JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Coronavirus Pandemic

Anthrax toxins-producing *Bacillus* spp. isolated from handwashing stations during COVID-19 pandemic in Lagos, Nigeria

Tajudeen A Bamidele[1], Bamidele T Odumosu[2], Princess T Adenola[2], James Ameh[3], Olaide K Kareem[1], Babatunde Osoba[4], Oliver C Ezechi[1], Babatunde L Salako[1]

[1] *Nigerian Institute of Medical Research, Yaba-Lagos, Nigeria*
[2] *University of Lagos, Akoka-Lagos, Nigeria*
[3] *University of Queensland, School of Veterinary Science, Brisbane, Australia*
[4] *Ministry of Health, Lagos State, Nigeria*

Abstract

Introduction: The virulence binding factor, protective antigen (*pag*) and poly-D-γ-glutamate capsular (*cap*) genes, peculiar to *Bacillus anthracis* are located in the pXO1 and pXO2 plasmids which are transferable horizontally to related species called “cereus group”. The cereus group are usually isolated from the environmental/food samples and have been implicated in debilitating human and animal anthrax-like diseases. This study was designed to investigate the presence of the anthrax virulence genes in different *Bacillus* spp. isolated from handwashing facilities during COVID-19 pandemic in Lagos, Nigeria.

Methodology: The *Bacillus anthracis* (OK316847), *B. thuringiensis* (OK316855), *B. amyloliquefaciens* (OK316857), *B. cereus* (OK316858) and *B. thuringiensis* (OK316859) previously isolated from rinsates and bowl water in two local government areas (LGAs) of Lagos state were further investigated by the polymerase chain reaction (PCR) amplification of the *pag* and *cap* genes using specific primers.

Results: *Bacillus anthracis* and *B. cereus* co-harboured the two 578 bp *cap* and 364 bp *pag* genes while *B. thuringiensis* only harboured the *cap* gene. Similarly, the non-cereus *B. amyloliquefaciens* was found to habour the *pag* gene.

Conclusions: The two anthrax toxin genes were amplified in the *Bacillus* spp isolated from rinsates and bowl water used in hand washing in the two study LGAs. Given that these virulence genes have a global consequence and are a potential threat to life, this study calls for an extensive surveillance, and reassessment of gene regulators and plasmid distribution among these strains in our environment.

Key words: anthrax; virulence; handwashing; COVID-19; *Bacillus*.

J Infect Dev Ctries 2023; 17(8):1076-1080. doi:10.3855/jidc.18228

(Received 16 March 2023 – Accepted 07 June 2023)

Copyright © 2023 Bamidele *et al*. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

Bacillus anthracis is a spore former, pathogenic and causes anthrax infection in man and animals through a combination of bacterial infection and toxemia. It has also been a top bioterrorism agent since the anthrax attacks in the USA in 2001 [1].

The bacterium contains 2 large extrachromosomal plasmids, pXO1 (182 kb) and pXO2 (96 kb) which are essential for its full virulence [2,3]. The pXO1 encodes the 3 anthrax exotoxins components which are protective antigen (PA or pag, 83 KDa), lethal factor (LF, 89 KDa), and edema factor (EF, 90, 90 KDa), while pXO2 encodes proteins which synthesize the unique poly-D-γ-glutamic acid capsule (cap) conferring resistance to phagocytosis. Although, PA, LF and EF components are non-toxic individually, they pair up to form the 2 major virulence factors; lethal toxin (LT; LF + PA) and edema toxin (ET; EF + PA) [4], thus PA is the cellular binding moiety while LF and EF are catalytic parts of the toxins.

The biology and taxonomy of the closely related species, referred to as “cereus” group, comprising of *B. cereus*, *B. anthracis*, *B. thuringiensis*, *B. mycoides*, *B. pseudomycoides*, and *B. weihenstephanensis* have been reported, while there seems to be lack of consensus whether they should be considered separate spp. or not [5-12]. Few reports exist on the ability of certain cereus groups to harbor anthrax toxin genes and even cause severe and fatal pneumonia resembling those caused by *B. anthracis*. For instance, *B. cereus* was previously reported to carry virulence similar to the anthrax lethal factor toxemia [13-16], while [17] reported *B. thuringiensis* bearing the genes for producing poly-D-γ-glutamic acid capsule of *B. anthracis*.

