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POLYMERASE CHAIN REACTION (PCR) PROVIDES A SUPERIOR TOOL FOR THE DIAGNOSIS OF PNEUMOCOCCAL INFECTION IN BURKINA FASO

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ABSTRACT

Purpose of study: The aim of this study was to determine the value of real-time Polymerase Chain Reaction (rt-PCR) in the routine surveillance of pneumococcal meningitis in Burkina Faso, compared to standard methods of culture, Gram stain and latex agglutination assay.

Materiel and methods: A total of 385 specimens of cerebrospinal fluid were analyzed by the three standard bacteriological methods (Gram stain, latex agglutination assay, and culture) and real-time Polymerase Chain Reaction.

Results: Of 385 specimens analyzed by these methods, 204 *S. pneumoniae* were detected by one or more methods. Gram stain detected 36.4% (140/385) Gram positive encapsulated diplococci; 37.7% (145/385) and 20.8% (80/385) of the specimens were positive for pneumococci by latex agglutination assay and culture. These specimens were tested with rt-PCR, which confirmed 51.2% (197/385) *S. pneumoniae* positive. The sensitivity and specificity of culture were 54.4% and 31.5%, respectively, and the sensitivity and specificity of rt-PCR were 96.6% and 100%, respectively. These results showed that rt-PCR was more sensitive than Gram stain ($p=0.0235$), latex agglutination assay ($p=0.0442$)and culture ($p=0.0006$).The culture is the gold standard method; however, the result showed that rt-PCR had specificity and was as specific as Gram stain ($p=0.3405$) and latex agglutination assay ($p=0.7745$).

Conclusion: rt-PCR was highly sensitive and specific. It could be used as a complementary diagnostic tool to improve case confirmation of bacterial meningitis. However,its high cost, the qualification of the technical staff and infrastructures required for its implementation, constitute obstacles to its widened use in countries with limited resources.

Keywords: *Streptococcus pneumoniae*, meningitis, rt-PCR, standard bacteriological methods

REACTION DE POLYMERISATION EN CHAINE, UN OUTIL SUPERIEUR POUR LE DIAGNOSTIC DES INFECTIONS PNEUMOCOCCIQUES AU BURKINA FASO

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RESUME

Objectif: Le but de cette étude était de déterminer la place de la rt-PCR dans la surveillance de routine de méningitespneumococciquesau Burkina Faso et la comparée avec les méthodes de la bactériologie classique: Culture, coloration de Gram et l'agglutination au latex.

Matériel etméthodes: Au total, 385 échantillons deliquides céphalorachidiens (LCR)étaient analysés par les trois méthodes de la bactériologie classique (coloration de Gram, agglutination au latex, culture) et la PCR en temps réel.

RESULTATS: **Parmi 385 échantillons analysés, 204 cas de *Streptococcuspneumoniae* étaient détectés par une ou plusieurs méthodes. La coloration de Gram adétecté 36,4% (140/385) diplocoques encapsulés à Gram positif (DGP); 37,7% (145/385) et 20,8% (80/385) d'échantillons étaient positifs aux pneumocoques par l'agglutination au latex et la culture. Ces échantillons étaient aussi testés par rt-PCR qui a confirmé 51,2% (197/385) cas positifs de *S. pneumoniae*. La sensibilité et la spécificité de la culture étaient respectivement de 54,4% et 31,5%, et la sensibilité et la spécificité de rt-PCR étaient respectivement de 96,6% et 100%. Ces résultats ont montré que la rt-PCR était plus sensible que la coloration de Gram (*p=0,0235*), l'agglutination au latex (*p=0,0442)* et la culture(*p=0,0006*).La culture est une méthode de référence; cependant, le résultat a montré que rt-PCR était plus sensibleet aussi spécifique que la coloration de Gram ((*p=0,3405*)et l'agglutination au latex (*p=0,7745*).**

Conclusion : :rt-PCR était plus sensible et plus spécifique. Elle pourrait être utilisée comme un outil de diagnostic complémentaire pour améliorer les cas de confirmation de méningites bactériennes. Cependant, ces coûts de réalisation, la qualification de techniciens et les matériels pour son application constituent des obstacles pour sa vulgarisation dans les pays à ressources limités.

