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Original Research Article

The Value of Sputum Gram Stains in the Diagnosis of Childhood Community-Acquired Pneumonia

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Abstract

The usefulness of sputum Gram stain and culture in guiding microbiological diagnosis of community-acquired pneumonia is controversial. This study assessed the usefulness of Gram-stained sputum specimens and sputum culture in the microbiological diagnosis of community-acquired pneumonia in children at Tikur Anbassa University Hospital, Addis Ababa, Ethiopia through June 2008 to January 2009. Two hundred five children, aged 6 months to 12 years, with community-acquired pneumonia were enrolled in this study. Clinical diagnosis of community-acquired pneumonia was made based on a clinical observation of signs and symptoms associated with a lower respiratory tract infection, pulmonary consolidation by physical examination or abnormalities in chest X-ray. Expectorated sputum samples were collected from 170 patients. Forty-five good quality sputum samples were found based on macroscopic and microscopic examinations. Sputum cultures were processed according to standard protocols. Evidence of bacterial infection was detected in 36 of the 45 (80%) children who provided good quality sputum samples. A single bacterial infection was found in 17 of the 45 (38%) children. Two or more bacteria were found in 19 of the 45 (42%) children and no pathogen was found in 9 of the 45 (20%) sputum cultures. A total of 63 bacteria were isolated from 36 patients with community-acquired pneumonia. The isolated bacteria were identified by using API bacterial identification kits. Overall, 32 of 63 (50.8%) were Gram-positive bacteria and 31 of 63 (49.2%) were Gram-negative bacteria. This study showed the limited value of Gram staining of sputum as a diagnostic tool in the initial evaluation of patients with CAP, as a PM could be identified in only 13 of the 45 (28.9%) patients with good-quality sputum samples. And the sensitivity of sputum Gram stain (55.6%) for diagnosis of pneumococcal pneumonia was also low.

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Introduction

Pneumonia causes about 1 in 5 under five deaths worldwide: more than 2 million children each year. It kills more children than any other disease; more than AIDS, malaria and measles combined (UNICEF, 2006). In Africa, 21% of the 4.4 million deaths of children less than five years of age per year are caused by pneumonia. The annual incidence of pneumonia in children younger than 5 years of age is 34 to 40 cases per 1000 in Europe

and North America, higher than at any other time of life, except perhaps in adults older than 75 or 80 years of age. In the developing world, pneumonia is not only more common than it is in Europe and North America; it is also more severe and is the largest killer of children (McIntosh, 2002). Pneumonia is the sixth leading cause of death in the United States of America and is the most common infectious cause of death. The mortality rate is reported to be 1% in the outpatient setting but may increase up to 25% in those requiring hospital admissions (Marrie, 1999).

Pneumonia is important in tropical and developing countries, due to its high lethality in children under 5 years old, especially among infants (March and Sant' Anna, 2005; Yoshimine et al., 2001). In developed countries, this can be verified by the radiological finding of consolidation. In the developing world a more practical term-acute lower respiratory infection is preferred, reflecting the difficulties in obtaining a chest radiograph (BTS, 2002). The WHO (1994) has defined pneumonia solely on the basis of clinical findings obtained by visual inspection and timing of the respiratory rate.

Community-acquired pneumonia (CAP) remains a major cause of morbidity and mortality.

A causative agent is identified in 30% to 40% of cases, and the most common is *Streptococcus pneumoniae*. The clinical and radiographic microbiological diagnoses of pneumonia lack accuracy, cultures take at least 24 hours to produce a positive result, and specific rapid tests based on the detection of soluble antigens of *S pneumoniae* or *Legionella pneumophila* in body fluids are not always available. Therefore, initial antibiotic therapy is usually empirically chosen (Garcia-Vazquez et al., 2004).

Current criteria of diagnosis are based on sputum Gram stain and sputum culture (Gleckman, 1991). Although a sputum culture has the advantage of high sensitivity; routine laboratories are not able to perform this test for various reasons (Ozyilmaz et al., 2005). As no specific cause is found in 25-50% of patients with CAP, large number of patients needs to be treated empirically (Guclu et al., 2005). Even when a bacterial pathogen is identified, antimicrobial susceptibility information is frequently delayed for a few days, necessitating empiric treatment decision (Metlay et al., 2004). But empirical antimicrobial choice is complicated by the increasing prevalence of antibiotic resistance of some of the common lower respiratory tract pathogens (Ozyilmaz et al., 2005).

