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Case diagnosis and characterization of suspected paralytic shellfish poisoning in Alaska

Jennifer S. Knaack [a,1,2], Kimberly A. Porter [b,1], Justin T. Jacob [c], Kate Sullivan [d], Matthew Forester [e], Richard Y. Wang [a], Vera L. Trainer [f], Steve Morton [g], Ginny Eckert [h], Ernest McGahee [a], Jerry Thomas [a], Joseph McLaughlin [i], Rudolph C. Johnson [a,*]

[a] Emergency Response Branch, Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Highway, MS F44, Atlanta, GA 30341, USA
[b] Epidemic Intelligence Service, (Alaska Section of Epidemiology), Centers for Disease Control and Prevention, 1600 Clifton Rd, Atlanta, GA 30333, USA
[c] Oak Ridge Institute for Science and Education Fellow at the Centers for Disease Control and Prevention, MC-100-44, P.O. Box 117, Oak Ridge, TN, 37831-0117, USA
[d] Fisheries Technology, University of Alaska Southeast, 2600 7th Ave, Ketchikan, AK 99901, USA
[e] Alaska Environmental Health Laboratory, Alaska Department of Environmental Conservation, 5251 Dr. Martin Luther King Jr. Ave., Anchorage, AK 99507, USA
[f] Marine Biotoxins Program, Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Seattle, WA 98112 USA
[g] National Centers for Coastal Ocean Science, National Oceanic and Atmospheric Administration, Marine Biotoxins Program, 219 Fort Johnson Road, Charleston, SC 29412, USA
[h] School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, 17101 Point Lena Loop Rd., Juneau, AK 99801, USA
[i] Section of Epidemiology, Alaska Department of Health and Social Services, 3601 C Street, Suite 540, Anchorage, AK 99503, USA

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ABSTRACT

Clinical cases of paralytic shellfish poisoning (PSP) are common in Alaska, and result from human consumption of shellfish contaminated with saxitoxin (STX) and its analogues. Diagnosis of PSP is presumptive and based on recent ingestion of shellfish and presence of manifestations consistent with symptoms of PSP; diagnosis is confirmed by detection of paralytic shellfish toxins in a clinical specimen or food sample. A clinical diagnostic analytical method using high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) was used to evaluate the diagnosis of saxitoxin-induced PSP (STX-PSP) in 11 Alaskan patients using urine specimens collected between June 2010 and November 2011. Concentrations of urinary STX were corrected for creatinine concentrations to account for dilution or concentration of urine from water intake or restriction, respectively. Of the 11 patients with suspected PSP, four patients were confirmed to have STX-PSP by urine testing (24–364 ng STX/g creatinine). Five patients had clinical manifestations of PSP though no STX was detected in their urine. Two patients were ruled out for STX-PSP based on non-detected urinary STX and the absence of clinical findings. Results revealed that dysphagia and dysarthria may be stronger indicators of PSP than paresthesia and nausea, which are commonly used to clinically diagnose patients with PSP. PSP can also occur from exposure to a number of STX congeners, such as gonyautoxins, however their presence in urine was not assessed in this investigation. In addition, meal remnants obtained from six presumptive PSP cases were analyzed using the Association of Official Analytical Chemists' mouse bioassay. All six samples tested positive for PSP toxins. In the future, the clinical diagnostic method can be used in conjunction with the mouse bioassay or HPLC–MS/MS to assess the extent of STX-PSP in Alaska where it has been suggested that PSP is underreported.

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Abbreviations: Cr, creatinine; HPLC–MS/MS, high performance liquid chromatography tandem mass spectrometry; LRL, lowest reportable limit; STX-PSP, saxitoxin-induced paralytic shellfish poisoning.

* Corresponding author.
E-mail addresses: Knaack_JS@Mercer.edu (J.S. Knaack), RJohnson6@cdc.gov (R.C. Johnson).

[1] These authors contributed equally to this investigation.

[2] Present address: Department of Pharmaceutical Sciences, Mercer University, 3001 Mercer University Dr., DV-114, Atlanta, GA 30341, USA.

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1. Introduction

The increasing concentration, duration, and frequency of HABs over the past few decades have been documented in many coastal regions of the world (Anderson, 1995; Burke et al., 2000). HABs cause significant ecosystem, human health, and economic impacts (Anderson, 2000; Hoagland and Scatasta, 2006; McFarren et al., 1960) and have become a national and international research focus within the past decade. Paralytic shellfish poisoning (PSP) currently is the primary health concern caused by harmful algal blooms (HABs) in Alaska. Around the world, PSP is caused by ingestion of seafood containing bioaccumulated toxins produced by certain species of marine dinoflagellates in the genera *Alexandrium, Gymnodinium*, and *Pyrodinium* (McFarren et al., 1960; Riegel et al., 1948; Schantz, 1961; Schantz et al., 1966; Wiese et al., 2010). Though commercially harvested shellfish are tested and considered safe, shellfish harvested for subsistence and recreational consumption are not routinely tested in Alaska, resulting in this state having one of the highest incidences of reported PSP in the world (Gessner and Schloss, 1996). Between 1973 and 1996, more than 200 incidences of PSP were reported in Alaska from 70 outbreaks, with the majority occurring in southeast Alaska (Gessner and Schloss, 1996). These cases likely represent a fraction of actual PSP cases because not all affected people seek medical treatment and there is no rapid and readily accessible diagnostic test or biomarker for assessing human exposure.

