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Antimicrobial Resistance and Serotype Distribution of *Streptococcus pneumoniae* Strains Causing Childhood Infection in Burkina Faso

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Abstract: In Burkina Faso, a Western African country, reports on pneumococci carriage, resistance patterns and serotypes are inconsistent. The present study was conducted in order to evaluate these parameters. Thus 860 nasopharyngeal swabs were collected from children attending vaccination centers for pneumococci isolation, identification and serotype determination. The susceptibility to 16 antibiotics was assayed as recommended by the National Committee for Clinical Laboratory Standard (NCCLS). The results revealed that the majority of children were of 2 to 24 months age and 73.4% of children were well vaccinated. A carriage rate of 50.6% was recoded among the children. The main serotypes were: 6 (22.22%); 23 (16.67%); 7 and 9 (3.70%); 4, 11, 14, 15, 20 and 24 (1.85%). Serotypes19, 23, 6, 7 and 18 were linked to penicillin resistance. Globally, high resistance rates to: amikacin, tetracyclin, pefloxacin, cotrimoxazol and penicillins (resistance rates greater than 25%) were recorded; however the following antibiotics remained active on the strains: rifampicin, ceftriaxone, erythromycin, spectinomycin, chloramphenicol, vancomycin, lincomycin and ciprofloxacin.

Key words: *Streptococcus pneumoniae*, antibiotics, resistance, serotypes, Burkina Faso

INTRODUCTION

Streptococcus pneumoniae is the leading agent of childhood pneumonia by causing 20 to 40% of the global annual 4 million Respiratory Tracts Infections (RTI)-related deaths of children below 5 years (Saha *et al*., 1999). This microorganism is also the common cause of meningitis among children in developing countries (Yaro *et al*., 2006; Traore *et al*., 2009; WHO, 2009). For the treatment of such infections clinicians have long time relied on the use of penicillin, ampicillin and cotrimoxazole in developing countries as recommended by the World Health Organization.

In Burkina Faso as in many of these countries, empirical therapy is still the rule rather than exception, indeed, health care workers give cotrimoxazole tablets to patients with community acquired pneumonia. However, information about the antimicrobial susceptibility from most of developing world which could potentially benefit the design of RTI control programs is severely deficient. In fact, the majority of these countries have no antibiotic resistance surveillance system, of course in Burkina Faso few studies have reported the susceptibility of urinary tract infection bacteria (Karou *et al*., 2009), gastroenteritis bacteria (Bonfiglio *et al*., 2002; Simpore *et al*., 2009), resistance pattern and genotyping of *Streptococcus pneumoniae* (Bere *et al*., 2005, 2006) but there is a lack of data on *Streptococcus pneumoniae* serotypes distribution and their susceptibility to antibiotic in the country.

It is evident that resistance to β-lactams, macrolides and trimethoprim-sulfamethoxazole (SXT) continues to increase among clinical isolates of *Streptococcus pneumoniae*. This trends coupled with the potential for increasing resistance to fluoroquinolones has prompted surveillance studies in developed countries (Karnezis *et al*., 2009; Campa *et al*., 2009; Hanage *et al*., 2009). Although, evolving patterns in antimicrobial

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resistance suggest an exigency for such surveillance studies, the frequency with which these studies are needed has not been addressed (Saha *et al*., 1999). Since developing countries have not appropriate monitoring systems to conduct nationwide studies, institutional or city levels studies are the only means for the moment to ascertain for the current situation of antibiotic resistance, in order to prescribe adequate therapy. We herein report the antibiotic resistance and the serotypes distribution of *Streptococcus pneumoniae* strains causing childhood infections in Bobo-Dioulasso town in Burkina Faso.

MATERIALS AND METHODS

Antimicrobial tests were conducted using Muller Hinton agar disk diffusion as recommended by the national Committee for Clinical Laboratory Standards (NCCLS, 2003).

Serotype determination was performed by capsular swelling method using antisera of Statens-serum Institute (Copenhagen, Denmark) (Sørensen, 1993).

