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Induced sputum as an adequate clinical specimen for the etiological diagnosis of community-acquired pneumonia (CAP) in children and adolescents

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ABSTRACT

Objectives: This study aimed to evaluate the utility of induced sputum (IS) for the diagnosis of community-acquired pneumonia (CAP) in pediatric population.
Methods: This cross-sectional study included pediatric population aged between 1 month and 17 years who were hospitalized with a diagnosis of CAP in 13 hospitals in Colombia, in whom an IS sample was obtained. Gram staining, aerobic bacterial and mycobacterial culture tests, and polymerase chain reaction (PCR) for 6 atypical bacteria and 15 respiratory viruses were performed. We evaluated the quality of IS samples.
Results: IS samples were collected in 516 of 525 children included in this study. The median age was 32 months, 38.6% were younger than 2 years, and 40.9% were between 2 and 5 years. Two patients had transient hypoxemia during the procedure. The quality of the IS obtained was good in 48.4% and intermediate in 24.5%. Identification of a respiratory pathogen was achieved with an IS sample (with Gram staining, culture test, and PCR) in 372 of 516 children with CAP.
Conclusion: Our study shows that IS is an adequate sample for the diagnosis of CAP in pediatric population that required hospitalization. The procedure was safe, well tolerated, and with better diagnostic yields compared with the rest of the samples obtained.

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Introduction

Pneumonia remains the main cause of mortality among children aged between 1 and 59 months and constitutes 12.8% of deaths among this age group worldwide (Liu et al., 2016). The annual incidence is 0.22 episodes per child per year, equivalent to 155 million new cases per year worldwide, of which, 10%-17% require hospitalization (Rudan et al., 2008).

The community-acquired pneumonia (CAP) diagnosis in children is based on clinical findings of fever, cough, abdominal pain, chest pain, rales, lethargy, vomiting, headaches, tachypnea, tachycardia, hypoxemia, and respiratory distress in a previously healthy child. None of the previously mentioned signs and symptoms was considered specific for this diagnosis (Bradley et al., 2011; Harris et al., 2011).

Despite the availability of molecular and antigen detection techniques for the identification of pathogens in CAP, it is often not easy to determine whether the identified organism is colonizing the respiratory tract or is a true pathogen (Zar et al., 2017). This accounts for the frequent overuse or misuse of antibiotics in case of viral infections, resistant microorganisms, or mixed coinfections, with a negative effect on the microbiota, thus increasing the risk for antibiotic resistance and adverse effects.

To determine the etiological agents in pneumonia, the ideal sample would be the one obtained directly from the lung, such as bronchoalveolar lavage. However, this sample is invasive, expensive, and requires highly trained specialists and specialized equipment, all of these are not readily available for routine use (Zar et al., 2017). Although obtaining spontaneous sputum samples in younger children is difficult and they often swallow the respiratory secretions, it is possible to induce an adequate sputum sample with a procedure that results in the movement of secretions from the lower respiratory tract and that induces effective cough using nebulization with hypertonic saline solution (Grant et al., 2012). This procedure has been described in microbiological studies of tuberculosis (TB), cystic fibrosis, *Pneumocystis jirovecii* pneumonia, and *Cryptosporidium* spp. infections and to assess airway inflammatory response in asthma (D'Sylva et al., 2017; Licari et al., 2020; Nyangulu et al., 2021; Ognibene et al., 1989).

Therefore, IS represents an adequate and feasible diagnostic tool for obtaining lower respiratory tract samples to determine etiological agents, thus allowing for an accurate and prompt diagnosis of CAP in children and adolescents. Sputum induction is a procedure that is less expensive, safe, and can be performed at all levels of hospital care (Zar et al., 2017).

In this study, we evaluated the use of IS in establishing the etiology of CAP in children and adolescents aged between 1 month and 17 years and describing the microscopic quality of the samples obtained.

Materials and methods

Type of study

This is a cross-sectional study that collected samples between August 2011 to September 2012.

Institutions

The patients were enrolled in 13 intermediate and high complexity care hospitals in 4 cities in Colombia (Medellín, Itagüí, Bello, Envigado): E.S.E. Metrosalud Unidad Hospitalaria San Javier, Clínica Infantil Santa Ana, Clínica Sagrado Corazón, Clínica León XIII, Hospital Universitario San Vicente Fundación, Hospital General de Medellín, Hospital Pablo Tobón Uribe, Clínica Las Américas, Hospital San Rafael de Itagüí, Clínica CES, Hospital Marco Fidel Suárez, Clínica SOMA, and Hospital Manuel Uribe Ángel.

