論　文　内　容　の　要　旨
Synopsis of Thesis

氏　　名 Name	Delly Chipta Lestari
論文題名 Title	Clonality Analysis of *Streptococcus pneumoniae* in Clinical Specimens (臨床検体における *Streptococcus pneumoniae* のクロナリティー解析)

論文内容の要旨

〔目　的(Purpose)〕

Streptococcus pneumoniae, also known as pneumococcus, is generally known to be the leading bacterium causing community-acquired pneumonia (CAP). However, pneumococcus can also reside in the upper respiratory tract as a commensal. This study proposes a novel approach, i.e. clonality analysis to discriminate pneumococcus between causative agents and commensal organisms (oropharyngeal colonizers) directly from sputum specimens of adult CAP patients. The clonality analysis was focused on the *cpsB* gene. The *cpsB* gene is one of the important virulence factors in pneumococcus and is involved in the biosynthesis of the capsule polysaccharide. The presence of serotype-associated polymorphisms in *cpsB* is the basis for a molecular biology-based approach to serotyping.

〔方法ならびに成績(Methods/Results)〕

Method: A retrospective study was conducted on clinical sputum specimens collected from adult patients diagnosed with CAP. Real-time PCR and metagenomics were used to complement a culture-based method for diagnosing pneumococcus as the etiological agent of CAP. Metagenomics results were analyzed for clonality analysis by performing low-frequency variant detection using the CLC Genome Workbench®, then Sanger sequencing of the PCR product of the *cpsB* gene and investigating gene sequences in the sequence read archive (SRA) were performed as confirmatory.

Results: In one sample with a pneumococcus-positive culture and the lowest Ct value among all samples, our clonality analysis revealed that no two SNPs appeared in the same position. Hence, pneumococcus in this sample was classified as a single clone. Analysis of the housekeeping genes also displayed similar findings to the analysis of the *cpsB* gene. We also discovered a significant association between the clonality status and the cycle threshold (Ct) value of real-time PCR for pneumococci among specimens. By Fisher's exact test analysis, we found that the clonality status (either single or multiple) of pneumococcus correlates with the Ct value of real-time PCR. Specifically, when the Ct value was below 22, there was a high probability that pneumococcus existed as a single clone (p-value 0.0007). Our method was supported by public datasets accessible at the SRA, that the pneumococcus responsible for causing lower respiratory tract infections, confirmed with clinical symptoms and culture results, was present as a single clone. Moreover, as a respiratory tract colonizer/commensal, pneumococci were present as multiple clones.

〔総　括(Conclusion)〕

This study demonstrated the possible correlation between pneumococcal clonality and bacterial load in clinical specimens. The clonality analysis approach we employed has the potential to be applied to other bacteria and various types of infections. With technological advancements, particularly next-generation sequencing (NGS), a metagenomic approach can be employed for clonality analysis. which has not yet been extensively explored for infectious diseases. Understanding bacterial clonal dynamics could be useful in developing targeted interventions and treatment strategies for infectious diseases.

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本研究でデリ・チプタ・レスタリ氏は、市中肺炎と診断された成人患者から採取した喀痰検体中に存在する肺炎球菌のクローナリティー解析を行った。まず、次世代シーケンシング（NGS）を用いてショットガンメタゲノミクスを実施し、*cpsB*遺伝子を対象としたクローナリティー解析を行った。ショットガンメタゲノミクスでのクローナリティー解析に十分なリード数を示す検体が限られていたため、*cpsB*遺伝子のPCRアンプリコンに対するサンガー法による解析も行った。加えて肺炎球菌に対するリアルタイムPCRを実施し、リアルタイムPCRの結果と肺炎球菌のクローナリティーとの相関関係を分析した。その結果、リアルタイムPCRのCt値が22未満の場合は、肺炎球菌が単一クローンとして存在する可能性が高いことが明らかになり、これは統計学的に有意であった（p値0.0007）。また、遺伝子データベースの公開データを利用して*cpsB*遺伝子を対象にしたクローナリティー解析法について検証した。本研究は、細菌のクローナリティー解析が病因菌か定着菌かの区別に利用できる可能性を示唆したものであり、博士（医学）の学位授与に値すると考える。