Discussion
Milestones in understanding the complex infectious processes leading to morbidity and mortality in acute respiratory infections in children are reached slowly. The process starts insidiously, most often in the child’s natural surroundings. What makes it difficult to understand is the multiplicity of pathogens, overlapping clinical syndromes and various risk factors. In this study, an attempt was made to look at some bacterial agents associated with ARI.

GROUP A BETA-HEMOLYTIC STREPTOCOCCAL PHARYNGITIS IN SCHOOL CHILDREN

In the first phase of the study involving school children from urban and rural Pondicherry, ambulatory cases with pharyngitis were screened for the presence of Group A betahemolytic streptococci. This was done with specific objective of finding out the extent of GABHS association with pharyngitis in these children. Being ambulatory, they come into close contact with other healthy children in the close confines of a class room, therefore forming potential source of infection to the others. Although outbreaks of pharyngitis due to non-GABHS have been reported, only Group A Streptococcus (GAS) has been associated with serious sequelae such as rheumatic fever and glomerulonephritis\textsuperscript{259}. Hence the importance of establishing this infection in children cannot be overemphasized.
In the school survey conducted among urban and rural children (between 5-15 years) an overall incidence of 9.4% infection by GABHS was detected in 617 children with pharyngitis. This was within the range of 5 to 20% in children reported by other studies in India\textsuperscript{67,68}. Rural children with an incidence of 10.8% were found to be slightly higher than the urban group. In a similar study in South India by Koshi et al\textsuperscript{260}, a higher incidence of 22.2% was reported among rural children, between 5-15 years of age. Significantly higher incidence of 13% among children between 5-9 years age group was noted in our study, compared to 6.5% in the older age group. Streptococcal infection is known to replace viral URI around 5 years of age\textsuperscript{251}. Higgins has reported the highest incidence of Streptococcal pharyngitis in children between 5-9 years in a long term observation of Streptococcal pharyngitis over a period of 3 years\textsuperscript{261}. Children start school at the age of 5 years and are therefore at an increased risk of acquiring GABHS and manifesting the disease. Older children by virtue of increased exposure are expected to develop certain amount of immunity hence less infection. Increasing carriage rates in lower school grade has also been reported by Nicolle et al\textsuperscript{262} from Manitoba.

Female children with pharyngitis were found to be significantly lower in number than their male counterparts in both the urban and rural schools. Higgins has reported a higher incidence of infection among female children in contrast to our study. Gupta et al\textsuperscript{263} have also reported a similar finding from Delhi. Filter paper strip method for collection of throat swabs was found convenient and gave
comparable results with that reported by Koshi et al\textsuperscript{260}. Field studies involving collection of large number of samples can be undertaken using filter paper strips for transportation, as the organisms remain viable for variable lengths of time.

Antibiotic susceptibility pattern of the strains isolated from urban and rural school children gave an uniform pattern. 4.3\% strains showing resistance to erythromycin was found among isolates from urban children. An incidence of 5.1\% has been reported by Prakash et al\textsuperscript{199}. Resistance of upto 20\% to 44\% have been reported in some parts of the world\textsuperscript{195,198}. Reports from Japan have shown increased resistance to erythromycin\textsuperscript{248}. Increasing tolerance to penicillin is being reported from cases with treatment failures following penicillin therapy\textsuperscript{194,195}, although no in vitro resistance to penicillin has so far been reported from clinical isolates\textsuperscript{196}. This makes it necessary to study the susceptibility pattern of clinical isolates to other antibiotics. Thirty four percent of the strains isolated in our study were resistant to cotrimoxazole. Ranges of 8\% to 76\% have been reported in literature\textsuperscript{200}. Cotrimoxazole is a widely used antibiotic and is available without a prescription, over the counter. This widespread use may have resulted in a high percentage of strains becoming resistant to the antibiotic, both in urban and rural Pondicherry. Tetracycline resistance of 10\% is found to be slightly lower than approximately 20\% of strains showing resistance by Jacoby\textsuperscript{193} and Weildmann et al\textsuperscript{200}. Clindamycin which is not in common use was sensitive in 100\% of the isolates. With increasing use of cephalosporins, a greater eradication of the infection is expected, however
pressure emergence of resistant strains of other flora have to be borne in mind. Newer macrolide Azithromycin, which has shown promising results in the west has not yet come into general use in India. Hence, susceptibility to cephalosporins and newer macrolides were not undertaken.

Rapid diagnostic methods are being increasingly used either to replace older time consuming and labour intensive standard isolation methods, or more often, to give a quick result to the clinician for a meaningful management. This study was done to determine the feasibility of antigen detection for rapid diagnosis of Streptococcal pharyngitis in rural children. Swabs were collected in the school, transported to the laboratory and stored at 4°C. Test could be done after 24 hours, but the results were available immediately, on that day. Thus a report was available 24 hours earlier than the conventional culture. Visuwell Kit for direct antigen detection gave a sensitivity of 92.5% and specificity of 89.4% (n = 150, in a prevalence of 18%). These were comparable to the results obtained by various other groups\textsuperscript{130-132}. Using Visuwell Kit, Drulak\textsuperscript{131} has compared their results with culture and has reported sensitivity of 88% and specificity of 92.4%. Positive predictive value of 65% was comparable to their value of 70%, and negative predictive value of 98% obtained by us, with 97.4% by the authors. Cross reaction with organisms other than Group A Streptococci in the oropharynx was negligible by this kit. Major limiting factor of this method was the prohibitory cost of each kit (Rs.7000/= for a Kit of 48 tests). A short shelf life and availability were other factors
which limited the use of this Kit as a rapid diagnostic tool in the field. Indigenous preparation of antigen detection methods with strict quality control could obviate this difficulty in the developing countries.

Detection of antibodies to Streptolysin O is important to diagnose non-suppurative sequelae. We undertook the study to determine ASLO levels in children with Streptococcal pharyngitis to determine the extent of infection, reflected by high antibody titres. Thirty nine percent of children with culture proven Streptococcal pharyngitis had high ASLO levels \( \geq 300 \text{ IU} \). Latex agglutination test for determining the ASO was used. Contrary to the expectation that older children have higher antibody levels following repeated exposure, our study revealed 20% of children between 5-9 years of age with high antibody levels as compared to 11% in the older children corroborating the culture results showing higher infection in younger children. Sensitive antibody detection methods such as anti-DNase B and antistreptozyme test may have been able to detect antibodies in a greater proportion of infected children. Sixty one percent of infected children who did not have detectable antibody levels, may have been in the acute phase of the infection or more likely to have been carriers.
BACTERIAL COLONIZATION OF UPPER RESPIRATORY TRACT OF CHILDREN WITH ACUTE RESPIRATORY INFECTION ATTENDING PRIMARY HEALTH CENTRES

Following the study on school children with Streptococcal pharyngitis, we undertook the second and third phase of our work to get an insight into the bacteriological association with acute respiratory infection in children at the primary health centre and those with acute lower respiratory infection admitted to the referral hospital.