Keywords: *Streptococcus pneumoniae*, méningites, rt-PCR, méthodes de la bactériologie classique

INTRODUCTION

Streptococcus pneumonia (pneumococcus) is a common pathogen associated with benign or severe infections including acute otitis media, meningitis, septicemia and pneumonia (1). According to WHO annual reports, 1.6 million cases of fatal pneumococcal disease occurredworldwide annually, mostly in infants and elderly(2, 3). However, many countries have implemented pneumococcal conjugate vaccine during the last few years and number of cases has declined significantly (4-7). *S. pneumonia* is fastidious and sensitive to temperature variation and thereforerequires rapid and accurate identification for proper and timely antibacterial therapyand epidemiologic surveillance. Bacterial etiology is typically identified and characterized usingstandard methods such as Gram stain, latex agglutination assay, culture, susceptibility to optochin (ethyl hydrocupreine hydrochloride)and bile solubility(8-10).The probability to detect pneumococcus in CSF by Gram stains and culture is determined by the bacterial concentration in a CSF specimen (8). Gram stain is a simple, rapid and inexpensive method but the probability to detect pneumococcus in CSF by this methodwas determined by the bacterial concentration in a CSF specimen (8). Although, the culture is cheap, it is difficult to perform on every specimen collected. In many developing countries, surveillance for bacterial meningitis is hampered by limited use of culture and a high frequency of negative cultures (11). Toovercome the limitations of the standard bacteriological methods, molecular methodswere introduced in many countries to improve the detection of etiologic agents. In Africa, particularly in Burkina Faso, multiplex PCR was implemented to identify *Neisseria meningitidis*, *Haemophilusinfluenzae* serotype b and *S. pneumonia* simultaneously in an effort to evaluate the usefulness of PCR technology in meningitis surveillance(12). In another study, PCRidentified 27% pneumococci fromCSF specimens but cultureand latex agglutination assayonly detected 9% and 15%, respectively (13): the results reported in these studies showed that PCR were more sensitive than culture and latex agglutination assay. The aim of this study was to determine the value of rt-PCR in the routine surveillance of pneumococcal meningitis in Burkina Faso, compared to standard methods of culture, Gram stain and latex agglutination assay.

MATERIAL AND METHODS

Study sites

The study was conducted in the Bacteriology and Virology Departmentof the Teaching Hospital YalgadoOuedraogo (TH-YO)in Ouagadougou, Burkina Faso. The specimens were collectedfrom ninemedicalregions of Burkina Faso: Center, Centre-West, Center-South, Center-North, Central Plateau, East, North, Boucle of Mouhoun and the Sahel, from March 2010 toDecember 2012.

Clinical specimen collection

The cerebrospinal fluid (CSF) was obtained from patients with suspect meningitis. A case of suspect meningitis was defined by rapid onset of fever (>38.5°C rectal or >38.0°C axillary) followed by stiff neck, headache, altered consciousness, bulging fontaneland convulsion for infant, vomiting and coma, and a confirmed meningitis that was laboratory-confirmed by growing (culturing) or identifying (by Gram stain, antigen detection, or PCR) a bacterial pathogen (*S. pneumoniae*) in CSF of patients with a clinical syndrome consistent with bacterial meningitis (14). Lumbar puncture was performed for the cases of suspect meningitis and three or five milliliters (3-5mL) of CSF specimen werecollected and transferred into asterile tube for laboratory confirmation. The specimens collected at the teaching hospitalof Ouagadougou weretransported to the laboratory in less than one hourafter collection.

For the specimens coming from other medical regions, 1 mL of CSFwas inoculated intoTrans-Isolate (T-I) medium for cultureand 0.5mL of CSF into cryotubes for PCR in less than one hour after collection. All cryotubes were stored at -80°C until their analyses.

Analyses of CSF specimens

Gram stain

Gram stain was conducted either in the department of bacteriology and virology laboratory on CSFs collected at the teaching hospital YalgadoOuedraogo or in the local laboratories on CSFs collected in other medical regions. The Gram stain results were reported on the notification forms before transferring the CSFs and forms to the bacteriology and virology department in the teaching hospital.

A CSF was considered positive for *S. pneumonia* if Gram positive diplococcic and/or cocci encapsulated in short chains were observed during the microscopic examination with a clear halo around the bacterial cells. A Gram stain was considered negative if no bacteria or any other types of bacterial cell morphology wereobserved.

