G.A. Syrogiannopoulos, A. Vogiatzi, K. Douros, M. Slack, M.L. Everard, Bacterial bronchitis caused by Streptococcus pneumoniae and nontypable Haemophilus influenzae in children: the impact of vaccination, Chest 143 (1) (2013) 152–157.
[19] D.L. Hamilos, Host-microbial interactions in patients with chronic rhinosinusitis, J. Allergy Clin. Immunol. 133 (3) (2014) 640–653 e4.
[20] L. Hall-Stoodley, F.Z. Hu, A. Gieseke, L. Nistico, D. Nguyen, J. Hayes, M. Forbes, D.P. Greenberg, B. Dice, A. Burrows, P.A. Wackym, P. Stoodley, J.C. Post, G.D. Ehrlich, J.E. Kerschner, Direct detection of bacterial biofilms on the middle-ear mucosa of children with chronic otitis media, JAMA (2006), 12;296(2):202–211.
[21] L.O. Bakaletz, Bacterial biofilms in the upper airway - evidence for role in pathology and implications for treatment of otitis media, Paediatr. Respir. Rev. 13 (3) (2012 Sep) 154–159.
[22] A. Foreman, A.J. Psaltis, L.W. Tan, et al., Characterization of bacterial and fungal biofilms in chronic rhinosinusitis, Am. J. Rhinol. Allergy 23 (2009) 556–561.
[23] B.P. Conlon, E.S. Nakayasu, L.E. Fleck, M.D. LaFleur, V.M. Isabella, K. Coleman, S.N. Leonard, R.D. Smith, J.N. Adkins, K. Lewis, Activated ClpP kills persisters and eradicates a chronic biofilm infection, Nature 503 (7476) (2013) 365–370.
[24] P.S. Stewart, J.W. Costerton, Antibiotic resistance of bacteria in biofilms, Lancet 358 (9276) (2001) 135–138.
[25] C.A. Fux, J.W. Costerton, P.S. Stewart, P. Stoodley, Survival strategies of infectious biofilms, Trends Microbiol. 13 (1) (2005) 34–40.
[26] A.A. Chavez-Dozal, L. Lown, M. Jahng, C.J. Walraven, S.A. Lee, In vitro analysis of finasteride activity against Candida albicans urinary biofilm formation and filamentation, Antimicrob. Agents Chemother. 58 (10) (2014) 5855–5862.
[27] J.D. Bryers, R.A. Jarvis, J. Lebo, A. Prudencio, T.R. Kyriakides, K. Uhrich, Biodegradation of poly(anhydride-esters) into non-steroidal anti-inflammatory drugs and their effect on Pseudomonas aeruginosa biofilms in vitro and on the foreign-body response in vivo, Biomaterials 27 (29) (2006) 5039–5048.
[28] S. Roveta, A.M. Shito, E.A. Debbia, et al., Confronto tra gli effetti di N-acetilcisteina, Ambroxol, Bromexina e Sobrerolo sui biofilm di Staphylococcus aureus, GIMMOC 8 (2004) 131–142.
[29] C. Pérez-Giraldo, A. Rodriguez-Benito, F.J. Moràn, et al., Influence of N-acetylcysteine on the formation of biofilm by Staphylococcus epidermidis, JAC 39 (1997) 643–646.
[30] M.A. El-Feky, M.S. El-Rehewy, M.A. Hassan, H.A. Abolella, R.M. Abd El-Baky, G.F. Gad, Effect of ciprofloxacin and N-acetylcysteine on bacterial adherence and biofilm formation on ureteral stent surfaces, Pol. J. Microbiol. 58 (3) (2009) 261–267.
[31] A. Marchese, M. Bozzolasco, L. Gualco, E.A. Debbia, G.C. Schito, A.M. Schito, Effect of fosfomycin alone and in combination with N-acetylcysteine on *E. coli* biofilms, Int. J. Antimicrob. Agents 22 (Suppl. 2) (2003 Oct) 95–100.
[32] A. Mohsen, A. Gomaa, F. Mohamed, R. Ragab, M. Eid, A.H. Ahmed, A. Khalaf, M. Kamal, S. Mokhtar, H. Mohamed, I. Salah, R. Abbas, S. Ali, R.M.A. El-Baky, Antibacterial, anti-biofilm activity of some non-steroidal anti-inflammatory drugs and N-acetyl cysteine against some biofilm producing uropathogens, Am. J. Epidemiol. Infect. Dis. 3 (1) (2015) 1–9.
[33] S. Aslam, R.O. Darouiche, Role of antibiofilm-antimicrobial agents in controlling device-related infections, Int. J. Artif. Organs 34 (9) (2011) 752–758.
