Review Article

Non-cystic fibrosis bronchiectasis: The long road to multidrug resistant bacteria

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ABSTRACT

Bronchiectasis is a common progressive respiratory disease characterized by exacerbations and recurrent chest infections with high morbidity and reduced quality of life. Cole's vicious cycle model explains the evolution of this disease, in which an initial insult in the bronchi, often on a background of impaired mucociliary clearance or bactericidal activity, results in persistence of microbes in the sinobronchial tree and microbial colonization. Microbial overgrowth then causes infection and chronic inflammation, resulting in tissue damage, and impaired mucociliary motility. Subsequent antimicrobial treatments, microbiota interactions, and hypermutation can favor the development of resistance and the appearance of multidrug-resistant (MDR) bacteria. In this paper, we summarize the current knowledge on how bacteria become MDR in noncystic fibrosis bronchiectasis, and which are the most common bacterial pathogens, excluding *Mycobacteria*.

Key words: Disease progression, multidrug-resistant bacteria, noncystic fibrosis bronchiectasis

INTRODUCTION

Bronchiectasis is a suppurative lung disease which is defined by a permanent and abnormal dilatation of the bronchi and is usually diagnosed on axial images of high-resolution computed tomography scans of the chest. It can be considered the result of a variety of different factors, although most cases are idiopathic. Bronchiectasis is a common progressive respiratory disease characterized by exacerbations and recurrent chest infections with high morbidity and reduced quality of life. Cole's vicious cycle model explains the evolution of this disease, in which an initial insult in the bronchi, often on a background of impaired mucociliary clearance or bactericidal activity, results in persistence of microbes in the sinobronchial tree, and microbial colonization. Microbial overgrowth then causes infection and chronic inflammation, resulting in tissue damage, and impaired mucociliary motility. This then leads to more infection with a cycle of progressive inflammation causing lung damage.[1-3]

Subsequent antimicrobial treatments can favor the development of resistance and the appearance of multidrug-resistant (MDR) bacteria. Unlike in the case of

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cystic fibrosis, there is scarce information about MDR in noncystic fibrosis bronchiectasis (NCFB), but evidence from recent studies shows that these bacteria have a considerable impact on exacerbations and community-acquired pneumonia.[4]

In this review, we will talk about bacteria involved in these processes and how they can become MDR, excluding *Mycobacteria*.

MICROBIOLOGY OF NONCYSTIC FIBROSIS BRONCHIECTASIS

Until recently, the lower respiratory tract (LRT) was considered a sterile area that could be colonized briefly by microorganisms from the oropharynx or chronically in lungs with some underlying condition.[5-7] This concept has been modified following the development of new-generation molecular biology techniques, particularly the massive sequencing of regions of 16S ribosomal RNA, which made it possible to detect and identify microorganisms that normally do not grow on routine culture media.

Each person has a unique microbiota, with differences in species and proportions that is acquired at the time of delivery or even before birth, through the placenta.[8,9] Certain species are found in most healthy individuals at a higher rate and are called core microorganisms; the rest are known as satellite microorganisms.

Segal *et al*. demonstrated that the microbiota of the LRT differs from the higher respiratory tract in composition and number.[10] Supraglottic core microbiota included larger numbers of Gram-negative anaerobes as (*Veillonella* and *Prevotella*), while the pulmonary core microbiota had Gram-positive bacteria (*Propionibacterium* and *Staphylococcus*). In addition, the flora of the oropharynx was fifty times more abundant than that of the LRT. However, there is continuity between the microbiota of both anatomical sites that would be part of an interactive gastrointestinal respiratory axis.[7,11]

The study of the microbiota in chronic diseases such as NCFB has become a crucial issue and is changing the perspective of pathogenesis. In fact, numerous articles find associations between alterations in the microbiota and illness.[5,12,13]

The role of the microbiota as an inducer or maintainer of chronic respiratory diseases is gaining in importance. Moreover, damage to the lung tissue may begin years before its effects are detected. Lal *et al*. observed in two cohorts of newborns that the differences in the microbiota at birth was the common factor to developed bronchopulmonary dysplasia, regardless of birth weight, prematurity, and maternal antimicrobial intake, with a decrease in Firmicutes, especially *Lactobacillus*, and an increase in Proteobacteria, especially *Enterobacteriaceae*.[8] The scant effect of prior antibiotic consumption endorses other studies showing that the composition of the microbiota is stable and difficult to alter using antimicrobial treatments, although both the structure of the microbial community and the amount of each species are affected, especially anaerobes.[14,15] In any case, NCFB patients have complex polymicrobial microbiota with population densities similar to patients with cystic fibrosis.

