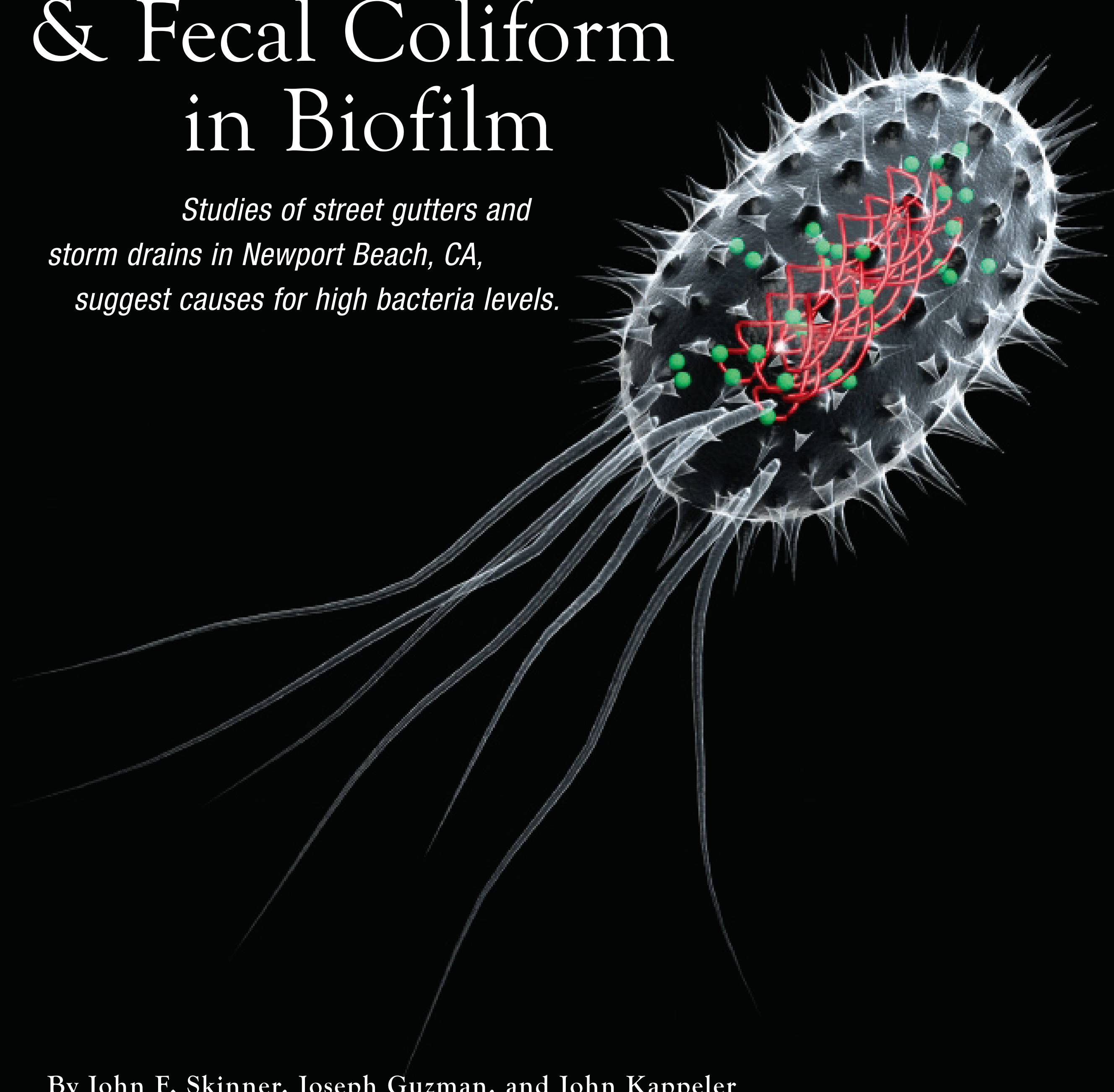
Regrowth of Enterococci & Fecal Coliform in Biofilm

Studies of street gutters and storm drains in Newport Beach, CA, suggest causes for high bacteria levels.



