Short Communication
A Correlation Between the Genes Responsible for Penicillin and Erythromycin Resistance in *Streptococcus Pneumoniae* and the Minimum Inhibitory Concentration (MIC) Values: A Potential Approach for Molecular Detection of Susceptibility

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Summary

The correlation of *pbp 2b* restriction fragment length polymorphism (RFLP) patterns of fifty clinical isolates of Malaysian *Streptococcus pneumoniae*, as well as the distribution of determinant genes for erythromycin resistance; *mefE* and *ermB*, with the respective minimum inhibitory concentration (MIC) values for penicillin and erythromycin (determined by agar dilution method) were examined. Strains with penicillin MIC of ≥ 0.5 μg/ml had unique *pbp 2b* RFLP patterns that were absent in the penicillin-susceptible strains. The prevalence of *ermB*, either alone or together with *mefE*, was frequently observed in strains with higher MIC values (> 8 μg/ml) whereas *mefE* alone was observed in strains with lower MIC values (1 μg/ml - 4 μg/ml). This showed that the *pbp 2b* RFLP patterns and the distribution of *mefE* and *ermB* genes followed certain patterns in relation to the susceptibility. This correlation may thus be used as a diagnostic criterion for molecular detection of resistance.

Keywords: *Streptococcus pneumoniae*, Penicillin, Erythromycin, MIC

Pneumococcus is still one of the leading causative agents of community-acquired pneumonia in the world and, in spite of the availability of modern antimicrobial therapy and intensive care facilities, remains an important cause of morbidity and mortality. Penicillin has been the drug of choice for treatment but therapeutic failure began to be reported in patients with pneumococcal meningitis due to the emergence of penicillin-resistant strain (PRSP) [1]. In addition, PRSP also tends to be multi-drug resistant. For example, resistance to erythromycin, an alternative drug for respiratory infections in patients allergic to penicillin, is frequently observed among PRSPs [2]. At the nucleotide level, penicillin resistance is due to alterations in the penicillin-binding protein gene (*pbp*) sequence which result in amino acid sequence alterations and thus the inability of penicillin to recognize its target sites. Depending on the degree of the alterations, this gives rise to elevated minimum inhibitory concentration (MIC) values for penicillin as well as for many of the β-lactam antibiotics. Resistance to erythromycin is due to the presence of *mefE*, *ermB* or both genes [3]. The former encodes a membrane-bound efflux protein that pumps the antibiotic out of bacterial cells while the latter encodes a ribosomal methylase that alters the ribosomal target site of erythromycin [3]. We have reported earlier the diverse restriction fragment length polymorphism (RFLP) patterns of *pbp* genes in PRSPs due to the gene alterations as compared to penicillin-susceptible *S. pneumoniae* (PSSP) strains that have uniform RFLP patterns among fifty clinical isolates of pneumococci obtained from the University of Malaya Medical Centre, Kuala Lumpur, Malaysia [4]. Here, we extend the study to determine the correlation of *pbp 2b* RFLP patterns of the fifty isolates, as well as the distribution of the determinant genes for erythromycin resistance (*mefE* and *ermB*) with the related MIC values (determined by agar dilution method) for penicillin and erythromycin respectively. It was shown that the *pbp 2b* RFLP patterns and the distribution of the *mefE* and *ermB* followed certain patterns in relation to the susceptibility. Therefore, they may be used as a diagnostic criterion for molecular detection of resistance.

The *pbp 2b* genes were amplified from all the fifty pneumococcal isolates as a 1.5 kb fragment with five distinct RFLP patterns upon digestion with *Hinf*I [4]. The correlation of the five RFLP patterns to the penicillin MIC values is illustrated in Figure 1. Taking the uniform *pbp 2b* gene pattern of the PSSPs (pattern 5) to represent the unaltered gene sequence, it was then observed that alterations, as shown by the different RFLP patterns, took place in strains with penicillin MIC of ≥ 0.5 μg/ml;

