

Letter to the Editor

Sensitization of a child to Cyanobacteria after recreational swimming in a lake

To the Editor:

Here, we report a case of an 11-year-old white girl who had an allergic reaction manifesting as severe facial swelling with periorbital edema and an erythematous pruritic blistering rash over her arms and hands (Fig 1) after playing and swimming in Lake Ontario at Presque Isle State Park. After repeated handling of algae making “bird’s nests,” the patient put sunscreen on her arms and face and then went swimming in the lake for 20 minutes. The exact time of the onset of the reaction is unknown because it began a few hours after she went to sleep. The next morning, she awoke with erythematous itchy swollen arms, face, and eyes and over time had facial and periorbital edema. Soon thereafter, she lost consciousness for approximately 30 seconds.

She was taken to an emergency department in Ontario, where she was given diphenhydramine and a dose of cetirizine. Her body temperature was normal, and over 1 to 2 hours, her rash and swelling began to subside. However, she still required diphenhydramine every 4 to 6 hours because her rash appeared to worsen as the previous dose wore off. The photograph of this initial reaction was taken 2 days after onset (Fig 1, A), demonstrating a red facial rash with edema.

On the third day after the initial exposure, she awoke with a worsening rash and again was taken to the emergency department, where she was prescribed oral corticosteroids for 5 days and advised to continue the use of diphenhydramine every 4 to 6 hours or cetirizine. One day after discontinuing the corticosteroids, approximately 8 days after the initial reaction, she experienced itching, and blisters appeared on her hands (Fig 1, B). She was seen by an allergist who prescribed an epinephrine autoinjector and topical corticosteroids for the hands; the rash and blisters completely resolved after 10 days.

Her mother subsequently confirmed with Canadian environmental health officials that the lake had an overgrowth of freshwater Cyanobacteria. Skin prick test responses to common aeroallergens were negative. Patch testing was also performed to exclude a reaction to the sunblock, and a thorough history was obtained to exclude other underlying causes. Interestingly, despite being nonatopic, a serum sample sent to our laboratory showed increased specific IgE levels to Cyanobacteria extract. This study’s objective was to determine whether the patient’s specific IgE to Cyanobacteria was functionally relevant and to compare the allergenicity of various Cyanobacteria species.

Cyanobacteria (also known as blue green algae) are a phylum of bacteria that grow in brackish and fresh water environments and are capable of producing toxins. Apart from their toxicity, Cyanobacteria are sensitizing in susceptible subjects.¹ Sensitization has been attributed to several Cyanobacteria genera and species, including *Arthrospira* species, *Oscillatoria* species, *Lyngbya* species, *Anabaena* species *Synechocystis* species, *Aphanizomenon flos-aquae*, and *Microcystis* species.^{1–5} More recently, severe cases of anaphylaxis have been reported after ingestion of spirulina tablets containing the Cyanobacteria genus *Arthrospira*.^{2,3} In all of these cases, the causes of sensitization have been diverse in

terms of the Cyanobacteria species implicated, raising the possibility of cross-reactivity of various Cyanobacteria species. In Lake Ontario, for example, a number of Cyanobacteria genera and species have been detected, including *Synechococcus* species, *Oscillatoria* species, *Anabaena* species, *Aphanizomenon* species, and *Microcystis aeruginosa*, with the latter being the most dominant species.⁶ Previously, phycocyanin of *M aeruginosa* was shown to be responsible for causing sensitization in a subset of patients with chronic rhinitis, and the toxicity of these organisms is inversely proportional to their allergenicity.^{1,7} Because all Cyanobacteria species produce phycocyanin, we investigated whether the patient was sensitized to 1 or more species of Cyanobacteria. After obtaining an informed consent approved by the University of Cincinnati which allows analysis of serum for a spectrum of allergic conditions, we performed an IgE-specific ELISA with serum from the patient and extracts from a variety of laboratory strains of Cyanobacteria. The results indicate varying degrees of cross-reactivity with the majority of Cyanobacteria species (Fig 2, A). Although extracts from the microcystin-nonproducing strain of *M aeruginosa* (MC[–]; no. 2) and *Synechococcus* species (no. 5) showed the most reactivity, the extracts from *Nostoc* species (no. 3) and *Scytonema* species (no. 8) did not possess any IgE reactivity. Interestingly, IgE-specific reactivity was also observed to *Arthrospira* species (no. 10), which, as previously mentioned, has been associated with anaphylaxis.^{2,3}

M aeruginosa phycocyanin shows greater than 70% sequence similarities with phycocyanin from various species of Cyanobacteria (see Table E1 in this article’s Online Repository at www.jacionline.org). To test whether the allergen in these species is the same as the allergen found in MC(–), we performed an ELISA inhibition assay using plates coated with *Synechococcus* species lysate (solid phase), with MC(–) lysate as the serum inhibitor (solution phase). The results show that the MC(–) extract dose-dependently inhibited binding of *Synechococcus* species lysate to specific IgE in the patient’s serum, indicating that the same allergenic peptide, phycocyanin, is present in both lysates (Fig 2, B).