The role of sputum culture as a rapid diagnostic tool that could direct antimicrobial treatment of CAP is a matter of controversy. Some of its limitations are the difficulty to obtain good quality samples, its lack of reliability due to possible sputum contamination by the flora of the upper airways, its low diagnostic yield (i.e. sensitivity), and, therefore, its low impact on treatment decisions. There are lacks of studies analyzing the usefulness of sputum gram staining in the diagnosis of childhood CAP. The aim of this study of inpatients and outpatients was to evaluate the usefulness of sputum gram staining and sputum culture in the microbiological diagnosis of CAP in children.

Materials and methods

Study design

A hospital based cross-sectional study was conducted at the Pediatric OPD in Tikur Anbassa Hospital, Addis Ababa, Ethiopia from June 2008 through January 2009. The study was reviewed and approved by the department of Microbiology, Immunology, and Parasitology ethical committee and Institutional Review Board of the Medical Faculty of Addis Ababa University. Signed informed parental consent and the child's assent (if the child was < 10 years old) were obtained. All the information obtained was kept confidential. If patients were not interested in the study, they had the right to withdraw from the study.

Study populations

Patients were recruited in the paediatric outpatient department of Tikur Anbassa Hospital, Addis Ababa, Ethiopia. The samples were collected from children aged 6 months to 12 years with suspected CAP. The population at large is served in this health facility that comes from the city of Addis Ababa and different parts of Ethiopia. Two hundred five children with CAP were included in the study. All children aged 6 months to 12 years admitted to Pediatric OPD emergency room with suspect community-acquired pneumonia were included in the study.

Eligibility and exclusion criteria

Community-acquired pneumonia was defined as

pneumonia that has been acquired in the community in a patient who has not been hospitalized within 14 days prior to onset of symptoms or has been hospitalized for less than 4 days prior to the onset of symptoms. Patients eligible for inclusion in the study met the following criteria:

I. Children aged 6 months to 12 years with a primary (putative) diagnosis of CAP made within 24 hr of admission; a medical and clinical history of pneumonia
II. Clinical observation of two or more signs and symptoms associated with a lower respiratory tract infection (i.e. body temperature >37.8^{O}C, new or increased cough, production of purulent sputum) or at least two of the minor conditions, which include: pleuritic chest pains, dyspnoea, altered mental status, pulmonary consolidation by physical examination.
III. Children with CAP who have been hospitalized less than 4 days prior to the onset of symptoms.

Patients in the following categories were excluded from the study: children < 6 months of age; and admission from a nursing home or a hospital within a month prior to the study so as to avoid the inclusion of possible nosocomial case.

Sample collection and transport

Within 24 hr of admission, a physician examined the patients and recorded the findings on standardized case record forms. Nurse collected samples of sputum from children with suspected CAP in sterile, screw top sputum cups. The expectorated sputum was collected, ideally a minimum volume of 1ml. by asking the patient to cough deeply into the container, followed by immediate screwing on the cup. No special procedures were performed to obtain sputum samples if they could not be obtained spontaneously. Samples were transported to the laboratory within two hrs of collection and processed immediately as explained by Yamazaki et al. (2005) and Nagalingam et al. (2005). Sample processing and Microbiological examination was done at Bacteriologic Unit of Tikur Anbassa University Hospital.

Microbiological examinations

Microscopy: Sputum Gram stains

A smear was prepared from the most purulent materials in the sputum and a Gram stain was done using standard methods (Bauer et al., 1966). The quality of the sputum specimen was assessed by evaluating the Gram stain slide under light microscope (X10). Polymorphonuclear leucocytes and squamous epithelial cells were counted. Sputum samples were considered of good quality if they had less than 10 squamous epithelial cells and more than 25 Polymorphonuclear neutrophil per low power field. Otherwise, the sputum sample was considered contaminated by saliva and discarded (Garcia-Vazquez et al., 2004; Roson et al., 2000; Nagalingam et al., 2005).

Good quality specimens were then screened for a predominant bacterial morphological type at oil immersion field (X100). Quantitation of these parameters was accomplished using the following criteria: (0, none seen; 1+, 1 - 5; 2+, 5 - 10; 3+, 11 - 25; and 4+, > 25 per field) (Nagendra et al., 2001). A predominant morphotype was defined as the presence of a single morphotype that accounted for > 75% of the organism seen (Roson et al., 2000).

