Εθνικό και Καποδιστριακό Πανεπιστήμιο
Αθηνών Ιατρική Σχολή
Μεταπτυχιακό Πρόγραμμα Σπουδών
«Παιδιατρική Λοιμωξιολογία»

POST GRADUATE PROGRAM:

«PAEDIATRIC INFECTIOUS DISEASES»

NATIONAL AND KAPODISTRIAN UNIVERSITY OF ATHENS

MEDICAL SCHOOL

MASTER THESIS

TITLE: CHRONIC SUPPURATIVE LUNG DISEASES IN CHILDREN MICROBIOLOGY AND DIAGNOSTIC TOOLS

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ATHENS

2022

ΜΕΤΑΠΤΥΧΙΑΚΟ ΠΡΟΓΡΑΜΜΑ:

«ΠΑΙΔΙΑΤΡΙΚΗ ΛΟΙΜΩΞΙΟΛΟΓΙΑ»

ΕΘΝΙΚΟ ΚΑΙ ΚΑΠΟΔΙΣΤΡΙΑΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ

ΙΑΤΡΙΚΗ ΣΧΟΛΗ

ΜΕΤΑΠΤΥΧΙΑΚΗ ΔΙΑΤΡΙΒΗ

ΤΙΤΛΟΣ: ΧΡΟΝΙΕΣ ΠΥΩΔΕΙΣ ΠΝΕΥΜΟΝΟΠΑΘΕΙΕΣ ΣΤΑ ΠΑΙΔΙΑ ΜΙΚΡΟΒΙΟΛΟΓΙΑ ΚΑΙ ΔΙΑΓΝΩΣΤΙΚΗ ΠΡΟΣΕΓΓΙΣΗ

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ΑΘΗΝΑ

2022

Summary

Bronchiectasis (BE), protracted bacterial bronchitis (PBB) and chronic suppurative lung disease (CLSD) are serious diseases with important harmful impacts on children, their families, the healthcare sector, and the broader community [Chang et al. 2008]. They overlap clinically, and diagnoses can be confirmed only after further investigations and long-term follow-ups [Chang et al. 2008]. It has been assumed (however stays unconfirmed) that PBB, CLSD, and BE are part of a medical continuum and share common triggers and/or pathophysiologies while being distinguished by their respective degrees of severity [Chang et al. 2008; Kalu et al. 2010]. Shared themes of suppurative pulmonary situations are persistent and continuing contagion, poor clearance of infected material and extreme neutrophilic inflammation [Redding and Carter, 2017]. It is broadly acknowledged that insistent bacterial infections are damaging to the airway [Stockley 1998]. It is important to establish the most accurate diagnostic tools to accurately identify the bacterial/viral pathogens involved in the pathogenesis or acute exacerbation of these diseases. Accurately identifying the etiology is critical to ensuring that the most effective treatment is instituted. Currently, the standard care for children within the spectrum of CLSD diseases involves the regular surveillance of airway microbiology and airway microbiology during acute pulmonary exacerbations. However, those children are frequently unable to expectorate sputum, even if vigorously coughing throughout an acute exacerbation. Consequently, it seems that efficacious sampling regarding lower airway microbiology is challenging, although it is essential if contagions are to be successfully managed or prohibited [Forton 2015; Ronchetti et al. 2018]. Sputum induction constitutes a good method to getting lower airway sampling from individuals who are not impulsively productive, and its utilization in tuberculosis monitoring in children is well-addressed [Iriso et al. 2005; Zar et al. 2000]. The role of sputum induction when treating young children within the spectrum of CLSD diseases has not been systematically addressed.

The oropharyngeal cough swab process contains asking the child to cough whereas a swab is put in the oropharynx. This is a very non-invasive, routine and straightforward process and is improbable to affect the outcome of a succeeding induced sputum. Nevertheless, oropharyngeal cough swabs cultures are a poor surrogate for cultures from lower airway samples taken throughout a coexisting BAL [Rosenfeld et al. 1999].

Despite the current knowledge regarding the microbiology of the lower airways in children suffering from CLSD, several challenges exist in the process of recognizing the pathogens involved in pathogenesis, such as in an acute respiratory exacerbation of CLSD. As previously stated, impulsive or induced sputum constitutes a trustworthy and available foundation of specimens regularly utilized for microbiologic examination in adult population. Nevertheless, gathering sputum from young children is quite challenging, because they have difficulties in expectorating. Though oropharyngeal cough swabs might constitute a valuable alternative, in practical way, the organism accountable for an exacerbation is hardly recognized and doctors usually trust empiric data to handle PBB or respiratory exacerbations in children with CLSD/ BE.

In part the issue is that notwithstanding their regular utilization in clinical practice, upper airway secretions composed by cough or throat swabs do not consistently forecast what are the organisms that exist in the lower airways, particularly possible pathogens of interest (*M. catarrhalis*, NTHI and *S. pneumoniae*), since these are likewise usually detected in the upper airway spaces of children, who are healthy. A possible problem could be the infenction with upper airway microflora, the time when lower airway specimens are attained for bacterial cultures [Loens et al. 2009]. Preferably, recurrent bronchoscopies with multi-lobar BAL could be applied to gather lower airway specimens before, during, and following acute exacerbations in young children who are too young to consistently expectorate sputum. However, this method is impractical given its invasive nature, as it would require repeated sedation or general anesthesia. Additionally, though it is a safe technique, contamination could still happen throughout BAL gathering in young children, since the used tube is extremely narrow for the protected brush process. Therefore, the information needed to assertively assign causation in investigation and patient treatment are partial [Singleton et al. 2014]. Therefore, more examinations are required in order to elucidate the association between these three methods (cough swabs, induced sputum, and BAL) when applied to children with CLSD [Pizzutto et al. 2017]. According to the above discussion, an assay capable of quickly and accurately detecting multiple pathogens, including viral pathogens, is highly desirable. Cough swabs and induced sputum are non-invasive airway-sampling approaches, which if shown to be effective, could contribute to the reduction of the number of bronchoscopies that children with CLSD need to undergo and routine infection observation. Further research is required to recognize if the association between these three

methods holds for CLSD patients. If it does, it would enable practitioners to better manage, monitor, and treat this condition. The objectives of the present research were to decide whether the microbiology results from cough swabs samples provided by children with PBB and children with acute exacerbation of CLSD or BE are accurate and can be used for further clinical decisions, and to evaluate the accuracy of the Film array Biofire pneumonia panel plus, through assessment of agreement with standard methods.

Περίληψη

Η βρογχεκτασία, η χρόνια βακτηριακή βρογχίτιδα και η χρόνια πυώδης πνευμονοπάθεια αποτελούν σοβαρές νόσους στα παιδιά με σημαντικές επιπτώσεις σε αυτά, στις οικογένειές τους, στον τομέα της υγείας και στην ευρύτερη κοινότητα [Chang et al. 2008]. Αλληλεπικαλύπτονται κλινικά και η διάγνωσή τους μπορεί να είναι βέβαιη μόνο μετά από μακροχρόνια παρακολούθηση των ασθενών [Chang et al. 2008]. Έχει προταθεί (ωστόσο δεν έχει επιβεβαιωθεί) ότι οι παραπάνω ασθένειες αποτελούν μέρος μιας κλινικής συνέχειας και οι πιο πάνω κλινικές καταστάσεις μοιράζονται κοινή παθοφυσιολογία, ενώ διακρίνονται από τους αντίστοιχους βαθμούς σοβαρότητάς τους [Chang et al. 2008; Kalu et al. 2010]. Κοινά θέματα των χρόνιων πνευμονοπαθειών είναι η επίμονη ή υποτροπιάζουσα λοίμωξη του κατώτερου αναπνευστικού, η αναποτελεσματική κάθαρση του μολυσμένου υλικού από το κατώτερο αναπνευστικό και η σοβαρή ουδετεροφιλική φλεγμονή [Redding and Carter, 2017].

Η χρόνια βακτηριακή βρογχίτιδα δεν αποτελεί μια νέα κλινική οντότητα, καθώς κλινικές καταστάσεις παρόμοιες με αυτήν, έχουν αναφερθεί βιβλιογραφικά, συχνά κατά τα προηγούμενα χρόνια [Kantar et al. 2017; Taussig et al. 1981]. Ωστόσο, μόλις πρόσφατα χαρακτηρίστηκε επαρκώς κλινικά [Chang et al. 2008; Donnelly et al. 2007] και αποτελεί πλέον μια καλά αναγνωρισμένη κλινική οντότητα. Χαρακτηριστικά, τα παιδιά με χρόνια βακτηριακή βρογχίτιδα είναι μικρά σε ηλικία (κάτω των πέντε ετών, μέσος όρος ηλικίας = 3 έτη) [Marchant et al. Chest 2006; Donnelly et al. 2007] με κυρίαρχο κλινικό σύμπτωμα τον χρόνιο υγρό βήχα [Chang et al. 2008]. Παρόλο που αρκετοί γονείς έχουν δηλώσει επίσης την παρουσιά συριγμώδους αναπνοής (41-81% [Donnelly et al. 2007; Darelid et al. 1993]), σπάνια επιβεβαιώθηκε η διάγνωση χρόνιας βακτηριακής βρογχίτιδας σε αυτά τα παιδιά [Kantar et al. 2017]. Οι αρνητικές επιδράσεις της νόσου στην υγεία των παιδιών είναι ελάχιστες και τα παιδιά με χρόνια βακτηριακή βρογχίτιδα είναι φαινομενικά υγιή με φυσιολογική ανάπτυξη για την ηλικία τους και δεν παρουσιάζουν σημεία κύριας χρόνιας πυώδους πνευμονοπάθειας [Chang et al. 2008]. Υπάρχουν περιπτώσεις που τα παιδιά με χρόνια βακτηριακή βρογχίτιδα λόγω μη αναπόκρισης στη θεραπεία με βρογχοδιασταλτικά, μπορεί λανθασμένα να διαγνωσθούν με σοβαρό άσθμα [Marchant et al. Chest 2006; Donnelly et al. 2007]. Τέτοιες διαγνώσεις περιπλέκονται περαιτέρω σε περιπτώσεις, όπου η χρόνια βακτηριακή βρογχίτιδα και το άσθμα συνυπάρχουν. Η διάκριση των υποτροπιάζοντων και μη υποτροπιαζοντων επεισοδίων βήχα σε ένα παιδί είναι σημαντική, επειδή τα άτομα με πιο τακτικά περιστατικά παρατεταμένου υγρού βήχα μπορεί να διατρέχουν

μεγαλύτερο κίνδυνο να μεταπέσουν σε χρόνια πυώδη πνευμονοπάθεια και στη συνέχεια σε βρογιεκτασία. Αυτή η υπόθεση επιβεβαιώθηκε σε ενήλικες πληθυσμούς που ταυτοποιήθηκαν πρόσφατα με βρογχιεκτασία και οι περισσότεροι από τους οποίους έχουν στο παρελθόν μακράς διάρκειας υγρό βήχα που σε κάποιους χρονολογείται από τα παιδικά τους χρόνια [Pasteur et al. 2000; King et al. 2006].

Η βρογχεκτασία, που δεν συνδέεται με την κυστική ίνωση, αποτελεί σοβαρή νόσο και έχει μεγάλη νοσηρότητα [Kapur et al. 2009; Chang, Grimwood et al. 2002; Singleton et al. 2000]. Ο ορισμός της βρογχεκτασίας που δόθηκε από τον Laenec το 1819 βασίστηκε στη μεταθανάτια ιστοπαθολογία των πνευμόνων ασθενών με βρογχεκτασία [Lae¨nnec, 1819]. Αργότερα, τα βρογχογραφήματα, τα οποία ορίστηκαν το 1951 για πρώτη φορά [Mannes, 1951], έγιναν η εξέταση εκλογής, προτού αντικατασταθούν κυρίως από σαρώσεις αξονικής τομογραφίας θώρακα υψηλής ανάλυσης (chHRCT). Σήμερα, η βρογχεκτασία περιγράφεται ως η «μόνιμη διάταση των περιφερικών αεραγωγών» [Westcott 1991; Webb et al. 2001] και είναι μια κατάσταση όπου οι βρόγχοι των πνευμόνων καταστρέφονται μόνιμα και διευρύνονται. Η βρογχεκτασία είναι το αποτέλεσμα μακροχρόνιων λοιμώξεων των αεραγωγών με αποτέλεσμα την απώλεια της δομικής ακεραιότητας του τοιχώματός τους που δημιουργεί τη μόνιμη διάτασή τους. Μπορεί να λάβει χώρα εστιακά μετά από σοβαρές οξείες αναπνευστικές λοιμώξεις ή σε περίπτωση μετα-αποφρακτικής πνευμονίας λόγω ξένων σωμάτων ή άλλων ενδοβρογχικών βλαβών (φυματίωση, όγκος) ή και στα πλαίσια άλλων σοβαρών νόσων (π.χ. πρωτοπαθής δυσκινησία κροσσών και κυστική ίνωση). Η βρογχεκτασία μπορεί επιπλέον να αναπτυχθεί μετά από σοβαρές ιογενείς λοιμώξεις του κατώτερου αναπνευστικού συστήματος [Colom et al. 2015; Trenholme et al. 2013]. Γνωστοί παράγοντες κινδύνου για βρογχεκτασία στα παιδιά περιλαμβάνουν τη σοβαρή και υποτροπιάζουσα πνευμονία [Valery et al. 2004; Eastham et al. 2004]. Ωστόσο, οι αρχικοί παθογενετικοί μηχανισμοί που οδηγούν σε βρογχεκτασία είναι άγνωστοι. Η βλάβη που προκαλείται στους πνεύμονες από τις βρογχεκτασίες είναι μόνιμη, αλλά η θεραπεία μπορεί να βοηθήσει στην ύφεση των συμπτωμάτων και να σταματήσει την επιδείνωση των βλαβών. Το κυρίαρχο σύμπτωμα της βρογχεκτασίας είναι η εμφάνιση ενός παρατεταμένου, παραγωγικού υγρού βήχα. Πρόσθετα συμπτώματα περιλαμβάνουν σοβαρή δύσπνοια, υποτροπιάζουσες λοιμώξεις αναπνευστικού, επίμονο υγρό βήχα με απόκριση στα αντιβιοτικά, συμπτωματολογία αντιδραστικής νόσου των αεραγωγών (asthma like condition) και υπολειπόμενη ανάπτυξη. Στα παιδιά, η αιμόπτυση συμβαίνει σπάνια, εξαιρουμένων των τελικών

σταδίων της νόσου. Πρόσθετα κλινικά σημεία της βρογχεκτασίας περιλαμβάνουν παραμόρφωση του θωρακικού τοιχώματος, πληκτροδακτυλία, διάταση του θωρακικού τοιχώματος και επιπρόσθετους ήχους κατά την ακρόαση του θώρακος [de Blic et al. 2000; Οι Kalu et al. 2010].

Σε αντίθεση με τη βρογχεκτασία, ο όρος χρόνια πυώδη πνευμονοπάθεια περιγράφει την κλινική κατάσταση που βασίζεται στην κλινική συμπτωματολογία της βρογχιεκτασίας χωρίς τα ακτινολογικά ευρήματα της βρογχιεκτασίας στην cHRCT. Το κύριο σύμπτωμα των ασθενών με χρόνια πυώδη πνευμονοπάθεια είναι ο παρατεταμένος υγρός βήχας. Εκτός από την απουσία των ευρημάτων στην cHRCT, η συμπτωματολογία των ασθενών με χρόνια πυώδη πνευμονοπάθεια είναι παρόμοια με εκείνη της βρογχιεκτασίας. Σε αντίθεση, οι ασθενείς με χρόνια βακτηριακή βρογχίτιδα παρουσιάζουν ένα επεισόδιο μεμονωμένου υγρού βήχα (που δεν περιέχει συμπτωματολογία και σημεία βρογχεκτασίας ή χρόνιας πυώδους πνευμονοπάθειας) [Chang et al. 2008]. Τα παιδιά με χρόνια πυώδη πνευμονοπάθεια αντιμετωπίζονται με την ίδια θεραπευτική αγωγή που αντιμετωπίζονται τα παιδιά με βρογχιεκτασία. Αξίζει να σημειωθεί ότι τα παιδιά με χρόνια πυώδη πνευμονοπάθεια διαφέρουν από τα παιδιά με χρόνια βακτηριακή βρογχίτιδα στο ότι η κατάστασή τους δεν μπορεί να αντιστραφεί πλήρως μετά τη χορήγηση σχημάτων αντιβιοτικών. Η χρόνια πυώδη πνευμονοπάθεια είναι βέβαιη εάν οι ασθενείς παρουσιάζουν την τυπική συμπτωματολογία των ασθενών με βρογχιεκτασία χωρίς να παρουσιάζουν τα χαρακτηριστικά ακτινολογικά ευρήματα που παρουσιάζουν οι ασθενείς με βρογχιεκτασία στην cHRCT [Chang et al. 2008]. Η μη σωστή αντιμετώπιση της χρόνιας πυώδους πνευμονοπάθειας μπορεί να οδηγήσει στην εξέλιξη σε βρογχεκτασία λόγω αθροιστικής βλάβης των αεραγωγών από επαναλαμβανόμενη ή επίμονη βακτηριακή λοίμωξη [Chang et al. 2008]. Οι οξείες παροξύνσεις της χρόνιας πυώδους πνευμονοπάθειας και της βρογχεκτασίας σχετίζονται με τη σοβαρότητα της νόσου [Wang et al. 2015]. Αν και δεν υπάρχουν επαρκή βιβλιογραφικά δεδομένα για τη βρογχεκτασία ή τη χρόνια πνευμονοπάθεια στα παιδιά, οι παροξύνσεις τους είναι πιθανό να επηρεάσουν αρνητικά την πνευμονική λειτουργία. Παράγοντες που επιδρούν αρνητικά στην πνευμονική λειτουργία σε ενήλικές ασθενείς είναι η συχνότητα των παροξύνσεων, οι αυξημένοι φλεγμονώδεις δείκτες και η λοίμωξη με *Pseudomonas aeruginosa* [Martinez-Garcia et al. 2007].

Όπως η βρογχεκτασία και η χρόνια πυώδη πνευμονοπάθεια, η χρόνια βακτηριακή βρογχίτιδα συνδέεται με επίμονες/υποτροπιάζουσες βακτηριακές λοιμώξεις στους αεραγωγούς [Marchant et al. Στήθος 2006; Donnelly et al. 2007] και είναι ευρέως αποδεκτό ότι τέτοιες

βακτηριακές λοιμώξεις βλάπτουν τους αεραγωγούς [Stockley 1998; Οι Chang et al. 2008]. Η διάγνωση του βακτηριακού παράγοντα που προκαλεί αναπνευστική λοίμωξη σε αυτούς τους ασθενείς είναι εξαιρετικά δύσκολη καθώς είναι γνωστό ότι τα παιδιά δεν είναι ικανά για απόχρεμψη ώστε να ληφθεί ικανό αναπνευστικό δείγμα για περαιτέρω εργαστηριακό έλεγχο. Είναι συνεπώς σημαντικό, να καθοριστούν τα πιο ακριβή διαγνωστικά εργαλεία για τον ακριβή εντοπισμό των βακτηριακών/ιικών παθογόνων που εμπλέκονται στην παθογένεση ή την οξεία παρόξυνση αυτών των νόσων. Ο ακριβής προσδιορισμός της αιτιολογίας των πιο πάνω παροξύνσεων είναι πολύ σημαντικός γιατί με αυτό τον τρόπο επιλέγεται η καταλληλότερη αντιβιοτική θεραπεία. Σήμερα, η καθιερωμένη καθημερινή κλινική πρακτική για τα παιδιά εντός του φάσματος των χρόνιων πυώδων πνευμονοπαθειών περιλαμβάνει την τακτική παρακολούθηση της μικροβιολογίας των αεραγωγών αλλά και της μικροβιολογίας των αεραγωγών κατά τη διάρκεια οξείων παροξύνσεων. Ωστόσο, αυτά τα παιδιά συχνά αδυνατούν για απόχρεμψη ακόμα κι αν υπάρχει υγρός βήχας. Κατά συνέπεια, φαίνεται ότι η αποτελεσματική δειγματοληψία σχετικά με τη μικροβιολογία των κατώτερων αεραγωγών αποτελεί πρόκληση, αν και είναι ουσιαστικής σημασίας για την επιτυχή διαχείριση και αντιμετώπιση των λοιμώξεων [Forton 2015; Ronchetti et al. 2018].

Τα προκλητά πτύελα αποτελούν μια καλή μέθοδο για τη λήψη δειγμάτων αντιπροσωπευτικών του κατώτερου αναπνευστικού από άτομα που δεν είναι ικανά για απόχρεμψη και η χρήση τους στην αντιμετώπιση της φυματίωσης στα παιδιά είναι καλά αποδεδειγμένη [Iriso et al. 2005; Zar et al. 2000]. Ο ρόλος των προκλητών πτυέλων στη θεραπεία μικρών παιδιών εντός του φάσματος των ασθενειών CLSD δεν έχει μελετηθεί συστηματικά. Οι συγγραφείς μιας μελέτης του 2018 [Ronchetti et al. 2018] συνέστησαν την τακτική εφαρμογή προκλητών πτυέλων σε παιδιά με κυστική ίνωση. Η πρόταση αυτή χαρακτηρίσθηκε από την ευρεία αποδοχή της διαδικασίας από όλες τις ηλικιακές ομάδες, την αποδοχή των γονέων και ασθενών, την ευκολία επαναληψιμότητάς της, την επιτυχή λήψη δειγμάτων, την εφαρμογή τόσο σε εσωτερικούς όσο και εξωτερικούς ασθενείς, το μεγάλο ποσοστό των ανιχνεύσιμων παθογόνων και τη σημαντική οικονομική εξοικονόμηση σε σύγκριση με τη βρογχοσκόπηση. Οι συγγραφείς πρότειναν ωστόσο, ότι τόσο τα προκλητά πτύελα όσο και η λήψη BAL έξι λοβών θα πρέπει να χρησιμοποιείται ως τυπική πρακτική για την πλήρη ανίχνευση παθογόνων κατώτερου αεραγωγού σε παιδιά με κυστική ίνωση.

Η λήψη cough swabs περιλαμβάνει τη διαδικασία μέσα από τη οποία ζητάμε από το παιδί να βήξει ενώ ένας στειλεός τοποθετείται στο οπίσθιο τοίχωμα του στοματοφάρυγγα χωρίς να το αγγίξει. Αποτελεί μη επεμβατική και απλή διαδικασία και είναι απίθανο να επηρεάσει την έκβαση μιας ενδεχόμενης διαδικασίας προκλητών πτυέλων που θα ακολουθήσει. Ωστόσο, οι καλλιέργειες επιχρισμάτων cough swabs δεν δείχνουν καλή συσχέτιση αν συγκριθούν με καλλιέργειες από δείγματα BAL [Rosenfeld et al. 1999]. Εν μέρει το ζήτημα είναι ότι παρά την τακτική χρήση τους στην κλινική πράξη, οι πιθανές επιμολύνσεις από τις εκκρίσεις των ανώτερων αεραγωγών έχουν ως αποτέλεσμα να μην μπορεί με βεβαιότητα να χαρακτηρισθεί ο απομονωμένος μικροοργανισμός ως πιθανό άιτιο λοίμωξης του κατώτερου αναπνευστικού. Επιπρόσθετα, πιθανά παθογόνα ενδιαφέροντος (*M. catarrhalis*, NTHI και *S. pneumoniae*), ανιχνεύονται επίσης συνήθως στο ανώτερο αναπνευστικό των παιδιών, τα οποία είναι υγιή. Ένα πιθανό πρόβλημα θα μπορούσε να είναι η μόλυνση με μικροχλωρίδα του ανώτερου αεραγωγού, τη στιγμή που λαμβάνονται δείγματα κατώτερου αεραγωγού για βακτηριακές καλλιέργειες [Loens et al. 2009].

