International Journal of Advanced Research in Biological Sciences
ISSN : 2348-8069
www.ijarbs.com

Research Article

Detection of Neuroaminidase genes of *S. pneumoniae* isolated from patients with pneumonia in Najaf Province/ Iraq

Assist. Prof .Dr. Hawraa A. A. Al-Dahhan

Lab. Investigator Dept. College of Science / Kufa university
*Corresponding author: *hawraa20012012@yahoo.com*

Abstract

S.pneumoniae expresses neuraminidase that cleaves sialic acid containing substrates and is thought to promote pneumococcal colonization by exposing host cell receptors . The *S. pneumoniae* genome codes for up to three NA proteins: NanA, NanB and NanC. In this study, three genes of neuraminidase enzyme were detected by using monoplex –PCR technique in 74 *P.aeruginosa* isolates that isolated from 600 patients with clinical symptoms of pneumonia. the *nanA*gene was found in all *S.pneumoniae* isolates (100%) , While the results amplification of *nanB* gene primer by PCR revealed that most (62.2%) of *S.pneumoniae* isolates have *nanB* gene, except four of isolates (S_{11}, S_{12}, S_{17}, S_{20}) gave negative result (37.8%).

Keywords: *S.pneumoniae*, neuraminidase, nana , nan B, nanC, monoplex –PCR, pneumonia.

Introduction

Streptococcus pneumoniae is globally a significant pathogen and causes a wide range of diseases such as pneumonia, meningitis, otitis media, bacteraemia, and other less-frequent infections such as endocarditis and arthritis (Hausdorff *et al.,* 2005). *Its* commonly called the pneumococcus, is responsible for high rates of morbidity and mortality worldwide (Rudan *et al.*, 2008). *S. pneumoniae* normally colonizes the human nasopharynx, nose, and throat asymptomatically and such carriage is considered essential for subsequent development of disease in susceptible individuals (particularly infants, the elderly, and the immune-compromised (LeMessurier, *et al*., 2006). The incidence of pneumococcal disease and the occurrence of antibiotic- resistant isolates have been positively correlated to the levels of carriage (Paul, 1997).

S.pneumoniae expresses a variety of protein virulence factors that allow colonization of different human mucosal surfaces. Among these factors are neuraminidases (NA), or sialidases, that cleave terminal sialic acids from glycoconjugates. Based on substrate specificity and catalytic mechanism NAs can be separated into three different classes (Lue *et al.*, 1999).

NanA, which contributes to nasopharyngeal colonization and development of otitis media in a chinchilla animal model (Tong *et al.*, 2000), and respiratory tract infection and sepsis in mice (Manco *et al.*, 2006). NanA is proposed to aid pathogenesis by revealing carbohydrate receptors for adherence, providing a carbon source for the bacteria, modifying the surface of other bacteria in the same niche, and affecting the function of host defense molecules (King *et al.*, 2006 ; Burnaugh *et al.*, 2008 ; Yesilkaya *et al.*, 2008).

Influenza virus encodes the neuraminidase NA, which is similar to *S.pneumoniae* NanA in substrate specificity (Suzuki, 2005 ;Xu *et al.*, 2008). NanA cleaves N-acetylneuraminic acid (sialic acid) residues

on red blood cells, platelets and endothelial cells leading to the exposure of the Thomsen–Friedenreich antigen (TA) and allowing normally circulating anti-T antigen antibodies to react with the exposed TA on cells (Cochran *et al.*, 2004 ; Scheiring *et al.*, 2010).NanA may help promote colonization through desialylation of host proteins that mediate bacterial clearance, such as lactoferrin or immunoglobulin A2 (King *et al.* 2004). NanA also has been shown to desialylate lipopolysaccharides of *Neisseria meningitidis* and *Haemophilus influenzae* strains (Shakhnovich *et al.*, 2002). The desialylation of lipopolysaccharide may give pneumococci a competitive advantage over *N. meningitidis* and *H.influenzae*, which reside in the same host niche, by making them more susceptible to complement mediated clearance.

Much less is known about the 78 kDa NanB protein, which has low sequence identity (24%) with NanA (Berry *et al.*, 1996). Recent investigations suggest that NanB plays an important role during pneumococcal infection of the respiratory tract and sepsis (Manco *et al.*, 2006) as well as playing a role in bacterial nutrition (Burnaugh *et al.*, 2008).The third putative NA, NanC, present in less than 50% of pneumococcal strains has high sequence identity to NanB (46%) but remains to be characterized (Pettigrew *et al.*, 2006).

Pneumococci have been shown to have at least two distinctly separated appearances when grown on a transparent medium, these two appearances are referred to as either transparent or opaque, how these different morphological appearances are accomplished remains to be explained, but is generally considered to depend on protein expression and capsular thickness. Transparent phenotypes have been demonstrated to express a higher amount of neuraminidase, a fact that has suggested this as an explanation for the observed enhanced adhesion of transparent phenotypes in colonization (Melegaro and Edmunds,2004 ; Millar *et al*., 2006).

Materials and Methods

Sample collection and processing

Sputum samples were collected from 600 out- and inpatients suffering from lower respiratory tract infection (LRTI) attending to the Chest Unit in Al-Sadder Medical City, Al-Hakeem General Hospitaland Clinic Consultive Center for Chest Disease and Al-Zahra'a Hospital for Childbirth and Children in Al-Najaf province during the period from February 2013-Aprile 2014. The patients included both sex (male and female) and the age range (1-80 years).

Pneumococcal Identification

Sputum Ziehl-Neelsen Stain Method was performed according to Macfaddin (2000). With a special care, sputum was homogenized for a few minutes with a clean wooden stick. Gram stained sputum preparations were used for polymorphonuclear neutrophils (PMNs) and epithelial cells. If the sputum contain too many squamous epithelial cells (more than 10 cells per lower powered field) (100x) the specimen was considered not useful , sputum samples were considered valuable if no more than 10 squamous epithelial cells and more than 20 neutrophils per low-power field were visible and were considered positive for pneumonia infections(Murray,1975; Miriam and Buenviaje,1988).

sputum cultures were made for each specimen according to sputum gram stain for pneumonia infections. Sputum specimens were homogenized with an equal volume of normal saline on a vortex mixer. Blood agar and Chocolate agar were inoculated with 0.1 ml of homogenized specimen. Plates were incubated in (5-10)% CO_2candle jar at 37 C° overnight. The identification of *S.pneumoniae* was achieved according to morphological staining, culture characters and biochemical reactions that described in Macfaddin, .biochemical tests that confirmed the identification of isolates *S. pneumoniae* such as optochin sensitivity , 2% deoxycholate solubility and α-haemolysis. STREPTO-SYSTEM 9R for *S.pneumoniae* identification was used according to the recommendation of company product (Liofilchem, England). The final identification of *S. pneumoniae* was performed with VITEK-2 compact system using GP cards which contained 43 biochemical tests and one negative control well . The results of this test have been showed to be compatible with results of STREPO-SYSTEM 9R test in which all 74 *S. pneumoniae* isolates that diagnosed in STREPO-SYSTEM 9R gave positive results in VITEK-2 test.

Extraction and Isolationof DNA

S.pneumoneae isolates were cultured on tryptic soy agar supplemented with 5% sheep blood and inoculated individually into TSB and incubated at 37˚C/24h. Genomic DNA Extraction Kit (Geneaid) was used for DNA extraction. Geneaid Mini kit (USA) include the following: Gram+ Buffer (30ml),GB Buffer (40ml),W1 Buffer (45ml), Elution Buffer (30ml),GD Column (100pcs),2ml Collection Tube(200pcs),Wash Buffer(25ml) (add ethanol) (100ml), Loding dye, Nuclease Free water, Ethidium bromide, TBE(Tris- Borate EDTA)buffer ,TE (Tris – EDTA)buffer Agarose,1kb DNA Ladder (DNA marker),100 bp DNA Ladder (DNA marker).

Gel electrophoresis was used for detection of DNA by UV transilluminator according to Sambrook *et al.* (2001).

PCR assay was performed in monoplex patterns in order to amplify three genes of neuraminidase (nanA,nanB,nanC) under study for detecting of Staphylococcal virulence factor genes.

Monoplex Master Mix Mixture

Type	Description	Purpose	Origin
KABA2G FastHotStart Ready Mix (2x)	The 2X Ready Mix contains KABA2G Fast HotStartDNA polymerase, KABA2G Fast HotStart PCR buffer, dNTPs (0.2mM each dNTP at 1X), MgCl2 (1.5mM at 1X) and stabilizers**.**	Monoplex PCR	KAPA Biosystem (USA)

PCR Primers of neuraminidase

Target Gene	DNA sequence(5'-3')	Product Size (bp)	References
nanA	F:ATA GAC GTG CGC AAA ATA CAG AAT CA R:GTC GAA CTC CAA GCC AAT AAC TCC T	550	Pettigrew *et al.* (2006)
nanB	F:ACT ACG AGG TGT TAA TCG TGA AGG R:CCA ATA CCC GCA GGC ATA ACA TC	500	Pettigrew *et al.* (2006)
nanC	F:TGG GGT AAG TAC AAA CAA GAG G R:CTA ATG GTA CTG GCG AAA ATC A	500	Pettigrew *et al.* (2006)

PCR Cycling Conditions

PCR reaction tubes were centrifuged briefly to mix and bring the contents to the bottom of the tubes, and placed into thermocycler PCR instrument where DNA was amplified as indicating in below:

Table (3.7): Program used to amplify the *nanA* and *nanB*

Stage	Temperature (time)	
Initial denaturation	94C° for 3min	
Denaturation	94C° for 1min	35cycle
Annealing	52C° for 1min	
Extension	72C° for 1.5min	
Final extension	72C° for 10min	

Table (3.8): Program used to amplify the *nanC*

Stage	Temperature (time)	
Initial denaturation	94C° for 5min	
Denaturation	98C° for 30sec	35cycle
Annealing	51C° for 30sec	
Extension	72C° for 1min	
Final extension	72C° for 10min	

Results and Discussion

In this study, the *nanA* gene was found in all *S. pneumoniae* isolates (100%) as shown in Figure (1). While the results amplification of *nanB* gene primer by PCR revealed that most (62.2%) of *S. pneumoniae* isolates have *nanB* gene, except four of isolates (S_{11}, S_{12}, S_{17}, S_{20}) gave negative result (37.8%) figure (2).

