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ORIGINAL ARTICLE

Identification of the serotypes of bacterial meningitis agents; implication for vaccine usage

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ABSTRACT

Background and Objectives: Bacterial meningitis is one of the most serious infections and should be treated as emergency. As it has significant morbidity and mortality throughout the world, every country should have precise information regarding the etiological agents of disease and populations at risk to design public health prevention strategy. In the present study in addition of evaluation of common etiological agents (*Haemophilus influenzae*, *Neisseria meningitidis*, and *Streptococcus pneumoniae*) in bacterial meningitis cases, we sero-grouped or serotyped the obtained agents in order to predict the usefulness of existing vaccines against bacterial meningitis.

Materials and Methods: Cerebrospinal fluid of 182 suspected meningitis patients were collected, from which 114 cases were approved by biochemical, microbiological and molecular tests as bacterial meningitis. The isolated bacteria were serogrouped or serotyped to determine the dominant serotypes.

Results: *Streptococcus pneumoniae* accounted for 36%, *Haemophilus influenza* for 26% and *Neisseria meningitidis* for 14% of cases. From 13 serogroups of *N. meningitides*, the most frequent serogroups, were meningococcus group B (51%), C(24%) A (18%), Z(2%), W135 (1%) and 3% was not identified. In *H. influenzae* group only serotype b (100%) have been identified and in pneumococcal meningitis the most common serotype among our cases were 18C (44%) followed by14 (17%), 19A (13%), 6A (9%), 7F (4%), 4(3%), 3 (3%), 9V (2%), 8 (2%), 23f (2%), 5(1%).

Conclusion: Since there is no nationwide mass immunization program for common agents of bacterial meningitis in Iran, the result of this study can be used to improve the existing vaccines to cover the detected serotypes and consequently reduce the incidence of bacterial meningitis.

Keywords: *Bocterial meningitis, Neisseria meningitids, Streptococcus pneumoniae, Huemophilus inflhenzae, Vaccine*

INTRODUCTION

Inflammation of the brain and spinal cord protective coatings causes a disease called meningitis. It is caused by broad spectrum of factors, out of them bacterial meningitis (BM) is acute and fatal and needs to be treated urgently (1).

The disease is one of relatively common diseases for infants, so that, if it wouldn't be diagnosed urgently and correctly, healing timely would be along with fatality and many troubles like deafness, blindness, hydrocephalus, neurologic disorders, paraplegic and general paralysis, mental retardation, skin necrosis and many other cases (2-3). Additionally, ability of developing epidemic by specific causative organisms without precise evaluation of patients and emergency reporting to special care organization has made it problematic (3).

The main organisms meningitis are *S. pneumoniae*,

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Haemophilus influenza type b (Hib), and *Neisseria meningitidis* (4). Group B streptococci (GBS) and Gram-negative enteric bacilli are the main causative agents of this cerebral inflammation in neonates (4-6). The other less common causative agents are *Escherichia coli* and other Gram-negative enteric bacilli, including *Klebsiella*, *Enterobacter* and *Salmonella*, but they occur more commonly in nosocomial outbreaks in developing countries. In neonates, pathogens like *Listeria monocytogenes*, *Enterobacter sakazakii* and *Citrobacter koseri* occasionally give rise to this disease, especially during outbreaks (7-9).

Neisseria meningitidis is a Gram-negative, kidney shaped, encapsulated diplococcus, carried in the nasopharynx of adolescents and adults with low carriage rates in children <10 years of age. The peak incidence of invasive disease is between the ages of 6 months and 2 years, and ranges from mild disease to fulminate septicemia leading to death (10).

Risk of meningococcal meningitis increase with being immunosuppressive e.g. Asplenic, Complement system problems, or HIV patients and under treatment with immunosuppressive drugs. The crowded places like army, university dormitories are at risk for developing meningococcal meningitis, therefore *N. meningitidis* has potential to cause large epidemics (11-12). Meningococcus is classified into 12 serogroups based on the polysaccharide capsule. Five serogroups (A, B, C, W135 and Y) are causing agents of all meningococcal disease (10-11).

In developed countries, the incidence of invasive disease has been reported ~1–5 per 100 000, but incidences are much higher in the developing countries with poor populations (6, 11-12).