There have been no studies on ARI in children attending PHC in India. As majority of the children population in rural areas have access only to PHC, we decided to study the group. With limited or absence of any microbiological diagnostic facilities in this set up, management of ARI in children at the primary health care delivery system depends only on the medical officers clinical diagnosis. This makes etiological diagnosis of ARI in the outpatient, one of the most formidable tasks faced by a clinician. In the absence of culture facilities and other diagnostic aids, most doctors in the primary health centres have to rely on their clinical acumen, with little else to go by. Their task becomes even more difficult in choosing an appropriate antibiotic, while not having any inkling to the susceptibility pattern of the offending pathogen. In institutions with limited choice of antibiotics this situation is further worsened. With these factors in mind the present study was undertaken to
find out the extent of colonization of common bacterial respiratory pathogens and determine their antibiotic susceptibility pattern.

Children are first brought to the primary health centres during any ill-health. Hence, selected PHCs were chosen in the union territory, to cover most of the areas in rural as well as urban Pondicherry. Government General Hospital, although not a primary care centre, often gets patients from the town as a first preference, in the choice of seeking medical care. Samples were therefore collected from the paediatric OPD of this hospital.

Total of 488 nasopharyngeal and throat swabs from urban and 571 samples from rural settings were collected from children with ALRI. Peak age at which most of ALRI cases were observed between 1-4 years (13-48 months) age. This is similar to the pattern observed in Haryana. However, most other studies have reported a higher incidence in infancy, including a previous study from Pondicherry. Shabong et al made this observation in children hospitalised in JIPMER, which is a tertiary care referral centre, therefore catering to severely ill children, that includes a large number of infants. Very few studies have been made, among children attending primary health centres in rural and urban India. Most studies in urban India have shown higher incidence in the lower age group. In our study, incidence decreased in children ≥ 5 years of age. Very much lower peak ages have been reported from other developing countries, 6 to 11 months being reported from
Phillippines, Papua New Guinea, Argentina and Kenya. Our figures are based on clinical observations and colonization only, and not supported by X-ray or blood cultures.

In our study a male preponderance was noticed in all age groups excepting the 5-9 years group, where female children outnumbered the males in both urban and rural settings with rates of 27.1% and 34% in the urban and rural female children, as compared to 21% and 27% respectively in the corresponding male children. Increased incidence in females in this group alone cannot be explained. An overall incidence of 56% of males in urban and 51.8% in rural areas is comparable to 55-60% of cases in other South East Asian countries. Average male to female ratio of 1.7 to 1 in India has been reported by Nawur. Our study showed a male : female ratio of 1.2 : 1 in the urban and 1.09 : 1 among the rural children. Rural children did not have a marked gender predisposition as compared to urban children, a similar observation has been reported by Walia et al from Chandigarh.
Bacterial colonization of upper respiratory tract in children attending primary health care centres including State Government Hospital OPD

Throat swab isolations of GABHS from urban and rural children showed a distinct pattern of colonization starting after one year of age (only one child was found to be colonized with GABHS at seven months of age). Sixty one percent of all isolates were found in children above 5 years of age. Our findings are in agreement with the observations by others, that, incidence of GABHS infections of the throat is lower in children less than 5 years of age. Amir et al\textsuperscript{264} have noted a rise in GABHS infection rate in children $\geq$ 5 years of age. They did not find infection by this organism in children less than 2 years.

*S. pneumoniae* carriage rate of 9.2\% in the upper respiratory tract of children with ARI was found to be similar to that reported by Aino K Takala\textsuperscript{265} who found 8.7\% carriage rate in children 3 years of age in Finland. In contrast, in a study from another part of South India, a very low rate of $< 1\%$ colonization of the upper respiratory tract by *S. pneumoniae* was observed by Sivadasan\textsuperscript{266}.

Colonization was seen in children as young as two months of age in our study. A similar finding was observed by Montgomery\textsuperscript{267}, who reported colonization of *S. pneumoniae* by 3 months of age. Colonization rate declined by 5 years of age. There was a peak of almost 16\% between 3-4 years of age which was found to be
statistically significant ($p > 0.001$). This has not been reported by any other group. More studies involving various risk factors have to be conducted before any predisposition of this age group alone can be verified.

*H. influenzae* colonization was observed from the age of 2 months onwards, with 18.9% of all isolation occurring below 12 months of age. Children below 5 years of age had a 5.5% carriage rate. This was lower than the observation made by Mastro et al. from Pakistan, who reported 37.4% carriage in children below 5 years of age, who were bacteraemic. As we did not undertake blood culture in this group, the bacteraemic status of the children cannot be commented upon. Sivadasan reported a rate of 11%. As carriage rate is known to increase with invasive infection, our rates may have been low, in the absence of invasive infection by *H. influenzae*. This could reflect the normal carriage rate of 5.5% in children below 5 years and a rate of 7.4% in children below 15 years of age.

51.8% of all *H. influenzae* strains isolated belonged to type b. This was found to be higher than reported by Gratten in Australian aboriginal children hospitalized with ALRI and also from a study in Papua New Guinea, with a rate of 16% and 8.3% respectively. Sivadasan in his study in South India did not find *H. influenzae* type b colonization in the throat swabs of hospitalized as well as outpatient children. A high rate of colonization with type b strains by our children could predict a greater risk of developing invasive *Haemophilus influenzae* infection. Nontypeable strains of
*H. influenzae* were found to be 32.9% of all the isolates. High levels of nonserotypeable strains of *H. influenzae* have been reported from other developing countries. Seventy four percent of all isolates were found nontypeable by Gratten et al in Papua New Guinea. Children in the west are less colonized with nontypeable strains, 40% of children in the west carried Hib in their upper respiratory tract within the first 5 years of their life as shown in a longitudinal study.

Colonization of nontypeable *H. influenzae* was seen in infants after 6 months of age. 88.4% of isolates were found between 7 months to 48 months of age. Only 11% of children above 5 years of age were colonized with nontypeable strains. A similar finding has been reported in Papua New Guinea.