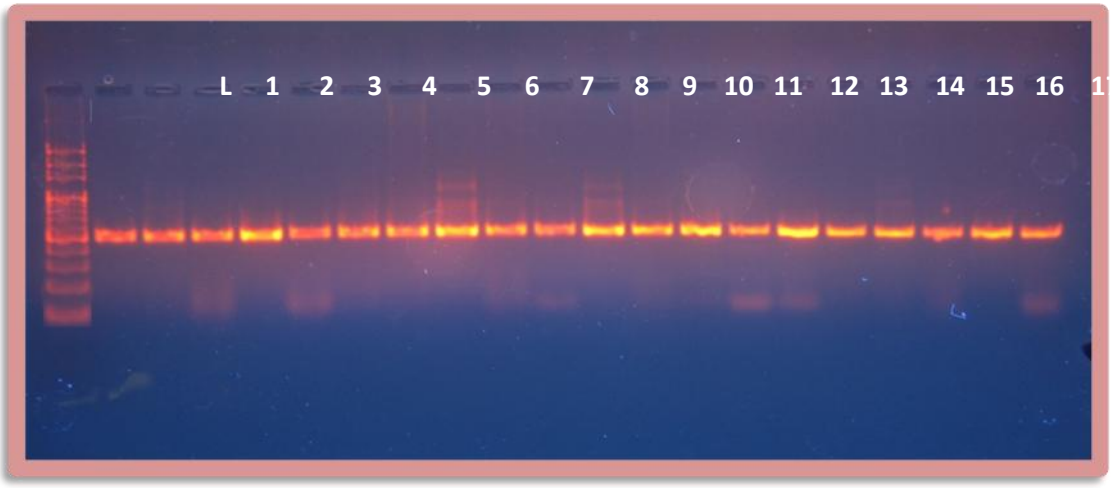

Figure (1): Gel electrophoresis of PCR product of *nanA* gene primers with product 550 bp.
Lane (L), DNA molecular size marker (2000-bp ladder), Lanes (1-20) show positive results with *nanA* gene**.**

Figure (2): Gel electrophoresis of PCR product of *nanB* gene primers with product 500 bp. Lane (L),
DNA molecular size marker (2000-bp ladder), Lanes (11, 12, 17) show negative results with *nanB* gene.

S. pneumoniae is believed to produce more than one form of neuraminidase, but there has been uncertainty as to whether this is due to posttranslational modification of a single gene product or the existence of more than one neuraminidase-encoding gene. The pneumococcus genome encodes up to three neuraminidase proteins that have been shown to be important virulence factors: *NanA*, *NanB* and *NanC* (Berry *et al*., 1988). Neuraminidase cleaves terminal sialic acid residues from a wide variety of glycolipids, glycoproteins, and oligosaccharides on cell surfaces or in body fluids, and such activity hasthe potential to cause great damage to the host (Krivan *et al*., 1988). Histochemical studies of organs from mice dying after intraperitoneal administration of partially purified pneumococcal neuraminidase have indicated marked decreases in the sialic acid contents of the kidneys and liver compared with those of controls (Kelly *et al*., 1970) and contributes to nasopharyngeal colonization and development of otitis media in a chinchilla animal model (Tong *et al*., 2000) and respiratory tract infection and sepsis in mice (Manco *et al*., 2006).

Two neuraminidases, encoded by *nanA* and *nanB*, have been described for *S. pneumoniae*. *NanA* is proposed to aid pathogenesis by revealing carbohydrate receptors for adherence, providing a carbon source for the bacteria and facilitating bacterial adherence by removing terminal sialic acid residues from glycoconjugates. Additionally, *NanA* also has been shown to desialylate lipopolysaccharides of *Neisseria meningitidis* and *Haemophilus influenzae* strains (King *et al*., 2004). The desialylation of lipopolysaccharide may give pneumococci a competitive advantage over *N. meningitidis* and *H. influenzae*, which reside in the same host niche, by making them more susceptible to complement-mediated clearance. (Shakhnovich *et al*., 2002), as well as antibacterial components of human airway secretions (King *et al*., 2004), potentially reducing pneumococcal clearance whilst promoting the clearance of competing bacteria (affecting the function of host defense molecules) (Shakhnovich *et al*., 2002 ; King *et al*., 2006 ; Burnaugh *et al*., 2008). *NanA* may help promote colonization through desialylation of host proteins that mediate bacterial clearance, such as lactoferrin or immunoglobulin A2 (Shakhnovich *et al*., 2002). A *nanB* homolog, *nanC*, has also been identified, but its expression and activity have not been described. The third putative *NA*, *NanC*, present in less than 50% of pneumococcal strains has high sequence identity to *NanB* (46%) but remains to be characterized (Chou *et al*., 1996).

The benefits to a pneumococcus of production of two distinct neuraminidases are unclear. Apart from their difference in size, the two enzymes have widely different pH optima, which imply that these enzymes may assist exploitation of distinct environmental niches. Although a clear difference in specific activity was observed with MUAN as the substrate, this may not hold for other potential substrates. *NanA* and *NanB* are both exported proteins, with typical signal peptides, but unlike *NanB*, *NanA* contains a C-terminal cell surface anchorage domain.

Proteolytic cleavage without loss of enzymic activity may be important for controlled release of surface-bound *NanA*. The possible involvement of neuraminidase in pneumococcal pathogenesis has been suggested by Berry *et al*., (2014) that purified *NanA* is a partially protective immunogen in mice (Lock *et al*., 1988). However, it has not been possible to assess the contribution of neuraminidase to pneumococcal virulence by molecular genetic techniques, as *NanA* deficient mutants have residual enzymic activity because of production of *NanB*.

LeMessurier *et al*., (2006) revialed that the expression of *nanA* was significantly elevated in the nasopharynx of infected mice compared to the other niches examined. And these results provide further support for an important role for *NanA* in colonization of the nasopharynx by pneumococci. Influenza virus encodes the neuraminidase *NA*, which is similar to S. pneumoniae *NanA* in substrate specificity (Xu *et al*., 2008) *NanA* cleaves N-acetylneuraminic acid (sialic acid) residues on red blood cells, platelets and endothelial cells leading to the exposure of the Thomsen–Friedenreich antigen (TA) and allowing normally circulating anti-T antigen antibodies to react with the exposed TA on cells (Scheiring *et al*., 2010).

The results of the current study were compatible with Pettigrew *et al*., (2012) results and found *nanA* was present in all strains of *S. pneumoniae* isolates, while *nanB* and *nanC* were present in 96% and 51% of isolates, respectively. The distribution of *nanC* varied among the strain collections from different tissue sources and suggested that the presence of *nanC* may be important for tissue-specific virulence. Studies that both incorporate MLST and take into account

additional virulence determinants will provide a greater understanding of the pneumococcal virulence potential.

Previously less is known about the 78 kDa NanB protein, which has low sequence identity (24%) with NanA. Recent investigations suggest that NanB plays an important role during pneumococcal infection of the respiratory tract and sepsis as well as playing a role in bacterial nutrition. Gut *et al.*, (2008) reported that the first structure of a neuraminidase from *S. pneumoniae*, the crystal structure of NanB in complex with its reaction product 2, 7-anhydro-Neu5Ac., and showed that NanB differs in its substrate specificity from the other pneumococcal neuraminidase NanA. Gut *et al*., (2008) also, confirmed this finding and establish that free Neu5Ac (the reaction product of pneumococcal NanA) can act as a substrate for NanB. In addition, our biochemical assays clearly demonstrate the strict specificity of NanB towards a2-3 glycosidic substrate linkages and highlight the differences in substrate specificity between NanA and NanB.

Interestingly, hybridization analysis indicated that these two neuraminidase genes are different and that individual pneumococcal isolates contain both genes (Camara *et al*., 1994). Berry *et al*., (2014) demonstrated that *nanB*, a gene encoding a second *S. pneumoniae* neuraminidase, is located on the pneumococcal chromosome approximately 4.5 kb downstream of *nanA*. *nanB* appears to be part of a large operon consisting of at least six ORFs. Janapatla *et al*., (2012) isolated and characterized a second neuraminidase gene (designated *nanB*), which is located close to *nanA* on the pneumococcal chromosome (approximately 4.5kb downstream). *nanB* was located on an operon separate from that of *nanA*, which includes at least five other open reading frames. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis suggested that *NanB* has a molecular size of approximately 65 kDa.

Pettigrew *et al*., (2010) from USA reported that among the invasive isolates *nanB* and *nanC* were present in 95% and 58% of the isolates, respectively; Recently, Imai *et al*., (Lami *et al*., 2011) from Japan reported that among the 156 pneumococcal isolates recovered from adult patients with community-acquired pneumonia *nanC* was present in 35.9% of the isolates. A recent study showed that *NanA* but not *NanB* was necessary for TA exposure on red blood cells in mice (Coats *et al*., 2011). Nevertheless, Janapatla *et al*., (2012) suggests that *NanC* could provide an additive effect to *NanA* and *NanB* in the overall activity of pneumococcal neuraminidases to expose Thomsen–Friedenreich antigen on various cells in patients with hemolytic uramic syndrome (complications of invasive pneumococcal infection).

In summary, the results of this study affirm that *nanA* is essential for virulence and that having *nanC* may predispose strains for invasion of the lung. Studies that incorporate MLST and take into account the presence or absence of additional virulence determinants will provide a greater understanding of the virulence potential of pneumococcal strains.

References

Chandler, L. J., B. S. Reisner, G.L. Woods, and A. K. Jafri. (2000). Comparison of four methods for identifying *Streptococcus pneumoniae*. Diagn. Microbiol. Infect. Dis. 37:285–287.

Kearns, A. M., J. Wheeler, R. Freeman, P. R. Seiders, J. Perry, A. M. Whatmore, and C. G. Dowson. (2000). Pneumolysin detection identifies atypical isolates of *Streptococcus pneumoniae*. J. Clin. Microbiol. 38:1309–1310

17. Bidossi A, Mulas L, Decorosi F, Colomba L, Ricci S. (2012). A functional genomics approach to establish the complement of carbohydrate transporters in *streptococcus pneumoniae*. PLoS One 7: e33320.

79. Soong, G., A. Muir, M. I. Gomez, J. Waks, B. Reddy, P. Planet, P. K. Singh, Y. Kaneko, M. C. Wolfgang, Y. S. Hsiao, L. Tong, and A. Prince. 2006. Bacterial neuraminidase facilitates mucosal infection by participating in biofilm production. J. Clin. Investig. 116:2297–2305

Abdeldaim G, *et al*. (2009). Is quantitative PCR for the pneumolysin (*ply*) gene useful for detection of pneumococcal lower respiratory tract infection. Clin. Microbiol. Infect. 15:565–570. doi:10.1111/j.14690691.2009.02714.x

Abdeldaim GM, Stralin K, Olcen P, Blomberg J, Herrmann B. (2008). Toward a quantitative DNA-based definition of pneumococcal pneumonia:a comparison of *Streptococcus pneumonia* targetgenes, with special reference to the *Spn9802* fragment.Diagn.Microbiol.Infect.Dis. 60:143–150.

