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ORIGINAL RESEARCH

A Prospective Study on Etiopathogenesis of Various Pneumococcal Serotypes in Invasive Respiratory Tract Infections in a Tertiary Care Centre in Western Uttar Pradesh

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ABSTRACT

Background: Acute respiratory tract infections are a major cause of under-five childhood morbidity and mortality across the world. Knowledge of pathogenicity and invasiveness of serotypes can help in predicting the course of disease and will permit necessary interventions to be taken in time. This will result in better opportunities of care and significant decrease in morbidity and mortality associated with pneumococcus. The aim of this study to identify the various pneumococcal serotypes affecting children as pneumococcal invasive disease and to study the clinical presentation, invasiveness and the morbidity and mortality caused by them.

Materials & Methods: It was a hospital based prospective analytical study done at K.D. Medical College, Mathura. All patients within the age group of six months to eighteen years were enrolled in the study. All patients admitted with community acquired pneumonia were screened for evidence of sepsis with the help of routine investigations and acute phase reactants (CRP and Procalcitonin). Clinical profile was studied, and an attempt was made for identification of causative organism by means of Blood culture and DNA PCR.

Results: Out of 716 cases admitted with LRTI, 316 were considered to be bacterial in origin based on clinical presentation, X-Ray findings (classical Consolidation/Empyema), Neutrophilic Leukocytosis, raised Acute Phase Reactants (CRP/PCT) or Blood Culture positivity. Causative organism was identified on basis of blood culture in 28.4% cases. Pneumococcal infection was documented by blood culture in 0.69 % cases while evidence of invasive pneumococcal disease in form of identification of serotype was obtained in 30% cases. 21 different pneumococcal serotypes were identified. Serotype 1 was most common isolate. Other common isolates were serotype 14, 6B, 7F, 9B, 19F, 9N, 18C, 19A, 20, 10F and 5. Serotypes 18C and 19A were associated with maximum mortality. Serotype 14 was another most virulent serotype.

Conclusion: We concluded that most common serotypes in children less than 5 years were- Serotype 1, 7F, 6B, 14, 19F and 9V. Above 5 years- Serotype 1, 14 and 23F were more common. Serotype 18C and 19A having the maximum mortality. Pneumococcal organisms can be better picked up by RT-PCR. Procalcitonin is useful in predicting bacterial origin and can be used as a guide for selecting samples for RT-PCR.
Key words: Pneumococcal Invasive Disease, Serotypes, Pneumonia, DNA PCR.

INTRODUCTION

Acute respiratory tract infections are a major cause of under-five childhood morbidity and mortality across the world. Pneumonia, the most serious presentation, is singly responsible for almost one fifth of total mortality in this vulnerable age group.[1]

Streptococcus pneumoniae infection is a serious problem worldwide.[2,3] It is a common cause of pneumonia, meningitis, and septicaemia, and the case fatality rate remains high.[4] Globally, India has the highest number of deaths caused by pneumococcal infections among children below 5 years of age.[3] The incidence, severity and mortality of the disease, depends not only on host factors such as age, underlying disease, co-morbid conditions, and immunosuppression, but also on the properties of the organism.[5]

Pneumococcal serotypes have varying abilities to cause invasive disease. The serotype distribution varies over time and with geographic location, and local surveillance is therefore needed to determine the regional spread.[6]

A definite diagnosis of pneumococcal pneumonia using conventional methods is currently difficult to establish. The isolation of *Streptococcus pneumoniae* from blood or pleural fluid, the gold standard tests, is made in only 15–40% of cases, and the results obtained from sputum lack specificity.[7,8]

The knowledge of DNA/RNA sequences of most bacteria allow us to obtain etiologic diagnosis and perform bacterial sero-typing even where standard culture methods fail.[9-11] The development of the polymerase chain reaction (PCR) test holds promise as a rapid and specific test that can detect very small amounts of a specific micro-organism.[12] Nucleic acid amplification tests such as PCR do not require viable bacteria for a positive assay, and are generally considered to be highly sensitive in comparison to culture.[13]

The inclusion of the pneumococcal conjugate vaccine in the routine pediatric immunization schedule in western countries has markedly decreased the incidence of invasive pneumococcal disease. Unfortunately, pneumococcal vaccines are not a part of India's routine immunization programmes resulting in continued mortality and morbidity due to invasive pneumococcal disease.