Study area and children: The present study was conducted from September to December 2000 and from January to June 2001, in Bobo-Dioulasso, the second town of Burkina Faso in western Africa. The town is located in western Burkina faso in sub Saharan climatic zone, the population is about 500 000 habitants. Eight hundred and sixty healthy children aged from 0 to 5 years regardless of sex, attending the two major maternal and child health centre of Bobo-Dioulasso for vaccination were included in the study after free agreement consent of the parents. A written consent to screen children was obtained from parents; they latter completed a short questionnaire related to family size, age of sibling, travel, hospital admissions and antibiotics use in the preceding 3 months. A child was considered as well vaccinated in accordance with his age; he received all vaccines Vaccination Program of Burkina Faso. Local public-health authorities gave ethical approval for the study.

Microbiological methods

Microorganism isolation: From each child, anterior nares were swabbed with sterile calcium alginate swabs which were plated immediately onto 5% sheep blood agar +5 mg L^{-1} of gentamicin. Samples were transported within the 3 hours after collection to laboratory. Specimens were then plated onto Columbia agar plates supplemented with 5% sheep-blood agar and 5 mg L^{-1} gentamicin (Becton Dickinson, Milan, Italy) and plates were afterwards incubated at 37°C in 5% CO_2 atmosphere for 24 h. Alpha-hemolytic colonies were identified as pneumococci by their typical colony morphology, susceptibility to optochin disk (ethylhydrocupreine) and positive bile solubility test. Strains were subcultured for 18 to 24 h and from subcultured strains; an overnight growth specimen was suspended in serum glycerol freezing medium and stored at-70°C for serotyping studies. The rests of subcultures were directly used for the susceptibility tests.

Antibiotic susceptibility tests: Antimicrobial tests were conducted using Muller Hinton agar disk diffusion as recommended by the national Committee for clinical laboratory standards (NCCLS, 2003). Plates were incubated overnight at 37°C in 5% CO_2. The antimicrobial activity was assayed by measuring the inhibition zone diameter around the disk. Interpretation was made using the NCCLS breakpoints (NCCLS, 2003). All isolates were screened for penicillin G resistance using 1 μg of oxacillin diskc. The following antibiotics were tested: oxacillin, lincomycin, ampicillin, spectinomycin. Pefloxacin, ciprofloxacin, amikacin, amoxcillin+clavulanic acid, amoxicillin, vancomycin, chloramphenicol, ceftriaxon, erythromycin, tetracyclin, cotrimoxazol and rifampicin. All the disks were purchased from Bio-Merieux or Sanofi Diagnostics Pasteur. *Streptococcus pneumoniae* A.T.C.C. 49619 was used as control. Strains that resisted to at least three antibiotic classes were considered as multidrug resistant (MDR).

Determination of serotypes: Frozen strains were transported to Lausanne (Switzerland) hospital for identities confirmation using inhibition zone diameter around optochin disk and by positive bile solubility test. Serotype determination was performed by capsular swelling method using antisera of Statens-serum Institute (Copenhagen, Denmark).

Among our research samples, we had strains that were not typed as:

- The strains non-typable serotype (NTS) that had the capsules already inflated because our typing technique uses the swelling of the capsule followed by a reading at the inverted microscope
- The non-vaccine serotypes (NVS) were not also taken into consideration because the antibodies that we used mainly targeted vaccine serotypes

Statistical analysis: Data's processing and analysis were performed using Epi-Info version 6.01. Statistical significance was set at $p<0.05$.

RESULTS

Epidemiological and clinical data: A total of 860 children coming for vaccination in the two centers were diagnosed for the presence of pneumococci. Indeed 860 swabs were

Table 1: Percentage of isolated *Streptococcus pneumoniae* serotypes according to children age groups

Serotypes	0-2 (n = 8)	2-24 (n = 216)	24-60 (n = 8)
4	50	1.85	-
6	-	22.22	-
7	-	3.70	-
9	-	3.70	-
11	-	1.85	-
14	-	1.85	-
15	-	1.85	-
17	-	5.56	-
18	-	5.56	-
19	-	3.70	-
20	50		-
23	-	16.67	-
24	-	1.85	-
NT	-	16.67	-
NV	-	12.97	100