Noninvasive *H. influenzae* infection is known to occur by nontypeable strains. It is hypothesized that host's defence mechanisms act in a different manner to that seen by capsulated strains. Localized damage to epithelial cells by viral infections have been shown to act synergistically. Other factors contributing to alteration in mucus production occur in response to smoke of open fires in primitive cooking (a practise widely followed in rural India and urban slums), and together with bacterial toxins (LPS of *H. influenzae*) results in a conducive environment for contiguous spread of the organism. Bacteraemic spread by nontypeable strain therefore is not the primary mode of spread. Otitis media, sinusitis, and pneumonia are known to occur due to nontypeable *H. influenzae*. High carriage rate of nontypeable strains
among all isolates in infants and young children in this study and others from developing countries is a point to be noted in designing vaccines for use in the developing world.

Type a was the third common serotype isolated in our study (13% of all isolates) which was equally distributed in children from 6 months of age to 5 years. Gratten et al\textsuperscript{269} have reported similar results from highland children in Papua New Guinea.

\textit{Moraxella catarrhalis} which was once considered a nonpathogenic commensal of the respiratory tract has gained notoreity as a potential pathogen in the past decade. This part of our study was done to determine the colonization of \textit{M.catarrhalis} and detect resistant strains among them. Concurrent presence of this organism in the upper respiratory tract along with other potential pathogen is seen in greater proportions of children than adults. Nine percent of the children examined were found to harbour \textit{M.catarrhalis}. Colonization was observed by one month of age. 3.1\% of all isolates were seen in infants less than 6 months of age. In a study of children less than 5 years of age Wood et al\textsuperscript{91} have found 36\% colonization. Only 9\% of the isolates were considered significant or probably significant. In our study, in the absence of other supportive investigations (ear swab, sinus fluid and blood cultures) it is difficult to opine on the importance of these isolations. Sputum is considered a valuable specimen to indicate infection. Nasal and nasopharyngeal
swabs are not useful indicators of pathogenic significance of *M. catarrhalis*, as colonization rates of 54% to 78% have been reported with regards to children, significantly lower rates of 2% to 3% have been reported from adults.

Compared to the isolates of GABHS from school children, a higher rate of resistant strains were isolated from children attending health care centres with regards to erythromycin. 4.3% among school children as compared to 12.5% in children seeking medical care. Highest MIC level detected in the resistant strains was \( \geq 8 \mu g/ml \). MIC level of resistant strains however was much lower than the levels reported by Prakash et al. who have shown MIC levels of \( \geq 100 \mu g/ml \) in some of their strains isolated from cases of pharyngitis. Resistance to cotrimoxazole and tetracycline was found in 37.5% each. Again tetracycline resistance was seen to be slightly lower in the school children than in children with ARI.

For many years, pneumococcus was uniformly susceptible to penicillin G with MICs of \( \leq 0.1 \mu g/ml \). Penicillin was and still is the drug of choice in primary pneumococcal pneumonia. But over the last twenty years number of isolates relatively resistant to penicillin have been reported. Our study showed 16.3% of isolates from the nasopharynx to be resistant to penicillin with MIC levels of \( \geq 1 \mu g/ml \). Highest MIC levels recorded was \( \geq 8 \mu g/ml \). High levels of penicillin resistance have been reported from Spain, Chile, South Africa and Alaska. In a study in Durban 12% of nasopharyngeal colonizers were found to be resistant.
Lower rates of resistance in *S. pneumoniae* strains have been reported in several studies from US and UK\textsuperscript{200,204}. As we could not undertake serotyping of our strains, type specific resistance patterns could not be observed. A stepwise acquisition of penicillin resistance through alteration in the penicillin binding protein gene is responsible for the resistance. Youngest age at which penicillin resistant strain of *S. pneumoniae* was isolated in our study was at 3 months of age. These findings have important therapeutic implications for selection of antimicrobials to be initially used in empirical treatment of critically ill patients.

MIC cannot be done as a routine laboratory procedure for screening all isolates of *S. pneumoniae* from clinical specimens, therefore oxacillin (1 μg) disc diffusion has been recommended\textsuperscript{253}. By this method, we observed resistance in 18.3% of our isolates. However, MIC values of ≥ 1 μg/ml of penicillin were seen in 16.3% of isolates. Oxacillin resistant strains susceptible to penicillin have been documented\textsuperscript{249}. It is therefore recommended to determine MIC levels of penicillin in all isolates with reduced susceptibility to oxacillin. We did not find any difference in the antibiotic susceptibility pattern among strains isolated from rural and urban children. Pondicherry has a well distributed health care facility, with easy access by the rural population. Prescription patterns and antibiotic usage do not vary to a great degree among the rural and urban population. This may have reflected on the uniform pattern of susceptibility in rural and urban isolates. A lower rate of resistance was observed in rural isolates in a study conducted in Pakistan\textsuperscript{268}.
Erythromycin and chloramphenicol resistance rates did not vary between urban and rural isolates. MIC value of both these antibiotics were found to be $\geq 8 \mu g$ and $\geq 4 \mu g/ml$ respectively. Our values were lower than those reported from Korea by Hoan-Jong Lee et al\textsuperscript{272} who had an MIC range of $\leq 0.08 - > 128 \mu g/ml$, where as range of $\leq 0.03 - > 8 \mu g/ml$ were observed in our study. Highest MIC observed (in the Korean study) to chloramphenicol was 16 $\mu g/ml$ as compared to $\geq 4 \mu g/ml$ in our isolates. Ten percent of penicillin resistant strains were found to be concomittantly resistant to erythromycin and chloramphenicol (6%).

A high (59.8%) incidence of resistance to cotrimoxazole was observed among our isolates. MIC range of $\leq 4$ to $\geq 16 \mu g/ml$ was observed in trimethoprim and $\leq 8 - \geq 128 \mu g/ml$ of sulphamethoxazole. 39.7% of strains isolated from Hungary have been reported to be resistant to cotrimoxazole. A lower incidence of 6.1% resistance has been reported from Switzerland\textsuperscript{273}.

A high incidence of resistance to cotrimoxazole in our study could reflect the widespread use of this drug for all types of infections including those of upper respiratory tract, urinary tract, enteric fever and gastrointestinal infections. Cotrimoxazole, due to its ease of administration and good patient compliance has also been chosen as the first line of treatment in the National ARI Control Programme and recommended by WHO in its ARI Control Programme in developing countries\textsuperscript{2}. An increasing incidence of resistance to this drug by a common
respiratory pathogen calls for redesigning the treatment protocol by programmers for
the control of ARI.