Τα οφέλη των προκλητών πτυέλων έναντι των επιχρισμάτων cough swabs έχουν αναγνωριστεί σε ενήλικες και μεγαλύτερα παιδιά με κυστική ίνωση. Ωστόσο, καμία μελέτη δεν έχει συγκρίνει τις δύο προσεγγίσεις σε ασθενείς με CLSD. Τα αποτελέσματα ότι οι καλλιέργειες cough swabs μπορούν να χρησιμοποιηθούν αντί για BAL και ότι αποτελούν αξιόπιστα δείγματα του κατώτερου αναπνευστικού προέρχονται από πέντε αναφορές από ασθενείς με κυστική ίνωση [Armstrong et al. 1996; Avital et al. 1995; Ramsey et al. 1991; Rosenfeld et al. 1999; Jung et al. 2002]. Στοιχεία από πέντε μελέτες δείχνουν ότι τα προκλητά πτύελα είναι πιο αποτελεσματικά από τα cough swabs για τον εντοπισμό βακτηριακών παθογόνων σε ασθενείς με κυστική ίνωση [Al-Saleh et al. 2010; Suri et al. 2003; De Boeck et al. 2000; Henig et al. 2001; Ho et al. 2004]. Μια μικρή εξέταση 10 ενηλίκων ατόμων με κυστική ίνωση έδειξε συγκρίσιμες μικροβιολογικές αποδόσεις όταν χρησιμοποίησαν αυθόρμητα προκαλούμενα πτύελα, προκλητά πτύελα και BAL. Δύο μεγαλύτερες μελέτες συνέκριναν cough swabs με προκλητά πτύελα σε άτομα με κυστική ίνωση (που αφορούσαν 43 και 94 παιδιά, αντίστοιχα). Και οι δύο εξετάσεις αναγνώρισαν περαιτέρω οργανισμούς στα προκλητά πτύελα στο 30% και 42% των περιπτώσεων, αντίστοιχα.

Παρά την τρέχουσα γνώση σχετικά με τη μικροβιολογία των κατώτερων αεραγωγών σε παιδιά που πάσχουν από CLSD, υπάρχουν αρκετές προκλήσεις στη διαδικασία αναγνώρισης των παθογόνων που εμπλέκονται σε μια οξεία αναπνευστική παρόξυνση της νόσου. Όπως

αναφέρθηκε προηγουμένως, η συλλογή πτυέλων από μικρά παιδιά είναι αρκετά δύσκολη, επειδή έχουν δυσκολίες στην απόχρεμψη. Αν και τα cough swabs μπορεί να αποτελούν μια πολύτιμη εναλλακτική, πρακτικά, ο μικροοργανισμός που ευθύνεται για την παρόξυνση δεν αναγνωρίζεται και συνήθως η αντιμετώπιση των παροξύνσεων γίνεται εμπειρικά. Κατά προτίμηση, θα μπορούσαν να εφαρμοστούν επαναλαμβανόμενες βρογχοσκοπήσεις για λήψη BAL για τη συλλογή δειγμάτων αντιπροσωπευτικών των κατώτερων αεραγωγών πριν, κατά τη διάρκεια και μετά από οξείες παροξύνσεις σε μικρά παιδιά που δεν είναι ικανά για απόχρεμψη. Ωστόσο, αυτή η μέθοδος δεν είναι πρακτική, δεδομένου του επεμβατικού χαρακτήρα της, καθώς θα απαιτούσε επαναλαμβανόμενη γενική αναισθησία. Ως εκ τούτου, οι πληροφορίες που απαιτούνται για να αποδοθεί με βεβαιότητα η αιτιολογία του μικροβιολογικού παράγοντα που ευθύνεται για την οξεία παρόξυνση της νόσου είναι λίγες και η θεραπεία του ασθενούς αναγκαστικά γίνεται εμπειρικά [Singleton et al. 2014]. Συμπερασματικά, απαιτούνται περισσότερες μελέτες προκειμένου να διαλευκανθεί η συσχέτιση μεταξύ αυτών των τριών μεθόδων (cough swabs, προκλητά και BAL) όταν εφαρμόζονται σε παιδιά με CLSD [Pizzutto et al. 2017].

Πέρα από το πρόβλημα ύπαρξης κατάλληλου αναπνευστικού δείγματος, που πρέπει να είναι αντιπροσωπευτικού του κατώτερου αναπνευστικού, απαιτείται και μια μικροβιολογική τεχνική που να ανιχνεύει γρήγορα και με ακρίβεια το παθογόνο που εμπλέκεται στη λοίμωξη με σκοπό την έγκαιρη έναρξη στοχευμένης αντιβιοτικής θεραπείας. Ως γνωστόν οι κλασσικές μέθοδοι μικροβιολογίας (καλλιέργειες) απαιτούν τουλάχιστον 48 ώρες για την απομόνωση βακτηρίων και απαιτείται περαιτέρω χρονικό διάστημα για την διεκπεραίωση του αντιβιογράμματος.

Έτσι, σύμφωνα με την παραπάνω συζήτηση, μια εξέταση ικανή να ανιχνεύει γρήγορα και με ακρίβεια πολλαπλά παθογόνα, συμπεριλαμβανομένων των ιικών παθογόνων, είναι πολύ επιθυμητή. Επίσης τα cough swabs και τα προκλητά πτύελα είναι μη επεμβατικές προσεγγίσεις δειγματοληψίας αεραγωγών, οι οποίες εάν αποδειχθούν αποτελεσματικές, θα μπορούσαν να συμβάλουν στη μείωση του αριθμού των βρογχοσκοπήσεων που πρέπει να υποβληθούν τα παιδιά με CLSD.

Σκοπός της παρούσας μελέτης ήταν να προσδιορισθεί η εγκυρότητα των μικροβιολογικών αποτελεσμάτων που προκύπτουν από τα cough swabs (αντιπροσωπευτικά δείγματα κατώτερου αναπνευστικού) και κατά πόσον αυτά μπορούν να χρησιμοποιηθούν ως βάση για περαιτέρω

κλινικές αποφάσεις. Στόχος ακόμη ήταν, να προσδιορισθεί η εγκυρότητα των αποτελεσμάτων που προκύπτουν από την τεχνική Film array Biofire pneumonia panel plus με βάση τα αποτελέσματα των reference standard μεθόδων (καλλιέργεια) και να καθορισθούν οι μικροβιολογικοί αιτιολογικοί παράγοντες της οξείας παρόξυνσης χρόνιας πυώδους πνευμονοπάθειας, βρογχιεκτασίας και χρόνιας βρογχίτιδας (εάν τα cough swabs δείγματα αποδειχθούν αξιόπιστα δείγματα αντιπροσώπευσης κατώτερου αναπνευστικού).

Οι υποθέσεις ήταν ότι τα μικροβιολογικά αποτελέσματα των δειγμάτων cough swabs που λαμβάνονται από ασθενείς με οξεία παρόξυνση χρόνιας πυώδους πνευμονοπάθειας, βρογχιεκτάσιας και σε ασθενείς με χρόνια βορογχίτιδα είναι έγκυρα και αντιπροσωπεύουν το κατώτερο αναπνευστικό και δεν αντανακλούν ευρήματα φυσιολογικής χλωρίδας ανώτερου αναπνευστικού και ότι τα μικροβιολογικά αποτελέσματα του Film array Biofire pneumonia panel plus από τα cough swabs δείγματα είναι αξιόπιστα και το μικροβιολογικό φάσμα που ανιχνεύει η συγκεκριμένη εξέταση είναι μεγαλύτερο από το μικροβιολογικό φάσμα που ανιχνεύει η καλλιέργεια.

Για να ελεγχθούν οι πιο πάνω υποθέσεις, από 23 ασθενείς 2-16 χρονών, που πληρούσαν τα κριτήρια οξείας παρόξυνσης χρόνιας πυώδους πνευμονοπάθειας, βρογχεκτασίας και χρόνιας βρογχίτιδας καθώς και από 17 προηγουμένως υγιή παιδιά που νοσηλεύθηκαν για λοίμωξη ανώτερου αναπνευστικού (age matched) λήφθηκαν cough swabs και έτυχαν περαιτέρω μικροβιολογικής διερεύνησης με την χρήση καλλιέργειας και της τεχνικής film array.

Με βάση τα αποτελέσματα της μελέτης τα cough swabs δεν αντιπροσωπεύουν αξιόπιστα δείγματα προερχόμενα από το κατώτερο αναπνευστικό και για αυτό το λόγο δεν μπορούν να χρησιμοποιηθούν περαιτέρω στην καθημερινή κλινική πρακτική για λήψη κλινικών αποφάσεων. Επίσης, λαμβάνοντας υπόψιν τα αποτελέσματα, η τεχνική BioFire FilmArray Pneumonia Panel επιτρέπει την ταχεία ανίχνευση παθογόνων και παρέχει μεγαλύτερο μικροβιολογικό φάσμα εάν συγκριθεί με τη reference standard μκροβιολογική μέθοδο που είναι η καλλιέργεια και επίσης χαρακτηρίζεται από υψηλή ευαισθησία και μέτρια προς υψηλή ειδικότητα στην ανίχνευση αναπνευστικών παθογόνων. Ένα αρνητικό αποτέλεσμα με την τεχνική film array σε δείγμα cough swab αποκλείει την ύπαρξη του ως πιθανού αιτιολογικού παράγοντα της λοίμωξης (υψηλή NPV) ενώ ένα θετικό αποτέλεσμα δεν μπορεί να επιβεβαιώσει την παρουσία του ως πιθανού αιτιολογικού παράγοντα της λοίμωξης (χαμηλή PPV).

Τέλος, συμπεραίνουμε ότι, είναι επιτακτική η ανάγκη για διενέργεια μελετών για την αποτελεσματικότητα της τεχνικής film array χρησιμοποιώντας όλα τα διαθέσιμα αναπνευστικά δείγματα.

ABSTRACT

Introduction Protracted bacterial bronchitis, chronic suppurative lung disease and are severe illnesses with damaging adverse influences on children, their families, the healthcare sector, and the broader community. Shared themes of suppurative pulmonary circumstances are extreme neutrophilic inflammation, tenacious and continuing contamination and poor clearance of infected material. Dissimilar respiratory sampling methods take place to recognize lower airway pathogens in individuals with chronic suppurative lung diseases, of which expectorated sputum and bronchoalveolar lavage are thought to be the "gold standard." The diagnosis of lower respiratory tract contaminations in non-expectorating patients is perplexing, since bronchoalveolar lavage is not able to be repeated boundless. Some additional sampling methods are cough swab, nasal swab and induced sputum. Our aims were to determine whether the microbiology results from cough swabs samples provided by children with PBB and children with acute exacerbation of CLSD or BE are accurate and can be used for further clinical decisions, and to evaluate the accuracy of the Film array Biofire pneumonia panel plus, through assessment of agreement with standard methods. **Materials and Method** Children aged 2 to 16 years old were eligible for the study if they had a definitive diagnosis of PBB, BE or CLSD and they were in acute exacerbation phase. Age-matched previously healthy children who were hospitalized with evidence of upper respiratory infection were also enrolled in study, comprising the healthy control group. Cough swabs were collected from all the patients and controls. Cough swab samples were educated for 48 hours utilizing routine microbiological processes and all the samples tested for several bacterial and viral pathogens using the BioFire FilmArray Pneumonia Panel plus test. The data were analyzed by using statistical software IBM SPSS 24. Chi square test was utilzed to test the the dependence between group category (patients, control) and existence of microorganism (yes, no). McNemar test was used to test differences between film array and culture technique for patients, regarding the existence of microorganism. Sensitivity, Specificity, PPV and NPV was used for film array and culture technique. **Results** No statistically significant differences using film array technique between patients and control group were indicated ($p \geq 0.113$). Statistically significantly differences between film array and culture technique for patients appeared, in microorganisms *Haemophilus influenzae* ($p=0.008$) and *Moraxella catarrhalis* ($p=0.016$) where the microbiological yield was higher for film array technique comparing with culture. The utilization of the film array PN Panel led to a 114.29%

grow in the total number of bacterial targets detected The Film array test presented high Sensitivity (1.00) and NPV (1.00) when compared to bacterial culture, medium specificity (0.78) and low PPV (0.50) when the results compared with the results of reference standard method the culture. **Conclusions** Cough swab samples do not represent reliable samples from lower respiratory tract so, they are not useful samples to support/confirm etiological diagnose of LRTi in CLSD patients in every day practice. The BioFire FilmArray Pneumonia Panel enables fast recognition of pathogens and provides greater microbiological yield than the reference standard method, culture. A high sensitivity (100%) and high specificity (78%) was found for film array assay for the discovery of normal respiratory microorganisms. A negative cough swab oropharyngeal film array result can effectively rule out a possible inferior airway contagion (NPV is high), whereas a positive cough swab oropharyngeal film array result does not reliably confirm a possible lower airway infection (PPV is low). More examinations are required in order to assess the possible clinical efficacy of the FA-Pneumo assay with several specimen kinds.

Keywords: Protracted bacterial bronchitis (PBB), Bronchiectasis (BE), chronic suppurative lung disease (CLSD), Bronchoalveolar lavage (BAL), cough swabs, induced sputum.

To the memory of my father, Marios Neocleous

(†13 August 2018)

Acknowledgments

I would like to acknowledge some individuals, who contributed to the completion of my master thesis.

I especially thank **Professor Vasiliki Papaevangelou** for pointing out to me the topic of the thesis, as well as for giving me the opportunity to gain knowledge and experience. I am thankful to her for believing in me and being supportive throughout the writing of this thesis.

I would also like to acknowledge **Professor Spyros Pournaras** and **Associate Professor Konstandinos Douros** for their advice and their keenness on answering to every question I had.

I am also thankful to the staff of the 3rd Department of Pediatrics NKUA and of the Microbiology laboratory of the University hospital of ATTIKON for contributing to the completion of this study.

Finally, I would like to thank my family for their unconditional love, support and patience throughout the years. Without them, I could not have made it through.

Table of Acronyms

PBB	Protracted Bacterial Bronchitis
BE	Bronchiectasis
CLSD	Chronic Suppurative Lung Disease
ALRI	Acute Lower Respiratory Infections
BAL	Bronchoalveolar Lavage
CF	Cystic Fibrosis
ERS	European Respiratory Society
NTHi	Non typeable *Haemophilus Influeza*
CT	Computed Tomography
HAdV	Adenovirus
NNT	Number needed to treat
VCD	Validated verbal category descriptive
BTS	British Thoracic Society
BAR	Bronchoarterial Ratio
Chrct	Chest high-resolution computerized tomography
NP	Nasopharyngeal
URT	Upper Respiratory Tract
COPD	Chronic Obstructive Pulmonary Disease
CXR	Chest radiograph
OM	Otitis Media
IPD	Invasive Pneumococcal Disease
ERS	European Respiratory Society
OTC	Over the counter
FilmArray PP	FilmArray Pneumonia Panel
RML	Right Middle Lobe
LLi	Left Lingular
RLL	Right Lower Lobe
RUL	Right Upper Lobe
LLL	Left Lower Lobe
LUL	Left Upper Lobe

CONTENTS

INTRODUCTION

Bronchiectasis (BE), protracted bacterial bronchitis (PBB), and chronic suppurative lung disease (CLSD) are severe diseases with noteworthy harmful influences on children, their families, the healthcare sector, and the broader community [Chang et al. 2008]. They overlap clinically, and diagnoses can be confirmed only after further investigations and long-term follow-ups [Chang et al. 2008]. It has been assumed (nevertheless stayes unconfirmed) that BE CLSD and PBB are part of a clinical spectrum and share common triggers and/or pathophysiologies while being distinguished by their respective degrees of severity [Chang et al. 2008; Kalu et al. 2010]. Shared themes of suppurative pulmonary circumstances are extreme neutrophilic inflammation, insistent and recurring infection and poor clearance of infected material [Redding and Carter, 2017]. It is extensively recognized that tenacious bacterial infections are damaging to the airway [Stockley 1998].

1. Protracted Bacterial Bronchitis (PBB)

PBB does not constitute a novel entity, as PBB-like circumstances were stated throughout the preceding era [Kantar et al. 2017; Taussig et al. 1981]. However, it has only recently been adequately clinically characterized (by Bronchoalveolar lavage (BAL)) [Chang et al. 2008; Marchant et al. Chest 2006; Marchant et al. Thorax 2006; Donnelly et al. 2007]. PBB is now a well-recognized clinical entity [Wurzel 2005; Gibson et al. 2010; Shields 2008; Dinwiddie 2013; Irwin 2006; Pohunek and Svobodova 2013].

The innovative classification of PBB, which was addressed in 2006, involved the subsequent principles: the occurrence of bacterial infection of the lower airways as displayd on BAL, extended wet cough that last more than weeks and the resolution of coughing within two weeks of appropriate antibiotic therapy [Marchant et al. Chest 2006]. However, the classification of PBB has progressed since then [Marchant et al. Chest 2006; Wurzel 2005]. Today, PBB is considered present when a continuous chronic (duration >4 weeks) productive or wet cough exist; signs or symptoms (i.e., particular cough indicators) suggestive of other grounds of wet or the cough is determined after two to four weeks of a proper oral antibiotic cure and productive cough are not present [Kantar et al. 2017].

For augmented likelihood and applicability to primary and tertiary care situations, the BAL element was absent in the reviewed description of PBB [Gibson et al. 1010]. This was additionally maintained by a randomized controlled trial on children with prolonged cough (n=50) that displayed considerably advanced cough-resolution rates in children getting amoxicillin clavulanate antibiotics for two weeks (48%) compared to those receiving a placebo (16%) [Wurzel 2005; Marchant et al. 2012]. PBB has been accepted in an official way by the British Thoracic Society, Thoracic Society of New Zealand and Australia 5 [Chang et al. 2008; Shields et al. 2008]. PBB is likewise acknowledged by European Respiratory Society (ERS) and the American College of Chest Physicians [Chang et al. Chest 2017] as a clinical body as specified by its assimilation into the ERS pediatric syllabus in 2012 and a task force manuscript [Kantar et al. 2017; Chang AB and Marchant 2019]. PBB constitutes a main reason of enduring wet cough in children in Australia, UK and US, and it is perceived to be the topic of investigation made by numerous groups [Donnelly et al. 2007; Kompare and Weinberger 2012; Zgherea et al. 2012].

1.1 Pathobiology

The lower airway profile of BE and PBB [Chalmers et al. 1018] is described by extreme neutrophilic infection with noticeable proinflammatory mediator reactions (e.g. matrix metalloproteinase-9, interleukin (IL)-8 and IL-1β) [Marchant, Gibson et al. 2008; Baines et al. 2014]. Validation cohorts and experimental and of children with PBB have revealed expressively greater levels of genetic factor and protein expression of α-defensin, IL-1β, CXCR2 and IL-1 pathway members, than non-PBB illness controls [Baines et al. 2014]. The expression of the gene of some IL-1β signaling molecules (i.e., IL-1 receptor-associated kinase 2 and Pellino-1) were considerably advanced in children with persistent PBB (more than episodes per year) than in those without this condition [Baines et al. 2014], as 85% of those in the former group eventually had BE within two years [Wurzel et al. 2016]. In alignment with microbial data and clinical this likewise proposes a dose-response phenomenon with BE at one end and PBB at the other.

Furthermore, simultaneous experimentations displayed that the pathobiological reactions of lower airway specimens of children with BE and PBB and share some notable resemblances and are considerably different from those of controls. Damaged apoptosis and efferocytosis to non-typeable Haemophilus influenza (NTHi) [Hodge et al. 2016] and gene expression levels of NTHi-stimulated BAL airway cells [Chen et al. 2018] in children with PBB were between the values observed in controls and children with BE [Chang AB and Marchant 2019].

1.2 Clinical profile of children with PBB

Children with PBB are characteristically young (below five years old, median age = 3 years [Marchant et al. Chest 2006; Donnelly et al. 2007]). Also, they present a chronic wet cough [Chang et al. 2008]. Although several parents have stated 'ever wheeze' (41-81% [Donnelly et al. 2007; Darelid et al. 1993]), doctors rarely diagnosed these children with auscultation-confirmed wheeze [Kantar et al. 2017]. General effects are normally negligible or non-particular, and children with PBB normally seem to be healthy—they usually do not exhibit wheezing during clinical assessments [Chang et al. 2008]. They also exhibit standard development and progress and are short of signs of principal chronic suppurative lung disease (CSLD), like chest wall deformity, digital clubbing and adventitial auscultatory chest outcomes [Darelid et al. 1993]. However, crackles and 'rattly chest' are infrequently detected [Goyal et al. 2016; Chang et al. 2008; Kantar et al. 2017]. When appropriate treatment is provided, these symptoms typically recover before the cough resolves. Meanwhile, symptoms deteriorate throughout inter-current viral contagions, and the mixture of a persistent "cough at night" and viral exacerbations normally result in misdiagnoses of asthma.

Because children with PBB do not react to bronchodilator treatment, they are occasionally erroneously diagnosed with severe asthma [Marchant et al. Chest 2006; Donnelly et al. 2007]. Such diagnoses are further complicated in cases when bacterial bronchitis and asthma exist together. Like children with an enduring cough, children with PBB have high morbidity [Chang et al. 2008]. According to previous research, in relation to 'illness controls' who underwent bronchoscopy for non-cough-related circumstances (e.g., apnoea ot stridor), children with PBB were more probable to have joined childcare (odds ratio (OR)=8.4, 95% confidence interval (CI)

2.3-30.5), though the tobacco smoke exposure rates of the two groups (~30% [Marchant et al. Chest 2006; Darelid et al. 1993]) were similar [Darelid et al. 1993].

Children with and without PBB also show similarities in terms of the dominance of atopic features (systemic and airway eosinophilia, eczema, positive radioallergosorbent test results or elevated IgE) [Darelid et al. 1993; Kantar et al. 2017]. The coughs of children with PBB resolve only after extended treatment (10–14 days at least) with proper antibiotics. PBB diagnosis ought to be established only the time that the reaction to cure is dramatic—meaning, if the child does not have symptoms.

1.3 Recurrent versus non-recurrent PBB

Distinguishing non-recurrent and recurrent PBB and in an individual child is important because those with more regular incidents of protracted wet cough might be at higher danger of CLSD and, subsequently, BE. This notion is validated by examinations on adult population recently identified with BE, most of whom (60-80%) have a past of long-lasting wet cough dating to their childish years [Pasteur et al. 2000; King et al. 2006]. Concomitant neutrophilic inflammation of the lower airways and enduring bacterial infection (which are important elements of PBB) are chief components of Cole's vicious circle theory regarding the pathogenesis of BE. Destruction to airway epithelia (e.g., secondary to chronic or acute respiratory infection) damages mucociliary clearance and predisposes an individual to chronic bacterial infection. This initiates an inflammatory reaction that additional hinders mucociliary clearance, leading to a phenomenon of a vicious circle [Cole 1986].

Young children with PBB regularly have recurrent occurrences. A current retrospective appraisal of 44 children (median age 2.7 years), 33 of whom suffered from PBB, 25 (76%) had additional relapse/s over an undetermined median follow-up period [Pritchard et al. 2014]. A previous evaluation of 81 children with clinical PBB displayed that 41 (51%) were without symptoms following two courses of antibiotics; nevertheless, 11 (13%) required six or more courses of antibiotics [Donnelly et al. 2007]. The association between recurring episodes of PBB and future BE diagnoses in children has not been studied until now [Wurzel 2015].

1.4 Imagine screening tests

Chest X-rays might be described as "typical" nonetheless frequently display peribronchiolar alterations [Marchant et al. Chest 2006; Donnelly et al. 2007]. Although hyperinflation is rare, its presence should raise concern when observed in children with asthma with PBB or asthma alone [Chang et al. 2008]. Computer tomography (CT) scans must be kept until after an ineffective treatment trial, since results of BE might happen following acute respiratory contaminations nonetheless resolve some months later. Additionally, the perseverance and severity of symptomatology do not essentially relate to CT alterations [Chang et al. 2008]. Whenever an irregularity, either in the spirometry or the chest radiograph (other than peri-bronchial alterations), presents itself in a child who have chronic wet cough, further examinations regarding its underlying origin are recommended. When such investigations are performed, both respiratory and spirometry system [De Baets et al. 2012] resistance and reactance, assessed by the forced oscillatory method (unpublished), were likewise typical [Kantar et al. 2017].

1.5 Microbiology of PBB

1.5.1 Bacterial

Like BE and CSLD, PBB is linked to insistent bacterial infections in the airways [Marchant et al. Chest 2006; Donnelly et al. 2007], and it is broadly acknowledged that such bacterial infections are damaging to the airways [Stockley 1998; Chang et al. 2008]. Examinations regarding the lower airways of children are not many since children are not normally capable of expectorating sputum; henceforth, we rely on lower airway sampling practices.

Current examinations regarding the lower airways of children suffering from chronic coughs has been restricted to retrospective examinations and small cohorts. The primary examination referring to PBB noticed normally documented M. *catarrhalis* (26%)) and respiratory pathogens (H. *influenzae* (47%), S. *pneumoniae* (35%) in BAL cultures at great bacterial loads ($>10^5$ colony-forming units/mL) [Marchant et al. Chest 2006]. Subsequent studies

supported these findings (**Table 1**) [Kantar et al. 2017]. The development of one or more bacterial types at a density consistent with the infection (e.g., $\geq 10^4$ CFU/mL) was identified in the lower airways of 30-50% of affected children [Donnelly et al. 2007; Chang, Eastburn et al. 2005; Chang, Van Asperen et al. 2015; Marchant, Gibson et al. 2008; Seear and Wensley 1997] with PBB [Kantar et al. 2017].

