Streptoccoccus pneumoniae carriage among healthy and sick pediatric patients before the generalized implementation of the 13-valent pneumococcal vaccine in Morocco, 2010-2011.

Jroundi I[1,2#.],Mahraoui C[3.], Benmessaoud R[1.], Moraleda C[1.], Carmen Munoz Almagro[4],Seffar M[3], Tligui H[3], Cherif Kettani S[3.], Benjellon B.S[3.], Alonso PL[1.], Bassat Q[1,5.]

1. ISGlobal, Barcelona Ctr. Int. Health Res. (CRESIB), Hospital Clinic- Universitat de Barcelona. Barcelona. Spain

2. Unit of training and research in public health, School of Medicine and Pharmacy of Rabat. University Mohamed V, Rabat.

3. Hopital d´Enfants de Rabat, Centre Hospitalier Universitaire IbnSina Rabat.

4. University Hospital Sant Joan de Déu, Esplugues, Barcelona, Spain.

5. Centro de investigação em Saúde de Manhiça (CISM), Maputo, Mozambique.

Corresponding author:

Imane Jroundi, MD, MPH, PHD

Address: Unit of training and research in public health, School of Medicine and Pharmacy of Rabat. University Mohamed V, Rabat.AV Mohamed Belarbi El Alaoui Rabat Institut, BP 6203 Rabat, Morocco.

Email: imane.jroundi@cresib.cat

Abstract

Nasopharyngeal carriage studies provide insights into the local prevalence of circulating pneumococcal serotypes. These data are critical for vaccination monitoring purposes, as they allow predicting and assessing their impact. Scarce data are available on the carriage of pneumococcal serotypes in Morocco. Here, we describe the prevalence of *Streptococcus pneumonia* carriage and serotype distribution among195 healthy infants and young children recruited at a vaccination clinic and 697 pediatric patients aged from 2 to 59 months admitted to a Moroccan reference hospital with severe pneumonia. Carriage rates were 40.5% (79/195) for healthy children and 22.8% (159/697) among sick ones. Most frequent circulating serotypes included 6A, 6B and 19F all included in the current 13-valent anti-pneumococcal conjugate vaccine recently introduced in the country. Monitoring of circulating serotypes remains necessary after vaccine introduction to assess whether serotype replacement is occurring.

Key Word: *Streptococcus pneumoniae*, nasopharyngeal carriage, children, serotypes, Pneumococcal conjugate vaccines.

Background

Pneumococcal disease is the leading cause of vaccine preventable deaths and *Streptococcus pneumoniae* is estimated to be responsible for 11% of all deaths in children less than five years of age worldwide, mostly due to community-acquired pneumonia[1].Nasopharyngeal colonization is known to play an important role in the development and transmission of pneumococcal disease. Infants and young children are considered to be the main reservoir of this pathogen[2].Studies show that it is essential to monitor prospectively the pneumococcal circulating serotypes to predict and assess the impact of the vaccine introduction in a given community and also to allow the demonstration of whether serotype replacement may be occurring[3].

In Morocco, a middle-income country in Northern Africa, pediatric pneumonia remains a major public health challenge. A recent study conducted in 2010 in a tertiary hospital among admitted children under five years of age with clinically severe pneumonia, reported that the etiologies of pneumonia were mostly viral, and that bacterial pathogens were rarely isolated, affecting only 3.5% of the patients[4]. The Moroccan Ministry of Health introduced in 2010 the pneumococcal (13-valent) conjugate vaccines into its expanded program of immunization (EPI) schemes among other control measures[5]. Due to the scarce available data in Morocco[6], we aimed to investigate pneumococcal carriage and serotype distribution among healthy infants recruited at vaccination clinics and sick children admitted with a diagnosis of severe pneumonia at a referral tertiary hospital in Morocco.

Material and methods

Study area and population

The study was conducted at the *Hôpital d'Enfants de Rabat* (HER)- the only public hospital dedicated to children in the region- and at four heath centers located in the area of Rabat-Salé-Zemmour-Zair (RSZZ Rabat province). The proportion of children under five in the region of RSZZ has been estimated at 5.66%[5].

Study design

The study population was recruited from HER among patients admitted for severe clinical pneumonia according to WHO definition[7]during a one-year survey from November 2010 to December 2011, as part of a wider research protocol trying to define the epidemiology and etiology of respiratory distress at HER[4]. Healthy children who were visiting primary health care centers of the province of Rabat for routine vaccination were also recruited from February to March 2011. These sites were selected randomly from the list of the primary health care of the region RSZZ. The sample size for the community study (n=200) was calculated based on the preliminary carriage rates found at HER in children with WHO-defined severe clinical pneumonia[4].