Also, current pneumococcal conjugated vaccines are effective against serotypes included in the vaccines, but do not protect against all pneumococcal serotypes. To evaluate the health impact of a national immunization program with pneumococcal conjugate vaccine, it is crucial to assess disease burden and serotype distribution before vaccine introduction.

Identification of serotypes is important for early diagnosis and treatment of disease. Knowledge of pathogenicity and invasiveness of serotypes can help in predicting the course of disease and will permit necessary interventions to be taken in time. This will result in better opportunities of care and significant decrease in morbidity and mortality associated with pneumococcus. Identification of serotypes will also help in evaluating incidence of a particular serotype and also detection of any new strain paving path for development of newer and improved vaccines.

Multiple studies have been published worldwide regarding prevalence of different pneumococcal serotypes. Certain serotypes are much more likely to be associated with nasopharyngeal carriage than to cause invasive disease.[14]

As per a review of 169 studies from 70 countries including more than 60,000 strains, Johnson et al opined that Seven serotypes (1, 5, 6A, 6B, 14, 19F, 23F) were the most common globally; and based on year 2000 incidence and mortality estimates these seven serotypes accounted for >300,000 deaths in Africa and 200,000 deaths in Asia.[15]

Limited information is available on the outline of invasive diseases caused by *Streptococcus pneumoniae* among the underprivileged children in India. Data on serotype prevalence and antimicrobial resistance of IPD have however been documented in some studies in India.[16-19]

Recently, in a hospital-based study on invasive pneumococcal disease the common serotypes identified were 6A, 14, 5, 6B, 1, 18C, 19A and 9V.[20]

Western Uttar Pradesh has hitherto remained uncharted with respect to identification of serotypes of invasive pneumococcal disease. We hereby present our study on pneumococcal serotypes and their prevalence in radiologically proven pneumonia among children within the age group of 6months to 18 years. The serotypes were determined by real time PCR, their invasiveness was judged by correlating with the morbidity and mortality in the affected children.

MATERIALS AND METHODS

It was a hospital based prospective analytical study done at K.D. Medical College, Mathura. All patients within the age group of six months to eighteen years were enrolled in the study. In all these patients, detailed clinico-epidemiological details were collected including age, gender, locality, religion, presenting complaints with duration, any significant family or past history and immunisation status. All the cases were subjected to a battery of tests including complete Hemogram, C - reactive protein, Procalcitonin, Chest X-Ray and Blood Culture.

Blood sample for culture was collected before the first dose of empirical antibiotics was given. The site was prepared with spirit and application of povidone iodine for minimum of 60 seconds and up till complete drying. The blood was collected directly into the blood culture bottles taking utmost aseptic precautions. The sample to be tested was inoculated into the vial which was inserted into the BACTEC fluorescent series instrument for incubation and periodic reading. Each vial contained a sensor which can detect increase in CO_2 produced by the growth of microorganisms. The sensor was monitored by the instrument every ten minutes for an increase in its fluorescence, which is proportional to the amount of CO2 present. A positive reading indicated the presumptive presence of viable microorganisms in the vial.

C-Reactive Protein (CRP) was done by CRP reagent kit (Rapid Diagnostic Pvt. Ltd, Aspen Laboratories, Delhi) based on latex agglutination method and Procalcitonin (PCT) was determined by a QDx Instacheck kit (Boditech Med Inc. Korea, imported by DiaSys Diagnostics India Pvt. Ltd) based on the principle of immunoassay.