The time of evaluating the bacteriology of PBB, it is imperative to address that not all BAL examinations are straight comparable, since only some of them utilized a quantitative bacterial culture to define their BAL outcomes [Van Asperen et al. 2015; Seear and Wensley 1997]. Moreover, the spreading of bacteria in the lungs is inconsistent. One retrospective examination addressed that sampling from a single lobe missed a total of 17 dissimilar organisms in 15 of 50 individuals, eight of whom would then not have had any organisms cultured [Seear and Wensley 1997]. However, this examination utilized non-quantitative bacterial culture techniques and did not succeed to address bacterial densities between the countless lobes.

A summary of data on the bacteria addressed in PBB is stated in **Table 1** [Kantar et al. 2017]. Likewise, children with PBB frequently have damaging bacterial cultures in their BAL [Marchant et al. Chest 2006]. This is probably because antibiotic exposure during sampling can suppress colony counts to below the threshold required to be categorized as a ‘contamination’ or because an insufficient BAL method with reduced return or very localized sampling was used, which might miss the infested portion of the lung [Narang et al. 2014]. Finally, sub-optimal sample handling (e.g., a delay in processing, plating or insufficient storage), might prevent an organism that is present from forming a culture. This could be especially relevant in the case of fastidious organisms, such as NTHi [Van Eldere et al. 2014].

Table 1: Summary of bacteriology on children with PBB

Publication year/country	*H. Influenzae* (Hi)%	*S. pneumonia* (Spn)%	*M. catarrhalis* (Mcat)%	Other	>=2 organisms	Quantitative or Non-quantitative
2015/China	47	37	0	E. coli=6% Enterobacter=5%	NR	NR
2014/Australia	72	39	43		50%	Quantitative
2014/England	63	23	51	Sa=19%	48%	Non-Quantitative
2014/England	50	16	28	Sa=22%	39%	Non-Quantitative
2014/Australia	Adv+: 68 AdV-: 47	35 22	35 19	 Sa=10.2%	NR	Quantitative * Some cohorts had BE
2014/Australia	50	NR	NR	Sa=16%	NR	Quantitative * PBB cohort only
2013/Greece and England	Greek grp: 61	27.6	32	Sa=6%	39%	Quantitative
2012/Australia	38	24	19		NR	Quantitative
2008/Australia	45	32	24		NR	Quantitative
2007/England	81	37	NR		30%	Cough swab n=50 BAL n=19

1.5.2 Viruses

In earlier studies on PBB [Marchant et al. Chest 2006], children suffering from respiratory viruses in BAL were not included; therefore, most examinations on PBB have concentrated on bacterial infections of the airways [Marchant et al. Chest 2006; Marchant et al. 2008; Donnelly et al. 2007; Kompare and Weinberger 2012; Zgherea et al. 2012].

Only one examination methodically searched for infections [Darelid et al. 1993]. The presence of at least one infection was detected in 38% of BAL samples taken from 104 children with PBB [Darelid et al. 1993], which was considerably greater than a group of 49 children with other long-lasting respiratory illnesses (38% vs. 9%; OR=6.3, 95%CI 2.1-19.1). The most shared virus observed in children with PBB was human adenovirus (HAdV) (23%); the most representative species was the HAdV-C species (detected in 96% of HAdV+ children) [van der Gast et al. 2014].

Furthermore, a prolonged molecular-founded diagnostic panel regarding 17 respiratory viruses was done on a subgroup of 27 children. Rhinovirusfound was detected in 11 (41%), while human coronavirus and human bocavirus were each noticed in one (4%) participant. Nonetheless, the dominance of these further viruses was comparable to the control group. The

similar examination presented that H. influenzae contamination in the lower airway (>10^4 CFU/mL) was significantly linked to HAdV co- contamination [Darelid et al. 1993].

1.5.3 Viral Bacterial co-infection

Although the possible synergy between bacteria and viruses has been recognized in other acute respiratory illnesses like pneumonia [Korppi et al. 1991] and RSV 22 bronchiolitis [Thorburn et al. 2006], few clinical studies have correlated viral bacterial co-contamination and PBB in children. Viral-bacterial coinfection is associated with heightened neutrophilic inflammation of the lower airways in children [Wurzel et al. 2014; Wurzel, Marchant et al. 2014]. Thus, it is postulated that microbial synergy and viral-bacterial co-infection are additional key features of PBB.

However, there are many mechanisms by which viral contaminations upsurge the possibility of secondary bacterial infections. For instance, in the upper respiratory tract (URT), viral infections predispose the tract to secondary bacterial contamination by interrupting the mucociliary functioning, impairing the epiglottal-cough reflex and disrupting the ciliary ultrastructure by decreasing the numbers of ciliated cells and cilia [Bakaletz 1995]. Alterations to cilial beat occurrence likewise damage the elimination of particles, fluid and bacteria from sinuses, the middle ear and lower respiratory tract [Suzuki K and Bakaletz 1994; Ohashi et al. 1991; Park et al. 1993]. Additionally, to producing structural—and, accordingly, functional—impairments, specific viruses (e.g., herpes viruses and adenoviruses) are able to control the host's immune reaction, thus allowing the perseverance of the virus in the airways [Mandell et al. 2010].

1.6 Treatment of PBB

PBB is normally treatable, unlike asthma, Treatments involve eradicating the bacteria with antibiotics, improving coughing's efficiency, and keeping the airways without being infected to permit curing process. However, no published randomized trials have dealt with PBB specifically. Nevertheless, two studies [Darelid et al. 1993; Gottfarb and Brauner 1994] on extended wet coughing summarized in a Cochrane appraisal (although restrictions exist) defined

that the wet cough reacted to antibiotics, with some of them required to treat (NNT) of 3 (95% CI) [McMillan et al. 2004]. Nevertheless, it was uncertain how many of the 140 children suffered from PBB [McMillan et al. 2004]. The development of the disease (stated by the requisite for additional antibiotics) was considerably inferior in the group that used antibiotics, with an NNT of 4 (95% CI) [Marchant, Morris et al. 2005]. Initial antibiotics treatments targeting the organisms stated before generally differ from two to four weeks. However, children with recurring PBB (more than episodes each year) ought to be assessed for BE (e.g., functional antibody responses to vaccinations, bronchoscopy, evaluation of immunoglobulins, sweat test, full blood count, HRCT scan) [Chang et al. 2008].

The time that a usual course (five days) of antibiotics is utilized, the cough either declines within two to three days, or it somewhat diminishes but does not stop totally. Conversely, the short course of antibiotics needed to treat community-acquired pneumonia in well children is five to seven days [Agarwal et al. 2004].

1.6.1 Use of co-amoxiclav

In a randomized controlled trial in Australia with 50 children (median age = 1.9 years) suffering from long-lasting wet cough for more than three weeks, 25 children were consigned to get either two weeks of twice-daily oral co-amoxiclav (22.5 mg per kg per dose) or a placebo. The principal end-point observed was ‘cough resolution,’ described as either (i) a >75% decrease in the validated verbal category descriptive (VCD) cough score in 14 days of cure in relation to baseline scores or (ii) the cessation of coughing for more than three days. A significantly greater cough resolution rate (48%) was distinguished in children who tooj co-amoxiclav than in children who took the placebo (16%). Furthermore, the median VCD score (post-treatment) in the co-amoxiclav group was significantly lower than in the placebo group (0.5 vs. 2.2). There was no significant dissimilarity in the BAL data between the two populations.

This examination delivered the first high-level proof supporting the inclusion of antibiotics in pediatric cough-particular strategies as a treatment for PBB [Marchant et al. 2012]. Although the British Thoracic Society (BTS) proposes a four- to six-week sequence of antibiotics [Shields et al. 2008], a two- to three-week course might be used in clinical practice at the first discussion so that the child’s situation can be reviewed at the end of the antibiotic therapy [Paul et al. 2013].

1.6.2 Use of macrolide antibiotics

A Cochrane review failed to find that macrolide antibiotics like azithromycin were superior to co-amoxiclav for curing children suffering from chronic cough [Panpanich et al. 2008]. Nevertheless, these antibiotics can be used to treat children with PBB, especially those who may be allergic to penicillin-containing antibiotics. Azithromycin furthermore has a dosing advantage, since it requires to be managed only one time during a day. It might be prescribed to be taken three days per week for three weeks [Shields et al. 2008]. Thus, merely nine doses of oral azithromycin have to be provided, whereas 42 doses of co-amoxiclav are required over two weeks. Azithromycin is also useful for treating young children, for whom adherence might be a concern. The rest of macrolide antibiotics, containing clarithromycin and erythromycin, are correspondingly efficacious, however several doses have to be offered to children [Shields et al. 2008].

1.7 Significance of PBB

In the 1940s, a possible connection between BE chronic and bronchitis was proposed [Finke 1948], containing proposals that this could be disturbed by serious antibiotic treatment [Finke 1948; Field 1949]. After some decades, in the 1980s, a retrospective assessment of 20 children with chronic bronchitis testified bronchoscopic evidence of bronchial wall infection and purulent bronchial secretions (mostly encompassing *Haemophilus influenzae*), most of whom enhanced after antibiotic treatment [Smith et al. 1985]. This study overlapped with the journal of Cole's 'vicious circle' theory of long-lasting bacterial infection and inflammation triggering BE, [Cole 1986], which assisted to deliver a theoretical background for PBB as a possible pre-BE condition in some children [Chang et al. 2008; Kantar et al. 2017].

However, no examinations until now exist that have prospectively inspected the temporal association between BE and PBB and to deliver definitive support for this suggested connection. Therefore, the mechanisms supporting the natural history of PBB and expansion are still not known. [Shields 2006; Chang et al. 2008], as are the medium-term outcomes of PBB [Shields 2006]. PBB is undoubtedly dissimilar from acute bronchitis, as indicated, for instance, by the fact that the cough is of a shorter duration in pediatric acute bronchitis (two weeks) [Chang and

Glomb 2006; Chang et al. 2006]. Whether PBB is precursor to BE in some children is unidentified, and its assessment is vital [Marchant et al. Thorax 2006; Donnelly et al. 2007; Shields 2006]. Children with PBB do not have proven BE, since those with proven BE frequently have a dissimilar clinical profile and are improbable to recuperate after 10–14 days of oral antibiotics. Nonetheless, as formerly specified, there might be a connection between BE and PBB as suggested by the vicious circle hypothesis [Cole 1986] and experimentally supported by long-standing natural history data [Phelan 1984].

The pathognomonic chest CT element of BE is an irregular dilatation of the airways, stated as an augmented bronchoarterial ratio (BAR) of >0.8 in children [Chalmers et al. 2018]. BAR increases as the severity of BE increases [Chalmers et al. 2018]. Meaning, from a normal mean BAR of 0.5–0.6 in children [Kapur et al. 2011], a steady upsurge—other than for congenital types of BE (e.g., Mounier-Kuhn syndrome)—happens the time that the airway insults (typically contamination and inflammation) are uncurtailed. Repeated or insistent contaminations are part of the "vicious cycle" of airway injuries associated with BE [Chalmers et al. 2018]. At some degree throughout this procedure, the lung damage becomes so progressive that the tissue alterations become irreversible (irreversible BE). It is logically and biologically plausible that PBB signifies the initial variety of chronic endobronchial suppurative illness, while irretrievable BE represents the late end (**Figure 1**) [Chang AB and Marchant 2019].

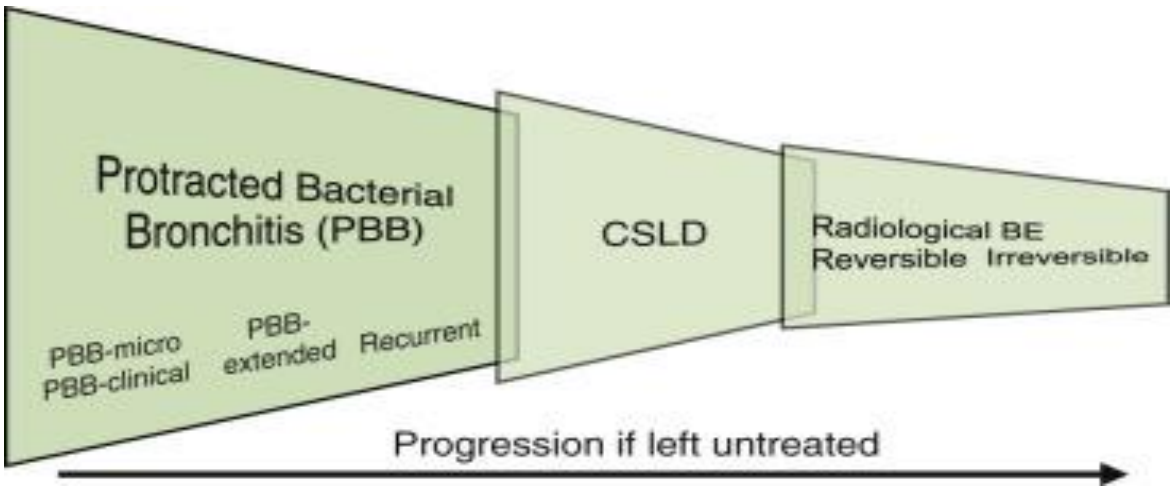


Figure 1. The spectrum of chronic suppurative lung diseases.

Thus, we support intervening in children with PBB and not wait until BE grows [Chang et al. 2008]. Mild radiological BE if cured early in children, is reversible, which allows these children to avoid a future progressive decrease in the function of the lung [Chang, Bush, et al. 2018]. Adults who experienced BE symptoms from their childish years suffer more extreme diseases and have poorer prognoses than adult-onset patients [King et al. 2009]. The pathobiology (e.g., airway microbiota [van der Gast et al. 2014]) is different for severe BE (e.g., adults with established BE) than for mild BE. However, more longstanding cohort examinations and more detailed examinations are required [Chang AB and Marchant 2019].

The progression of PBB disease utilizing the pathobiological model, PBB, CSLD, and radiographically confirmed bronchiectasis probable represent dissimilar ends of a continuum with related fundamental mechanisms of endobronchial bacterial infection, airway neutrophilia and damaged mucociliary clearance. Without treatment, it is probable that some (nonetheless not all) children with PBB will finally grow CSLD; a few will finally grow bronchiectasis, which will be primarily revocable but consequently irreversible if untreated. There is a degree of connection between each of the units (replicated with permission from the publisher [Chang, Upham et al. 2016]).

2. Bronchiectasis (BE)

BE, unconnected to cystic fibrosis (CF), is a significant impact of respiratory morbidity in developing states and is progressively being recognized in indigenous individuals in prosperous states [Kapur et al. 2009; Chang, Grimwood et al. 2002; Singleton et al. 2000]. The definition of BE provided by Laenec in 1819 was founded on post-mortem histopathology [Lae¨nnec 1819]. Later on, bronchograms, which were defined in 1951 for the first time [Mannes 1951], became the gold standard before being mostly substituted by chest high-resolution computerized tomography (cHRCT) scans.

Presently, BE is described as the “unalterable dilatation of peripheral airways” and is typically diagnostically recognized radiologically by cHRCT scans [Westcott 1991; Webb et al. 2001]. In the affected areas extra mucus tends to shape and pool, rendering them prone to infection and chronic inflammation [Hare 2014; Cole 1986]. In most developed countries, the frequency of childhood BE has reduced significantly over time. This reduced incidence has been

described as due to declined crowding, enhanced immunization curriculums, improved hygiene and nourishment, and primary admission to medicinal care [Nikolaizik and Warner 1994; Saynajakangas et al. 1997]. However, BE remains common in poorer countries [Karakoc 2001; Callahan and Redding 2002; Sethi and Batra 2000] and among deprived indigenous groups in industrialized nations like in the USA in the Alaskan Yupik children [Singleton et al. 2000], indigenous children in Australia [Chang, Masel et al. 2003], and Maori and Pacific Islanders in New Zealand [Edwards et al. 2003; Chang et al. 2008]. Today, the frequency of BE is increasing because of the widespread use of HRCT scanning [Cohen and Sahn 1999].

BE diagnosis in children is definitive if:

1. The previously published radiological criteria are fulfilled.
2. The clinical symptoms are consistent with BE [Chang et al. 2008; Wurzel et al. 2014].

All children with definitive BE must have evidence of cylindrical BE via a cHCT scan [Chang et al. 2008; Wurzel et al. 2014]. The key components of BE in HRCT scans are amplified bronchi in the periphery of the lung, lack of tapering and a bronchial wall thickening [Webb and Muller 2001; Naidich et al. 1982]. A bronchus is thought to be dilated if, on the CT scan, the broncho-arterial ratio with the adjacent supplementary artery surpasses [Kasarabada et al. 2012]. However, the normal airway-to-vessel diameter ratio is age-dependent in typical children [Redding and Carter 2017; Kapur et al. 2011]. The pathognomonic chest CT in children is stated as an augmented BAR of >0.8 [Chalmers et al. 2018]. BAR increases as the severity of BE increases [Chalmers et al. 2018]. Meaning, from a normal mean BAR of 0.5–0.6 in children [Kapur et al. 2011], a slow upsurge (other than for congenital types of BE, like Mounier–Kuhn disorder) happens when the airway insults (typically inflammation and contamination) are not condensed [Chang and Marchant 2019]. Other elements contain decreased attenuation in expiratory scans, a linear array or cluster of cysts and mucous plugging [Webb and Muller 2001].

The prevailing symptom of BE constitutes the occurrence of an exceptionally extended wet cough. In some other children, the cough might be purulent and productive. Additional symptoms comprise of exceptional dyspnoea, recurring chest contaminations, persistent wet cough in response to antibiotics, symptomatology of reactive airway illness (asthma-like circumstance), and development failure. In children, hemoptysis seldom happens, excluding

progressive stages of the illness. Additional clinical signs of BE contain chest wall deformity, clubbing, hyperinflation and adventitious sounds on chest auscultation [de Blic et al. 2000; Kalu et al. 2010].

Of note, the word CSLD is utilized to define a diagnostic verdict with clinical symptomatology of BE but without cHRCT evidence [Chang et al. 2008]. Untreated CSLD may progress to BE due to a cumulative airway damage from recurring or a constant bacterial contamination [Chang et al. 2008]. In its most severe type, BE can grow to end-stage pulmonary failure during adulthood [Stafler and Carr 2010].

2.1 Pathogenesis

BE is the outcome of long-lasting airway infections resulting in loss of the structural integrity of the airway wall that creates airway expansion. It might take place focally following severe acute respiratory contaminations or post-obstructive pneumonia because of foreign bodies or endobronchial lesions (tuberculosis and tumor) or during the lung in a patchy distribution (e.g., Primary ciliary dyskinesia and CF). BE might additionally grow following extreme lower respiratory viral contaminations, particularly in indigenous groups of people [Colom et al. 2015; Trenholme et al. 2013].

There are not many information regarding the pathogenesis of BE in children [Morrissey et al. 2003; Chang and Bilton 2008; King et al. 2006; Watt et al. 2004]. In current decades, significant tries have been attempted to comprehend the airway inflammation liked to BE. There are a number of parallels between BE and other long-lasting respiratory illnesses, containing chronic obstructive pulmonary disease (COPD) and CF regarding airway infection. As a consequence, the inflammatory procedures contained in COPD and CF are occasionally utilized to comprehend the pathophysiology of BE. Nevertheless, COPD and CF have noticeably dissimilar causes than BE in children; thus, data should be extrapolated with caution, particularly in pediatric settings [Pizzutto et al. 2017].

Known risk factors for BE in children include severe and recurrent pneumonia [Valery et al. 2004; Eastham et al. 2004]. However, the initial pathogenetic mechanisms that lead to BE are unknown. Recurrent microaspiration into the lower airways of pathogenic bacteria coming from the nasopharynx might be one possibility that BE is caused [Ben-David et al. 2005; Bogaert, de

Groot and Hermans 2004; Thach 2008]. It is also possible that the repeated aspiration of relatively large inocula of bacteria-laden nasopharyngeal (NP) secretions during acute respiratory infections may overwhelm local pulmonary defenses, thus initiating endobronchial contagion, inflammation, and airway damage.

This notion is dominant to Cole's 'vicious circle' theory regarding the roots of BE [Cole 1986] (**Figure 2**). Constant neutrophilic inflammation and airway colonization might progress into airway inflammation, prolonged mucus hypersecretion and chronic cough. In a number of circumstance, cumulative airway injuries due to recurring or insistent bacterial infections might result in BE. This can occur extremely fast if the airway injury is severe, like following adenoviral ALRIs, or it might be slow if caused by recurrent (nonetheless less contagious) ALRIs [Chang et al. 2008].

Nevertheless, both chronic respiratory situations propose that BE ascends from dysregulated inflammation or exaggerated in reaction to challenges from respiratory pathogens. Therefore, the "vicious circle" theory of inflammation, self-perpetuating contamination and tissue damage mentioned by Cole [Cole 1986] stays the most probable clarification for the pathogenesis of BE. However, a critical question remains regarding what produces the highly controlled immune reaction to become dysregulated [Pizzutto et al. 2017].

The innate inflammatory reaction constitutes a fast reaction unit of the immune system. When pathogens enter physiological barriers, like the epithelium, a series of extreme controlled cellular and non-cellular events synchronize to quickly encompass the contamination. Additional to its role as a first-reaction component, the inflammatory reaction composes the beginning of the pathogen-specific adaptive reaction. When functioning in the best manner, the primary inflammatory procedure resolves as fast as it starts preserving tissue homeostasis. Irregulation whether a delayed response in resolution or an overstated primary reaction, can lead to an accumulation of potent cytotoxic compounds that might impair host tissues and deliver an environment conducive to additional contamination [Pizzutto et al. 2017].

No innate immune examinations exist with children having non-CF BE. The significance of innate immunity dysfunction is progressively acknowledged in pulmonary illness [Hartl et al. 2007]. Nonetheless, the duration and nature of that immune dysfunction has not been stated, nor is it obvious if the dysfunction is precise to the lower airways or more generalized whereas likewise involving circulating leukocytes.

The pathogenesis of development of PBB to BE and CSLD is still not known. Nevertheless, it has been speculated that untreated PBB intensifies airway neutrophilia and leads to succeeding airway damage, progressing to CSLD and later BE.

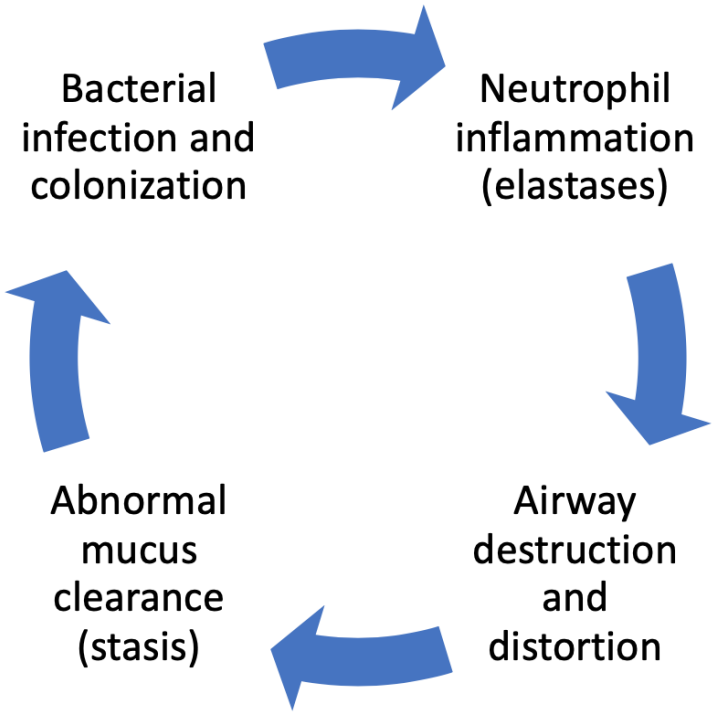


Figure 2. BE Cole's 'vicious circle' pathogenesis.

