Editorial

Microscopic analysis and quality assessment of induced sputum from children with pneumonia

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Community acquired pneumonia (CAP) in children especially under five years age is more common in developing nations with estimated incidence approximately 0.026 episodes per child year accounting for 95% of all episodes pneumonia in young children worldwide (1). It is leading cause of mortality in children below 5 years of age. Interventions such as empirical antibiotics in children with suspected pneumonia has led to significant reduction in mortality due to pneumonia (2). World Health Organization has suggested identification of pneumonia by rapid respiration and respiratory difficulty and administration of antibiotics in countries with high infant mortality (2). This approach has raised concerns about development of drug resistant organisms. It is observed that majority of children with non-severe pneumonia diagnosed by using WHO criteria had normal X ray films suggesting that they possibly did not have bacterial pneumonia (3). This was supported by randomized controlled trials comparing antibiotics and placebo in non-severe pneumonia diagnosed by WHO criteria; showing no difference in outcome (4,5). These observations suggest that administration of antibiotics may be lifesaving in children with pneumonia caused by bacteria but empirical antibiotics may be responsible for development of drug resistant organisms and their adverse consequences. Therefore it is important to identify causative agents for pneumonia and decide about antibiotics.

Routinely used methods to identify etiological microbiological agents in pneumonia are: Respiratory tract specimens for microscopy and culture, identification of antigens in respiratory specimens and urine, serological studies for certain agents, blood culture, polymerase chain reaction (PCR) (6).

The procedure of respiratory tract specimen collection especially from lower respiratory tract has an enormous influence on microscopy and culture results and interpretation. Commonly used specimen includes throat swabs, nasopharyngeal aspirates, nasopharyngeal washings, sputum, induced sputum, bronchoalveolar lavage, bronchoscopic protected brush sampling and non bronchoscopic lavage. Throat swab cultures are established method for diagnosis of bacterial pharyngitis (7). Nasopharyngeal aspirates and nasopharyngeal washings are used for identification of viral agents and atypical agents (8), Bronchoscopic and non bronchoscopic lavage as well as protected brush sampling are useful specimen but are invasive and need expertise therefore indicated only in selected cases. Sputum examination is commonly used for microbiological investigations in adults with pneumonia. In children sputum sample representing lower airway sample is difficult to obtain as most of time it gets contaminated with throat and airway colonizers. Induced sputum has been used successfully in hospitalized and ambulatory children with tuberculosis (9) and for identification of pneumocystis jiroveci in immunocompromised children with pneumonia (10). There are enough evidence to suggest that induced sputum has good correlation with lower airway microbial agents (bacterial agents i.e., *pseudomonas spp*., *staphylococcus*) in children with cystic fibrosis (11). However, there are limited studies on use of induced sputum in children with CAP.

Important steps in obtaining induced sputum includes: induction, collection, assessing its quality, processing for identification of microbial agents using multiple techniques.

Induction of sputum and collection are well established as it is being used for diagnosis of tuberculosis in children. Interpretation of sputum for organisms that are not found in oropharynx such as Mycobacterium tuberculosis, atypical agents, PCP etc is easy. However it is difficult for bacterial agents because of high rates of colonization with common bacterial agents that are also responsible for causing pneumonia in children. This is particularly true for sputum (expectorated and induced) which may lead to the incorrect conclusion that upper respiratory tract colonizers are pneumonia pathogens.

Hence, sputum specimens should be checked for quality before processing them for culture. Quality check typically includes calculating the number of squamous epithelial cells (SECs) and polymorphonuclear cells (PMNs) in a Gram stain smear of the specimen.

Murray *et al.* examined expectorated sputum grossly and microscopically to decide their appropriateness for bacterial culture. The mean number of bacterial species isolated was 2.7 from specimens with <10 SECs per field, 2.4 from transtracheal aspirates and more than 4 from specimens >10 SECs per field. Potential bacterial pathogens were isolated in less than 15% of specimens which had more than 10 SECs per field. The bacterial flora of specimens with fewer than 10 SEC per field was similar to that of transtracheal aspirates (12). Musher et al examined gram stain and culture of sputum specimen from 105 patients with pneumococcal pneumonia proven by blood culture. They defined adequate sputum specimen as ≥10 WBCs for each SEC at magnification of 400×. Performance of culture in those samples yielded correct diagnosis >80% of cases with pneumococcal pneumonia (13). Hence, presence of >25 PMNs and < 10 SECs per low-power field or ≥10 leukocytes for each SEC is regarded as a high quality expectorated sputum specimen in adults (12,13). Specimen which contain relatively high number of SECs and low number of PMNs are regarded as poor quality specimen and represent contamination with upper respiratory tract flora. However, Mycobacterium tuberculosis, Legionella, majority of fungi and viruses do not produce PMNs in sputum and hence, specimen quality can be better measured by assessing the number of SECs per low-power field (14).

