

## **REPORT**

# **Colonization rates and immune status of children against *Streptococcus pneumoniae* before the introduction of pneumococcal conjugate vaccine**

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**Abstract:** Pneumococcal infections contribute to high morbidity and mortality in Pakistan. Pneumococcal colonization rates and immune status of children before and after vaccination can serve as an important indicator to determine the success of immunization program. Aim of this study was to determine the pneumococcal colonization rate and immune status of children (<3-years) before the introduction of Pneumococcal immunization in EPI program. A cross sectional study of 3-months duration was conducted in collaboration with EPI Centre of NICH, Karachi. A total of 192 healthy children aged 3-months to 3-years were included in the study. Children with chronic or recurrent pulmonary disease were excluded. Demographic data and presence of any risks for pneumococcal colonization or immunity was recorded. Nasopharyngeal swabs were collected to determine the presence of *S. pneumoniae* and sensitivity pattern of the isolates. Blood samples were collected to determine immune status of these children. Sixty (31%) children were found to carry *S. pneumoniae* in their nasopharynx. Resistance to cotrimoxazole, erythromycin, chloramphenicol, penicillin and vancomycin was 100%, 30%, 25%, 22% and 0% respectively. Mean anti-PCP titre of children was 23.3mg/L. Mean anti-PCP titre in children who had previously suffered from RTI was significantly increased (p-value 0.039). This study may serve as baseline report for evaluating the success of pneumococcal immunization in future. A significant drop in nasopharyngeal colonization rates post vaccination would depict the success of pneumococcal immunization.

**Keywords:** Pneumococcal conjugate vaccine, Pneumococcal colonization, antibiotics sensitivity, vaccine

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## **INTRODUCTION**

Pneumococcal infections contribute to high morbidity and mortality throughout the world (Rajalakshmi *et al.*, 2001). It is estimated that pneumococcal disease claims 1.6 million lives each year worldwide (WHO., 2007). Moreover a large number of patients who survive undergo severe neurological sequelae including unilateral and bilateral deafness (Komelisse *et al.*, 1995). In Pakistan 10 million cases of pneumonia are observed each year and *S. pneumoniae* is found associated with 10% of the cases (Rudan *et al.*, 2008 and Ghaffor *et al.* 1990).

Though associated with serious life threatening infections it is also important to note that *S. pneumoniae* constitute the commensal flora of upper respiratory tract which colonizes the nasopharyngeal niche (Bogaert *et al.*, 2004). An important claim made by researchers in this context is that pneumococcal disease may not occur without preceding nasopharyngeal colonization of the homologous strain of *S. pneumoniae* (Frank *et al.*, 1997). Highest rates of colonization are reported among children of <5years, with the peak rates in those 3-years of age (Frank *et al.*, 1997). This is the reason that highest

frequency of pneumococcal infections is observed in children and hence preventive strategies like vaccination also focus on prevention of nasopharyngeal colonization especially among young children (Komelisse *et al.*, 1995).

Currently four types of pneumococcal vaccines are available including PCV7, PCV10, PCV13 and 23-valent pneumococcal polysaccharide vaccines. These vaccines are designed on the basis of epidemiological data of diseases causing serotypes of pneumococci in different parts of the world particularly in the western countries. Pneumococcal vaccine causes significant reduction in the nasopharyngeal carriage of pneumococci resulting in decreased morbidity (Dagon *et al.*, 2002) and a significant reduction in pneumococcal colonization rates after vaccination is used as an indicator for effectiveness of vaccination program (Dagon *et al.*, 2002 and Laval *et al.*, 2006). Varied responses to pneumococcal vaccination programs in terms of efficacy have been reported among different populations which are attributed to the differences in ethnicity, crowding, environmental features and socioeconomic features (Frank *et al.*, 1997).

The persistent high burden of invasive and noninvasive pneumococcal infections and rapid emergence of multi drug resistant pneumococcal strains have contributed to

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the increased interest of health authorities in Pakistan towards preventive strategies against pneumococcal infections. But, baseline data regarding actual burden, disease distribution, colonization rates, current immune status and serotype distribution of *S. pneumoniae* in our population is scarce. In the absence of such data it is impossible to assess and hence difficult to estimate as to what extent we may control pneumococcal infections if such a vaccine is introduced in Pakistan, especially when reports from neighboring countries suggest poor serotype coverage of vaccine (Saha *et al.*, 2003). This study was conducted as an attempt to generate some baseline data regarding pneumococcal colonization rates and immune status of children (less than 3-years of age) against pneumococcus to help authorities in the future assessment of pneumococcal vaccination program in Pakistani children.