2.2 Initiation of lower respiratory infection

Since lower airway contaminations come from the aspiration of pathogenic bacteria coming from the upper airways [Bogaert, de Groot and Hermans 2004], dissimilarities in URT carriage in dissimilar populations might be worth exploring. Four of the five chief bacteria associated with BE (*P. aeruginosa* being the exclusion) are shared colonizers of the URT in children. Nasal or NP carriage of NTHi *M. catarrhalis* and *S. pneumonia* is frequent in young children, predominantly among those who attend day care centers [De Lencastre et al. 1999; Stubbs et al. 2005] and who live in low-income countries [Adegbola et al. 2014]. Nevertheless, extreme high rates (80–90%) have been stated in Australian Aboriginal children [Hare et al. 2011; Stubbs et al. 2005] and children in a number of developing nations like Papua New Guinea

(PNG) [Gratten et al. 1989] and The Gambia [Obaro et al. 1996; Kwambana et al. 2011], which likewise have high rates of pneumonia mortalit and morbidity [Greenwood 1992; Lehmann 200;]. Gambian and Australian Aboriginal toddlers obtain NP carriage of all three chief respiratory pathogens at a very early age [Kwambana et al. 2011; Leach et al. 1994], and the URT weight is greater in Indigenous Australian children than in non-Indigenous children [Stubbs et al. 2005; Watson et al. 2006].

The high level of concordance amongst bacterial strains (pneumococcal serotypes and NTHi ribotypes) in the nasopharynx and lungs of Indigenous Australian children with BE, concurrent carriage, and lower airway contagion [Hare et al. 2010] suggests the current aspiration of NP secretions based on the high turnover of NP strains. Nevertheless, several strains of *S. pneumonia and* NTHi have constantly been observed more regularly in BAL than in NP specimens [Hare et al. 2010; Hare et al. 2012]. This recommends the gathering of strains in the lower airways resulting from recurring aspiration and failure to eradicate prior strains.

Several NTHi strains have likewise been testified in sputum microbiology from adult people with chronic respiratory circumstances [Sethi et al. 2004; King 2007]. The high and early burden of pathogens in the nasopharynx of children probably leads to the high burden of acute and chronic lower respiratory contaminations (**Figure 3**) [Pizzutto et al. 2017]. **Figure 3**, adjusted from Cole's original model [Cole 1986] and revised to elucidate chronic lung illness [Hare KM, Smith-Vaughan et al. 2010], illustrates the "prolonged vicious circle" theory, which attempts to clarify the high rates of long-lasting endobronchial illnesses like BE [Pizzutto et al. 2017].

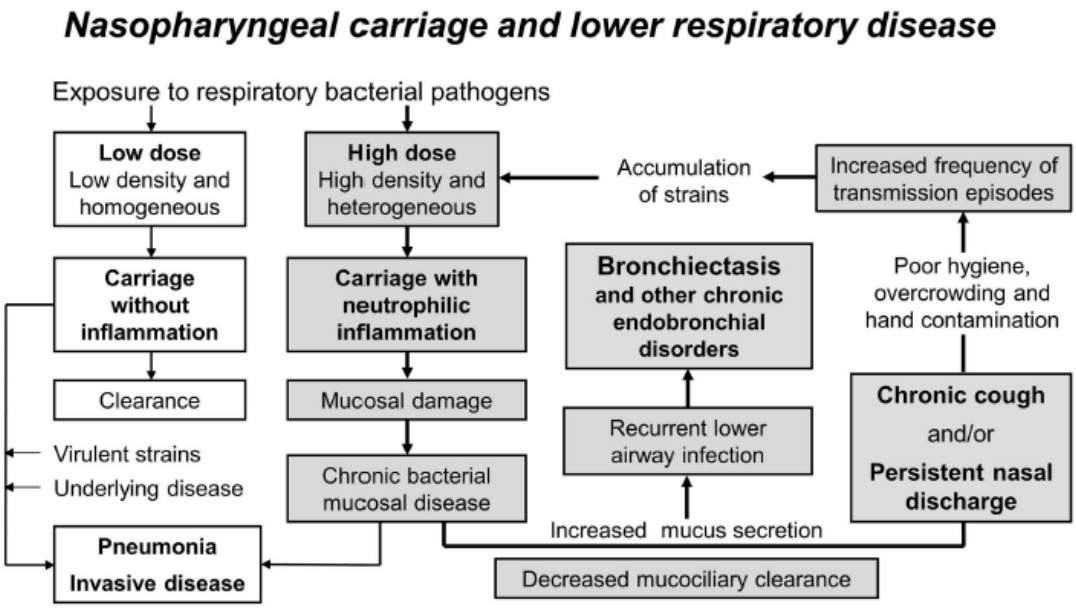


Figure 3. Extended vicious circle hypothesis.

2.3 Causes of BE

BE can be differentiated depending on whether it is because of systemic, anatomic illnesses, congenital, idiopathic or post-infectious, causes (**Table 2**) [Barker 2002, O'Donnell 2008]. CF is considered the most substantial etiology of clinically noteworthy BE in developed nations, as non-CF BE has declined due to improved hygiene and nutrition, better vaccine coverage, and the early administration of antibiotic treatment [Kapur and Karadag 2011]. However, with estimated prevalences of 1 in 6000 in New Zealand [Edwards, Asher et al. 2003] and 1 in 5800 in Britain [Eastham et al. 2004], pediatric non-CF BE is only slightly less common than CF BE, even in developed countries [Hare 2014].

BE might be categorized into three forms, based on the cHRCT,: varicose BE, cylindrical BE and saccular or cystic BE (**Figure 4**). In cylindrical BE, the bronchi cannot taper as the bronchi progress peripherally. Varicose BE has an unbalanced and beaded appearance and seems as "a string of pearls." Cystic BE or Saccular emerges as a group of cysts without identifiable bronchial structures distal to the sacs (Webb, WR, High resolution CT chest of the Lung 2009) [Kasarabada et al. 2012].

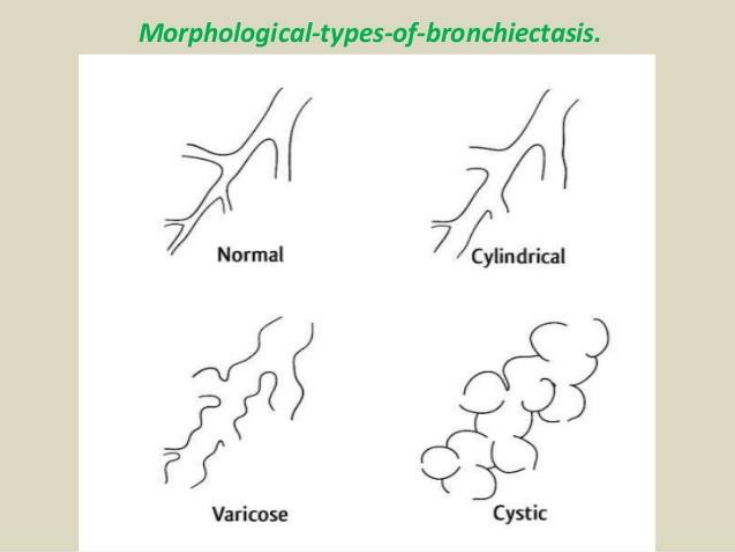


Figure 4. Morphological types of bronchiectasis.

Table 2: Differential diagnoses of known etiologies of bronchiectasis

Post-Infectious Lower respiratory tract Granulomatous infections Necrotizing pneumonias Other respiratory infections	**Inhalational and Obstruction** Severe gastroesophageal reflux disease Chronic aspiration pneumonia Toxic or thermal inhalational injury
Primary Immune Disorders Hypogammaglobulinemia Waldenstrom's Other humoral/cellular disorders Neutrophil abnormalities	**Cystic Fibrosis** **Young's Syndrome** **Alpha1-Antitrypsin Deficiency**
Heritable Structural Abnormalities Ciliated epithelium such as primary Ciliated dyskinesia Cartilage or connective tissue (Tracheobronchiomegaly; Williams-Campbell) Sequestration, agenesis, hypoplasia	**Allergic Bronchopulmonary Aspergillosis** Or Other Mycosis **Post-obstruction** Foreign body Tumor (benign and malignant)
Idiopathic Inflammatory Disorders Sarcoidosis Rheumatoid arthritis Systemic lupus erythematosus Sjogren's syndrome Inflammatory bowel disease Relapsing polychondritis	**Miscellaneous** HIV infection/AIDS Yellow Nail Syndrome Radiation Injury

2.4 Clinical profile of children with BE

The leading symptom of BE is the occurrence of extremely extended wet cough. In older children, coughing might be purulent and productive. Some other symptoms contain exceptional dyspnea, persistent chest infections, recurrent wet cough in response to antibiotics, symptoms of reactive airway illness (asthma-related conditions), and development failure [Chang et al. 2008]. Children with BE have acute exacerbations of respiratory disease, and several require hospitalization when oral treatments do not succeed; however, no known forecasters appear in published research [Saynajakangas et al. 1998].

Persistent exacerbations might result in the advanced worsening of lung function [Ellerman and Bisgaard 1997] and are one of the strongest predictors of poor quality of life among BE patients [Wilson et al. 1997; Kapur et al. 2009]. In children, hemoptysis rarely happens excepting when the illness reaches advanced stages. Clinical signs contain chest wall deformity, clubbing, adventitious sounds in chest auscultation, and hyperinflation [Chang et al. 2003; Chang et al. 2002]. However, the nonappearance of those signs does not necessarily show that BE itself is absent.

When the disease reaches advanced stages, signs of long-lasting hypoxemia and pulmonary hypertension can arise. The outcomes of BE range from augmented mortality [Karakoc et al. 2001; Keistinen et al. 1997], morbidity from the disease itself (e.g., augmented hospitalization and medicinal necessities, low quality of life) [Martinez-Garcia et al. 2005; Nicotra et al. 1995; Saynajakangas et al. 1998], and increased rates of co-morbidities (e.g., pulmonary hypertension, asthma, cardiac disease, malnutrition) [King et al. 2005; Khalid et la. 2004]. Moreover, people with BE tend to exhibit a rapid decline in lung function [King et al. 2005] and accelerated death [Keistinen et al. 1997].

The impacts of BE spread beyond the respiratory structure. For instance, systemic [Hill et al. 1988], cardiac (e.g., left ventricular diastolic function [AkalIn et al. 2003], and psychological (anxiety and depression) [O'Leary et al. 2002] effects have been observed in BE patients. Additionally, in adult population, long-lasting bronchitis/respiratory infection constitutes an autonomous risk element regarding coronary heart disease and atherosclerosis [Simons et al. 1996; Kiechl et al. 2001]. The effective management of BE reduces both the short-term [Hill et al. 1986] and long-standing [Dogru et al. 2005] morbidity associated with the disease, as well as

mortality [Dogru et al. 2005]. Thus, the inhibition, primary diagnosis, and proactive controlling of BE are encouraged [Chang et al. 2003; Callahan and Redding 2002; Singleton et al. 2000; Edwards et al. 2003].

2.5 Imagine screening tests

As previously stated, according to a recent definition, BE is the "irretrievable dilatation of peripheral airways" and is frequently diagnostically recognized radiologically by cHRCT scans [Westcott 1991; Webb et al. 2001]. c-HRCT remains a diagnostic gold standard [Thoracic Society of Australia and New Zealand. Clinical Practice Guideline, 2014]. The key elements of BE in cHRCT scans are dilated bronchi in the bronchial wall thickening, thr periphery of the lung and a lack of tapering [Webb et al. 2001; Naidich et al. 1982]. A bronchus is perceive to be expanded if a CT scan displays a broncho-arterial ratio with the adjacent accompanying artery exceeds [Kasarabada et al. 2012]. However, the normal ratio of the airway to the vessel diameter in normal children is age-dependent, as previously stated [Redding and Carter 2017; Kapur et al. 2011]. As the primary radiographic criteria of broncho-arterial ratio in individuals without lung illness is also age-dependent [Matsuoka et al. 2003], child-specific criteria [Kapur et al. 2011] should be considered [Thoracic Society of Australia and New Zealand. Clinical Practice Guideline, 2014].

Pathognomonic chest CTs in children is addressed as an augmented BAR of >0.8 [Chalmers et al. 2018]. BAR increases as the severity of BE increases [Chalmers et al. 2018]. Meaning, starting from a normal mean BAR of 0.5–0.6 [Kapur et al. 2011], the gradual increase (excluding congenital forms of BE, such as Mounier–Kuhn syndrome) happens in children when the airway insults (typically inflammation and infection) are not curtailed [Chang and Marchant, 2019]. Other components comprise of a linear array or cluster of cysts, reduced attenuation shown in expiratory scans, and mucous plugging [Webb et al. 2001]. However, a c-HRCT reassembled from a multi-detector (MDCT) scan is considerably more sensitive than a conventional c-HRCT [Hill et al. 2010]. As children are at a high risk of suffering from radiation-induced cancers later in life [Chang, Bell et al. 2010; Mathews et al. 2013], the c-HRCT protocol ought to safeguard the lowest potential radiation exposure to get a satisfactory valuation [Thoracic Society of Australia and New Zealand. Clinical Practice Guideline, 2014].

Chest radiographs have been employed to monitor BE, but they have poor sensitivity [Currie et al. 1987]. Moreover, there is not enough available data to draw any assumptions from CXR (merely 30% of exacerbations had a CXR taken). Nevertheless, present outcomes propose that CXR alterations are less significant than clinical symptoms when describing a respirational exacerbation [Kapur et al. 2009].

On a scientific level, this radiology-founed description is problematical, particularly as it regards children, for the following reasons:

1. Many children present clinical syndromes of BE, but their cHRCT scans do not confirm the criteria for radiological BE. It is unidentified at which phase of the illness the HRCT signs of BE happen. Though HRCT is the present standard, it is less sensitive than bronchography in adults [Silverman and Godwin 1987; Young et al. 1991]. Incorrect negative outcomes are especially probable to happen when the illness is mild and concentrated [Silverman and Godwin 1987]. As children are probable to have less extreme BE than adult people, the CT scans in a subgroup of children with clinical symptoms of BE might not indicate radiological BE.
2. HRCT BE conclusions have been produced from examinations on adults [Gaillard et al. 2003], but scans taken from adults are not essentially equal to those from children. Modifications to the airways and morphologic alterations in the lung happen with development and aging [Roberts et al. 1991; Rains et al. 1992]. One of the critical HRCT signs of BE is an augmented bronchoarterial ratio (i.e., the diameter of the bronchial lumen separated by the diameter of its accompanying artery) of >1–1.5. This ratio, as previously stated, is impacted by age (r′O0.768, p <0.0001). This was defined previously by Matsuoka et al. in an examination on 85 adult people without cardiopulmonary disease [Matsuoka et al. 2003]. Therefore, it is probable that the typical bronchoarterial ratio is inferior in children than in adult people; hereafter a lower ratio is vital to describe abnormalities that indicate BE in children.
3. A minimum of two HRCT scans are needed in order to accurately accomplish the criteria of “irreversible dilatation,” Making more than one HRCT scan just for diagnostic motives (as opposed to for management matters) in children is controversial due to (a) augmented cancer danger from CTs in children [Brenner 2002] and (b) implications regarding costs.

4. CHRCT scans made throughout dissimilar conditions of "wellness" might yield dissimilar outcomes. Although HRCT scans are preferably executed in a "non-acute condition," this condition is hard to describe. Moreover, the "non-exacerbation condition" is not essentially identical to the "post-treatment" condition. For an extensive time, experts have understood that this is a noteworthy drawback, and it has been lately established by Gaillard et al. [Gaillard et al. 2003]. The Liverpool group stated that post-medical treatment bronchial dilatation totally resolved in six of 21 children with BE [Gaillard et al. 2003].

2.6 The microbiology of BE

2.6.1 Bacteria

Studies citing the various bacteria linked to BE in children are recorded in **Table 3**. *H. influenza* (stated as NTHi in three examinations) was the most shared pathogen recognized, followed by *Moraxella catarrhalis* and *S. pneumonia* [Edwards, Asher et al. 2003; Kapur, Grimwood et al. 2012; Banjar 2007; Eastham et al. 2004; Hare et al. 2010; Karadag et al. 2005; Li et al. 2005; Zaid et al. 2010]. These three classes are normally related to acute exacerbations in adult population suffering from BE [King, Holdsworth et al. 2007; Kelly et al. 2003]. Though it is not that common, united outcomes from all pediatric examinations show that *S. aureus and P. aeruginosa* have a comparable dominance as *M. catarrhalis,* though it is not easy to associate the outcomes because of the dissimilar sampling types utilized (**Table 3**).

The dominance of *H. influenzae, M. catarrhalis and S. pneumoniae,* in BE shape the foundation of empiric antibiotic treatment regimens to manage acute exacerbations in children [Chang, Bell et al. 2010]. Nevertheless, these data have chiefly a basis on cross-sectional examinations of clinically stable children based on retrospective chart reviews [Pizzutto et al. 2017]. In children's BE, the pathogen separation rate from sputum or BAL ranges from 53% to 67% [Ruchaud-Sparagano et al. 2013]. However, molecular examinations designed to distinguish between dissimilar strains are still essential [Wark et al. 2000].

Despite previous developments regarding the microbiology of the lower airways in children with BE, various difficulties remain in terms of recognizing the pathogens accountable for a respiratory exacerbation. Impulsive or induced sputum is a trustworthy and available source

of specimens regularly utilized for microbiologic examinations in adult population. Nevertheless, gathering sputum from young children is difficult, because children find it hard to clear out. Though NP swabs might be beneficial for this purpose, in practical procedure, the organism accountable for an exacerbation is seldom recognized, and doctors normally depend on empiric data to treat respiratory exacerbations in children with bronchiectasis.

Additionally, infection with upper airway microflora is a possible difficulty when lower airway specimens are acquired for a bacterial culture [Loens et al. 2009]. Even when great care is taken, infection might happen throughout BAL collection in young children as the tube utulized is too narrow for the protected brush technique [Pizzutto et al. 2017].

Table 3: Bacterial pathogens associated with bronchiectasis in children

Setting	**Number (n)**	**Age (years)**	**Specimen**	***Haemophilus influenza* (%)**	***Streptococcus pneumonia* (%)**	***Moraxella catarchalis* (%)**	**Pseudomonas aeruginosa (%)**	**Staphylococcus aureus (%)**
New Zealand	60	1–17 (md 10)	Sputum	55 (NTHi)	10	5	2	0
United Kingdom	93	1.6–18.8 (md 7.2)	Various	48	22	17	6	8
Turkey	111	1–17.5 (md 7.4)	Sputum	39	23	6	11	17
United Kingdom	136	3–18 (md 12.1)	Various	39	17	2	11	4
Saudi Arabia	151	7.3 ± 4.1	NP swab, sputum	37	17	9	16	7
Ireland	92	1.5–13 (md 6.4)	Sputum and BAL	54	37	10	9	15
Australia (Indigenous)	104	0.4–12.9 (md 2.4)	BAL $>10^4$ CFU/mL	31 (NTHi)	16	12	0	3
Australia	113	2.7–16 (md 5.3)	BAL $\geq 10^5$ CFU/mL	32 (NTHi)	14	8	2	5
Six countries	860	0.4–18.8	Various	40	20	8.5	7.9	7.6

2.6.2 Viruses

The purpose of viral infections in BE is not well defined [Grimwood 2011; King 2009], and there are few data available that can be considered when characterizing the significance of viral contamination in the pathogenesis of BE in children. Adenoviruses may be a significant cause of post-infectious BE in childhood [Becroft 1971] and are the most commonly reported

[Alharbi et al. 2012; Diaz et al. 1999; Edwards, Asher and Byrnes 2003; Hogg et al. 1989; Kapur et al. 2011].

Recent data from two separate prospective Australian studies on children with BE indicate that attention to viruses is warranted. For example, respiratory viruses were linked to 48% of exacerbations in 69 Queensland children with BE [Kapur, Mackay et al. 2014] and noticed in the BAL of 44% of 68 clinically steady (mainly Indigenous) children in the Northern Territory [Pizzutto et al. 2015]. Additionally, in a research of BE made in 58 adults in Guangdong, China, respiratory viruses were distinguished in 49% of 100 exacerbations [Gao et al. 2014]. Rhinovirus was the most frequent identified virus in the two examinations of children, whereas coronavirus, after rhinovirus and influenza, were the most shared in the adult examination. Whether this dissimilarity in viral domination is demographically focused or related to the severity of illness is not known. Nevertheless, in both adults and children, the occurrence of a virus throughout respiratory exacerbation is linked to more extreme symptomatology. In other chronic respiratory diseases, containing COPD and asthma, respiratory viruses are furthermore a notable cause of exacerbations [Kurai et al. 2013; Yerkovich 2012].

It has been assumed that viruses might change immune reactions and endorse respiratory exacerbations produced by bacterial contaminations [Beadling and Slifka 2004]. Respiratory viruses are probable an under-identified element contributing to acute exacerbations and insistent airway inflammation in children with BE. Therefore, large, population-founded examinations on children exploring the influence of viruses on airway immunopathology are needed to completely comprehend the involvement of viruses to long-lasting inflammation and the pathogenesis of BE in children [Pizzutto et al. 2017].

2.6.3 Bacterial virus co-infections

BE is linked to human T-cell lymphotropic virus type 1 infection in Jamaican children [La Grenade 1996] and Indigenous Australian adults [Einsiedel et al. 2012]. Furthermore, respiratory syncytial virus [Diaz et al. 1999; Edwards, Asher and Byrnes 2003], human immunodeficiency virus [Berman et al. 2007; Jeena et al. 1998; Masekela et al. 2012], influenza [Kapur et al. 2011; Laraya-Cuasay et al. 1977] and parainfluenza [Eastham et al. 2004; Kapur et al. 2011] have been detected in children with BE. Respiratory viral infections often lead to

bacterial superinfection by mechanisms including the promotion of bacterial adhesion to respiratory epithelial cells [Peltola and McCullers 2004]. Therefore, it is feasible that viral infections contribute to the initiation of BE and subsequent exacerbations in susceptible hosts [Hare 2014].

2.6.4 Persistent infection and BE

Respiratory pathogens occupy various approaches to evade clearance by host resistance mechanisms. When efficacious, these approaches obstruct hosts from efficiently clearing infections and contributing to a setting that fosters long-lasting infections and related inflammation. A number of the most prominent tactics by shared respiratory pathogens contain establishing protective structures like biofilm (*Staphylococcus aureus, Streptococcus pneumoniae and H. influenzae*, *Pseudomonas aeruginosa*,) [Bjarnsholt et al. 2009; Hall-Stoodley and Stoodley 2009], secreting immune-blocking agents like IgA proteases (*H. influenzae*) [Fernaays et al. 2006], and releasing toxins that harm mucus-clearing assemblies (containing cilia) of the epithelium [Bailey et al. 2012]. Secreted proteases might harm the construction of the bronchial wall, containing cilia, thus hindering sputum clearance from the lungs and endorsing inflammatory procedures by the host. In the lower airways of children with BE biofilm has been observed [Marsh et al. 2015] and can prevent antibiotics from having the desired effect [Starner et al. 2006]. A number of pathogens linked chronic respiratory contaminations, containing *Mycobacterium tuberculosis and H. influenza*, might stop the host's humoral reaction by hijacking antimicrobial mechanisms, controlling the host's phagocytic cells and creating an intracellular niche [Wolf et al. 2008].

2.6.5 Biofilm

Bacterial biofilms (**Figure 5**) have long been linked to persistent infections [Costerton et al. 1999]. Biofilm formation has been demonstrated in *H. influenza* [Cardines et al. 2012; Starner et al. 2006], *S. aureus* [Molina et al. 2008] and *P. aeruginosa* [Lutz et al. 2012; Murray, Egan et al. 2007] from CF patients; *H. influenzae* in the sputum of individuals with COPD [Murphy and Kirkham 2002]; and *S. pneumoniae* [Allegrucci et al. 2006], NTHi, and *M. catarrhalis* [Pearson

et al. 2006] in chronic and recurrent OM [Murphy, Bakaletz et al. 2009]. While many studies were performed *in vitro* or using animal models, biofilm formation has been demonstrated in the middle ear mucosa of children with prolonged OM [Hall-Stoodley et al. 2006], patients with chronic suppurative OM (smears from children and biopsies from adults) [Homoe et al. 2009], and in the middle ear effusions of children with recurrent acute OM [Thornton et al. 2013]. Biofilms likely also form in the lower airways of children with non-CF BE [Hare 2014].

Bacteria form biofilms as a survival mechanism, meaning that biofilms are ubiquitous. Antoni van Leeuwenhoek, in 1683, detected and defined biofilms on matter collected from his own teeth using a primitive microscope. Nevertheless, the biofilm lifestyles of microorganisms did not concern medical microbiologists until the early 1970s, the time that Nils Høiby detected a connection between the aggregates of bacteria and the causes of an insistent infection in CF patients [Høiby 2017]. Since that time, biofilms have been documented as contained in several clinical contaminations [Costerton et al. 1999; Hall-Stoodley and Stoodley 2009], and the evidence that biofilms contribute to the pathogenesis, particularly in long-lasting contaminations, continues to grow [Bjarnsholt 2013; Vestby et al. 2020].