The congeners of saxitoxin (STX) have a common heterocyclic guanidine structure and they include STX, neosaxitoxin, gonyautoxins, and other derivatives that vary in toxicity based on structural determinants with different side chains and functional groups that affect their binding affinity to sodium channels (Henderson et al., 1974; Hille, 1975; Strichartz, 1984). Specifically, these toxins bind to the sodium channel pore, thereby blocking the influx of sodium into these cells (Hille, 1975). STX and neosaxitoxin are the most potent of the PSP toxins (Shimizu, 2000), however, biotransformation of toxins from low to high affinity isoforms (or vice versa) can occur in algae (reviewed in Wiese et al., 2010), shellfish (review in Bricelj and Shumway, 1998) or humans (Garcia et al., 2010; see discussion below). Clinical diagnosis of PSP is based on recent ingestion of shellfish and the presence of clinical manifestations consistent with toxicity, including nausea, vomiting, paresthesia (skin sensations such as tingling), dysarthria (abnormal speech), dysphagia (difficulty swallowing), and weakness that can lead to paralysis and respiratory failure (McFarren et al., 1960; Schantz, 1960; Tennant et al., 1955). The detection of a paralytic shellfish toxin in a clinical specimen, such as blood or urine, or an uneaten food sample also can be used to confirm the diagnosis of PSP in a patient (AOAC, 2005a,b) .

Saxitoxin has been detected in urine specimens collected from victims of PSP (Garcia et al., 2004; Gessner et al., 1997; Llewellyn et al., 2002; Rodrigues et al., 2012), although other congeners can be present at concentrations higher than STX or at the exclusion of STX. In one report, STX was detected at a higher concentration than neosaxitoxin in urine specimens from two patients with PSP from Alaska (Gessner et al., 1997). However, gonyautoxins (GTX2 and GTX3), and C1 toxins comprised a larger portion of the total measured toxin in these patients. Although these toxins can be detected in clinical specimens using high performance liquid chromatography with fluorescent detection (AOAC, 2005b; Garcia et al., 2004), this method is not commonly used for clinical investigations. Recently, a mass spectrometry-based clinical method was developed for diagnosing PSP by detecting STX in urine (Johnson et al., 2009). This is the only fully characterized diagnostic method available for clinical use and directly detects STX without the need to oxidize or conjugate the analyte.

The purpose of this investigation was to apply this diagnostic test for STX (Johnson et al., 2009) to urine samples from patients with suspected PSP in Alaska to confirm STX-induced PSP (STX-PSP). This method was previously used to evaluate two patients from Alaska in 2011 with presumed PSP (Centers for Disease Control and Prevention, 2011). In this investigation, these two patients are described in more detail with respect to their clinical manifestations. Results are documented using this diagnostic test in seven other Alaskan patients with presumptive PSP and two Alaskan patients who consumed contaminated shellfish but did not report symptoms. The clinical findings in the context of the urinary STX concentrations are presented, when available, in conjunction with the total paralytic shellfish toxin concentration in shellfish, assessed by mouse bioassay. Other STX congeners causing PSP were not assayed in this investigation.

2. Materials and methods

A human subject research determination was conducted at Centers for Disease Control and Prevention (CDC) in accordance with the Code of Federal Regulations, Title 45, Part 46. As a result, this dataset was determined not human subject research.

2.1. Study design and selection of participants

PSP is a mandatorily reportable condition in Alaska. Clinicians reporting a patient with suspected PSP to the Alaska Section of Epidemiology (SOE) provide clinical data and, if known, the patient's recent history of shellfish consumption. A case of presumptive PSP is one that is assessed by a physician to demonstrate clinical manifestations consistent with PSP (Gessner et al., 1997). Clinical specimens were obtained from 11 patients with presumptive PSP from June 2010 to November 2011 and were analyzed for STX in the National Center for Environmental Health, Division of Laboratory Sciences at the Centers for Disease Control and Prevention (CDC). Specimens from other patients with suspected PSP during this time were not collected by clinicians or were not sent for urinalysis. Urine specimens were stored and shipped frozen to the Alaska State Public Health Laboratory, repackaged, and shipped frozen to CDC for testing. When possible, unconsumed portions of shellfish were obtained from patient meals and analyzed by the Alaska Department of Environmental Conservation's environmental health laboratory for the presence of paralytic shellfish toxins.

2.2. Methods and measurements

Clinical data were obtained from the following sources: patient interview with a structured questionnaire, consultation with the patient's healthcare provider, and the patient's medical record. Data from medical records were summarized, including: time to onset of signs and symptoms since shellfish consumption; clinical manifestations of PSP including paresthesia, nausea, light-headedness, dysarthria, dyspnea, and dysphagia, and clinical course and outcome (Table 1). A case of confirmed STX-PSP was the detection of STX in the urine of a case of presumed PSP.

At CDC, urine specimens were analyzed for STX using a method compliant with the Clinical Laboratory Improvement Amendments Act of 1988 as previously described (Johnson et al., 2009). Saxitoxin was extracted from urine by solid phase extraction using a weak cation exchange sorbent and detected by high performance liquid chromatography coupled to tandem mass spectrometry (HPLC–MS/MS). The lowest reportable limit (LRL) for the analytical method, which was defined as the lowest standard in the assay, is 4.8 ng/mL (Johnson et al., 2009). Creatinine in urine (Cr) was