**Methodology**

This was a cross sectional study of 6-months duration conducted at the EPI Centre of National Institute of Child Health. A sample size of 191 children (<3-years of age) has been calculated at 7% precision and 95% confidence interval on the basis of previous study which shows that 55% of infants are colonized with pneumococci (Bogaert *et al.*, 2004). Healthy children attending EPI centre to receive routine immunization were enrolled in the study after taking informed consent from their parents. Children with chronic or recurrent pulmonary infection or who had received pneumococcal vaccine privately or those with the history of hospitalization for more than 1-week were excluded from the study. A predesigned structured proforma was used to record patient’s demographics, history of any illness, crowding at home or exposure to passive smoking. Nasopharyngeal swabs of patients were collected using flexible twisted wire Dacron cotton tipped swabs (ProLab, Canada) as described by Bogaert *et al.* (2004). These swabs were immediately put in sterile STTGA medium, vortexed and transported to the Microbiology lab of PMRC Research Centre, JPMC Karachi as per WHO guidelines. Blood sample (2-3cc) of these children was also collected by an experienced phlebotomist using aseptic measures to determine the presence of pneumococcal antibodies.

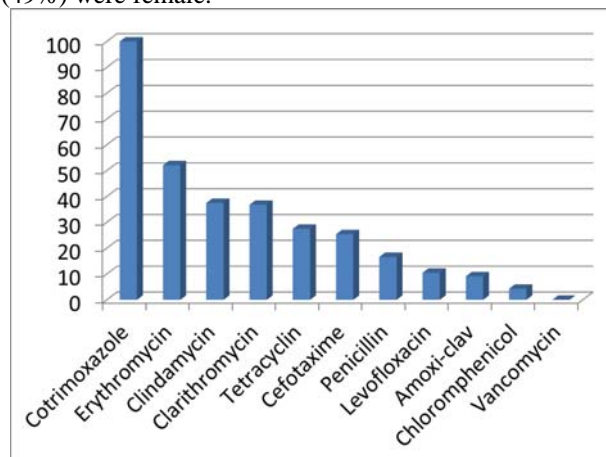
Culture of these swabs was done on blood agar supplemented with 5-7% sheep blood and plates were incubated at 37°C for 24-48 hours. Identification of Pneumococcus was done on the basis of colonial morphology and optochin susceptibility test. Susceptibility test of pneumococcal isolates against commonly prescribed antibiotics was also done by Kirby Bauer disk diffusion method using Mueller Hinton agar supplemented with 5-7% sheep blood. ELISA was performed for antibody determination against pneumococcus using PCP assay kits by The Binding Site, as per manufacturer’s guidelines.

**STATISTICAL ANALYSIS**

The data feeding and analysis was done on computer package SPSS (Statistical Packages for Social Sciences) version 19.0 to determine the clinical characteristics in terms of frequencies and percentages for categorical variables (colonization rates, gender, respiratory tract infections during last three months or since birth, hospitalization history, passive smoking etc.), mean±S.D for numerical variables (age, number of individuals in the household). Chi square test was used to determine the association of different variables with pneumococcal colonization and presence of pneumococcal antibody. P-value <0.05 was considered significant.

**RESULTS**

During the study period, parents of total 261 children who fulfilled the inclusion criteria were requested to participate in the study, out of these 69 declined to participate in the study hence a total of 192 children were enrolled in the study. The mean age of these children was 15±11months. Ninety eight (51%) were male while 94 (49%) were female.



**Fig. 1:** Resistance pattern of nasopharyngeal colonizing pneumococcal isolates.

Nasopharyngeal swab culture for pneumococcus was positive in 60 (31.25%) cases. Carrier rate with respect to age was also analyzed, peak colonization was observed between the age group of 6-12 months (table 1).

No statistically significant relation of the colonization rates with gender, history of RTI, average household number or exposure to passive smoking was observed (table 2).

Antibiotic sensitivity pattern of colonizing Pneumococcal isolates against commonly prescribed antibiotics is presented in fig. 1. Highest sensitivity (100%) was observed against Vancomycin while least sensitivity (0%) was observed against cotrimoxazole. Resistance to penicillin was observed in 16.6% isolates.

