RESEARCH PAPER

Naso-pharyngeal Carriage of Organisms in Children Aged 3-59 months Diagnosed with Severe Community-acquired Pneumonia

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Objective: To study the naso-pharyngeal carriage of organisms in children diagnosed with severe pneumonia.

Methods: Nasopharyngeal aspirate and swabs for microbiological analyses were collected from 377 children aged 3-59 months with severe pneumonia.

Results: 28.6% of the samples were positive for *S. pneumoniae*, 9.6% were positive for *H. influenzae*, and 8.5% were positive for both the organisms. Respiratory syncytial virus was detected in 27% of samples. The rate of isolation of *S. pneumonia* and *H. influenzae* was significantly more in the age group of 12-59 months.

Conclusions: In children with severe pneumonia, most common organisms isolated/detected from naso-pharyngeal aspirates were *S.pneumoniae* and Respiratory Syncytial Virus.

Keywords: *Etiology, Epidemiology, Respiratory Syncytial Virus, Streptococcus pneumoniae.*

Pneumonia is the single largest killer of children less than five years of age worldwide [1]; though, it is difficult to identify the etiological agents [2]. Nasopharyngeal aspirates can be used for identification of both bacterial and viral pathogens.

Nasopharyngeal colonization with *S.pneumoniae* usually precedes pneumonia. Though previous Indian studies have reported etiological agents causing pneumonia in children, only a single study reported the results based on culture of nasopharyngeal aspirates [3]. The present study reports the microbiological data from Chandigarh, which is part of IndiaCLEN multicenter study (ISPOT study) on oral amoxicillin in severe community acquired pneumonia (CAP) in children (Clinical trial Registry of India;CTRI/2010/000629).

METHODS

The present study was conducted over a period of 3 years (April 2008 to March 2011). Children aged 3 to 59 months fulfilling the criteria of WHO-defined severe CAP with the ability to take antibiotics orally, and without any radiological consolidation or effusion were included. Those with underlying chronic conditions, HIV, hospitalized for >48 hrs in the last 2 weeks, severe malnutrition, and antibiotic therapy for ≥48hrs prior to admission were excluded.

Nasopharyngeal swabs were collected and standard microbiological techniques were used to isolate *S. pneumoniae* and *H. influenzae*. Minimum inhibitory concentration (MIC) of amoxicillin and penicillin were measured by E test according to the manufacturer's instructions (AB Biodisk, Solna, Sweden). The nasopharyngeal aspirates were obtained soon after nasopharyngeal swabbing and tested for Respiratory Syncytial virus (RSV) antigen using ELISA method (Abbott Testpack for RSV from Abbott Diagnostics, Baar, Switzerland). Blood culture was not collected as stable, ambulatory patients were included.

Accompanying Editorial: Pages 115-16.

Nasopharyngeal swabs were cultured on 5% sheep blood agar with gentamicin for *S. pneumoniae* and modified chocolate agar with bacitracin (300 µg/ml) for *H. influenzae*. After overnight incubation at 36^0C, plates were observed for growth of *S. pneumoniae* and *H. influenzae*. All isolates were identified by standard microbiological methods [4]. For antimicrobial susceptibility testing, six antibiotic discs were tested – cotrimoxazole (2.5 µg trimethoprim and 23.5 µg sulfamethoxazole disc), ampicillin (10µg disc), erythromycin (15µg disc), tetracycline (30 µg disc), cefotaxime (30 µg disc), and ciprofloxacin (5 µg disc). Susceptibility testing was done on Muller hinton agar with 5% sheep blood for *S. pneumoniae*; that of *H.*

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influenzae was done by using the disk diffusion method on *Haemophilus* test medium. Strains were labeled as sensitive/resistant/intermediate susceptible based on Clinical and Laboratory Standards Institute (CLSI) guidelines [5]. American type culture collection (ATCC) reference strains of *S. pneumoniae* (ATCC 49619) and *H. influenzae* (ATCC 49247) were used as controls.

The collected data were analyzed and tabulated by using the statistical package, STATA software (version 12, college station, Texas, USA). Chi-square test was carried out to test the differences between proportions. *P*-value <0.05 was considered as significant.

RESULTS

A total of 430 children with severe CAP were screened, and 377 (237 males) were included. The exclusive breast-feeding rate was 66.6% till 6 months of age, and timely complementary feeding was started in 77.4% of children. Immunization status was up-to-date (as per history) in 92.8% children, and 35% children were having mild to moderate malnutrition. Auscultatory wheeze was present in 52% cases, and crackles in 76.6% cases. Infiltrates on chest *X*-ray was present in 63.9% cases.

Of the 377 nasopharyngeal swab (NPS) and aspirate (NPA) cultures, 27% were negative, 8.5% were positive for both *S.pneumoniae* and *H.influenzae*, 28.6% were positive for *S. pneumoniae* and 9.6% were positive for *H influenzae*. RSV antigen was detected in 27% cases (***Table* I**). Taking in account mixed infections, *S. pneumoniae* was present in 56.7% (214/377), *H influenzae* in 30.2% (114/377), and RSV in 53.3% (201/377).

The rate of isolation of the organisms according to different subgroups is presented in ***Table* II**. Regarding *S. pneumoniae*, the rates of isolation in 3 to <12months *vs.* 12 to 59 months were significantly different (38% *vs.* 70%, *P*=0.026), but the rates did not differ in other subgroups. The rates of isolation of *H. influenzae* in 3 to <12 months *vs.* 12 to 59 months (27.8% *vs.* 72.2%, *P*=0.039) were significantly different, but the rates did not differ in other subgroups.

