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Major article

Correlation between the growth of bacterial biofilm in flexible endoscopes and endoscope reprocessing methods

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Flexible endoscope
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Background: The purpose of this article was to investigate bacterial biofilm formed on endoscopes and to explore the possible correlation between endoscope reprocessing procedures and bacterial biofilm growth on endoscope channels.

Methods: Sixty-six endoscope suction and biopsy channels and 13 water and air channels were collected from 66 hospitals throughout China. Scanning electron microscopy was used to observe biofilm growth on the internal surface of these channels. Questionnaires were mailed to 66 endoscopy centers to investigate reprocessing procedures for endoscopes.

Results: Obvious biofilm growth was detected on 36 suction and biopsy channels (36/66, 54.6%) and 10 water and air channels (10/13, 76.9%). The percentage of manual cleaning in group B (n = 36, without detection of biofilms) was 92.3% (33/36), whereas it was 50.0% (15/30) in group A (n = 30, with detection of biofilms). Follow-up of group A (n = 30) showed that no biofilm was detected, whereas biofilm was detected in group B. The difference was statistically significant ($P = .001$). The proportion of detergent reuse in group B was 92.3% (33/36), and it was 61.5% in group A (18/30) ($P = .005$). The proportion of alcohol-air drying in group B was 38.9% (14/36), and it was 76.7% (23/30) in group A ($P = .002$).

Conclusion: The formation of endoscopic biofilm during clinical practice may be related to reuse of detergent, manual cleaning, and incomplete drying.

Endoscopic procedures are commonly indicated for the diagnosis and treatment of digestive diseases. In the United States, 20 million endoscopic procedures are performed annually.[1] Considering the huge population base in China, the estimated number of digestive endoscopy procedures performed per year may be much larger than that. Endoscopic procedure-related infections have been reported in China[2]; and the endoscopic procedure-related infection rate has been estimated at 1 in every 1.8 million procedures.[1] Endoscope reprocessing procedures in China have not received attention until recently. Because of the large population base and economic constraints in China, endoscope reprocessing is currently facing serious problems. To prevent endoscopic procedure-related infections, endoscope cleaning and disinfection is of particular importance.

The formation of bacterial biofilms is an important source of infection because of inadequate endoscope cleaning and disinfection. Biofilms are bacterial surface-associated communities attached to solid substrata, growing into a nutrient-containing water phase and embedded in a polymer matrix produced by the bacteria.[3] Biofilms are widespread and can be found on moist surfaces, including water pipes, ventilation pipes, and medical devices (eg, catheters, artificial heart valves, pacemakers, endoscope channels).[4] Pajkos et al[3] reported that biofilms were found on suction and biopsy channels and water and air channels of used endoscopes. They noted that there was still bacterial biofilms on endoscope channels even after thorough cleaning and decontamination as part of endoscope reprocessing. Endoscope channels with residual biofilms could lead to bacteria multiplying and regrowing and biofilms reforming. Contaminated endoscopes were found to cause infections in patients after endoscopic procedures.[3-5] Microorganisms might be protected from disinfectants by the output of thick masses of cells and extracellular materials in biofilms. When these masses form, microbes within them will be resistant to disinfectants through various mechanisms, which may include, but

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are not limited to, physical characteristics of older biofilms, genotypic variety of bacteria, microbial production of neutralizing enzymes, and physiologic gradients within the biofilms (hydrogen ion concentration). Bacteria within the biofilms are much more difficult to treat than the same bacteria in suspension,[6] which can result in failure of the decontamination process. For these reasons, research of biofilms is of particular importance to control postendoscopic infections. In a recent study, Vickery et al[7] found that biofilm removal detergent can effectively eliminate biofilms on endoscope channels. Ren et al also indicated that there was no significant difference in biofilm removal with different contact time of detergents, but nonenzymatic detergent could significantly reduce biofilms.[8]

To our knowledge, there is no study investigating the status of endoscope contamination of bacterial biofilms and its relationship with endoscope reprocessing procedures. Therefore, in this study, endoscope channel tubing samples collected from 66 hospitals were observed by scanning electron microscopy (SEM), and the corresponding endoscope reprocessing procedures from the 66 hospitals were surveyed to identify the correlation between bacterial biofilms and reprocessing procedures.

METHODS

Materials

Endoscopic suction and biopsy channels and water and air channels were provided by the endoscope repair centers of Olympus, Pentax, and Fujifilm in China. There were 66 suction and biopsy channels and 13 water and air channels, which were disassembled as part of the major repair of endoscopes in 66 endoscopic centers throughout China.