To demonstrate the functional relevance of the allergenic peptide, we assessed whether both the *Synechococcus* species and MC(–) lysates could release mediators using a rat basophil leukemic cell line that expresses high-affinity human IgE receptor. As shown in Fig 2, C, the patient’s serum released approximately 7% and 10% of the total β -hexosaminidase content for *Synechococcus* species and MC(–), respectively, which was significantly greater than that released by the control serum. This result indicates that both lysates could induce β -hexosaminidase release and confirms the functional relevance of specific IgE responses to these lysates.

Although *M aeruginosa* has been isolated in Lake Ontario,⁶ we were unable to obtain any test samples from the same lake where the child played. Therefore to test whether the patient could potentially be sensitized by exposure to natural sources of Cyanobacteria, axenic cultures of *M aeruginosa* and *Oscillatoria* species were isolated from environmental samples collected from Loch Norse (Northern Kentucky) and Great Lake St Mary (Northern Ohio), respectively (see Fig E1, A, in this article’s Online Repository at www.jacionline.org). Although a specific IgE ELISA

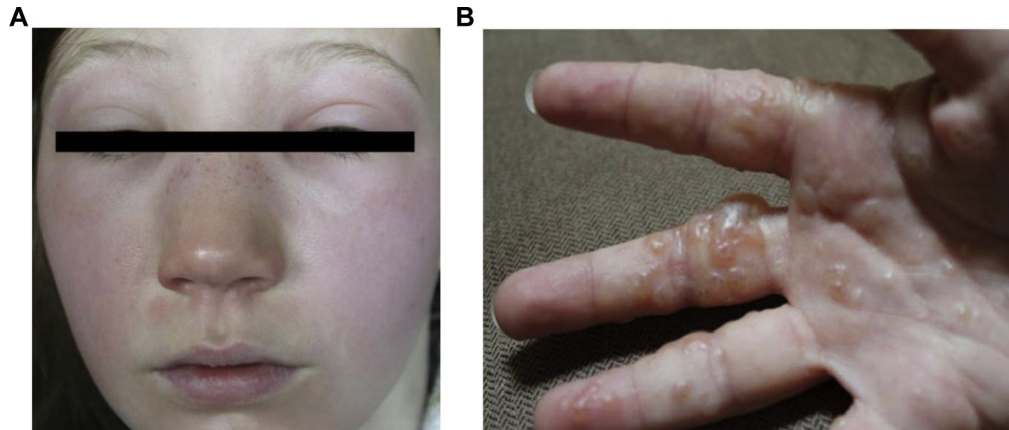


FIG 1. Facial rash with periorbital edema (A) and erythematous blistering rash on the child's hand (B) after exposure to Cyanobacteria.

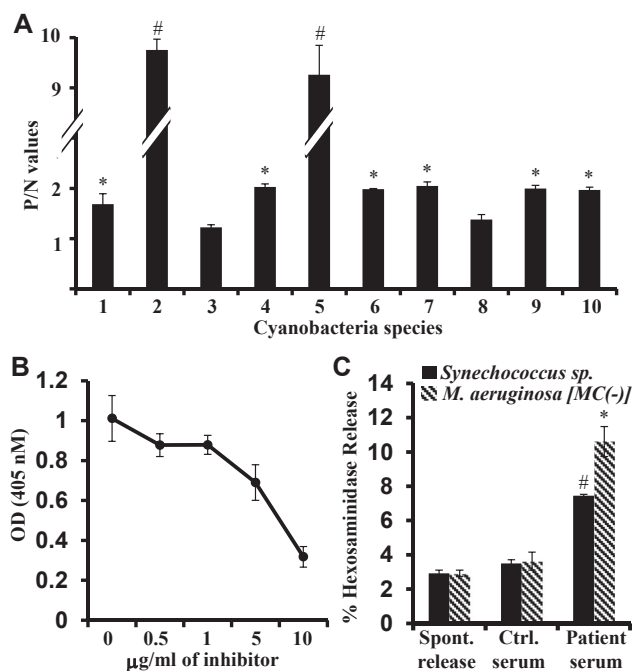


FIG 2. A, IgE-specific ELISA with extracts from various Cyanobacteria species: (1) toxic (MC[+]); (2) nontoxic (MC[−]) strains of *M aeruginosa*; (3) *Nostoc* species; (4) *Synechocystis* species; (5) *Synechococcus* species; (6) *Pseudanabaena* species; (7) *Oscillatoria* species; (8) *Scytonema* species; (9), *Lyngbya* species; and (10) *Arthrospira* species. These extracts were incubated with serum from the patient (P) and pooled control sera (N). Results from the patient/control sera (P/N) values for ELISA were then calculated. * $P < .001$ and # $P < 10^{-7}$, statistically significant difference between the patient's serum and pooled control sera. B, ELISA inhibition of the *Synechococcus* species lysate using *M aeruginosa* (MC[−]) extract. C, β -Hexosaminidase release assay using the patient's serum and extracts from *Synechococcus* species and *M aeruginosa* (MC[−]). * $P < .001$ and # $P < .01$, statistically significant difference between the patient's serum and pooled control sera.