Sensitivity testing of *S.pneumoniae* was standardised in this study and we
found reproducible results using Muller-Hinton lysed sheep blood agar as a
screening test using oxacillin (1 μg) discs in conjunction with other standard discs.
Muller Hinton ox blood agar was used for testing cotrimoxazole.

Forty nine percent of *Haemophilus influenzae* strains were resistant to
penicillin and 35% to ampicillin. Our rates are higher than 15-20% reported in other
studies. Respiratory isolates have been shown to demonstrate a higher
incidence of resistance to ampicillin. Betalactamase production was seen in
90.5% of the resistant strains. 9.5% of the strains which were resistant to ampicillin
did not produce betalactamase. This may have been due to altered penicillin
binding proteins. Such a mechanism has been described by Clarioux et al. It
forms the basis of non-betalactamase mediated resistance to betalactam antibiotics
by *H.influenzae*. 2.7% of our isolates had high MIC levels of 16 μg/ml. Sero type b
strains of *H.influenzae* were shown to have higher incidence of resistance to all the
antibiotics including ampicillin (39%). High level of erythromycin resistance to an
extent of 59% (with an MIC of ≥ 8 μg/ml) was seen among our strains. Erythromycin
and clarithromycin are only marginally effective against *H.influenzae*, therefore a
high resistance among our strains would not interfere with patient management.
Chloramphenicol resistance was seen in 27% of *H. influenzae* isolates. This is very often chosen for patient management, therefore increases rates of treatment failure in clinical practice. MIC values of $\geq 4 \ \mu g/ml$ were seen in these strains. Increased resistance to this antibiotic has been reported from Spain and other European countries\textsuperscript{218}.

Resistance to cotrimoxazole was found to be high among our isolates. Fifty percent of all isolates were resistant by the disc diffusion method and had MIC range of $\leq 0.5 \ \mu g/ml - \geq 32 \ \mu g/ml$ for trimethoprim and for sulphamethoxazole a range of $\leq 8 \ \mu g/ml - \geq 128 \ \mu g/ml$. Incidence of resistance of 7.3% have been reported from Europe, and a low level of 0.7% from US\textsuperscript{193}, against cotrimoxazole.

Resistance was seen to be higher in *H.influenzae* serotype b isolates, than others. Similar findings have been reported by Mortensen et al\textsuperscript{214}. The youngest age at which erythromycin resistant strain of Hib was isolated was 2 months. Appearance of ampicillin resistant *H.influenzae* occurred at 7 months of age. Nontypeable *H.influenzae* strains which were seen to colonize children between 3 months to 8 years of age, were found resistant to ampicillin, chloramphenicol and cotrimoxazole to a lesser degree than Hib strains. Betalactamase production was also seen in lesser number of nontypeable strains than type b strains. We did not find any difference between antibiotic resistance pattern among rural and urban
isolates of *H.influenzae*. No statistical significance was noticed among age distribution and frequency of colonization by drug resistant strains.

Apparent increase in the pathogenicity of *M.catarrhalis* has appeared to coincide with the emergence of increasing number of beta-lactamase producing strains. Prior to 1980 less than 10% of strains were beta-lactamase positive. Recent reports suggest a 90% positive rate\textsuperscript{91}. Beta-lactamase production among our isolates was found to be 48.9%, lower than that reported by Woods et al\textsuperscript{91}. Ampicillin resistance with an MIC range of more than 4 $\mu$g/ml - $\geq$ 32 $\mu$g/ml was seen in 34.3% of strains.

Around 80% of isolates resistant to ampicillin have been reported by Jorgensen et al\textsuperscript{215}. Resistance rates of 13.5% to erythromycin with MIC of $\geq$ 32 $\mu$g/ml were observed in our study. 41.6% of our strains were also resistant to cotrimoxazole (MIC 16/256 $\mu$g/ml). These findings are in contrast to those found in the West\textsuperscript{199}. Only rare isolates resistant to erythromycin (3%), chloramphenicol and cotrimoxazole (3%) have been reported by these authors. Almost all strains were reported to be sensitive to these antibiotics by Jorgensen et al\textsuperscript{215}. Resistant strains were found to colonize the nasopharynx of infants less than 6 months of age in our study. A high rate of colonization by resistant strains producing beta-lactamase could act as a potential donor of resistant genes to other pathogenic/commensal flora of the throat. It could also interfere with the action of beta-lactam antibiotics on pathogenic organisms of the throat. Its role in penicillin tolerance or treatment failures of Streptococcal pharyngitis to penicillin therapy needs to be studied. A high resistance among our strains could reflect the indiscriminate use of antibiotics among our population, giving rise to colonization by resistant resident flora of the upper respiratory tract.
Hospitalization of children with acute respiratory infection is often required for children less than 6 months of age, when there is significant respiratory distress, inability to hydrate or when the child does not respond to previous outpatient therapy. One of the major reasons for admission in developing countries is the inability of the family to provide therapy and supportive care. Very often children are brought to the hospital in a moribund stage, beyond the point of no return resulting in high death rate, inspite of adequate management. Very ill children form a major part of the admission to tertiary care centres, as JIPMER is.

Final phase of our study was to evaluate certain laboratory parameters which would help in confirming the diagnosis of acute respiratory tract infection of bacterial etiology, in children admitted to this hospital.
Among 329 cases admitted bronchopneumonia was diagnosed in 56.8% of cases and pneumonia in 19.1%. 5.6% had upper and 9.1% lower lobe involvement. Empyema was seen in 1.8% of cases. A male predominance was seen, which was more marked in children less than one year of age. A higher incidence in males is attributed to social character by most workers. There is tendency of seeking medical aid for the male child by most parents in the developing world. Earlier study in Pondicherry had reported a male : female ratio of 1:2 in the 0-5 years age group\textsuperscript{20,22}. Indian ratio is 1.7:1 as reported by Nawun\textsuperscript{21}. In the present study, a ratio of 1.2:1 was noted in children below 5 years of age.

Youngest age at which colonization of the upper respiratory tract with \textit{S.pneumoniae} was seen was one month, and \textit{H.influenzae} at 3 months. 18.5% of hospitalized children with ALRI were found to be colonized with \textit{S.pneumoniae}, a figure much lower than that of 79.5% seen in Australian aboriginal children, hospitalized with ALRI. Incidence of \textit{H.influenzae} colonization was even lower at a rate of 6% as compared to a very high rate of colonization (88.3%) in the Australian study. Ten percent of the children in our study had colonization with both the organisms. Seventy percent of \textit{H.influenzae} strains were type b as compared to 15% nontypeable strains. This was similar to that reported by Korppi from Finland (14%) nontypeable\textsuperscript{274}. None of the isolates in the Australian study\textsuperscript{269} were reported to be nontypeable. There are scanty reports from developing countries with regards to nasopharyngeal colonization by \textit{S.pneumoniae} and \textit{H.influenzae} among
hospitalized children. Hence, comparison of our data with those in the developing world could not be done to reflect the pattern in similar geographical area and socioeconomic conditions.