Serogroup A causes the majority of epidemic meningococcal infection in Africa and Asia. Serogroup B and C meningococci have been responsible for the most endemic meningococcal disease in European countries prior to vaccination (13). In the United States the approved state legislation which has begun on January 1, 2012 has declared that all entering college students have to be vaccinated or booster (if the vaccination is five years old) against bacterial meningitis before commencing their activity (14). This law is required for entering students at USA public and private colleges too. At present in United States, vaccines are approved and routinely used against serogroups C and Y A and W, but not B (15). In years 2002-2003, an outbreak of serogroup W135 has been developed amongst Hajj pilgrims and Burkina Faso outbreaks (16). After the epidemics caused by *N. meningitidis* W135 in 2000 and 2001, the Ministry of Health of Saudi Arabia required all pilgrims to be vaccinated with the tetravalent (A,C,Y,W135) polysaccharide vaccine and this has further highlighted the lack of availability of this vaccine (17-18).

Haemophilus influenzae type b is a Gram-negative coccobacillus that causes meningitis and respiratory infections, cellulitis, epiglottitis in all cases (adults and children) mainly in under 2 years old (19-20). In Many countries the rate of disease incidence has decreased markedly by including Hib conjugate vaccines in their routine childhood immunization programs and this pathogen as a cause of meningitis in young children in those countries has been eliminated. However, the vaccine is still too expensive (20-21)

Streptococcus pneumoniae is a major cause of community-acquired bacterial pneumonia, otitis media and meningitis (22). It has 91 known serotypes, with a limited number accounting for the majority of invasive disease isolates in specific geographic locations (23). The peak rate of both colonization and invasive disease occurs during the first 2 years of life, dropping during later childhood and rising again in old age (24). Mortality rates from meningitis are about 25-73% in children and 20-30% in adults of affected cases and are often characterized by neurological sequel in survivors.

isease rates are particularly high at the extremes of age, in patients with underlying chronic disease and in immunocompromised individuals, particularly those with HIV infection, where the incidence of disease is 50–100-fold higher (25-26). The cost of the conjugate vaccine is a disadvantage in developing countries, which carry the main burden of related disease (27).

Therefore, there is need for cheaper and more broadly cross-protective protein based pneumococcal vaccines is being pursued. In February 2000, a 7-valent pneumococcal protein–polysaccharide conjugate vaccine was licensed for use in USA. The first randomized controlled trial in >37 000 children showed that the 7-valent vaccine prevented 94% of invasive pneumococcal cases (27-28). Both this trial and a smaller study in Finland found a reduction of ~6% in cases of otitis media, with an increase in otitis media caused by non-vaccine type organisms. The 7-valent pneumococcal vaccine does not cover all

disease-causing serotypes, prompting the development of 9-, 11- and 13-valent vaccines (27). As mentioned before, worldwide, *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae* type b (Hib) most frequently give rise to bacterial meningitis in neonates and young children. Children older than 5 years of age and adolescents are mostly affected by meningitis which has been caused by *S. pneumoniae* and *N. meningitides* (28).

There has been reports of meningitis epidemic in our country in the years, 1966-7, 1981, 1996, arising from *N.* meningitidis (10%) in Tehran in which ages between 5 to 14 and male persons had the most percentage of affection (29). *Haemophilus influenza*, serotype b which is the most invasive type of *haemophilus* , has also been reported from more than 97% of meningitis among children Iran (30). Like countries, *Streptococcus pneumoniae* is one of other common agents of meningitis in Iran,. Considering that, no research has been conducted on the serotypes of etiologic agents of meningitis in Iran; this study has addressed this issue. The study samples has been collected in 19 months in collaboration with Departments of Infectious Diseases and Pediatric surgery of Mofid, and Takhti hospitals, Valiasr Infant's Medical Center, Shohada, and Akhavan hospitals

MATERIALS AND METHODS

Study design. An observational, descriptive study was intended to evaluate the prevalence of bacterial meningitis (BM) in a pediatric population in some hospitals of Tehran, during a 19 month period.

Patients. In coordination with the pediatric infectious disease unit of five hospitals in Tehran, the suspected patients clinical specimens (CSF) were received as emergency. All patients with clinical diagnosis of meningitis or with fever and leukocytosis or with increased levels of serum C-reactive protein were included in the study.

Acase of bacterial meningitis was defined as a child admitted to children's hospital in the study period with suspected meningitis and diagnosis of Streptococcus *pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae* type b using conventional bacteriologic and molecular methods. Clinical conditions preceding the present state, even vaccinations and any previous disease were recorded by cooperation of child parents or supporters.

