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Molecular characterization of bacteria strians isolated from "JIKO": A herbal preparation consumed in some parts of Kaduna metropolis, Nigeria

Ebipade Kereakede[1*], Mohammed Sani Abdulsalami[1], Nkechi Eucharia Egbe[1], Victoria Moltong Yilwa[2], Enimie Endurance Oaikhena[1], Elizabeth Omolola Oladapo[1], Asabe Danjuma[1] and Kingsley Onuh[1]

[1]Department of Biotechnology, Nigerian Defence Academy, Kaduna, Nigeria.
[2]Department of Biology, Nigerian Defence Academy, Kaduna, Nigeria.

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Herbal products are considered as phytomedicines which are used as complementary medicine and without adequate monitoring they are leased straightaway into the market. Preparation, transportation and handling of these products can lead to high microbial contamination which can be harmful to human health. The aim of this research was to molecularly characterize some multiple resistance bacteria obtained from JIKO which is a herbal preparation consumed in some parts of Kaduna metropolis. The genomic DNA was extracted from each bacteria isolates, 16s rRNA genes were amplified and sequenced. The sequenced obtained from the amplified genes were analyzed using Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Institute database to compare the genes with the exist genes with ones in GENbank. The results obtained confirmed the identities of four bacteria isolates: Three *Bacillus cereus* and a *Serratia* species. It was concluded that herbal products can contain some bacterial contaminants if not properly handled.

Key words: Herbal, phytomedicines, contaminants, genomic.

INTRODUCTION

Globally, herbal medicine is practiced, and most people rely on plants as source of food and for medical reasons since time immemorial. Most of the people in developing countries including Nigeria make use of herbal medicines to satisfy their health desire. Herbal preparations is also called botanical medicines or phytomedicines, herbs, herbal materials, herbal medicines, and finished herbal products that contain parts of plants or other plant materials as active ingredients (WHO, 2011). The plant materials used include seeds, berries, roots, leaves, bark or flowers (Ehrlich, 2010). Local herbal products and their preparations have been globally used for the thousands of years in developing and developed countries owing to its natural origin and lesser side effects (Anjoo, 2012). Herbal medicine contains active ingredients of plant materials which comprise different biological activities. Despite the widespread use of herbal preparations globally and their health benefits, they are not completely

*Corresponding author. E-mail: Ekereakede@nda.edu.ng.

harmless. The indiscriminate, irresponsible or non-regulated use of several herbal medicines may put the health of their consumers at risk of toxicity (Kloucek et al., 2005; Abt et al., 1995). Due to their excessive use and disposal, contaminants from environmental sources may even be present if an herb is organically grown (WHO, 2007). Harmful contaminants may also originate from the soil in which the herbs are cultivated, post-harvest treatment of herbal material e.g. fumigants, and finished product manufacturing stages (*e.g*. organic solvent residues) (Chan, 2003). Microbial contamination of herbs and/or products may result from improper handling during production and packaging process. The most likely sources of contamination are microbes like bacteria, fungi and virus from the soil and processing facilities (contaminated air, microbes of human origin). Cross contamination is also possible from extraneous materials such as packaging plastics and other materials which come in contact with herbal medicines, herbal preparations or products. Other contaminants may also be introduced during harvesting, handling and production of different herbal remedies since no awareness are made to decontaminate the herbs other than by washing them (Anyanwu, 2010). This study was aimed at molecularly characterizing some bacteria from herbal preparation consumed in some parts of Kaduna metropolis, Nigeria.

MATERIALS AND METHODS

Study site

The study was conducted in Kaduna metropolis, the capital of Kaduna State. Kaduna comprises two local government areas: Kaduna South and Kaduna North and also extends to Chikun and Igabi local government areas. The metropolis holds a region of about 260 km^2, and the distance between the Eastern and Western limits of the city is estimated 13.7 km (Figure 1).

Herbal sample collection

A total of 30 samples of JIKO herbal preparation were collected 15 from Kakuri and 15 from Ugwan Tanko market were collected into sterile bottles. Samples collected were transported to the laboratory of Biological Sciences Department, NDA in an ice-box container for bacterial isolation.

Preparation of media

Nutrient agar

Nutrient agar (Antec/USA) was prepared according to manufacturer's specification and sterilization of materials was done in an autoclave at 121°C for 15 min.