Patients having a positive PCT value and with typical X-ray findings were considered to be bacterial regardless of the culture results and were further evaluated by use of real time PCR to find the presence of pneumococcal DNA and determine the serotype of the incriminating pneumococcus causing the disease.[21]

RESULTS

A total of 316 samples were subjected for molecular diagnosis in the form of RT-PCR to determine the causative pneumococcal strain. 28.4% of the cases were found to be culture positive for all bacterial and fungal organisms. Blood culture was able to detect the presence of Pneumococcus in only 5 cases, however its presence was documented by RT-PCR in 95 cases and amongst these 21 different serotypes, spread over 17 serogroups were determined.

Maximum (82%) patients were under the age of 5 years and only18% were above 5 years. 62.3% were males and 38% were females. 87% of the patients were from a rural background

as compared to only 13% from urban locality. This distribution is in congruence with the general distribution in our outdoor and indoor patients.

Most common serotype in our study was serotype 1 followed by 14, 6B, 7F, 9V, 19F, 9N, 23F,5, 10F, 20, 11A, 15B, 22F, 3, 10A, 6A and 33F in that order. Most common serotypes in children less than 5 years were- Serotype 1, 7F, 6B, 14, 19F and 9V

Most common serotypes in children aged more than 5 years were-Serotype 1, 14 and 23F.

Serotype 1 (11.6 %), 14 (9.4%), 6B and 7F (8.4% each) were the more common serotypes detected. Patients with serotype 14 had a longer duration of stay with a mean value of 11.44 days. A higher morbidity was also associated with serotypes 11A, 9V and 18C as obvious by longer duration of their hospital stay. The higher mean duration of stay was generally associated with strains presenting with a high initial PCT value except for certain 11A and 6A where PCT levels were low despite increased morbidity reflected by a longer duration of stay. This could be due to a delayed presentation to us whereby PCT being an acute phase reactant falls and the pathology increases with passage of time.

The total mortality of our cohort was 11 out of 316 (3.5%). The mortality amongst RT-PCR positive cases (N=95) was 4.2% and was solely contributed by patients infected with serotype 18C, 19A and 14. The other serotypes had no associated mortality. Amongst the lethal serotypes 18C and 19A with 25% each showed a higher mortality as compared to serotype 14(22.22%). All mortality was in the </= 5 Year age group.

Table 1: Age wise distribution of Pneumococcal Serotypes

Serogroups/types	Total Frequency	Age Group	
		<=5 Years (N=259)	>5Years (N=57)
1	11	8	3
14	9	6	3
6B	8	7	1
7F	8	8	0
9V	6	6	0
19F	6	6	0
9N	5	5	0
18C	4	4	0
19A	4	3	1
20	4	4	0
10F	4	3	1
5	4	4	0
23F	5	3	2
22F	3	2	1
15B	3	2	1
11A	3	2	1
3	2	2	0
4	2	2	0
10A	2	1	1
6A	1	1	0
33F	1	1	0

Table 2: PCT levels and Morbidity Profile of different serotypes

Serotype	Frequency	Mean duration of stay	Mean PCT
1	11	6.54	26.05
14	9	11.44	17.42
6B	8	6.75	17.7

7F	8	7.1	10.89
9V	6	10.32	21.1
19F	6	7.5	6.18
9N	5	7.2	21.26
18C	4	9.25	2.39
19A	4	9	25.5
20	4	4.75	2.32
10F	4	5.75	25.66
5	4	5.25	10
23F	5	7	1.97
22F	3	4.67	1.2
15B	3	5.33	50.95
11A	3	10.67	0.786
3	2	10	52.32
4	2	6	6.13
10A	2	6	4.43
6A	1	8	0.62
33F	1	9	45.44

