Assessment of the Bacterial Contamination and Remediation Efficacy After Flooding Using Fluorometric Detection

ABSTRACT: In the aftermath of flooding, rapid response assessment and cleanup is critical to recovery. In this study, the level of contamination of bacteria on surfaces in flooded houses was estimated and compared to the level found on surfaces in non-flooded houses with a rapid field test based on hydrolase enzyme activity present in bacteria and by measuring endotoxin. Sampling was performed by swabbing a 9 cm² area using sterile cotton swabs wetted with a bacteriostatic buffer. A correlation between endotoxin levels and levels of hydrolase activity was seen (R² = 0.6469, P < 0.0001). The median value and the variance of the result distribution were higher in flooded buildings as compared to non-flooded buildings. In the non-flooded buildings, surfaces were divided into visually clean and visually dirty. As expected the level of bacteria was higher on the visually dirty surfaces, and overall the hydrolase activity correlated well with the visual inspection. Using the results from the visually clean surfaces in the reference buildings as the criteria for clean, four methods of cleaning were tested for their ability to reach these criteria.

KEYWORDS: bacteria, hydrolase activity, flooding, endotoxin, post remediation verification, cleaning

Introduction

Evaluating tools for assessment of bacterial contamination after flooding events or sewage overflow should address whether the measured parameter is relevant, reproducible, and rapid. Traditionally, assessment has been performed using cultivation of coliform and fecal indicator bacteria like Escherichia coli. Assessments have rightfully focused on the risk of infection as the main health concern. However, health effects of bacterial contamination are not solely a question of risk of infections from pathogenic bacteria. A high number of bacteria in general can cause other health effects such as hypersensitivity pneumonitis, allergy, and inflammation due to the influence of bacterial cell wall agents. One such example is endotoxin, which is part of the outer cell membrane of all Gram-negative bacteria. It can be released in water suspensions after lysis of the bacteria. During agitation of water and dust, it can become aerosolized and increased levels of endotoxin have been measured in many studies and related to health effects [1–4]. Thus measurements of total bacteria level as well as infectious bacteria are relevant for risk assessments.

Another issue with evaluating contamination and remediation cleaning efforts based on cultivation of E. coli is that this approach suffers from the fact that E. coli and many pathogenic bacteria typically have a very short survival time in the environment. Therefore, noninfectious bacterial reservoirs can remain undetected on furnishings, carpet, and other surfaces in the flooded buildings. Testing for endotoxin has an advantage over cultivation in that endotoxin is relatively stable and will remain detectable for a longer period even when many of the bacteria are dead or unculturable [5].

To quantify endotoxin, it needs to be extracted from the bacterial membrane. This is a relatively complex process, and the outcome is very dependent on the protocol used conferring an inherent variability to endotoxin assays [6,7]. Many protocols exist and with significant differences in yield of endotoxin extracted. Some important variables in the protocols are agitation rate and time, use of ultrasound, and addition of the surface active compound Tween. The lack of standardization makes it difficult to compare endotoxin levels between studies when different protocols are used. Furthermore, at present, endotoxin analysis is a time consuming, costly laboratory-based process, and there is no field detection kit for endotoxin.
In an attempt to improve the assessment of bacteria in environmental samples, this study compares a field detection method for fluorometric detection of hydrolase activity to endotoxin levels. Measuring enzymatic activity is simple and occurs without any extraction procedures. The fluorophore that is released upon hydrolyzation of a fluorogenic enzyme substrate is released extracellularly and can be measured directly. While cultivation of bacteria typically takes several days, hydrolase measurements can be performed on location in less than 1 h.

The present study has been conducted to evaluate if bacterial hydrolase activity could be used to measure of the level of bacterial contamination to evaluate whether the levels of bacterial hydrolase activity were significantly different between visually clean and visually dirty surfaces in reference buildings and between visually dirty surface in reference buildings and contaminated surfaces in flooded buildings. Finally, the study sought to evaluate whether different cleaning efforts in a flooded building could bring the level of bacteria from contaminated to clean where clean would refer to the level as found on visually clean surfaces in reference buildings.