In the era of COVID-19 pandemic, the waste water generated from various handwashing facilities was disposed of into the public drainage without further

treatment. Microbial study of this waste water indicated the presence of pathogenic bacterial morphotypes including the anthrax bacillus which was isolated from the communities hosting the markets for cattle and other ruminants in Lagos [18]. This study was therefore designed to investigate the possession or otherwise of anthrax protective antigen (*pag*) and capsule protein (*cap*) genes in *Bacillus* spp. isolated from handwashing stations during the COVID-19 pandemic in Lagos, Nigeria.

Methodology

Study site, bacterial cultural isolation

The *Bacillus* spp (*B. anthracis* (OK316847), *B. thuringiensis* (OK316855), *B. amyloliquefaciens* (OK316857), *B. cereus* (OK316858), and *B. thuringiensis* (OK316859)) were isolated from bucket water (bw) used in handwashing, and rinsates (rs) at two local government areas (LGAs) (Alimosho and Agege) in Lagos, Nigeria. The cultural isolation was done according to standard microbiological methods. Briefly, the water samples (bw, n = 26 and rs, n = 28) were transported on ice packs in screw capped sterile containers, and were serially diluted (10-fold), pour plated on Mueller Hinton (MH) agar plates and incubated aerobically at 37 °C for 24 h. The distinct Gram +ve rods, catalase producing colonies, showing hydrolysis of starch that are characteristic of *Bacillus* were sub-cultured for purity and stored in 20% MH-glycerol broth at -80 °C until further use.

Ethical/social consideration

This study did not involve any human participants. The approval (IRB/20/097) was obtained from the Institutional Review Board of the Nigerian Institute of Medical Research (NIMR-IRB). The permission to conduct the study was granted by the Lagos State Primary Health Care Board (LS/PHCB/MS/1128/VOL VIII/093) while community entry was facilitated by the respective Medical Health Officer (MHO) in each LGA.

Bacterial identification and 16S rRNA sequencing

Preliminary identification of the isolates suspected to be *Bacillus* spp. was carried out by a combination of colonial morphology and biochemical tests. The 16S rRNA amplification was performed on the distinct (single, pure) colonies and sequenced using primers targeting the hyper variable regions V5-V6-V7 which have been demonstrated to produce a high number of bacterial operating taxonomic units (OTUs) [29] (Table 1). The 16S rRNA amplicons were sent out to Inqaba Biotec, South Africa for commercial Sanger sequencing and the data subjected to basic local alignment search tool (BLAST) algorithm on National Center for Biotechnology Information (NCBI) GenBank. All the sequence data (16S rRNA) were submitted to the GenBank and accession numbers allocated.

Detection of virulence genes

The polymerase chain reaction (PCR) was performed for the amplification of anthrax virulence (*pag* and *cap*) genes in 20 µL reaction comprising the primer pair, ready to load master mix (SolisBiodyne, Tartu, Estonia), template DNA and distilled water. The amplicons were later resolved in duplicates alongside a 100 bp DNA ladder (ThermoFisher Scientific, Waltham, USA) on 1.5% agarose gel run at 100V for 1 hour and viewed under the UV trans-illuminator.