Latex agglutination assay

The PASTOREXTMMeningitis kit (Bio-Rad, France) was used country wide in Burkina Faso for the direct detection of the capsular antigen of *S. pneumoniae.* The test was performed according to the manufacturer's instruction.

Bacterial isolation and identification

To isolate *S. pneumoniae*, 1or 2 drops of CSF specimenor inoculated T-I medium wasplatedontoa chocolate agar plate and/or ontoa blood agar plate (Trypticase-soya agar supplemented with 5% sheep blood). All plates were incubated overnight at 37°C, with 5% CO_2.

Colonies with typical pneumococcal morphology (moist or sometimes mucoid, gray, central depression) were tested for catalase andα-hemolysis on blood agar plate or egg yolk-like hemolysis on chocolate agar plate. Susceptibility to optochin (5µg; Bio-Merieux, France)was performed on the catalase-negative and α-hemolytic colonies for the presumptive identification. Any isolates that produced an inhibition zone with diameter equalor largerthan 14mmwere considered susceptible.Isolates with smaller inhibition zone or without inhibition zone were tested with the bile solubility test using 2% sodium deoxycholate.

An isolate was identified as *S. pneumonia* if it was Gram-positive for pneumococci, producedα-hemolysis on blood agar plate or egg yolk-like hemolysis on chocolate agar plate, was catalase negative and optochin-susceptible or solubilized by bile salt solution when resistant to optochin. All *S. pneumonia* strains isolated were stored at -80°C in 10% Skim milk glycerol.

Real-time PCR (rt-PCR)

-DNA Extraction:100uL of saline containing 0.04g/mL of lysozyme (Sigma-L-6876) and75U/mlof mutanolysin (Sigma-M9901) was pipetted into themicrocentrifuge tube.Then, 200µl of CSF was added into the microcentrifuge tube. DNA extraction was performed using QIAamp® DNA Mini Kit (Qiagen S.A., France) according to the manufacturer instructions. Extracted DNA samples were stored at -20°C.

-DNA amplification: The *lytA*rt-PCR, targeting the autolysin gene*lytA*, was performed as described previously (15), with the following modifications. The assays were carried out in a final 25µl reaction volume and were performed using12.5µl of Master Mix TaqMan® Universal PCR (Applied Biosystems), with 2µl of sample extracted DNA. Forward primer, reverse primer, and probe for each target gene were used in concentrations of 200 nM(15). The PCR cycling conditions were 1 cycle of 50°C for 2 minutes, 1 cycle of 95°C for 10 minutes, followed by 50 cycles of 95°C for 15 seconds and 60°C for 1 minute. The thermal cycler Stratagene Mx3005PTM (Agilent La Jolla Technologies, California, USA) was used for PCR amplification. The results were analyzed by MxPro (Mx3000P/Mx3005P) software.

Interpretation of PCR results: A positive result was defined as a cycle threshold (Ct) value was ≤35 cycles; and in conclusive or equivocal result as the Ct was between >35cyclesand <40 cycles, and the negative result as a Ct value was ≥40 cycles. All inconclusive or equivocal results were diluted to 1:4 and 1:10and PCR were repeated on the dilutions. In order to assess the performance of rt-PCR in detection of *S. pneumoniae*, we considered the culture as the gold standard method to determine the sensitivity, the specificity, positive predictive value (PPV) and negative predictive value (NPV) of rt-PCR.Specimens that were culture positive for another organism were excluded.

Ethical aspects: All specimens were collected as part of the routine clinical management of patients, according to the national guidelines in Burkina Faso. The study was approved by the medical establishment committee of Teaching Hospital YalgadoOuedraogo.

Statistical analyses

Epi-Info version 3.5.1 and MedCal 11.0.1.0 were used to compare the performance of rt-PCR to those of standard bacteriological methods. The difference was statistically significant when the *p*-value was lower than .05 ($p<0.05$).