Isolation and Identification of bacterial pathogens

A proportion of another purulent area of the good quality sputum was used for microbiological analysis. Sputum cultures were processed immediately in blood agar, chocolate agar, and MacConkey agar media. The following organisms were considered potential pathogens: *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *S. aureus*, beta-haemolytic streptococci, Enterobacteriaceae, Pseudomonas and Acinetobacter sp. The organism was considered as the potential etiology based on the definition of Fang et al (1990).
Five percent sheep blood agar was used to isolate and identify Gram-positive organisms. The plates were incubated in a 5-10% candle extinction jar at 35-37^{O}C for 18-24hrs. On the next day, identification of colonies, Gram staining, and sub-culturing were done.

To isolate *S. pneumoniae*, the plates were examined and colonies that showed alpha haemolysis and button-shaped were sub-cultured onto 5% sheep blood agar, incubated at 35-37^{O}C and in 5-10% CO_2 overnight. For confirmation, suspected colonies were sub-cultured onto 5% sheep blood agar and an optochin disk was placed onto the streaks by using sterile forceps; these were incubated at 35-37% and 5-10% CO_2 for 24hrs to note zones of inhibition. Colonies exhibiting characteristic colonial morphology, alpha haemolytic after overnight incubation on 5% sheep blood agar, Gram-positive

lancet-shaped cocci that were sensitive to optochin (≥ 14 mm zone of inhibition around a 5μg disk) were identified as *S. pneumoniae*.

Those colonies with zone of inhibition 9-13 mm were tested by bile solubility (NCCLS, 2001). The bile solubility test was performed by taking several loops of the strain from the growth on 5% blood agar plate and making a suspension in 1.0 ml of sterile saline. The suspension of cells was adjusted to be equal to that of 0.5 McFarland density standards. The suspension was divided into two equal amounts (0.5 ml per tube) and 0.5 ml of saline was added to one tube and 0.25 ml of 2% deoxycholate (bile salts) was added to the other.

The tubes were then shaken gently and incubated at 35-37°C for up to 2 hrs. The tubes were examined periodically for lysis of the cells in the tube containing the bile salts. A clearing of the tube, or a loss in turbidity, was taken as a positive result (NCCLS, 2001). The strains which had <14 mm zone of inhibition of optochin and were not bile soluble were considered as Viridans Streptococci.

To isolate *S. pyogenes*, the BAP with beta-haemolysis, golden yellow or white or cream colonies was sub-cultured onto BAP and incubated at 35-37°C in 5-10% CO_2 overnight. For confirmation of *S. pyogenes*, suspect colonies from BAP were sub-cultured onto BAP and a bacitracin disk were placed onto the streaks by using sterile forceps; these plates were incubated at 35-37^{O}C for 24hrs. On the next day, the plates were examined and those colonies with any zone of inhibition of growth were considered as a positive result.

To isolate *S. aureus*, the BAPs were examined and colonies that were large beta-haemolytic, golden yellow or white or cream were sub-cultured onto BAP and incubated at 35-37^{O}C overnight. On the next day, a representative colony from overnight BAP was taken and catalase, slide and tube coagulase tests were performed. Those strains, which were Gram-positive cluster cocci, catalase positive, slide coagulase and/or tube coagulase positive were considered as *S. aureus*.

Chocolate agar was used to isolate and identify *H. influenzae* and *M. catarrhalis*. To isolate *H. influenzae*, the sputum sample was inoculated to CA by taking a loopful of specimen. The plates were incubated at 35-37^{O}C in 5-10% CO_2 for 18-24hrs. On the next day, the CA plates were examined and colonies that were white and glossy were sub-cultured onto CA and incubated at 35-37°C in 5-10% CO_2, overnight (Nagalingam et al., 2005). Confirmation was made by API 10NH system (BioMerieux, France) according to the manufacturer′s standard (Guclu et al., 2005) and by demonstrating satillitism with the x and v test. To isolate Moraxella species, after overnight incubation of the primary CA plates were incubated at 35-37°C in 5-10% CO_2 for 24 hrs. On the next day, the CA plates were examined and those colonies, which were grayish-pink opaque, were sub-cultured at 35-37°C in 5-10% CO_2 for overnight. Confirmation was made by API 10NH system (BioMerieux, France) according to the manufacturer′s standard (Guclu et al., 2005).