Results

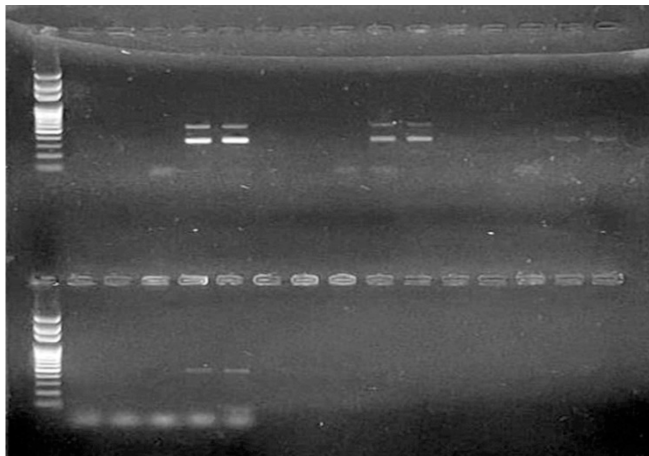
A total of 67 bacteria were isolated altogether; however, bacillus was identified based on preliminary identification and colonial morphology on the culture media. Thirteen (19.40%) bacilli consisting of six different species were identified based on 16S rRNA sequencing and basic alignment with 98-100% homology with those already deposited in the GenBank. The bacillus population was distributed in the following proportion: *B. cereus* (1; 7.69 %), *B. amyloliquefaciens* (2; 15.4%), *B. anthracis* (1; 7.69%), *B. subtilis* (5; 38.46%), *B. thuringiensis* (3; 23.10%), and *B. megaterium* (1; 7.69%). The *pag* and *cap* genes were distributed among the *Bacillus* spp in different proportions. *Bacillus anthracis* and *B. cereus* co-

Table 1. Primers, target genes, expected size, and cycling parameters used in polymerase chain reaction (PCR) amplification.

Primer (5’-3’)	Target gene	Size (bp)	Cycling parameters	Reference
799F - AACACGGATTAGATACCCG 1193R - ACGTCATCCCCACCTTCC	16S rRNA	394	94 °C for 3 minutes, 35 cycles of 94 °C for 1 minute, 53 °C for 1 minute, 72 °C for 1 minute. Final elongation at 72 °C for 10 minutes	[29]
F6 - CCTTGTGGCAGCTTATCCGA R6 - GTAGATTGGAGCCGTCCCAG	*pag*	364	94 °C for 30 seconds, 35 cycles of 94 °C for 30 seconds, 58 °C for 25 seconds, 72 °C for 1 min. Final elongation at 72 °C for 10 minutes	[30]
F8 - TCATCCGGATCCAGGAGCAATGAG R7 - GCAGGTAAAATACCTGTTCTTTCTG	*cap*	578		

harboured the *cap* and *pag* genes (Figure 1) while *B. thuringiensis* harboured the cap gene only but was negative for *pag*. Among the non-cereus bacillus, only *B. amyloliquefaciens* was positive for the amplification of *pag* gene while *B. megaterium* was negative for both anthrax genes (Table 2).

Figure 1. Gel images for amplified anthrax genes.



Lane 1: 100 bp marker (upper and lower), upper gel (lanes 5, 6): *pag*, *cap* genes of *B. anthracis*, (lanes 10, 11): *pag*, *cap* genes of *B. cereus*, (lanes 15, 16): *pag* gene of *B. amyloliquefaciens*. lower gel (lanes 5, 6): *cap* gene of *B. thuringiensis*.

Discussion

Our study focused on the occurrence of two virulence genes, *cap* and *pag*, in *Bacillus* spp isolated from handwashing stations at various public places during the COVID-19 pandemic. The study was carried out due to the public health risk and urgency in providing sufficient data for proper future surveillance in our region. We reported the diversity of microorganisms associated with COVID-19 handwashing stations in a previous study [18] and as a matter of interest, we carried out further investigation of the *Bacillus* spp that were identified in the same study. *Bacillus* spp, especially *B. cereus*, are well known and reported from food contamination causing spoilage and diarrhea [19,20], periodontal and opportunistic diseases in humans due to their production of enterotoxins [21,22]. To the best of our knowledge, this study is the first to report the anthrax virulence genes from community-associated samples in Nigeria.