Abdeldaim, G. M., Stralin, K., Olcen, P., Blomberg, J. and Herrmann, B. (2008). Toward a quantitative DNA-based definition of pneumococcal pneumonia: a comparison of *Streptococcus pneumoniae* target genes, with special reference to the Spn9802 fragment. Diagn Microbiol Infect Dis 60, 143–150.

Agron, P. G., Macht, M., Radnedge, L., Skowronski, E. W., Miller, W. and Andersen, G. L. (2002). Use of subtractive hybridization for comprehensive surveys of prokaryotic genome differences. FEMS Microbiol Lett 211, 175–182.

Alam MR, Saha SK, Nasreen T, Latif F, Rahman SR, Gomes DJ. (2007). Detection, Antimicrobial Susceptibility and Serotyping of *Streptococcus pneumoniae* from Cerebrospinal Fluid Specimens from Suspected Meningitis Patients. Bangladesh J Microbiol.24(1): 24-9.

Al-heety,A.(2005). Role of peptidoglycan in pathogenicity of *Staphylococcus saprophyticus*. .MSc. thesis. University of Baghdad. College of Science. Baghdad, Iraq.

Allegrucci M, Sauer K. (2008). Formation of *Streptococcuspneumoniae* non-phasevariable colony variants is due to increased mutation frequency present under biofilm growth conditions. J Bacteriol 190: 6330–6339.

Allegrucci, M., and K. Sauer. (2007). Characterization of colony morphology variants isolated from *Streptococcus pneumoniae* biofilms. J. Bacteriol. 189: 2030–2038.

Allegrucci, M., F. Z. Hu, K. Shen, J. Hayes, G. D. Ehrlich, J. C. Post, and K. Sauer. (2006). Phenotypic characterization of *Streptococcus*pneumoniae biofilm development. J. Bacteriol. 188:2325–2335.

Anderson, G. G., J. J. Palermo, J. D. Schilling, R. Roth, J. Heuser, and S. J. Hultgren. (2003). Intracellular bacterial biofilm-like pods in urinary tract infections. Science 301:105–107.

ArbiqueJC,*etal*. (2004).Accuracy of phenotypic and genotypic testing for identification of *Streptococcus pneumoniae* and description of *Streptococcus pseudopneumoniae* sp. nov. J. Clin. Microbiol. 42:4686–4696. doi:10.1128/JCM.42.10.4686-4696.

Balaban, N. Q., J. Merrin, R. Chait, L. Kowalik, and S. Leibler. (2004). Bacterial persistence as a phenotypic switch. Science 305:1622–1625.

Balsalobre, L., M. J. Ferrandiz, J. Linares, F. Tubau, and A. G. de la Campa. (2003). Viridans group streptococci are donors in horizontal transfer of topoisomerase IV genes to *Streptococcus pneumoniae*. Antimicrob Agents Chemother 47:2072-81

BeanD. C. and KlenaJ. D. (2002). Prevalence of *erm(A)* and *mef(B)* erythromycin resistance determinants in isolates of *Streptococcus pneumoniae* from New Zealand.Journal of Antimicrobial Chemotherapy, 50, 597–599 DOI: 10.1093/jac/dkf169.

BeanD. C. and KlenaJ. D. (2002).Prevalence of *erm(A)* and *mef(B)* erythromycin resistance determinants in isolates of *Streptococcus pneumoniae* from New Zealand. Journal of Antimicrobial Chemotherapy 50, 597–599 DOI: 10.1093/jac/dkf169.

Bidossi A, Mulas L, Decorosi F, Colomba L, Ricci S. (2012). A functional genomics approach to establish the complement of carbohydrate transporters in *Streptococcus pneumoniae*. PLoS One 7: e33320

Block SL, Harrison CJ, Hedrick JA, (1995). Penicillin-resistant *Streptococcus pneumoniae* in acute otitis media: risk factors, susceptibility patterns, and antimicrobial management. Pediatr Infect Dis J;14:751–9.

Bo¨ttger, E. C. (1990). Frequent contamination of Taq DNA polymerase with DNA. Clin. Chem. 36:1258–1259.

Boersma WG, Lowenberg A, Holloway Y, Kuttschrutter H, Snijder JA, Koeter GH.(1993). Rapid detection of pneumococcal antigen in pleural fluid of patients with community acquired pneumonia. Thorax;48: 160–2

Bogaert, D., R. de Groot, and P. Hermans. (2004). *Streptococcus pneumoniae* colonisation: the key to pneumococcal disease. LancetInfect.Dis.4:144–154.

Bogaert,D.,P.W.M.Hermans,P.V.Adrian,H.C.Rumke,andR.deGroot. (2004). Pneumococcal vaccines: an update on current strategies. Vaccine 22: 2209–2220.

Borek, P. A., D. C. Dressel, J. Hussong, and L. R. Peterson. (1997). Evolving clinical problems with *Streptococcus pneumoniae*: increasing resistance to antimicrobial agents, and failure of traditional optochin identification in Chicago, Illinois, between 1993 and 1996. Diagn. Microbiol. Infect. Dis. 29:209–214.

Borg, M.A.; Tiemersma, E.; Scicluna, E.; van de Sande-Bruinsma, N.; de Kraker, M; Monen, J.;

Grundmann, H. (2009). Prevalence of penicillin and erythromycin resistance among invasive *Streptococcus pneumoniae* isolates reported by laboratories in the southern and eastern Mediterranean region. Clin. Microbiol. Infec. 15(3), 232-237

Branda, S. S., S. Vik, L. Friedman, and R. Kolter. (2005). Biofilms: the matrix revisited. Trends Microbiol. 13:20–26.

Briles DE, Crain MJ, Gray BM, Forman C, Yother J. (1992). Strong association between capsular type and virulence for mice among human isolates of *Streptococcus pneumoniae*. Infect Immun 60: 111–116.

Briles, D. E., L. Novak, M. Hotomi, F. W. van Ginkel, and J. King. (2005). Nasal colonization with *Streptococcus pneumoniae* includes subpopulations of surface and invasive pneumococci. Infect. Immun. 73:6945–6951.

Brooks, G. ;Butel, J. and S. Morse.(2004). Medical microbiology 23 ed, Lange medical publishing division. New York, pp.211–212.

Brown SA, Palmer KL, Whiteley M. (2008). Revisiting the host as a growth medium. Nat Rev Microbiol 6: 657–666.

Buckwalter CM and King SJ. (2012). Pneumococcal carbohydrate transport: Food for thought Trends in Microbiology.

Buckwalter CM. and King SJ. (2012). Pneumococcal carbohydrate transport: Food for thought Trends in Microbiology.

Budhani, R. K., and J. K. Struthers. (1997). The use of Sorbarod biofilms to study the antimicrobial susceptibility of a strain of *Streptococcus pneumoniae*. J. Antimicrob. Chemother. 40:601–602.

Burnaugh, A. M., L. J. Frantz, and S. J. King. (2008). Growth of *Streptococcus pneumoniae* on human glycoconjugates is dependent upon the sequential activity of bacterial exoglycosidases. J. Bacteriol. 190:221–230.

Burne, R. A. (1998). Oral streptococci products of their environment. J. Dent. Res. 77:445–452.

Camilli R, Pantosti A, Baldassarri L. (2010). Contribution of serotype and genetic background to biofilm formation by *Streptococcus pneumoniae*. Eur J Clin Microbiol Infect Dis 30: 97–102.

Carvalho SM, Kloosterman TG, Kuipers OP, Neves AR. (2011). CcpA ensures optimal metabolic fitness of *Streptococcus pneumoniae* D39. Submitted 6: e26707

Carvalho SM, Kloosterman TG, Kuipers OP, Neves AR. (2011). CcpA ensures optimal metabolic fitness of *Streptococcus pneumoniae* D39. Submitted 6: e26707

Carvalho, M. da G. S., Tondella, M. L., McCaustland, K., Weidlich, L., McGee, L., Mayer, L. W., Steigerwalt, A., Whaley, M., Facklam, R. R. (2007). Evaluation and improvement of real-time PCR assays targeting *lytA*, *ply*, and *psaA* genes for detection of pneumococcal DNA. J Clin Microbiol 45, 2460–2466.

CarvalhoMG,*etal*. (2007).Evaluation and improvement of real-time PCR assays targeting *lytA*, *ply*, and *psaA* genes for detection of pneumococcal DNA. J. Clin. Microbiol. 45:2460–2466.

Casey R, Newcombe J, McFadden J, Bodman-Smith KB. (2008).The acutephase reactant C-reactive protein binds to phosphorylcholine-expressing *Neisseria meningitidis* and increases uptake by human phagocytes. Infect Immun 76: 1298–1304.

CDC.(1994). Prevalence of penicillin-resistant *Streptococcu spneumoniae* —Connecticut, 1992–1993. MMWR;43:216–7, 223.

Charalambous,B. M. ; S. L. Batt, A. C. Peek, H. Mwerinde, N. Sam, and S. H. Gillespie.(2003).Quantitative Validation of Media for Transportation and Storage of *Streptococcus pneumoniae*.journal of Clinical Microbiology, p. 5551–5556 Vol. 41, No. 12. American Society for Microbiology.

Chen, D. K., A. McGeer, J. C. de Azavedo, and D. E. Low. (1999). Decreased susceptibility of *Streptococcus pneumoniae* to fluoroquinolones in Canada. Canadian Bacterial Surveillance Network. New England Journal of Medicine 341:233-9.

Chiou, C. C. C., and M. C. McEllistrem. (2001). Novel penicillin-, cephalosporin-, and macrolide-resistant clones of *Streptococcus pneumoniae* serotypes 23F and 19F in Taiwan which differ from international epidemic clones. J. Clin. Microbiol. 39:1144–1147.

Claverys, J. P., M. Prudhomme, I. Mortier-Barriere, and B. Martin. (2000). Adaptation to the environment: *Streptococcus pneumoniae*, a paradigm for recombination-mediated genetic plasticity? Mol. Microbiol. 35:251–259.

Clinical Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Eighteenth Informational Supplement M100-S18. CLSI, Wayne, PA, USA, 2008.

Coffey, T. J., M. C. Enright, M. Daniels, J. K. Morona, R. Morona, W. Hryniewicz, J. C. Paton, and B. G. Spratt. (1998). Recombinational exchanges at the capsular polysaccharide biosynthetic locus lead to frequent serotype changes among natural isolates of *Streptococcus pneumoniae*. Mol. Microbiol. 27:73–83.