Inclusion criteria

Children and adolescents aged between 1 month and 17 years, hospitalized with a diagnosis of CAP, in 1 of the 13 participating institutions were included in the clinical study "Etiology and the Challenge of Diagnostic Testing of Community-Acquired Pneumonia in Children."

CAP was defined as the finding of alveolar or interstitial opacities on chest x-ray imaging in addition to 1 of the following signs and symptoms: 1) axillary temperature of >38.3°C; 2) tachypnea according to age; and 3) presence of rhonchi, rales, or wheezing. Children and adolescents with a diagnosis of asthma were included in the study as long as they had CAP.

Exclusion criteria

Children and adolescents who were hospitalized in the previous 15 days, had received antibiotics treatment for more than 72 hours at the time of admission, who had primary or acquired immunodeficiencies, neurological disorders (cerebral palsy, degenerative neuromuscular disorders) or psychiatric disorders that would prevent them from signing and assent to participate in the study, inborn errors of metabolism, bronchiolitis without radiological evidence of pneumonia, hematologic malignancies, neutropenia (<500 cell/mm^3), chronic lung disease (bronchiolitis obliterans, cystic fibrosis, disorder of the cilia), HIV or CD4 counts <15% in children aged <5 years or CD4 counts of <200 cells/mm^3 in children aged >5 years, and treatment with >1 mg/kg prednisolone for more than 8 days or other immunosuppressive drugs such as cyclosporine, methotrexate, mycophenolate mofetil, cyclophosphamide, azathioprine, and fluorouracil.

The sputum induction procedure was not performed in patients with major contraindications such as patients with severe asthma who had previous history of admission to an intensive care unit, if the primary doctor did not consider it appropriate because of the patient's clinical condition, imminent respiratory failure or hypoxemia (oxygen saturation <90%), hemoptysis, hemodynamic instability, thoracic, abdominal or cerebral aneurysms, pneumothorax, pulmonary embolism, rib fractures or thoracic trauma, recent eye surgery, or in patients in whom the parents, legal guardians or the patient did not accept the procedure.

Ethical considerations

This study had the approval of the ethics committee of the Medical School of the Universidad de Antioquia and the ethics committee of each institution.

The purpose of the study was explained to the parents and the child (when feasible according to the child's age); potential adverse effects, precautions and contraindications related to the procedure, and an estimate of the date when the results of microbiological assays would be available were clearly explained. Once we confirmed a clear understanding and acceptance of the study and procedures, a written consent was obtained from the parents and legal guardians of the patient, and an assent in children aged ≥7 years, in presence of 2 witnesses who would not be any health care worker to avoid undue pressure.

Procedures

Sputum induction was performed by general practitioners who were hired for this study and received training for this procedure; they used appropriate biosafety devices such as N95 face

masks, gloves, goggles, disposable long sleeve garments and collected samples with appropriate ventilation systems at the site. The procedure was performed in the child's room or in a special procedure room, when available, with prior assessment of child's oxygen saturation and after making sure that an adequate source of oxygen and crash carts were readily available in the event of any complications. Before the procedure, a minimum of 3 hours fasting was required, and the patient received treatment with 200 mcg of inhaled salbutamol (2 puffs for 10 seconds each with 1-minute interval between puffs) using an inhaler chamber appropriate for the child's age.

Afterward, the accompanying adult was asked to clean the child's mouth with water to clear food residues or any traces of the inhaler. Nebulization with 5 ml of 5% hypertonic saline was carried out 10 minutes after the completion of last inhalation with a maximum duration of 15 minutes.

After an understanding of the procedure, children older than 7 years of age were asked to perform slow and deep breaths through their mouths sitting in semi-Fowler position. If the child was able to produce effective cough, it was verified that a 3 to 5 ml sample was collected. If this was not possible, the procedure was repeated 30 minutes later.

The procedure was considered to be completed once a 3 to 5 ml sample was obtained, or if after 3 nebulizations, no sample was obtained.