Antibiotic resistance among these strains showed 21.3% of *S.pneumoniae* isolates to be resistant to penicillin. Range of MIC of penicillin among these strains was $< 0.5 \ \text{µg/ml} - \geq 8 \ \text{µg/ml}$. There was no difference between the MIC range of resistant strains isolated from cases and controls. Our study showed an overall increasing trend of the MIC values obtained for sensitive strains among the hospital isolates as compared to those from the community. 44.4% of the strains had penicillin MIC values of $\geq 1 \ \text{µg/ml}$.

*H.influenzae* resistant to ampicillin were seen in 20% of the isolates with 71% of these strains producing beta-lactamase. MIC value of $\geq 32 \ \text{µg/ml}$ of ampicillin was the highest level recorded among the isolates. Importance of these findings among hospitalized children lies in the fact that highly resistant strains have the potential to cause secondary infection in an already compromised host in the hospital set up. Cephalosporins, which form an important part of therapeutic regimen in the present day are also being reported to be ineffective against *H.influenzae*217. Strains of *H.influenzae* which are non-beta-lactamase producers, but are ampicillin resistant, do so by altering the penicillin binding protein. Such strains are found to be less susceptible to cefotaxime, cefuroxime and cefaclor and amoxycillin/clavulanate217.
Three of our isolates were found to have an increased MIC of 4 μg/ml against cefotaxime. All three isolates were serotype b. Thirty four percent of respiratory isolates from hospitalized patients have been recorded to produce beta-lactamase\textsuperscript{214}. Thirty percent of strains isolated in this study were non-beta-lactamase producers, resistant to ampicillin. Hence, there is a probability of these strains acquiring resistance to other cephalosporins, and amoxycillin/clavulanate.

Blood cultures are positive in 3-10% of ambulatory patients with pneumonia\textsuperscript{115} and increase to 36-50% in severe infections\textsuperscript{44}. 8.5% of hospitalized children with ALRI in our study were bacteraemic. The number of blood culture positive for bacterial pathogens is considerably lower than that reported in international literature and studies carried out under the BOSTID project in certain developing countries. It was, however, lower than 11.5% blood cultures reported by Hortal et al\textsuperscript{125} from Uruguay and 12.9% isolated by John et al from Vellore (South India)\textsuperscript{62}, and higher than 4% isolation by Wissenbacher\textsuperscript{114} from Argentina. Bacterial agents isolated from blood cultures in our study varies from other studies in some of the developing countries. Organisms belonging to the family Enterobacteriaceae were found to be 20% of all the isolates followed by Staphylococcus aureus (12.5%). 16.4% of our isolates were \textit{H.influenzae}. This was similar to the isolation rate reported from Pakistan by Ghafoor et al\textsuperscript{59}. A lower rate of 7.1% was reported by John\textsuperscript{62}. Eighty percent of the blood isolates in our study were type b and 20% were non-typeable. Study from Pakistan reported isolation of 36% nontypeable strains.
However, the sample size in the Pakistan study was 1,492 cases as compared to 329 in our study.

A few studies and substantial circumstantial evidence involving increased nasopharyngeal isolation and antigen detection from serum and urine of children with ALRI implicate *H.influenzae* as one of the most important pathogens contributing to morbidity and mortality associated with ALRI. With the limited data available, *H.influenzae* other than type b, mainly non-typeable strains account for a substantial number of ARI cases, perhaps the majority of cases in young infants in developing countries\textsuperscript{102}. Our study however revealed majority of *H.influenzae* pneumonia to be due to serotype b. But with limited number of cases studied with very low isolation, it is difficult to comment on the serotype distribution among *H.influenzae* pneumonia cases. Case fatality rates again based on limited data suggest that the rate is as high as 20\%\textsuperscript{20,44}, with highest rates in hospitalized children. Consistently higher case fatality rates have been associated with bacteraemic patients and those with empyema\textsuperscript{102}. None of our patients had a fatal outcome. Emerging resistant strains with beta-lactamase production as well as altered penicillin binding proteins pose a problem in treating pneumonia with beta-lactam antibiotics as well as non-beta-lactam antibiotics. Problem of resistant strains in the developing countries is further compounded by the indiscriminate use of antibiotics especially in upper respiratory infections leading to treatment failures in ALRI among children. Only one of our strains isolated from blood was found to be
resistant to ampicillin. With increasing isolation rates, this problem may be encountered more frequently.

Bacterial evidence of pneumonia can be conclusively proved in only 10% of cases\textsuperscript{249}. Lung aspirate studies before the administration of antibiotics in developing countries showed \textit{S.pneumoniae} to be the most frequently isolated bacteria. This organism is the most common cause of bacterial pneumonia in all age groups. Specific serotypes, relatively few in number, are responsible for most of the invasive infections. Twenty five percent of pneumococcal pneumonia can be diagnosed by blood culture as compared to 3-10% caused by other agents in ambulatory patients.

Isolation of \textit{S.pneumoniae} (10% of all blood culture isolates) in our study was found to be similar to than that reported by Ghafoor\textsuperscript{59} (10%) and John\textsuperscript{62}. All the blood isolates of \textit{S.pneumoniae} were sensitive to penicillin and other antibiotics including cotrimoxazole.

Changing patterns of susceptibility to currently available antibiotics by multi-drug resistant \textit{Streptococcus pneumoniae} requires frequent reconsideration of the drug for initial therapy. This is further compounded by limited and specific microbiologic diagnosis of pneumonia, hence antimicrobial susceptibilities are usually not available to the clinician. With increasing isolation rates of \textit{S.pneumoniae} resistant to penicillin as well as to alternative drugs such as
erythromycin, chloramphenicol, cotrimoxazole and cephalosporins, a dilemma is often faced by the clinician in choosing the antibiotic. This problem will be manifold in the developing countries in choosing alternatives. Non-availability and high cost of these drugs will keep it out of reach for the masses. With the isolation of *S.pneumoniae* from blood culture in our study, we failed to detect any resistant strains. However, colonization of multidrug resistant *S.pneumoniae* in the hospitalized children as well as in the primary health care, it will not be long before we encounter such strains in invasive pneumococcal disease.