Vaccination. In pneumococcal, Haemophilus and meningococcal vaccination, is not included in the vaccination program for the pediatric or adult population. National Vaccination for each one of mentioned bacteria is conditional and is sometimes suggested by individual physicians mainly for immune-compromised, travelers, pilgrims and soldiers.

Clinical specimens. CSF and blood specimens were obtained from children with symptoms of meningitis, as soon as they were admitted at hospital and were processed for culture and subsequent identification.

Microbiology. The isolated bacteria were identified using conventional techniques including Gram staining, growth in differential media (Tayer Martin, Chocolate agar, Blood agar, McCongey agar) and the morphology of the colonies and standard biochemical tests including Oxidase, Catalase, Sugar utilization, Optochin test and bile solubility, Bacitracin test, TSI, IMVIC, SIM and Urea) (31).

Latex agglutination test. In order to investigate soluble antigens in CSF specimen, Soluble antigens of *Haemophilus influenzae* type b, *Streptococcus pneumoniea*, *Nisseria meningitidis* serogroups in CSF were identified using Wellcogen™ Bacterial Antigen Kit (Murex Diagnostics Ltd, USA).

Serotyping. Serotyping of pneumococcus was performed using the capsular swelling procedure; quellung reaction (Statens Serum Institut, Copenhagen, Denmark) and in culture negative cases multiplex PCR method (SM-PCR) as described by Pai et al. in 2006 (32).

Statistical analysis. All variables were expressed as mean ± SD. Pearson's chi-square test and the McNemar test were used for statistical analysis. P values less than 0.05 were considered statistically significant. SPSS version 17 software was used for analysis of data.

RESULTS

Children with meningitis. One hundred eighty two children with suspected meningitis [102 males (56%) and 82 females (44 %)] were

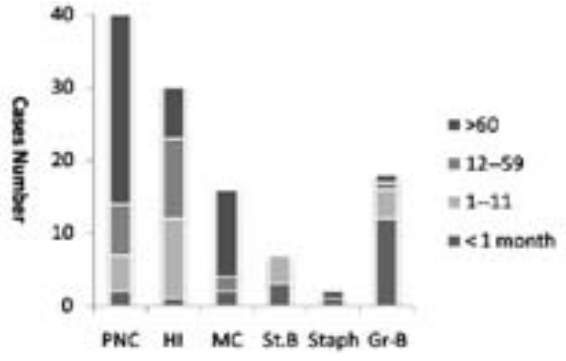

Fig. 1. Distribution of 114 cases of Bacterial meningitis according to age.

investigated in the study. Bacterial meningitis due to *N. meningitidis, H. influenzae,*and *S. pneumoniae* and group B streptococcus, and Gram negative bacteria were diagnosed in 114 patients admitted to 5 hospitals during 19 months. CSF specimens obtained from patients were analyzed by Biochemical and microbiological test according to the national protocol defined by the center of disease control of Iran (Table 1).

Of 182 cases of suspected meningitis, 114 [63 males (56 %), 51 (44 %) females] were diagnosed as having bacterial meningitis and 21 had got viral meningitis and the illness of remainder (n=47) were caused by diseases other than meningitis.

The agent most commonly associated with bacterial meningitis was *S. pneumoniae* (36% of cases), *H. influenza* (30 %) followed by Gram negative Baciili (17%) and *N. meningitidis* (14%), and group B streptococcus (6%). The case fatality rate of meningitis agents in the study were also verified (Table 2).

Age distribution and incidence of bacterial meningitis. The mean age of patients in this study was 61±40.2 months (range <1-166 months).The age distribution of Bacterial Meningitis BM patients is shown in Fig. 1. Larger studies are needed to give a correct estimate of BM prevalence in relation to patient age; however the present data suggest a higher frequency of BM when evaluated by different diagnostic method combinations (eg. biochemical, microbiology and PCR). On the other hand in our study we evaluated only patients admitted to five Tehran hospitals. There are other hospitals (governmental and private section) in Tehran which admit the patients too.

The incidence rate obtained by cultural methods was 91/114 (80%) cases which was lower than molecular methods (data not shown). The advantage of molecular method is because of the patients treated by antibiotics before diagnosis of BM and culture of CSF would be difficult to grow, but PCR in these types is valuable.