Corless, C. E., Guiver, M., Borrow, R., Edwards-Jones, V., Fox, A. J. and Kaczmarski, E. B. (2001). Simultaneous detection of *Neisseriameningitidis*, *Haemophilu sinfluenzae*, and *Streptococcus pneumoniae* in suspected cases of meningitis and septicemia using real-time PCR. J Clin Microbiol 39, 1553–1558

Corless, C. E., M. Guiver, R. Borrow, V. Edwards-Jones, E. B. Kaczmarski, and A. J. Fox. (2000). Contamination and sensitivity issues with a real-time universal 16S rRNA PCR. J. Clin. Microbiol. 38:1747–1752.

Cornaglia, G., G. Lo Cascio, L. Masala, (2000). The Italian Surveillance Group for Antimicrobial Resistance, and R. Fontana.. Macrolide resistance among *S. pneumoniae* isolates in Italy, p. 250–254. In S. H. Zinner, L. S. Young, J. F. Acar, and C. Ortiz-Neu (ed.), New considerations for macrolides, azalides, streptogramins, and ketolides. M. Dekker, Inc., New York, N.Y.

Cosgrove, S. E. (2006). The relationship between antimicrobial resistance and patient outcomes: mortality, length of hospital stay, and health care costs. Clin. Infect. Dis. 42(Suppl. 2):S82–S89

Costerton JW, Stewart PS, Greenberg EP. (1999). Bacterial biofilms: a common cause of persistent infections. Science 284: 1318–1322.

Cowan, S. T., and K. C. Steel. (1974). Manual for the identification of medical bacteria, p. 29. Cambridge University Press, London, England.

Cundell DR, Gerard NP, Gerard C, Idanpaan-Heikkila I, Tuomanen EI. (1995).*Streptococcus pneumoniae* anchor to activated human cells by the receptor for platelet-activating factor. Nature 377: 435–438.

Cvitkovitch, D. G., Y. H. Li, and R. P. Ellen. (2003). Quorum sensing and biofilm formation in streptococcal infections. J. Clin. Investig. 112:1626– 1632.

de la Campa AG, Ardanuy C, Balsalobre L. (2009). Changes in fluoroquinolone-resistant *Streptococcus pneumoniae* after 7-valent conjugate vaccination, Spain. Emerg Infect Dis; 15: 905–11.

Depardieu F. and Courvalin P. U. (2001).Mutation in 23S rRNA Responsible for Resistance to 16Membered Macrolides and Streptogramins in *Streptococcus pneumoniae*.Antimicrobial Agents and Chemotherapy,p. 319–323 Vol. 45, No. 1

Diekema, D. J., A. B. Brueggemann, and G. V. Doern. (2000). Antimicrobialdrug use and changes in resistance in *Streptococcus pneumoniae*. Emerg. Infect. Dis. 6:552–556.

Dobay O, Rozgonyi F, Hajdú E, Nagy E, Knausz M, Amyes SGB. (2003). Antibiotic susceptibility and serotypes of *Streptococcus pneumoniae* isolates from Hungary Journal of Antimicrobial Chemotherapy.51;887-93.

DomenechA., Ardanuy, C., Calatayud L., Santos, S. , Fe Tubau, Grau, I., Verdaguer, R., Dorca, J., Pallares R., Martin R. and Lin˜ares J. (2010). Serotypes and genotypes of *Streptococcuspneumoniae* causing pneumonia and acute exacerbations in patients with chronic obstructive pulmonary disease. J Antimicrob Chemother doi:10.1093/jac/dkq480.

Donlan, R. M., J. A. Piede, C. D. Heyes, L. Sanii, R. Murga, P. Edmonds, I. El-Sayed, and M. A. El-Sayed. (2004). Model system for growing and quantifying *Streptococcus pneumoniae* biofilms in situ and in real time. Appl. Environ. Microbiol. 70:4980–4988

Duchin JS, Breiman RF, Diamond A, (1995). High prevalence of multidrug-resistant *Streptococcus pneumoniae* among children in a rural Kentucky community. Pediatr Infect Dis J;14: 745–50.

Eagle, H., R. Fleischman, and A. D. Musselman. (1950). The bactericidal action of penicillin in vivo: the participation of the host, and the slow recovery of the surviving organisms. Ann. Intern. Med. 33:544–571

Edwards, M. C., and R. A. Gibbs. (1994). Multiplex PCR: advantages, development and applications. PCR Methods Appl. 3:S65–S75

Ehrlich,G.D.,R.Veeh,X.Wang,J.W.Costerton,J.D.Hayes,F.Z.Hu,B.J. Daigle, M. D. Ehrlich, and J. C. Post. (2002). Mucosal biofilm formation on middle-ear mucosa in the chinchilla model of otitis media. JAMA 287:1710– 1715.

Enright MC, Spratt BG, Kalia A, Cross JH, Bessen DE. (2001). Multilocus sequence typing of *Streptococcus pyogenes* and their relationships between emm type and clone. Infect Immun; 69: 2416-27.

Feigin RD, Cherry JD, Demmler GJ, Kaplan SL.(2004). Textbook of pediatric infectious diseases, 5th edn. Baltimore, MD: Saunders, 287.

Fica A, Fernande J, Ebensperger G, Cona E, Galanti A, Alonso C, (2003). Molecular epidemiology of a *Streptococcus pyogenes* related nosocomial outbreak in a burn unit. Rev Med Chil; 131: 145-54.

Fuller JD, Low DE. (2005). A review of *Streptococcus pneumoniae* infection treatment failures associated with fluoroquinolone resistance. Clin Infect Dis; 41: 118–21.

Fung, C. P., B. S. Hu, S. C. Lee, P. Y. Liu, T. N. Jang, H. S. Leu, B. I. Kuo, M. Y. Yen, C. Y. Liu, Y. C. Liu, Y. J. Lau, and K. W. Yu. (2000). Antimicrobial resistance of *Streptococcus pneumoniae* isolated in Taiwan. An island-wide surveillance study between 1996 and 1997. J. Antimicrob. Chemother. 45: 49–55

Garcia-Castillo M, Morosini MI, Valverde A, Almaraz F, Baquero F, (2007). Differences in biofilm development and antibiotic susceptibility among *Streptococcus pneumoniae* isolates from cystic fibrosis samples and blood cultures. J Antimicrob Chemother 59: 301–304.

Garcia-Medina, R., W. M. Dunne, P. K. Singh, and S. L. Brody. (2005). *Pseudomonas aeruginosa* acquires biofilm-like properties within airway epithelial cells. Infect. Immun. 73:8298–8305.

Garrett L. (1994). The coming plague: newly emerging diseases in a world out of balance. New York: Farrar, Straus, and Giroux:411.

Ghooi, R. B., and S. M. Thatte. (1995). Inhibition of cell wall synthesis—is this the mechanism of action of penicillins? Med. Hypotheses 44:127–131.

Giammarinaro P. and Paton JC. (2002). Role of RegM, a homologue of the catabolite repressor protein CcpA, in the virulence of *Streptococcus pneumoniae*. Infection and Immunity 70: 5454–5461.

Glover DT, Hollingshead SK, Briles DE. (2008).*Streptococcus pneumoniae* surface protein PcpA elicits protection against lung infection and fatal sepsis. Infect Immun 76: 2767–2776.

Goffin, C., and J. M. Ghuysen. (1998). Multimodular penicillin-binding proteins: an enigmatic family of orthologs and paralogs. Microbiol. Mol. Biol. Rev. 62:1079–1093

Gonzalez–Rey C, Belin AM, Jorbeck H, Norman M, Krovacek K , Henriques B, (2003). RAPD-PCR and PFGE as tools in the investigation of an outbreak of beta-haemolytic streptococcus group A in a Swedish hospital. Comp Immunol Microbiol Infect Dis; 26: 25-35.

Gossens, H. (2009). Antibiotic consumption and link to resistance. Clin. Microbiol. Infec. 15(suppl.3), 12-15.

Gotz, F. (2002). *Staphylococcus* and biofilms. Mol. Microbiol. 43:1367–1378.

Gray, B. M., M. E. Turner, and H. C. Dillon. (1982). Epidemiologic studies of *Streptococcuspneumoniae* in infants. The effects of season and age on pneumococcal acquisition and carriage in the first 24 months of life. Am. J. Epidemiol. 116:692–703.

Guiver, M., R. Borrow, J. Marsh, S. J. Gray, E. B. Kaczmarski, D. Howells, P. Boseley, and A. J. Fox. (2000). Evaluation of the Applied Biosystems automated TaqMan™ PCR system for the detection of meningococcal DNA. FEMS Immunol. Med. Microbiol. 1208:1–7

Hager, H. L., T. W. Woolley, and S. L. Berk. (1990). Review of recent pneumococcal infections with attention to vaccine and nonvaccine serotypes. Rev. Infect. Dis. 12:267-272

Hall-Stoodley L, Hu FZ, Gieseke A, Nistico L, Nguyen D, (2006). Direct detection of bacterial biofilms on the middle-ear mucosa of children with chronic otitis media. JAMA 296: 202–211.

Hall-Stoodley, L., F. Z. Hu, A. Gieseke, L. Nistico, D. Nguyen, J. Hayes, M. Forbes, D. P. Greenberg, B. Dice, A. Burrows, P. A. Wackym, P. Stoodley, J. C. Post, G. D. Ehrlich, and J. E. Kerschner. (2006). Direct detection of bacterial biofilms on the middle-ear mucosa of children with chronic otitis media. JAMA 296:202–211.

Hammerschmidt S, Wolff S, Hocke A, Rosseau S, Muller E. (2005). Illustration of pneumococcal polysaccharide capsule during adherence and invasion of epithelial cells. Infect Immun 73: 4653–4667.

Hammerschmidt, S., S. Wolff, A. Hocke, S. Rosseau, E. Muller, and M.Rohde. (2005). Illustration of pneumococcal polysaccharide capsule during adherence and invasion of epithelial cells. Infect. Immun. 73:4653–4667

Hammerschmidt,S.,A.Muller,H.Sillman, M. Muhlenhoff, R. Borrow.(1996). Capsule phase variation in *Neisseria meningitidis* serogroup B by slipped-strand mispairing in the polysialyltransferase gene (siaD):

correlationwithbacterialinvasionandtheoutbreakofmeningococcaldisease.Mol. Microbiol. 20:1211–1220.

Hava, D. L., J. LeMieux, and A. Camilli. (2003). From nose to lung: the regulation behind *Streptococcus pneumoniae* virulence factors. Mol. Microbiol. 50:1103–1110.

