

# MOLECULAR SCREENING OF CHEWING STICKS AND SPONGES FOUND ON THE GHANAIAN LOCAL MARKET FOR DIARRHOEA-CAUSING MICROBES- A PILOT STUDY

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## ABSTRACT

Chewing sticks and sponges are used for oral hygiene in Ghana and other African countries. In addition to their affordability, they have additional advantages of anti-microbial and anti-plaque properties. They are usually sold on the open market under low hygienic conditions, exposing them to environmental pathogens. Since they are mostly not sterilized before use, it is important to screen for the presence of pathogens of public health importance on randomly selected samples. This preliminary study used molecular assays to screen for Rotavirus A, *Salmonella typhi*, *Vibrio cholerae* and *Escherichia coli* on 10 chewing stick and sponge samples purchased randomly from the Agbobbloshie market in Accra. The samples were incubated in sterile distilled water overnight at room temperature to dislodge pathogens. Dislodged pathogens were captured using the Nanotrap Microbiome A kit. Total nucleic acids were extracted from concentrates using the RADI prep DNA/RNA kit. All PCR assays were performed using 2X SYBR Green Mix and pathogen specific primers. Out of the four pathogens screened, only *E. coli* was detected (40% and 60% of chewing sponge and stick samples respectively). Despite the advantages of chewing sticks and sponges, the detection of *E. coli* on samples is a cause for concern since they indicate faecal contamination and can cause diarrhoeal diseases. It is recommended that chewing sticks and sponges should be washed clean before used for oral health. An alternative is to train local producers and retailers on improved hygienic packaging and handling of these essential cleaning agents.

**Keywords:** oral hygiene, diarrhoea , chewing stick, chewing sponge , molecular detection local market

## Introduction

The oral cavity, with the optimum humidity and temperature for the growth of microbes, could contain over 700 different types of bacteria, hence the need for the frequent removal of these bacteria to prevent infections (Kim *et al.*, 2018). Maintenance of good oral health is regarded as very important for a healthy life (Riasat *et al.*, 2021). Mechanical and chemical methods such as toothbrushes, toothpaste and

chewing sticks are the most common methods used for the removal of debris and keeping the mouth germ-free. The toothbrush market was estimated at USD 6.78 billion in 2021 and projected to be about USD 9.14 billion by 2029 globally (Riasat *et al.*, 2021; Fortune Business Insights, 2021).

The use of toothbrushes, chewing sticks and other oral cleaning agents for the maintenance of oral hygiene exists in both

developed and developing countries (Umoh *et al.*, 2020). Over 180 different plant species can be used as chewing stick with the common ones being *S. persica* (Peelu), *Olea europaea* (Zaitoon), *Capparis aphylla* (Khiran) and *Azadirachta indica* (Neem). In Ghana, the main users of chewing sticks and sponges are either those that lack access to toothbrushes, or those that use them in conjunction with orthodox tooth brushes (Mensah *et al.*, 2017). The most common types of traditional teeth cleaning agents in Ghana are the chewing sticks, made commonly from plants such as *Garcinia mannii* (Sokodua) and *Azadirachta indica* (Neem tree), and chewing sponges (locally called Sawere by Akans) made from *Acacia kamerunensis* (Debrah, 2022; Mensah *et al.*, 2017). These chewing sticks and sponges have been shown to have several beneficial properties including antioxidant, and antimicrobial properties and the ability to remove dental plaques and carries (Akaji & Otakhoigbogie, 2020; Essuman *et al.*, 2021). There is generally little to no awareness about microbial contamination of toothbrushes and oral care products as a result of poor storage conditions during their marketing and usage. They are usually stored in bathroom or with other toothbrushes of other members of the household. Consequently, toothbrushes become easily contaminated with microorganisms (Kim *et al.*, 2018). In the case of chewing sticks and sponges sold on the Ghanaian market, there is a similar pattern

of improper storage and handling to prevent microbial contamination.

Diarrhoeal diseases, although treatable and preventable, are the leading cause of death and malnutrition in children aged five years and below and are estimated to result in about 525,000 deaths in this age group per year globally (World Health Organization, 2017). Diarrhoea refers to a medical condition where an individual passes three or more loose, watery stools per day (Manetu *et al.*, 2021). It is caused by microbes, helminths and toxins that lead to an imbalance in the systems responsible for the regulation of the absorption of electrolytes and water in the gut, consequently leading to dehydration (Essuman *et al.*, 2021).

Rotavirus and *Escherichia coli* are regarded as the most common pathogenic agents of diarrhoea with rotavirus accounting for about 40% of all hospital related diarrhoea cases in children worldwide (Manetu *et al.*, 2021; World Health Organization, 2017). Other major microbes that have been shown to cause diarrhoea are *Salmonella*, *Vibrio cholerae*, *Shigella*, *Cryptosporidium* and *Campylobacter* (Manetu *et al.*, 2021).