In NCFB patients, *Haemophilus* spp., *Pseudomonas* spp., and *Streptococcus* spp. are the dominant microorganisms of the core microbiota although proportions vary between stable and exacerbation periods. While some studies link exacerbations with increased density of anaerobes, such as *Veillonella*, others find associations with multiple genres and decreased diversity. Adaptive changes of the main pathogens in the core microbiota may also trigger exacerbations.[12,14,15]

Furthermore, the microbiota modulates the inflammatory response. A "healthy" microbiota is associated with a balance between inflammation (Th17) and regulation (ThReg) while an abnormal microbiota is associated with an inflammatory state sustained by the direct action of the microorganisms themselves or the metabolites produced.[16]

Thus, the NCFB microbiota is altered and can determine disease progression. *Haemophilus influenzae* and *Pseudomonas aeruginosa* play an important role as core microbiota in NCFB. Both are found in patients with more exacerbations and worse evolution, and both influence the diversity of the accompanying microbiota. Interestingly, when one of them prevails, the other is absent or present in very small numbers.[15,17,18]

NONSPECIALIZED ANTIMICROBIAL RESISTANCE: BIOFILMS AND ESCAPE MECHANISMS

Microorganisms exhibit various strategies to survive in hostile environments. Two of these strategies are the production of biofilms and small colony variants (SCVs), both involved in chronicity of infections and antimicrobial treatment failure.

Microorganisms in nature live mainly in biofilms and only a small percentage live as planktonic bacteria. Within a biofilm, there are multiple environments and even specialized cells; some authors therefore consider this type of organization a multicellular organism.[19,20]

Furthermore, expression of virulence factors, membrane proteins, nutrient acquisition, metabolic pathways, and antimicrobial resistance is very different from planktonic cells. This is achieved through cooperation and coordination among community members, through chemical signals known as quorum sensing. This arrangement allows

microorganisms to have a greater amount of genetic, phenotypic, and metabolic resources.

Clinically, it is important to note that biofilms are a therapeutic challenge because such structures are resistant to elimination: Phagocytosis is inhibited by matrix components, and antimicrobials cannot act for being inactivated, presenting a poor diffusion or not finding metabolically active cells on which to act.[19,21,22]

In addition, some pathogenic species such as *Candida* spp., *S. aureus*, and *P. aeruginosa* form biofilms more rapidly in the presence of polymorphonuclear leukocytes and take advantage of neutrophil extracellular traps (NETs) to build biofilms.[21,23]

The ultimate goal of biofilms is survival; therefore, a strong immune response would be against the interests of its members. Intriguingly, the interaction between species can reduce the expression of virulence factors, such as *Candida* spp. on *P. aeruginosa*, or modulate the inflammatory response, thereby altering both local inflammation and phagocytosis.[23-25]

SCV appear spontaneously but are selected in response to stress, whether environmental, immune, or antimicrobial. SCV microorganisms present a slower rate of growth, express fewer virulence factors, respond worse to quorum sensing molecules, produce fewer antigenic molecules, and interestingly, invade host cells more effectively and survive in intracellular environments, even escaping from phagosomes.[26,27] These variants are responsible for recolonization by pathogenic microorganisms after antimicrobial treatments, usually because they are too short or ineffective to remove them.