Hee Kuk Park, Sang-Jae Lee, Jang Won Yoon, Jong Wook Shin,4 Hyoung-Shik Shin, Joong-Ki Kook, Soon Chul Myung and Wonyong Kim(2010). Identification of the *cpsA* gene as a specific marker for the discrimination of *Streptococcus pneumoniae* from viridans group streptococci. Journal of Medical Microbiology, 59, 1146–1152 DOI 10.1099/jmm.0.017798-0

Ho, P. L., T. L. Que, D. N. C. Tsang, T. K. Ng, K. H. Chow, and W. H. Seto. (1999). Emergence of fluoroquinolone resistance among multiply resistant strains of *Streptococcus pneumoniae* in Hong Kong. Antimicrob. Agents Chemother. 43:1310–1313

Ho, P. L., W. C. Yam, T. K. Cheung, W. W. Ng, T. L. Que, D. N. Tsang, T. K. Ng, and W. H. Seto. (2001). Fluoroquinolone resistance among *Streptococcus pneumoniae* in Hong Kong linked to the Spanish 23F clone. Emerging Infectious Diseases 7:906-908.

Hoa M, Tomovic S, Nistico L, Hall-Stoodley L, Stoodley P, (2009). Identification of adenoid biofilms with middle ear pathogens in otitis-prone children utilizing SEM and FISH. Int J Pediatr Otorhinolaryngol 73: 1242–1248.

Hong, W., K. Mason, J. Jurcisek, L. Novotny, L. O. Bakaletz, and W. E. Swords. (2007). Phosphorylcholine decreases early inflammation and promotes the establishment of stable biofilm communities of nontypeable *Haemophilusinfluenzae* strain 86-028NP in a chinchilla model of otitis media. Infect. Immun. 75:958–965.

Hsieh YC, Hsueh PR, Lu CY, Lee PI, Lee CY, Huang LM.(2004) Clinical manifestations and molecular epidemiology of necrotizingpneumonia and empyema caused by *Streptococcus pneumoniae* in children in Taiwan. Clin Infect Dis. 38:830–5

Hsiu-Yuan Tsai, Po-Ren Hsueh, Lee-Jene Teng, Ping-Ing Lee,Li-Min Huang, Chin-Yun Lee, and Kwen-Tay Luh.(2000).Bacteremic Pneumonia Caused by a Single Clone of *Streptococcus pneumoniae* with Different Optochin Susceptibilities. Journal of Clinical Microbiology, p. 458–459 Vol. 38, No. 1.

Hsueh, P. R., Y. C. Liu, J. M. Shyr, T. L. Wu, J. J. Yan, J. J. Wu, H. S. Leu, Y. C, Chuang, Y. J. Lau, and K. T. Luh. (2000). Multicenter surveillance of antimicrobial resistance of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* in Taiwan during the 1998–1999 respiratory season. Antimicrob. Agents Chemother. 44:1342–1345

Irie, Y., A. Preston, and M. H. Yuk. (2006). Expression of the primary carbohydrate component of the *Bordetellabronchiseptica* biofilm matrix is dependent on growth phase but independent of Bvg regulation. J. Bacteriol. 188: 6680–6687.

Ispahani P, Slack RCB, Donald FE, Weston WC, Rutter N. (2004) Twenty year surveillance of invasive pneumococcal disease in Nottingham: serogroups responsible and implications for immunisation. Arch Dis Child 89: 757–762.

Iwalokun Bamidele A., Fowora, M., Akinloye Olubukola, Oluwadun Afolabi, Antonio M. and Adegbola, R. A. (2012).Full Length Research paper A retrospective study of clinical *Streptococcus pneumoniae* isolates from four health facilities in South-West Nigeria.International Journal of Medicine and Medical Sciences Vol. 4(8), pp.160-170.

Johnson, C. N., W. H. Benjamin Jr, Jr., S. A. Moser, S. K. Hollingshead, X. Zheng, M. J. Crain, M. H. Nahm, and K. B. Waites. (2003). Genetic relatedness of levofloxacin-nonsusceptible *Streptococcus pneumoniae* isolates from North America. J Clin Microbiol 41:2458-64.

Jones, R. N., and M. A. Pfaller. (2000). Macrolide and fluoroquinolone (levofloxacin) resistance among *Streptococcus pneumoniae* strains: significanttrends from the SENTRY Antimicrobial Surveillance Program (North America, 1997–1999). J. Clin. Microbiol. 38:4298–4299.

Judy C. Arbique, Claire Poyart, Patrick Trieu-Cuot, Gilles Quesne, Maria da Glo´ria S. Carvalho, Arnold G. Steigerwalt, Roger E. Morey, Delois Jackson, Ross J. Davidson, and Richard R. Facklam.(2004). Accuracy of Phenotypic and Genotypic Testing for Identification of *Streptococcus pneumoniae* and Description of Streptococcus pseudopneumoniae sp. nov Journalof Clinical Microbiology, p. 4686–4696 Vol. 42, No. 10. American Society for Microbiology.

Kadioglu, A., J. N. Weiser, J. C. Paton, and P. W. Andrew. (2008). The role of *Streptococcus*

pneumoniae virulence factors in host respiratory colonization and disease. Nat. Rev. Microbiol. 6:288–301.

Kaijalainen, T., S. Rintama ¨ki, E. Herva, and M. Leinonen. (2002). Evaluation of gene-technological and conventional methods in the identification of *Streptococcus pneumoniae*. J. Microbiol. Methods 51:111–118

Kamerling, J. P. (1999). Pneumococcal polysaccharides: a chemical view, p. 81–114. In A. Tomasz (ed.), *Streptococcus pneumoniae*: molecular biology and mechanisms of disease. Mary Ann Liebert, Inc., Larchmont, N.Y.

Karin Kverweg, Chris D. Pericone, Gerridina G. C. Verhoef, Jeffrey N. Weiser, Hugo D. Meiring, AD P. J. M. de Jong, Ronald de Groot, and Peter W. M. Hermans. (2000).Differential Protein Expression in Phenotypic Variants of *Streptococcus pneumoniae*. Infection and Immunity,p. 4604–4610 Vol. 68, No. 8. American Society for Microbiology.

Kawamura, Y. (1996). Evaluation and comparison of newly available identification kits for streptococci. J. Med. Technol. 40:409–416.

Kawamura, Y., Whiley, R. A., Shu, S. E., Ezaki, T. and Hardie, J. M. (1999). Genetic approaches to the identification of the mitis group within the genus *Streptococcus*. Microbiology 145, 2605–2613.

Keith ER, Podmore RG, Anderson TP, Murdoch DR. (2006). CharacteristicsofStreptococcuspseudopneumoniaei solatedfrompurulentsputum samples. J. Clin. Microbiol. 44:923–927. doi:10.1128/JCM.44.3.923927.2006.

Kellog, J. A., D. A. Bankert, C. J. Elder, J. I. Gibbs, and M. C. Smith. (2001). Identification of *Streptococcus*pneumoniae revisited. J. Clin. Microbiol. 39: 3373–3375

Kelly, R. T., and D. Greiff. (1970). Toxicity of pneumococcal neuraminidase. Infect. Immun. 2:115–117

Kikuchi, K., T. Enari, K. Totsuka, and K. Shimizu. (1995). Comparison of phenotypiccharacteristics,DNA-DNA hybridization results, and results with a commercial rapid biochemical and enzymatic reaction system for identification of viridans group streptococci. J. Clin. Microbiol. 33:1215–1222.

Kilic AO, Tao L, Zhang Y, Lei Y, Khammanivong A, (2004). Involvement of *Streptococcus gordonii* β-glucoside metabolism systems in adhesion, biofilm formation, and in vivo gene expression. Journal of Bacteriology 186: 4246–4253.

Kim JO, Weiser JN. (1998). Association of intrastrain phase variation in quantity of capsular polysaccharide and teichoic acid with the virulence of *Streptococcus pneumoniae*. J Infect Dis 177: 368–377.

Kim S. LeMessurier, Abiodun David Ogunniyi and James C. Paton. (2006). Differential expression of key pneumococcal virulence genes in vivo. South Australia 5005, Australia Microbiology, 152, 305–311 DOI 10.1099/mic.0.28438-0.

Kim, J. O., and J. N. Weiser. (1998). Association of intrastrain phase variation in quantity of capsular polysaccharide and teichoic acid with the virulence of *Streptococcus pneumoniae*. J. Infect. Dis. 177:368–377.

Kim, J. O., and J. N. Weiser. (1998). Association of intrastrain phase variation in quantity of capsular polysaccharide and teichoic acid with the virulence of *Streptococcus pneumoniae*. J. Infect. Dis. 177:368–377

Kim, W., Kim, J.-Y., Cho, S.-L., Nam, S.-W., Shin, J.-W., Kim, Y.-S. and Shin, H.-S. (2008). Glycosyltransferase – a specific marker for the discrimination of *Bacillus anthracis* from the Bacillus cereus group. J Med Microbiol 57, 279–286.

King SJ, Hippe KR, Gould JM, Bae D, Peterson S. (2004). Phase variable desialylation of host proteins that bind to *Streptococcus pneumoniae* in vivo and protect the airway. Mol Microbiol 54: 159–171.

King SJ. (2010). Pneumococcal modification of host sugars: A major contributor to colonization of the human airway? Mol Oral Microbiol 25: 15–24

Klugman, C. P. (1990). Pneumococcal resistance to antibiotics. Clin. Microbiol. Rev. 3, 171-96.

Krivan, H.C.,D.D. Roberts, and V. Ginsberg. (1988).Many pulmonary pathogenic bacteria bind specifically to the carbohydrate sequence GalNAcb14Gal found in some glycolipids. Proc. Natl. Acad. Sci. USA 85:6157–6161

Lanie JA, Ng WL, Kazmierczak KM, Andrzejewski TM, Davidsen TM. (2007). Genome sequence of avery's virulent serotype 2 strain D39 of *Streptococcus pneumoniae* and comparison with that of unencapsulated laboratory strain R6. Journal of Bacteriology 189: 38–51.

Lavender, H. F., J. R. Jagnow, and S. Clegg. (2004). Biofilm formation in vitro and virulence in vivo of

mutants of *Klebsiella pneumoniae*. Infect. Immun. 72:4888–4890

Levin, A.S.; Teixeira, L.M., Sesselogo, J.F.; Barone, A.A. (1996). Resistance of *Streptococcus pneumoniae* to antimicrobials in São Paulo, Brazil: clinical features and serotypes. Rev. Inst. Med. Tropical 38(3), 187-192.

Levy, S. B., and B. Marshall. (2004). Antibacterial resistance worldwide: causes, challenges and responses. Nat. Med. 10:S122–S129.