On the local Ghanaian market, it is observed that chewing sticks are tied with rubber bands and sold openly on tables or by hawkers. Chewing sponges are also sold spread out on mats and tables in markets (Figure 1), increasing the exposure and likelihood of microbial contamination possibly leading to diarrhoeal diseases.



Fig. 1: Chewing sponges sold at Agboghloshie in Accra

These pose a likely health hazard since chewing sticks and sponges are purchased and used for oral hygiene with no prior cleaning and treatment, justifying the need to investigate if there are any microbial contaminants that can affect overall health. This information will support the need for proper methods for storage and handling of chewing sticks and sponges. This study aims to assess the presence of selected diarrhoeal pathogens (*Rotavirus A*, *E. coli*, *Salmonella* and *V. cholerae*) on chewing sticks and chewing sponges sold as ready-to-use teeth cleaning agents on the Ghanaian market.

## Experimentation

### *Sampling procedure*

A total of 10 samples (5 chewing sticks and 5 chewing sponges) weighing between 15 to 20 g each were randomly purchased from the Agboghloshie market in Accra. These samples were transferred into sterile ziplock bags and labelled appropriately before being transported to the Biomedical and Public Health Research Unit of the CSIR-Water Research Institute for laboratory processing.

### *Sample processing*

Sterile 50ml falcon tubes were labelled as TS001-TS005 and CS001-CS005 to represent the 5 chewing sponge samples and 5 chewing sticks samples respectively. Each of these tubes was filled with 40 mL distilled water. For both chewing stick and sponges 3.5 g of samples were transferred into labelled tubes. These tubes were kept at room temperature for 24 hours with intermittent shaking by inversion to dislodge pathogens.

### *Pathogen capture and concentration*

A 10 mL of incubated distilled water above was transferred into sterile 50 mL falcon tube. A 10  $\mu$ L aliquot of inactivated Bovine respiratory syncytial virus (BRSV) was added to each sample to serve as extraction control. Pathogen capture and concentration of the sample was performed using the Ceres Nanotrap Microbiome A Particles kit (Ceres Nanosciences Inc., Manassas, VA, USA) according to the manufacturers protocol. Briefly, 400  $\mu$ L of Enhancement Reagent 1 was added to the spiked sample followed by the addition of 400  $\mu$ L of Nanotrap Microbiome A Particles. The solution was inverted 5 times to mix and placed on a 50 mL magnetic rack and allowed to sit for 10 minutes. The supernatant was discarded without dislodging the magnetic beads and 1 mL of nuclease free water was added to resuspend the beads. The mixture was then transferred to a 2mL magnetic rack and allowed to sit for 3 minutes to allow separation. A 500  $\mu$ L aliquot of MagMax™ Microbiome Lysis Buffer (Thermo Fisher Inc, Massachusetts, USA) was added to the beads and allowed to sit for 10 minutes. The tubes were then placed on a 2 mL magnetic rack. The supernatant from this step was used for nucleic acid extraction.

### Nucleic acid extraction

A 400 µL aliquot of the supernatant from the pathogen capture and concentration step was transferred into newly labelled 2 mL microcentrifuge tubes. Total nucleic acid extraction was performed on the aliquots using the RADI PREP Swab and Stool DNA/RNA KIT (KH Medical Co. Ltd., Pyeongtaek, Gyeonggi, Republic of Korea) according to the manufacturer's protocol. The isolated nucleic acid was further purified using One-Step Inhibitor Removal Kit (Zymo Research Corporation, Irvine, CA, USA). The purified nucleic acid was then used for the polymerase chain reaction (PCR) screening.

### Nucleic acid amplification

Polymerase Chain Reaction (PCR) was performed as previously described (Quarcoo

*et al*, 2022) with slight modifications to screen for the presence of rotavirus, *Escherichia coli*, *Salmonella* and *V. cholerae* in all extracted nucleic acids. The PCR reaction mixture was prepared using 5 µL of 2x Sybr Green Mix (Quanta Biosciences), 0.2 µL of 10µM of each pathogen specific forward and reverse primers (sequences listed in Table 1), 2.6 µL of nuclease free water and 2 µL of extracted nucleic acid as template. The PCR reaction was performed on an Eppendorf Nexus Gradient PCR machine (Eppendorf GA, Hamburg, Germany). Cycling conditions for PCR reactions were; 95 °C for 3 min, 45 cycles of 95 °C for 1 min, 56 °C (*Rotavirus*, *Salmonella typhi* and *V. cholerae*) / 57.7°C (*E. coli*) for 30 sec , 72 °C for 1 min and final extension at 72 °C for 5 min.