RESULTS

A total of 385CSFs specimens were collected from suspect casesfor meningitis. *S. pneumoniae* was detected in 204 (51.2%) CSFs specimens,by the three methods (latex agglutination assay, culture and rt-

PCR). As shown in Table 1, among 385 CSFs analyzed by the three confirmatory tests, 140 (36.4%) were Gram positive encapsulated cocci. All these 140 CSFs specimens were positive for *S. pneumonia* by latex agglutination assay, culture or/and rt-PCR. Latex agglutination assay, culture and rt-PCR detected 145 (37.7%), 80(20.8%), and 197 (51.2%)positive cases of *S. pneumoniae*respectively. The results showed (Table 1) that rt-PCR detected more cases than latex agglutination assay *(p=0.0058)* and culture *(p<0.0001)*.Of the 197 *S. pneumoniae* detected by rt-PCR, 57 (14.8%) *S. pneumoniae* were detected from CSFs that were negative by culture and latex agglutination assay (Table 2). Of the 385 CSFs tested by both culture, latex agglutination assay and rt-PCR, 204 (53.0%) were positive by at least one of the three methods (Table 2).

TABLE 1: *S. PNEUMONIAE*CASES DETECTED BY THE STANDARD BACTERIOLOGICAL METHODS AND RT-PCR

Test	Positive (%)	95%CI	Negative (%)	95%CI	*p value*
Gram stain	**140 (36.4)**	**31.2-40.9**	**245 (63.6)**	**56.2-74.9**	**<0.0001**
Latex agglutination	**145 (37.7)**	**32.4-42.2**	**240 (62.3)**	**55.7-74.1**	**<0.0001**
Culture	**80 (20.8)**	**16.6-24.8**	**305 (79.2)**	**69.3-92.6**	**<0.0001**
rt-PCR	**197 (51.2)**	**44.8-54.9**	**188 (48.8)**	**45.1-55.2**	***0,6835***

TABLE 2: CO-DETECTION OF *S. PNEUMONIAE* BY CULTURE, LATEX AGGLUTINATION ASSAY AND RT-PCR

Combination of methods	Numberdetected (%)
rt-PCR+/Culture+/Latex agglutination assay+	**76 (19.7)**
rt-PCR+/Culture– /Latex agglutination assay+	**62 (16.1)**
rt-PCR+/Culture–/Latex agglutination assay–	**57 (14.8)**
rt-PCR–/Culture+/Latex agglutination assay+	**2 (0.5)**
rt-PCR–/Culture+/Latex agglutination assay–	**0**
rt-PCR–/Culture–/ Latex agglutination assay+	**5 (1.3)**
rt-PCR+/Culture+/ Latex agglutination assay–	**2 (0.5)**
Total	**204 (53.0)**

Among the 197 rt-PCR positive specimens, 138 (70.1%) were *S. pneumoniae* positive by latex agglutination assay,and 78 (39.6%) positive by culture. All culture-positive CSF specimens were rt-PCR positive except for two specimens. These specimens were analyzed twice by rt-PCR, and the results remained negative (Table 3). *S. pneumoniae* was detected by culture and/or latex agglutination assay in 7CSFs that were rt-PCR negative.

Sensitivity and specificity of culture, latex agglutination assay and rt-PCR were evaluated using 385 analyzed samples (Table 4A). The rt-PCR assay was more sensitive (96.6%) than Gram stain (67.4%) (*p=0.02*), latex agglutination assay (70.4%) (*p=0.04*) and culture (54.4%) (*p=0.0006*). Their specificity and positive predictive value were 100% (Table 4B). Culture showed a lower specificity (31.5%) compared to latex agglutination assay (95.2%) (*p<0.0001*). These differences were statically significant. There was no significant statistical difference in thenegative predictive values (NPV) (*p=0.8*) between Gram stain (71.8%) and latex agglutination assay (75.0%), and the positive predictive values (83.5% for Gram stain *vs* latex agglutination assay (70.4%) (*p=0.2*).