Bacterial biofilms constitute groups of bacteria which attach to to each other or a surface and become inserted in a self-produced matrix. The biofilm matrix contains substances such as polysaccharides (e.g., alginate), proteins (e.g., fibrin) and eDNA. Additional to the safety provided by the matrix, bacteria in biofilms might employ numerous survival approaches to avoid their host's defense structures [Vestby et al. 2020]. Bacteria in biofilm formations are protected from phagocytosis and are resistant to antimicrobials, making biofilm infections harder to treat [Starner et al. 2006]. By remaining inactive and unseen from the immune system, they might lead to local tissue impairment and, eventually, an acute contamination. Within the biofilm, the bacteria adapt to environmental anoxia and nutrient limitations by changing their gene expression, metabolism and protein construction, which can lead to a lower metabolic rate and a decreased rate of cell division [Hall-Stoodley and Stoodley 2009; Donlan and Costerton 2002].

In addition, as previously mentioned, these adaptations make these bacteria more resilient to antimicrobial treatment, as they can deactivate the antimicrobial targets or decrease the requirements for the cellular function with which the antimicrobials inhibit. During a biofilm contamination, inherent and acquired host immune reactions might happen concurrently.

Nevertheless, neither kind of activity has the ability to eradicate the biofilm pathogen; but they accelerate collateral tissue impairment [Moser 2017]. Accordingly, biofilm-linked illnesses are characteristically tenacious and slow. Furthermore, they are infrequently resolved by the immune system, and their reactions to antimicrobial managements are unpredictable [Vestby et al. 2020]. This could explain the persistence of NTHi in COPD [Murphy et al. 2004], chronic OM, CF BE, and (theoretically) non-CF BE. It is likely that biofilms also form in the lower airways of children with non-CF BE [Hare 2014].

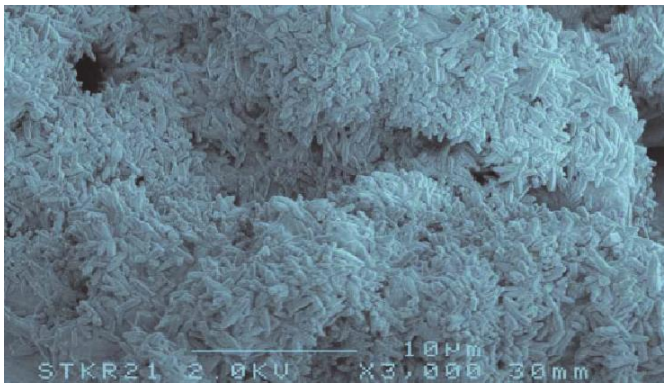


Figure 5. Scanning electron microscopy image of bacterial biofilm in an endoscope channel.

2.7 Treatment - Prevention

Antibiotic treatment constitutes the foundation of BE treatment in children. Antibiotics are given to inhibit exacerbations, decrease symptomatology and maintain lung function by decreasing lower airway bacterial load and infection [Grimwood et al. 2014]. Amoxicillin is still the dominant antibiotic regarding acute respiratory infections because of its extensive history of clinical accomplishment, restricted side effects, satisfactoriness and comparatively inferior cost. Big doses of amoxicillin might be given for penicillin-resistant pneumococci; however clinical failure might show another contaminating pathogen which is a β-lactamase creator. This approach of resistance might be overwhelmed by antibiotics enclosing β-lactamase inhibitors like clavulanic acid. Oral amoxicillin-clavulanate is frequently given as a primary empiric treatment for children with BE and mild-to-moderate exacerbations [Grimwood et al. 2014].

Three appraisals [Figueiredo and Ibiapina 2011; Masekela and Green, 2012; Gao et al. 2014] of preservation macrolide treatment (duration of 2–24 months) for BE have testified six RCTs in adult population and four in children [Koh et al. 1997; Valery et al. 2013; Yalcin et al.

2006; Masekela et al. 2013]. Macrolide treatment was linked to a decreased occurrence of exacerbations in adult population (risk ratio [RR] 0.42, $p < 0.001$) and children (RR 0.50, $p < 0.001$) [Gao et al. 2014], frequently with other enhancements in decreased sputum volume and pulmonary function.

At present, no commercially obtainable vaccine exists, particularly directing NTHi, the chief bacterial pathogen in childrens' BE. Oral vaccines which offer protection from NTHi infections have been produced to decrease the severity and number of exacerbations, and also carriage, in adult people with chronic bronchitis and COPD [Clancy et al. 1985; Foxwell et al. 2006; Lehmann et al. 1991; Tandon et al. 2010]. Nevertheless, a systematic examination displayed the advantage was too small to support the prevalent utilization of this oral NTHi vaccination in individuals suffering from COPD [Teo et al. 2014].

A more efficacious oral vaccine might advance mucosal safety [Clancy and Dunkley 2010] and decrease the occurrence or severity of respiratory contaminations produced by NTHi. Pneumococcal polysaccharide vaccines were advanced to inhibit pneumonia triggered by *S. pneumonia*; these vaccines contain serotypes triggering the most disturbing pneumococcal disease (IPD). The 23-valent polysaccharide vaccine was presented in 1983 [Austrian 1999] and is being utilized until recent years. Nevertheless, even though antibodies' reactions to immunization with polysaccharides are adequate in most individuals over two years old, the reaction is not that satisfactory among those aged between six months and two years and extremely poor in children, who are younger than six months old [Douglas and Riley 1979]. The conjugation of pneumococcal polysaccharides to a carrier protein advances immune reactions in toddlers. The initial certified pneumococcal conjugate vaccine (PCV7), presented in 2000, enclosed seven serotypes producing most IPD cases in the USA in children under five years old [Black et al. 2000].

Since that time, vaccines containing more serotypes have been created, containing a 10-valent pneumococcal NTHi protein D conjugate vaccine (PHiDCV) [Croxtall and Keating 2009] and PCV13, which was approved in 2010 [Centers for Disease Control and Prevention 2010]. PCVs have proven to be efficacious in decreasing the occurrence of IPD produced by vaccine serotypes [Roche et al. 2008; Palmu et al. 2013; Kaplan et al. 2013]. Nevertheless, PCVs have been less efficacious in relation to other kinds of treatment in decreasing pneumonia (a risk element regarding BE). Confirmed decreases of 20 to 22% in radiological (likely bacterial)

pneumonia have been described in some populations [Black et al. 2002; Tregnaghi et al. 2014], while other reports have cited no reduction [O'Grady et al. 2010]. The restricted influence on pneumonia occurrence might be due to the comparative influence of vaccine-type pneumococci in a situation where numerous other pathogens (containing pneumococcal serotypes not enclosed in the vaccine) are predominant.

Although vaccination is currently recommended, few published clinical trials have assessed the influence of PCVs in children with BE [Chang, Bell et al. 2010]. A clinical test of PHiDCV in children with expanded bacterial bronchitis, CLSD, and BE (together mentioned to as chronic endobronchial illnesses), with respiratory exacerbation as the chief result, is presently underway [O'Grady et al. 2013]. It was discovered that the carriage serotypes of *S. pneumonia* (mainly non-vaccine sorts in PCV-vaccinated individuals) have a comparable tendency to produce lower airway infections in Australian children with chronic endobronchial illnesses [Hare et al. 2017]. These data imply that PCVs do not have much influence on lower respiratory contaminations in this group of people. Nevertheless, PHiDCV might effectively protect people against mucosal contaminations with NTHi [Leach et al. 2014], and we expect the complete results of the clinical trial [O'Grady et al. 2013].

Inhaled anti-inflammatories perhaps are capable of managing the powerful and insistent airway inflammation linked BE. Corticosteroids and inhaled non-steroidal anti-inflammatories (NSAIDs) have been suggested as possible agents in the managing of BE [Chang, Marsh et al. 2012]. Nevertheless, two current systematic analyses of controlled randomized clinical trials did not find any proof to upkeep the efficiency of corticosteroids [Kapur, Bell et al. 2009] or inhaled NSAIDs [Pizzuto et al. 2016] in children with BE.

These appraisals highlight a gap in information concerning new therapeutics settled to handle airway inflammation in children with BE. Therefore, clear information displaying how gasped anti-inflammatories influence airway inflammation is essential. The longstanding influence of anti-inflammatories on persistent contamination in children with BE also requires more support before they can be introduced into the therapeutic regime.

3. CLSD

As opposed to BE, the term CSLD describes a diagnosis based on clinical symptomatology of BE without cHRCT indication of BE. The leading symptom of CSLD is an extremely lengthy moist cough. Other than the absence of cHRCT elements the symptomatology of CSLD are similar to those of BE.

In an opposite view, PBB is characterized by the manifestation of an isolated wet cough (not containing symptomatology and signs of BE or CSLD) [Chang et al. 2008]. Children with CSLD react to similar cure regimens utilized for childhood BE; a few writers even perceive them as a continuum of the same syndrome. Of note, children with CSLD diverge from children with PBB in that their situations cannot be fully inverted with various courses of antibiotics. In a two-year prospective examination, 43% of 161 children with PBB had more than three incidents of wet cough per year even though they have received antibiotic cure [Wurzel, Marchant et al. 2016].

The number of failures of treatments that establishes an alteration in diagnosis from PBB to CSLD has not been stated. In one study, 13% of children with PBB finally verified radiographic proof of BE [Wurzel, Marchant et al. 2016]. CSLD and PBB probable signify a continuum of long-lasting suppurative airway illness; thus, children with PBB who exhibit recurrent treatment “failures” might be considered as having CSLD or BE [Redding and Carter 2017]. To date, there are no clinical or treatment information obtained from childthat have obviously described CSLD [Goyal et al. 2016].

3.1 Microbiology

The microbiology observation of sputum or airway microbiology as in BE aids to lead antibiotic treatment regarding CSLD patients [Chan et al. 1992], especially if deterioration or an inadequate response to current treatment is detected. The most mutual pathogens recovered from children with BE are *Streptococcus pneumoniae*, NTHi and *Moraxella catarrhalis* [Twiss et al. 2005; Hare, Grimwood et al. 2010]. *Pseudomonas aeruginosa* and NTHi predominate in adults [King, Holdsworth et al. 2007], and about 25–45% of airway samples cannot develop pathogenic bacteria. As the illness growths, the microbiological flora change, with *P. aeruginosa* often

emerging in more progressive stages of the illness and forecasting a worse prognosis [King, Holdsworth et al. 2007]. Non-tuberculous mycobacteria species and aspergillus have been identified in a few individuals with bronchiectasis, though their pathogenic roles are frequently undefined [King, Holdsworth et al. 2007]. Nevertheless, non-tuberculous mycobacteria have been distinguished in exacerbations [Chan et al. 1992] and pulmonary deterioration [Wickremasinghe et al. 1997].

3.2 The overlap between PBB, CSLD and BE

The resemblances among these three situations contain the occurrence of a chronic wet cough (containing or not ruttles), endobronchial bacterial infection, neutrophilic airway inflammation and damaged mucociliary clearance. Furthermore, the kinds of micro-organisms associated with PBB and the early phases of CSLD/BE are similar. The key dissimilarities lie in the severity of signs and symptomatology, the reaction to two to four weeks of oral antibiotic administration, and cHRCT findings.

In the clinical model represented as "illness entities," there are strong connections between CSLD and PBB and between radiological BE and CSLD. It is not yet known whether these situations are dissimilar situations or different severities of the same condition (**Figure 2**). Nonetheless, it is believable that children with recognized BE had CSLD at some point earlier in the illness. Correspondingly, children with CSLD could have PBB at some earlier phase in the illness. The risk elements and amount of children with PBB who grow CSLD are still unidentified [Chang et al. 2008].

3.3 Global disparities in suppurative childhood lung diseases

Glaring disparities continue among children in dissimilar civilizations who are at danger of emerging CSLD. Admittance to vaccines for *pneumococci*, *Haemophilus* type b and *influenza* and complete longitudinal healthcare diverge considerably across and within nations. In prosperous states with efficacious public health organizations and positive well-child care programs, suppurative lung illness is rare and regularly happens because of an acknowledged pulmonary or immune host defense illness.

Nonetheless, as previously stated, among high-risk ethnic groups in New Zealand, Australia, Alaska and Argentina, post-infectious BE is very frequent. There is a continuing argument regarding the comparative roles of societal and environmental dangers versus immune deficits and irregular lung injuries as they concern the repair processes in different populations of children [Singleton et al. 2014; Hodge et al. 2016; Pizzutto, Yerkovich et al. 2014]. A great rate of consanguinity has been stated in numerous families whose children have idiopathic BE, suggesting that some groups have a genetic predisposition to BE [Banjar 2007; Karadag et al. 2005].

The worldwide weight of CSLD in children is unidentified. The geographic dominance of BE are partial relatively because of a lack of admission to the imaging technology required to have a diagnosis. Areas with the highest prevalence of tuberculosis, HIV infections, acute pneumonia, malnutrition, and tobacco and biomass combustion are the most probable to have recurrent cases of CSLD; this pattern specifies the disparities in occurrences of risk elements across nations and geographic districts [Pedding and Carter 2017].

3.4 Long-term consequences of suppurative lung disease

Most children having acute suppurative procedures (e.g., empyema, pneumonia, abscesses), if treated correctly, recuperate completely with the least lasting morbidity. Nonetheless, with the exclusion of CF, the continuing results of CSLD in children have not been well-defined. Sequential lung function trends for children with non-CF and CF post-infectious BE have been stated [Twiss et al. 2006]. Non-CF BE is related to worse lung function early in life (soon following the primary airway damage), nonetheless it growths much more gradually over time than CF-linked BE.

Symptoms of one-third of native Alaskan children with post-infectious BE completely resolve by adolescence [Singleton et al. 2000]. Though the mortality danger is superior in adults with BE than in others having asthma, it is lower than in adults with COPD (at least in industrialized states) [Keistinen et al. 1997]. Among adults with COPD, mortality is greater when BE is furthermore existent. Treatment regimens designed for transitioning youth and young adults with CSLD to adult providers have been most efficacious for individuals with CF and, more lately, for those with ciliary illnesses. Likewise, inclusive care models of non-CF-

linkedBE that stabilize and enhance lung function over time have been developed [Chang, Bell et al. 2010]. Nevertheless, the influence of smoking tobacco and other inflammable products among adolescents and young adults with BE has not been testified. Furthermore, these models have not accounted for ultimate alterations to adult treatment [Pedding and Carter 2017].

4. Acute pulmonary exacerbations

Acute pulmonary exacerbations of CLSD and BE are associated with disease severity [Wang et al. 2015]. Although no straight information for BE or CSLD in children are available, exacerbations are probable to reduce lung function. Elements of speeded lung function weakening in adult population having BE are the frequency of exacerbations that are hospitalized, augmented systemic inflammatory indicators and infection with *Pseudomonas aeruginosa* [Martinez-Garcia et al. 2007]. Among other features, augmented risks of mortality are linked to the degree of the damage of lung function [Loebinger et al. 2009].

Examinations conducted in the United Kingdom and Australia testified that children with regular lung function when they were diagnosed with BE, they preserved typical lung function five years later [Bastardo et al. 2009; Kapur, Masters et al. 2009; Kapur, Masters et al. 2010]. Children who have poor lung function the time of diagnosis were probable to still have poor lung function five years later (although with considerable enhancements), with the only noteworthy forecaster of pulmonary decrease being the regularity of exacerbations that were hospitalized [Kapur, Masters et al. 2009]. With each hospitalized exacerbation, the forced expiratory volume in 1-s percentage (FEV1%) estimate diminished by 1.95%, adjusted for time [Kapur, Masters et al. 2009]. Consequently, interventions that diminish exacerbation incidences are probable to be significant in the general managing of CLSD and BE [Chang, Bell et al. 2010].

In addition, preventing exacerbations could reduce economic and social costs. From 1993 to 2006, the median cost for adult inpatient treatment in the USA was US$7,827 [Seitz et al. 2010]. Hospitalization rates augmented considerably over the examination period, with an annual percentage upsurge of 2.4% for men and 3.0% for women [Seitz et al. 2010]. According to another report in 2004 from a New Zealand Hospital, half of the service's pediatric BE individuals needed at least one hospital admission yearly (range: 1–10) for exacerbations [Edwards et al. 2004]. The mean length of stay was one week (range: 1–25). 8% of these

children, to preserve respiratory status at a mean cost in 2004 of US$5,492 frequently had three to four monthly admittances for 14 days (this amount does not contain the cost of inserting peripherally inserted central catheter vascular lines or theater time) for each hospitalization [Edwards et al. 2004; O'Grady and Grimwood 2017].

4.1 Definition

As with BE and CSLD in children overall [Flight and Jones 2012], a dearth of literature describing an acute exacerbation exists. In adult individuals, acute exacerbations of chronic COPD are described by worsened, increased sputum volume, dyspnea and purulence [Stoller 2002]. The exacerbations of adults with BE have comparable elements to those of adults with COPD (i.e., augmented cough occurrences and purulence or sputum volume) and are frequently linked in sputum to culturing respiratory bacterial pathogens [European Respiratory Society. Bronchiectasis 2013].

In settings of study, predominantly in examinations for which exacerbations were considered the main result, the characterization might likewise contain the necessity for intravenous antibiotics and hospitalization [Grillo et al. 2015]. Presently clinical trials in children utilize comparable descriptions as are utilized in adult population, containing discrepancies in symptomatology (e.g., dyspnea, augmented coughing, color intensity or augmented sputum volume, new chest inspection or radiographic outcomes, deterioration in FEV1% forecasted >10%, or hemoptysis) [Valery et al. 2012; Chang, Grimwood et al. 2012; Chang, Grimwood et al. 2013; O'Grady et al. 2013].

However, not all definitions mention the necessity for intravenous antibiotics or hospitalization. In an examination of 30 children with 115 exacerbations of BE [Kapur, Masters et al. 2009], changes in cough characteristics (67%) and augmented cough frequency (88%) were the most frequent symptoms. Fever (28%), purulence (35%) and augmented sputum volume (42%) and worsening chest auscultatory (58%) were furthermore common, whereas alterations in spirometry values (in comparison with the stable condition) were not [Kapur, Masters et al. 2009].

Additional study was undertaken from this cohort's data (81 exacerbations were involved) to grow a standardized description of an exacerbation in children having BE. Wet cough and cough severity (score $\geq$2) over 72 h were the greatest forecasters of exacerbations [receiver

operating distinguishing area under the curve of 0.85 (95% CI 0.79, 0.92) and 0.84 (95% CI 0.77, 0.91), correspondingly] [Kapur, Masters et al. 2012]. Hemoptysis, sputum color, chest pain, dyspnea and chest signs were though as minor criteria. Moreover, the adding of amyloid-A, serum C-reactive protein (CRP) and interleukin-6 enhanced the specificity and positive predictive value of the definition [Kapur, Masters et al. 2012].

The authors subsequently categorized symptomatology and laboratory measures into three sets of criteria (minor, major and laboratory) and later devised three options that might be utilized to describe an exacerbation in BE/CLSD (**Table 4**). A noteworthy limitation of the study is that no objective gold standard exacerbation diagnosis in CLSD/BE was used. Instead, the criteria were measured against a pediatric pulmonologist's description, which involved changes in symptomatology and the necessity for extra treatment after patients reached a stable condition. More examinations confirming the definition in larger cohorts are ongoing [O'Grady and Grimwood 2017].

Table 4: Proposed criteria for defining a pulmonary exacerbation in children with BE

(I) Major criteria • Noteworthy cough frequency (median cough score ≥2) over 72 h • Wet cough for 72 h
(II) Minor criteria • Sputum color ≥2 on BronkoTest™ • Child/parent perceived breathlessness • Auscultatory crackles • Chest pain • Wheeze • Hypoxia (oxygen saturation ≤93% by pulse oxymetry)
(III) Laboratory criteria • CRP >3 mg/dlon high sensitive testing • Serum interleukin-6 >2 mg/L • Serum amyloid –A >5 mg/L • Raised peripheral blood neutrophil % (age-appropriate)
The three combinations considered the best to define an exacerbation • (Option-A) *One major* PLUS any one laboratory criteria positive [sensitivity 63%, specificity 94%, AUC 0.784, $p < 0.001$; positive predictive value (PPV) 91%, negative predictive value (NPV) 71.6%], OR • (Option-B) *Two major* criteria positive [sensitivity 92.6%, specificity 75.3%, AUC 0.84, $p < 0.001$; PPV 79%, NPV 91%], OR • (Option-C) *One major* PLUS and *two minor* criteria positive [sensitivity 95%, specificity 75%, AUC 0.84, $p < 0.001$; PPV 78.5%, NPV 93.75%]

4.2 Etiology

The precise etiology regarding the exacerbations in young children with BE or CSLD is not well-defined, and it is undistinguishable whether exacerbations represent a resurgence of a long-lasting infection, a new infection, or a mixture of both [European Respiratory Society. Bronchiectasis, 2013; Chang and Bilton, 2008]. Partially the problem is that notwithstanding their common utilization in clinical field, upper airway secretions gathered by cough swabs or throat do not consistently perceive organisms within the lower airways [Hare, Grimwood et al. 2010], especially considering that possible pathogens of interest (NTHi, *S. pneumoniae*, and *M. catarrhalis*) are likewise normally noticed in the upper airway spaces of healthy children [Jourdain et al. 2011].

Ideally, repeated bronchoscopies with multilobar BAL would be used to gather lower airway specimens before, during, and following acute exacerbations in children too young to reliably expectorate sputum. However, such methods are impracticable due to their aggressive nature, containing the requirement for repetitive general anesthesia or sedation. Therefore, the information needed to assuredly assign causality in investigation and patient care are restricted.

S. pneumoniae, *Haemophilus influenzae* and *M. catarrhalis* are regularly isolated at high densities ($\geq 10^4$ colony-forming units/mL) from BAL specimens gathered from children and adult individuals during acute exacerbations, though varied infections are additionally common [Hare, Grimwood et al. 2010; Kapur, Grimwood et al. 2012]. However, a number of examinations have examined systematically for viruses. *P. aeruginosa* and non-tuberculous mycobacteria are unusual in children with BE and, when existing, indicate the likelihood of undiagnosed CF and more extreme underlying BE and comorbidities [Kapur, Grimwood et al. 2012].

A small study on 69 children with BE (including 900 child-months of follow-ups) recognized at least one respiratory virus in nasopharyngeal aspirates in 48% of exacerbations; the most common was human rhinovirus (54% of virus-positive occasions) [Kapur, Mackay et al. 2014]. Compared to children with virus-negative exacerbations, children with virus-positive exacerbations were more probable to necessitate hospitalization (59% vs. 32.5%; $p = 0.02$), have a fever (OR 3.1, 95% CI 1.2, 11.1), develop hypoxia (OR 25.5, 95% CI 2.0, 322.6), show chest signs (OR 3.3, 95% CI 1.1, 10.2), or exhibit increased CRP (OR 4.7, 95% CI 1.7, 13.1) [Kapur, Mackay et al. 2014].

In 2014, a cross-sectional examination was conducted on 245 children having mild BE or PBB undergoing bronchoscopy and BAL for clinical indication (median age = 30 months) [Wurzel, Mackay et al. 2014]. The researchers performed a typical respiratory panel for viruses utilizing polymerase chain reaction assays on all specimens. Human adenovirus (HAdV) was noticed to be the most shared virus, as it was detected in 40 children. Meanwhile, influenza virus was distinguished in three children, parainfluenza virus was found in 12 children, respiratory syncytial virus was observed in 11 children, and human metapneumovirus was reported in five children [Wurzel, Mackay et al. 2014]. HAdV finding was more common in the young age individuals (p = 0.001) than the older age groups and was positively linked to each of the three chief bacterial pathogens [Wurzel, Mackay et al. 2014]. Nevertheless, this connection vanished following adjusting for age. Furthermore, given the lack of control participants and the cross-sectional nature of the inspection, a causal connection between clinical disease and these viruses and at that time of bronchoscopy could not be decided. In fact, a small number of examinations have examined risk elements of CSLD or BE exacerbations.

A previous study revealed that 74% of children of 93 Indigenous children from Alaska and Australia with BE or CSLD experienced more than two exacerbations over three years [Redding et al. 2014]. In the current examinations, the elements linked to continuing episodes were young age (no more than three years old), hospitalization for an acute exacerbation during the first year of life, and hospitalization for an acute exacerbation or pneumonia in the year prior to admission [Redding et al. 2014].