-: Indicates no isolates obtained, NTS: Non-typable serotype, NVS: Non-vaccinable serotype

collected and analyzed; 440 were collected in the first centre and 420 in the second. Total carriage rate in the global sample was 50.6% (N = 436) and distributed as follows: 48.18% (N = 212) and 53.33% (N = 224) for the first and the second centre, respectively. Microbiological analyses for serotype determination and antibiotic susceptibility were performed on the 232 well stored samples. This allowed us to divide the positive pneumococci children into three age groups: 0 to 2 months: 8 children, 2 to 24 months: 216 children and 24 to 60 months: 8 children. The medium age was 13±0.19 months. The determination of serotypes revealed that 13 major serotypes could be identified from a total of 232 strains tested. These were serotypes 4, 6, 7, 9, 11, 14, 15, 17, 18, 19, 20, 23 and 24. Table 1 lists the identified serotypes according to age groups. According to the table, in the 0 to 2 months children only two serotypes, serotype 4 and serotype 20 were identified. The majority of identified serotypes were in 2 to 20 months aged children. In this age group, serotype 6 followed by serotype 23, non typable serotypes and non vaccinable serotypes were identified as the most represented and being at the same time the most represented serotypes in the study. Only non vaccinable serotypes were isolated from children aged from 24 to 60 months. For the vaccinal status, 73.4% of children were considered as well vaccinated according to the National Vaccination Program and this status was highly correlated with the susceptibility to oxacillin.

Susceptibility to antibiotics: A total of 16 antibiotics were tested in the present study. For the interpretation all intermediary resistant strains were considered as totally resistant. The percentages of resistant strains and the serotypes linked to the resistance are displayed in Table 2. Globally, resistance to penicillins tetracycline, cotrimoxazole, amikacine and pefloxacine were common in the serotypes with resistance rate greater than 25; however few antibiotics such as rifampicin, vancomycin, erythromycin, ceftriaxon, spectinomycin and ciprofloxacin remained active on the pneumococci with resistance rates under 10%. Among the tested serotypes, Serotypes 19, 23, 6, 7 and 18 were those who have developed multirestance to antibiotics. Serotype 6 was linked to resistance to all the tested antibiotics.

Table 2: Percentage of resistant strains and serotypes liked to antibiotic resistance

Antibiotics	Resistant strains (n = 232)	Serotypes
Ampicillin	26.81	6, 7, 18, 19, 23, 24
Amoxicillin	25.00	6, 7, 18, 19, 23, 24
Amoxicillin/clavulanic acid	25.00	6, 7, 18, 19, 23, 24
Oxacillin	3.85	6, 7, 18, 19, 23, 24
Ceftriaxone	6.74	
Tetracycline	65.30	4, 6, 7, 9, 14, 17, 18, 19, 20, 23
Cotrimoxazole	31.70	6, 14, 18, 19, 20, 23, 24
Erythromycin	6.10	6
Lincomycin	17.30	6, 23
Amikacin	95.19	6, 7, 18, 19, 23, 24
Spectinomycin	7.70	6, 23
Ciprofloxacin	12.22	6, 23
Pefloxacin	54.80	6, 7, 18, 19, 23, 24
Chloramphenicol	8.70	4, 6, 19, 23
Rifampicin	1.28	6
Vancomycin	12.33	6

DISCUSSION

In our previous research, we have rarely found resistance to vancomycin and rifampicin. While in this study, we isolated the serotype 6, which is resistant to both types of antibiotic. In addition, we found that serotype 6 is also resistant to other 16 antibiotics tested. Thus, our results are different from those of previous study carried out in Burkina Faso, because our investigation shows pneumococcal strains that are susceptible or resistant to antibiotics tested (Table 2).

