Letter to Editor

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Zmpb Gene in *Streptococcus Pneumoniae* Causing Meningitis

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Streptococcus pneumoniae is among the leading and most aggressive (around 30% of attributable mortality) causes of meningitis (Van de Beek et al. [1] O'Brien et al. [1]. and the pathogenesis of this infection is not yet clearly understood. The capsular polysaccharide plays a central role in the pathogenesis of pneumococcal infection; however, other virulence factors need to be considered. An intense inflmmatory response is observed in infections due to *S. pneumoniae* and this is at least partially attributed to ZmpB, a zinc metalloprotease present in virtually all pneumococcal strains, ZmpB likely to serve as an important but unknown housekeeping function associated with the human host. There are four zinc metalloprotease described in *S. pneumoniae* (IgA1 protease, ZmpB, ZmpC, and ZmpD) that may be important for virulence in humans (Bek-Thomsen et al. [2]. It was demonstrated that levels of proinflammatory cytokine tumor necrosis factor alpha in lung tissue were significantly lower and the survival time increased in an animal model when the challenge was performed with a mutant strain lacking zmpB gene (Blue et al. [3]. Results of a study suggest that ZmpB is a potential candidate for pneumococcal vaccine antigen, since immunization with zmpB-based vaccine showed protection against pneumococcal disease in mice (Gong et al.) [4]. Besides this, in a model of complicated pneumonia in mice, the influence of sequence variation of zmpB gene was established; isolates originally obtained from children with complicated pneumonia presented sequences with high identity to those of a well-defined virulent strain of *S. pneumoniae* (TIGR4- genBank access number: AE005672.3) and were capable to induce a stronger inflammatory response in the animal model (Hsieh et al.) [5]. The influence of this allelic variation was not investigated so far in other forms of invasive pneumococcal disease, and we aimed to study the DNA sequences of zmpB gene of pneumococcal isolates obtained from meningitis cases. A total of nine pneumococcal isolates from cerebrospinal fluid of patients with meningitis attended in two hospitals in Porto Alegre, Brazil, was used in this study. DNA extraction was performed according to previously described (http://www.cdc.gov/ncidod/biotech/files/pcr-DNA-extraction-culture-May2009.pdf). DNA was employed for serotyping (Dias et al.) [6,7] and for amplification of zmpB, the reaction was carried out in a final 25 µL volume using Buffer 1X, MgCl2 2.0 µM, dNTPs 0.4 µM, with 1.0 µL of DNA sample, primers final concentration of 0.4 µM (5'-AGCTGCTTCATTCAAACATA, 5'-TCGTTGCTTTATCTTTAACTTC), and Taq DNA polymerase 1 U (Platinum, Invitrogen) using the following cycling parameters: 95°C for 5 min, followed by 35 cycles of 95°C for 45 s, 48°C for 45 s and 72°C for 1 min. After the zmpB amplification step, DNA was purified (Purelink PCR Purification Kit, Invitrogen) and sequenced in an ABI 3130 (Applied Biosystems) apparatus. Sequencing was performed by using primer forward (PHRED values=25) and the FASTA format of sequences were compared to the zmpB of TIGR4 (http://blast.ncbi.nlm.nih.gov/Blast.cgi). It was sequenced a 224 bp fragment of the C-terminal region, which harbored the target sequence with the higher degree of variability: 5'-TCATCACTTAGAACAGACTCACCATCTGTTTTAGATTGTTGTTTATATTTATTTGAAGCATAACCTAAGAACCCATTT. Among the nine pneumococcal isolates, serotype distribution was as follows: 7F (n=2), 9V (n=1), 12F (n=1), 14 (n=2), 20 (n=1), 23F (n=1), and 34 (n=1). Eight isolates presented 97-100% of identity with the zmpB sequence of TIGR4; while one isolate, belonging to serotype 20, presented a lower level (84%) of identity. Our results indicate that there is a low degree of diversity in the sequence of zmpB analyzed among pneumococci obtained from meningitis, independent of the serotype and a high identity with TIGR4 is expected. This may suggest that this particular allele of zmpB not only induces a stronger inflammatory response (not verified in this study), but may also provide conditions for pneumococci to cross the defenses of the blood-brain barrier and invade the meninges. Further investigation is necessary to better elucidate the role of allelic variation of zmpB in the pneumococcal meningitis, including studies with animal models. Besides this, a comparative approach of zmpB sequences of pneumococci obtained from the nasopharynx of healthy carriers would permit other insights about this issue.

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