TABLE I FREQUENCY OF ORGANISMS IN NASOPHARYNGEAL SECRETIONS IN CHILDREN WITH COMMUNITY-ACQUIRED SEVERE PNEUMONIA

Organisms	*Home (n=184)*	*Hospital (n=193)*
S. pneumoniae	52 (28.2)	56 (29)
H. influenzae	17 (9.2)	19 (9.8)
Both organisms	15 (8.1)	17 (8.8)
RSV	49 (26.6)	53 (27.5)
H. influenzae + RSV	14 (7.6)	11 (5.7)
S. pneumoniae + RSV	25 (13.6)	28 (14.5)
All three organisms	12 (6.5)	9 (4.7)

RSV – Respiratory syncytial virus; values in no. (%).

Of the 108 *S. pneumoniae* isolates, 20.4% were susceptible to co-trimoxazole, 31.5% to tetracycline, and 100% to ampicillin, erythromycin, cefotaxime, and ciprofloxacin. Of the 36 *H. influenzae* isolates, 27.8% were susceptible to co-trimoxazole, 38.9% to tetracycline, and 100% to ampicillin, erythromycin, cefotaxime, and ciprofloxacin.

DISCUSSION

In the present study, the most common organisms isolated/detected from nasopharyngeal swab (NPS) and aspirate (NPA) cultures were *S. pneumoniae* and RSV. All the isolates of *S. pneumoniae* and *H. influenzae* were

TABLE II ORGANISMS FROM NASOPHARYNGEAL SECRETIONS BY SUBGROUPS

Characteristics	*S. pneumoniae (+)*	*H. influenzae (+)*	*S. pneumoniae & H. influenzae (+)*	*RSV (+)*
Male	57 (52.7%)	23 (63.9%)	17 (53.1%)	54 (52.9%)
Female	51 (47.3%)	13 (36.1%)	15 (46.9%)	48 (47.1%)
3 mo – <12 mo	40 (37%)	10 (27.8%)	14 (43.8%)	56 (54.9%)
12 mo – 59 mo	68 (63%)	26 (72.2%)	18 (56.2%)	46 (45.1%)
Auscultatory wheeze	52/196 (26.5%)	17/196 (8.7%)	19/196 (9.7%)	64/196 (32.6%)
No auscultatory wheeze	56/181 (30.9%)	19/181(10.5%)	13/181 (7.2%)	38/181 (21%)
Crackles	85/289 (29.4%)	27/289 (9.3%)	24/289 (8.3%)	71/289 (24.6%)
No crackles	23/88 (26.1%)	09/88 (10.2%)	08/88 (9%)	31/88 (35.2%)
Infiltrates on chest *X*-ray	76/241 (31.5%)	23/241 (9.5%)	20/241 (8.3%)	68/241 (28.2%)
Normal chest *X*-ray	32/136 (23.5%)	13/136 (9.6%)	12/136 (8.8%)	34/136 (25%)

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WHAT THIS STUDY ADDS?

- In children aged 3-59 months with severe community-acquired pneumonia, most common organisms isolated from naso-pharyngeal specimens are *S. pneumoniae* and RSV.
- *S pneumoniae* and *H influenzae* show 100% susceptibility to ampicillin, erythromycin, cefotaxime, and ciprofloxacin.

susceptible to ampicillin, erythromycin, cefotaxime, and ciprofloxacin.

Limitations of present study include: only children presenting to the health facility were recruited, only nasopharyngeal aspirate was used to identify the organisms, a single center data was presented, and some other organisms were not studied for identification. As the carriage of an organism does not necessarily correlate with the etiology of the pneumonia, non-inclusion of simultaneous age-matched controls is also a limitation. The details of vaccines received by children were also not studied.

Present study results are consistent with some previous results from India. The rate of isolation in previous Indian studies for *S. pneumoniae* was 9% to 40%, for RSV was 13% to 25%, and for *H. influenzae* was 7.6% to 22.7% [2,3,6-12]. Present study also noted a higher carriage rate of the organisms in the 12-59 months age group compared to the infants (<12 months), and there was no sex predilection. It has been debated that nasopharyngeal colonization may or may not translate into disease itself. Though this is true, studies have shown nasopharyngeal colonization to be a risk factor for development of pneumonia. In a study from China, compared to controls, the isolation of *S. pneumoniae* was more in children with radiologically-confirmed pneumonias [13].

Previous Indian studies [7,14,15] have shown the susceptibility pattern of both the organisms as follows: *S. pneumoniae* being sensitive to penicillin/ampicillin (65%-98.7%), co-trimoxazole (9.1%-81.8%), chloramphenicol (83.4%-94.6%), tetracycline (39.2%), erythromycin (87.5%-89.1%), and ceftriaxone (85%-100%). *H.influenza* being sensitive to penicillin/ ampicillin (59%-81.1%), co-trimoxazole (32.7%-48%), chloramphenicol (45%-81.5%), tetracycline (75%), erythromycin (72.4%), and ceftriaxone (100%). Present study found 100% susceptibility rate of both the organisms to ampicillin, erythromycin, cefotaxime, and ciprofloxacin.

Future research should focus on comparing the organisms isolated from healthy children to those having pneumonia. Simultaneous blood culture to know the exact etiology/pathogenecity of the organism isolated, newer methods of isolation of organisms, serotyping of the bacterial isolates, and testing of additional bacterial isolates should be done to facilitate future policy decisions regarding management of pneumonia in under-five children.

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