Technical Note: Contamination of Some Kratom Products with *Salmonella*

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Abstract. ***Objective***. Recently, the US Food and Drug Administration investigated the contamination of kratom (Mitragyna speciosa) by *Salmonella*, an event that caused an outbreak in 20 states after its consumption. Therefore, we investigated 16 different bags of kratom submitted for testing for potential contamination with *Salmonella* and other microorganisms within the Public Health Laboratory for the state of South Carolina. ***Materials and Methods.*** All kratom powders collected were analyzed for potential contamination with bacteria by an in-house modified Food and Drug Administration's Bacteriological Analytical Manual *Salmonella* procedure. ***Results.*** Out of 16 products analyzed, six brands have unknown manufacturers, but manufacturer information was available for the other 10 products. In total, three brands of kratom showed presence of any *Salmonella species*. ***Conclusions.*** Recently analyzed kratom products show a presence of *Salmonella*.

Key words: Kratom, *Salmonella*, Microorganisms, Contamination.

Introduction

Kratom (Mitragyna speciosa) is a plant indigenous to Southeast Asia. The leaves and tea brewed from the plant have been used by people to relieve fatigue and to manage pain. Initially, kratom was classified as a legal herbal product by the US Drug Enforcement Administration (DEA), but in August 2016, the DEA announced plans to classify kratom and its mitragynine constituents as Schedule 1 controlled substances because of their high abuse potential [1]. These products are sold in clandestine markets as "legal high" [2].

The pharmacologically active alkaloids isolated from kratom are mitragynine and 7-hydroxymitragynine. These compounds have CNS stimulation effects in low concentration, but act as CNS depressant at higher concentrations. The pharmacological effects are primarily mediated through monoaminergic and opioid receptors [3]. Kratom alkaloids exert opioid and α-2 receptor agonistic effects, as well as anti-inflammatory and parasympathetic-impeding effects. Human pharmacologic, pharmacokinetic, and clinical data are sparse, with no conclusion regarding its safety and abuse potential being reached at this time. Although respiratory depression has not been commonly reported, kratom causes a host of adverse effects without clear guidance for how they should be treated. There are numerous assessments where people have been unable to stop using kratom therapy, with its withdrawal signs and symptoms being evident and problematic. Kratom cannot be detected during routine drugs of abuse testing. As a result, potential overdoses of kratom in a patient may not be recognized. Thirty-six deaths have been attributed to kratom, and the Food and Drug Administration issued a public health warning about the substance in November 2017 [4].

As of May 24, 2018, 199 people were infected with outbreak strains of *Salmonella* were reported from 41 states. Illnesses started on dates ranging from January 11, 2017, to May 8, 2018. However, no deaths were reported. Epidemiologic and laboratory evidence indicate that kratom was the likely

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Table 1. *Kratom products contaminated with Salmonella.*

Container #	Sample/Product	Brand	Microorganisms isolated
1	Kratom powder	Maeng Da	*Salmonella* enterica ser. Weltevreden, *Salmonella* enterica ser. Chingola and *Salmonella* enterica ser. Javiana
2	Kratom powder	Maeng Da	*Salmonella* enterica ser. Weltevreden, *Salmonella* enterica ser. Javiana and *Salmonella* enterica ser. Thompson
6	Kratom powder	Maeng Da	*Salmonella* enterica ser. Weltevreden

source of this multi-state outbreak because 76 of 103 people (74%) available for interview reported consuming kratom in pills, powder, or tea. Most people reported consuming the powder form. People who reported consuming kratom purchased it from retail locations in several states, and from various online retailers [5]. Because the contamination of kratom is a public health issue, we analyzed 16 kratom products for possible contaminations of *Salmonella*.

Materials and Methods

One set of samples came from a source in a small plastic container, with brown packages labeled by the name of the kratom type, stamped on the packaging. A second set of samples were received in the manufacturer's original packaging, with the name Earth Kratom on the exterior. The third set of samples was in sealed plastic bags that were marked with the names of the type of kratom. All samples had the same visual appearance and consistency as the samples that came either in or with original packaging. Empty original packaging was sampled using a sterile swab that was placed in 10 mL of BPW for incubation.

Testing for potential contamination with bacteria was performed using an in-house modified Food and Drug Administration's Bacteriological Analytical Manual *Salmonella* procedure [6]. The in-house for *Salmonella* isolation began with making a 25 gram per 225 milliliter buffered peptone water primary enrichment sample solution for each specimen. This solution was incubated at room temperature for 1 hour, followed by pH measurement using Hydrion indicator strips (Catalog #9400, Micro Essential Laboratory, Brooklyn, NY) to ensure the pH is within 6.8 +/- 0.2 range. The primary enrichment samples were incubated at 35 +/- 2 degrees Centigrade for 24 +/- 2 hours. Secondary enrichment was performed in a selective enrichment step using two broth media. In a tube, 1 mL of the primary enrichment sample was added to ten mL of tetrathionate (TT) broth. In a separate tube, 0.1 mL of primary enrichment sample was added to 10 mL of Rappaport-Vassiliadis (RV) broth. The inoculated broth tubes were placed in a circulating, thermostatically controlled water bath for 24 +/- 2 hours at 42 +/- 2 degrees Centigrade. A subculture was performed separately from the secondary enrichment tubes on 3 selective and differential media plates, for each specimen. This resulted in 6 subculture plates per specimen. Subculture to Hektoen Enteric (HE) agar, Xylose Lysine Desoxycholate (XLD) agar, and Bismuth Sulfite (BS) agar was performed by streaking a 10 µL loop using a sterile loop from each respective sample to the respective agar plate. The plates were incubated at 35 +/- 2 degrees for 24 +/- 2 hours, then read for colony morphology. BS plates were incubated for an additional 24 +/- 2 hours and re-read for colony morphology.