If the child was not able to expectorate or was younger than 5 years of age, respiratory secretions were aspirated after maneuvers to stimulate cough (thoracic percussions). In children aged <1 year, nasal washing was performed before suction (0.25 to 0.5 ml with 0.9% saline), and in children aged >1 year it was performed with 0.1 ml/kg. During the suction for sputum collection, the child was immobilized and nasopharyngeal aspiration was performed with a suction catheter (6 to 7 F-fringe in young infants, 8 to 9 F in children aged 3 to 12 years, and 10 to 12 F in adolescents) introduced through the nostrils and connected to an aspirator (MEDIPUMP of THOMAS®) applying negative pressure (60 to 80 mm Hg in young infants and 80 to 110 mm Hg in children aged 2 years). The catheter was removed with a twisting motion and suctioning for a maximum of 15 seconds. The aspirator was connected to a Lukens trap where the sample was collected with no risk of contamination. Finally, washing through the port with 5 ml of sterile water was carried out to remove the sputum that remained in the latex catheter until the sample reaches the trap.

The clinicians evaluated each child before, during, and 30 and 60 minutes after the procedure through monitoring respiratory rate, oxygen saturation, skin color, and hemodynamic parameters (blood pressure and heart rate) every 3 minutes. In the event of any sign of an adverse effect such as bronchospasm, persistent cough, dyspnea, tachypnea, cyanosis, wheezing, hypoxemia with <90% oxygen saturation, nauseas, and/or vomiting, the procedure was stopped. In case of bronchospasm, 1 treatment with inhaled salbutamol was administered, and 10 minutes later, the procedure was reattempted if the child was stable and had adequate oxygen saturations. All the patients were observed until discharged from the hospital.

In Supplementary Material 1, a step by step description of the materials and equipment used in the study and a description of the IS procedure are provided, and in Supplementary Material 2, a description about the aspiration of secretions is provided.

Definitions and variables

Demographic characteristics (age, sex, weight, and height) and findings in physical examinations and comorbidities recorded by the general practitioners or pediatricians at the time of admission to the emergency departments of the institutions were recorded by the investigator.

Gram staining was performed on all the sputum samples to determine their quality, defined as finding <10 epithelial cells per low-power field (LPF) as good quality and finding between 10-25 cells/LPF as intermediate quality (Murray and Washington, 1975).

In addition, multiplex PCR (mPCR) for the detection of 6 bacterial species, namely *M. pneumoniae, L. pneumophila, C. pneumoniae, S. pneumoniae, H. influenzae*, and *B. pertussis* (Seeplex® PneumoBacter ACE Detection, Seegen) and 15 respiratory viruses, namely adenovirus (AdV), coronavirus 229E/NL63 (229E/NL63), y OC 43 (OC43), parainfluenza 1 (PIV1), 2 (PIV2), 3 (PIV3), and 4 (PIV4), rhinovirus A/B/C (HRV), respiratory syncytial virus A (RSVA) and B (RSVB), influenza A (FluA) and B (FluB), bocavirus 1/2/3/4 (HBoV), metapneumovirus (MPV), and enterovirus (HEV) (Seeplex® RV 15 OneStep ACE Detection, Seegen) was performed. Only those samples with <25 epithelial cells/LPF were plated into different culture media (blood agar, chocolate agar, and MacConkey agar).

For identification of *Mycobacterium tuberculosis* auramina-rodamine staining and culture of sputum samples in solid culture media (Lowenstein-Jensen and thin-layer agar), and liquid media (BD Bactec™ MGIT™ 960) was performed.

As part of another study protocol "Etiology and the Challenge of Diagnostic Testing of Community-Acquired Pneumonia in Children" blood cultures were also performed (411 samples), culture of pleural fluids were performed when available (14), and blood samples were collected for paired serological studies (491 patients) of atypical bacteria (*Mycoplasma pneumoniae, Chlamydophila pneumoniae, Legionella pneumophila*, and *Coxiella burnetii)* and respiratory viruses (AdV, FluA and FluB, PIV1, PIV2, PIV3, and respiratory syncytial virus); urine samples (515 patients) were collected for the detection of urinary antigens (*S. pneumoniae* and *L. pneumophila*); nasopharyngeal samples (520 samples) were collected for immunofluorescence staining for the detection of antigens of AdV, FluA, FluB, PIV1, PIV2 and PIV3, and RSV.

To define a microbiological diagnosis of pneumonia from a sputum sample the following criteria were established:

Streptococcus pneumoniae: isolation of *S. pneumoniae* from sputum sample (with positive Gram stains and good/intermediate quality of the sample) or sputum samples with <25 epithelial cells/LPF in which a Gram stain had a high percentage of compatible bacterial forms (gram-positive cocci or diplococci) with or without isolation of *S. pneumoniae*.