Data collection

Children were recruited both at the wards of HER or at the health centres only after an informed consent was signed by parents or legal guardians. The study team would then administer standardized questionnaires and obtain nasopharyngeal samples from the children using a mini culturette extra-thin flexible wire swab with its tip, in addition to other procedures explained elsewhere[4].

Laboratory methods

The nasopharyngeal cultures were injected into Stuart transport medium tubes. Samples were processed on a daily basis: homogenized, aliquoted, and frozen at -70ºC. Nasal samples were cultured using conventional methods and bacterial isolates were identified by colony

morphology and biochemical tests. Molecular capsular typing of pneumococcal strains isolated by conventional culture was investigated using sequential multiplex Real Time PCR targeting 24 common serotypes (1, 3, 4, 5, 6A,6B, 7F/A, 8, 9V/A/N/l, 14, 15B/C, 18C/B, 19A, 19F/B/C, 23A and 23F)[8].

Data analysis

Study variables were counted and summarized in frequency tables. Quantitative variables are summarized as Means with their corresponding standard deviations. Qualitative variables are presented as a percentage with 95% confidence interval. As the inclusion of patients from the peripheral health posts was only conducted to provide a baseline assessment of carriage rates among theoretically healthy children, but was not designed to infer differences or associations, no formal statistical comparisons are presented between both groups.

Results

Seven hundred patients were admitted for severe clinical pneumonia during the one- year survey and were eligible for the study, and 200 healthy children were recruited from primary health care centers during one month. The proportion of infants less than 1 year of age, was 30.6% (214/700) among the admitted patients and 70.7% (138/195) in children from the community. Three samples from the hospitalized group and five from the outpatient group were not suitable for analyses. Thus, nasopharyngeal carriage *of streptococcus pneumonia* among admitted patients was 22.8% (159/697), and 40.5% (79/195) among children attending the primary care centers.

Among admitted patients, monthly distribution of carriage was significantly different (data not shown, $p<0.001$), and carriage was significantly more frequent during spring and summer

(~31-32%) than during autumn or winter (~19-20%) (p=0.003). Seasonality could not be assessed among outpatients as recruitment lasted only one month.

Carriage rates also varied, albeit no significantly, according to number of doses received of pneumococcal vaccine. Thus, in infants, carriage rates were 21.2%, 24.5%, 31.3% and 33% among those not having received any dose, 1, 2 or three doses respectively. 24.0% and 31.6% of the children aged over one year and not having received any dose or one dose, respectively, were carriers, while none of those having received at least two doses were positive.

Serotype distribution was assessed by molecular methods in 74.2% (118/ 159) and 58.2% (46/79) of the individuals having test positive for *pneumococcus*. The most frequent circulating serotypes included 6A, 6B and 19F/B/C. Their proportion among healthy children was 2to 8 times higher in children less than 12 months of age; while19F/B/C was twofold higher among children having received at least one dose of the 13-valent pneumococcal conjugate vaccine. In this population serotype, 6A was similarly frequent, but 6B was only isolated among incompletely (one or two doses) vaccinated children. Vaccine coverage for the circulating serotypes among children for which individual serotype data were available was 55.9% (66/118) among hospitalized children and 52.1% (24/46) for healthy children. Certain serotypes for which inter-group differentiation was not possible because of the diagnostic method utilized (i.e. 19 F/B/C) were also observed. Table 1 shows the characteristics of the children included in this study and the distribution of serotypes according to the status group.

Discussion

This study showed a moderately low nasal carriage of pneumococci, ranging from 23% to 40% in two very distinct pediatric populations, namely sick children with clinical severe pneumonia and healthy children. This analysis gives baseline information of the current status of pneumococcal carriage among children in Morocco, although it is worth noting that the two

groups studied do differ significantly in some important variables, such socioeconomic status or exposure to antibiotics or smoking. These differences probably reflect different patterns of health seeking behavior at the health post and hospital level.