Musher *et al.* showed that microscopic examination of sputum samples before antibiotics administration yielded correct diagnosis in more than 80% of patients with pneumococcal pneumonia (13).One study done on both adults and children to identify respiratory pathogens with TaqMan array card technology concluded that identification of pathogenic bacteria and viruses is better in high quality sputum compared to oropharyngeal or nasopharyngeal swabs (15). Lahti *et al.* studied 101 children aged 6 months to 15 years with CAP over 15 months. 76 children could produce high quality sputum and possible causative organism could be identified in 90% who produced high quality sputum (16).

Nearly 75% of adults with pneumonia can produce adequate amount and quality of sputum for microbiological diagnosis (17,18). In adults, the sensitivity of sputum examination was reported to be more than 75% for identification of bacterial pathogens. However, prior antibiotic exposure and contamination with oral and oropharyngeal bacteria to be kept in mind (19). Usually it may not be practical to collect sputum from infants and children due to various reasons. Often, children tend to swallow the sputum specimen rather than expectorate it. Compared to adults, high chances of sputum contamination with colonizing bacteria like *Streptococcus pneumoniae* and *Haemophilus influenzae* during collection. It is also known that the carriage prevalence of Streptococcus pneumoniae is 6–14% in adults as compared to 57–65% in children aged 5 years, and the carriage prevalence of *Haemophilus influenzae* is 3% in adults compared with 26% in children aged 5 years (20).

Safety of induced sputum also needs to be considered in children especially those with less than 5 years age. Usually it is done in children with severe or very severe pneumonia requiring hospitalization to identify microorganism. In older children 6–16 yrs age with asthma, induced sputum is shown to be a non-invasive and relatively safe procedure except for few patients experienced moderate bronchospasm, nausea, sore throat, tingling sensation in palms and soles (21,22). Recently two studies done in Kenya and New Caledonia assessed safety of sputum induction among children less than 5 years age who were admitted with severe or very severe pneumonia. Only one child had adverse event in the form of seizures during hypertonic saline nebulisation (23,24). Hence, induced sputum can be performed in children with severe or very severe pneumonia safely.

Murdoch *et al.* (25) examined 3772 induced sputum specimens from children aged 1 month to 59 months with severe pneumonia who were hospitalized from seven sub African and Asian countries. They intended to get induced sputum prior to initiation of antibiotics and followed standardized protocol at all centres to assure uniformity across the centres. Two thousand six hundred and eight (69%) sputum samples had <10 SECs per low-power field, 2,350 (62%) samples had >25 PMNs per low power field

and 1,509 (40%) had both <10 SECs and >25 PMNs per low power field. Samples with SECs <10 per LPF had low quantities of oropharyngeal flora and higher prevalence of potential pneumonia causing pathogens making SECs <10 per LPF as high quality marker of sputum. As the number of SECs increased the prevalence of *Hemophilus influenzae*, *Streptococus pneumoniae*, and *Moraxella catarrhalis* decreased in sputum specimen. However, prevalence of gram-negative bacteria, *Staphylococcus aureus* increased with number of SECs. The quantity of oropharyngeal flora increased with the presence of greater numbers of SECs, but there was no clear association could be observed with numbers of PMNs. Sputum specimens with SECs <10 per LPF were associated with a lower odds of culturing larger quantities of oropharyngeal flora 0.23 (95% CI, 0.19–0.29) whereas specimens with PMNs >25 per LPF was associated with an increased odds of culturing larger quantities of oropharyngeal flora 1.60 (95% CI, 1.34–1.91) however the effect size was small. This study is one of the few studies that tried to identify etiological agents for pneumonia in children. There are several limitations, including lack of gold standard for comparison, influence of prior antibiotic use on sputum culture and probable discrepancies in the reporting of sputum cultures and Gram stain across different countries where the study took place.

Sputum with <10 SECs and >25 PMNs per LPF have long been regarded as high quality sample (12), however, the prerequisite for large numbers of PMNs has been an issue as some pneumonias are not definitely associated with elevated PMNs (26). On contrary, conditions associated with elevated PMNs in sputum specimen apart from pneumonia like cigarette smoking, pollutants like ozone endotoxins, steroid resistant asthma (27) needs to be kept in mind while assessing sputum quality.

If induced sputum can be used for identification of etiological agents of pneumonia in children, it may be a great step towards rationale use of antibiotics in children. There is need to show reproducibility of results found in study by Murdoch *et al.* (25) by performing more studies. Equally important is to train paediatric nurses and paediatric physician to induce sputum properly and transport to laboratory. Apart from bacterial culture, there is enormous scope to use molecular technology to identify various etiological agents within few hours.

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Footnote

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