BACTERIAL PATHOGENS IN NONCYSTIC FIBROSIS BRONCHIECTASIS

According to the current data, NCFB microbiota associated with a worse evolution is mainly dominated by two microorganisms: *P. aeruginosa* and *H. influenzae*. Nevertheless, recent investigations have provided a broader view and shown that other microorganisms are involved in NCFB progression, exacerbation, and prognosis, especially MDR.[4,28]

The risk of MDR in NCFB seems different depending on the origin of the microorganism. Thus, those adapted to the respiratory tract such as *S. pneumoniae* and *H. influenzae* are rarely MDR and are easier to eradicate. In contrast, those that come from different environments, such as *Enterobacteriaceae*, Gram-negative nonfermenters, and *S. aureus*, develop resistance and are associated with worse clinical conditions, exacerbations, and community-acquired pneumonia.[4,28] A 1-year study conducted on sputa from NCFB patients at hospital La Fe, Valencia, Spain, detected 23.2%, 5.4%, and 7.7% of MDR among *S. aureus*, *P. aeruginosa*, and *S. pneumoniae*, respectively; *Achromobacter xylosoxidans* and *Stenotrophomonas maltophilia* were also isolated, and all were considered MDR (personal communication).

Pseudomonas aeruginosa

P. aeruginosa is an opportunistic pathogen often associated with colonization and infection, higher number of hospital admissions, older patients, worse lung function, increased mortality, and a preference for infecting the upper lung lobes.[15,29,30]

Its virulence factors facilitate survival in the lung, where its presence triggers a global response for its elimination. Virulence factors are in the bacterial membrane (pili, flagella, and lipopolysaccharide), inoculated directly into human cells using type III and IV secretion systems, or excreted to defend itself or get nutrients (alginate, exotoxin A, phospholipase, exoproteases, rhamnolipids, etc.). Exoproteases include elastase, a zinc-dependent metalloenzyme capable of breaking down different proteins found in the extracellular matrix, cytokines and chemokines, and pulmonary surfactant. All these allow *P. aeruginosa* to survive in a harsh environment, avoid phagocytosis, and cause more invasive infections.[31-34]

Moreover, this microorganism responds to human endocrine signals. In particular, it was noted that noradrenaline activates the expression of virulence factors, and transforms *P. aeruginosa* into an invasive phenotype.[35] Hypothetically, it could link stress to NCFB exacerbations.

Of note, *P. aeruginosa* isolates from patients with an NCFB presented a different phenotype from cystic fibrosis or wild strains, exhibiting an inhibition of virulence factors.[36]

This microorganism presents intrinsic resistance to many antibiotics, mainly by modifying enzymes, impermeability, or efflux pumps. Mutations, loss of porins (OprD), or acquisition of plasmids with resistance determinants such as metallo-β-lactamases, make this microorganism a real threat, leaving very few active drugs against it.[37] Despite this and the high rates of colonization of this microorganism, the emergence of MDR is lower than expected and a high percentage of patients achieve eradication.[29] However, it has been observed that patients with NCFB have a high prevalence of hypermutable strains that leads to the development of resistance mechanisms and justifies age and antimicrobial treatments as selective factors for MDR.[36,38] It would be advisable to investigate whether more virulent MDR clones with a worse prognosis are circulating among NCFB patients as described in patients with cystic fibrosis.[39]

Haemophilus influenzae

H. influenzae is related to infections and colonization in younger patients with better lung function and a preference for infecting the lower lung lobes.[12,17,30]

H. influenzae has different mechanisms that allow it to survive in the lung such as the secretion of IgA proteases, adhesion proteins to lung epithelium (HIF, Hmw1/2, Hap, Hia/Hsf, OMP-2.5, OAPA, PCP, proteins E, and F), molecules that allow it to survive phagocytosis and produce invasive infections (msfA1-14), stress response systems that improve survival in biofilms (DPS), and the production of outer membrane vesicles that act as decoys for the immune response.[40-45]

Unlike *P. aeruginosa*, this opportunist commensal of the normal flora seems designed to thrive in the airway causing minimal damage. Patients with the prevalence of *H. influenzae* have fewer and milder exacerbations than patients with *P. aeruginosa* and require less hospitalization.[17] Surprisingly, patients with the prevalence of *H. influenzae* have higher levels of tissue metalloproteinase (MMP-2, MMP-12, and MMP-8 activity), which degrades the extracellular matrix and causes local damage, than patients with the prevalence of *P. aeruginosa*. Nevertheless, both groups of patients presented higher proteolytic activity than NCFB patients with other bacteria and healthy individuals.[46,47] The induced proteolytic activity may be related to the alleged involvement of *H. influenzae* in the development of NCFB.[48] Alternatively, the proteolytic activity of bacterial origin may be important in NCFB with the prevalence of *P. aeruginosa*.