Current levels of penicillin resistance are not associated with increased mortality in patients treated with penicillin or ampicillin, but there are reports of failure of erythromycin and tetracycline in the management of pneumococcal pneumonia caused by resistant strains\(^{204}\). There is a grave concern about the high levels of cotrimoxazole resistance among Pneumococci and *H.influenzae* strains as this agent is recommended by World Health Organization as the first drug of choice for the management of acute respiratory infections in children, in the developing world.

One isolate of *H.influenzae* from the blood culture in our study was a beta-lactamase producer showing resistance to ampicillin. This belonged to serotype b. A single non-typeable strain was found to be sensitive to all the antibiotics tested.
Serotype distribution of pneumococcal strains could not be undertaken in this study, hence knowledge regarding the prevalent serotype of *S. pneumoniae* in invasive infection is lacking.

*Staphylococcus aureus*, which was isolated from 12.5% of our cases has also been reported from various other studies\(^ {59,62}\). *Staphylococcal pneumonia* was recorded, in infants less than 5 month of age among our patients. Empyema was associated in 2% of these patients. Our results were similar to that obtained by John et al\(^ {62}\) from Vellore.

Earliest age at which Gram negative organisms were isolated from children was less than 5 months of age. These organisms were *Escherichia coli*, Klebsiella pneumoniae, *Citrobacter diversus* and Enterobacter species. One of our patients had *Salmonella typhi* infection, presenting with pneumonia. Enteric pathogens have been isolated from various studies\(^ {62,125}\). These organisms are difficult to eradicate, being resistant to most of the antibiotics. Neonatal pneumonias are predominantly caused by enteric-gram negative bacilli and *Escherichia coli*. Most of our isolates were seen in the younger age group in the study population.

Group A beta-hemolytic Streptococcal pneumonia is uncommon in children. Viral infections such as measles, influenza or chicken pox may precede the pneumonia, but it can also occur in healthy children. Six percent of our isolates
were GABHS, recovered each from a child less than 5 months old (one strain), one year old and 3 years old. Five months old child also had empyema. None of these children had associated toxic shock or necrotizing fascitis, conditions which predict an increasing frequency of pneumonia due to GABHS. 

First step in the identification of bacterial pathogens responsible for ALRI is their isolation from relevant site. Blood culture and lung aspirates are the most useful samples to arrive at a specific diagnosis. However, sensitivity of blood culture is often very low even in the most sophisticated of laboratories. Lung aspiration, although very sensitive, gives variable results in many studies. One of the major factors contributing to low results is the presence of antibiotics in specimens being tested. In most developing countries, where antibiotics are available without prescription and over the counter, results of blood culture isolation rates are dismally low. In this study, a history of prior antibiotic therapy was found in 61 (18%) of the children. Serum of 32.6% children (out of 101 tested) had bactericidal activity. When correlated with history of prior antibiotic therapy it was found to be statistically significant. Therefore, our findings suggest as did Shann from Papua New Guinea and Mariana Catalano from Argentina, that positive reports by the parents are generally correct regarding prior use of antibiotics.

Blood culture outcome when compared with serum bactericidal activity was also shown to be significantly affected by the antibacterial activity of the serum,
reflecting on the low rate of isolation. Similar results had been obtained by us in a previous study conducted in this department\textsuperscript{126}. In a larger context of total blood culture however, a negative report by the parents did not reflect on the outcome, hence a negative report by the parents cannot be relied upon by the clinician. This may be due to ignorance of parents regarding prior medication or the reluctance to disclose earlier treatment.

Bactericidal activity of serum and urine to assess prior antibiotic therapy has been shown to give varying results. Negative reporting by parents was seen to be misleading in a study by Catalano et al\textsuperscript{127} who assayed urine from children with ALRI for bactericidal activity. We found that collection of urine was not feasible in very young infants and children, as we had to rely on the mothers for collection of the samples. Serum on the other hand proved a useful sample, as it could be collected simultaneously during blood collection for culture and other laboratory investigations.

Frequent courses of antimicrobial therapy has been shown to increase carriage of penicillin resistant pneumococci\textsuperscript{275}. We could not elicit history of frequent courses of previous antibiotic therapy among our patients. Prior use of antibiotics (single course) however did not have any influence with the nasopharyngeal colonization among the hospitalised children with ALRI.
Specific diagnosis of bacterial pneumonias is accomplished by isolation of the causative organisms from blood or lung aspirates. However, there are several problems with this approach, which include the low sensitivity of blood cultures, inherent risks in performing a lung aspiration and nonavailability of culture methods in many primary and district head quarters hospital of developing countries. Antigen detection from body fluids of commonly encountered pathogens holds promise.

In our study, antigen detection using a latex agglutination kit and coagglutination assay for *H. influenzae* and *S. pneumoniae* circulating antigens in the serum had varied results with proven bacteremia. A low sensitivity ranging from 15% (Directigen) to 8.5% (co-agglutination for *H. influenzae* antigen) and 4.4% (co-agglutination for *S. pneumoniae* antigen) was recorded. Specificities of these three tests were 99.3%, 99.6% and 99.6% respectively. An earlier study from Pondicherry, sensitivity and specificity of antigen detection from serum was found to have similar results\(^\text{159}\). An interesting fact noted in our study was that all pneumococcal and *H. influenzae* bacteraemic samples were positive for antigen detection from the serum by the respective techniques. In addition, 2 samples, one each with *S. pneumoniae* and *H. influenzae* isolated from blood were positive by both co-agglutination assays. Cross reaction between pneumococcal polysaccharide and *H. influenzae* type b lipopolysaccharide has been noted by Requezo et al\(^\text{153}\). Witt et al\(^\text{154}\), in a study from Gambia detected cross reaction between pneumococcus type 8 and Hi b. As we did not serotype our pneumococcal isolates
a comment on this cannot be made. However, the blood isolate of *H. influenzae* was type b. Cross reaction between pneumococcal co-agglutination with GABHS and *Staphylococcus aureus* isolation from blood was also noted in two cases. None of the literature reviewed from BOSTID studies carried out in the developing countries have reported similar findings.

GABHS and *Staphylococcus aureus* both being gram positive, there may have been some cross reaction between capsular polysaccharide of pneumococcus and these organisms. Cell wall polysaccharide and capsular polysaccharide of *Streptococcus* species are known to cross react with each other. Pneumococcus is known to possess a surface protein A (*Pneumococcal surface protein A - PSP A*) with structural and antigenic variability. With diverse surface antigens, it is therefore possible for polyvalent pneumococcal antisera to detect certain circulating antigens belonging to other streptococcal species and possibly *Staphylococcus aureus*.