Light RW.(1985). Parapneumonic effusions and empyema. Clin Chest Med;6:55–62

LigozziM., BerniniC., BonoraM. G., de FatimaM., ZulianiJ., andFontana R. (2002).Evaluation of the VITEK 2 System for Identification and Antimicrobial Susceptibility Testing of Medically Relevant Gram-Positive Cocci. journal of clinical microbiology, p. 1681–1686 Vol. 40, No. 5.

Lizcano A, Chin T, Sauer K, Tuomanen EI, Orihuela CJ. (2010). Early biofilm formation on microtiter plates is not correlated with the invasive disease potential of *Streptococcus pneumoniae*. Microb Pathog 48: 124–130.

Llull D, Lopez R, Garcia E. (2006). Characteristic signatures of the *lytA2* gene provide a basis for rapid and reliable diagnosis of *Streptococcus pneumoniae* infections. J. Clin. Microbiol. 44:1250–1256. doi:10.1128/ JCM.44.4.1250-1256.2006.

Louie J, Jean C, Chen TH. (2009). Bacterial coinfections in lung tissue specimens from fatal cases of 2009 pandemic influenza A (H1N1) United States. MMWR, 58: 1071–1074.

Madhi S, Kuwanda L, Cutland C, Klugman KP. (2005). The impact of a 9valent pneumococcal conjugate vaccine on the public health burden of pneumonia in HIV-infected and uninfected children. Clin Infect Dis; 40: 1511–1518.

Madhi SA, Klugman KP, the Vaccine Trialist Group.(2004). A role for *Streptococcus pneumoniae* in virus-associated pneumonia. Nat Med; 10: 811–813.

Madhi SA, Levine OS, Cherian T. (2008) Pneumococcal conjugate vaccine is efficacious and effective in reducing the burden of pneumonia. Bull World Health Organ 86: A–C.

Madhi SA, Ludewick H, Kuwanda L. (2006). Pneumococcal coinfection with human metapneumovirus. J Infect Dis, 193: 1236–1243.

Magee, A. D., and J. Yother. (2001). Requirement for capsule in colonization by *Streptococcus pneumoniae*. Infect. Immun. 69:3755–3761

Manetti, A. G., C. Zingaretti, F. Falugi, S. Capo, M. Bombaci, F. Bagnoli, G. Gambellini,G. Bensi,M.Mora,A. M.Edwards,J. M. Musser,E.A. Graviss, J. L. Telford, G. Grandi, and I. Margarit. (2007). *Streptococcus pyogenes* pili promote pharyngeal cell adhesion and biofilm formation. Mol. Microbiol. 64:968–983.

MarcosMA,JimenezdeAntaMT,delaBellacasa JP. (2003).Rapid urinary antigen test for diagnosis of pneumococcal community-acquired pneumonia in adults. Eur Respir J;21:209–14.

Marion C, Aten AE, Woodiga SA, King SJ. (2011). Identification of an ATPase, MsmK, which energizes multiple carbohydrate ABC transporters in *streptococcus pneumoniae*. Infection and Immunity 79: 4193–4200.

Marion C, Burnaugh AM, Woodiga SA, King SJ. (2011). Sialic acid transport contributes to pneumococcal colonization. Infection and Immunity 79: 1262– 1269.

Martín-Galiano,A.J.,L.Balsalobre,A.Fenoll,andA.D.delaCampa. (2003). Genetic characterization of optochin-susceptible viridans group streptococci. Antimicrob. Agents Chemother. 47:3187–3194. 40.

Maruyama T, Gabazza EC, Morser J, Takagi T, D'Alessandro-Gabazza C, (2010). Community-acquired pneumonia and nursing home-acquired pneumonia in the very elderly patients. Respir Med 104: 584–592.

Massey, R. C., A. Buckling, and S. J. Peacock. (2001). Phenotypic switching of antibiotic resistance circumvents permanent costs in *Staphylococcusaureus*. Curr. Biol. 11:1810–1814.

May T. Fleih , Harith J.F. Al-Mathkhury and Zahraa S. Mahmod. (2007).Thepathological effect of peptidoglycan on rats' lungs part one: pathogenic bacteria *Streptococcus pneumoniae*. Journal of Al-Nahrain University,Science, Vol.10(2), pp. 87-93

McAllister LJ, Ogunniyi AD, Stroeher UH, Paton JC. (2012). Contribution of a genomic accessory region encoding a putative cellobiose phosphotransferase system to virulence of *Streptococcus pneumoniae* PloS One 7: e32385.

McAvin, J. C., Reilly, P. A., Roudabush, R. M., Barnes, W. J., Salmen, A., Jackson, G. W., Beninga, K. K., Astorga, A., McCleskey, F. K. (2001). Sensitive and specific method for rapid identification of *Streptococcus pneumoniae* using

real-time fluorescence PCR. J Clin Microbiol 39, 3446–3451.

McAvin,J.C.,P.A.Reilly,R.M.Roudabush,W.J.Barnes, A.Salmen,G.W. Jackson, K. K. Beninga, A. Astorga, F. K. McCleskey, W. B. Huff, D. Niemeyer, and K. L. Lohman. (2001). Sensitive and specific method for rapid identification of *Streptococcus pneumoniae* using real-time fluorescence PCR. J. Clin. Microbiol. 39:3446–3451

McCool, T. L., T. R. Cate, G. Moy, and J. N. Weiser. (2002). The immune response to pneumococcal proteins during experimental human carriage. J. Exp. Med. 195:359–365.

McCullers J. (2006). Insights into the interaction between influenza virus and pneumococcus. Clin Microbiol Rev; 19: 571–582.

McEllistrem, M. C., J. V. Ransford, and S. A. Khan. (2007). Characterization of in vitro biofilm-associated pneumococcal phase variants of a clinically relevant serotype 3 clone. J. Clin. Microbiol. 45:97–101.

McGee, L., C. E. Goldsmith, and K. P. Klugman. (2002). Fluoroquinolone resistance among clinical isolates of *Streptococcus pneumoniae* belonging to international multiresistant clones. Journal of Antimicrobial Chemotherapy 49:173-176.

McGee, L., Klugman, K. P., Wasas, A., Capper, T. and Brink, A. (2001). Serotype 19F multiresistant pneumococcal clone harboring two erythromycin resistance determinant s*erm(B)* and *mef (A)*in South Africa. Antimicrobial Agents and Chemotherapy 45, 1595–8.

McKessar SJ. and Hakenbeck R. (2007). The two-component regulatory system TCS08 is involved in cellobiose metabolism of *streptococcus pneumoniae* R6. Journal of Bacteriology 189: 1342–1350.

Mendonça-Souza C. R.V.,CarvalhoDa G. S.,Barros R. R.,Dias C. A.,Sampaio J L M.,Castro A C.D. , Facklam R R., andTeixeira L M. (2004). Occurrence and Characteristics of Erythromycin-Resistant *Streptococcus pneumoniae* Strains Isolated in Three Major Brazilian States. Microbial Drug Resistance, Volume 10, Number 4, ©) Mary Ann Liebert, Inc.

Mendonça-Souza, C.R.V.; Carvalho, M.G.S.; Barros, R. R.; Dias, A. D.; Sampaio, J.L.M.; Castro, A.C.D.; Facklam, R.R.; Teixeira, L.M. (2004). Occurrence and characteristics of erythromycin-resistant *Streptococcus pneumoniae* strains isolated in three major Brazilian States. Microb. Drug Resist. 10(4), 313-320.

Mhyre,A. ; J.Stuestol, M.Dahle, G. Overland, C.Thiemermann, S.Foster, P.Lilleaasen, A.Aasen, and J.Wang,(2004). Organ injury and cytokine release caused by peptidoglycan dependent on the structural integrity of the glycan chain, Infect. Immun, Vol. 72, No.3, pp. 1311-1317.

Michel N, Watson M, Baumann F, Perolat P, Garin B.(2005). Distribution of *Streptococcus pneumoniae* Serotypes Responsible for Penicillin Resistance and the Potential Role of New ConjugateVaccines in New Caledonia. Journal of clinical microbiology.43(12):6060–3.

Michelow IC, Lozano J, Olsen K. (2002). Diagnosis of *Streptococcus pneumoniae* lower respiratory infection in hospitalized children by culture, polymerase chain reaction, serological testing, and urinary antigen detection. Clin. Infect. Dis., 34:e1–11.

Mitchell TJ. (2003).The pathogenesis of streptococcal infections: From tooth decay to meningitis. Nat Rev Microbiol 1: 219–230.

Mitchell,T.J. (2003).The pathogenesis of streptococcal infections: from tooth decay to meningitis. Nat. Rev. Microbiol. 1:219–230.

MontanariM. P., Mingoia M., Giovanetti M., and VaraldoP. E. (2001). Differentiation of Resistance Phenotypes among Erythromycin-Resistant. Journal of clinical microbiology,p. 1311–1315 Vol. 39, No. 4.

Moore DP, Klugman K, Madhi SA. (2010). Role of *Streptococcus pneumoniae* in hospitalization for acute community-acquired pneumonia associated with culture-confirmed Mycobacterium tuberculosis in children: a pneumococcal conjugate vaccine probe study. Pediatr Infect Dis J; 29: 1099–1104.

Morrison, K.E., Lake, D., Crook, J.,Carlone, G. M.,Ades, E.,Facklam, R. and Sampson, J. S. (2000). Confirmation of *psaA* in all 90 serotypes of *Streptococcus pneumoniae* by PCR and potential of this assay for identification and diagnosis. J Clin Microbiol, 38, 434–437.

Moscoso M, Garcia E, Lopez R. (2006). Biofilm formation by *Streptococcus pneumoniae*: role of choline, extracellular DNA, and capsular polysaccharide in microbial accretion. J Bacteriol 188: 7785–7795.

Moscoso, M., E. Garcia, and R. Lopez. (2006). Biofilm formation by *Streptococcus pneumoniae*: role of choline, extracellular DNA, and capsular polysaccharide in microbial accretion. J. Bacteriol. 188:7785–7795

Mun˜oz-Elías, E. J., and J. D. McKinney. (2002). Bacterial persistence: strategies for survival, p. 331–355. In S. H. E. Kaufmann, A. Sher, and R. Ahmed (ed.), Immunology of infectious diseases. ASM Press, Washington, DC.

Mun˜oz-Elías, E. J., Joan Marcano, and Andrew Camilli. (2008).Isolation of *Streptococcus pneumoniae* Biofilm Mutants and Their Characterization during Nasopharyngeal Colonization. Infection and Immunity, p. 5049–5061 Vol. 76, No. 11 0019-9567/08/$08.00 0 doi:10.1128/IAI.00425-08.