Isolation of bacteria

Serial dilution of the herbal samples was done in sterile distilled water. Then 0.1 ml of the sample in 10^{-2} and 10^{-4} dilution was transferred into nutrient agar plates and spread on the agar surface using sterile bent glass rod. This was done in triplicate and was incubated at 37°C for 24 h. After incubation, the bacterial colonies on the agar plates were sub-cultured and pure culture obtained.

Molecular identification of the bacteria isolates

Extraction of DNA

The DNA of the bacteria isolates were extracted using phenol chloroform (Sambrook et al., 2012).

Amplification of 16S rRNA Gene using PCR

The amplification of the 16S rRNA gene from extracted bacteria DNA was done using the primer pairs Forward 5'-GGACTACAGGGTATCTAAT-3' and Reverse 3'-AGAGTTTGATCCTGG-5'. The amplification was carried out in an Eppendorf thermocycler TTC-100^{TM} (USA) using the following parameters: predenaturation at 90°C for 5 min, 25 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 7 min. The amplified 16S rRNA gene of each isolate was visualized by agarose gel electrophoresis.

RIBOSE-1 GGACTACAGGGTATCTAAT 16S Primer and Forward RIBOSE-2 AGAGTTTGATCCTGG 16S Primer reverse

Sequencing of PCR products

The amplified 16S rRNA gene of each isolate was processed for sequencing. The sequencing kit (Applied Biosystems) with the product was analyzed with ABI prism DNA sequence (ABI). The gene sequence of each of the isolate obtained in this study was confirmed by similarity of 16s rRNA gene sequence with that in the Gene Bank database as described by Jyothi et al. (2012).

RESULTS

16S rRNA gene amplification of bacteria isolates

The band size of 800 bp was observed on the agarose gel for the bacteria isolates from Kakuri and Unguwan-tanko (Plate 1). BLAST analysis confirmed the identities of the bacteria isolates.

16S rRNA gene sequences

The BLAST uses the National Center for Biotechnology Information. BLAST analysis of the gene sequence from three of the isolates showed 99.73, 97.45 and 98.86% similarity with *Bacillus cereus*, respectively while the fourth sequence showed 86.26% similarity with *Serratia* species shown in Table 1

DISCUSSION

Local herbal products and herbal remedies have been globally used for the thousands of years in developing and developed countries owing to its natural origin and