The *cap* and *pag* virulence genes were prevalent in all the investigated *Bacillus* species. All the investigated bacilli except *B. megaterium* harboured either *cap* or *pag* gene while *B. anthracis* and *B. cereus* haboured both the virulence genes. Uneven distribution of these genes among the isolates may suggest the loss or gain of plasmids for virulence among the bacteria as recently reported elsewhere [23]. *Bacillus anthracis*, is thought to differ from other species of same genus by the presence of the virulence genes borne on the plasmids, pXO1 and pXO2 encoding the lethal toxin genes that cause the severe inhalation anthrax illness. It has also been established by some researchers that there is a high genome similarity of this species to *B. cereus* and other closely related species [7,8,24,25]. Based on this similarity, some researchers have even considered *B. cereus* to be a pathogenic variant of *B. anthracis* [6,13-15]. The *B. cereus* isolated in this study from bowl water, is of similar identity (99.72%) with *B. thuringiensis* which corroborates genomic similarity between these bacteria. In this study, the capsular protein (*cap*) was amplified in *B. thuringiensis* while *B. anthracis* and *B. cereus* harboured both *cap* and *pag* virulence genes (Table 2). This is in agreement with some studies elsewhere; anthrax toxin- expressing *B. cereus* strain BcFL2013, from an anthrax like eschar (a human facial lesion), Bcbva, JF3964 from cattle and *B. thuringiensis* strain producing a polyglutamate capsule similar to that of *B. anthracis* were reported [13,17,26,27]. *Bacillus amyloliquefaciens* was also found harbouring the *pag* gene in the present study (Table 2). *B. amyloliquefaciens* is a non-member of the *B. cereus* group and normally would not be expected to share the same virulence genes unless it was transferred horizontally. Our results corroborate an earlier suggestion by [13] on possible transmission of plasmids from *B. anthracis* to *B. cereus*. Although the present study did not investigate the presence of plasmids among the species because we have a justification from literature that the investigated genes are plasmid borne,

Table 2. The sources of the Bacillus spp. with the amplified anthrax genes.

***Bacillus* spp.**	**Accession number**	**Sample source/Local government area (LGA)**	**Amplified gene**
B. anthracis	OK316847	bw/Al	*pag, cap*
B. thuringiensis	OK316855	rs/Ag	*cap*
B. amyloliquefaciens	OK316857	bw/Al	*pag*
B. cereus	OK316858	bw/Al	*pag, cap*
B. thuringiensis	OK316859	rs/Ag	*cap*
B. megaterium	OK316839	bw/Al	Nil

bw: bowl water; rs: rinsates; Al-Alimosho; Ag: Agege; *cap*: capsule gene; *pag*: protective antigen.

our results are in line with the reported possible transmission of the virulence genes across the species.

Based on our results, we can also affirm that focusing on *B. anthracis* as the only agent of anthrax disease can be deceptive because, genetically related species, such as we have identified in the present study, may harbor the virulence genes specific for anthrax as previously reported [13,14,17].

The occurrence of *B. anthracis* and *B. cereus* in bowl water used for handwashing suggests contamination with the spore and if this gets to the food or water for public consumption, it could cause an outbreak. It is also noteworthy that the region, Alimosho LGA, is the most populated in the state [28] with large abattoir and cattle market in close proximity to human residential areas.

The presence and transmission of anthrax virulence genes *pag* and *cap*, among community acquired isolates of bacillus in this study calls for great deal of caution and serious surveillance. The *B. amyloliquefaciens* isolated in this study, is not known to share genomic similarity with the "cereus" group, neither has it been reported to harbour anthrax genes. The amplification of *pag* gene in this bacterium is surprising and to the best of our knowledge, this is the first of such report in the Sub-Saharan region. The fact that the virulence genes are borne on plasmid and are transferred horizontally make the acquisition possible by even non-related bacteria.

Conclusions

The two anthrax-specific toxin genes, *pag* and *cap*, were amplified in the *Bacillus* spp isolated from rinsates and bowl water used in hand washing in the two study LGAs. Given that these virulence genes have a global consequence and are a potential threat to life, this study calls for an extensive surveillance, and reassessment of gene regulators and plasmid distribution among these strains in our environment.

Acknowledgements

The staff members of the Department of Molecular Biology and Biotechnology, Nigerian Institute of Medical Research (NIMR) are acknowledged for their participation during the execution of this work, especially during the PCR. This study was partly funded by the COVID-19 intervention Grant (NMG-CIF-07-0025) provided by the Federal Government of Nigeria through NIMR.

Authors' contributions

The contributions of all authors are follows; TAB, OCE: concept and literature review; TAB, BTO, PTA, OKK: planning/design, sample collection and analysis; TAB, BTO, JA: PCR and analysis; all the authors were involved in manuscript writing; BLS: mentorship and administrative support.