GNEB were isolated from the sputum sample by plating onto a MacConkey agar plate, incubated at 35-37°C aerobically for 24hrs. Using the standard microbiological and biochemical techniques GNEB and Pseudomonas were identified based on both microscopically and macroscopically (Shah et al., 2002). Confirmation was made by API 20E system (BioMerieux, France) according to the manufacturer′s standard (Guclu et al., 2005).

Standard control reference strains

The reference control organisms used were *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27833) and *Escherichia coli* (ATCC 25922) which were obtained from EHNRI. All *in vitro* tests were done strictly according to the research protocol, and a 4-day quality control run-in period was followed by actual *in vitro* studies.

Statistical analysis of data

The data obtained from this study were entered into a computer and all statistical values were analysed using SPSS soft-ware package, version 15. Descriptive data were presented as means ± SD for continuous variables and as rates for categorical variables. Statistical comparisons of categorical variables were made by Chi-square analysis or the Fisher exact test, when appropriate. Statistical significance was defined as $P < 0.05$ (2-tailed). Performance characteristics of Gram-positive diplococci identification for culture of *S. pneumoniae* in sputum were also analyzed. Diagnostic parameters such as sensitivity, specificity, and positive and negative predictive values were calculated according to standard equations

Results

Patient demographics

A total of 205 children with community-acquired pneumonia met the inclusion criteria for enrollment (Fig. 1). Eleven cases of the 205 patients were radiographically confirmed to have community-acquired pneumonia, as diagnosed by radiologists in Tikur Anbassa Hospital. The diagnosis of lower respiratory involvement was based on clinical signs and symptoms which were made by the attending physicians in pediatric OPD. Of these patients, 98 (47.8%) were males. Ages ranged from 6 months to 12 years with mean of 5.13 years. Seventy-three (36.0%) were 6 months to < 2 years old, 37 (18.0%) were 2 to < 5 years old, and 95 (46.0%) were ≥ 5 years to 12 years old (Table 1). Most had been ill for less than two weeks. The mean duration of illness prior to coming to hospital was 7.9 days. One hundred and thirty-three (64.9%) patients had received antibiotics prior to admission. The most common antibiotics used were penicillin 63 (47.4%), ceftriaxon 31 (23.3%), ampicillin 16 (12.0%), amoxicillin and gentamycin 15 (11.3%) and chloramphenicol 8 (6.0%).

The principal presenting symptoms and signs at admission are also shown in Table 1. The most common symptoms and signs which were observed in the patients were: cough 163 (79.5%), difficulty breathing 127 (62.0%), fever 123 (60.0%), chest pains 10 (4.9%) and convulsion 9 (4.4%).

Sputum examination result: Macroscopic and Microscopic

Sputum samples were obtained from 170 patients (82.3% of the total) (Fig. 1). By macroscopic examination at the appearance of the 170 collected sputum specimens, 101 (59.4%) were found to be serous, 49 (28.8%) purulent, 14 (8.2%) mucoid and 6 (3.5%) seropurulent. Concerning their color, 15 (8.8%) of the samples were blood-streaked sputum, 54 (31.5%) yellow-green and 101 (59.4%) clear and colorless. One hundred and sixty-nine (99.4%) of them did not have putrid odor (Table 2). By observing the status of PMNs and the epithelial cells in a Gram-stained smear, 45 of 170 (25.3%) collected sputum specimens were determined to be suitable for culture. Thus, the final study populations consisted of 45 children from whom good quality sputum samples were obtained (Fig. 1).