Collection and processing of endoscope channels

After collection, endoscope channels were immediately placed in sterile, sealed bags and then sent to the National Center for Nanoscience and Technology for SEM. All scans were finished within 12 hours of collection. One centimeter segments of endoscope channels were taken from suction and biopsy channels and water and air channels at a distance of 10 cm from the apex (portion 1), from the intermediate portion (portion 2), and at 10 cm from the push button portion at the bottom (portion 3). The sizes were 1×1 cm, and they were placed in sterile bags for SEM testing.

SEM

First, sample segments were fixed in sterile phosphate-buffered saline (10 mM potassium phosphate, 0.15 M natrium chloride, hydrogen ion concentration 7.0) containing 3% glutaraldehyde for 1.5 hours at room temperature and were washed with phosphate-buffered saline 3 times. Then, graded alcohol (30%, 50%, 70%, 90%, 100%) was used to dehydrate sample segments step by step; the samples were allowed to dry overnight. SEM (S-4800, HITACHI, Tokyo, Japan) was used to examine the interior surface of the sample fragments with a voltage of 10 kV. Representative images were collected for subsequent analysis.

Design of follow-up questionnaire and statistical analysis

A questionnaire with 13 questions was designed according to guidelines in China and abroad.[9] Then, the questionnaire was sent to 66 hospitals. EpiData version 3.0 software (EpiData, Odense, Denmark) was used for data entry and management. SPSS version 10.0 (SPSS Inc, Chicago, IL) software was used for statistical analysis. All results were categorized according to whether biofilms were detected in the individual hospitals. Fisher exact and χ^2 tests were applied. Statistical significance was set at 2-tailed $P < .05$.

RESULTS

Analysis of detection of biofilms on endoscopic suction and biopsy channels and water and air channels

SEM results from suction and biopsy channels

SEM was used to observe biofilm growth on the inner surface of suction and biopsy channels of endoscopes used in the endoscopic centers. The results are shown in Figure 1. Figure 1A (200 μm) shows that the endoscope suction and biopsy channels were completely clean without biofilm growth; Figure 1B (10.00 μm) shows that biofilm formed on the inner surface of suction and biopsy channels with a single bacteria moving freely; Figure 1C (50.0 μm) shows sheet-like biofilms covering the inner surface of endoscope suction and biopsy channels; and Figure 1D (100 μm) shows a sheet of biofilms growing on the inner surface of an endoscope suction and biopsy channel.

A total of 66 suction and biopsy channels were scanned, and 36 (54.6%) were found to have obvious biofilm growth. In some channels, biofilm grew in all 3 sites. In other channels, a large sheet of biofilms was found to grow only in the middle portion (portion 2), whereas there were little or no biofilms in portions 1 and 3.

SEM results from water and air channels

Thirteen water and air channels were observed with SEM, of which there were 10 (76.9%) with obvious biofilm structure. Other unidentified impurities on the water and air channels were also relatively common.

Results of the survey of the hospitals

The responses of the questionnaires of the 66 hospitals were collected (Table 1). The 66 hospitals were divided into 2 groups based on whether biofilms were detected on endoscopes used. Group A (n = 30) included hospitals without detection of biofilms on endoscopes; group B (n = 36) consisted of hospitals with detection of biofilms. There was no significant difference between groups A and B in endoscopic procedures performed per day ($P = .239$). The percentage of manual cleaning in group B was 92.3% (33/36) and 50.0% (15/30) in group A ($P = .001$). Eight hospitals in group A used a biofilm removal detergent, and this indicated a significant difference between the 2 groups ($P = .003$). Enzymatic detergents were used exclusively in group B. The proportion of detergent reuse in group B was 92.3% (33/36), whereas it was 61.5% (18/30) in group A ($P = .005$). The proportion of hospitals using alcohol and air drying after reprocessing in group B was 38.9% (14/36), whereas it was 76.7% (23/30) in group A ($P = .002$). The proportions of complete suctioning of all endoscope channels in groups A and B were 90.0% (27/30) and 83.3% (30/36), respectively. The proportion of hospitals using sterile water for rinsing in groups A and B was 60.0% (18/30) and 61.1% (22/36), respectively. There was no statistical difference between the 2 groups for these 2 operations ($P = .670$ and .927, respectively).

DISCUSSION

In this study we investigated the formation of biofilms on the channels of gastrointestinal endoscopes used in 66 gastrointestinal departments in China. For suction and biopsy channels and water and air channels scanned, the detection rates of biofilm formation were 54.6% (36/66) and 76.9% (10/13), respectively. The incidence of