Nasopharynx of children is the main ecological reservoir of pneumococci and other germs such as *Haemophilus influenzae* and *Moraxella catarrhalis*. From this reservoir *Streptococcus pneumoniae* can become invasive and cause sepsis, meningitis, osteomyelitis or can spread to adjacent mucosal tissue and provide infections such as otitis, sinusitis and pneumonia. *Streptococcus pneumoniae* can also be transmitted by direct contact or through aerosols from other individuals. Therefore many individuals are colonized by pneumococci at any given time and most children are colonized at some point during the first years of life. The present study aimed to evaluate the pneumococci carriage rate, their serotype distribution and

their resistance patterns among young children in Bobo-Dioulasso. Our results revealed that the carriage rate was similar to that found by Ronchetti *et al.* (1998) who reported that over 95% of children had a nasopharyngeal carriage of *S. pneumoniae* before 2 years and 30 to 60% of children have it at any time. We noticed that prevalence rate of penicillin-resistant principally ampicillin and amoxicillin strains was over 25% and many serotypes were multiresistant.

Resistance to penicillin in pneumococci has been commonly reported in 1970s, however their resistance to vancomycin and rifampicin is more recent (Guillemot and Carbon, 1999). Present results showed that 2.73 and 1.2% of isolates resisted to vancomycin and to rifampicin, respectively. Epidemiological monitoring of resistance and rational use of antibiotics are then necessary in Burkina Faso in order to avoid the spread of resistance.

Several serotypes were isolated, according to their prevalence they could be classed as follows 6, 23, 17, 18, 7, 9, 4, 11, 14, 15, 20 and 24. Similar findings were reported by Sniadack *et al.* (1995). According to their results, serotypes 6, 14, 8, 5, 1, 19, 9, 23, 18, 55 are the most frequently isolated serotypes in developing countries and serotypes 14, 6, 19, 18, 9, 23, 7, 4, 1 are mostly found in invasive diseases, mucosal infections and carriage in developed countries.

These data indicate that serotypes responsible for frequent infections in developed countries differ from those in developing countries. This difference may probably be due to extreme susceptibility of children in developing countries; susceptibility which may be linked to associated germs and diseases such as *Haemophilus* and *Plasmodium* and poor hygiene and low sanitation. The main serotypes were 6 and 23, both representing approximately 58.33% of all isolates. Most of resistant strains belonged to these limited numbers of serotypes which were also among the most common causes of paediatric infections (Dagan *et al.*, 1994; Sniadack *et al.*, 1995). Serotype 6 was linked to resistance to vancomycin, rifampicin, erythromycin alone and it was both with serotypes 19, 23 associated to resistance to penicillin and chloramphenicol. The two serotypes 3 and 23 were more likely linked to resistance to penicillin and to multidrug resistance. The same finding were reported in Spain (Fenoll *et al.*, 1998).

This study showed clearly that 92.3% of serotypes found in Burkina Faso belonged to serotypes covered by 23 valences polysaccharides vaccine. However, even if we have a good coverage of serotypes, because of their low immunogenicity, polysaccharides vaccines are not indicated for young children. In addition, these vaccines have no effect on the nasopharyngeal carriage and have a limited impact on invasive strains in children below 2 years (Dagan *et al.*, 1999).

Conjugate vaccine with 7 valences covered 53.84% of isolated serotypes in Burkina Faso. This vaccine is immunogenic in children and has a significant impact on carriage and invasive strains (Klein and Eskola, 1999; Obaro *et al.*, 1996). This could be beneficial in areas of high resistance to penicillin such as Burkina Faso, but the disadvantage of this vaccine is that it contains very few serotypes isolated in Africa (Dagan *et al.*, 1999). This fact was noticed by Hausdorff *et al.* (2000), who found that conjugate vaccine with 7 valences contained only 4 of the 7 serotypes/serogroups most frequently isolated in Asia and Africa.

It may be concluded that, considerable antimicrobial resistance has been developed by *S. pneumoniae* strains carried by children attending vaccination centres in Burkina Faso. Nationwide usual surveillances are necessary to extend theses findings to invasive strains and to other parts of the country. The issue of these studies may also allow rationale antibiotics prescribing in the community in order to limit the emergence and the spread of resistance as we found for vancomycin.

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