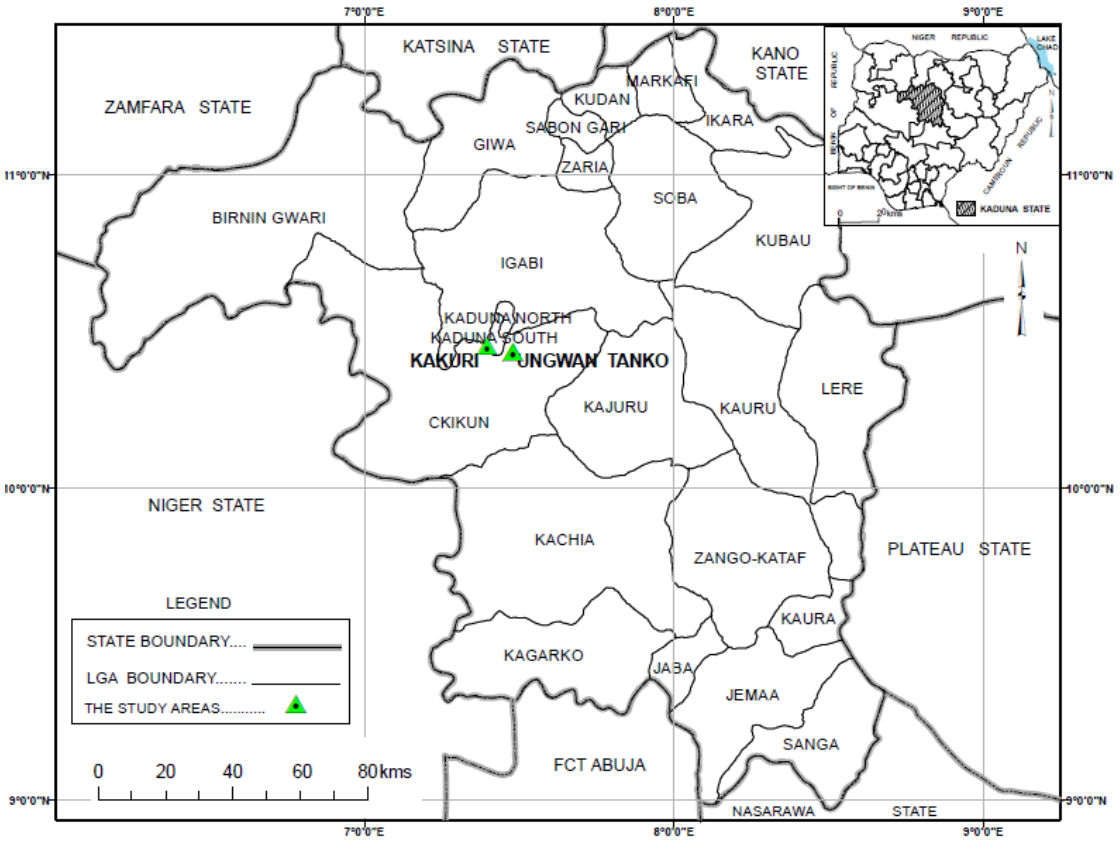

Figure 1. Map of Kaduna State showing the study area (Kakuri and Ugwan-Tanko).
Source: Geography Department NDA Kaduna.

lesser side effects (Anjoo, 2012). However herbal preparations usually carry enormous number of microorganisms originating from the soil. Microorganisms of different types are usually attached to roots, leaves, seeds, stems and flowers.

In the present study, four bacterial isolates obtained from the local herbal preparation (JIKO) were characterized by molecular techniques. The 16s rRNA gene sequence of the bacteria were aligned to the sequences in the GenBank using the BLAST tool. *B. cereus* was revealed as the dominant bacteria isolate. Similarly, Oyetayo (2008) and Heejin Ham (2017) recorded microbial contamination of agricultural herb products with *B. cereus* as the most dominant pathogenic species. This result is also in agreement with Nwankwo and Olime (2019) who reported that among the *Bacillus* species, the most prevalent was *Bacillus subtilis,* this was followed by *B. cereus* in their herbal preparation.

B. cereus is a widespread aerobic spore-forming bacteria naturally occurring micro-flora of medicinal plants and it is widely distributed in soil and water. The presence of *B. cereus* probably come from the soil, since the herbal preparation usually contain more than one plant or plant parts that have been procured from multiple harvest sites, from plants grown on soil which is the natural habitat for *Bacillus*. Other possible sources of contaminants could be improper drying, unhygienic handling of product as well as water, those processing the herbs and other contaminated equipment. Microbial contamination can render plant materials toxic either by transforming the chemicals in the plant material or from the production of toxic compounds by the microbes.

Also, *Serratia* spp. was recovered from the herbal drink. This finding agrees with the work of Abdela et al., (2016) which revealed that bacterial isolates such as *Serratia, Shigella*, *Streptococcus*, *Staphylococcus, Bacillus*, *Escherichia*, *Klebsiella*, *Clostridium*, *Enterobacter*, *Clostridium*, *Pseudomonas*, *Salmonella* and *Citrobacter* was recovered from herbal medicinal preparation. Balvindra and Neelam (2019) recorded *Serratia*, *Salmonella, Klebsiella,* and *Proteous* species from fruit juice.

Serratia spp. are widely spread in the environment, but they are not usual constituents of the human faecal flora (Carrero et al., 1995).

The microflora of the final product may represent contaminants from the raw materials, equipment, water, and atmosphere and from personnel (Oyetayo, 2008).