The predominant serotypes identified in these population were 6A, 19F/B/C, 6B, 23A and 23F serotypes. This result could be explained by the fact that the samples were obtained shortly after the introduction of the vaccine in the EPI schedule. Similar results were found in the Region of Marrakech among healthy children[9] and admitted patients in Casablanca[10]. At least half of the individual serotypes identified are included in the current vaccine used by the national immunization program introduced in November 2010[11]. Actual coverage of circulating serotype of this vaccine in Morocco may be even higher, as some of the grouped serotypes identified are also included in the vaccine. Consequently, the wider implementation of this vaccine, for which coverage rates were still low among admitted patients with severe pneumonia at the time of the study, may not only contribute to the observed decreased pneumococcal associated severe disease, but also asymptomatic carriage[12]. The potential public health impact of this vaccine, once it has been adequately deployed throughout the country, seems therefore significant. In low and middle-income countries, and under natural conditions of exposure, children become quickly colonized by pneumococci, and it is not uncommon to find moderate to high carriage rates (often exceeding 2/3 of the children) in countries that have not yet implemented vaccinations program, particularly among young infants[13]. In our study, we found lower than expected carriages rates of those serotypes most commonly described in the literature[13]. Continuous monitoring and repeated investigations for the serotype distribution of pneumococcal carriage and invasive disease remains necessary in Morocco, to adequately detect the emergence of new circulating serotypes[14] and prevent further morbidity and mortality as well as assess the impact of the recently implemented vaccine.

Funding:

This work was supported by the Spanish Agency of International Cooperation for Development (AECID) through the grant 07-CO1-021 awarded to *Fundació Clínic per a la Recerca Biomèdica* (Convenio de Fortalecimiento del sistema nacional de salud, con especial atención a la salud maternoinfantil, Marruecos, 2008–12). The research leading to these results has also received funding from the People Programme (Marie Curie Actions) of the European Union's seventh Framework Programme FP7/2007-2013 under REA grant agreement n° 621216. QB has a fellowship from the program Miguel Servet of the ISCIII (Plan Nacional de I+D+I 2008-2011, grant number: CP11/00269).

Conflict of interest:

The author(s) declare that they have no competing interests.

Acknowledgments:

The authors are greatly indebted to the children and their parents and also the MOH staff in the primary health care centers for their contribution in the realization of this study.

We also want to acknowledge the facilitating role played by Pascal Andignac, Eva Lopez, Younes Ben Azzouz, Maria JoséLópez, Robert Álvarez and the rest of the team at *Fundació Clínic*Maroc, together with the nursing and clinical personnel at HER, Rabat (Emergency ward, P1 and Intensive Care Unit), and the technical personnel at the research laboratory of CHIS). We also thank Dr Jordi Vila, who contributed to the general set-up of laboratory methods at the research laboratory.

Author´s contributions:

IJ conceived of the study, carried out the literature search, collected the data and drafted the first and subsequent versions of the manuscript. QB conceived the study, helped with the coordination, analyzed the data, contributed to the drafting of all versions of the manuscript, and edited the English language. CMo, helped to collect the data, revised critically the manuscript and approved the final manuscript. CMa contributed significantly to the coordination of the data collection, review of the clinical cases and the construction of the database. SEK and BSB helped to collect the data. RB and CMA designed and set-up the laboratory experiments and methodologies and performed all the laboratory procedures and analyses. MS and TH participated in the laboratory analyses and validated the biological results. PA contributed to the design of the study and critically revised the manuscript. All authors read and approved the final manuscript.

Table 1: Baseline characteristics and microbiological finding among admitted children with clinical severe pneumonia and healthy patients.

Patient's variables		**Hospitalized patients N=700**	**Healthy Community Controls N=200**
		n/N (%)	n/N (%)
Basic demographics	Female gender	251/700 (35.9)	99/195 (50.8)
	Age in months: Mean (SD)	21.5 (14.5)	8.9 (11.1)
	Infants (<1 year)	214/700 (30.6)	138/195 (70.8)
	Both parents unemployed	120/691 (17.4)	9/195 (4.6)
	Both parents employed	58/691 (8.4)	48/195 (24.6)
	Medical insurance	181/696 (26.0)	92/193 (47.2)
	Proportion of children living in house>6 people	268/700 (38.3)	49/195 (25.1)
	Number of rooms in house: Mean (SD)	2.2 (1.1)	1.8 (0.9)
	Breastfeeding >6months	422/697 (60.5)	56/195(28.7)
	Prior admission due to respiratory condition	227/700 (32.4)	18/194 (9.3)
	Smokers at home	282/699 (40.3)	58/195 (29.7)
	Pre-recruitment use of antibiotics	206/700 (29.4)	34/195 (17.4)
	At least one dose of Pneumococcal vaccine	117/700 (16.7)	137/195 (70.3)

Bacterial carriage in the nasopharynx	Positive *pneumococcus* in NPA	159/697 (22.8)	79/195 (40.5)
Streptococcus pneumoniae serotypes distribution	Non-typable pneumococci	41/159(25.8)	33/79(41.8)
	Typable pneumococci	118/ 159(74.2)	46/79 (58.2)
	18 C/B	5/118 (4.2)	1/79 (1.3)
	19 F/B/C	22/118(18.6)	11/79 (13.9)
	23 A*	6/118(5.1)	5/79(6.3)
	23 F	11/118(9.3)	3/79(3.8)
	4	-	1/79(1.3)
	6A	34/118(28.8)	13/79(16.5)
	6B	21/118(17.8)	10/79(12.7)
	8*	-	1/79 (1.3)
	9V/A/N/L	6/118(5.1)	1/79 (1.3)
	19A	10/118(8.5)	-
	7 FA	2/118(1.7)	-
	3	1/118(0.8)	-

*Serotypes not included in the 13-valent pneumococcal conjugate vaccines.