Exacerbations are likewise common in severe BE. One examination conducted on 111 children displayed that severe medical interventions decreased the yearly exacerbation rate by 56%. Nonetheless, children still experienced a mean of 2.9 episodes every year [Karadag et al. 2005; O'Grady and Grimwood 2017].

4.3 Prevention

Averting exacerbations and decreasing their severity are significant objectives when treating children with BE or CSLD to help them preserve their lung health and augment their quality of life [Chang, Bell et al. 2010]. A critical aspect of CSLD handling is the mixture of airway clearance methods and antibiotic treatment, with or without other treatments like

bronchodilators and anti-inflammatory agents [European Respiratory Society. Bronchiectasis, 2013; Chang and Bilton, 2008; Chang, Grimwood et al. 2008]. Nevertheless, moderate or high-quality examinations in children for either BE or CSLD (containing those addressing wider methodologies like health promotion, vaccines and chronic illness management approaches) are inadequate [Welsh et al. 2015].

4.3.1 Antibiotics

A lack of evidence supporting prophylactic antibiotics was displayed by a Cochrane review of antibiotic effectiveness for preventing repeated LRTi in high-risk children aged under 12 years [Onakpoya et al. 2015] and highlighted the necessity for high-quality trials. In an Australian study [Valery et al. 2013], 89 children with either BE or CSLD were randomly assigned to get either azithromycin (30 mg/kg once weekly) or a placebo for up to 24 months. Children getting azithromycin had considerably lower exacerbation rates (incidence rate ratio 0.50; 95% CI 0.35, 0.71) than the control group [Valery et al. 2013]. Nevertheless, children in the azithromycin group additionally grew considerably higher carriages of azithromycin-resistant bacteria (19 of 41, 46%) than those getting the placebo (4 of 37, 11%; $p = 0.002$) [Valery et al. 2013].

In other research, more macrolide-resistant organisms were acquired in those who were poorly adherent (76%) than those who complied with the treatment regimen (52%; OR 2.94, 95% CI 1.23, 7.14). Furthermore, their post-intervention *Staphylococcus aureus* strains stayed resilient to macrolides [Hare, Grimwood et al. 2015]. These findings support the usefulness of directly observed therapy programs for people and societies where adherence might be suboptimal. Those programs will subsequently assist to confirm the benefits of long-term antibiotics are appreciated while diminishing the possible harm caused by obtaining antibiotic-resistant organisms.

A dose-response connection between azithromycin utilization and increased macrolide-resistant strains of *S. aureus* and *S. pneumoniae* in the nasopharynx was detected in a prospective examination of 79 remote children with CSLD or bronchiectasis in Australia, conducted between 2004 and 2008 [Hare, Singleton et al. 1013]. These results are in consistence with the results of the 2015 Cochrane evaluation [Hnin et al. 2015], which stated a threefold upsurge in antibiotic-

resistant bacteria. Concerning short-term antibiotics (duration <4 weeks), another Cochrane review recognized no examinations in children and, consequently, displayed that there was no evidence to support this attitude for decreasing the severity and occurrence of exacerbations in children with BE or CLSD [Wurzel, Marchant et al. 2011].

4.3.2 Expectorants, Mucolytics, and Mucokinetics

Retained mucus in the lower airways endorses continuing airway inflammation, bacterial development and bronchial wall injury [Rubin, 2007]. Therefore, decreasing mucus retention is an important characteristic of airway clearance methods. Mucoactive agents contain mucolytics, expectorants and mucokinetic agents. Expectorants' goal is to upsurge the volume of airway water or secretion to upsurge the efficiency of coughing [Nair and Ilowite 2012]; these contain over-the-counter (OTC) cough medications and inhaled hyperosmolar saline and mannitol, as well.

However, there is no solid date supporting the utilization of OTC cough drugs and expectorants in children [Smith et al. 2014], and worries regarding negative events, containing a number of reports of toddler deaths [Hampton et al. 2013], have led several countries to recommend abstaining from giving OTC cough medications to young children. Mucolytics (which are obtainable both in oral and inhaled forms) make mucus less viscous in order to be expectorated more easily. However, no pediatric trials of mucolytics in children with CSLD or BE have been conducted [Wilkinson et al. 2014].

Furthermore, RhDNase is not suggested in adults or children having BE in accordance with a research in adult individuals stating its harmful impacts on exacerbation rates and lung function [O'Donnell et al. 1998]. Mucokinetics (one class of which is aerosol surfactants) increase the efficiency of coughing, either by growing expiratory cough airflow or by eliminating secretions from the airway walls; [Nair and Ilowite 2012]. Nonetheless, as with the other pharmacologic airway clearance treatments defined above, there is no high-level data confirming their utilization in children with CSLD or BE [Strickland et al. 2015].

4.3.3 Bronchodilators

BE might be linked to a hindering ventilatory defect, which could deteriorate during an exacerbation [AL-Shirawi et al. 2006]. Although patients with BE might also have asthma [Pasteur et al. 2010] and even though the presence of BE might deteriorate asthma exacerbations [Amalakuhan et al. 2015], small adult examinations propose the airflow limitation is not easily reversible [Murphy, Reen et al. 1984]. However, information regarding the occurrence of asthma in children with CSLD or BE is scarce, though it has been suggested that these children have been misdiagnosed as having asthma [Chang, Bell et al. 2010]. There are no trials of bronchodilators in children with BE or CSLD in the nonappearance of an established diagnosis of asthma, and their utilization in this group of children is not currently suggested [Chang, Bell et al. 2015].

4.3.4 Corticosteroids

Airway infection is a defining feature of both BE and CSLD, and the subsequent symptomatology might be quite the same to those of asthma; thus, CSLD and BE are often falsely misdiagnosed as asthma [Kapur, Bell et al. 2009]. According to limited data, the presence of asthma-like symptomatology in the presence of BE has been linked to faster declines in lung function [Keistinen et al. 1997; Saynajakangas et al. 1997; Field 1969].

Inhaled corticosteroids are frequently utilized to regulate asthma symptomatology and inhibit exacerbations. Adherence is critical for this strategy to be effective [McCullough et al. 2014]. While data regarding children with CSLD or BE are limited, non-adherence to inhaled corticosteroids regimens in adult individuals with BE has been recognized [McCullough et al. 2014; 2015] and is acknowledged to be challenging in children (particularly adolescents) suffering from asthma. A Cochrane review of inhaled corticosteroids in children and adult people with BE recognized no pediatric examinations; therefore, there is no evidence supporting their utilization [Kapur, Bell et al. 2009].

4.3.5 Vaccines

Concerning pathogens linked to respiratory diseases, pediatric vaccines are presently offered for influenza, *S. pneumonia* (23 valent polysaccharide vaccines and 7-, 10-, and 13-valent pneumococcal conjugate vaccines), *H. influenzae* type b, *Bordetella pertussis*, measles, and varicella. The 10-valent pneumococcal vaccine utilization protein D (an outer membrane protein derivative from NTHi strains) as the conjugate.

There is presently no evidence showing that any of these vaccines inhibit acute exacerbations of CSLD or BE in children [O'Grady, Chang and Grimwood 2014], primarily because the necessary trials have not been done. Notwithstanding the lack of data for inhibiting exacerbations, children with chronic lung illnesses have greater risk of growing severe contaminations from most of these organisms. Therefore, illness management ought to guarantee that children get on-time and age-appropriate immunizations in all nations where these vaccines are suggested through national immunization curriculums [Aigbogun et al. 2015; Pelton et al. 2014; Montella et al. 2007].

4.4 Chronic Disease Management Plans

The difficulty of treating individuals suffering from chronic illnesses demands a multi-disciplinary methodology that contains collective decision-making and contains the patient and their carers/families when creating plans regarding treatment [van Dongen, van Bokhoven et al. 2016; van Dongen et al. 2016]. Personalized chronic illness treatment plans include joint goal-setting and agreement on what actions are essential to attain those objectives [Coulter et al. 2015].

A systematic evaluation of 19 examinations containing 10,856 adult individuals with a range of chronic health situations determined that modest enhancements might be made through personalized management plans in psychological and physical parameters, and also in patients' capacity to self-manage their situations [Coulter et al. 2015].

There seems to be an absence of available examinations investigating the effectiveness of chronic illness management plans in inhibiting exacerbations of BE or CSLD in children. A systematic review of action plans for adult people with COPD that involved limited self-

management education acknowledged enhancements in the appreciation and beginning of cure for acute exacerbations. However, no evidence was found for reduced healthcare utilization or improved health-related QoL [Walters et al. 2010]. An appraisal of the common care of individuals with chronic illnesses between primary and specialty health services determined that, other than improved prescribing, there was inadequate data for the benefits of that methodology [Smith et al. 2007].

5. Microbiology evaluation/samples for lower airway microbiology in PBB, CLSD and BE

It is important to establish the most accurate diagnostic tools to accurately identify the bacterial/viral pathogens involved in the pathogenesis or acute exacerbation of these diseases. Accurately identifying the etiology is critical to ensuring that the most effective treatment is instituted. Currently, the standard care for children within the spectrum of CLSD diseases involves the regular surveillance of airway microbiology and airway microbiology during acute pulmonary exacerbations. However, these children are frequently unable of expectorating sputum, even if actively coughing throughout an acute exacerbation. Therefore, efficacious sampling for lower airway microbiology is difficult yet vital if infections are to be successfully treated or prohibited. [Forton 2015; Ronchetti et al. 2018].

5.1 Bronchoalveolar lavage (BAL)

BAL is considered the gold standard for sample lower airway microbiology [Brennan et al. 2008]. Though the global community is concerned witg BAL-based microbiology investigation programs, there is little data supporting this invasive routine as part of a standard CLSD care approach [Wainwright et al. 2011; Jain et al. 2013]. BAL is commonly reserved for children with CF who have not reacted to proper or empirical antibiotic treatments and for whom oropharyngeal cultures do not explain the perseverance of symptomatology.

No agreement exists on which BAL techniques should be used, and practice differs. Strategies for children with CF recommend two-lobe BAL, managed as follows: three-aliquot BAL from the right middle lobe and a single-aliquot BAL from the lingular (or the most affected) lobe [Brennan et al. 2008]. A study published in 2011 [Gilchrist et al. 2011] showed

that comprehensive six-lobe BAL is well-tolerated, safe and superior to single-lobe [de Blic et al. 2000] or two-lobe [Gilchrist et al. 2011] BAL, proposing that bacterial communities may be compartmentalized in the lung [Ronchetti et al. 2018].

Furthermore, a research relating the microbiological yield from BAL gathered from the right middle lobe and lingula presented dissimilarities, proposing that bacterial distribution is heterogeneous within the lung. In 2000, the European Respiratory Society (ERS) task force published a guide for implementing BAL methods for children [de Blic et al. 2000]. It proposed taking a single BAL from the most impacted lobe or (if there was a diffuse illness) from the right middle lobe. An effort to establish a global consensus in 2007 produced adjusted strategies for the BAL surveillance of lower airway microbiology in children with CF. It was recommended that a single-aliquot BAL from the lingular (or the most affected) lobe and a three-aliquot BAL should be taken from the right middle lobe and [Brennan et al. 2008].

5.2 Sputum induction

Sputum induction is a harmless method to finding lower airway samplings from individuals who are not spontaneously productive, and its utilization in tuberculosis surveillance in children is well-displayed [Iriso et al. 2005; Zar et al. 2000]. The role of sputum induction when treating young children within the spectrum of CLSD diseases has not been systematically addressed. Nevertheless, evidence from 27 studies on adults and two studies on children shows that induced sputum is as good as bronchoscopy and BAL for having lower airways samples.

More specifically, studies on adults have compared the two methods in terms of their capability to elucidate and screen diagnostic cytology in sarcoidosis [Fireman et al. 1999; Moodley et al. 2000; Mroz et al. 2007, Mroz et al. 2002; Tsiligianni et al. 2002; Tsiligianni et al. 2005], asthma [Fahy et al. 1995; Grootendorst et al. 1997; Keatings et al. 1997; Pizzichini et al. 1998; McGarvey et al. 2000; Siergiejko 2003], CF [McGarvey et al. 2000], pulmonary fibrosis, interstitial lung disease and hypersensitivity pneumonitis [Mroz et al. 2002; Antoniou et al. 2005; Sobiecka et al. 2008]. Microbiological yields determined utilizing the two methods have been compared in invidiuals suffering from TB [Anderson et al. 1995; Conde et al. 2000; McWilliams et al. 2002; Saglam 2005; Brown et al. 2007; Schoch et al. 2007] and HIV [Rush et al. 1989; Huang et al. 1995; Skot et al. 1995; Silva et al. 2007].

Meanwhile, two reports have made comparisons in induced sputum with bronchoscopy and BAL in children [Reinhardt et al. 2003; Kim et al. 2009]. Both studies observed patients with asthma and CF and compared inflammatory indices rather than microbiological yields. A small examination containing 11 adult people having CF, compared the microbiological yields from sputum induction, spontaneous sputum and BAL [Henig et al. 2001]. Test-specific recognition rates for the chief CF pathogens displayed similarity between the three approaches in terms of isolation sensitivity, with a non-significant trend indicating superior sensitivity for sputum induction.

Finally, the authors of a 2018 study recommended regularly implementing sputum induction for children with CF. This proposal was maintained by the acceptability of the process in all age groups of people, the acceptability among parents and patients, the ease of repeatability, the accomplishment of finding samples, the applicability to both inpatient and outpatient settings, the great proportion of identified pathogens, and the noteworthy economic savings in comparison with bronchoscopy. The authors suggested that both sputum induction and six-lobe BAL generate autonomous, sizeable gains in pathogen discovery when linked to the current gold-standard two-lobe BAL. They proposed that a mixture of sputum induction and six-lobe BAL should be used as a typical treatment for comprehensive lower airway pathogen detection in children with CF (**Figure 6**). In symptomatic individuals, inducing sputum before BAL helps to appropriately define the lower airway pathogen environment in nearly two-thirds of patients. Moreover, if utilized regularly, it might significantly decrease the number of bronchoscopy processes needed (**Figure 6**) [Ronchetti et al. 2018].

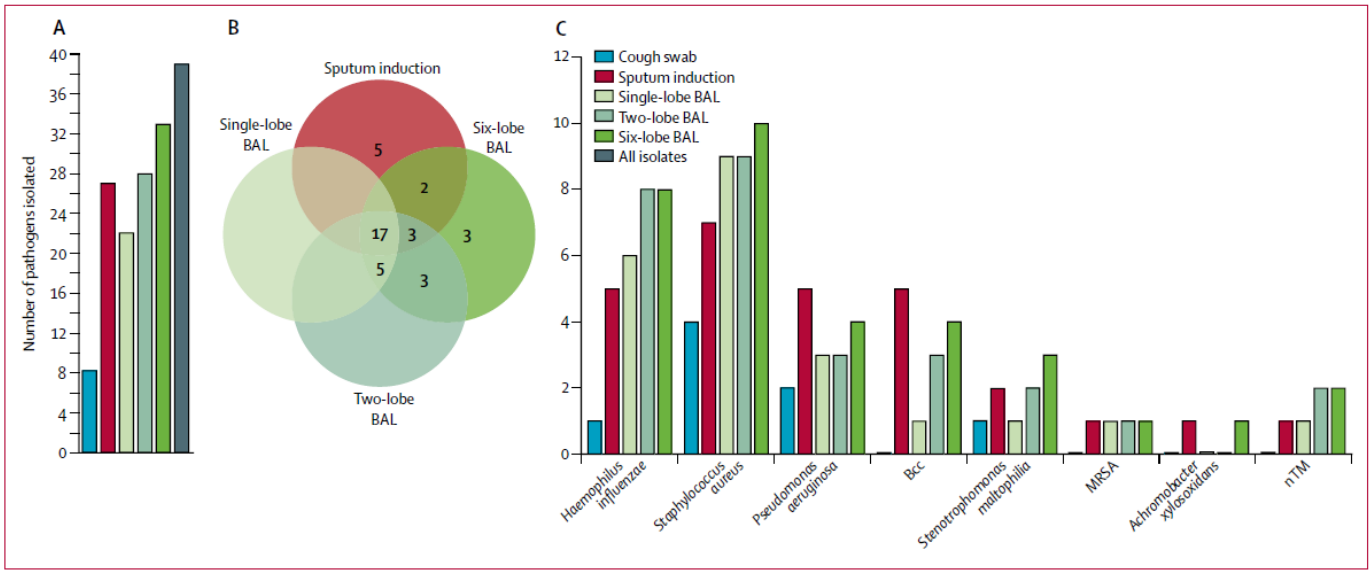


Figure 6. Six lobe bronchoscopy.

5.2.1 Safety and tolerability of sputum induction maneuver

Induced sputum is a well-established and harmless method to having lower airway samplings either for cultures or for assessing inflammation and cytology. The data showing that sputum induction is harmless in children comes from 22 examinations within the contexts of asthma [Araujo et al. 2011; Covar et al. 2004; Jones et al. 2001; Lex et al. 2005; Palomino et al. 2005; Li et al. 2006; Reining et al. 2000; Rytila et al. 2000; Twaddell et al. 1996], tuberculosis [Iriso et al. 2005; Zar et al. 2000; Zar et al. 2005; Qureshi et al. 2011], pneumonia [Zar et al. 2003; Lahti et al. 2009], and CF-22 [Ronchetti et al. 2018]. Together, these examinations display the tolerability of induced sputum in 1834 children.

No important side effects were reported in any of these studies, however 5% of all individuals could not endure the process and, thus, did not complete it. Most studies included patients who were above the age of six years and, thus, could consistently perform spirometry in order for the negative outcomes to the sputum induction process could be observed. Six reports examined the utilization of sputum induction in children under the age of five years [Zar et al. 2000; Zar et al. 2005; Qureshi et al. 2011; Zar et al. 2003; Lahti et al. 2009; Mussaffi et al. 2008].

Three of these studies (all published by the same group) focused on the utilization of sputum induction in toddlers and very young children. These high-profile, large examinations evaluated sputum induction in individuals with pneumonia and tuberculosis in an HIV-prevalent area [Zar et al. 2000; Zar et al. 2005; Zar et al. 2003]. The three examinations contained a combined 602 patients. The median ages in the examinations ranged from six to 13 months. Induced sputum was very well-accepted in this age group, even in acutely unwell children. Wheezing, vomiting, or hypoxia happened in only 3% of cases. Mild epistaxis was likewise observed in some children.

Six examinations looking at the safety and acceptability of induced sputum in children with CF-22 enrolled relatively old children who can perform reliable spirometry. These six examinations contained a total of 211, of whom 16 could not complete the sputum induction protocol after displaying symptoms (7.5%). In the one examination focused on children from a wide age range (i.e., eight months to eight years old), fewer side effects were reported in the young age group [Mussaffi et al. 2008]. No examinations have concentrated on the tolerability and safety of sputum induction in children with CLSD.

5.2.2 Oropharyngeal cough swab

The oropharyngeal cough swab procedure contains requesting the child to cough whereas a swab is positioned in the oropharynx. This is a very non-invasive, routine and uncomplicated process and is improbable to impact the outcome of a subsequent induced sputum. However, oropharyngeal cough swabs cultures are a poor surrogate for cultures from lower airway samples taken during a coexisting BAL [Rosenfeld et al. 1999].

The benefits of sputum induction over oropharyngeal cough swabs have been recognized in adults and older children with CF. However, no studies have compared the two approaches in CLSD patients. Evidence that cough swab oropharyngeal cultures might be utilized as a replacement for cultures from lower airway samplings in non-expectorating patients comes from five reports from patients with CF [Armstrong et al. 1996; Avital et al. 1995; Ramsey et al. 1991; Rosenfeld et al. 1999; Jung et al. 2002].

Rosenfeld reviewed three examinations containing 141 children with CF under five years old. Cough swab oropharyngeal cultures supported a specificity of 95%, a sensitivity of 44%,

PPV 44%, and NPV 95%. Children within this age range tend to have mild lung disease, and there are few pathogen isolations. Thus, it is suggested that an adverse cough swab oropharyngeal culture might efficiently dismiss the possibility of a lower airway contamination, while a positive cough swab oropharyngeal culture does not consistently confirm a lower airway contamination.

The NPV and PPV of cough swab oropharyngeal cultures in adult groups who are patients (for whom there is a great dominance of airway pathogens) change respectively. In this condition, a negative cough swab oropharyngeal culture has not the ability to efficiently rule out a lower airway contamination (NPV is low), whereas a positive cough swab oropharyngeal culture does consistently confirm a lower airway contamination (PPV is high) [Equi et al. 2001].

Evidence from five studies indicates that induced sputum is more effective than cough swab oropharyngeal samples for identifying bacterial pathogens in CF patients [Al-Saleh et al. 2010; Suri et al. 2003; De Boeck et al. 2000; Henig et al. 2001; Ho et al. 2004]. A small examination of 10 adult people having CF displayed comparable microbiological yields when utilizing spontaneously induced sputum, expectorated sputum and BAL. Two small examinations in children detected developed yields regarding induced sputum than a cough swab [Suri et al. 2003; De Boeck et al. 2000]. Two larger examinations compared cough swabs with induced sputum in individuals having CF (involving 43 and 94 children, respectively. Both examinations recognized further organisms on induced sputum in 30% and 42% of cases, respectively [Al-Saleh et al. 2010; Ho et al. 2004].

6. Challenges and limitations

Despite the current knowledge regarding the microbiology of the lower airways in children with CLSD, there are various difficulties faced when recognizing the pathogens involved in pathogenesis, such as in an acute respiratory exacerbation of CLSD. As previously stated, spontaneous or induced sputum is a consistent and available source of specimens routinely utilized for microbiologic analysis in adult population. Nevertheless, gathering sputum from young children is challenging, as they find it problematic to expectorate. Though oropharyngeal cough swabs might be a useful alternative, in practice, the organism accountable for an

exacerbation is seldom recognized, and doctors normally trust empiric evidence to manage PBB or respiratory exacerbations in children with CLSD/ BE.

Partially the difficulty is that notwithstanding their typical utilization in clinical practice, upper airway secretions gathered by throat or cough swabs do not consistently forecast what organisms are present in the lower airways, particularly possible pathogens of interest (NTHI, *M. catarrhalis* and *S. pneumonia),* and as these are additionally commonly detected in the upper airway spaces of healthy children. Contamination with upper airway microflora is a possible problem when lower airway specimens are attained for bacterial cultures [Loens et al. 2009].

Ideally, recurrent bronchoscopies with multi-lobar BAL would be applied to gather lower airway specimens before, during, and following acute exacerbations in young children who are too young to consistently expectorate sputum. However, this method is impractical given its invasive nature, as it would require repeated sedation or general anesthesia. Additionally, though it is a safe technique, contamination could still happen during BAL gathering in young children, as the utilized tube is too narrow for the safe brush method. Henceforth, the data needed to assuredly assign causality in investigation and patient care are restricted [Singleton et al. 2014]. Therefore, more examinations are required to clarify the association between these three methods (cough swabs, induced sputum, and BAL) when applied to children with CLSD [Pizzutto et al. 2017].

Quantitative cultures are used to exclude sparse bacteria in BAL fluid because of upper airway contamination; nonetheless, the threshold ustilized to define lower airway contamination diverges between examinations. As others have done, we utilized a cutoff of 10^4 colony shaping units (CFU)/mL BAL in children [Hare, Grimwood et al. 2010; De Schutter et al. 2011; Wurzel, Marchant et al. 2014]. Nevertheless, others have utilized dissimilar cutoffs—for example, 10^3 CFU/mL in examinations with adult population [Angrill et al. 2002; Loens er al. 2009] and 10^5 CFU/mL in studies on infants with CF [Armstrong et al. 1996]. Although the validation threshold of 10^4 CFU/mL was used to describe lower airway infections based on correlations with airway neutrophilia and quantitative PCR alternative thresholds need investigation [Hare, Marsh et al. 2013].

Identifying which pathogens are accountable for lower airway contaminations is more complex by restrictions in laboratory procedures. Although conventional microbial-detection techniques are accurate, they are time- and labor-intensive, have a limited range of detection, and

can be subjective since they rely heavily on the practitioner's technical expertise. Additionally, direct fluorescent antibody assays and immunochromatographic antigen testing, despite their rapid nature, have poor sensitivity when used to detect most viruses. Another issue is that, as previously stated, research on the microbiology of CLSD in children has primarily concentrated on bacterial pathogens, while there is a lack of investigation on viral pathogens [Wurzel et al. 2014; Joish et al. 2013].