performed with *Oscillatoria* species showed immunoreactivity, similar to the laboratory strains (Fig 2, A), the *M aeruginosa* species from Great Lake St Mary did not show any reactivity (see Fig E1, B). Further analysis revealed that this *M aeruginosa* species produced a high amount of microcystin (271 ng/mL), which might have inhibited its allergenicity.⁷ These results indicate that specific IgE in the patient's serum reacts not only to

extracts of laboratory strains but also to naturally occurring strains of Cyanobacteria. The identity of the species causing the patient's symptoms remains unclear, but assumptions can be made from the Cyanobacteria (*M aeruginosa*) samples isolated from Lake Ontario in the summers of 2001, 2003, and 2004. In the summer of 2004, data collected along the shores of Lake Ontario showed the presence of high levels of phycocyanin and low levels of microcystin.⁸ In addition, sequence analysis studies have identified unusual microcystin sequence features that might inhibit the production of microcystin in this particular strain.⁶ Under these circumstances, it is reasonable to speculate that the *M aeruginosa* from Lake Ontario produces less toxin and more phycocyanin, rendering them more allergenic.⁷

One major limitation lies in the fact that we were unable to perform skin patch tests to confirm that the pruritic blistering rash (Fig 1, B), which developed 8 days after exposure, was due to a delayed-type hypersensitivity reaction to Cyanobacteria. However, the initial facial reaction (Fig 1, A), which was the focus of this study, was consistent with an immediate-type hypersensitivity reaction to Cyanobacteria, which was confirmed by specific IgE reactivity to various Cyanobacteria species. Although other conditions, such as reactions to topical medications, sunscreens, and plant material (phototoxic reactions), might be responsible for blister formation, a skin patch test result with sunscreen was negative, and a phototoxic reaction to Cyanobacteria is less likely, as opposed to a delayed hypersensitivity reaction, because the appearance of blisters occurred a week after the initial exposure. Cross-reactivity to multiple Cyanobacteria species and selective inhibition assays indicates that phycocyanin is the major protein eliciting these allergic reactions. Because Cyanobacteria can act similarly to anemophilous pollinating plants by being dispersed large distances, resulting in remote outdoor and indoor exposure, sensitization in this child could have resulted from numerous potential sources. For example, Cyanobacteria has been found to be more abundant in dust from the homes of asthmatic children compared with dust from the homes of nonasthmatic children, suggesting a possible association between Cyanobacteria and asthma.⁹ To our knowledge, this is the first case report confirming the functional relevance of Cyanobacteria sensitization in a child after recreational exposure and that phycocyanin is the likely sensitizing protein. This case emphasizes the importance of Cyanobacteria as an unrecognized ubiquitous allergen in

allergic and nonallergic subjects and that recreational exposure of sensitized subjects to freshwater Cyanobacteria can lead to severe allergic reactions. In this case the patient was advised not to swim in fresh bodies of water (ie, lakes), where Cyanobacteria growth was possible, or play with seaweed on the beach, and she was prescribed 10 mg/d cetirizine to attenuate any reactions that could occur because of inadvertent exposures elsewhere. Further investigation into the prevalence of Cyanobacteria sensitization in the general population is warranted given the high levels of Cyanobacteria found in house dust samples and the amount of recreational time spent by Americans around freshwater lakes.

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METHODS

Cultivation of environmental samples of Cyanobacteria

Environmental samples were collected from Loch Norse and Great Lake St Mary. Growth of Cyanobacteria cultures and preparation of crude cell extracts has been described elsewhere.^{E1} Cyanobacteria were rendered axenic by plating on complete BG11 agar plate (BG11 medium supplemented with 1.8 mmol/L NaNO₃ and 10 mmol/L NaHCO₃ with 1% agarose). Single well-isolated colonies were picked and propagated in liquid complete BG11 medium. Microcystin content of Great Lake St Mary *Microcystis* species was determined by means of ELISA (Microcystin Quantiplate; Envirologix, Portland, Me).