[34] S. Aslam, B.W. Trautner, V. Ramanathan, R.O. Darouiche, Combination of tigecycline and N-acetylcysteine reduces biofilm-embedded bacteria on vascular catheters, Antimicrob. Agents Chemother. 51 (4) (2007) 1556–1558.
[35] A.C. Olofsson, M. Hermansson, H. Elwing, N-acetyl-L-cysteine affects growth, extracellular polysaccharide production, and bacterial biofilm formation on solid surfaces, Appl. Environ. Microbiol. 69 (8) (2003) 4814–4822.
[36] J. Lea, A.E. Conlin, I. Sekirov, V. Restelli, K.G. Ayakar, L. Turnbull, P. Doyle, M. Noble, R. Rennie, W.E. Schreiber, B.D. Westerberg, In vitro efficacy of N-acetylcysteine on bacteria associated with chronic suppurative otitis media, J. Otolaryngol. Head. Neck Surg. 7 (43) (2014) 20.
[37] L. Drago, E. De Vecchi, R. Mattina, C.L. Romanò, Activity of N-acetyl-L-cysteine against biofilm of Staphylococcus aureus and Pseudomonas aeruginosa on orthopedic prosthetic materials, Int. J. Artif. Organs 36 (1) (2013) 39–46.
[38] T. Zhao, Y. Liu, N-acetylcysteine inhibit biofilms produced by Pseudomonas aeruginosa, BMC Microbiol. 12 (10) (2010) 140.
[39] J.H. Moon, E.Y. Jang, K.S. Shim, J.Y. Lee, In vitro effects of N-acetyl cysteine alone and in combination with antibiotics on Prevotella intermedia, J. Microbiol. 53 (5) (2015) 321–329.
[40] A. Roveta, E. Debbia, G. Schito, A. Marchese, Comparison of the activity of N-acetylcysteine, ambroxol, bromexine and sobrerol on Staphylococcus aureus biofilms, GIMMOC 8 (1) (2004) 12.
[41] M. Bozzolasco, E.A. Debbia, G.C. Schito, Rilevanza dei biofilm batterici nelle infezioni respiratorie: problematiche terapeutiche e possibili soluzioni, GIMMOC VI (3) (2002) 203–215.
[42] B. Leite, F. Gomes, P. Teixeira, C. Souza, E. Pizzolitto, R. Oliveira, Combined effect of linezolid and N-acetylcysteine against Staphylococcus epidermidis biofilms, Enferm. Infecc. Microbiol. Clin. 31 (10) (2013) 655–659.
[43] S. Kirmusaoğlu, S. Yurdugül, M.E. Koçoğlu, The effect of N-acetylcysteine on growth and biofilm formation in Staphylococcus epidermidis strains, Turk J. Med. Sci. 42 (4) (2012) 689–694.
[44] F. Gomes, B. Leite, P. Teixeira, J. Azeredo, R. Oliveira, Farnesol in combination with N-acetylcysteine against Staphylococcus epidermidis planktonic and biofilm cells, Braz. J. Microbiol. 43 (1) (2012 Jan) 235–242.
[45] M. Venkatesh, L. Rong, I. Raad, J. Versalovic, Novel synergistic antibiofilm combinations for salvage of infected catheters, J. Med. Microbiol. 58 (Pt 7) (2009) 936–944.
[46] S.Y. Quah, S. Wu, J.N. Lui, C.P. Sum, K.S. Tan, N-acetylcysteine inhibits growth and eradicates biofilm of Enterococcus faecalis, J. Endod. 38 (1) (2012) 81–85.
[47] R.M.A. El-Baky, D.M.M.A. El Ela, G.F.M. Gad, N-acetylcysteine inhibits and eradicates Candida albicans biofilms, Am. J. Infect. Dis. Microbiol. 2 (5) (2014) 122–130.
[48] N. Kashef, S. Karami, G.E. Djavid, Phototoxic effect of hypericin alone and in combination with acetylcysteine on Staphylococcus aureus biofilms, Photodiagnosis Photodyn. Ther. 12 (2) (2015) 186–192.
[49] G. Del Prado, V. Ruiz, P. Naves, et al., Biofilm formation by Streptococcus pneumoniae strains and effects of human serum albumin, ibuprofen, N-

acetylcysteine, amoxicillin, erythromycin, and levofloxacin, Diagn. Microbiol. Infect. Dis. 67 (2010) 311–318.