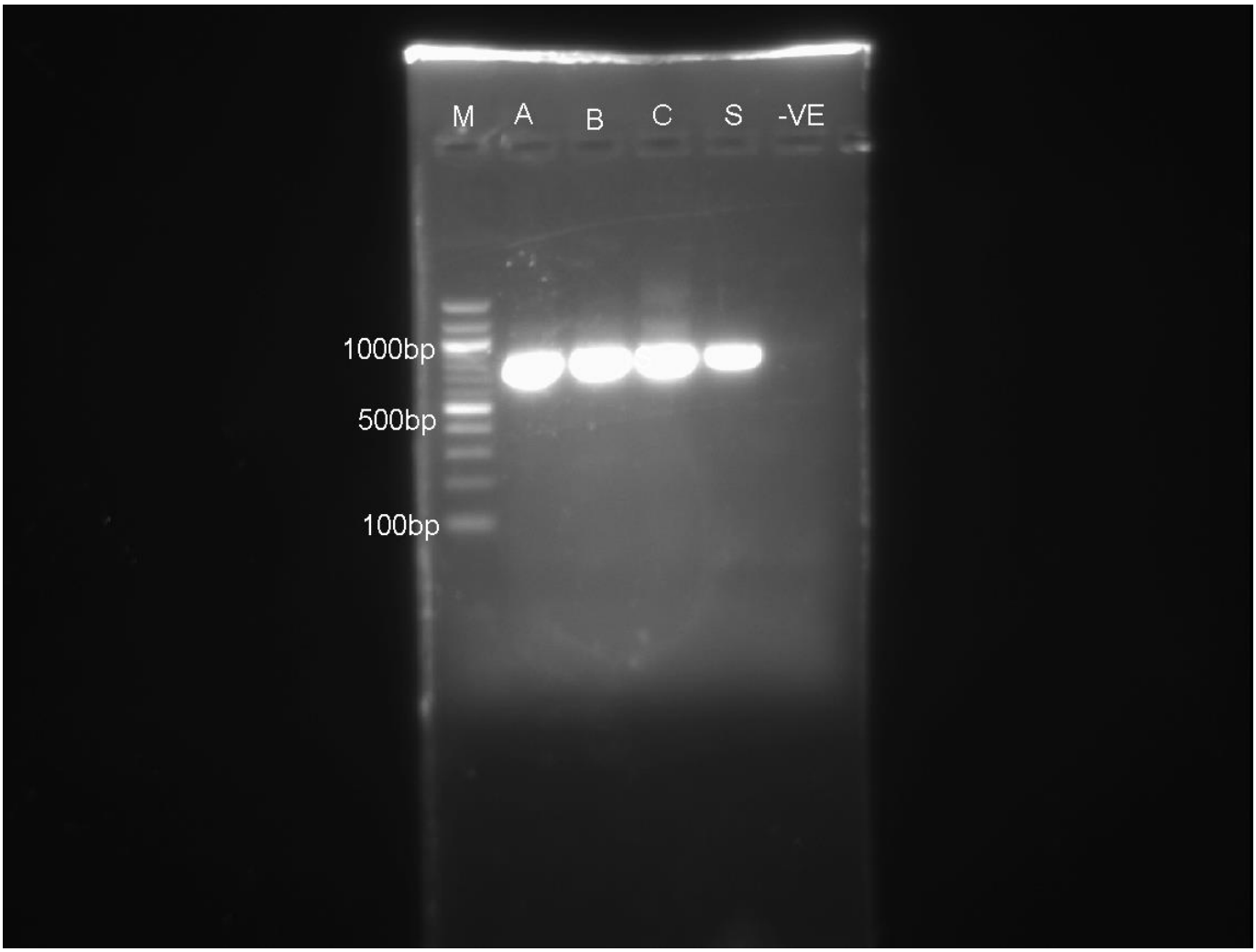

Plate 1. Agarose gel electrophoresis pattern showing PCR amplification of 16S rRNA gene of four bacteria isolates from JIKO from Kakuri and Ungwan-Tanko. M: DNA marker, -VE, 800base pair, A-2A, B-2B, C-2C, S- 2S, Negative control.

Table 1. BLAST results of 16S rRNA sequenced gene for bacteria identification.

Isolate code	Accession number	Length	Identity (%)	Identified organism
A-2A	KY435707.1	741	99.73	*Bacillus cereus*
B-2B	MH938327.1	692	97.45	*Bacillus cereus*
C-2C	KP701021.2	729	98.86	*Bacillus cereus*
S-2S	HQ238869.1	447	86.26	*Serratia* spp.

Conclusion

Herbs are extensively used in Nigeria because some plants possess vital curative properties, which can actually be used to treat human and animal diseases. The sequenced analysis of the 16S rRNA genes suggested that molecular identification of bacteria is the most accurate approach, especially for identifying organisms. Therefore, plants should be properly screened and also Good Manufacturing Practice (GMP) should be adopted by producers to ensure safe and good hygiene of herbal products.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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