Mundy, L. S., E. N. Janoff, K. E. Schwebke, C. J. Shanholtzer, and K. E. Willard. (1998). Ambiguity in the identification of *Streptococcus pneumoniae*: optochin, bile solubility, quellung, and the Accu Probe DNA probe tests. Am. J. Clin. Pathol. 109:55–61.

Munoz, R., A. Fenoll, D. Vicioso, and J. Casal. (1990). Optochin resistant variants of Streptococcus pneumoniae. Diagn. Microbiol. Infect. Dis. 13:63– 66.

Munoz-Elias EJ, Marcano J, Camilli A. (2008). Isolation of *Streptococcus pneumoniae* biofilm mutants and their characterization during nasopharyngeal colonization. Infect Immun 76: 5049–5061.

Nandi s., GangulyN. K., KumarR., BakshiD. K., Sagar V.andChakrabortiA. (2008). Genotyping of group A streptococcus by various molecular methods.Indian J Med Res 127, January, pp 71-77.

Navarro D, Garcı´a-Maset L, Gimeno C, Escribano A, Garcı´a-de-Lomas J.(2004). Performance of the Binax NOW *Streptococcus pneumoniae* urinary antigen assay for diagnosis of pneumonia in children with underlying pulmonary diseases in the absence of acute pneumococcal infection. J Clin Microbiol;42:4853–5

Neidhardt, F. C., and R. A. VanBogelen. (1987). *Escherichiacoli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, DCHeat shock response, p. 1334–1345. In F. C. Neidhardt (ed.).

Nelson, A. L., A. M. Roche, J. M. Gould, K. Chim, A. J. Ratner, and J. N. Weiser. (2007). Capsule enhances pneumococcal colonization by limiting mucus-mediated clearance. Infect. Immun. 75:83–90.

Nester,E. ; D.Anderson, C. Roberts Jr, N.Pearsall, and M. Nester.(2001). Microbiology a human perspective 3r ed, MacGraw Hill, pp. 62, 564.

Nielsen, S. V., and J. Henrichsen. (1992). Capsular types of *Streptococcus pneumoniae* isolated from blood and CSF during 1982-1987. Clin. Infect. Dis. 15:794-798.

O'Brien KL, Wolfson LJ, Watt JP, Henkle E, Deloria-Knoll M, (2009). Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. Lancet 374: 893–902.

O'Neill, A. M., Gillespie, S. H. and Whiting, G. C. (1999). Detection of penicillin susceptibility in *Streptococcus pneumonia* by pbp2b PCR restriction fragment length polymorphism analysis. J Clin Microbiol 37, 157–160.

O'Toole, R. D., L. Goode, and C. Howe. (1971). Neuraminidase activity in bacterial meningitis. J. Clin. Invest. 50:979–985.

Obaro, S. K. (2000). Confronting the pneumococcus: a target shift or bullet change? Vaccine 19:1211–1217.

Oggioni MR, Trappetti C, Kadioglu A, Cassone M, Iannelli F, (2006). Switch from planktonic to sessile life: a major event in pneumococcal pathogenesis. Mol Microbiol 61: 1196–1210.

Oggioni, M. R., C. Trappetti, A. Kadioglu, M. Cassone, F. Iannelli, S. Ricci, P. W. Andrew, and G. Pozzi. (2006). Switch from planktonic to sessile life: a major event in pneumococcal pathogenesis. Mol. Microbiol. 61:1196–1210.

Orihuela CJ, Gao G, Francis KP, Yu J, Tuomanen EI. (2004). Tissue-specific contributions of pneumococcal virulence factors to pathogenesis. J Infect Dis 190: 1661–1669.

Pallares R, Lin˜ares J, Vadillo M. (1995). Resistance to penicillin and cephalosporin and mortality from severe pneumococcal pneumonia in Barcelona, Spain. N Engl J Med; 333: 474–80.

Parker D, Soong G, Planet P, Brower J, Ratner AJ, (2009). The NanA neuraminidase of Streptococcus pneumoniae is involved in biofilm formation. Infect Immun 77: 3722–3730.

Paul, J. (1997). HIV and pneumococcal infection in Africa. Microbiological aspects. Trans. R. Soc. Trop. Med. Hyg. 91:632–637

Pernot, L., et al. (2004). A PBP2x from a clinical isolate of *Streptococcus pneumoniae* exhibits an alternative mechanism for reduction of susceptibility to beta-lactam antibiotics. J. Biol. Chem. 279:16463–16470

Rai GP, Zachariah K, Sharma R, Phadke S, Belapurkar KM. (2003). Pneumococcal antigen detection in cerebrospinal fluid: a comparativestudy on counter immunoelectrophoresis, latex agglutination and coagglutination. Comp Immunol Microbiol Infect Dis;26:261–7

Reid SD, Hong W, Dew KE, Winn DR, Pang B, (2009).*Streptococcus pneumoniae* forms surface-attached communities in the middle ear of experimentally infected chinchillas. J Infect Dis 199: 786–794.

Revenge of the killer microbes: losing the war against infectious disease. Time 1994; 144(11):62–9.

Richard N, Komurian-Pradel F, Javouhey E. (2008). The impact of dual viral infection in infants admitted to a pediatric intensive care unit associated with severe bronchiolitis. Pediatr Infect Dis J; 27: 213– 217.

Riedel S.; Beekmann S.E.; Heilmann K.P.; Richter S.S.; Garcia-deLomas J.; Ferech M.; Goosens H.; Doern G.V. (2007). Antimicrobial use in Europe and antimicrobial resistance in *Streptococcus pneumoniae*. Eur. J. Clin. Microbiol. Infect. Dis. 26, 485–490.

Rijal B, Tandukar S, Adhikari R, Tuladhar NR, Sharma PR, Pokharel BM, Gami FC, Shah A, Sharma A, Gauchan P, Sherchand JB, Burlakoti T, Upreti HC, Lalitha MK, Thomas K, Steinhoff M. (2010).Antimicrobial susceptibility pattern and serotyping of *Streptococcus pneumoniae* isolated from Kanti Children Hospital in Nepal. , Vol. 8, No. 2, Issue 30, 164-168.

Rosenow, C., P. Ryan, J. N. Weiser, S. Johnson, P. Fontan, A. Ortqvist, and H. R. Masure. (1997). Contribution of novel choline-binding proteins to adherence, colonization and immunogenicity of *Streptococcuspneumoniae*. Mol. Microbiol. 25:819–829.

Roush SW, Murphy TV. (2007). Historical comparisons of morbidity and mortality for vaccine-preventable diseases in the United States. JAMA 298: 2155–2163.

Rubins JB, Charboneau D, Paton JC, Mitchell TJ, Andrew PW. (1995). Dual function of pneumolysin in the early pathogenesis of murine pneumococcal pneumonia. J Clin Invest 95: 142–150.

Rudan I, Boschi-Pinto C, Mulholland K, Campbell H (2008) Epidemiology and ethiology of childhood pneumonia. Bull World Health Organ 86: 408–416.

Sagar V, Bakshi DK, Nandi S, Ganguly NK, Kumar R, Chakraborti A. (2004). Molecular heterogeneity among north Indian isolates of group A *Streptococcus*. Lett Appl Microbiol; 39: 84-8.

Saha SK, Rikitomi N, Ruhulamin M, Masaki H, Hanif M, Islam M, (1999). Antimicrobial Resistance and Serotype Distribution of *Streptococcus pneumoniae* Strains Causing Childhood Infections in Bangladesh, 1993 to 1997. Journal of Clinical Microbiology. 37(3):798-800.

Sanchez C. J., Nikhil Kumar, Anel Lizcano, Pooja Shivshankar, Julie C. Dunning Hotopp, James H. Jorgensen, Herve ´ Tettelin, Carlos J. Orihuela.(2011). *Streptococcus pneumoniae* in Biofilms Are Unable to Cause Invasive Disease Due to Altered Virulence Determinant Production. PLoS ONE6(12): e28738. doi:10.1371/journal.pone.0028738.

Sanchez CJ, Shivshankar P, Stol K, Trakhtenbroit S, Sullam PM, (2010). The pneumococcal serine-rich repeat protein is an intra-species bacterial adhesin that promotes bacterial aggregation in vivo and in biofilms. PLoS Pathog 6.

Schmitz, F. J., Perdikouli, M., Beeck, A., Verhoef, J. & Fluit, A. C. (2001). Molecular surveillance of macrolide, tetracycline and quinolone resistance mechanisms in 1191 clinical European *Streptococcus pneumoniae* isolates. International Journal of Antimicrobial Agents 18, 433–6.

Schooling, S. R., and T. J. Beveridge. (2006). Membrane vesicles: an overlooked component of the matrices of biofilms. J. Bacteriol. 188:5945–5957.

Schrag, S. J., B. Beall, and S. F. Dowell. (2000).Limiting the spread of resistant pneumococci: biological and epidemiologic evidence for the effectiveness of alternative interventions. Clin.Microbiol. Rev. 13:588–601.

Scott G, Mlacha Z, Nyiro J. (2005). Diagnosis of invasive pneumococcal disease among children in Kenya with enzyme-linked immunosorbent assay for immunoglobulin G antibodies to pneumococcal surface adhesin A. Clin Diagn Lab Immunol, 12: 1195–1201.

Scott JA, Obiero J, Hall AJ, Marsh K. (2002). Validation of immunoglobulin G enzyme-linked

immunosorbent assay for antibodies to pneumococcal surface adhesin A in the diagnosis of pneumococcal pneumonia among adults in Kenya. J Infect Dis; 186: 220–226.

Selinger, D. S., and W. P. Reed. (1979). Pneumococcal adherence to human epithelial cells. Infect. Immun. 23:545–548.

Seppala H, He Q, Osterblad M, Huovinen P.(1994). Typing of group A streptococci by random amplified polymorphic DNA analysis. J Clin Microbiol; 32 : 1945-8.

Service RF. Antibiotics that resist resistance. Science (1995);270:724–7.

Shafeeq S, Kloosterman TG, Kuipers OP. (2011). CelR-mediated activation of the cellobiose-utilization gene cluster in *Streptococcus pneumoniae*. Microbiology (Reading, England) 157: 2854–2861.

Shafeeq S, Kloosterman TG, Kuipers OP. (2011). CelR-mediated activation of the cellobiose-utilization gene cluster in *Streptococcus pneumoniae*. Microbiology (Reading, England) 157: 2854–2861

Shafeeq S, Kloosterman TG, Rajendran V, Kuipers OP. (2012). Characterization of the ROK-family transcriptional regulator RokA of *Streptococcuspneumoniae* D39 Microbiology (Reading, England) 158 (Pt 12), 2917–2926

Sheppard CL, Harrison TG, Smith MD, George RC. (2011). Development of a sensitive, multiplexed immunoassay using xMAP beads for detection of serotype-specific *Streptococcus pneumoniae* antigen in urine samples. J Med Microbiol; 60: 49–55.