*Haemophilus influenzae* type b antisera used in our co-agglutination assay cross reacted with one serum sample from a patient whose blood culture had yielded gram negative enteric bacteria (*Citrobacter diversus*). This again may be explained by antigenic diversity of haemophilus surface polysaccharide. Cross reaction of *H.influenzae* type b capsular antiserum has been documented with antigen of *Escherichia coli* K100, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus faecalis*²⁷⁶.
Most of their studies were based on antigen detection in urine using either counter immuno electrophoresis (CIE) or latex agglutination\textsuperscript{60,125}. Use of serum antigen detection was evaluated by Witt et al with a sensitivity of 63% and specificity of 100%.

Antigen detection from serum in our study did not give very promising results as compared to some of the other studies, regarding the sensitivity. However, we obtained reasonably high negative predictive values (86.1% to 94.7% by the various techniques). Use of this test would therefore result in ruling out bacterial pneumonia in around 90% of cases. However, its use in diagnosing either \textit{Streptococcal pneumonia} or pneumonia due to \textit{H.influenzae} is limited. Further purification of the antigens or use of more specific antisera could improve the sensitivity of the tests.

Using a monovalent latex agglutination reagent Harrison et al have evaluated antigen detection from alkalinised unconcentrated urine. A sensitivity of 46% and a specificity of 88% was recorded. They also concluded that antigen detection has a limited role in patient care and clinical research. Use of gene probe techniques or highly sensitive DNA amplification techniques although attractive do not yet find a role in majority of the developing countries. More work therefore is necessary to improve the simple antigen detection assays based on co-agglutination or latex agglutination.
We found use of serum as a source for antigen detection superior to urine, because of the feasibility of the procedure in our set up. However, in a primary health care set up, urine would be a better sample, due to the ease of collection without any intervention and transportation to a laboratory if need be, however, one of the major drawbacks would be contamination of the samples due to the difficulty in collecting urine from very young children and infants in the absence of urine bags.

Antigen in the serum has been shown to correlate positively with nasopharyngeal carriage of the potential pathogens in very few studies. However, association of antigenuria and pharyngeal colonization by Hartal et al\textsuperscript{125} did not show any positive correlation but Wilts et al\textsuperscript{154} found percentage of positive reactions was highest for children carrying \textit{H.influenzae} type b (83\%) intermediate for those carrying \textit{S.pneumoniae} type 6 (35\%) and least for those carrying neither organism (11\%). In our study, it was found that 40\% of all positive children to harbour \textit{H.influenzae} type b and 26\% of all children positive by co-agglutination assay for pneumococcal antigen detection to harbour \textit{S.pneumonia} in their nasopharynx. Our figures although slightly lower than that of Witts et al does show some association of pharyngeal carriage and antigenaemia. However, further studies have to be done with specific markers to establish an accurate relationship between antigen assays and pharyngeal carriage and to evaluate the significance of such a finding. Pharyngeal carriage in children with antigenaemia could help in favouring a bacterial etiology of ALRI. Although antigen detection holds a great deal of promise as a
rapid and simple diagnostic tool, it has its limitations with regards to sensitivity, positive predictive value and reproducibility. In primary health care centres, field studies have to be done before antigen detection can be widely used in developing countries. Cost of preparing specific antisera, availability of the same and standardising the tests have to be undertaken, before antigen detection can be relied upon. Most of the commercially available kits are meant for detecting antigen from CSF. Directigen Kit used in this study for detection H.influenzae antigen although found to be specific (99.3%) had a very low sensitivity. The test Kit was meant for antigen detection from CSF although it could be used to detect antigen from serum according to the manufacturers instructions. Cost of such kits are high, and hence these tests are not cost-effective. Indigenous preparation of antigen detection assays have to be done. We have evaluated co-agglutination assays for the detection of H.influenzae and pneumococcal antigen in the serum, and found it to be less sensitive than the commercially available kit. Further work needs to be done to improve upon the present assay methods.
LABORATORY DIAGNOSIS OF ACUTE RESPIRATORY INFECTION

Identification of respiratory tract pathogens is possible in very limited number of cases even in the most advanced medical settings. With emerging drug resistant, potential respiratory pathogens, it is important to identify the offending agent and determine its susceptibility pattern for effective management, bearing in mind that although bacterial pneumonias occur less commonly than viral, complications and mortality is higher with these agents.

Bacterial agents causing upper respiratory tract infection including otitis media and Streptococcal pharyngitis need to be isolated in the laboratory due to increasing tolerance to penicillin and resistance to alternate antibiotics such as erythromycin, tetracycline and cotrimoxazole.

Antigen detection

Rapid antigen detection techniques used in this study to detect GABHS pharyngitis proved useful, however its prohibitive cost could limit the use in developing countries.

Throat Swab

Transport: Transport of specimen on filter paper strips wrapped with aluminium foil was found to be a simple and convenient way of bringing the sample to the laboratory from field surveys. This technique can be adopted at the PHC, to send clinical material of upper respiratory tract to the regional reference laboratory.

Culture: Double layered sheep blood agar incubated anaerobically gave consistently good result, with regards to clear wide haemolysis, as there was no confusion with α-prime haemolysis being mistaken for β-haemolysis.
Antibiotic susceptibility testing on Mueller-Hinton blood agar used in our study can be adopted in most laboratories for routine testing of GABHS from respiratory specimens.

**Typing:** Although various methods have been described for GABHS antigen extraction, we found the micronitrous acid technique to be simple, rapid and reproducible in this study. A latex agglutination method for grouping the isolates was used.

**Isolation and Identification of Lower Respiratory Bacterial Pathogens**

**Blood culture:** Blood cultures are positive in a very low percentage of children with ALRI. Techniques to improve the isolation rates are needed. Most often children are brought to the hospital following antibiotic therapy. Timing the collection therefore is very important. Preferably collection should be made before the start of therapy or just before the next dose, if antibiotic has already been given. Immediate incubation of the blood following collection is needed for better yield.

In our study blood culture bottles containing brain heart infusion broth were incubated at 5% CO₂ after loosening the cap. Subcultures after 24 and 48 hours of incubation need to be done. *S.pneumoniae*, due to the presence of enzyme autolysin, undergoes autolysis on prolonged incubation. Hence, a subculture on to
chocolate or blood agar is necessary after overnight incubation, for isolating *S.pneumoniae* from the blood.