TABLE 3: COMPARISON BETWEEN RT-PCR AND THE STANDARD BACTERIOLOGICAL METHODS FOR THE DETECTION OF *S. PNEUMONIAE*

Test		rt-PCR		
		Positive (%)	Negative (%)	Total (%)
Gramstain	Positive	126/197 (64.0)	14/188 (7.4)	140/385 (36.4)
	Negative	71/197 (36.0)	174/188 (92.6)	245/385 (63.6)
Latex agglutination assay	Positive	138/197 (70.1)	7/188 (3.7)	145/385 (36.9)
	Negative	59/197 (29.9)	181/188 (96.3)	240/385 (63.1)
Culture	Positive	78/197 (39.6)	2/188 (1.1)	80/385 (20.8)
	Negative	119/197 (60.4)	186/188 (98.9)	305/385 (79.2)

DISCUSSION

Identification of *Streptococcus pneumonia* by culture is essentially based on colony appearance, bacterial cell morphology in Gram stained smears,biochemical characteristics including the optochin susceptibility (ethyl-hydrocupreine hydrochloride) and bile solubility. Molecular tests have been developed in the past several years,to overcome the limitations of standardbacteriologicalmethods (Gram stain, culture and latex agglutinationassay) in order to distinguish pneumococcus (*S. pneumoniae*) from other *Streptococcus* species such as *Streptococcus oralis* and S*treptococcus mitis*.

The advantage of using PCR over culture and Gram stain is that PCRis turnaround time or being able to detect a non-viable organism where a patient may have been on antibiotic treatment at the time of specimen taking. More importantly, PCR is more sensitive than other bacteriological methods. Although culture is still considered as the gold standard (16), its sensitivity can be low due to many factors such as prior antibiotic treatment, the timing of specimen transport, and media quality. According to our results, only20.4% of suspect cases were culture positive; 79.6% samples selected in this study were culture negative.

The low sensitivity of culture entrained a difficulty to identify the infection etiology. In fact, PCR can effectively confirm the results of the standard bacteriological methods and improve the confirmation of pneumococcal disease (17). In our study, rt-PCR showed 96.6% sensitivity and 100% specificity. The positive predictive value was 100%. These results were comparable with those obtained from other studies where the PCR sensitivitywas estimated from 88 to 100% and the specificity from 75 to 100% (18-22). The fact that PCR has shown high specificity and positive predictive value in our study indicates that PCR is unlikely to produce false positive results, and therefore can be used as a reliable tool for pneumococcal meningitis diagnosis. However, a negative PCR result does not indicate the absolute absence of infection as no single test has demonstrated 100% sensitivity; it is possible in thissituation, either the primers were not adequate to the target gene, the probe or the primers were damaged, or there was an inhibitor.

Other PCR platforms such as multiplex PCR were used in other studies to identify the common bacterial meningitis pathogens (12, 19, 20, 23) and reported to produce reliable results with improved turnaround time. The target gene for the detection of *S. pneumonia* used in these PCR assays was the pneumolysin gene (*ply*) (24, 25), which is present in all Streptococcusspecies and not specific to *S. pneumoniae* especially in carriage studies (25). PCR assays targeting genes such as *sod*A (superoxide dismutase A gene) (26), *sp*9802 (fragment 9802 gene) (21), and *psa*A (pneumococcal surface adhesion A gene) (25,27) were less specific than pneumococcal *lytA*PCR (15, 17, 28, 29). Our data showed that rt-PCR targeting autolysin gene *lyt*A was acomplementary method for identification of *S. pneumonia* during the epidemic seasons in Burkina Faso.

TABLE 4: SENSITIVITY AND SPECIFICITY OF DIFFERENT METHODS

A.

		Culture		
		Positive	**Negative**	**Total**
Gram stain	**Positive**	**132**	**26**	**158**
	Negative	**64**	**163**	**227**
	Total	**196**	**189**	**385**
Latex agglutination assay	**Positive**	**140**	**9**	**149**
	Negative	**59**	**177**	**236**
	Total	**204**	**186**	**390**
rt-PCR	**Positive**	**197**	**7**	**204**
	Negative	**0**	**181**	**181**
	Total	**197**	**188**	**385**

B.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Gramstain	**67.4**	**86.2**	**83.5**	**71.8**
Latex agglutination assay	**70.4**	**95.2**	**70.4**	**75.0**
Culture	**54.4**	**31.5**	**32.9**	**52.8**
rt-PCR	**96.6**	**100**	**100**	**96.3**

