Penicillin-binding protein 2B gene (pbp2b) based PCR and sequencing for screening Streptococcus pneumoniae infection and predicting its susceptibility in cerebral spinal fluid from paediatric bacterial meningitis patients.

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Background: To evaluate the penicillin-binding protein 2B gene (pbp2b) based PCR and sequencing for screening Streptococcus pneumoniae infection and predict its susceptibility in cerebral spinal fluid from paediatric bacterial meningitis patients.

Methods: A nested PCR targeting pbp2b and another two S. pneumoniae specific PCR targeting pneumolysin (ply) and autolysin (lytA) were developed for detection of S. pneumoniae in cerebral spinal fluid from bacterial meningitis patients. The PCR results of three different genes and culture were compared. The consistency of penicillin susceptibility PCR (using resistant and susceptible primers respectively), sequencing and culture based phenotypic penicillin resistant results were compared.

Result: Of the 161 specimens studied, there were totally 25 S. pneumoniae infection confirmed by different methods (16 by lytA PCR, 14 by ply PCR, 16 by pbp2b PCR and 9 by cerebrospinal fluid culture). Of the 16 pbp2b positive specimens, penicillin sensitive and resistant sequence types account for half respectively. 4 of the 16 pbp2b positive specimens had culture based phenotypic penicillin-resistant result. 3 of 4 were consistent with penicillin susceptibility PCR result. The result of susceptibility PCR targeting pbp2b was consistent with sequencing result. There were no new point mutations but new sequence types were found in these strains when compared with GenBank. Penicillin resistant in pneumococcal meningitis was 66.67% (6/9) by culture phenotype and 50% (8/16) by PCR and sequencing when culture was negative.

Conclusion: pbp2b can be served as a good target gene to detect S. pneumoniae and predict its penicillin susceptibility, especially important when culture was negative.

In vitro activity of antibiotics on biofilm producing isolates of Pseudomonas aeruginosa

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Objectives: Pseudomonas aeruginosa is a known biofilm producer especially in the lung of patients suffering from Cystic fibrosis. We undertook this study to check for biofilm producing capability of Pseudomonas aeruginosa isolated from various clinical specimens and also to check their susceptibility pattern against anti-pseudomonal antibiotics in single and in various combinations.

Methods: The present study was conducted in the Department of Microbiology on 100 consecutive clinically significant isolates of Pseudomonas aeruginosa. Isolates were identified and their antibiogram was performed following standard methods. All these isolates were tested for biofilm production following the method described by Stepanovic et al. Minimum inhibitory concentration against Ceftazidime, Ciprofloxacin, Rifampicin and Colistin was determined by Agar dilution method. A total of ten biofilm producing isolates were randomly selected to study the effect of various combinations of antibiotics at various concentrations by Microbroth dilution method.

Results: Out of hundred consecutive isolates of Pseudomonas aeruginosa isolated from clinical samples, 78% were positive for biofilm production. Out of these, 41.66% were strong biofilm producers, 25% were moderate and 11.66% were weak producers. Colistin and Rifampicin demonstrated synergy in 9/10 isolates whereas Ceftazidime and Ciprofloxacin showed synergy only in 3/10 strains tested.

Conclusion: Biofilm formation is commonly associated with Pseudomonas aeruginosa isolated from various clinical samples. Colistin and Rifampicin was the most effective combination followed by Colistin and Ceftazidime against biofilm producing isolates.