**Antigen detection:** Rapid tests to detect bacterial antigens of *S.pneumoniae* and *H.influenzae* show variable results in serum and urine. In our study antigen detection from serum showed a low sensitivity, but high specificity. Antigen detection from serum was found feasible as the serum was collected during collection of blood for culture and other investigations. Urine collection had logistic problems with extremely small infants and children.

Availability of standard Pneumococcal and Haemophilus antisera often pose a problem in most of the developing countries. High cost of these reagents is another hindering factor. Adopting Staphylococcal co-agglutination and latex coagglutination techniques can obviate some of these difficulties.

Coating the reagents on to Staphylococcal Protein-A which has an Fc receptor of IgG, will result in increasing the amount of resultant reagent. In this study, the method of co-agglutination for antigen detection was found very useful. Large number of samples could be studied using the reagents prepared in the laboratory. The reagent could be kept in the refrigerator for one month without losing its potency. This can form a useful, rapid diagnostic test in the primary health centres where culture facilities are not available.
Antibiotic susceptibility testing

All isolates of *H.influenzae* and *S.pneumoniae* along with other pathogens need to be subjected to antimicrobial susceptibility testing. We have carried out the susceptibility testing of *S.pneumoniae* on Mueller-Hinton lysed blood agar, and found it to give reproducible results. Oxacillin (1 μg) discs were used routinely to screen for penicillin resistant pneumococci. This has become necessary due to increasing emergence of penicillin resistant strains in clinical isolates.

Testing *H.influenzae* for antibiotic susceptibility has to be standardised in the laboratory before reproducible results can be achieved. Various media which can be used are Iso-Sensitest agar with supplement, Haemophilus Test Media and Mueller-Hinton Chocolate agar. In our study, the Iso-Sensitest Agar with supplement was used and gave satisfactory result. However, a strict quality control with standard strains, inoculum size, media thickness and incubation have to be maintained when testing these fastidious organisms.

Beta-lactamase production by the clinical isolates also needs to be detected. It becomes important in the absence of antibiotic susceptibility testing, the mere detection of beta-lactamase can help the clinician avoid beta-lactam antibiotics.

Nitrocefin, which is a chromogenic cephalosporin, has been used in this study to detect beta-lactamase in all the *H.influenzae* isolates. Other methods which can be adopted in most laboratories is the iodometric or acidometric method. Use of some simple techniques or modification of existing ones, an attempt can be made to improve the diagnostic ability of most laboratories. This is very essential in developing countries, where lack of infrastructure in many laboratories or absence of sophisticated facilities in others limits the usefulness of these in influencing patient care and management.
Group A beta-hemolytic Streptococci: GABHS was isolated from 9.4% of school children with pharyngitis. 2.4% of children attending the primary health centre with acute respiratory tract infection were seen to be colonized with this organism. Antibiotic susceptibility testing of GABHS against cotrimoxazole revealed 27.5% resistance among the isolates from school children and 37.5% among children attending the PHCs. Erythromycin resistance was found to be higher among children attending the PHC than those isolated from school children. Ten percent of all isolates from blood culture in hospitalised children with ALRI were found to be GABHS. These isolates were found to be sensitive to erythromycin and cotrimoxazole. In this study, we have detected erythromycin and cotrimoxazole resistant GABHS for the first time in Pondicherry. Our results will have a bearing on the treatment of children with pharyngitis who fail to respond to penicillin. Isolation of GABHS from throat swabs and subjecting them to antibiotic susceptibility testing has to be done for effective management.
**Streptococcus pneumoniae:** 9.25% of children with acute respiratory infection attending the PHCs were found to be colonized with *S.pneumoniae* as compared to 18.5% of hospitalised children. Colonization in hospitalized children with bacterial flora from the hospital environment have been well established. Increased *S.pneumoniae* carriage in these children as compared to children in the community attending PHCs bears this out. Penicillin resistance among hospital strains were seen to be slightly more than the community strains (21.3% as compared to 18%). Eight percent of strains isolated from hospitalised children were found to be resistant to chloramphenicol as compared to 4.8% strains from the community. Around 57% of strains from the community were found to be resistant to cotrimoxazole as compared to 39% in the hospital strains. This reflects a wider use of the drug in the community than in the hospital. An increased minimum inhibitory concentration of most of the antibiotics was observed against *S.pneumoniae* isolated from the hospitalised children as compared to the community isolates.

*S.pneumoniae* isolated from the blood of children with ALRI were found to be susceptible to the antibiotics tested. Antibiotic resistance was found among the nasopharyngeal colonizers of children attending primary health centres in the community, as well as those hospitalised with ALRI.
**Haemophilus influenzae:** Colonization of *H. influenzae* in the upper respiratory tract of children with ARI in the community was found to be 7.4% as compared to 6% in the hospitalised children. A higher percent of hospitalised children however carried serotype b *H. influenzae* (70% as compared to 51.8% of strains from children in the PHC). All the blood isolates of *H. influenzae* were found to be type b excepting a single nontypeable strain. Colonization of the more virulent type b *H. influenzae* among the hospitalised children may lead to increased risk of developing bloodstream infection in moribund children.

Antibiotic resistance among the hospital strains and community isolates did not differ to any great extent. Cotrimoxazole resistance again was seen to be higher among the community isolates than hospital strains (50% and 40% respectively). Ampicillin resistance was seen to be around 35% with most of the isolates producing beta-lactamase among colonizers isolated from hospitalised children and those attending PHCs. A higher percentage of hospital strains (35%) showed resistance against cefotaxime than community strains. This reflects an increased use of cefotaxime provided by the hospital to the patient in this setting. On the other hand, high cost and mode of administration limits its use in the PHC. Thereby restricting the emergence of cefotaxime resistant *H. influenzae* in the community. Cefotaxime resistant *H. influenzae* in the hospital is worrisome, because of the ease with which it can spread among hospitalized children and its potency to cause infection.
**Moraxella catarrhalis**: Emergence of *M. catarrhalis* as a potential pathogen in the past decade prompted us to look at this organism in the upper respiratory tract of children with ARI. Nine percent of children with ARI attending PHCs were colonized with this organism, 48.9% of these strains were beta-lactamase producers. Ampicillin resistance was found in 34.3% of the isolates, and 41% resistance to cotrimoxazole. Infants less than 6 months of age were found to be colonized by resistant *M. catarrhalis*. A high rate of colonization by strains producing beta-lactamase could act as potential donors of resistant genes to other pathogenic bacteria of the throat. Its presence could also interfere with the action of beta-lactam antibiotics on pathogenic organisms of the throat. Role of beta-lactamase producing *M. catarrhalis* in penicillin tolerance or treatment failures in Strptococcal pharyngitis needs to be studied.