Materials and Methods

Selecting Monitoring Sites

Buildings that had been flooded due to heavy rain and with suspected sewage contamination were termed flooded buildings. Sampling was performed when drying had been completed or was in the late phase.

Buildings with no prior history of flooding were visually inspected for moisture problems or other signs of water damage. If no problems were discovered, the building was used as a non-flooded or reference building.

Sampling

Sterile cotton swabs on wooden handles were moistened with a buffer containing a bacteriostat. Moistened swabs have a greater sampling efficiency [9], and the bacteriostat prevents growth on the cotton swab without affecting the hydrolase activity of the bacteria present. For each sample, an area of 9 cm² was swabbed. The swabs were then used to analyze for hydrolase activity and endotoxin.

Enzyme Activity

Bacterial hydrolase activity was measured typically less than 24 h after sampling using the standard operating procedure specified by the manufacturer’s specifications (Mycometer A/S, Copenhagen, Denmark). The swabs were transferred to a tube containing 2 ml of the fluorogenic 4-methylumbelliferone hydrolase enzyme substrate and allowed to react for approximately 30 min. The exact reaction time is dependent on the ambient temperature and was determined according to a table listed in the manufacturer’s manual. The fluorescence generated during the reaction time was measured by withdrawing 100 μl of the reactant and adding it to 2 ml of alkaline buffer in a disposable polystyrene cuvette (Sarstedt AG, Nümbrecht, Germany). The fluorescence was measured on a Picofluor fluorometer (Turner Designs, Sunnyvale, CA) using an excitation wavelength of 365 nm and measuring emission at 445 nm. Calibration of the instrument was performed prior to analysis using two standards of known fluorescence. The calibration was acceptable if the instrument reading of the fluorescence standard deviated less than 1% from the fluorescence value of the standard. Enzyme substrate blanks were measured and subtracted from sample values. Hydrolase activity is measured in arbitrary fluorescence units (AFU). Analytical grade chemicals and pyrogenic free water was used for preparing the buffers.

Endotoxin

Samples for endotoxin analysis were frozen at −50°C until all samples had been collected. Endotoxin was extracted from the swabs by agitating for 15 min in pyrogenic free water. The extracts were diluted 10³ to 10⁶ times in pyrogenic free water before analysis. Five sterile swabs were extracted by the same procedure and the mean value used as the blank value.

The amount of endotoxin in the liquid extracts was determined using the Limulus Aemobocyte Lysate method with specific lysate and the chromogenic, kinetic version (Associates of Cape Cod, MA).
405 nm absorbance of the samples was measured during a 30 min reaction time on a SK 601 Wellreader (Seikagaku Corporation, Tokyo, Japan). The incubation temperature was 37°C. Four different endotoxin concentrations were prepared according to the manufacturer’s specifications and used as internal standards. All glassware was baked at 270°C for 3 h before use. The results are given in nanograms endotoxin.

Results and Discussion

Bacterial Hydrolase Activity and Heterotrophic Plate Count (HPC)

A small amount of yeast extract (125 mg/l) was added to tap water to simulate an organic pollution. The indigenous bacteria in the tap water were allowed to proliferate reaching a maximum after approximately 30 h. A dilution series was prepared, and samples were withdrawn for HPC and hydrolase activity. Figure 1 shows that the activity of hydrolase activity correlates to the level of bacteria determined by HPC in water samples with different levels of indigenous bacteria ($R^2 = 0.988$).

Bacterial Hydrolase Activity and Endotoxin

Parallel samples were collected in both flooded and non-flooded reference buildings and used to analyze for hydrolase activity and endotoxin. Figure 2 shows a correlation between the two bacteria indicators ($R^2 = 0.6469$, $P < 0.0001$). The two parameters correlate despite that endotoxin is only present in gram negative bacteria while the hydrolase activity is present in both gram negative and gram positive bacteria (unpublished result).