The main goal of this study was to evaluate the serotypes (and serogroups) of common agents of BM in the five governmental pediatric hospitals in Tehran and no other causes responsible of meningitis cases were assayed. The BM incidence relative to the different disease suspected to BM studied is mentioned in Fig .1.

Serotyping. Microbiological culture methods allowed serogroup or serotyping (Tables 3-5) in 91./114 patients (80 %). For the other 23 (20%; 23/114) culture-negative cases, the etiology was determined either by LAT or PCR . Of these 23 cases 2 were meningococcus A and 2 Haemophilus b, and 13 pneumococcus (17/23, 74%) by LAT. As there was sufficient amount of CSF of these 19 (13/23 pneumococcus and 6 unknown cases) unidentified samples, we performed conventional PCR and then multiplex PCR with 12 primer pairs for serotypes 23f,7c, 7f ,18c, 17f ,14 ,20 ,6a ,19a ,8 ,3 and 1 for serotyping the culture nagative pneumococcus strains (PCR of *cps* as internal control. was positive for 16 /16 ,100%) the result calculated as Table 5. Three (3/23, 13%) CSF samples were not positive with

Table1. Concentration of CSF Glucose, Protein and WBC, Neut, Ln cont.(%), and Serum CRP conc., and CSF to serum Glucose ratio.

Group \ Tests	CSF Glucose	CSF Protein	CSF WBC	%Neutrophil	%Lymphocyt	Serum CRP Conc.	CSF/Serum Glucose Ratio
						Mean ± SD	
Bacterial Meningitis	29.9[b]23.6±	268.6[a]333±	2858[a]3925±	79.5[a]19±	19.718.3±	125.4[a]88.9±	0.27[b]0.22±
Not bacterial Meningitis	**72.6±11**	**19.55.3±**	**1.852.17±**	**00±**	**00±**	**<80±**	**0.710.07±**

There was a significant increase between bacterial group and other groups. [a]P <0.001
There was a significant decrease between bacterial group and other groups. [b]P <0.001

Table 2. Causes of 114 Bacterial Meningitis cases and case fatality.

Organism	Number Of patients	Percentage*	Fatality
Streptococcus pneumoniae	40	36	5
Haemophilus influenzae	30	26	1
Gram negative- Bacilli	19	17	2
Neisseria meningitidis	16	14	3
Group B streptococcus	7	6	1
Other	2	2	0

*Frequency of isolated bacteria from CSF meningitis patients

neither PCR of *cps* nor multiplex PCR.

Serotype distribution of *Haemophilus Influenzae*, *Neisseria meningitidis* and *S. pneumoniae* causing meningitis in Tehran in a. 19 months study period is shown in Tables 3, 4, 5.

Based on these data only serotype b of *Haemophilus influenzae* is involved in bacterial meningitis. This serotype is one of the main causes of meningitis in children 1 to 60 months in our country. In our study, serotype B of *N. meningitdis* and serotype 18 of *S. pneumoniae* were involved in 50% and 44% of cases with meningitis.

DISCUSSION

Bacterial meningitis is a severe infection. Although the majority of cases with meningitis recover, it can cause serious complication, e.g. brain damage, deafness. It may cause even death unless the disease is treated successfully. Appropriate antibiotic treatment of meningitis would reduce the risk of death from meningitis, however the risk remains higher among young infants and the elderly population. There are other factors which reduce success of treatment, like resistance to antibiotics which should be noticed (33).

Use the recommended vaccines is the most effective way to protect children and adults against bacterial meningitis cuased by *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* type b (Hib) (34-35). However, limited information is available for bacteria causing meningitis in Iran. In this study, 114 patients were diagnosed with bacterial meningitis and the most frequent bacteria isolated were *S. pneumoniae* (36 %), which is similar with previous research by Ataee et al (36). However, a frequency of 11% for *Streptococcus pneumoniae* in CSF samples of meningitis patients have been reported in developed world (28, 34). The rational for this reduced rate could be due to their vaccination programs (34).