H. influenzae has intrinsic resistance to macrolides, lincosamides, streptogramin B, and ketolides associated with an acrAB-like efflux pump; although macrolides may be clinically effective, especially azithromycin. The prophylactic use of macrolides facilitates the acquisition of erm and mef genes (encoding ribosomal RNA methylases and efflux pumps, respectively) or promotes mutations that cause high-level resistance to these antimicrobials.[49,50] *H. influenzae* can also acquire conjugative plasmids with antibiotic resistance genes, such as TEM or ROB β-lactamases, or develop mutations as those described in GyrA and ParC, or PBP3, which makes it resistant to quinolones and β-lactams, respectively.[51,52]

Hypermutable strains have been described in patients with cystic fibrosis although no studies have been carried out in patients with NCFB.[53]

Methicillin-resistant *Staphylococcus aureus*

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a common and important pathogen involved in nosocomial and health-care-related infections. Pneumonia by this microorganism is clinically important because of its severity, the high incidence of complications and the increased mortality that is usually associated with inadequate initial antibiotic therapy.

MRSA has also emerged as an increasingly important cause of community-acquired bacterial infection, and these strains frequently carry Panton–Valentine leukocidin genes.[54]

S. aureus has a number of mechanisms to evade the immune response. These include a capsule, surface proteins, exotoxins, and Panton–Valentine leukocidin. The capsule inhibits phagocytosis and promotes adherence to surfaces. Protein A, collagen adhesin, and staphylococcal clumping factor inhibit opsonization and complement activation. *S. aureus* also produces a wide array of extracellular toxins such DNAse, which breaks down NETs, or Panton–Valentine leukocidin, which specifically targets leukocytes and produces pores in their membrane, resulting in cell death.[55]

Resistance is acquired by horizontal transfer or chromosomal mutation.[56] Methicillin resistance is carried by the chromosome cassette mecA, which is integrated into orfX, a *S. aureus* gene of unknown function. This cassette codes a mutant PBP (PBP2a), which has low affinity for β-lactams and makes these bacteria resistant to almost all agents in this group. Interestingly, bacteria with this gene produce better biofilms but are less virulent, a possible explanation for its survival in chronic lung diseases.[57]

This microorganism can asymptomatically colonize people and be part of the normal flora. Its interactions with the host and neighbor microbiota are complex; for example, *S. aureus* downregulates inflammation induced by *P. aeruginosa* by decreasing interleukin-8.[58] Nevertheless, it has been related to exacerbations and community-acquired pneumonia in NCFB.[4,28]

Streptococcus pneumoniae

S. pneumoniae is the leading cause of community-acquired pneumonia worldwide and may be considered an airway specialist pathogen, but it is also associated with exacerbations in NCFB.[4] Beyond its well-known virulence factors, such as pneumolysin, new pathogenic mechanisms are being discovered, including the upregulation of virulence factors in coinfections with respiratory syncytial virus.[59]

MDR are rare and one of the principal means of acquiring resistance factors are biofilm-associated hyperrecombinant strains.[60,61] Exposure to antimicrobials and poor compliance are decisive in the development and selection of resistance.[62-64]

Enterobacteriaceae

Enterobacteriaceae are important members of the gut microbiota that can be the cause of infections, most frequently by species of *Escherichia*, *Klebsiella*, *Enterobacter*, *Proteus*, *Providencia*, and *Serratia*. Outside their natural environment, they can behave differently and present more antimicrobial resistance mechanisms; for example, *Escherichia coli* isolated from respiratory samples tend to be more resistant.[65] MDR *Enterobacteriaceae* due to extended-spectrum β-lactamases or AmpC enzymes, and

those producing carbapenemases have spread throughout the world in recent decades. Many of these isolates are also resistant to other agents, such as fluoroquinolones or aminoglycosides. The very limited therapeutic options available for these organisms are a real challenge and although, once associated with nosocomial infections, they are now found in the community.[66]

An in-depth study of *Enterobacteriaceae* is beyond the scope of this review, but these microorganisms are relevant in chronic obstructive pulmonary disease and NCFB exacerbations and are community-acquired.[4,67]