Nonspecific and uncharacteristic growth patterns were noted on negative plates. For samples 1, 2 and 6, HE plates presented with typical blue-green colonies and black centers; XLD plates presented with typical red colonies and black centers, while BS plates presented gray colonies with a metallic sheen.

Colonies were picked from the selective plates and streaked on blood agar plates (BAP) for isolation prior to biochemical analysis. These colonies were also sub cultured to Triple Sugar Iron (TSI) (K/A H2S+/-), Lysine Iron Agar (LIA) (K/K H2S+/-) and Urea (negative) slants where K=alkaline and A=acid. These results were used to determine which isolates met the testing criteria for further workup and identification.

Further testing was performed on colonies from BAP using the BioMereuix Vitek 2 (Marcy-l'Étoile, France) for rapid identification of bacteria using a phenotypic algorithm to analyze the biochemical results. Salmonella

Group was determined with a 96% - 99% probability for confirmation by serological tests. Forty-seven biochemicals were performed by the Vitek 2 instrument for each colony. The results from the Vitek 2 indicated that Salmonella was present in 3 unique samples.

Results and Discussion

We analyzed a total of 16 kratom samples. Four of these specimens were labelled as "Earth Kratom", while another six were labelled as "Maeng Da". The remaining six specimens had no label, but were identified as kratom. We identified *Salmonella* species only in three brands. All three specimens that showed the presence of *Salmonella* belong to the "Maeng Da" brand kratom product. Kratom products contaminated with *Salmonella* are listed in **Table 1**.

Although 13 out of 16 products were not contaminated with *Salmonella*, our findings that three products were contaminated are still troublesome because the risk of *Salmonella* infection continues to exist after consuming kratom. As of April 19, 2018, the FDA reported that out of 66 specimens of Kratom analyzed, 33 specimens have been found positive for Salmonella. The FDA warns people that eating raw kratom can cause people to obtain *Salmonella*. Such products might cross-contaminate surrounding surfaces, possibly exposing others to *Salmonella* [7]. To our knowledge, this is the first non-FDA study indicating contamination of kratom product with *Salmonella*.

Unlike many of the nontyphoidal *Salmonella* serovars, *Salmonella* Typhi is unique in that it causes life-threatening typhoid fever in humans [8]. The FDA study did not report any *Salmonella* typhi in kratom products, which may explain why no death has been associated at this point. We also did not isolate *Salmonella* typhi in any kratom product. Nevertheless, nontyphoidal *Salmonella* organisms can cause severe illness, including possible hospitalization and potential fatality [9]. Therefore, we warn people not to consume kratom products.

References

1. Prozialeck WC. Update on the Pharmacology and Legal Status of Kratom. J Am Osteopath Assoc. 2016;116:802-809
2. Swogger MT, Walsh Z. Kratom use and mental health: A systematic review. Drug Alcohol Depend. 2018;183:134-140.
3. Warner ML, Kaufman NC, Grundmann O. The pharmacology and toxicology of kratom: from traditional herb to drug of abuse. Int J Legal Med. 2016;130:127-138
4. White CM. Pharmacologic and clinical assessment of kratom. Am J Health Syst Pharm. 2018: 75:261-267.
5. CDC Report May 24, 2018, Multistate Outbreak of Salmonella Infections Linked to Kratom (Final Update): available online-https://www.cdc.gov/Salmonella/kratom-02-18/index.html
6. Center for Food Safety and Applied Nutrition. (n.d.). Laboratory Methods - Bacteriological Analytical Manual (BAM) Chapter 5: Salmonella. Available online https://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm070149.htm
7. FDA Investigated Multistate Outbreak of Salmonella Infections Linked to Products Reported to Contain Kratom: available online https://www.fda.gov/Food/RecallsOutbreaksEmergencies/Outbreaks/ucm597265.htm
8. Chong A, Lee S, Yang YA, Song J. The role of typhoid toxin in Salmonella typhi virulence. Yale J Biol Med. 2017;90:283-290.
9. Uche IV, MacLennan CA, Saul A. A Systematic Review of the Incidence, Risk Factors and Case Fatality Rates of Invasive Nontyphoidal Salmonella (iNTS) Disease in Africa (1966 to 2014). PLoS Negl Trop Dis. 2017;11:e0005118