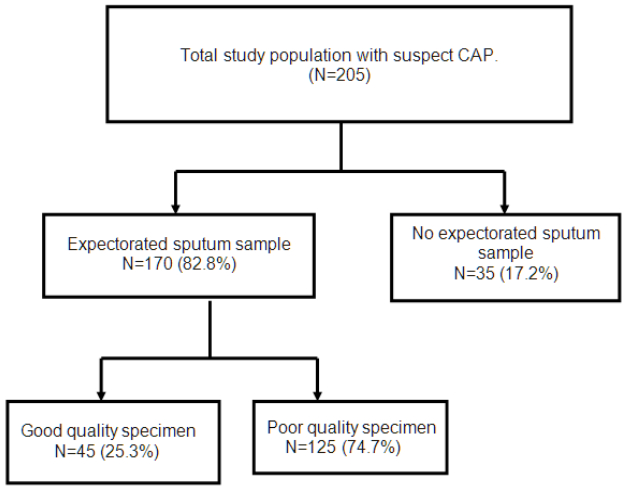

Fig. 1: Flow diagram of study patients and screening sputum specimens for microscopy and culture.

Table 1. Patient characteristics and clinical findings of children with CAP.

Characteristics	Children with CAP (N=205) n (%)
Age	
6 months to < 2yrs	73 (35.6)
2yrs to < 5yrs	37 (18.1)
5yrs to 12yrs	95 (46.3)
Gender	
Male	98 (47.8)
Symptoms and signs	
Cough	163 (79.5)
Difficulty breathing	127 (62.2)
Fever	123 (60.0)
Chest pains	10 (4.9)
Convulsion	9 (4.4)
Ongoing antibiotic treatment on admission	**n=133 (%)**
Penicillin	63 (47.3)
Ceftriaxon	31 (23.3)
Ampicillin	16 (12.0)
Amoxicillin and Gentamycin	15 (11.3)
Chloramphenicol	8 (6.0)

CAP: Community Acquired Pneumonia

Thirteen of the 45 good-quality samples (28.9%) showed PMs on sputum Gram stains. Eight of them (61.5%) were Gram-positive diplococci, 3 of them (23.1%) Gram-positive cocci and 2 of them (15.4%) were Gram-negative bacilli (Table 3). Results for patients with previous antibiotic treatment are also shown in Table 3.

Table 2. Macroscopic examination results of sputum samples (N=170).

Characteristics of sputum	No. (%)
Consistency and appearance of sputum	
Serous /liquid	101 (59.4)
Mucoid	14 (8.2)
Seropurulent	6 (3.5)
Mucopurulent/purulent	49 (28.8)
Color of sputum	
Bloody/rust colored sputum	15 (8.8)
Yellow/green sputum	54 (31.8)
Clear and colorless	101 (59.4)
Odor	
Putrid odor	1 (0.6)
Odorless	169 (99.4)

Table 3. Bacterial distribution in sputum Gram stains of 170 patients with CAP.

Gram stain result	Patients who provided sputum (N=170) n (%)	Patients with PAT and who provided sputum (N=119) n (%)
Good quality samples	45 (25.3)	31 (26.1)
Predominant morphotype	13 (28.9)	6 (19.4)
Gram-positive diplococci	8 (61.5)	3 (50.0)
Gram-positive cocci	3 (23.1)	2 (33.3)
Gram-negative bacilli	2 (15.4)	1 (16.7)

CAP: Community-acquired pneumonia, PAT: Previous antibiotic treatment. Statistical significance was defined as $p<0.05$.

Etiologic agents

The bacteria isolated from sputum collected for this study are shown in Tables 4 and 5. Evidence of bacterial infection was detected in 36 of the 45 (80.0%) children who provided good quality sputum samples. A single bacterial infection was found in 17 of the 45 (38.0%) children. Two or more bacteria were found in 19 of the 45 (42.0%) children and no pathogen was found in 9 of the 45 (20.0%) sputum cultures (Table 4).

Sixty-three isolates were cultured from 36 patients with community-acquired pneumonia. Overall, 32 of 63 (50.8%) were Gram-positive organisms and 31 of 63 (49.2%) were Gram-negative bacteria.

S. pneumoniae and *S. pyogenes*, each accounted for 14.3% of the total isolated bacteria, followed by *S. aureus* (12.5%), *E. cloacae* (11.1%), Viridans Streptococci (9.5%), *Acinetobacter* spp (7.9%), *E. coli*, *S. marcescens* and *Klebsiella* spp. (*K. pneumoniae* and *K. oxytoca*) [6.3%], *M. catarrhalis* (4.8%), and *P. aeruginosa*, *P. mirabilis*, *C. freundi* and *Aeromonas* spp. (1.6%) (Table 5).

Table 4. Identified causative bacteria from 45 expectorated sputa cultures of cases with CAP.