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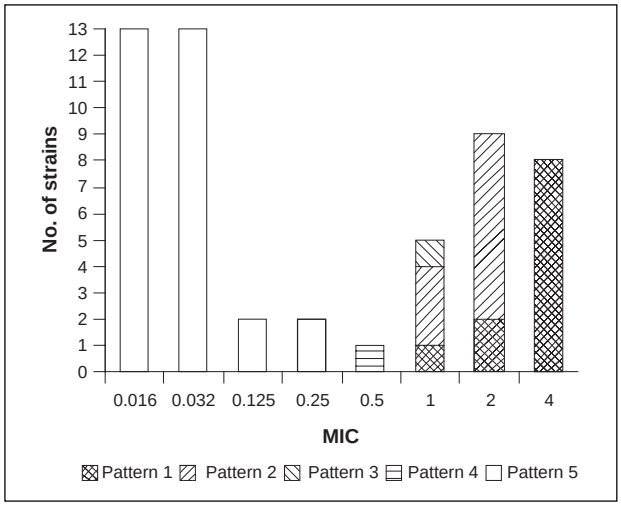

Figure 1: Distribution of the five *pbp 2b* RFLP patterns in relation to the MICs (μg/ml) of penicillin. Numbers on the Y-axis represent the frequency of strains with the respective RFLP patterns at the corresponding penicillin MIC values on the X-axis.

the upper intermediate category of penicillin susceptibility. This finding is concurrent with reports by Smith & Klugman [5] and Beall *et al* [6] who stated that alterations in the *pbp 2b* gene were usually observed among strains with penicillin MIC values of ≥ 0.1 μg/ml. This suggests that alterations in this gene could play a vital role in the expression of decreased susceptibility towards penicillin.

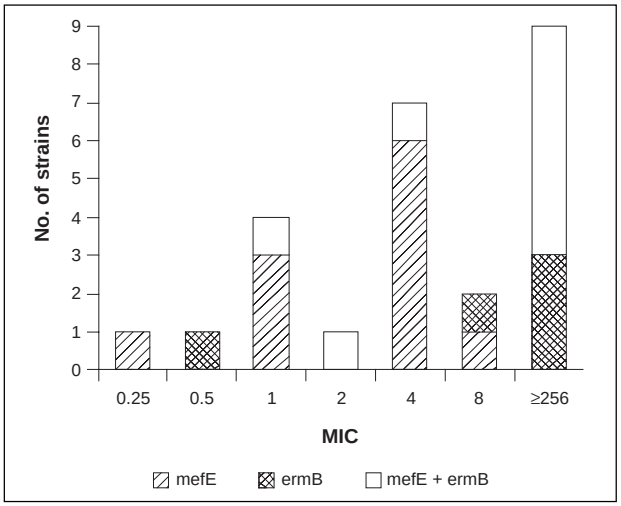

Figure 2: Distribution of erythromycin-resistance determinant genes in relation to the MICs (μg/ml) of erythromycin. Numbers on the Y-axis represent the frequency of strains with the respective distribution of the *mefE* and *ermB* genes at the corresponding erythromycin MIC values on the X-axis.

Genes encoding determinants for erythromycin resistance, *mefE* and *ermB* were amplified as 348 bp and 639 bp fragments respectively. As shown in figure 2, the two genes were detected either alone or together in all twenty three strains that were erythromycin-resistant; MIC values ≥ 1 μg/ml, while the rest of the strains that were erythromycin-susceptible; MIC values ≤ 0.25 μg/ml, were PCR-negative for both genes with the exception of one strain positive for *mefE* alone (This strain had a MIC value at the upper limit of the erythromycin-susceptible category). The prevalence of *ermB*, either alone or together with *mefE*, was frequently observed in strains with higher MIC values (> 8 μg/ml) whereas *mefE* alone was observed in strains with lower MIC values (1 μg/ml - 4 μg/ml). Such a correlation was also observed by Johnston *et al* [7] and Luna *et al* [8]. In this study, none of the strains having the *mefE* gene alone had erythromycin MIC values of > 8 μg/ml.

Because of the genetic conservation of the *pbp 2b* gene in PSSP, any pneumococcal strain with *pbp 2b* RFLP pattern different from that in PSSP may be anticipated as to have decreased susceptibility to penicillin. If the scope of this study is extended to include more strains from various locations, it might also be possible to apply statistical analysis to reliably find common patterns that might be associated with certain level of susceptibility (e.g. intermediate or resistant). The presence of either one or both of the determinant genes for erythromycin resistance might infer that the strain has decreased susceptibility to erythromycin. In addition, the presence of *ermB* might be presumptive that the strain has a high level of resistance as this gene is commonly found in strains with higher MICs. This calls for a study at a large scale to look for consistency in the *pbp 2b* RFLP patterns or distribution of the erythromycin-resistance determinant genes so that they can be developed as a criterion for molecular diagnosis of susceptibility. Since conventional culture-based method might be tedious and time consuming, this molecular approach may provide a faster and reliable result.

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