PPV: Positive Predictive Value; NPV: Negative Predictive Value

Our results also showed that rt-PCR improved diagnosis of *S. pneumonia* compared to standard bacteriological methods (Gram stain, latex agglutination assay,culture), particularly when thesemethods were negative or inconclusive; however, other tests were still valuable for pneumococcal diagnosis in laboratories where PCR technology was not available. In spite of its low sensitivity (54.4%) and specificity (31.5%), culture remained the gold standard method in the pathogenic diagnosis of this bacteriumand todetermine its sensitivity to antibiotics. Isolates were especially valuable to study the molecular epidemiology of *S. pneumonia* and to identify target genes for developing diagnostic tools. With a sensitivity of 70.4% and a specificity of 95.4%, latex agglutinationassay can be also considered as an alternative in absence of PCR. These results were similar to those of McAvin et *al* (2001) who obtained sensitivity and a specificityof latex agglutinationassayof 96% and 85% respectively (17). These results prove the importance of latex agglutination assayin the diagnosis of pneumococcal meningitis particularly in emergency situations. rt-PCR provides a rapid and reliable diagnostic tool(16). However, this method should not replace the standardbacteriological methods, particularly the culturethat contributesto provide data about the germ susceptibility to antibiotics:there are all complementary in the improvement of the surveillance of pneumococcal infections.

The cost of the rt-PCR constitutes an important limit for its implementation in low-income countries (12). This cost could be minimize by implementing the technique in central laboratory or in national reference laboratory which would receive CSF specimens collected for the confirmations of cases suspected in various sanitary regions of the country.

CONCLUSION

The implementation of rt-PCR in Burkina Fasoallowsconfirmation of more suspect cases of pneumococcal meningitis compared to culture andlatex agglutination assay. Although the standard bacteriological methods remain essential for the diagnoses of pneumococcal meningitis, rt-PCR represents an improvement on the standard bacteriological method's performance because of its sensitivity and specificity. PCR is a rapid and reliable method and can be used as a complementary method for pneumococcal detection. PCR has proven to be a valuable tool in medical laboratories of West Africa countries such as Burkina Faso where culture-based bacterial detection is challenging due to contamination, delay in transport and inappropriate storage of CSF specimens collected in rural medical centers.

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REFERENCES

1. Picard C., et Casanova, J.L.Prédisposition génétique aux infections à pneumocoques. *Rev.Mar. Mal.Enf*.2008; 18:72-76
2. WHO. Pneumococcal conjugate vaccine for childhood immunization-WHO position paper. WER 2007; 82(12): 93-104
3. WHO: 23-valent pneumococcal polysaccharide vaccine WHO position paper. WER 2008; 83(42): 373-384
4. O'Brien, K.L., Wolfson, L.J., Watt, J.P., Henkle, E., Deloria-Knoll, M., McCall, N., Lee, E., Mulholland, K., Levine, O. S., Cherian, T. Burden of disease caused by *Streptococcus pneumoniae*in children younger than 5 years: global estimates.*Lancet*.2009; 374: 893–902
5. Tin TinHtar, M, Madhava, H., Balmer, P., Christopoulou, D., Menegas, D., Bonnet E. A Review of the Impact of Pneumococcal Polysaccharide Conjugate Vaccine (7-valent) on Pneumococcal Meningitis. *Adv.Ther*.2013; 30:748–762.
6. Mackenzie, G.A., Plumb, I.D., Sambou, S., Saha, D., Uchendu, U.,Akinsola, B., Ikumapayi, U.N., Baldeh, I., Usuf, E., Touray, K., Jasseh, M., Howie, S.R.C., Wattiaux, A., Lee, E., Knoll, M.D., Levine, O.S., Greenwood, B.M., Adegbola, R.A., Hill,P.C.Monitoring the introduction of pneumococcal conjugate vaccines into West Africa: design and Implementation of a population-based surveillance system. *PLoS Med*.2012;91): e1001161. doi:10.1371/journal.pmed.1001161
7. Pilishvili, T.,Lexau, C., Farley, M.M., Hadler, J., Harrison, L.H., Bennett, N.M.,Reingold, A., Thomas, A., Schaffner,W., Craig, A.S., Smith, P.J., Beall, B.W., Whitney, C.G., and Moore, M.R. for the Active Bacterial Core Surveillance/Emerging Infections Program Network, Sustained Reductions in Invasive PneumococcalDisease in the Era of Conjugate Vaccine.*J. Infect. Dis*.2010; 201:32–41

8.Gray, L.D., andFedorko, D.P.Laboratory diagnosis of bacterial meningitis. *Clin.Microbiol. Rev*.1992; 5(suppl 2):130

