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Original article

Streptococcus pneumoniae invasive infections in Burkina Faso, 2007 to 2011

Infections invasives à Streptococcus pneumoniae *au Burkina Faso, de 2007 à 2011*

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Abstract

Objective. – We had for aim to determine the epidemiology of meningeal and lung invasive infections due to *Streptococcus pneumoniae* in Burkina Faso.

Material and methods. – We screened for *S. pneumoniae* with the usual bacteriology techniques and with real time polymerase chain reaction (rt-PCR) in 7917 samples of cerebrospinal fluid (CSF) and pleural fluid (PF) collected in the Ouagadougou Yalgado Ouedraogo Teaching Hospital, from 2007 to 2011.

Results. – *S. pneumoniae* was identified in 476 (6%) samples including 455 (5.7%) in CSF and 21 (0.3%) in PF. Sixty-seven percent of invasive infections occurred in patients 15 years of age or less, without any significant sex ratio difference. The infections occurred most frequently between January and August, with the first and most important peak between January and May (dry season) and the second peak between June and August (at the beginning of rain season). The introduction of rt-PCR proved the under diagnosing of invasive infections by usual bacteriological methods (latex agglutination assay and culture).

Conclusion. – Invasive pneumococcal infections occur mainly in patients 15 years of age or less, without any difference in sex ratio and with peaks in the dry season. Vaccinal schedules should include all age ranges in Burkina Faso.

Keywords: *Streptococcus pneumoniae*; Invasive infections; Burkina Faso

Résumé

Objectif. – Le but de ce travail était de déterminer les caractéristiques épidémiologiques des infections invasives méningées et pulmonaires dues à *Streptococcus pneumoniae* au Burkina Faso.

Matériel et méthodes. – *S. pneumoniae* a été recherché par des techniques de bactériologie classique et par PCR en temps réel (rt-PCR) dans 7917 échantillons de liquides céphalorachidiens (LCR) et de liquides pleuraux (LP) collectés au CHU Yalgado de Ouagadougou de 2007 à 2011.

Résultats. – *S. pneumoniae* a été identifié dans 476 (6 %) des échantillons analysés dont 455 (5,7 %) dans les LCR et 21 (0,3 %) dans les LP. Soixante-sept pour cent des infections invasives confirmées sont survenus chez les patients de 15 ans et moins. Aucune différence significative n'était associée au sexe des patients. Les cas surviennent entre janvier et août principalement, avec un premier pic entre janvier et mai (saison sèche) plus important que le second observé entre juin et août (début d'hivernage). L'introduction de la rt-PCR a montré une sous-notification des cas confirmés d'infections invasives pneumococciques par les techniques bactériologiques classiques (détection de l'antigène capsulaire soluble par des particules de latex sensibilisées et culture).

Conclusion. – Les infections invasives pneumococciques surviennent dans toutes les tranches d'âge, avec une fréquence plus élevée chez les 15 ans et moins, sans distinction de sexe et avec des pics en saison sèche. Les perspectives vaccinales devraient prendre en compte toutes les tranches d'âges des populations du pays.

Mots clés : *Streptococcus pneumoniae* ; Infections invasives ; Burkina Faso

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1. Introduction

Streptococcus pneumoniae is a major cause of morbidity and mortality, especially in children less than 2 years of age and geriatric patients [1,2]. It is responsible for invasive infections (pneumonia, meningitis, bacteremias, pleurisy, peritonitis) and non-invasive infections (acute otitis media, sinusitis) [1–6]. The number of pneumococcal infection was estimated at 14.5 million cases in children less than 5 years of age in 2000, including 95.6% due to pneumonia [2]. According to the WHO, pneumococcal infections cause 1.6 million deaths every year, including 0.7 to 1 million in children 5 years of age or less [1,7]. *S. pneumoniae* became the main bacterial cause of infections in children less than 5 years of age, after introducing the vaccine against *Haemophilus influenzae* b in developing countries [8,9]. The incidence of pneumococcal infections is usually higher in developing countries than in developed countries; 416 and 779 cases for 100,000 individuals respectively in children less than 5 years of age less and infants less than 3 months of age were reported in the South of Mozambique, from 2001 to 2003, compared to 3.1/100,000 individuals in children 4 to 5 years of age in Portugal, and 90.6/100,000 individuals in children less than 5 years of age in Spain, between 2002 and 2004 [10–12]. The yearly incidence of pneumococcal meningitis in children less than 5 years of age was 33/100,000 individuals in Burkina Faso, whereas it was 15 to 26/100,000 individuals in Ghana for all age ranges [13,14].