Gram-negative nonfermenters

Gram-negative nonfermenters such as *S. maltophilia* and *A. xylosoxidans* are usually associated with cystic fibrosis. However, these microorganisms have been isolated in NCFB and pose a serious challenge due to their intrinsic resistance profiles, which makes them MDR pathogens.[68,69]

Effects of antimicrobial treatment on multidrug resistance

Long-lasting antimicrobial treatment during the stable phase of NCFB aims to prevent acute exacerbations and slow the progression of the disease; but presents an important drawback: antimicrobial resistance can arise. Until recently, few studies focused specifically on NCFB; therefore, treatments were extrapolated from studies on cystic fibrosis - some with clinically untoward results.[69-72]

Recently, clinical trials suggest that the use of antibiotics for extended periods may decrease the symptoms, number of exacerbations, and slow reduction in forced expiratory volume in 1 s. Although results are disparate between studies, the development of resistance was a possible outcome in some trials; however, a Cochrane systematic review on this subject by Welsh *et al*. found no clear evidence to support this, mainly because of the heterogeneity of populations and the lack of stratification by microbiota composition and antimicrobials used.[73-78]

Importantly, the analysis of these studies did not discriminated patients based on the dominant microorganism in their microbiota. *P. aeruginosa* is usually susceptible to most antimicrobials studied-aminoglycosides, fluoroquinolones, or colistin-, but it is not to macrolides, which were tested in many of these studies and obtained good results. This may be due to the effect of this group of antibiotics on other members of the microbiota, the inhibitory effect on alginate production and quorum sensing molecules, or its intrinsic immunomodulatory effects.[79]

With regard to exacerbations and community-acquired pneumonia, identifying risk factors to predict the presence of MDR organisms is critical for initiating adequate antimicrobial therapies.[80] Several studies have identified various risk factors in patients with community-acquired pneumonia that may be extrapolated to NCFB. Among these, age, prior antimicrobial treatments, recent hospitalization and institutionalization, colonization by MDR microorganisms, and chronic diseases are crucial.[81-84]

CONCLUSION

Although few studies have evaluated the risk factors related to the emergence of MDR microorganisms in NCFB, we can reconstruct the evolutionary history from what we already know [Figure 1].

An alteration in the lower airway favors changes in the normal microbiota and the emergence of potentially pathogenic microorganisms. These would thrive to quantitatively displace other local microorganisms and become the main component of the microbiota. From here, a cycle of tissue destruction, infection, immune response, and inflammation would be established. This would induce the appearance of hypermutant microorganisms, SCV forms, and pathogenic biofilm generation (mucoid forms).

Added to this, vicious cycle is the selective factor of antimicrobials once clinical signs and symptoms appear. Microorganisms with increased tolerance or those that reside in areas where drugs fail to reach would be selected and after treatment ended, recolonize the lower airway and eventually, produce new exacerbations. Moreover, the elimination of the initial microbiota would allow the acquisition of nosocomial microorganisms. The end result would be a damaged lung colonized by MDR.

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Conflicts of interest

There are no conflicts of interest.

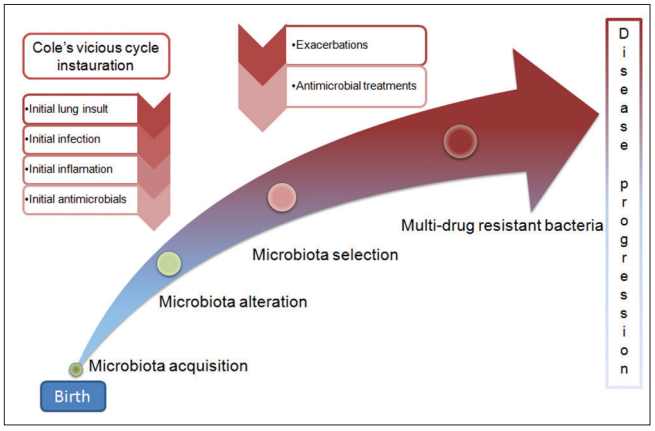

Figure 1: Microbiota evolution toward antimicrobial multiresistance

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