9. Arbique, J.C., Poyart, C., Trieu-Cuot, P., Quesne, G., Carvalho, M-G.S., Steigerwalt, A.G., Morey, R.E., Jackson, D., Davidson. RJ, Facklam, R.R. Accuracy of Phenotypic and Genotypic Testing for Identification of *Streptococcus pneumonia* and Description of *Streptococcus pseudopneumoniaesp*.*J.Clin.Microbiol*.2004; Vol.42 (N°10): 4686-4696.DOI: 10.1128/JCM.42.10.4686–4696
10. Llull, D., Lopez, R., Garcia, E. Characteristic Signatures of the *lytA*Gene Provide a Basis for Rapid and Reliable Diagnosis of *Streptococcus pneumoniae*Infections. *J.Clin.Microbiol*.2006; Vol44 (N°4): 1250-1256.doi:10.1128/JCM.44.4.1250–1256
11. Corless, C.E., Guiver, M., Borrow, R., Edwards-Jones, V., Fox, A.J., and Kaczmarski, E.B. Simultaneous detection of *Neisseria meningitidis*, *Haemophilusinfluenzae*, and *Streptococcus pneumoniae* in suspected cases of meningitis and septicemia using real-time PCR. *J.Clin.Microbiol*.2001; 39: 1553–1558.
12. Parent du Châtelet, I., Traoré, Y., Gessner, B.D., Antignac, A., Naccro, B., Njanpop-Lafourcade, B-M., Ouedraogo, M.S., Tiendrebeogo, S.R., Varon, E., Taha, M.K.Bacterial Meningitis in Burkina Faso: Surveillance Using Field-Based Polymerase Chain Reaction Testing. *Clin. Infect. Dis*. 2005; 40:17-25
13. Traoré, Y., Tameklo, T.A., Njanpop-Lafourcade, B-M., Lourd, M., Yaro, S., Niamba, D., Drabo, A., Mueller, J.E., Koeck, J-L., Gessner, B.D.Incidence, Seasonality, Age Distribution,and Mortality of Pneumococcal Meningitisin Burkina Faso and Togo. *Clin.Infect. Dis*. 2009; 48:S181-189
14. World Health Organization (WHO/V&B/03.01). Recommended standards for surveillance of selected vaccine-preventable diseases, the Department of Immunization, Vaccines and Biologicals thanks the donors whose unspecified financial support has made the production of this document possible, Geneva.2003; pp1-62.
15. Carvalho, M. da G.S., Tondella, M.L., Mc Caustland, K., Mc Gee, L.W.L., Mayer, L.W., Steigerwalt, A., Whaley, M., Facklam, R.R., Fields, B., Carlone, G., Ades, E.W., Dagan, R., Sampson, J.S., Evaluation and Improvement of Real-Time PCR Assays Targeting *lytA*, *ply*, and *psaA*Genes for Detection of Pneumococcal DNA. *J.Clin.Microbiol*. 2007; Vol. 45 (N°8)2460-2466. DOI: 10.1128/JCM.02498-06.
16. Sheppard, C.L., Harrison, T.G., Morris, R., Hogan, A., George, R.C.Autolysin-targeted Light Cycler assay including internal process control for detection of *Streptococcus pneumoniae* DNA in clinical samples. *J. Med.Microbiol*.2004; 53:189-195
17. Mc Avin, J.C., Reilly, P.A., Roudabush, R.M., Barnes, W.J., Salmen, A., Jackson, G.W., Beninga, K.K., Astorga, A., Mc Cleskey, F.K., Huff, W.B., Niemeyer, D., Lohman, K.L.Sensitive and Specific Method for Rapid Identification of *Streptococcus pneumoniae*Using Real-Time Fluorescence PCR. *J.Clin.Microbiol*.2001; Vol.39

