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**Title:** Bacteriological assessment of healthcare-associated pneumonia (HCAP) using 16S rRNA gene sequencing analysis

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**Body:** Background ATS/ IDSA guideline for HCAP was released in 2005. HCAP seems to be closer to HAP than CAP, but few reports has been documented according to the bacteriological incidence in HCAP. Molecular biological methods have been used in addition to ordinary cultivation methods, and we evaluated bacterial incidence of HCAP using molecular methods in addition to cultivation. Patients and Methods From April 2010 to December 2011, patients with HCAP were enrolled, and the bronchial washing were obtained from the pathological lesions using bronchoscopy. The partial 580 bp of 16S rRNA gene were amplified by PCR, and clone libraries were constructed. Then 96 clones in each sample were randomly chosen, and sequences of 16S rRNA gene were determined. Homology of the sequences was searched using BLAST. Results Thirty patients (22 males and 8 females, average age 70.9 (45-84)) with HCAP were enrolled. In relation to severities of pneumonia, 13.3% were mild, 63.3% were moderate and 23.3% were severe using Pneumonia Severity Index. First dominant phylotypes were *P. aseruginosa* (13.3%), *S. pneumoniae* (10%), *H. influenzae* (7.0%), *S. aureus* (6.7%), and about 40% of these patients showed anaerobes (14%) and oral streptococci (24%). Ordinary cultivation could not detect some organisms detected by this molecular method, especially anaerobes (0 in cultivation and 4 in molecular analysis) and oral streptococci (2 and 7, respectively). Conclusion It is speculated that anaerobic pathogens and oral streptococci were important in addition to potentially drug-resistant pathogens (*P. aseruginosa* and *S. aureus*), and oral streptococci were more important than previously reported in patients with HCAP.