Figure I: Serotype distribution and mortality amongst different Serotype

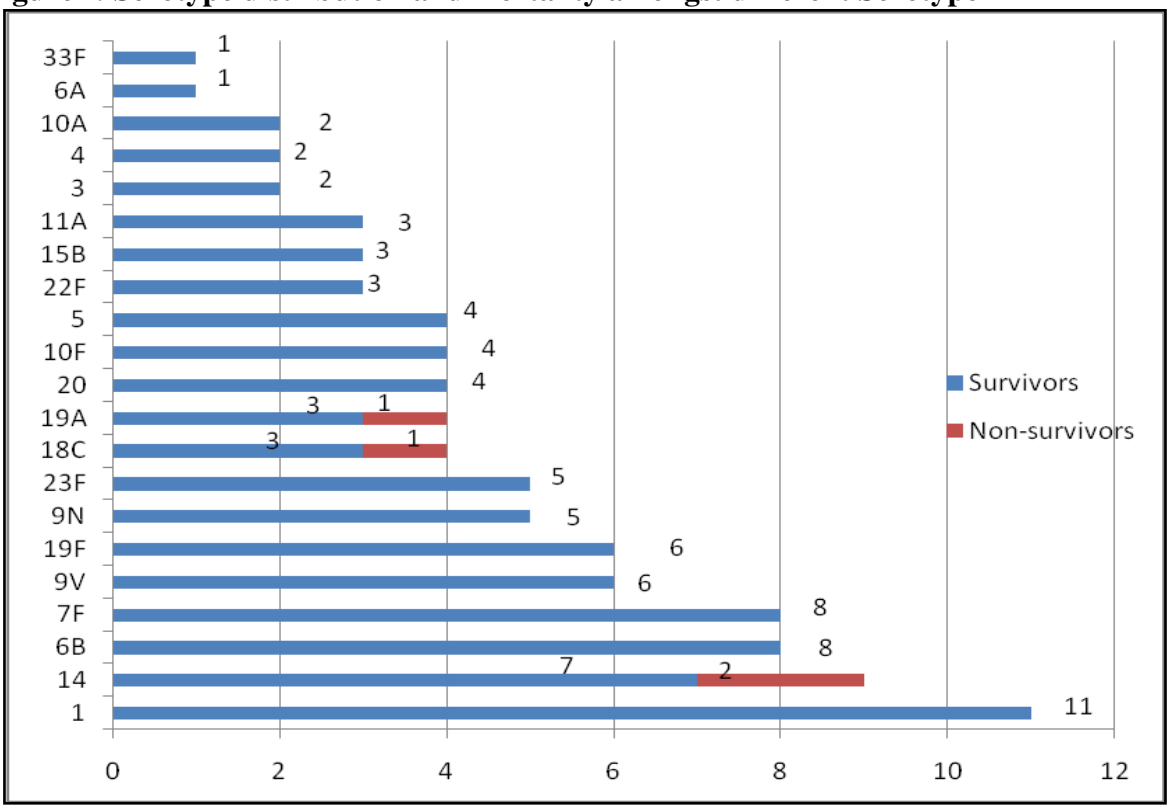

Table 3: Outcome of patients

Serotype	**Age Group**			
	<= 5 Years		**>5 Years**	
	Survivors	**Non-Survivors**	**Survivors**	**Non-Survivors**
RT-PCR Negative N=221 (No Serotype detected)	174	5	40	2

Serotype 18C	3	1	0	0
Serotype 19A	2	1	1	0
Serotype 14	4	2	3	0

DISCUSSION

This prospective study examined the burden of invasive pneumococcal disease and *S. pneumoniae* serotype distribution among young children admitted in a tertiary care centre of Jodhpur city. Notably, this is the first prospective study to document the prevalence of pneumococcal serotypes in children of Western Rajasthan with invasive pneumococcal disease. 82% of the cases were under the age of five years.

A total of 21 different serotypes were detected. Serotype 1 was the most common isolate. Other common serotypes were 14, 6B, 7F, 9V and 19F. In a review study conducted by Hausdorff et al[14], it was found that only 10 sero-groups are responsible for most paediatric infections whereas in our study 17 sero-groups were identified. As per Hausdorff et al[14], sero-groups 1, 6, 14, 19 and 23 were the major encountered sero-groups in each continent around the world in paediatric population. Our study found a similar higher incidence of Serotype 1, 6, 14 and 19 but we also detected a high incidence of Group 9 and 7 in our study.