Isolated bacteria	No. of cases (%) (N=45)
Single pathogen	**17 (38.0)**
S. pneumoniae	5 (29.4)
S. aureus	4 (23.5)
Viridans Streptococci	3 (17.6)
S. pyogenes	2 (11.8)
K. pneumoniae	1 (5.9)
E. cloacae	1 (5.9)
S. marcescens	1 (5.9)
Multiple pathogens	**19 (42.0)**
S. pyogenes + *E. coli*	2 (10.5)
S. pneumoniae + *P. aeruginosa*	1 (5.3)
S. pneumoniae + *S. pyogenes*	1 (5.3)
S. pneumoniae + Viridans Streptococci	1 (5.3)
S. pneumoniae + *S. marcescens*	1 (5.3)
S. pyogenes + Viridans Streptococci	1 (5.3)
S. pyogenes + *Aeromonas* spp	1 (5.3)
S. aureus + *E. coli*	1 (5.3)
M. catarrhalis + *K. oxytoca*	1 (5.3)
E. cloacae + *Acinetobacter* spp	1 (5.3)
S. aureus + *M. catarrhalis* + *E. cloacae*	1 (5.3)
S. aureus + *P. mirabilis* +Viridans Streptococci	1 (5.3)
S. aureus + *E. coli* + *Acinetobacter* spp	1 (5.3)
S. pyogenes +*E. cloacae* +*Acinetobacter* spp	1 (5.3)
S. pyogenes+*K. oxytoca*+*Acinetobacter* spp	1 (5.3)
E. cloacae+*S. marcescens*+*Acinetobacter* spp	1 (5.3)
E. cloacae + *K. pneumoniae*+*S. marcescens*	1 (5.3)
E. cloacae + *M. catarrhalis* + *C. freundi*	1 (5.3)

9 of the 45 cultures were negative (i.e. 20.0%)

Table 5. Pathogen distribution in the sputum samples of 170 patients with CAP.

Characteristics	Patients who provided sputum (N=170) n (%)	Patients with PAT and who provided sputum (N=119) n (%)
Gram positive bacteria	**32 (50.8)**	**19 (40.4)**
S. pneumoniae	9 (14.3)	5 (10.6)
S. pyogenes	9 (14.3)	5 (10.6)
Viridans Streptococci	6 (9.5)	4 (8.5)
S. aureus	8 (12.5)	5 (10.6)
Gram negative bacteria	**31 (49.2)**	**28 (59.6)**
Moraxella catarrhalis	3 (4.8)	3 (6.4)
Acinetobacter spp.	5 (7.9)	5 (10.6)
E. cloacae	7 (11.1)	6 (12.8)
E. coli	4 (6.3)	3 (6.4)
S. marcescens	4 (6.3)	4 (8.5)
K. pneumoniae	2 (3.2)	2 (4.3)
K. oxytoca	2 (3.2)	2 (4.3)
P. aeruginosa	1 (1.6)	0 (0)
P. mirabilis	1 (1.6)	1 (2.1)
C. freundi	1 (1.6)	1 (2.1)
Aeromonas spp.	1 (1.6)	1 (2.1)
Total	**63 (100.0)**	**47 (100.0)**

CAP: community-acquired pneumonia, PAT: Previous antibiotic treatment, Statistical significance was defined as $p<0.05$.

Correlation of Gram stain and sputum culture for *Streptococcus pneumoniae*

Overall, sputum cultures were positive in 36 of 45 (80.0%) good-quality samples. *Streptococcus pneumoniae* was cultured in only 9 samples. In the case of patients with previous antibiotic treatment, sputum cultures were positive in 26 of 31 (83.9%) good quality samples ($p < 0.05$) (Table 4). However, in these cultures, 33.3% of the PMs were Gram positive cocci ($p<0.05$), compared with 23.1% in the general samples (Table 3).

Therefore, sputum culture contributed to identify *Streptococcus pneumoniae* in 9 of the 205 (4.5%) patients. In correlating Gram-positive diplococci as PM in sputum Gram stain with the sputum culture recovery of *Streptococcus pneumoniae*, the presence of Gram-positive diplococci as PM in Gram stained samples was resulted in a sensitivity of 55.6% and specificity of 91.6% for the presence of *Streptococcus pneumoniae* in sputum culture (with a positive predictive value of 62.5%) (Tables 6 and 7).