During our study period, *N. meningitidis* were only isolated from CSF of 16 (14%) patients who

Table 3. Serotype distribution of *Haemophilus Influenza* in 114 cases

H.Influenza serotype	a	b	c	d	e
Percent (%)	0	100	0	0	0

Table 4. Serogroup distribution of *Neisseria meningitis* in this study

Meningococcal serotype	A	B	C	W135	Z	Ntype
Percent (%)	19	51	24	1	2	3

Table 5. Serotype distribution of pneumococcus causing meningitis

Pneumococcal serotype	23f	5	19A	18C	14	9v	8	7F	6A	4	3
Percent (%)	2	1	13	44	17	2	2	4	9	3	3

were younger than mandatory vaccination age. This suggests that reduction in incidence of meningitis at the ages prior to vaccination program has occurred. It can be speculated that this discrepancy may be due to several reasons; including the poor laboratory detection of *N. meningitidis* as well as the mandatory vaccination programs for military recruits, pilgrimage to Haj. Accordingly, the mandatory military vaccination program should yield lower rates of meningitis in military aged-male subjects. But surprisingly the highest rate of meningitis is reported in 15-35 year old males (37-39).

An explanation for this dilemma, which is also supported by our results, could be the etiologic role of *Streptococcus pneumoniae* rather than *Neisseria meningitidis* in the reported rate of meningitis. On the other hand, there is an increase in bacterial meningitis after trauma (e.g. ear or sinus infection, car accidents, skull fractures, some surgeries) in children that mainly caused by *S. pneumoniae* including drug resistant strains (33). There are more than 91 types of pneumococcal bacteria and presently three PCV vaccines available on the global market: Prevnar (PCV-7), Synflorix(PCV-10) and Prevnar 13 **(**PCV-13**)**. The pneumococcal conjugate vaccine (PCV7) protects against 7 of them (4, 6B, 9V, 14, 18C, 19F, and 23F). These bacteria types are responsible for most common severe pneumococcal infections among children (34).

The PCV-10 vaccine contains ten serotypes of pneumococcus (1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F) which are conjugated to a carrier protein. The PCV-13 is a tridecavalent vaccine, meaning that it contains thirteen serotypes of pneumococcus (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F) which are conjugated to a carrier protein (40). This is the first time pneumococcal serotypes responsible for meningitis are being reported from Iran, despite earlier data from *Ataee et al*, who reported serotypes from invasive Pneumococcal Disease (IPD) (36).

According to the previous research by Ataee et al., in 2011, and present study the most frequent bacteria isolated were *Streptococcus pneumoniae* (35.67% in 2011, 36% 2013). Various researchers have reported different frequency of isolation of *Streptococcus pneumoniae* in developed countries. The reason for this difference may be due to vaccination programs. The incidence of pneumococcal meningitis declined significantly after the pneumococcal conjugate vaccine was added to the world infant immunization schedule in the developed countries (1-7). With respect to developed countries success in preventing the increase of pneumococcal infections and serotypes coverage of PCV13 , the vaccination with mentioned vaccine in our region would be highly effeicient.

In Vaccination with PCV13, the number of cases with meningitis and otitis media caused by *S. pneumoniae* would be reduced. Our data concerning pneumococcal meningitis serotypes is similar with Surveillance studies of invasive pneumococcal serotypes causing disease in other developing countries that confirmed the 13-valent pneumococcal vaccine is effective against meningitis (34). Therefore, the use of the 13-valent pneumococcal conjugate vaccines for wider coverage of pneumococcal serotypes could be suggested (41-42]. The most detected serotypes in our study were 18C:44% and then 14:17% ,19A:13% which are included in PCV13. Our data indicate that vaccintion of PCV13 would prevent a large proportion of serious pneumococcal diseases meningitis.

In conclusion, Iran is among middle income countries but does not have the adequate infrastructure for conjugate vaccine production. This could be due to lack of precise incidence and surveillance data, which weakens the obligation for vaccination, whilst mortality or treatment expenses increases. The cost of the mentioned vaccines is a disadvantage in developing countries, which carry the main burden of related disease. Therefore there is need for cheaper and more widely cross-protective vaccines for children in Iran. According to our study results we suggest:

A. The use of vaccination in children under 7 years old which are exposed to Hib meningitis. The Hib vaccine has had a major impact on the incidence of Hib meningitis in developed countries. This vaccines have to be implemented in our country where disease burden is not low but unidentified.

B. In children >50 months (>4years) of age which are at risk of developing bacterial meningitis after accidents and truma the vaccination of these groups with pneumococcal conjugate vaccine would be beneficial.

