Capturing the misidentified *Streptococcus pseudopneumoniae*: A retrospective analysis of the non-typeable *Streptococcus pneumoniae*

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ABSTRACT

Introduction: *Streptococcus* species, particularly *Streptococcus pneumoniae*, are significant human pathogens responsible for respiratory infections. However, pitfalls laboratory diagnosis, certain species such as *Streptococcus pseudopneumoniae* are occasionally misidentified as *S. pneumoniae* due to similarities in classical microbiological characteristics like colony morphology, Gram stain results, optochin susceptibility, and bile solubility. Notably, *S. pseudopneumoniae* exhibits a higher resistance rate to antimicrobial agents, which warrants the need for accurate identification. This study aims to re-evaluate diagnostic methods to accurately identify and differentiate *S. pseudopneumoniae* from non-typeable *S. pneumoniae*. **Materials and Method:** *Streptococcus pneumoniae* isolates were received at the Bacteriology Unit, Institute for Medical Research between the year 2017 to 2022. The database was screened for isolates that were non-typeable using the Quellung method. Out of 121 isolates archived at -80°C, only 10 were viable isolates to be subjected to phenotypic (hemolysis, Gram stain, bile solubility, optochin test) and genotypic analysis (PCR screening for cpsA, lytA, AliB-like ORF2 and ypdB genes) to detect *S. pseudopneumoniae*. **Results:** A total of 6 α-hemolytic, Gram-negative diplococci that were resistant to optochin and bile insoluble were presumptively identified as *S. pseudopneumoniae*. Further genotypic analysis confirmed 4 of these as *S. pseudopneumoniae* which were cpsA and lytA gene negative. The isolates were AliB-like ORF2 and ypdB gene positive. All 4 confirmed *S. pseudopneumoniae* were isolated from the respiratory specimens of adult patients (aged 30 to 49) from West Malaysia. Further antimicrobial susceptibility testing indicated a MIC_{90} for penicillin at 0.50 µg/mL and resistance to erythromycin and clindamycin. **Conclusion:** The findings underscore the necessity of further characterizing pneumococcal isolates that are optochin-resistant and bile insoluble to accurately identify *S. pseudopneumoniae*. Correct identification is crucial for ensuring accurate surveillance to better understand the epidemiology and clinical impact of this bacterium in Malaysia.