[50] G.C. Riise, I. Qvarfordt, S. Larsson, V. Eliasson, B.A. Andersson, Inhibitory effect of N-acetylcysteine on adherence of Streptococcus pneumoniae and Haemophilus influenzae to human oropharyngeal epithelial cells in vitro, Respiration 67 (5) (2000) 552–558.
[51] C.H. Zheng, K. Ahmed, N. Rikitomi, G. Martinez, T. Nagatake, The effects of S-carboxymethylcysteine and N-acetylcysteine on the adherence of Moraxella catarrhalis to human pharyngeal epithelial cells, Microbiol. Immunol. 43 (1999) 107–113.
[52] A.C. Olofsson, M. Hermansson, H. Elwing, Use of a quartz crystal microbalance to investigate the antiadhesive potential of N-acetyl-L-cysteine, Appl. Environ. Microbiol. 71 (5) (2005) 2705–2712.
[53] J. Tam, E.F. Nash, F. Ratjen, E. Tullis, A. Stephenson, Nebulized and oral thiol derivatives for pulmonary disease in cystic fibrosis, Cochrane Database Syst. Rev. 7 (2013) CD007168.
[54] M. Skov, T. Pressler, J. Lykkesfeldt, H.E. Poulsen, P.Ø. Jensen, H.K. Johansen, T. Qvist, D. Kræmer, N. Høiby, O. Ciofu, The effect of short-term, high-dose oral N-acetylcysteine treatment on oxidative stress markers in cystic fibrosis patients with chronic P. aeruginosa infection – a pilot study, J. Cyst. Fibros. 14 (2) (2015) 211–218.
[55] C. Conrad, J. Lymp, V. Thompson, C. Dunn, Z. Davies, B. Chatfield, D. Nichols, J. Clancy, R. Vender, M.E. Egan, L. Quittell, P. Michelson, V. Antony, J. Spahr, R.C. Rubenstein, R.B. Moss, L.A. Herzenberg, C.H. Goss, R. Tirouvanziam, Long-term treatment with oral N-acetylcysteine: affects lung function but not sputum inflammation in cystic fibrosis subjects. A phase II randomized placebo-controlled trial, J. Cyst. Fibros. 14 (2) (2015) 219–227.
[56] M. Cazzola, L. Calzetta, C. Page, J. Jardim, A.G. Chuchalin, P. Rogliani, M.G. Matera, Influence of N-acetylcysteine on chronic bronchitis or COPD exacerbations: a meta-analysis, Eur. Respir. Rev. 24 (137) (2015) 451–461.
[57] H.N. Tse, L. Raiteri, K.Y. Wong, K.S. Yee, L.Y. Ng, K.Y. Wai, C.K. Loo, M.H. Chan, High-dose N-acetylcysteine in stable COPD: the 1-year, double-blind, randomized, placebo-controlled HIACE study, Chest 144 (1) (2013) 106–118.
[58] P.N. Dekhuijzen, Antioxidant properties of N-acetylcysteine: their relevance in relation to chronic obstructive pulmonary disease, Eur. Respir. J. 23 (4) (2004) 629–636.
[59] Zuin R1, A. Palamidese, R. Negrin, L. Catozzo, A. Scarda, M. Balbinot, High-dose N-acetylcysteine in patients with exacerbations of chronic obstructive pulmonary disease, Clin. Drug Investig. 25 (6) (2005) 401–408.
[60] C. Grassi, F. De Benedetto, Recent clinical evidence of the efficacy and safety of thiamphenicol glycinate acetylcysteinate and thiamphenicol glycinate, J. Chemother. 14 (3) (2002) 279–284.
[61] A.T. Hill, S. Welham, K. Reid, et al., British Thoracic Society national bronchiectasis audit 2010 and 2011, Thorax 67 (2012) 928–930.
[62] A. Serra, G.C. Schito, G. Nicoletti, G. Fadda, A therapeutic approach in the treatment of infections of the upper airways: thiamphenicol glycinate acetylcysteinate in sequential treatment (systemic-inhalatory route), Int. J. Immunopathol. Pharmacol. 20 (3) (2007) 607–617.
[63] A. Macchi, P. Terranova, P. Castelnuovo, Recurrent acute rhinosinusitis: a single blind clinical study of N-acetylcysteine vs ambroxol associated to corticosteroid therapy, Int. J. Immunopathol. Pharmacol. 25 (1) (2012) 207–217.
[64] A. Smith, F.J. Buchinsky, J.C. Post, Eradicating chronic ear, nose, and throat infections: a systematically conducted literature review of advances in biofilm treatment, Otolaryngol. Head. Neck Surg. 144 (3) (2011) 338–347.