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Assessment of bacterial bio-films on the middle-ear mucosa of children with chronic otitis media at St. Paul Millennium College Specialized Hospital, Addis Ababa, Ethiopia

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**Introduction:** Chronic otitis media (OM) is a common pediatric infectious disease which can lead to significant hearing loss in children. In Ethiopia, as most developing countries, there is little information on chronic otitis media in children. Previous studies demonstrated that metabolically active bacteria exist in negative pediatric middle-ear effusions which results in the formation of adherent mucosal bio-films infection.

**Objective:** To assess bacterial bio-film as the major cause of chronic otitis media (OM) in humans, at St Paul Millennium College Specialized Hospital (St. PMCSH), Addis Ababa Ethiopia.

**Hypothesis:** Chronic middle-ear disorders are caused by bacterial bio-film.

Methodology: A one year (September 202012 to August 2013) hospital based cross sectional study was conducted to analyze the major causes of chronic otitis media (OM) in St. PMCSH otolaryngology practice. A total of 62 children Middle-Ear Mucosa (MEM) biopsy specimens who undergo tympanostomy tube placement for treatment of OM with effusion and recurrent OM were included in the study. In addition, socio-demographic data were collected using a semi- structured questionnaire. The samples were analyzed using microbiological culture, polymerase chain reaction (PCR), direct microscopic examination, fluorescence in situ hybridization and immunostaining. A total of 9 control MEM specimens were included in the study. We used confocal laser scanning microscopic (CLSM) images were obtained from MEM biopsy specimens and evaluated for bio-film morphology using basic stains and species-specific probes for *Proteus species*, *H. influenzae*, *Streptococcus pneumoniae* and *Moraxella catarrhalis*. Effusions, when present, were evaluated by PCR and culture for evidence of pathogen-specific nucleic acid sequences and bacterial growth, respectively.

**Results:** Of the 62 children (mean age of 4.9 years) undergoing tympanostomy tube placement, 38(61.3%) had OME, 42(67.7%) had recurrent OM and 14 (22.6%) had both diagnoses; 54 of 124(43.5%) of the ears had effusions, 54 of 54 effusions were PCR-positive for at least 1 OM pathogen and 31(57.4%) of 54 effusions were culture-positive for any pathogen. Mucosal bio-films were visualized by CLSM on 56 (90.3%) of 62 MEM specimens from children with OME and recurrent OM using pathogen-specific and nonspecific probes. Bio-films were not observed on 9 control MEM specimens.

**Conclusion:** Direct detection of MEM biopsy specimens from children with OME and recurrent OM revealed that these chronic middle-ear disorders ware bacterial bio-film-related.

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