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By John F. Skinner, Joseph Guzman, and John Kappeler

Recently the city of Newport Beach, CA, and the Orange County (CA) Health Care Agency Water Quality Laboratory have completed studies presenting evidence that biofilm regrowth of enterococci and fecal coliform bacteria is occurring in street gutters and storm drains. This may explain the occasional high levels of these bacteria in runoff water flowing from residential areas into nearby Newport Bay. If these findings of regrowth are duplicated by others, the health threat to recreational swimmers resulting from nonpoint sources may be overestimated (Colford et al. 2007).

The city of Newport Beach has implemented a number of measures to be certain that raw sewage is not entering the city's urban runoff system, including a comprehensive fiber-optic scoping program to check for sewage/storm drain cross-connects, and to identify any breaks in the integrity of the city's sewer system.

Previous studies indicate that biofilms provide a safe environment for enhanced bacterial replication; supply nutrients and water for biofilm bacteria; and offer protection against microbial predators, ultraviolet (UV) light, drying, and disinfectants (Coghlan 1996, Costerton et al. 1995, Donlan and Costerton 2002, Donlan 2002).

Bacteria have been observed detaching

from the surface of biofilms and entering the overlying water column as single planktonic bacteria or small clumps of bacteria attached to fragments of biofilm (Figure 1). The rate of detachment of these bacteria is related to factors such as water flow velocity, shear forces, nutrient availability, and aging of biofilm.

In 2006, the Orange County Health Care Agency's Water Quality Laboratory staff performed studies that determined that enterococci and fecal coliform were multiplying in bacterial biofilms in the Dover Drive storm drain located in Newport Beach (Ferguson 2006).

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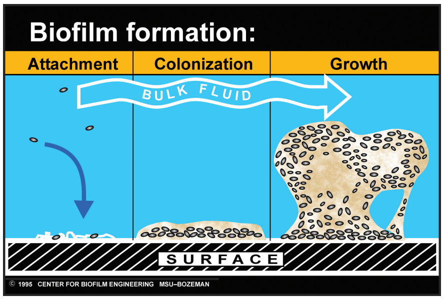

Figure 1. Process of biofilm formation

In the 2006 study, biofilm in the Dover Drive storm drain contained up to 4.6 million enterococci and 1.8 million fecal coliform/100 grams or 100 milliliters of biofilm. Enterococci and *E. coli* were grown in the laboratory under simulated natural conditions using filtered stormwater. These bacteria grew on the glass slides as microcolonies and secreted extracellular polymeric substances (EPS), a marker of biofilm formation. The presence of this EPS was validated using Calcofluor stain (Polysciences Inc., Warrington, PA). The multiplication of enterococci and *E. coli* in biofilm was documented by using PNA FISH (peptide nucleic acid probes and fluorescence in situ hybridization) (AdvanDx Inc., Woburn, MA) and visualized using fluorescent microscopy.

Subsequently, sections of PVC pipe and concrete coupons were placed in the Dover Drive storm drain for two weeks before removal. Some of the enterococci and fecal coliform were adherent to the pipe and coupons and could not be removed by vigorous rinsing or washing. However, sonication freed up these adherent bacteria. These findings are consistent with biofilm formation.

In 2009, the city of Newport Beach and the staff of the Orange County Health Care Agency Water Quality Laboratory performed water-quality studies in a residential neighborhood where street gutters flow directly into the Dover Drive storm drain just upstream from the site where the earlier 2006 study was performed.

The goal of the current studies was to determine the sources of high numbers of enterococci and fecal coliform found in street gutter runoff flowing from residential areas.

Initially, studies were performed to determine the levels of fecal indicator bacteria entering street gutters from a nearby residence. Bacteria-free hose water was used to wash down a driveway and a sidewalk for testing. Runoff water from flooding a residential front lawn was also

Figure 4. Street sweeper

Figure 2. Sprinklers overshooting lawn onto street

Figure 3. Sample collection 100 meters downstream

analyzed. Runoff from a front yard garden where the runoff water exited through a hole cut through the curb and drained directly into the gutter was studied. Finally, a water sample from lawn sprinklers was tested to be certain it was bacteria free.