According to the above discussion, an assay capable of quickly and accurately detecting multiple pathogens, including viral pathogens, is highly desirable. Cough swabs and induced sputum are non-invasive airway-sampling approaches, which, if shown to be effective, could contribute to reduce the number of bronchoscopies and routine contamination observation that children with CLSD need to undergo. Further research is required to recognize if the association between these three methods holds for individuals having CLSD. If it does, it would enable practitioners to better manage, monitor, and treat this condition.

OBJECTIVES

Our objectives were:

(1) to determine whether the microbiology results from cough swabs samples collected from children with PBB and children with acute exacerbation of CLSD or BE are accurate and can be used for further clinical decisions,

(2) to evaluate the accuracy of the Film array Biofire pneumonia panel plus, through assessment of agreement with standard methods,

(3) to evaluate the microbiology of PBB, CLSD, BE (if cough swabs samples prove to be reliable samples from lower respiratory tract).

Hypotheses

- Microbiology results from cough swabs samples collected from CLSD, PBB and BE patients are accurate and do not reflect upper respiratory flora.
- The Film array Biofire pneumonia panel plus assay provides accurate microbiological results and greater microbiology yield than cultures in CLSD, PBB and BE patients.

METHODOLOGY

Study setting

The study held from November 2018 to December 2019 at the Department of Microbiology and the Department of Medical Pediatrics at the ATTIKON University Hospital, Athens, Greece. The study was accepted by the hospital's institutional review board.

Subject eligibility

Children aged 2 to 16 years old were eligible for the study if they had a definitive diagnosis of PBB, BE, or CLSD (see clinical definitions) and they were in acute exacerbation phase (see table 4). Age-matched previously healthy children who were hospitalized with evidence of upper respiratory infection were also enrolled in study, comprising the healthy control group. Written informed consent was provided from the parents of all children enrolled in the study.

Exclusion criteria

Children diagnosed with PBB, BE, or CLSD and controls suffering from an impaired immune system—as indicated, for example, by B-cell deficiencies (IgG subclass deficiencies, IgG deficiencies, IgA deficiencies), chronic granulomatous illness, combined variable immunodeficiency, or T-cell deficits—were excluded from the study. Patients and controls and who had received antibiotic therapy in the previous two weeks they also excluded.

Clinical Definitions

- PBB diagnosis was definitive in children who satisfied the subsequent criteria: a history of chronic (≥4 weeks) wet cough, (b) prospective evidence (supported by diaries of cough) of responses to two weeks of antibiotic treatment, and (c) the nonexistence of clinical pointers proposing an alternative etiology for the cough [Marchant, Masters et al. 2006; Wurzel et al. 2014].
- BE diagnosis in children was definitive when the radiological criteria were fulfilled and clinical symptomatology constant with BE were present. All children with definitive BE had evidence of a cylindrical BE cHCT scan [O'Grady et al. 2017; Kalu et al. 2010].

- CLSD diagnosis was definitive when clinical symptoms of BE were present without cHRCT evidence of BE. The leading symptom of CSLD is the occurrence of an extremely persistent moist cough. Besides the deficiency of cHRCT elements, the symptomatology of CSLD are the same to those of BE. The main symptom of BE is the presence of an extremely prolonged wet cough. In older children, coughing might be purulent and productive. Other symptomatology contains recurring chest contaminations or a persistent wet cough in reaction to exrtional dyspnea, antibiotics, symptomatology of reactive airway illness (asthmalike state), and development failure. In children, hemoptysis seldom happens except in progressive stages of the illness. Clinical signs contain chest wall deformity, clubbing, adventitious sounds in chest auscultation, and hyperinflation [Kapur et al. 2012; Faniran et al. 1999].

1. Procedures

1.1 Cough Swabs

The oropharyngeal cough swab process includes querying the child to cough whereas a swab is located in the oropharynx. This is a simple, routine, and very non-invasive process. Oropharyngeal swabs were gathered utilizing a commercially obtainable nylon flocked swab (Mrk Tech., Shenzhen, China). The swab was located into the posterior pharynx, with iderect contact with the oropharyngeal mucosa and the individuals were requested to cough. Then the swab was placed in 2 mL VTM (Hopebio Technologies, Qingdao, China). The sampling was kept at -70 °C for the pathogen nucleic acid extraction.

1.2 Microbiology

Cultures

Cough swab samplings were cultured for 48 hours utilizing routine microbiological processes.

Film array test

A Filmarray test was utilized to identify a panel of respiratory viruses and bacteria in the lower respiratory tract in all collected samples. The BioFire FilmArray Pneumonia Panel (FilmArray PP) test is an emerging diagnostic method that detects multiple respiratory pathogens very quickly. It is a multiplexed nucleic acid test for the simultaneous quantitative identification and recognition of numerous bacterial and viral respiratory microorganisms and antimicrobial resistance gene targets linked to lower respiratory tract contaminations as well. This test permits for the quick recognition of multiple RNA or DNA targets in a single tube containing complicated respiratory pathogens. This technique is run on the BioFire FilmArray System, a US FDA-, CE-IVD-, and TGA-certified multiplex PCR system. The system assimilates sample preparation, nucleic acid extraction and purification, recognition, strengthening, and analysis into one simple system that needs just two minutes of hands-on time and a total run time of almost one hour. The new panel complements the current BioFire FilmArray Respiratory 2 Plus Panel to provide a complete diagnostic tool for detecting pneumonia and other lower respiratory tract contaminations. The ability to rapidly and accurately identify the causative agent of community- and healthcare-linked respiratory contaminations might assist to advance patient treatment by contributing to well-timed and efficacious antibiotic or antiviral treatment. In the meantime, the BioFire FilmArray Pneumonia Plus Panel tests for 18 bacteria (11 Gram-negative, 4 Gram-positive, and three atypical), seven antibiotic resistance indicators, and nine viruses that lead to pneumonia and other lower respiratory tract contaminations (**Table 5**). Furthermore, it provides an overall sensitivity and specificity for BAL-like samples of 96.2% and 98.3%, respectively; for sputum samplings, it yields a sensitivity and specificity of 96.3% and 97.2%, respectively. The panel of examined microorganisms includes *Klebsiella oxytoca, Acinetobacter, Enterobacter cloacae, Enterobacteraerogenes, E.coli, Haemophilus influenzae, Klebsiella pmeumoniae* group, *Moraxella, Proteus, Pseudomonas aeruginosa, Serratia, Staph. aureus, Srept. agalactiae, Strept. pneumoniae, Strept. pyogenes, Chlamydia pneumoniae,* human *Rhinovirus*/*enterovirus, Legionella, Mycoplasma, Adenovirus, Corona,* human *Metapneumovirus, Influenza* A/ B, *Parainfluenza* and RSV.

Table 5: The Film Array Pneumonia Panel. *Sample type: Sputum, Endotracheal Aspirate and Bronchoalveolar Lavage.*

Panel Menu		
Bacteria ***Semi-Quantitative Bacteria***	**Atypical Bacteria** ***Quantitative Bacteria***	**Antimicrobial Resistance Genes**
Acinetobacter calcoaceticus-baumanni complex	*Legionella pneumophila*	mecA/C and MREJ
Serratia marescens	*Mycoplasma pneumoniae*	KPC
Proteus spp.	*Chlamydia pneumoniae*	NDM
Klebsiella pneumoniae group		Oxa48-like
Enterobacter aerogenes	**Viruses**	CIX-M
Enterobacter cloacae	Influenza A	VIM
Escherichia coli	Influenza B	IMP
Haemophilus Influenzae	Respiratory Syncytial Virus	
Moraxella catarrhalis	Human Rhinovirus/Enterovirus	
Pseudomonas aeruginosa	Human Metapneumovirus	
Staphylococcus aureus	Parainfluenza virus	
Staphylococcus pneumoniae	Adenovirus	
Klebsiella oxytoca	Coronavirus	
Streptococcus pyogenes	Middle East Respiratory Syndrom Coronavirus	
Streptococcus agalactiae		

2. Statistical analysis

The data were analyzed by utilizing statistical software IBM SPSS 24. Data were presented with frequencies and percentages. Significance was set at 5%. Chi square test was utilized to test the dependence between nominal variables and specifically the dependence between group category (patients, control) and existence of microorganism (yes, no). McNemar test was used for related samples in nominal variables and in particular, to test differences between film array and culture technique for patients, regarding the existence of microorganism [Field, 2017]. Sensitivity, Specificity, PPV and NPV was used for film array and culture technique.

RESULTS

Demographic data of patients

A total of 23 children with the diagnosis of PBB, CLSD or BE were enrolled in our study, 13 males and 10 females, mean age 8,6 years. Ten were fulfilled the criteria of the diagnosis of PBB (7 males and 3 females) mean age 7 years, 8 of CLSD (3 males and 5 females) mean age 8 years and 5 of BE (3 males and 2 females) mean age 6,6 years. Seventy patients were 6–14 years old (school aged patients) and six patients between 3-6 years (preschool aged patients) (**Table 6**). All the 13 children diagnosed with CLSD or BE were fulfilled the criteria of acute exacerbation. A total of 17 age-matched, previously healthy children, who were hospitalized with evidence of upper respiratory infection were enrolled in study comprising the healthy control group.

Table 6: Demographic Characteristics of the 23 patients

CHARACTERISTICS	**N**
Gender M / F (*mean age*)	13 / 10 *(8.6 years)*
Age	
6-14	17
3-6	6
Diagnosis	
PBB, M / F *(mean age)*	10, 7 / 3 *(7 years)*
CLSD, M / F *(mean age)*	8, 3 / 5 *(8 years)*
BE, M / F *(mean age)*	5, 3 / 2 *(6.6 years)*

Clinical characteristics of patients

Nineteen individuals had a duration of cough more than 4 weeks and 4 patients had 4 weeks period of cough. The longest duration was 12 weeks. All of the children displayed wet cough, containing 7 patients with purulent sputum, and 22 patients were coughing both day and night. 11 patients accompanied by mild to moderate fever, seven individuals accompanied by wheezing and 19 patients had nasal symptoms, containing runny nose, nasal congestion and sneezing. Eleven individuals accompanied by rhinitis and 5 patients had sinusitis. Physical examination presented that 20 individuals had crackles, 7 individuals accompanied by wheezes and crackles (**Table 7**).

Table 7: Clinical Characteristics of the 23 patients

CLINICAL CHARACTERISTICS		**N**
Symptoms		
1.	Cough	23
	Wet	23
	Dry	0
	4 weeks	4
	4-12 week	19
	purulent sputum	7
	Day	0
	Night	1
	day / night	2
2.	Wheezing	7
3.	Fever	11
4.	Nasal symptoms	19
Physical examinations		
Crackles		20
crackles/wheezing		7
Comorbidities		
Rhinitis		11
Sinusitis		5

Cough swabs

A total of 23 good quality induced sputum samples were gathered from 23 CLSD patients and 17 cough swabs from 17 controls.

1. Microbiological yield using Film array Biofire pneumonia panel plus assay

A total of 70 pathogens were identified in 23 patients (induced sputum) in contrast of 46 pathogens that identified from 17 children from control group (cough swabs).

Patients

In 23 patients were identified 15 (65.2%) *Haemophilus influenzae*, 9 (39.1%) *Moraxella catarrhalis*, 9 (39.1%) *Staphylococcus aureus*, 7 (30.4%) *Streptococcus pneumoniae*, 2 (8.7%) *Streptococcus pyogenes*, 3 (13%) *Pseudomonas aeruginosa*, 16 (69.6%) *Human Rhinovirus/enterovirus*, 6 (26.1%) *Parainfluenzae virus* and 3 (13%) *Adenovirus* using film array test.

Control group

In 17 children (control group) were identified 8 (47.1%) *Haemophilus influenzae*, 6 (35.3%) *Moraxella catarrhalis*, 4 (23.5%) *Staphylococcus aureus*, 7 (41.2%) *Streptococcus pneumoniae*, 5 (29.4%) *Streptococcus pyogenes*, 10 (58.8%) *Human Rhinovirus/enterovirus*, 2 (11.8%) *Parainfluenzae virus*, 2 (11.8%) *Adenovirus* and 2 (11.8%) *Respiratory Syncytial Virus* using film array test (**Table 8**).

2. Microbiological yield using conventional sputum cultures

A total of 21 pathogens were identified in 23 patients (induced sputum) in contrast of 12 pathogens that identified from 17 children from control group (cough swabs).

Patients

In 23 patients were identified 7 (30.4%) *Haemophilus influenzae*, 2 (8.7%) *Moraxella catarrhalis*, 5 (21.7%) *Staphylococcus aureus*, 4 (17.4%) *Streptococcus pneumoniae*, 1 (4.3%) *Streptococcus pyogenes*, 2 (8.7%) *Pseudomonas aeruginosa*.

Control group

In 17 children (control group) were identified 4 (23.5%) *Haemophilus influenzae*, 2 (11.8%) *Moraxella catarrhalis*, 1 (5.9%) *Staphylococcus aureus*, 3 (17.7%) *Streptococcus pneumoniae*, 2 (11.8%) *Streptococcus pyogenes* (**Table 8**).

Table 8: Microorganisms using film array and culture techniques in patients and control group.

Microorganisms	Microorganisms in PBB, BE, CLSD patients (n=23) (% of patients) film array	Microorganisms in PBB, BE, CLSD patients (n=23) (% of patients) cultures	Microorganisms in controls (n=17) (% of control) film array	Microorganisms in controls (n=17) (% of control) cultures
Haemophilus influenzae	15 (65.2%)	7 (30.4%)	8 (47.1%)	4 (23.5%)
Moraxella catarrhalis	9 (39.1%)	2 (8.7%)	6 (35.3%)	2 (11.8%)
Staphylococcus aureus	9 (39.1%)	5 (21.7%)	4 (23.5%)	1 (5.9%)
Streptococcus pneumoniae	7 (30.4%)	4 (17.4%)	7 (41.2%)	3 (17.7%)
Streptococcus pyogenes	2 (8.7%)	1 (4.3%)	5 (29.4%)	2 (11.8%)
Pseudomonas aeruginosa	3 (13%)	2 (8.7%)	0 (0.0%)	0 (0.0%)
Human Rhinovirus/enterovirus	16 (69.6%)	N/A	10 (58.8%)	N/A
Parainfluenzae virus	6 (26.1%)	N/A	2 (11.8%)	N/A
Adenovirus	3 (13%)	N/A	2 (11.8%)	N/A
Respiratory Syncytial Virus	0 (0%)	N/A	2 (11.8%)	N/A

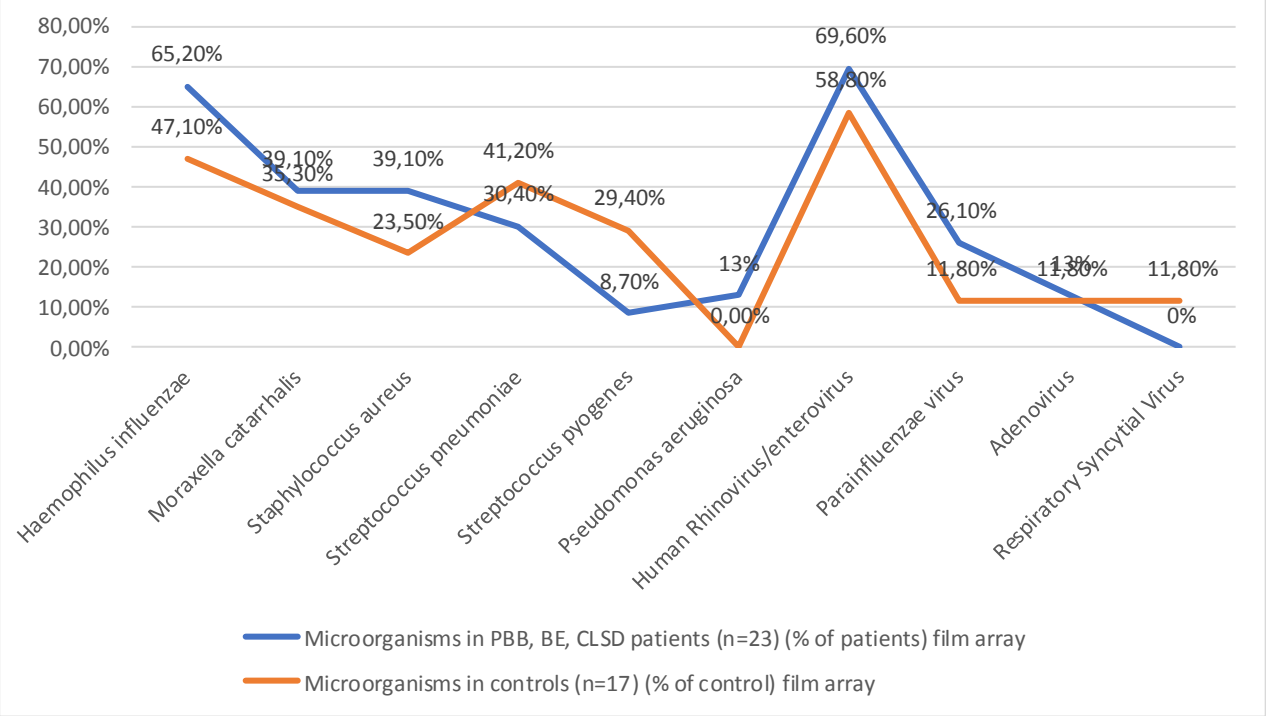
3. Differences using film array technique between patients and control group

Table 9 and **Graph 1** present the results of chi-square test using film array technique between patients and control group where no statistically significant differences appeared in any microorganism (p≥0.113).

Table 9: Chi square test using film array technique between patients and control group.

Microorganisms	Microorganisms in PBB, BE, CLSD patients (n=23) (% of patients) film array	Microorganisms in controls (n=17) (% of control) film array	X^2 (1)	p-value
Haemophilus influenzae	15 (65.2%)	8 (47.1%)	1.319	0.251
Moraxella catarrhalis	9 (39.1%)	6 (35.3%)	0.061	0.804
Staphylococcus aureus	9 (39.1%)	4 (23.5%)	1.085	0.298
Streptococcus pneumoniae	7 (30.4%)	7 (41.2%)	0.496	0.481
Streptococcus pyogenes	2 (8.7%)	5 (29.4%)	2.906	0.113+
Pseudomonas aeruginosa	3 (13%)	0 (0.0%)	2.397	0.248+
Human Rhinovirus/enterovirus	16 (69.6%)	10 (58.8%)	0.496	0.521
Parainfluenzae virus	6 (26.1%)	2 (11.8%)	1.253	0.428+
Adenovirus	3 (13%)	2 (11.8%)	0.015	1.000+
Respiratory Syncytial Virus	0 (0%)	2 (11.8%)	2.848	0.174+

+Fisher exact test



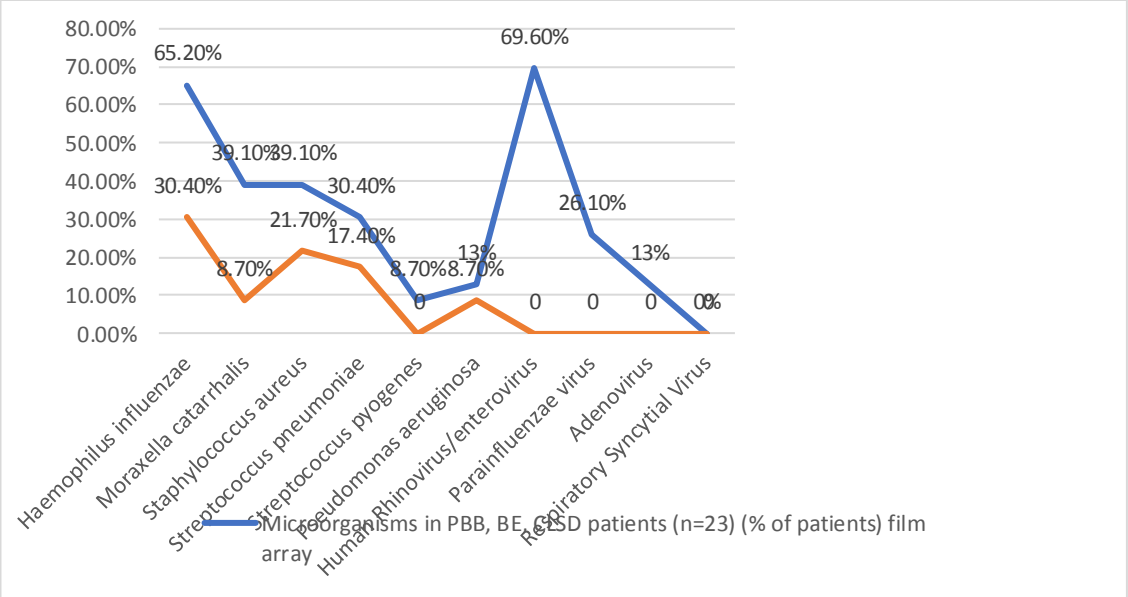
Graph 1: Film array technique between patients and control group.

4. Differences between film array and culture technique for patients

Table 10 and **Graph 2** present the results of McNemar test between film array and culture technique for patients, where there was a statistically significant difference in microorganism *Haemophilus influenzae* (p=0.008) where the microbiological yield was higher for film array technique (65.2%) comparing with culture (30.4%) and *Moraxella catarrhalis* (p=0.016) where similarly higher levels of microbiological yield appeared for film array technique (39.1%) than culture (8.7%).

Table 10: McNemar test between film array and culture technique for patients.

Microorganisms	Microorganisms in PBB, BE, CLSD patients (n=23) (% of patients) film array	Microorganisms in PBB, BE, CLSD patients (n=23) (% of patients) cultures	p-value
Haemophilus influenzae	15 (65.2%)	7 (30.4%)	0.008
Moraxella catarrhalis	9 (39.1%)	2 (8.7%)	0.016
Staphylococcus aureus	9 (39.1%)	5 (21.7%)	0.125
Streptococcus pneumoniae	7 (30.4%)	4 (17.4%)	0.250
Streptococcus pyogenes	2 (8.7%)	1 (4.3%)	1.000
Pseudomonas aeruginosa	3 (13%)	2 (8.7%)	1.000
Human Rhinovirus/enterovirus	16 (69.6%)	N/A	N/A
Parainfluenzae virus	6 (26.1%)	N/A	N/A
Adenovirus	3 (13%)	N/A	N/A
Respiratory Syncytial Virus	0 (0%)	N/A	N/A



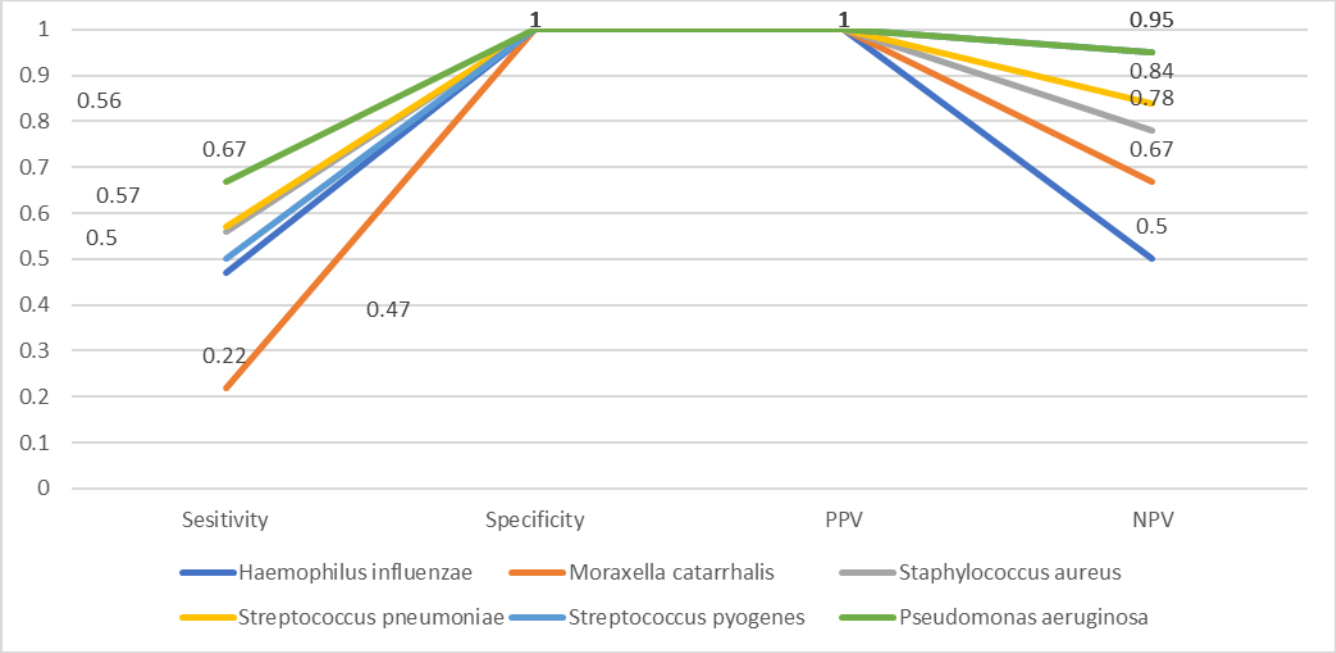
Graph 2: Film array and culture technique for patients.