Earlier studies conducted in India in early twenties[22], have shown the presence of sero-groups 6,1,19,14,4,5,45, 12 and 7 amongst Indian Children. Serotypes 1 and 5 accounted for 29% of all disease. A systemic review has reported the common vaccine serotypes from India as 14, 5, 1, 19F and 6B.[23]

In our study the major serotypes were 1, 14, 6B, 7F, 9V, 19 F, 9N, 23F, 18C, 19A, 20, 10F and 5. Out of these the strains causing disease in children under five years of age were Serotype 1, 7F, 6B, 14, 19F and 9V. There was considerable difference in the serotypes detected by us and those reported in this study amongst under five children. We found prevalence of 1,7F and 9V while serotype 5 was not that common. The serotypes identified in our study were similar to earlier studies conducted by Balaji et al[24], except that serotype 5 was a newer serotype incriminated in this age group according to our study.

The common presence of sero-groups 1,6,14 and 9 is similar to the observations made by Hausdorff et al[14] in their review article. The result is also similar to a study conducted at CMC, Vellore by Molander et al[19], which concluded the most common serotypes in their study to be 1, 5, 19F, 6B, 14 and 3. However the study was conducted on an adult population. In addition to these we have also found a very high presence of sero-group 7 amongst the pneumococcal organisms causing invasive disease in children in Western Rajasthan.

In a study from Asian countries by Kim et al[25], published in 2011, the serotypes detected were 19F [43.5%], 23F [4.3%], 6B [8.7%], 1 [4.3%], 5 [13%], 7F [8.7%], 19A [13%], 15 [4.3%], which were similar to those detected in our study. However, the age group of the patients was not uniform as both pediatric and adult age group patients were included.

In a recent study done by Shariff et al[26] from three hospitals in Delhi published in 2013, out of 108 *S. pneumoniae* isolates tested, serotypes detected were - 19 (26%), 6 (11%), 7 (10%), 1 (9%), 14 (7%), 9 (5%), 33 (4%), 17 (4%), 11 (2%), 3 (2%), 18 (1%), 23 (1%), 12 (1%), 32A (1%), 15B (1%), 22F (1%), 5 (1%) and 29 (1%). The most common serotypes according to this study were serotype 19 and 6 as compared to serotypes 1 and 14 in our study.

In a recent study of Pneumococaal invasive disease in Bangalore, 6A was the most commonly encountered serotype whereas in Rajasthan over a similar period of time and age group we found serotype 1 and 14 as the most common serotypes. This underscores the diversity of serotypes found in population groups emanating from different geographical locations.

Serotype 18C and 19A were found to have highest mortality in our study, each contributing to 9.09% of total cohort mortality and 25% of individual mortality. This was in contrast to a

study conducted by Molander et [a19l] which concluded that maximum mortality was seen in Serotype 6B (25%) and 19F (18%). Patients infected with serotype 14 of Pneumococcus had a longer duration of stay in hospital, hence attributing highest morbidity to serotype 14. Children in whom serotype 11A, 9V and 18C were detected also had a prolonged hospital stay. The final outcome was also poorer for serotype 18C. Children infected with 11A and 9V had a good recovery despite a longer stay. A different result was obtained in study by Molander et al[19], in which Serotypes with higher morbidity were serotypes 1, 5 and 7.

Apart from identifying the prevalence of various serotypes in pneumococcal invasive disease in Western Uttar Pradesh, our study also brings out their individual morbidity and mortality. The study also underscores the practical limitation of blood culture as a tool to detect the causative organism in pneumococcal pneumonia and finds Procalcitonin as an important marker which can help identify the patients to be subjected to RT-PCR analysis for better results.

Streptococcus pneumoniae is a fastidious organism which is difficult to pick on Blood culture and carries a high burden in the form of Invasive Pneumococcal disease in the Paediatric age group. The serotypes are variable over time and over geographical areas.

CONCLUSION

Most common serotypes in children less than 5 years were- Serotype 1, 7F, 6B, 14, 19F and 9V. Above 5 years- Serotype 1, 14 and 23F were more common. Serotype 18C and 19A having the maximum mortality. Pneumococcal organisms can be better picked up by RT-PCR. Procalcitonin is useful in predicting bacterial origin and can be used as a guide for selecting samples for RT-PCR.

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