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REFERENCE

1. Scheld WM, Koedel U, Nathan B, Pfister HW. Pathophysiology of bacterial meningitis: mechanism(s) of neuronal injury**.** *J Infect Dis* 2002; 186 Suppl 2:S225-233.
2. Osrin D, Vergnano S, Costello A. Serious bacterial infections in newborn infants in developing countries**.** *Curr Opin Infect Dis* 2004; 17:217-224.
3. Grimwood K, Anderson VA, Bond L, Catroppa C, Hore RL, Keir EH, et al. Adverse outcomes of bacterial meningitis in school-age survivors. *Pediatrics* 1995; 95**:**646-656.
4. WHO (2012) *Bacterial meningitis*. www.who.int/nuvi/meningitis.
5. Tunkel AR. Clinical Trials Report**.** *Curr Infect Dis Rep* 2001; 3**:**347-351.
6. Laving AM, Musoke RN, Wasunna AO, Revathi G. Neonatal bacterial meningitis at the newborn unit of Kenyatta National Hospital**.** *East Afr Med J* 2003; 80**:**456-462.
7. Stoll BJ, Hansen N, Fanaroff AA, Lemons JA. *Enterobacter sakazakii* is a rare cause of neonatal septicemia or meningitis in VLBW infants**.** *J Pediatr* 2004; 144**:**821-823.
8. Etuwewe O, Kulshrestha R, Sangra M, Riordan A. Brain abscesses due to Citrobacter koseri in a pair of twins**.** *Pediatr Infect Dis J* 2009; 28:1035.
9. Schuchat A, Robinson K, Wenger JD, Harrison LH, Farley M, Reingold AL, et al. Bacterial meningitis in the United States in 1995. Active Surveillance Team**.** *N Engl J Med* 1997; 337**:**970-976.
10. Pollard AJ. Global epidemiology of meningococcal disease and vaccine efficacy**.** *Pediatr Infect Dis J* 2004; 23**:**S274-279.
11. Miller E, Salisbury D, Ramsay M. Planning, registration, and implementation of an immunisation campaign against meningococcal serogroup C disease in the UK: a success story**.** *Vaccine* 2001; 20 Suppl 1**:**S58-67.
12. Dawson KG, Emerson JC, Burns JL. Fifteen years of experience with bacterial meningitis**.** *Pediatr Infect Dis J* 1999; 18**:**816-822.
13. Trotter CL, Andrews NJ, Kaczmarski EB, Miller E, Ramsay ME. Effectiveness of meningococcal serogroup C conjugate vaccine 4 years after introduction**.** *Lancet* 2004; 364**:**365-367.
14. Cohn AC, MacNeil JR, Clark TA, Ortega-Sanchez IR, Briere EZ, Meissner HC, et al. Prevention and control of meningococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP)**.** *MMWR Recomm Rep* 2013; 62:1-28.
15. University health service. *Bacterial Meningitis Vaccination Requirement* 2013 http://www.healthyhorns.utexas.edu/requiredvaccine/
16. Nathan N, Rose AM, Legros D, Tiendrebeogo SR, Bachy C, Bjorlow E, et al. Meningitis serogroup W135 outbreak, Burkina Faso, 2002**.** *Emerg Infect Dis* 2007; 13:920-923.
17. WHO.int/CSR (2003) *MeningGAR*. www.who.int/csr/resources/publications/.../MeningGAR_2003_10.pdf
18. Ahmed QA, Arabi YM, Memish ZA. Health risks at the Hajj. *Lancet* 2006; 367:1008-1015.
19. Davies EG, Elliman D, Hart AC, Nicoll A, Rudd P. Manual of childhood infections for the royal college of paediatrics and child health. W.B. Saunders (London) 2001; paper back, 514 pages.
20. McVernon J, Trotter CL, Slack MP, Ramsay ME. Trends in Haemophilus influenzae type b infections in adults in England and Wales: surveillance study. *BMJ* 2004; 329:655-658.
21. McVernon J, Howard AJ, Slack MP, Ramsay ME. Long-term impact of vaccination on Haemophilus influenzae type b (Hib) carriage in the United Kingdom**.** *Epidemiol Infect* 2004; 132:765-767.
22. Wardlaw TM JE, Hodge M .Pneumonia: the forgotten killer of children. pp. 40: World Health Organization; UNICEF; 2006:40.
23. Park IH, Pritchard DG, Cartee R, Brandao A, Brandileone MC, Nahm MH. Discovery of a new capsular serotype (6C) within serogroup 6 of *Streptococcus pneumoniae***.** *J Clin Microbiol* 2007; 45:1225-1233.
24. O'Brien KL, Wolfson LJ, Watt JP, Henkle E, Deloria-Knoll M, McCall N, et al. Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates**.** *Lancet* 2009; 374:893-902.
25. Boisier P, Mainassara HB, Sidikou F, Djibo S, Kairo KK, Chanteau S. Case-fatality ratio of bacterial meningitis in the African meningitis belt: we can do better**.** *Vaccine* 2007; 25 Suppl 1:A24-29.
26. Shields B. Prevnar (heptavalent pneumococcal conjugate vaccine): disease prevention in infants and children**.** *J Pediatr Health Care* 2001; 15**:**203-208; quiz 209-210.
27. Segal S, Pollard AJ. Vaccines against bacterial meningitis**.** *Br Med Bull* 2004; 72:65-81.
28. Jones ME, Draghi DC, Karlowsky JA, Sahm DF, Bradley JS. Prevalence of antimicrobial resistance in bacteria isolated from central nervous system specimens as reported by U.S. hospital laboratories from 2000 to 2002**.** *Ann Clin Microbiol Antimicrob* 2004; 3**:**3.
29. Tehran University of Medical Science protocols (1389) *Guideline of Meningitis surveillance*. http://health.tums.ac.ir/fa/620.aspx.
30. Nakhjavani FAH, F. Bonakdar Kalani, M. T. Kazemi, B. Detection of Type B in cerebrospinal fluid of suspected children with meningitis by PCR**.** *Medical Journal of The Islamic Republic of Iran* 2005; 19.
31. Brooks G, Caroll K, Butel J, Morse SA, Mietzner TA. *Jawetz Melnick&Adelbergs Medical Microbiology* 26/E edn.The McGraw-Hill Companies,Inc.N.Y 2013.
32. Pai R, Gertz RE, Beall B. Sequential multiplex PCR approach for determining capsular serotypes of