Table 6. Results of Gram stain of sputa for Gram positive diplococci and isolation of *Streptococcus pneumoniae* from 45 sputum cultures.

GPD seen as PM in Gram stain	Pneumococcal	Culture No. (%)	Total
Total	**Positive 9 (20.0)**	**Negative 36 (80.0)**	**45 (100.0)**
Positive	5	3	**8**
Negative	4	33	**37**

GPD: Gram-positive diplococci; PM: Predominant morphotype.

Table 7. Sputum Gram stain.

Sputum Gram stain	Ratio	Percentage
Sensitivity	5/9	55.6%
Specificity	33/36	91.6%
Positive predictive value	5/8	62.5%
Negative predictive value	33/37	89.18%
Accuracy	38/45	84.44%

Discussion

The By observing the status of Polymorphonuclear neutrophils and the epithelial cells in a Gram-stained smear, 45 of 170 (25.3%) collected sputum specimens were determined to be suitable for culture.

The result of this study showed a limited value of sputum Gram stain as a diagnostic tool in the initial evaluation of patients with community-acquired pneumonia, as a predominant morphotype could be identified in only 13 of the 45 (28.9%) patients with good-quality sputum samples. Therefore, the identification of a predominant morphotype by the microbiologist could have guided initial antibiotic choice in only 13 of the 205 (6.3%) pediatric patients who were evaluated. It is also of consideration that in patients with atypical community-acquired pneumonia, mixed infections can occur. Thus, the identification of a predominant morphotype in a Gram-stained sample cannot rule out an atypical community-acquired pneumonia and selective antipneumococcal empirical treatment might not be appropriate. The presence of Gram-positive diplococci in Gram-stained samples was less sensitive (55.6%) but with a specificity of 91.7% for the presence of *S. pneumoniae* in sputum culture. The rates of good-quality samples and PM in the Gram-stained sample were similar in patients who had taken antibiotic medications before collection of the sputum sample and in the overall group of patients who could produce sputum. Roson et al. (2000) found out a

sensitivity of 34.0% and a specificity of 100% in a study correlating Gram positive diplococci in Gram stain with the culture recovery of *S. pneumoniae*, which is comparable to the results of this study.

Sputum Gram stain had a low diagnostic yield and did not contribute significantly to initial community-acquired pneumonia patient management. Authors like Ewig et al. (1996), Woodhead et al. (1991), and Lentino and Lucks (1987) have pointed out that sputum Gram staining has a low value as a diagnostic tool in pneumonia because of its lack of sensitivity and specificity. More recently, Ewig et al. (2002) have shown that in primary care hospitals sputum Gram staining has low diagnostic yield (9%) and does not contribute significantly to patient management. Besides, the yield of Gram staining has proven to be highly dependent on skilled Microbiologist applying strict criteria (Boerner and Zwadyk, 1982). Garcia-Vazquez et al. (2004) found out the low diagnostic yield (44%) of sputum Gram staining as a diagnostic tool in the initial evaluation of patients with CAP and recommended that the value of sputum samples is for epidemiological study as a tool in providing information about microbiological and antibiotic susceptibility trends in patients with community-acquired pneumonia, but not in the emergency department.

In this particular study, from the 45 sputum specimens which were determined to be suitable for culture, 36 of them (80.0%) found to be culture-positive. The most common pathogen was *S. pneumoniae*, which was isolated from 9 patients. The finding of 9 *S. pneumoniae* isolates represents 14.3% of the total isolated bacteria. This result is related to the 8% prevalence of *S.pneumoniae* in HIV-uninfected persons in Uganda (Yoshimine et al., 2001), 6% prevalence of *S.pneumoniae* in Addis Ababa (Aderaye, 1994) and 18% in Zimbabwe (Nascimento-Carvalho, 2001). Other studies have revealed comparable values, as was found in Turkey, where an isolation rate of 25.5% was found amongst 98 patients (Ozyilmaz et al., 2005). However, the detected *S. pneumoniae* from sputum of the pneumonia cases in this study is less frequent than in some published studies where other methods of detection were used. Bacterial findings in studies from Africa by using lung aspirates showed prevalence of 35% in Nigerian children and 51% in Gambian children for *S. pneumoniae* (Nascimento-Carvalho, 2001). Since most studies employed 2 or 3 different diagnostic methods in combination to find this bacterium, caution must therefore be taken when comparing prevalence data on *S. pneumoniae*. This is a low yield as is the case in most developing countries where culture facilities are either unavailable or grossly inadequate. Besides, 64.9% of the population included in the study had taken antibiotics prior to coming to hospital and this might have compounded the problem.