**Table 1:** Nasopharyngeal pneumococcal colonization rates in different age groups.

Age (months)	Total n	Culture positive n (%)	Culture Negative n (%)	p-value
≤6	37	11(29.7)	25 (70.3)	>0.05
6-12	67	22 (32.8)	45 (67.2)	
12-18	37	12 (32.4)	25 (67.6)	
18-24	16	5 (31.25)	11 (67.7)	
24-30	13	4 (30.7%)	9 (69.3)	
30-36	22	6 (27.3%)	16 (72.7)	
Total	192	60 (31.1%)	133 (68.9)	

**Table 2:** Nasopharyngeal carriage rate of *S. pneumoniae* with reference to different factors.

Category	Sub-category	Total	Culture +ve n=60	Culture -ve n=132	p-value
Mean anti-PCP titre (mg/L)	-	-	17.6±20.12	25.3±48.13	0.286
Gender	Male	98	26 (26.5%)	72 (73.4%)	0.240
	Female	94	33 (33.3%)	61 (66.6%)	
History of RTI in last 3-months	Yes	78	24 (30.8%)	54 (69.2%)	0.835
	No	109	32 (29.4%)	77 (70.6%)	
History of RTI since birth	Yes	96	30 (31.2%)	66 (68.7%)	0.726
	No	90	26 (28.8%)	64 (71.2%)	
Average No. of individuals in household	-	-	8.3±6.47	7.6±4.97	0.420
Average No. of children (<3-years) in household	-	-	2.5±1.52	2.2±1.36	0.192
Exposure to passive smoking	Yes	64	18 (28.1%)	46 (71.9%)	0.589
	No	122	39 (31.9%)	83 (69.1%)	

**Table 3:** Effect of different factors on mean anti-PCP titre of children

Category	Sub-category	Total	Mean Ab titre	p-value
Gender	Male	84	18.9±35.09	0.202
	Female	77	27.4±48.03	
History of RTI in last 3-months	Yes	60	23.3±43.6	0.993
	No	98	23.2±41.6	
History of RTI since birth	Yes	76	30.45±56.67	0.039*
	No	81	16.5±19.74	
Average No. of individuals in household	≤5	46	22.7±31.2	0.411
	5-10	93	27.2±51.1	
	>10	45	16.1±29.3	
Average No. of children (<3-years) in household	1	39	25.5±47.7	0.653
	1-3	100	23.7±43.2	
	> 3	16	14.1±16.7	
Exposure to passive smoking	Yes	56	19.8±28.39	0.425
	No	100	25.4±48.58	

Out of the 192 children included in the study, blood sample of 31 children could not be drawn in single prick hence blood sample of 161 children was available for anti-PCP titre. Mean anti-PCP titre of children was 23.3 mg/L.

Effect of different factors on mean antibody titre of participants was also evaluated (table 3). The only factor which showed a significant association (p-value: 0.03) with antibody titre was the history of RTI since birth, Mean antibody titre was significantly raised in children who had a history of respiratory tract infection.

## DISCUSSION

The decision regarding the need of immunizing a particular population against a particular disease is mainly based on morbidity and mortality rates of that disease in the population. In the absence of such data it becomes harder to establish the efficacy of that vaccine in that population. Pneumococcal vaccine has been introduced in the routine immunization program of Pakistan, EPI since April, 2013. This decision is based on *estimated* burden of Pneumococcal infections in our population as, the data regarding actual burden of the pneumococcal infections

was not available due to the inherent problem of developing countries (including Pakistan) to obtain etiological diagnosis of pneumonia. Nasopharyngeal colonization rates of *S. pneumoniae* are also widely used as an indicator of the vaccine efficacy as pneumococcal infection is always followed by nasopharyngeal colonization and the vaccine claims to reduce the colonization rates, in turn reducing the disease burden.

The present study showed colonization rate of 31.25% in healthy Pakistani children, prior to the inclusion of pneumococcal vaccine in EPI program. A previous study conducted in Pakistan showed nasopharyngeal colonization rates of 62% but this study included only those children who had symptoms of acute respiratory tract infection (ARI) hence they observed a higher rate (Mastro I *et al.*, 1993). We could not find any study on healthy children from Pakistan but studies from neighboring and other countries including Bangladesh, India, Indonesia, Japan and Taiwan have shown the carrier rates of *S. pneumoniae* which vary between 6.5% to 60.3%. Lowest rates (6.5%) have been reported from New Dehli, India (Wattal *et al.*, 2007), this was the only study which exactly matched our inclusion criteria in terms of age, sample size and sampling technique but they have observed 5-times lower rates as compared to our study. A recent study from rural area of Assam, India reported 12.8% of children aged 0-14 years carried *S.pneumoniae* in their nasopharynx (Devi *et al.*, 2012) while a similar study enrolling school going children from Lucknow, India reported a carrier rate of 53.6% (Jain *et al.*, 2005). A nasal carriage rate of 47% was reported from Bangladesh among children of <5years (Saha *et al.*, 2003). Similarly studies from Lombok Island (Saewignjo *et al.*, 2001) and Java Island (Farida *et al.*, 2014) of Indonesia report carriage rates of 48% and 43% respectively. Masuda *et al* from Japan have reported highest rates (60.3%) of nasopharyngeal colonization among the children (<5years) attending day care centers (Masuda *et al.*, 2002). This variation among different population, is attributed to the geographical differences.