Streptococcus pneumoniae isolates**.** *J Clin Microbiol* 2006; 44:124-131.
33. Aspa J, Rajas O, Rodriguez de Castro F, Blanquer J, Zalacain R, Fenoll A, et al. Drug-resistant pneumococcal pneumonia: clinical relevance and related factors**.** *Clin Infect Dis* 2004; 38**:**787-798.
34. Hsu HE, Shutt KA, Moore MR, Beall BW, Bennett NM, Craig AS, et al. Effect of pneumococcal conjugate vaccine on pneumococcal meningitis**.** *N Engl J Med* 2009; 360:244-256.
35. Davis S, Feikin D, Johnson HL. The effect of *Haemophilus influenzae* type B and pneumococcal conjugate vaccines on childhood meningitis mortality: a systematic review**.** *BMC Public Health* 2013; 13 Suppl 3**:**S21.
36. Mehrabi Tavana A, Ataee RA. Invasive pneumococcal dsease (IPD) serotype frequency in Iranian patients**.** *Iran Red Crescent Med J* 2013; 15:740-742.
37. Mosavi-Jarrahi A, Esteghamati A, Asgari F, Heidarnia M, Mousavi-Jarrahi Y, Goya M. Temporal analysis of the incidence of meningitis in the Tehran metropolitan area, 1999-2005**.** *Popul Health Metr* 2009; 7:19.
38. Tavana AM AR. Meningococcal meningitis control in Iran: five year comparative study 2000-2004**.** *J Med Sci* 2009; 9**:**51-54.
39. Mehrabi Tavana A, Hosseini- Shokoh MJ, Gouya M, Mahmmodi Farahani M; Parhisgar SH; MD,Ansari M; MSc. The effects of vaccination against Meningococcal meningitis in Islamic Republic of Iran Military Forces during the years 1981 to 2009**.** *JAUMS* 2010; 8:186-192.
40. Miller E, Andrews NJ, Waight PA, Slack MP, George RC. Effectiveness of the new serotypes in the 13-valent pneumococcal conjugate vaccine**.** *Vaccine*; 29:9127-9131.
41. Zaidi AK, Khan H, Lasi R, Mahesar W. Surveillance of pneumococcal meningitis among children in Sindh, southern Pakistan**.** *Clin Infect Dis* 2009; 48 Suppl 2**:**S129-135.
42. Falade AG, Lagunju IA, Bakare RA, Odekanmi AA, Adegbola RA. Invasive pneumococcal disease in children aged <5 years admitted to 3 urban hospitals in Ibadan, Nigeria**.** *Clin Infect Dis* 2009; 48 Suppl 2**:**S190-196.