Haemophilus influenzae: isolation of *H. influenzae* from sputum sample (with positive Gram stains and good/intermediate quality of the sample) or sputum samples with <25 epithelial cells/LPF in which a Gram stain had a high percentage of compatible bacterial forms (gram-negative bacilli or coco bacilli) with or without isolation of *H. influenzae*.

Isolation of other bacteria such as *Moraxella catarrhalis, Bordetella pertussis, Staphylococcus aureus*, enterobacteria, and other gram-positive cocci or gram-negative bacilli): isolation in sputum culture of a microorganism known to cause pneumonia (with a compatible Gram stain and good/intermediate sample quality) or isolation from a sterile site (such as cerebrospinal fluid, pleural fluid, or blood) without an adequate explanation.

Tuberculosis (TB): isolation of *M. tuberculosis* in sputum culture (Lowenstein-Jensen, thin-layer agar, and/or MGIT).

We reported the proportion of sputum samples in which an etiological diagnosis was obtained and the quality of sputum samples.

Results

An IS sample was obtained in 516 of 525 patients who met the inclusion criteria. In 9 patients, the procedure was not performed

Table 1
Clinical characteristics of pediatric population with community-acquired pneumonia that required hospitalization and in which induced sputum was obtained.

Variable	Children with IS (N = 516)
Age in months, median (IQR)	32 (14–54)
Time from the onset of symptoms in days, median (IQR)	5 (3–8)
	n/N (%)
Age by groups	
0-23 months	199/516 (38.6)
2-4 years	211/516 (40.9)
> 5 years	106/516 (20.5)
Males	261/516 (50.6)
Females	255/516 (49.4)
History of asthma	138/516 (26.8)
History of antibiotic use in the last 48 hours	37/516 (7.2)
Symptoms	
Cough	504/516 (97.7)
Sputum production	
Mucoid	187/504 (37.1)
Purulent	317/504 (62.9)
Hemoptysis	0
Fever	476/516 (92.2)
Subjective fever	209/475 (44.0)
Objective (38.3°C) fever	266/475 (56.0)
Signs	
Tachypnea*	250/509 (49.1)
Pulse oximetry %. median (IQR) (n=447)	92 (88 – 95)

* Indicates >60 breaths/min for children aged <2 months, >50 breaths/min for children aged between 2 and 11 months, >40 breaths/min for children aged between 2 and 5 years, >30 breaths/min for children aged between 5 and 12 years and >20 breaths/min for children aged >12 years.
IQR: interquartile range, IS: induced sputum.

because they had moderate to severe respiratory distress and were admitted to intensive care unit without mechanical ventilation.

At the time of enrollment, all IS samples were obtained in the patient's room or emergency department cubicles, and only 1 institution had procedure rooms available. There were no cases that required cardiopulmonary resuscitation equipment, and in only 2 patients (0.3%) the procedure was put on hold because of desaturation and mild respiratory distress that resolved with inhaled salbutamol. Once the patient was stable, we continued with the procedure.

In most of the patients, the procedure elicited coughing starting from the first minute, allowing a productive cough and adequate samples. Of the patients who underwent IS procedure, 48.1% had mild, self-limited epistaxis owing to small lacerations of the nasal mucosa. Finally, an IS sample was obtained in 516 patients. After the procedure, no adverse effects were reported. There were no differences in the presence of complications in patients with a history of asthma.

Among the 516 children and adolescents with IS samples, 79.5% were aged <5 years and the median of the duration of symptoms was 5 days (IQR 3.0-8.0). Cough (97.7%) and fever (92.2%) were the most frequent symptoms, and 26.8% of the patients had a history of asthma (Table 1).

There were 52 of 516 patients with IS samples in whom Gram staining was not performed. Among the 465 IS samples with Gram staining, 339 (73%) were considered of good/intermediate quality and were cultured (Table 2).

Cultures of the 339 IS samples with good/intermediate quality allowed a bacterial microbiological diagnosis in 114 cases (33.6%) for *S. pneumoniae, H. influenzae, M. catarrhalis, S. aureus, E. coli, S. paucimobilis*, and *M. tuberculosis* (Table 3).

In 86% of the 525 patients of the main study (451/525), a respiratory pathogen was identified (through any of the diagnostic methods described or by a combination of paired-serology, cultures, urinary antigens, molecular assays, or blood cultures). mPCR performed in 516 IS samples detected an etiological agent in 258 samples (50% of the studied samples). Results of mPCR that were positive for *S. pneumoniae* or *H. influenzae* as a sole finding were not considered diagnostic because it is not possible to differentiate colonization from real infection; therefore, these were not included in this number. Table 4 shows the pathogens that were most often detected by mPCR.