References

1. O'Brien KL, Wolfson LJ, Watt JP, Henkle E, Deloria-Knoll M, McCall N, Lee E, Mulholland K, Levine OS, Cherian T: **Burden of disease caused by Streptococcus pneumoniae in children younger than 5 years: global estimates**. *Lancet* 2009, **374**(9693):893-902.
2. **Pneumococcal vaccines WHO position paper--2012**. *Releve epidemiologique hebdomadaire / Section d'hygiene du Secretariat de la Societe des Nations = Weekly epidemiological record / Health Section of the Secretariat of the League of Nations* 2012, **87**(14):129-144.
3. Weinberger DM, Malley R, Lipsitch M: **Serotype replacement in disease after pneumococcal vaccination**. *Lancet* 2011, **378**(9807):1962-1973.
4. Jroundi I, Mahraoui C, Benmessaoud R, Moraleda C, Tligui H, Seffar M, Kettani SC, Benjelloun BS, Chaacho S, Maaroufi A *et al*: **The epidemiology and aetiology of infections in children admitted with clinical severe pneumonia to a university hospital in Rabat, Morocco**. *Journal of tropical pediatrics* 2014, **60**(4): 270-8.
5. [http://www.hcp.ma/downloads/Annuaires-statistiques-regionaux_t11956.html.]
6. Jroundi I, Mahraoui C, Benmessaoud R, Moraleda C, Benjelloun B, Bassat Q: **Knowledge gaps on paediatric respiratory infections in Morocco, Northern Africa**. *Archives of public health = Archives belges de sante publique* 2015, **73**(1):28.
7. Mulholland EK, Simoes EA, Costales MO, McGrath EJ, Manalac EM, Gove S: **Standardized diagnosis of pneumonia in developing countries**. *Pediatr Infect Dis J* 1992, **11**(2):77-81.
8. Tarrago D, Fenoll A, Sanchez-Tatay D, Arroyo LA, Munoz-Almagro C, Esteva C, Hausdorff WP, Casal J, Obando I: **Identification of pneumococcal serotypes from culture-negative clinical specimens by novel real-time PCR**. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2008, **14**(9):828-834.
9. Warda K, Oufdou K, Zahlane K, Bouskraoui M: **Antibiotic resistance and serotype distribution of nasopharyngeal isolates of Streptococcus pneumoniae from children in Marrakech region (Morocco)**. *Journal of infection and public health* 2013.
10. Belabbes H, El Mdaghri M, Redouani A, Benbachir M: **Serotypes and antimicrobial susceptibilities of Streptococcus pneumoniae isolated in Casablanca between 1994 and 1997**. *Maroc medical* 2000, **22**(4):265-270.
11. Nuorti JP, Whitney CG: **Prevention of pneumococcal disease among infants and children - use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine - recommendations of the Advisory Committee on Immunization Practices (ACIP)**. *MMWR Recommendations and reports : Morbidity and mortality weekly report Recommendations and reports / Centers for Disease Control* 2010, **59**(Rr-11):1-18.
12. Zuccotti G, Mameli C, Daprai L, Garlaschi ML, Dilillo D, Bedogni G, Faccini M, Gramegna M, Torresani E, Ballerini E *et al*: **Serotype distribution and antimicrobial susceptibilities of nasopharyngeal isolates of Streptococcus pneumoniae from healthy children in the 13-valent pneumococcal conjugate vaccine era**. *Vaccine* 2014, **32**(5):527-534.
13. Adegbola RA, DeAntonio R, Hill PC, Roca A, Usuf E, Hoet B, Greenwood BM: **Carriage of Streptococcus pneumoniae and other respiratory bacterial pathogens in low and lower-middle income countries: a systematic review and meta-analysis**. *PloS one* 2014, **9**(8):e103293.
14. Gladstone RA, Jefferies JM, Tocheva AS, Beard KR, Garley D, Chong WW, Bentley SD, Faust SN, Clarke SC: **Five winters of pneumococcal serotype replacement in UK carriage following PCV introduction**. *Vaccine* 2015, **33**(17):2015-2021.