Resistance pattern of pneumococcal isolates showed 100% resistance against cotrimoxazole. This antibiotic was once used as a first line treatment for pneumococcal infections but in the current era of antibiotic resistance, it is no more recommended for pneumococcal infections in Asian countries. Penicillin is nowadays considered as the treatment of choice for pneumococcal infections but resistance against penicillin is also emerging rapidly. We observed 16.6% resistance among nasopharyngeal isolates against penicillin, A recent study from Indonesia reported 24% resistance, Saha *et al* from Bangladesh reported 9.5% resistance against penicillin and 79.5% resistance against cotrimoxazole among carriers (Saha *et al.*, 2003), 14.5% and 6.4% resistance against penicillin is reported from Gambian (Kisanya *et al.*, 2006) and Chinese (Yao *et*

*al.*, 2007) pneumococcal carriers. High rates of resistance were also observed against macrolides, clindamycin and tetracyclin in the present study, these results are in concordance with the resistance rates reported from Italy (Marchisio I *et al.*, 2002), and China (Yao *et al.*, 2007) but these resistance rates are quite higher as compared to India (Jain *et al.*, 2005), Bangladesh (Saha *et al.*, 2003) and Indonesia (Farida *et al.*, 2014). Here it is also pertinent to mention that though the evidence regarding bacteriological failure of macrolide resistance suggests that these antibiotics are ineffective in-vivo against in-vitro resistant strains but clinical relevance of in-vitro penicillin resistance of pneumococci is still not established and it is still considered the antibiotic of choice for treating pneumonias (Chiou *et al.*, 2006). Resistance rates against levofloxacin, amoxicillin-clvulanic acid, chloromphenicol and vancomycin also related well with other studies (Yao *et al.*, 2007 & Parra *et al.*, 2012).

Mean antibody titre of children was found to be 23.3 mg/L. No cut off values for pneumococcal antibody titre has yet been established showing the normal ranges or protective immunity for a collective response to multiple pneumococcal serotypes (Balmer *et al.*, 2007). This is because of the diversity of pneumococcal polysaccharide capsule types, different vaccine formulations including polysaccharide and conjugated vaccines, diverse pneumococcal serological assays, lack of immunogenicity data for the conjugate vaccine in different groups and complex vaccine schedule (Yao *et al.*, 2007). Pneumococcal antibody titres are performed to see the response to vaccination; studies suggest a two to four fold increase in the serum antibody titres after vaccination compared to baseline which shows an adequate response to vaccination (Fiorino *et al.*, 2012). Hence based on these suggestions it may be inferred that a significant increase in pneumococcal antibody titres in post vaccination era may depict adequate response to vaccination in our population.

The analysis of different factors which have previously been shown to affect the carriage rate or acquisition of natural immunity against *Pneumococcus* did not yield any significant results in the present study except for the history of RTI. Children who had a history of RTI since birth had a significantly raised pneumococcal antibody titre which shows that probably RTI episode was due to pneumococcal infection which in turn resulted in the development of antibodies in these children.

This study has some limitations including *i.e.* this study was conducted in vaccination clinic instead of community and inclusion of a modest number of participants but this was due to the lack of time and resources to conduct the community study as pneumococcal vaccination was about to be included in the EPI program in near future but we made sure that our study yields generalizable results as

we conducted the study in a vaccination clinic which was based in the largest public sector children hospital and people from all the areas of Karachi come here to get their children vaccinated. Another limitation of the study was the use of multiplex serology to quantitate pneumococcal antibody instead of calculating antibody concentrations against individual pneumococcal serotypes which requires sophisticated equipments and is too costly.

## CONCLUSIONS

The study provides baseline information regarding the pneumococcal carriage rates and immune status of children in the pre vaccination era which were found 31% and 23.3mg/L. The efficacy of pneumococcal vaccine in our population can be established if we observe a significant drop in the nasopharyngeal colonization rates and a significant increase in the pneumococcal antibody titres in post vaccination era.

## ACKNOWLEDGMENT

We are grateful to Pakistan Medical Research Council for providing us funds to carry out this study. Further I would also like to extend my thanks to Dr. Huma Qureshi for her guidance and great support in conducting the study and all my colleagues and staff at EPI centre, NICH for their assistance and support in carrying out this study.

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