Table 2
Characteristics of induced sputum samples in pediatric population with community-acquired pneumonia that required hospitalization

Quality of the sputum*	n/N (%)
Good	225/465 (48.4)
Intermediate	114/465 (24.5)
Bad	126/465 (27.1)

* Based on the number of epithelial cells (<10 cell per low-power field = good quality and 10-25 cell per low-power field = intermediate quality).

In total, a pathogen was identified (through PCR, Gram staining or sputum culture) in 372 of 516 children and adolescents with CAP from an IS sample (72.1%).

Discussion

An etiological diagnosis of pneumonia in children and adolescents has always been considered a challenge owing to the difficulty in obtaining an adequate respiratory sample for microbiological studies. With sputum induction, it is possible to obtain a sputum sample in children of all ages, through a procedure that can be performed by trained clinicians in health care institutions and hospitals with different levels of care. Although sputum induction is a procedure that requires trained health care personnel, the use of biohazard measures and equipment necessary to handle life-threatening complications, when used appropriately, can yield adequate samples from the lower respiratory tract, which can be

Table 3
Microbiological diagnosis of bacterial infection in pediatric population with community-acquired pneumonia, with samples obtained from induced sputum with good and intermediate quality.

Bacteria	Number of children with positive culture + compatible Gram staining
Streptococcus pneumoniae	32
Haemophilus influenzae	18
Moraxella catarrhalis	36
Staphylococcus aureus	9
Mycobacterium tuberculosis	6
Escherichia coli	1
Enterobacter cloacae	1
Sphingomonas paucimobilis	1
Haemophilus influenzae and *Moraxella catarrhalis*	1
Streptococcus pneumoniae and *Haemophilus influenzae*	1
Streptococcus pneumoniae and *Moraxella catarrhalis*	2
Haemophilus parainfluenzae and *Moraxella catarrhalis*	1
Total	109/339 (33.6%)

Table 4
Microbiological diagnosis in 516 children with pneumonia with multiplex PCR performed with induced sputum samples

Respiratory pathogen	Positive mPCR (%)
Respiratory syncytial virus A and B	111 (21.14)
Rhinovirus A/B/C	111 (21.14)
Parainfluenza 1, 2, 3, 4	68 (12.9)
Mycoplasma pneumoniae	54 (10.2)
Influenza A and B	47 (8.9)
Metapneumovirus	37 (7.0)
Coronavirus 229E/NL63 and OC 43	15 (2.8)
Bordetella pertussis	8 (1.5)
Enterovirus	6 (1.1)
Adenovirus	4 (0.76)
Moraxella catarrhalis	2 (0.38)
Bocavirus	2 (0.3)
Chlamydophila pneumoniae	1 (0.2)

used for the diagnosis of CAP in children and adolescents. Therefore, we believe that this study of IS for the diagnosis of CAP in children and adolescents is relevant and can be implemented in any hospital to achieve a rapid microbiological diagnosis and targeted treatment of CAP in pediatric population requiring hospitalization.

The technique for obtaining IS in pediatric population is safe and well tolerated and it can be performed in health care settings with limited resources (DeLuca et al., 2017). Although one-third of our patients had a history of asthma, none of them had an exacerbation of symptoms or adverse effects. The only 2 events presented with hypoxemia and mild respiratory distress resolved promptly with inhaled bronchodilator. These findings are similar to the few side effects reported in other studies, all of which resolved with appropriate medical attention by trained health care personnel (DeLuca et al., 2017; Sheehan et al., 2017; Zar et al., 2003).

From the IS samples, we were able to identify an etiological agent in 72.1% of the patients with CAP enrolled in this study. This proves that Gram staining, microbial culture tests, and multiplex PCR will yield the majority of etiological diagnosis in CAP when compared with other diagnostic tests. IS had better diagnostic performance than other specimens in the main study and explains the higher percentage of etiological identification when compared with other studies (Ferrari C et al., 2007). In previous studies, 75%-91% of IS samples were considered good quality (Bart et al., 2016; Lahti et al., 2009). In our study, 73% of IS samples were considered to be of good quality and appropriate to undergo testing with different microbiological procedures.