(N°10): 3446-3451.DOI: 10.1128/JCM.39.10.3446–3451

18. Sacchi, C.T., Fukasawa, L.O., Goncalves, M.G., Salgado, M.M., Shutt, K.A., Carvalhanas, T.R., Ribeiro, A.F., Kemp, B., Gorla, M.C.O., Albernaz, R.K., Marques, E.G.L., Cruciano, A., Waldman, E.A., Brandileone, M.C.C., Harrison, L.H., Sao Paulo RT-Surveillance Project Team.Incorporation of Real-Time PCR into Routine Public Health Surveillance of Culture Negative Bacterial Meningitis in Sao Paulo, Brazil. *PLoSONE.* 2011; 6(6): e20675. doi:10.1371/journal.pone.0020675
19. Tzanakaki, G., Tsopanomichalou, M., Kesanopoulos, K., Matzourani, R., Sioumala, M., Tabaki, A., Kremastinou, J.Simultaneous single-tube PCR assay for the detection of *Neisseria meningitidis, Haemophilusinfluenzae* type b and *Streptococcus pneumoniae*. *Clin. Microbiol. Infect.*2005; 11:386-390
20. Failace, L., Wagner, M., Chesky, M., Scalco, R., Jobim, L.F.Simultaneous detection of *Neisseria meningitidis, Haemophilusinfluenzae*and *Streptococcus* sp. by polymerase chain reaction for the diagnosis of bacterial meningitis. *Arq.Neuropsiquiatr*.2005; 63(suppl 4):920-924
21. Abdeldaim, G.M.K., Stralin, K., Korsgaard, J., Blomberg, J., Welinder-Olsson, C., Herrmann, B. Multiplex quantitative PCR for detection of lower respiratory tract infection and meningitis caused by *Streptococcus pneumoniae*, *Haemophilusinfluenzae* and *Neisseria meningitidis.BMC Microbiol*. 2010; 10:310
22. Kennedy, W.A., Chang, S-J., Purdy, K., Kilgore, P.E., Kim, J.S., Anh, D.D., Huong, P.L.T., Dong, B.Q., Tan, D.M., Clemens, J.D., Ward, J.I.Incidence of bacterial meningitis in Asia usingenhanced CSF testing: polymerase chain reaction, latex agglutination and culture. *Epidemiol. Infect*.2007; 135:1217-1226.
23. Ceyhan, M., Yildirim, I., Balmer, P., Borrow, R., Dikici, B., Turgut, M., Kurt, N., Aydogan, A., Ecevit, C., Anlar, Y., Gulumser, O., Tanir, G., Salman, N., Gurler, N., Hatipoglu, N., Hacimustafaoglu, M., Celebi, S., Coskun, Y., Alhan, E., Celik, U., Camcioglu, Y., Secmeer, G., Gur, D., Gray, S.A prospective study of etiology of childhood acute bacterial meningitis, Turkey. *Emerg. Infect. Dis.* 2008; 1089-1096
24. Rintamaki, S., Saukkoriipi, A., Salo, P., Takala, A., Leinonen, M.Detection of *Streptococcus pneumoniae* DNA by using polymerase chain reaction and microwell hybridization with Europium-labelled probes. *J.Microbiol.Meth*.2002; 50:313–318
25. Messmer, T.O., Sampson, J.S., Stinson, A., Wong, B., Carlone, G.M., Facklam, R.R., Comparison of four polymerase chain reaction assays for specificity in the identification of *Streptococcus pneumoniae*. *Diag.Microbiol. Infect. Dis*.2004; 49:249-254
26. Kawamura, Y., Whiley, R.A., Shu, S-E., Ezaki, T., Hardie, J.M.Genetic approaches to the identification of the mitis group within the genus *Streptococcus*. *Microbiol*.1999; 145:2605-2613
27. Morrison, K.E., Lake, D., Crook, J., Carlone, G.M., Ades, E., Facklam, R., Sampson, J.S.Confirmation of *psaA*in all 90 serotypes of *Streptococcus pneumoniae*by PCR and potential of this assay for identification and diagnosis. *J.Clin.Microbiol*.2000;Vol. 38 (N°1): 434-437
28. Messmer, T.O., Whitney, C.G., Fields, B.S. Use of polymerase chain reaction to identify pneumococcal infection associated with hemorrhage and shock in two previously healthy young children. *Clin. Chem*.19997; 43 *(6 Pt1):*930-935
29. Azzari, C., Moriondo, M., Indolfi, G., Massai, C., Becciolini, L., Martino, M., Resti, M.Molecular detection methods and serotyping performed directly on clinical samples improve diagnostic sensitivity and reveal increased incidence of invasive disease by *Streptococcus pneumoniae* in Italian children. *J.Med.Microbiol*.2008; 57:1205-1212