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The endotoxin levels measured in this study varied from below the detection limit to a maximum level around 2800 ng/cm². Surprisingly studies describing the level of endotoxin on surfaces in buildings were not found in the literature search. The quite extensive research on endotoxin in the environment focuses mainly on air measurements and to some extent dust samples. The levels found in the present study are quite high, e.g., compared to the guidelines proposed for “no-effect level” for environmental endotoxin [8]. This guideline describes health effects of increasing seriousness with endotoxin concentrations ranging from 10 to 200 ng/m³. With a maximum concentration of $2.8 \times 10^7$ m² found in the present study and assuming an average ceiling height of 2.5 m, 1 m² of the floor surface feeds approximately 2.5 m³ of air above. This means that less than one thousands of a percentage of the endotoxin on the surface would have to become airborne to exceed the high level (200 ng/m³).

**Flooded Versus Reference Buildings and Categories**

Swab samples were taken from surfaces in buildings with flooding or sewage spills. For comparison, samples were also taken from reference buildings with no flooding or sewage spills. Samples from the reference buildings were divided into samples from visually clean surfaces and from surfaces with varying levels of visual dirt. The results from the visually clean surfaces were used as criteria for clean after remediation. The results from the visually dirty surfaces in the reference building were used to describe dirty but not contaminated surfaces. For a surface to be classified as contaminated in the flooded buildings, the level of hydrolase activity should be above the level found on the dirty surfaces in the non-flooded buildings.

Table 1 shows a marked difference in the hydrolase activity from the different categories of surfaces. A logarithmic normal distribution was seen with samples from both flooded and non-flooded buildings. The visual clean surfaces in the reference buildings had a median value of 5 AFU, which is also the detection limit of the method, and all values were below 20 AFU. The median value, mean value and the variance were higher on visually dirty surfaces in non flooded buildings and much higher in flooded buildings.

**Measuring Cleaning Efficacy**

Measurements were made in a living room of a house that had been flooded after heavy rain. The floor had been removed, and the room was dried out. Four areas (1 m² each) of the concrete foundation were cleaned using four different cleaning methods. All areas were vacuumed with a vacuum cleaner with HEPA filter. Three of the parcels were then cleaned thoroughly with one of three cleaning chemicals.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Before cleaning, AFU (STD)</th>
<th>n</th>
<th>After cleaning, AFU (STD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steam cleaning</td>
<td>2</td>
<td>434(26)</td>
<td>2</td>
<td>7(0.0)</td>
</tr>
<tr>
<td>Rodalon (quaternary ammonium)</td>
<td>2</td>
<td>408(88)</td>
<td>2</td>
<td>2.0(1.4)</td>
</tr>
<tr>
<td>Biowash (quaternary ammonium)</td>
<td>2</td>
<td>419(76)</td>
<td>2</td>
<td>10.5(2.1)</td>
</tr>
<tr>
<td>Peroxy tabs (peroxide)</td>
<td>2</td>
<td>875(222)</td>
<td>2</td>
<td>44.5(4.9)</td>
</tr>
</tbody>
</table>
Rodalon, (a quaternary ammonium chloride), Biowash (a quaternary ammonium chloride), and peroxide tabs (potassium persulfate, oxidizing agent). The fourth parcel was cleaned with a steam method. Steam, at 6 bars with temperature from 130 to 150°C, was blown onto the surface. This procedure was repeated a second time with the steam passing through a microfiber cloth that is in contact with the surfaces and absorbs released dirt/bacteria. Finally, all four areas were vacuumed again before testing for enzyme activity. The results are shown in Table 2. All treatments significantly reduced the bacterial level. Three of four treatments were able to reduce the level of bacteria to a level not distinguishable from visually clean surfaces in the reference buildings.

References