TABLE 1

List of primer sequences and expected band sizes

Pathogen	Forward Primer (5-3')	Reverse Primer (5-3')	Size (bp)
BRSV	GCAATGCTGCAGGACTAGGTATAAT	ACACTGTAATTGATGCCCATCTCT	124
Rotavirus	CAGTGGTTGATGCTCAAGATGGA	TCATTGTAATCATATTGAATACCCA	131
<i>S. typhi</i>	TGTGGTAAAGGAACTCGGTAA	GACTCCGATACCGGATAATG	109
<i>V. cholerae</i>	CGCTTTATTGTTGATGCGTTA	ACTCGGTTATCGTCAGTTTGG	121
<i>E. coli</i>	ATGAAAGCTGGCTACAGG	GGTTTATGCAGCAACGAGACG	256

### Gel electrophoresis

PCR amplicons were mixed with 2 µl of Gel Loading Dye, Purple (6X) (New England Biolabs, Ipswich, USA) and separated using the electrophoresis technique on a 2% agarose gel prepared using 1X TBE and stained with Ethidium Bromide dye. Samples were run alongside a 100bp DNA ladder (New England Biolabs, Ipswich, USA). The electrophoresis setup was run at 100V for an hour followed by visualization of agarose gels under UV light

using a Fusion FX Spectra equipment (Vilber Lourmat, Upper Swabia, Germany).

### Results

Data from the molecular analysis indicated that there were no positives identified for Rotavirus A, *Salmonella spp* and *V. cholerae*. However, *E. coli* was detected (Figure 2) in 40% of chewing sponge and 60% of chewing stick samples (Figure 3). We could not detect any significant association between sample

type and the presence of *E. coli* (Fisher's Exact test; p-value = 1.000)



Fig. 2: Agarose gel image showing amplification of *E. coli* target DNA. Expected band size was 256 bp. DL represents a 100 bp ladder; PC and NC are positive and negative controls respectively. Samples TS003, TS004, CS002, CS004 and CS005 were positive.

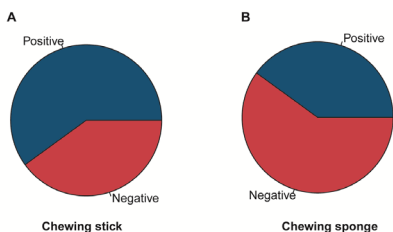


Fig. 3: Positivity rates of *E. coli* among chewing stick and sponge samples screened.

### Discussion

This is a preliminary study aimed at assessing the contamination of chewing sticks and sponges sold on the local Ghanaian market with diarrhoea-causing microbes. Chewing sticks and sponges are traditional teeth cleaning agents which are used by a majority of people in Ghana due to their availability, low cost and other health properties. In this study, the pathogens of interest were Rotavirus A, *Salmonella*, *V. cholerae*, and *E. coli*. The results obtained showed contamination of chewing sticks or sponges with *Salmonella*, Rotavirus-A or *V. cholerae*. However, there was evidence of contamination of both chewing sticks and sponges with *E. coli*. Although this is a preliminary study and only a few samples were collected, the fact that

50% of the samples were contaminated with *E. coli* should be a cause for concern. The sponges and chewing sticks are meant for oral hygiene but these are mostly handled without much care on our local markets. One major factor that could have led to this high level of contamination is the poor washing, sanitation and hygienic (WASH) conditions in the market. The market has limited facilities and sometimes handwashing might be done with only water after using toilet facilities. This results in remnants of pathogens on the hands which lead to contamination after handling chewing sticks and sponges.

The presence of *E. coli* is an indication of faecal contamination of samples collected (Odonkor & Mahami, 2020). Although our current study did not detect other diarrhoea causing pathogens on chewing stick and sponge samples, this does not rule out the likelihood of contamination with other pathogens such as norovirus and soil-transmitted helminths (Chard *et al.*, 2019). Soil-transmitted helminthes can be transmitted though the oral route, and is an important neglected tropical disease that affects about 1.5 billion people worldwide, causing 36.8 million disability-adjusted life years (DALYs).

*E. coli* infections result in symptoms such as diarrhoea and vomiting which consequently results in dehydration and dizziness (Qamar *et al.*, 2023). This can greatly affect overall productivity of individuals and even limit the attendance of school children (McMichael, 2019). Since chewing stick and sponges are mainly used for their potency in maintaining oral hygiene, infections from their usage will reduce patronage thereby preventing individuals from obtaining the benefits and reducing the earnings of most traders in the business.

### Conclusion

Data from this study show that chewing sticks and sponges sold on the local market in Accra are contaminated with pathogenic *E. coli*. Contamination of these local tooth cleaning agents with *E. coli* is not associated with the type of traditional tooth cleaning agents used. Based on the study findings, it is recommended that proper storage and hygienic measures be put in place by sellers in order to help curb contamination as well as appropriate regulations to control this. Further studies with larger sample size need to be carried out to properly establish and address this public health concern. This should include studies focusing on diarrhoea patients and usage of chewing sticks and sponges. Chewing sticks and sponges are good agents for maintaining oral hygiene but if care is not taken in their handling and storage, they can be potent carriers of diarrhoea-causing pathogens and other infectious pathogens such as soil-transmitted helminths.

### Author contributions

Conceptualization: SA; Investigation: GT, SA; Methodology: SA, GT, EA; Resources: SA; Data analysis: GT; Writing original draft: GT; Writing, review & editing: GT, EA, SA. All authors read, approved the final version of the manuscript and have consented to its submission to Ghana Journal of Science.

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