The following results were obtained: Bacteria counts in runoff from washing the sidewalk were 220 enterococci/100 ml and 180 fecal coliform/100 ml. Washoff water from the driveway was 160 enterococci/100 ml and 9 fecal coliform/100 ml. Runoff from flooding the grass contained 1,250 enterococci/100 ml and 2,000 fecal coliform/100 ml. Water draining directly into the gutter through a hole cut through the curb grew out 70 enterococci/100 ml and 100 fecal coliform/100 ml.

Most of the water entering the street gutters originated from misdirected sprinklers that sprayed directly onto the streets (Figure 2). Surprisingly, it was rare to see water entering the gutters from overwatering lawns. The amount of water in the usual sprinkler cycle apparently did not oversaturate lawns and cause runoff.

Flows from holes in the curb directly into the gutter usually indicate drainage either from backyard and side yard patios or from roof gutter drains plumbed to flow directly into the street gutter. Repeated checks of these curb holes during the summer and fall study period did not identify any other than the one measurable flow described above. There is need to gather more information to determine if these occasional flows contain high levels of enterococci or fecal coliform bacteria.

No dog excrement was observed during the time that the bacterial samples were obtained. However, a number of dog walkers were observed bagging their dogs' fecal material for proper disposal.

Table 1. Results of Wet Biofilm Samples

Date	Enterococci/100 ml	Fecal coliform/100 ml	Comments
10/8	9,000,000	6,000,000	Before rain
10/8	1,410,000	1,230,000	Before rain
10/14	Rainy day—all biofilm flushed from gutter		
10/16	41,000	1,330,000	Two days after rain
10/16	All biofilm manually scraped from stretch of gutter		
10/20	120,000	10,000	Biofilm patches a.m.
10/20	870,000	460,000	Biofilm patches p.m.
10/21	2,060,000	10,000	Diffuse patches
10/27	200,000	100,000	
11/19	670,000	24,000	

Further studies were performed to determine if enterococci and fecal coliform bacteria were growing in the street gutters and could be responsible for high indicator bacteria counts found in gutter water.

The first study was performed on July 8, 2009, and was designed to measure fecal indicator bacteria concentrations in a street gutter draining from 10 residential homes. Bacteria-free hose water was introduced into a dry street gutter and tested for enterococci and fecal coliform at 10 meters, 45 meters, and 100 meters downstream when the flow from the hose water reached those locations. There was a progressive rise of both enterococci and fecal coliform bacteria with the increased distance of flow. The levels of fecal indicator bacteria were 26,000 enterococci/100 ml and 14,000 fecal coliform/100 ml when the water reached the 100-meter test site, the last testing station (Figure 3). The source of these high numbers of bacteria is suspected to be coming from regrowth in the street gutters.

The EPA's single sample standard is 104 enterococci/100 ml.

The second study was performed on September 18, 2009, and utilized the same protocol as the first study to determine the impact of street sweeping on these high fecal indicator bacteria counts. Street sweeping of the 100-meter stretch of street gutter was performed by the city of Newport Beach using a street sweeper equipped with rotating brushes and vacuum cleaning equipment to pick up particulates in the gutter (Figure 4). Again, bacteria-free

Figure 5. Slime or biofilm in street gutter

hose water was introduced into the same street gutter. Water samples collected at the 100-meter sampling station revealed markedly reduced fecal indicator levels of 1,550 enterococci/100 ml and 870 fecal coliform/100 ml.

The third study took place between October 5, 2009, and October 27, 2009, and was designed to determine if the high fecal bacterial counts found in the street gutter water were due to replication of these bacteria growing in street gutter biofilm.

It was noted that the street gutter across the street from the previous testing site had a more abundant growth of slime or suspected biofilm (Figure 5), because street sweepers had not been able to clean that street gutter for weeks. This street gutter drains a separate watershed of 30 homes, with all runoff flowing four blocks before emptying into the Dover Drive storm drain near the site of the 2006 biofilm study.