We had for objective to study the evolution of some epidemiological parameters of common invasive meningeal and pulmonary infections due to *S. pneumoniae*, in some sanitary districts of Burkina Faso.

2. Material and methods

2.1. Collecting samples

CSF samples collected on sterile dry tubes or in Trans-Isolate (T-I) transport medium, and in sterile cryotubes were received in the Bacteriology-Virology Department of the Ouagadougou Yalgado Ouedraogo Teaching Hospital (YO-TH), in Burkina Faso, for confirmation of suspected cases of acute bacterial meningitis. They were sent from 9 of the 13 sanitary districts of Burkina Faso: Center, Center-West, Center-South, Center-North, Central Plateau, East, North, Mouhoun loop, and Sahel. Some CSF samples were also received from medical units of the YO-TH.

Pleural fluid (PF) samples collected on sterile dry tubes were sent by healthcare institutions of the Center Region. The samples were collected in compliance with standard operating procedures defined by the Ministry of Health in Burkina Faso. The sample collection was made from January 2007 to December 2011.

2.2. Collecting socio-demographic and clinical data

Each sample came with an analysis bulletin including the patient's documented socio-demographic and clinical data, and a personal form of case notification.

2.3. Cytobacteriological examination of samples

CSF samples collected in dry tubes were examined macroscopically, microscopically after Gram staining, and submitted to quantitative and qualitative cytological examination when necessary. Each CSF sample was inoculated systematically on chocolate agar enriched by Polyvitex® (Bio-Mérieux, France) to screen for common agents of acute bacterial meningitis (*Neisseria meningitidis*, *S. pneumoniae*, and *H. influenzae* b). The medium was then incubated at 37 °C in humid air enriched by 5 to 10% of CO_2 for 18 to 24 h.

Screening for soluble bacterial antigens in the CSF was performed with a latex particle agglutination test with the PASTOREX meningitis kit (Bio-Rad, France) to allow detecting capsular antigens of *N. meningitidis* A/B/C/Y/W135, *Escherichia coli* K1, *H. influenzae* b, *S. pneumoniae*, and *S. agalactiae*.

After receiving and recording the inoculated T-I medium, they were aired and incubated at 37 °C before microscopic examination to screen for bacteria and reseeded on appropriate media to isolate the pathogens, specifically chocolate agar for encapsulated Gram-positive diplococci suggesting pneumococci.

PF samples were analyzed by the same procedures as for CSF with, nevertheless, additional inoculation on Columbia agar when necessary.

The fine colonies yielding egg yolk-like hemolysis on chocolate agar supplemented or not by Polyvitex™ (Bio-Mérieux, France) were considered as suspicious: they were examined microscopically after Gram staining to screen for encapsulated Gram-positive diplococci. The identification of these colonies suspected to be encapsulated Gram-positive diplococci was completed by studying their susceptibility to optochin 5 μg (Bio-Mérieux, France) on blood agar, and the lysis of cultures by a solution of sodium deoxycholate (LABOSI, France). The other bacteria were identified according to standard operating procedures used in the YO-TH Bacteriology Department.

The identified *S. pneumoniae* strains were kept at −80 °C in a sterile solution of skim milk and glycerol.

2.4. Molecular analysis of CSF samples

Eight hundred and eight CSF samples in sterile cryotubes were received by the laboratory during the study period, and analyzed by rt-PCR adapted according to the Atlanta CDC protocol (GA, USA) [15]. The various steps of this analysis were as follows:

- *DNA extraction*: the bacterial DNA was extracted from 200 μl of CSF according to the Qiagen kit protocol (QIAamp® DNA Mini Kit, Ref. 51306. Qiagen SA, France);
- *preparation of the reagent mixture and DNA amplification*: 25 μl of the reagent mixture containing 2 μl of each of the 2 primers were pipetted into each of the micro plate's 96 wells: sense (CDC, Ref. No. 1019290) and antisense (CDC, Ref. No. 1019291), 2 μl of probe (CDC, Ref. No. 1019301), 12.5 μl of TaqMan® Universal PCR Master Mix (ABI, No. 4304437), 4.5 μl PCR quality water