Shivshankar P, Sanchez C, Rose LF, Orihuela CJ. (2009). The *Streptococcus pneumoniae* adhesin PsrP binds to Keratin 10 on lung cells. Mol Microbiol 73: 663–679.

Shortridge, V. D., Doern, G. B., Brueggemann, A. B., Beyer, J. M. and Flamm, R. K. (1999). Prevalence of macrolide resistance mechanisms in *Streptococcus pneumoniae* isolates from a multicenter antibiotic resistance surveillance study conducted in the United States in 1994–1995. Clinical Infectious Diseases 29, 1186–8. 3.

Shortridge, V.D., Doern, G.V.; Brueggemann, A.B.; Beyer, J.M.; Flamm, R.K. (1999). Prevalence of macrolide resistance mechanisms in *Streptococcus pneumoniae* isolates from a multicenter antibiotic resistance surveillance study conducted in the United States in 19941995. Clin. Infect. Dis. 29, 1186–1188.

Shundi L, Surdeanu M, Damian M. (2000). Comparison of serotyping, ribotyping and PFGE for distinguishing group A *Streptococcus* strains isolated in Albania. Eur J Epidemiol; 16 : 257-63

Sloan, G. P., C. F. Love, N. Sukumar, M. Mishra, and R. Deora. (2007). The *Bordetella* Bps polysaccharide is critical for biofilm development in the mouse respiratory tract. J. Bacteriol. 189:8270–8276

Song, J.; Chang, H.; Suh, J.Y.; Ko, K.S.; Jung, S.; Oh, W.S.; Peck, K.R.; Lee, N.Y.; Yang, Y.; Chongthaleong, A.; Aswapokee, N.; Chiu, C.; Lalitha, M.K.; Perera, J.; Yee, T.T.; Kumararasinghe, G.; Jamal, F.; Kamarulazaman, A.; Parasakthi, N.; Van, P.H.; So, T.; Keung, T. (2004). Macrolide resistance and genotypic characterization of *Streptococcus pneumoniae* in asian network for surveillance of resistant pathogens (ANSORP). J. Antimicrob. Chemother. 53, 457-463.

Steinhoff MC. (1999). Pulmonary diseases. In: Strickland T, ed. Hunter's tropical medicine, 8th edn. Baltimore: Saunders, 1–7.

Sutcliffe, J.; Grebe, T.; Tait-kamradt, A.; Wondrack. (1996). Detection of erythromycin-resistant determinants by PCR. Antimicrob. Agents Ch. 40(11), 2562-2566.

Tait-Kamradt A., ClancyJ., Cronan M., Wondrack F. Dib-Hajj, Lillian, Wei Yuan, and Sutcliffe J. (1997).*mefE* Is Necessary for the Erythromycin-Resistant M Phenotype in *Streptococcus pneumoniae*.Antimicrobial agents and chemotherapy, p. 2251–2255 Vol. 41, No. 10.

Tapiainen T, Kujala T, Kaijalainen T, Ikaheimo I, Saukkoriipi A. (2010). Biofilm formation by *Streptococcus pneumoniae* isolates from paediatric patients. APMIS 118: 255–260.

Tarafdar K, Rao S, Recco RA, Zaman MM.(2001). Lack of sensitivity of the latex agglutination test to detect bacterial antigen in the cerebrospinal fluid of patients with culture-negative meningitis. Clin InfectDis; 33:406–8

Thornsberry,C. (1985).Automated procedures for antimicrobial susceptibility tests, p. 1015–1018. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.

Tong HH, James M, Grants I, Liu X, Shi G. (2001). Comparison of structural changes of cell surface carbohydrates in the eustachian tube epithelium of chinchillas infected with a *Streptococcus pneumoniae* neuraminidased eficient mutant or its isogenic parent strain. Microb Pathog 31: 309–317.

Tran T. Dang-Hien ; Kwon Hyog-Young ; Kim, Eun-Hye ; Kim, Ki-Woo ; Briles, David E. ; Pyo S., andRhee1, Dong-Kwon. (2011). Decrease in Penicillin Susceptibility Due to Heat Shock Protein ClpL in *Streptococcus pneumoniae*. Antimicrobial agents and Chemotherapy, p. 2714–2728 Vol. 55, No. 6 0066-4804/11/$12.00 doi:10.1128/AAC.01383-10.American Society for Microbiology.

Trappetti C, Kadioglu A, Carter M, Hayre J, Iannelli F, (2009). Sialic acid: a preventable signal for pneumococcal biofilm formation, colonization, and invasion of the host. J Infect Dis 199: 1497–1505.

Trappetti C, Ogunniyi AD, Oggioni MR, Paton JC. (2011). Extracellular Matrix Formation Enhances the Ability of *Streptococcus pneumoniae* to Cause Invasive Disease. PloS one 6: e19844.

Tyx RE, Roche-Hakansson H, Hakansson AP. (2011). Role of dihydrolipoamide dehydrogenase in regulation of raffinose transport in *Streptococcus pneumoniae*. Journal of Bacteriology 193: 3512–3524.

van Selm, S., M. A. B. Kolkman, B. A. M. van der Zeijst, K. A. Zwaagstra, W. Gaastra, and J. P. M. van Putten. (2002). Organization and characterization of the capsule biosynthesis locus of *Streptococcus pneumoniae* serotype 9V. Microbiology 148:1747–1755.

Vernet, G. ; S. Saha, C. Satzke, D. H. Burgess, M. Alderson, J.-F. Maisonneuve, B. W. Beall, M. C. Steinhoff and K. P. Klugman.(2011).Laboratory-based diagnosis of pneumococcal pneumonia: state of the art and unmet needs. Original Article, 10.1111/j.1469-0691. 03496.x. Clinical Microbiology and Infection. European Society of Clinical Microbiology and Infectious Diseases

Vila-Corcoles, A.; Bejarano-Romero, F.; Salsench, E.; Ochoa-Gondar, O.; de Diego, C.; Gómez-Bartomeu, F.; Raga-Luria, X.; Cliville-Guasch, X.; Arija, V. (2009). Drug-resistance in Streptococcus pneumoniae among Spanish middle aged and older adults with community-acquired pneumonia. BMC Infect. Dis. http://www.biomedcentral.com/14712334/9/36

Vu HT, Yoshida LM, Suzuki M. (2010). Association between nasopharyngeal load of *Streptococcus pneumoniae*, viral co- infection, and radiologically confirmed pneumonia in Vietnamese children. Pediatr Infect Dis J; 30(1): 11–18. Aug 3.

Vuori-Holopainen E, Salo E, Saxe´n H. (2002). Etiological diagnosis of childhood pneumonia by use of transthoracic needle aspiration and modern microbiological methods. Clin. Infect. Dis., 34:583–90.

Waite, R. D., J. K. Struthers, and C. G. Dowson. (2001). Spontaneous sequence duplication within an open reading frame of the pneumococcal type 3 capsule locus causes high-frequency phase variation. Mol. Microbiol. 42: 1223–1232

Weber,F. T Dias, C. da Costa M. (2010). Antimicrobial susceptibility of *Streptococcus pneumoniae* and genotypic characterization of erythromycin-resistant strains in porto alegre, Brazil. Brazilian Journal of Microbiology, 41: 1-5 ISSN 1517-8382

Weiser JN, Markiewicz Z, Tuomanen EI, Wani JH. (1996). Relationship between phase variation in colony morphology, intrastrain variation in cell wall physiology, and nasopharyngeal colonization by *Streptococcus pneumoniae*. Infect Immun 64: 2240–2245.

Weiser, J. N., Z. Markiewicz, E. I. Tuomanen, and J. H. Wani. (1996). Relationship between phase variation in colony morphology, intrastrain variation in cell wall physiology, and nasopharyngeal colonization by *Streptococcus pneumoniae*. Infect. Immun. 64:2240–2245.

Weiser,J.N.,R.Austrian,P.K.Sreenivasan,andH.R.Masure. (1994).Phase variation in pneumococcal opacity: relationship between colonial morphology and nasopharyngeal colonization. Infect. Immun. 62:2582–2589

Wester, C. W., D. Ariga, C. Nathan, T. W. Rice, J. Pulvirenti, R. Patel, F. Kocka, J. Ortiz, and R. A. Weinstein. (2002). Possible overestimation of penicillin resistant *Streptococcus pneumoniae* colonization rates due to misidentification of oropharyngeal streptococci. Diagn. Microbiol Infect. Dis. 42:263–268.

Whitney, C.G.; Farley, M.M.; Hadler, J.; Harrison, L. H.; Lexau, C.; Reingold, A.; Lefkowitz, L.; Cieslak, P.R.; Cetron, M.; Zell, E.R.; Jorgensen, J.H.; Schchat, A. (2000). Increasing prevalence of multidrugresistant *Streptococcus pneumoniae* in

the United States. New Engl. J. Med. 343(26), 1917-1924.

Widdowson, C. A., P. V. Adrian, and K. P. Klugman. (2000). Acquisition of chloramphenicol resistance by the linearization and integration of the entire staphylococcal plasmid pC194 into the chromosome of *Streptococcus pneumoniae*. Antimicrobial Agents & Chemotherapy 44:393-5.

Wolter N., SmithA. M., LowD. E., and Klugman K. P. (2007).High-Level Telithromycin Resistance in a Clinical Isolate of *Streptococcus pneumoniae*. Antimicrobial agents and chemotherapy, Mar., p. 1092–1095 Vol. 51, No. 3.

Xayarath, B., and J. Yother. (2007). Mutations blocking side chain assembly, polymerization, or transport of a Wzy-dependent *Streptococcus pneumoniae* capsule are lethal in the absence of suppressor mutations and can affect polymer transfer to the cell wall. J. Bacteriol. 189:3369–3381.

Yang, L., J. A. J. Haagensen, L. Jelsbak, H. K. Johansen, C. Sternberg, N. Hoiby, and S. Molin. (2008). In situ growth rates and biofilm development of *Pseudomonas aeruginosa* populations in chronic lung infections. J. Bacteriol. 190:2767–2776.

Zaidi AKM.(2003). Resistant respiratory infections threaten developing countries. The APUA Newsletter.21(3):1-2.

Zettler, E.W., Scheibe, R.M., Dias, C.A.G., Santafé, P., Santos, D.S., Moreira, J.D.; Fritsher, C.C. (2006). Determination of penicillin resistance in *Streptococcus pneumoniae* isolates from Brazil by PCR. Intern. J. Infect. Dis. 10, 110-115.