IS has been mainly used for the diagnosis of TB in countries with high prevalence of TB and seldom for diagnosis of pneumonia in pediatric population. This study shows the importance and utility of IS for the microbiological diagnosis of CAP in children and adolescents, allowing for a more targeted antibiotic therapy, avoiding antibiotic misuse and in turn contributing to antibiotic stewardship. IS had a better yield even when compared with other samples such as blood cultures, in which only 3.6% yielded positive results. These results are similar to those published by Lahti et al (Lahti et al., 2009).

The Pneumonia Etiology Research for Child Health (PERCH) study, a case-controlled study in 9 hospitals in 7 sub-Saharan Africa and South Asian countries, assessed the performance of IS in 3772 children with suspected pneumonia. Among these, 2608 (69.1%) sputum samples met the criteria of high-quality sample (<10 epithelial cell/LPF) but only 1162 children (44.5%) had chest x-ray images compatible with pneumonia. In that study, 80.6% of the patients with a chest x-ray finding compatible with pneumonia had received antibiotic treatment before providing the IS sample (only 7.3% in our study), which could in turn have affected the yield of their cultures. In addition, in a significant number of controls without pneumonia, it was not possible to establish the cause of their respiratory symptoms (Murdoch et al., 2017). In our study, only children and adolescents with confirmed clinical and radiological CAP and who had received less than 72 hours of antibiotics were included. The etiology was determined by a combination of 5 different diagnostic tests (paired serum serology samples, molecular assays, microscopy, immunofluorescence, and cultures), but the sample with the highest diagnostic performance was the IS.

The identification of causative pathogens such as *M. pneumoniae*, some viruses, bacteria, and fungi often requires a combination of tests to achieve a more reliable, accurate, and rapid diagnosis. It is important to highlight that a positive PCR in the sputum of patients with CAP can represent either normal or dysbiotic microbiome of the lower respiratory tract, and the identification of genetic material using PCR based assays does not necessarily translate to a viable agent, as has clearly been demonstrated in the context of SARS-CoV-2 (the patient can have a positive PCR with non-cultivable virus) (Singanayagam et al., 2020; Wölfel et al., 2020). Transient colonization and asymptomatic infection have also been reported with *S. pneumoniae, H. influenzae*, and respiratory viruses (Copete et al., 2020; Madhi et al., 2020; Self et al., 2016; Thors et al., 2019, Zar et al., 2016). Therefore, it is necessary to combine different samples and tests to optimize the etiological diagnosis of CAP in pediatric population.

Diagnosis of TB as a cause of CAP was made in 6 patients (1.15%) by isolating *M. tuberculosis* in liquid culture media of sputum samples, which is relevant for public health, because it shows that TB can have an acute presentation in pediatric population, and it is undistinguishable from a CAP. This finding also shows that IS is a useful tool in the diagnosis of TB in children, as described

in previous studies (López et al., 2012; Moore et al., 2017, 2011; Planting et al., 2014; Qureshi et al., 2011; Ruiz Jiménez et al., 2013; Zar et al., 2005, 2000).

We believe that the strengths of our study were obtaining IS from 516 of 525 patients in whom their clinical conditions allowed the procedure, being able to perform the procedure in all of the institutions, achieving good or intermediate quality in high percentage of samples, and that a low percentage of patients received antibiotics before the procedure. Our main limitation was that we did not have a separate data collection form exclusively for IS, and therefore, mild symptoms associated with the IS procedure were not recorded.

Because IS sample is produced from the most distal sections of the lungs, it better represents the alveolar contents when compared with samples obtained from nasopharyngeal or oropharyngeal washes, therefore, allowing better microbiological results and a more targeted and rational use of antibiotics in a population that is at risk for developing adverse effects to medications and problems with bacterial resistance. In conclusion, our study demonstrates the utility and applicability of IS for the diagnosis of CAP in hospitalized children and adolescents in institutions with all levels of care.

Competing interests

The authors have declared that no competing interests exist.

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Author's contributions

Substantial contributions to the conception or design of the work: all authors. Acquisition of data: MB, AR, OM, CR, CA, CG, MT. Processing of samples: YA, ARC, CV, MH. IS training: GIN, LFC. Analysis and interpretation of data: all authors. Drafting the article: ZVR, AR, CG, OM, CR, CA, MAM, JLA, LAV. Revising the article critically for important intellectual content: MB, LFL, LL, ARC, CV, MH, MRG, GIN. Final approval of the version to be published: all authors. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: all authors. Principal investigator and funding acquisition: LAV.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2022.01.026.

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