IgE-specific ELISA

IgE-specific ELISA was performed by using a previously described method.^{E1} Briefly, 96-well polystyrene microtiter plates were coated with 100 μ L per well of 10 μ g/mL cyanobacterial lysates in 50 mmol/L carbonate buffer (pH 9.6). The plates were incubated for 2 hours at room temperature (RT) and then overnight at 4°C. After overnight incubation, the wells were then emptied, washed 4 times for 5 minutes each with 0.05% Tween-20 in 0.01 mol/L (pH 7.4) PBS (PBS-T), and blocked for 1 hour with blocking buffer (2% BSA in PBS-T). One hundred microliters of the patient's serum and control sera (diluted 1:10 with blocking buffer) were added onto the wells and further incubated for 1 hour at RT and then overnight at 4°C. The wells were washed 4 times with blocking buffer and incubated with goat anti-human IgE (diluted 1:1000 with blocking buffer) for 2 hours at RT and then washed again and incubated with alkaline phosphatase-conjugated rabbit anti-goat IgG (1:1500). The plates were developed by using p-nitrophenyl

phosphate substrate Sigma-Aldrich (St Louis, Mo) and read at 405 nm with the Multiskan Ascent 96 Plate Reader (Thermo Scientific, Madison, Wis).

Multiple sequence alignments

Sequence alignments were performed with β -phycocyanin sequences from *M aeruginosa* and *Nostoc*, *Synechocystis*, *Synechococcus*, *Pseudanabaena*, *Oscillatoria*, *Scytonema*, *Lyngbya*, and *Arthrospira* species by using the clustal omega Web tool.

Statistical analysis

All data are reported as the sample mean $\pm$ SD. A paired Student *t* test was used to compare IgE reactivity of the patient's serum with various Cyanobacteria species, and an unpaired Student *t* test was used to calculate the difference between the patient's serum and control sera in the β -hexosaminidase release assay.

Reagents

Goat anti-human IgE and alkaline phosphatase-conjugated rabbit anti-goat IgG were purchased from KPL (Gaithersburg, Md). Microtiter ELISA plates were from Corning (Corning, NY), and p-nitrophenyl phosphate substrate was from Sigma-Aldrich.

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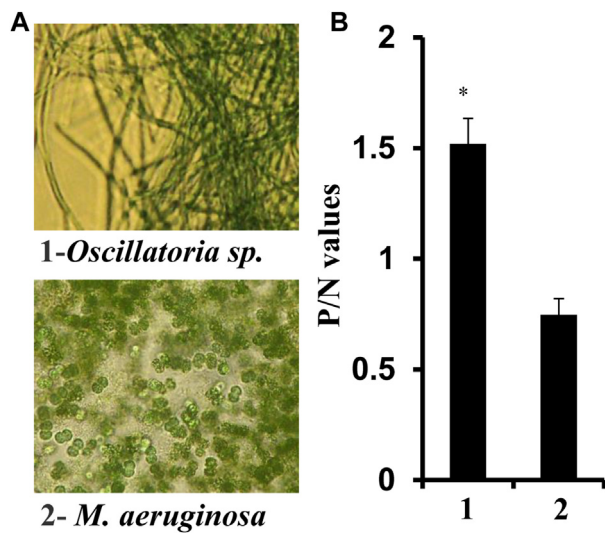


FIG E1. A, Microphotographs of axenic cultures of *Oscillatoria* species and *M. aeruginosa* collected from Loch Norse and the Great Lake St Mary lakes, respectively. B, IgE-specific ELISA using extracts from the environmental isolates: *Oscillatoria* species (1) and *M. aeruginosa* (2). Results using the patient/control sera (P/N) values for ELISA were then calculated. * $P < .001$, statistically significant difference between the patient's serum compared with pooled control sera.

TABLE E1. Percentage identity matrix of the β -phycocyanin protein sequences in various species of Cyanobacteria

Species		1	2	3	4	5	6	7	8	9
<i>M. aeruginosa</i>	1	100	77.3	82	75	79.7	82.6	70.4	84.3	83.7
<i>Nostoc</i> species	2	77.3	100	81.4	66.3	81.4	78.5	67.4	80.2	80.2
<i>Synechocystis</i> species	3	82	81.4	100	72.1	80.2	79.7	67.4	79.7	83.1
<i>Synechococcus</i> species	4	75	66.3	72.1	100	72.1	71.5	59.9	75.6	76.2
<i>Pseudanabaena</i> species	5	79.7	81.4	80.2	72.1	100	79.7	69.8	80.2	82
<i>Oscillatoria</i> species	6	82.6	78.5	79.7	71.5	79.7	100	65.7	80.2	79.7
<i>Scytonema</i> species	7	70.4	67.4	67.4	59.9	69.8	65.7	100	67.4	66.3
<i>Lyngbya</i> species	8	84.3	80.2	79.7	75.6	80.2	80.2	67.4	100	87.2
<i>Arthrospira</i> species	9	83.7	80.2	83.1	76.2	82	79.7	66.3	87.2	100