5. Sensitivity, Specificity, PPV and NPV of culture

Table 6 and **Graph 3** presents the results of Sensitivity, Specificity, PPV and NPV of culture technique for each microorganism with standard method the film array for patients. High levels of sensitivity appeared for *Pseudomonas aeruginosa* (0.67), medium for *Streptococcus pneumonia* (0.57), *Staphylococcus aureus* (0.56), *Streptococcus pyogenes* (0.50) and *Haemophilus influenzae* (0.47) and low for *Moraxella catarrhalis* (0.22). Specificity and PPV had the maximum value (1.00) in all microorganisms. Very high values of NPV appeared for *Streptococcus pyogenes* (0.95) and *Pseudomonas aeruginosa* (0.95), high for *Streptococcus pneumoniae* (0.84), *Staphylococcus aureus* (0.78) and *Moraxella catarrhalis* (0.67) while medium for *Haemophilus influenzae* (0.50).

Table 6: Results of Sensitivity, Specificity, PPV and NPV of culture with standard method the film array for patients.

Microorganisms	A	B	C	D	Positives	Negatives	Sesitivity	Specificity	PPV	NPV
Haemophilus influenzae	7	0	8	8	7	16	0.47	1	1	0.50
Moraxella catarrhalis	2	0	7	14	2	21	0.22	1	1	0.67
Staphylococcus aureus	5	0	4	14	5	18	0.56	1	1	0.78
Streptococcus pneumoniae	4	0	3	16	4	19	0.57	1	1	0.84
Streptococcus pyogenes	1	0	1	21	1	22	0.50	1	1	0.95
Pseudomonas aeruginosa	2	0	1	20	2	21	0.67	1	1	0.95



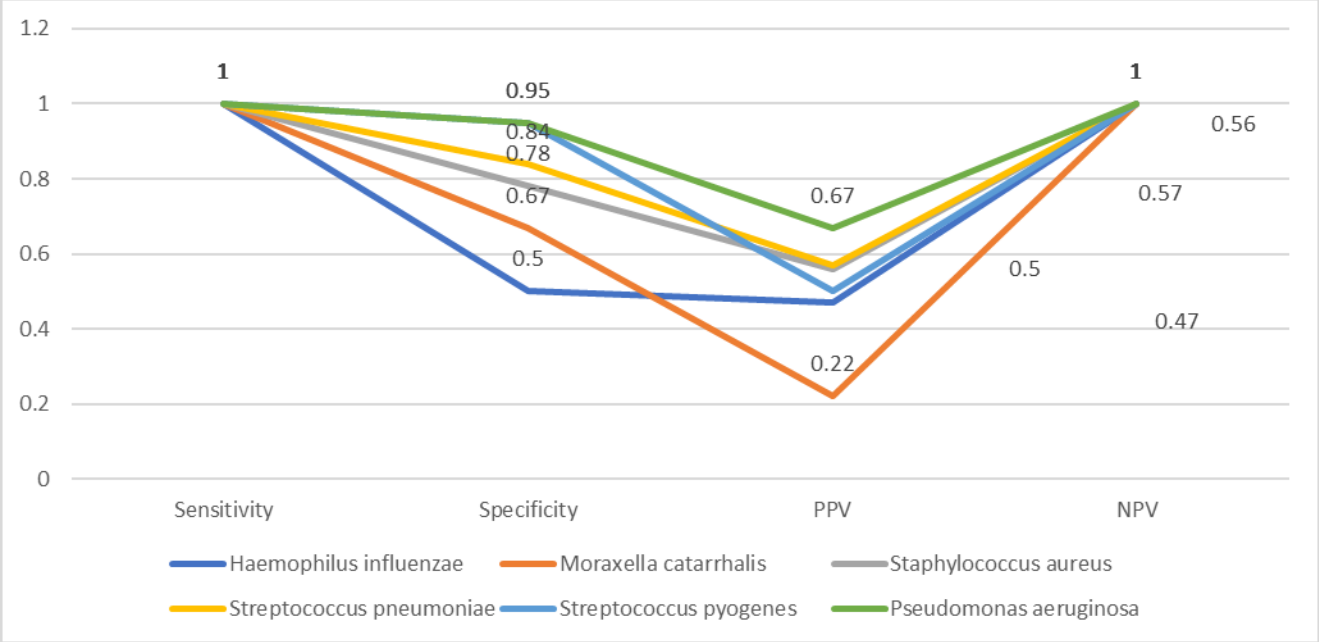
Graph 3: Results of Sensitivity, Specificity, PPV and NPV of culture with standard method the film array for patients.

6. Sensitivity, Specificity, PPV and NPV of film array

Table 7 and **Graph 4** presents the results of Sensitivity, Specificity, PPV and NPV of film array with standard method the culture for patients. Sensitivity and NPV had the maximum value (1.00) for all microorganisms. Very high values of Specificity appeared for *Streptococcus pyogenes* (0.95) and *Pseudomonas aeruginosa* (0.95), high for Streptococcus pneumoniae (0.84), *Staphylococcus aureus* (0.78) and *Moraxella catarrhalis* (0.67) while medium for *Haemophilus influenzae* (0.50). High levels of PPV appeared for *Pseudomonas aeruginosa* (0.67), medium for *Streptococcus pneumoniae* (0.57), *Staphylococcus aureus* (0.56), *Streptococcus pyogenes* (0.50) and *Haemophilus influenzae* (0.47) and low for *Moraxella catarrhalis* (0.22).

Table 7: Results of Sensitivity, Specificity, PPV and NPV of film array with standard method the culture for patients.

Microorganisms	A	B	C	D	Positives	Negatives	Sensitivity	Specificity	PPV	NPV
Haemophilus influenzae	7	8	0	8	15	8	1.00	0.50	0.47	1
Moraxella catarrhalis	2	7	0	14	9	14	1.00	0.67	0.22	1
Staphylococcus aureus	5	4	0	14	9	14	1.00	0.78	0.56	1
Streptococcus pneumoniae	4	3	0	16	7	16	1.00	0.84	0.57	1
Streptococcus pyogenes	1	1	0	21	2	21	1.00	0.95	0.50	1
Pseudomonas aeruginosa	2	1	0	20	3	20	1.00	0.95	0.67	1



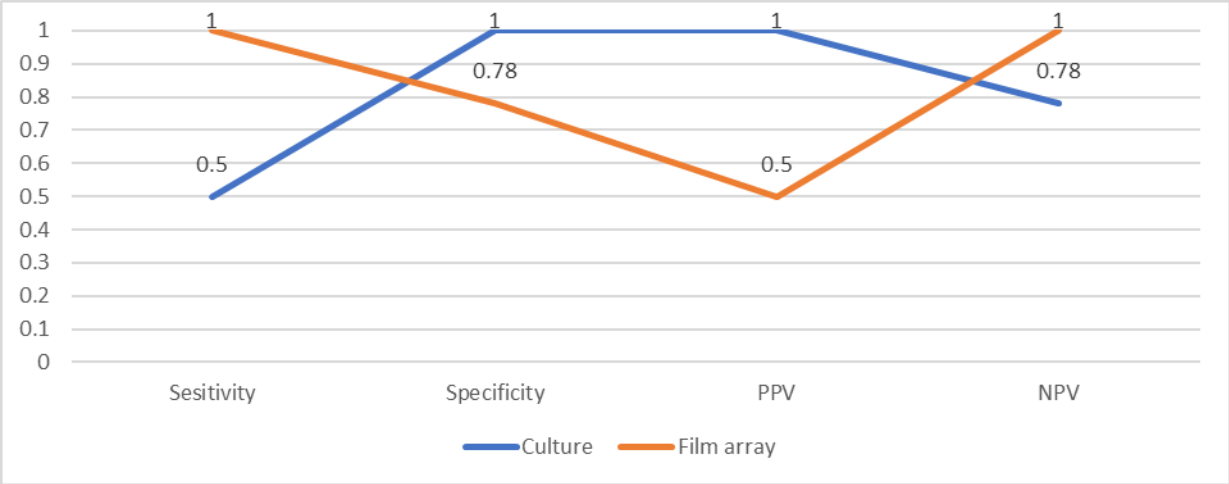
Graph 4: Results of Sensitivity, Specificity, PPV and NPV of film array with standard method the culture for patients.

7. Comparison of techniques

According to **Table 8** and **Graph 5**, culture technique presented Specificity and PPV maximum value (1.00), NPV was 0.78 and Sensitivity 0.50. Film array presented maximum value in Sensitivity and NPV (1.00), Specificity was 0.78 and PPV 0.50.

Table 8: Results of Sensitivity, Specificity, PPV and NPV of film array and culture technique for patients.

Technique	Sesitivity	Specificity	PPV	NPV
Culture	0,50	1,00	1,00	0,78
Film array	1,00	0,78	0,50	1,00



Graph 5: Results of Sensitivity, Specificity, PPV and NPV of film array and culture technique for patients.

DISCUSSION

Microbiological validation regarding infection of bacteria is seldom accomplished in children having acute lower-respiratory-tract infections (aLRTIs) due to the incapability to get material from the infection site (ie, the lung) [Hammitt et al. 2012]. Treatment is consequently, generally empirical, which irreversibly result in overtreatment and occasionally undertreatment. Notwithstanding the existing literature, queries regarding sampling approaches utilized for respiratory bacterial cultures in the non-expectorating children with evidences of LRTi continue to be not answered. Is the microbiological yield of cough swab, nasal swab and expectorated or induced sputum similar for each sampling technique, and in expectorating vs. non-expectorating patients? Are microbiological findings of these methods as sensitive and as precise as those of BAL? What is the clinical value regarding cough swab for the documentation of bacterial pathogens in the lower airways of the non-expectorating patient? Our aims were to determine whether the microbiology results from cough swabs samples provided by children with PBB and children with acute exacerbation of CLSD or BE are accurate and can be used for further clinical decisions, and to evaluate the accuracy of the Film array Biofire pneumonia panel plus, through assessment of agreement with standard methods.

Presently, samples for diagnostic aim by PCR contain NP aspirates, oropharyngeal (OP) swabs, nasopharyngeal (NP) swabs, OP suction and sputum. Though upper respiratory tract specimens are usually utilized in children with respiratory viral and some bacterial infections, there is worry whether the findings mirror the etiology of lower respiratory tract infection [Abanses et al. 2006]. Many studies have compared the yields of these upper respiratory tract specimens, and by PCR to recognize viral or bacterial infections by PCR, they have displayed that the sensitivity of aspirate (or suction) is bigger than that of swabs [Meerhoff et al. 2010; Lambert et al. 2008; Stensballe et al. 2002]. In comparison with specimen from upper airway, outstanding diagnostic sensitivity is perceived when sputum is accessible [Raty et al. 2005; Zampoli et al. 2016; Jeong et al. 2014; Lathi et al. 2009]. Nevertheless, for children, particularly young patients who are unable expectorate a sterile negative pressure suction catheter is applied to acquire OP suction. Young children and parents might find this quite disturbing and difficult process intolerable, therefore restricting its utilization in routine clinical practice [Zampoli et al. 2016; Chau et al. 2018]. Additionally, those oropharyngeal suction or sputa seemingly from or

contaminated by secretions from the upper respiratory tract [Thea et al. 2017; Jourdain et al. 2011; Wang et al. 2019]. In children having long-lasting wet/productive cough, quantitative bronchoalveolar lavage (BAL) culture is the gold-standard technique for diagnosing lower airway infection [Hare et al. 2019]. Even though flexible bronchoscopy is a comparatively harmless process with a low threat of serious problems (about 2%), it is still a disturbing process to implement, particularly in children [De Blic et al. 2002].

Consequently, a non-invasive and willingly accessible process is desired to forecast the causative organism of the PBB, CLS and BE and lead antibiotic treatment [Asseri et al. 2021]. Accordingly, cough swabs are regularly utilized to gain samples. The oropharyngeal cough swab procedure contains querying the child to cough whereas a swab is positioned in the posterior part of oropharynx. This is a repetitive, very non-invasive and simple process.

A number of examinations displayed dissimilar diagnostic approaches that compare upper- and lower airway bacteria in children with PBB, CF, lasting suppurative lung illness, and bronchiectasis [Hare et al. 2019; Harre et al. 2010; Armstrong et al. 1996; Ramsey et al. 1991; Taylor et al. 2006; D'Sylva et al. 2017; Jochmann et al. 2016; Rosenfeld et al. 1999]. These studies concluded that oropharyngeal cough swabs cultures are a poor surrogate for cultures from lower airway samples taken during a coexisting BAL and also that the nasopharyngeal swab is more prognostic and consistent in diagnosing lower airway infection in relation to the oropharyngeal technique [Asseri et al. 2021].

In The Lancet Respiratory Medicine, Katherine Ronchetti and colleagues made comparisons regarding the diagnostic yield of bacterial culture outcomes from 167 paired induced sputum and cough swab samplings in invidiuals aged 6 months to 18 years [Ronchetti et al. 2018]. In a subset of their examination they compared bacterial yield from induced sputum to bronchoalveolar lavage. Induced sputum samplings had a considerably higher bacterial yield than cough swab samples: 86 dissimilar pathogens were excluded in total from the samples, and 79 [92%] of these were acknowledged by sputum induction while 27 [31%] were on cough swab samples ($p<0{\cdot}0001$). This dissimilarity proposed that induced sputum has the ability to recognize pathogens from the lower airways that cough swabs cannot. When viewing these findings, induced sputum seems to be far superior to cough swab for recognition of lower airway pathogens. These results are helpful and compatible with other studies that displayed enhanced microbial yield of induced sputum over upper-airway sampling in children [Muloiwa et al. 2016;

Zampoli et al. 2016]. The poor diagnostic value of cough swabs is additionally in line with other studies tha showed that oropharyngeal swabs bear little-to-no association to lower-airway pathology in young children [Schultz and Caudri 2018].

Evidence that cough swab oropharyngeal cultures might be utilized as a substitute for cultures from lower airway samplings in non-expectorating individuals comes from five studies from patients with CF [Armstrong et al. 1996; Avital et al. 1995; Ramsey et al. 1991; Rosenfeld et al. 1999; Jung et al. 2002]. Rosenfeld reviewed three examinations contaning 141 children with CF under five years old. Cough swab oropharyngeal cultures carried a sensitivity of 44%, a specificity of 95%, PPV 44%, and NPV 95%. Children within this age range tend to have mild lung disease, and there are few pathogen isolations. Thus, it is suggested that a negative cough swab oropharyngeal culture might efficiently eliminate the possibility of a lower airway infection, whereas a positive cough swab oropharyngeal culture does not reliably confirm a lower airway contamination. The NPV and PPV of cough swab oropharyngeal cultures in adult population groups (for whom there is a high prevalence of airway pathogens) change correspondingly. In this situation, a negative cough swab oropharyngeal culture cannot successfully rule out a lower airway contamination (NPV is low), whereas a positive cough swab oropharyngeal culture does consistently confirm a lower airway contamination (PPV is high) [Equi et al. 2001].

The Infectious Diseases Society of America (IDSA) and American Thoracic Society (ATS) strategies propose routine utilization of sputum Gram stain and culture for adult individuals requiring admission to the intensive care unit for individuals with failure of outpatient antibiotic treatment and for individuals with pleural effusion [Mandell et al. 2007]. In children with LRTi, routine sputum analysis has not been suggested since young children cannot create sufficient sputum samplings and nasopharyngeal colonisation with bacteria producing LRTi is shared in healthy children [Harrison et al. 1999; Polack et al. 2000; Syrjanen et al. 2001; Bogaert et al. 2004; British Thoracic Society Standards of Care Committee 2002; Zemlickova et al. 2006].

Our findings indicate that cough swab samples could be easily collected in our ordinary, busy general hospital setting in all children with clinically assumed a LRTi. However, the major question was whether the cough swabs specimens were representative samples from the lower respiratory tract. Similar to other studies studies [Hare et al. 2019; Harre et al. 2010; Armstrong

et al. 1996; Ramsey et al. 1991; Taylor et al. 2006; D'Sylva et al. 2017; Jochmann et al. 2016; Rosenfeld et al. 1999], our findings indicated that there was no statistically significant differences in microbiological results using film array technique between patients (PBB, CLSD, BE) and control group (patients with upper respiratory infection) and probably, these cough swabs reflect upper respiratory flora. That means that maybe the cough swab sample was contaminated with the upper-respiratory tract normal flora. These findings indicate that these samples may not represent reliable samples and do not support our hypothesis that cough swabs are valuable to support etiological diagnoses of LRTi in CLSD patients in every day practice. Our results are in accordance with the results of previous studies [Hare et al. 2019; Harre et al. 2010; Armstrong et al. 1996; Ramsey et al. 1991; Taylor et al. 2006; D'Sylva et al. 2017; Jochmann et al. 2016; Rosenfeld et al. 1999] which found that cough swabs are a poor surrogate for cultures from lower airway samples taken during a concurrent BAL. On the other hand this could mean that an invasive infection by a colonizing bacterial pathogen had taken place, which is a known phenomenon in respiratory tract infections.

Bacterial respiratory pathogens are usually diagnosed utilizing Gram stain and culture, which are thought to be the reference standard methods. Nonetheless, bacterial culture needs knowledge-based practical expertise and long-lasting antibiotic susceptibility trials [Uzoamaka et al., 2017]. A number of multiplexed molecular assays have been established and applied in order to overcome these difficulties, regarding the precise and fast documentation of several microorganisms that produce respiratory contaminations. Lately, automated multiplex PCR assays like the Unyvero P55 Pneumonia LRT Panel (Curetis AG, Holzgerlingen, Germany) and FA-Pneumo have been presented to identify not only microorganisms, but similarly resistance indicators in lower respiratory specimens. Though the Unyvero assays have been assessed earlier in a number of examinations [Gadsby et al., 2019, Jamal et al., 2014, Papan et al., 2018], studies regarding the performance of FA-Pneumo are rare [Lee et al., 2019].

Our study shows discrepant results between culture and FA-Pneumo, with more microorganisms found utilizing the last technique. More specifically, a number of bacterial species, containing *H. influenzae* ($n = 8$), *Staphylococcus aureus* ($n = 4$), *Streptococcus pneumoniae* ($n = 3$), *Pseudomonas aeruginosa* ($n = 1$), were found utilizing FA-Pneumo however did not develop in noteworthy amounts in culture. Statistically significantly differences between film array and culture technique for patients appeared in microorganisms *Haemophilus*

influenzae and *Moraxella catarrhalis* where the microbiological yield was higher for film array technique comparing with culture. Seeing that the patients in this examination had not received antibiotics before sample and based on the discrepancy resolution of the particular organisms, the over-detection utilizing FA-Pneumo is probable because of a higher sensitivity rather and not due to false-positive consequences, and furthermore the capability to notice residual nucleic acids after management. According to our results, a negative cough swab oropharyngeal film array result can effectively rule out a possible lower airway contamination (NPV is high), whereas a positive cough swab oropharyngeal culture does not reliably confirm a possible lower airway contamination (PPV is low). These findings support our hypothesis that FilmArray PP assay provides a greater microbiology yield than cultures in CLSD, PBB and BE patients but still it is not possible to distinguish colonizing organisms from pathogens. Compared to culture, the film array method showed 100% sensitivity (positive coincidence rate) and 78% specificity (negative coincidence rate).

Nonetheless, trying to distinguish colonizing organisms from pathogens stays a challenge, since levels of bacteria below the culture threshold might deliver positive findings in FA-Pneumo. When compared to bacterial culture techniques, utilization of the film array PN Panel led to a 114.29% upsurge in the total number of bacterial targets detected (45 film array Vs 21 cultures). These specimens were stated as “negative/no growth” based on routine culture and reporting protocols. Our results are comparable to results available by Lee et al., who addressed a 70.3% upsurge in total bacterial targets perceived by the PN Panel among 59 BAL and endotracheal aspirate specimens [Lee et al. 2019], and Ozongwu et al., who reported a 129.4% upsurge in total bacterial targets found utilizing dissimilar complex molecular assay [Ozongwu et al. 2017]. In addition, filmArray PP detected 25 viral pathogens that were not possible to grow in cultures. The positive results described by molecular trials is not unanticipated and as previously said is probably because of the discovery of both viable and non-viable organisms, as well as recognition of low abundance targets and those not improved in culture because of fastidious development features [Buchan et al. 2020].

As with the rest of molecular approaches, differentiating whether the microbes spotted in the FilmArray analysis are causative pathogens or colonizers is not feasible [Self et al. 2016; Korten et. al 2016; Shanh 1986]. Consequently, the experts should be cautious when judging pathogens since the results are occasionally “false positive”. On the other hand, notwithstanding

the high recognition rate of FilmArray RP, a negative result does not mean the individual is not infected; furthermore, a positive result does not mean there is no other co-infecting agent. For this "false-negative" restriction, BioFire has a new pneumonia panel that also covers 9 shared viruses, and 15 bacteria, containing *Klebsiella pneumonia*, *Streptococcus pneumonia Haemophilus influenza* and *Staphylococcus aureus*. However, the FilmArray panel only targets to quickly deliver outcomes for possible pathogens as a reference. A more proper approach is to broadly consider the outcomes from other examinations, like routine C-reactive protein (CRP), culture and radiography, procalcitonin (PCT), blood testing, the erythrocyte sedimentation rate (ESR), as well as the patients' symptomatology, containing breathing, body temperature, heart rate, blood oxygen and psychological state [Li et al. 2018].

The possible benefit of these Film Array Pneumonia Panel discoveries is twofold. Initially, appreciation of a high-concentration (clinically significant) pathogen could hypothetically inhibit the initial termination of efficacious antibiotics. A negative culture outcome at 72 h might lead to early withdrawal and danger of relapse. Additionally, recognition of a particular pathogen(s) could permit adjustment (escalation or de-escalation) of empirical antibiotic treatment even in the face of a negative culture. For instance, a Film array Pneumonia Panel result of *H. influenzae* or *S. pneumoniae* might permit de-escalation of empirical broad-spectrum agents like vancomycin and cefepime or meropenem to a narrower spectrum beta-lactam regimen like amoxicillin or ceftriaxone. Proper reduction or interruption of antibiotic treatment based on quantitative culture results has been linked to a diminution in following infection with multi-drug resistant organisms (MDROs) [Raman et al. 2013]. Primary recognition of bacterial pathogens by the Film array Pneumonia Panel, even in negative cultures, might have related influence. Validation of these theories would necessitate a longitudinal comparison of patients whose antibiotics were held modified or continued broadly based on a positive Film array Pneumonia Panel however negative culture result. Unfortunately, the design of our examination did not permit the gathering of these data and this stays a field of noteworthy interest and investigation [Buchan et al. 2020].

Furthermore, it is suggested that initial diagnosis of pathogens in children with RTIs might diminish the length of hospitalization stay and decrease the mortality, particularly for various infections. Normally, antibiotics have been usually recommended for several children with RTIs. While the samples were distinguished with positive results by film array pneumonia

panel assay within 1 h, the experts would directly modify the therapeutic agenda for children. In accordance with the clinical data of these individuals, studies detected that children recognized with virus infections received or extended antivirus treatment and likewise decreased the inappropriate utilization of antibiotics throughout this procedure. A preceding study stated that the mean duration of antibiotic utilization was significantly shorter after application of film array pneumonia panel assay than that before the implementation [Rogers et al. 2015].

Conclusions

1. Cough swab samples do not represent reliable samples from lower respiratory tract so, they are not useful samples to support/confirm etiological diagnose oh LRTi in CLSD patients in every day practice.

2. The BioFire FilmArray Pneumonia Panel enables rapid detection of pathogens and provides greater microbiological yield than the reference standard method, culture.

3. A high sensitivity (100%) and high specificity (78%) was found for film array assay for the detection of typical respiratory bacteria.

4. A negative cough swab oropharyngeal film array result can effectively rule out a possible lower airway infection (NPV is high), whereas a positive cough swab oropharyngeal film array result does not reliably confirm a possible lower airway infection (PPV is low).

5. Additional studies are needed to evaluate the potential clinical effectiveness of the FA-Pneumo assay with various specimen types.

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