The zero prevalence (0.0%) of *H. influenzae* found in community-acquired pneumonia cases in this study is similar to published reports, such as 0.8% by Nagalingam et al. (2005), and 0% by Bochud et al. (2001) in Switzerland. Michelow et al. (2004) included 154 children in their study and used blood or pleural fluid cultures, pneumolysin-based polymerase chain reaction assays and serologic tests to clarify the epidemiology but *H. influenzae* was not found to be a causative pathogen of community-acquired pneumonia in their study. Since most patients who were included in this study did take antibiotic treatment before collection of sputum, due to this reason the isolation of *H. influenzae* from the specimens might become nil.

The prevalence of 12.5% was found for *S. aureus*. This prevalence of *S. aureus* is similar to the 12.0% found by Lahti et al. (2009). Bacteriologic findings of 9% prevalence of *S. aureus* in Nigeria and 10% prevalence of *S. aureus* in Zimbabwe (Nascimento-Carvalho, 2001) were found which are comparable to the findings of this study.

The prevalence of 4.8% for *Moraxella catarrhalis* in the present study is similar to the 6% prevalence of etiology of childhood community-acquired pneumonia in Europe using non-invasive diagnostic techniques (Nascimento-Carvalho, 2001). Ozyilmaz et al. (2005) found 12.2% prevalence of *Moraxella catarrhalis* in Turkey. Lahti et al. (2009) also found 28% prevalence by using induced sputum. *Moraxella catarrhalis* is considered as a less common agent of community-acquired pneumonia (Bartlett and Mundy, 1995).

Reported prevalence of community-acquired pneumonia in the general population due to Gram-negative enteric bacteria varies from 0% to 9% (Nagalingam et al., 2005). The prevalence of 11.1% for *Enterobacter cloacae* in the present study is comparable to the isolation of 17.8% prevalence reported by Buenviaje (1988).

The finding of 6.3% for *Klebsiella* spp. in this study is similar to the 8% prevalence which were found from a

study done in Uganda (Yoshimine et al, 2001) but higher than the 1% found by Ozyilmaz et al. (2005).

E. coli and *S. marcescens* accounted for 6.3% of the total isolated bacteria which are higher in studies done by Buenviaje (1988) who found 3.6% prevalence and Nagalingam et al. (2005) who found 1% prevalence of *E. coli* from sputum samples. The 1.6% prevalence of *P. aeruginosa* and *Proteus mirabilis* is comparable to the 3.6% prevalence of *Proteus* spp. found by Buenviaje (1988) and the 4% prevalence of *P. aeruginosa* found by Nagalingam et al. (2005). The 7.9% prevalence of *Acinetobacter* spp. is higher than when it is compared to the 3.6% prevalence found by Buenviaje (1988).

In the present study, *S. pneumoniae* was found to be the most frequent pathogen as the etiology of childhood pneumonia. Un-expected finding was that *S. pyogenes* was accounted for 14.3%. But, *S. pyogenes* is considered as a less common agent of community-acquired pneumonia (Bartlett and Mundy, 1995).

It is noteworthy that *E.cloacae* was the most frequently isolated Gram-negative enteric bacteria. There were more Gram-negative bacteria than Gram-positive bacteria isolated from specimens of patients with previous antibiotic treatment than those patients without previous antibiotic treatment which might indicate sampling prior to antibiotic treatment can influence the recovery of pathogens.

Conclusion

Forty-five cases (25.3%) with CAP were able to provide good quality specimens in the present study, which indicates that children cannot produce purulent sputum samples. Valid specimens from patients with CAP can be screened by Gram stain scoring of sputum, and a great deal of false positives could be avoided. But, the result of this study showed the limited value of Gram staining of sputum as a diagnostic tool in the initial evaluation of patients with CAP, as a PM could be identified in only 13 of the 45 (28.9%) patients with good-quality sputum samples. And the sensitivity of sputum Gram stain (55.6%) for diagnosis of pneumococcal pneumonia was also low.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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