Sampling of this suspected biofilm identified up to 9 million enterococci and 6 million fecal coliform per 100 grams (equivalent to 100 ml) of biofilm. These biofilm samples were sonicated to release entrapped bacteria, and the levels were validated with split sampling. Gutter water samples flowing over the biofilm contained 5,500 enterococci and 3,600 fecal coliform/100 ml.

To determine if this biofilm, or slime, was contributing bacteria to the runoff in the gutter, bacteria-free hose water was introduced into the dry gutter and was sampled 60 feet downstream. This test was performed to determine if the biofilm-like material was shedding enterococci or fecal coliform as the bacteria-free hose water flowed over the moist biofilm. Enterococci and fecal coliform levels in the water sampled 60 feet downstream were reported to contain 3,200 enterococci/100 ml and 230 fecal coliform/100 ml. It is suspected that these bacteria were free-floating planktonic forms of bacteria that were shed from the underlying biofilm.

At the time of testing, the biofilm-like slime had formed a coalescent film covering virtually the entire gutter surface.

On October 23, 2009, a gardener was seen washing off large paved areas at a home located 100 feet upstream from the gutter testing site. This water was seen flowing in the gutter for four blocks before entering the Dover Drive storm drain. There was no other water input from the side streets at that time. The bacterial counts in the gutter water just prior to entering the Dover Drive storm drain contained 38,000 enterococci/100 ml and 5,200 fecal coliform/100 ml, indicating that the gutter water apparently picked up more bacteria from the street gutter along the four-block flow path.

On October 14, 2009, there was a significant rain event that washed away nearly all of the slime/biofilm in the gutter. Subsequently, an 8-foot stretch of gutter was vigorously scraped with a putty knife to remove any remaining visible slime/biofilm from that section of gutter, and the gutter was observed over the next month (Figure 6).

Figure 6. Photo looking down at street gutter. The dark patches show biofilm re-forming in the gutter after it was scraped clean of biofilm two weeks earlier.

Four days after scraping the gutter, small patches of slime/biofilm were seen reforming on the scraped areas. Two small samples of biofilm were obtained and tested. The first contained 120,000 enterococci/100 grams and 10,000 fecal coliform/100 grams. The second sample contained 870,000 enterococci/100 grams and 460,000 fecal coliform/100 grams.

By five days after the slime removal, patches of the suspected biofilm growing in the gutter were larger and contained 2,060,000 enterococci/100 grams and 10,000 fecal coliform/100 grams. The last sample of new growth of biofilm was tested at one month after slime removal, and bacterial levels were 670,000 enterococci/100grams and 24,000 fecal coliform bacteria (Table 1).

The findings of these studies provide evidence that regrowth of both enterococci and fecal coliform bacteria are occurring in biofilm located in residential street gutters and storm drains in Newport Beach. It is suspected that these biofilm bacteria may be responsible for some of the high levels of enterococci and fecal coliform bacteria reaching Newport Bay from residential neighborhood runoff.

These findings raise important questions as to whether enterococci and fecal coliform bacteria replicating in biofilm located in street gutters and storm drains confound testing for fecal contamination and potential health issues. Health officials agree that enterococci and fecal coliform bacteria originating from human fecal sources indicate a health risk to swimmers not because of the presence of *E. coli* and enterococci but because of the presumed presence of human enteric viruses. It is the enteric viruses, including Enterovirus, Adenovirus, and Norovirus, that are believed to be the primary cause of swimmer-related gastrointestinal illnesses (Glass et al. 2009). These enteric viruses multiply in the human gut but not in the environmental biofilms such as those found in street gutters or storm drains.

If these study findings are substantiated by others, the focus of remediation should be on best management practices to reduce the bacterial biofilms in street gutters, catch basins, and storm drains.

Frequent street sweeping, cleaning out the catch basins of biofilm material, using storm drain filters to remove debris, reducing water usage for landscape irrigation, filling in pooling locations in residential street gutters where replication can occur, and focusing on proper placement of sprinklers to prevent water from being sprayed directly into street gutters all play an important role in reducing gutter biofilm growth.

The findings of these gutter studies provide a logical explanation for elevations of fecal coliform and enterococci found in urban runoff in the absence of human fecal contamination.

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