References

1. Hudson MJ, Beyer W, Bohm R, Fasanella A, Garofolo G, Golinski R, Goossens PI, Hahn U, Hallis B, King A, Mock M, Montecucco C, Ozin A, Tonello F, Kaufmann SHE (2008) *Bacillus anthracis*: balancing innocent research with dual-use potential. Int J Med Microbiol 298: 345-364. doi: 10.1016/j.ijmm.2007.09.007.
2. Leppla SH (2013) Chapter 281 - Anthrax lethal factor. In Rawlings ND, Salvesen GS, editors. Handbook of proteolytic enzymes. Academic Press. 1257-1261. doi: 10.1016/B978-0-12-382219-2.00282-9
3. Fouet A, Mock M (2006) Regulatory networks for virulence and persistence of *Bacillus anthracis*. Curr Opin Microbiol 9: 160-166. doi: 10.1016/j.mib.2006.02.009.
4. Moayeri M, and Leppla SH (2009) Cellular and systemic effects of anthrax lethal toxin and edema toxin. Mol Aspects Med 30: 439-455. doi: 10.1016/j.mam.2009.07.003.
5. Daffonchio D, Cherif A, Borin S (2000) Homoduplex and heteroduplex polymorphisms of the amplified ribosomal 16S-23S internal transcribed spacers describe genetic relationships in the "*Bacillus cereus* group". Appl Environ Microbiol 66: 5460-5468. doi: 10.1128/AEM.66.12.5460-5468.2000.
6. Helgason E, Caugant DA, Olsen I, Kolsto AB (2000a) Genetic structure of population of *Bacillus cereus* and *Bacillus thuringiensis* isolates associated with periodontitis and other human infections. J Clin Microbiol 38: 1615-1622. doi: 10.1128/JCM.38.4.1615-1622.2000.
7. Helgason E, Okstad OA, Caugant DA, Johansen HA, Fouet A, Mock M, Hegna I, Kolsto AB (2000b) *Bacillus anthracis*, *Bacillus cereus*, and *Bacillus thuringiensis*-one species on the basis of genetic evidence. Appl Environ Microbiol 66: 2627-2630. doi: 10.1128/AEM.66.6.2627-2630.2000.
8. Bavykin SG, Lysov YP, Zachariev V, Kelly JJ, Jackman J, Stahl DA, Cherni A (2004) Use of *16S rRNA*, *23S rRNA*, and *gyrB* gene sequence analysis to determine phylogenetic relationships of *Bacillus cereus* group micro-organisms. J Clin Microbiol 42: 3711-3730. doi: 10.1128/JCM.42.8.3711-3730.2004.
9. Harrell LJ, Andersen GL, Wilson KH (1995) Genetic variability of *Bacillus anthracis* and related species. J Clin Microbiol 33: 1847-1850. doi: 10.1128/jcm.33.7.1847-1850.1995.
10. Keim P, Kalif A, Schupp J, Hill K, Travis SE, Richmond K, Adair DM, Hugh-Jones M, Kuske CR, Jackson P (1997) Molecular evolution and diversity in *Bacillus anthracis* as detected by amplified fragment length polymorphism markers. J Bacteriol 179: 818-824. doi: 10.1128/jb.179.3.818-824.1997.
11. Chang YH, Shangkuan YH, Lin HC, Liu HW (2003) PCR assay of the *groEL* gene for detection and differentiation of *Bacillus cereus* group cells. Appl Environ Microbiol 69: 4502-4510. doi: 10.1128/AEM.69.8.4502-4510.2003.
12. Radnedge L, Agron PG, Hill KK, Jackson PJ, Ticknor LO, Keim P, Anderson GL (2003) Genome differences that distinguish *Bacillus anthracis* from *Bacillus cereus* and *Bacillus thuringiensis*. Appl Environ Microbiol 69: 2755-2764. doi: 10.1128/AEM.69.5.2755-2764.2003.
13. Marston CK, Ibrahim H, Lee P, Churchwell G, Gumke M, Stanek D, Gee JE, Boyer AE, Gallego-Candela M, Barr JR, Li

H, Boulay D, Cronin L, Quinn CP, Hoffmaster AR (2016) Anthrax toxin-expressing *Bacillus cereus* isolated from an anthrax-like eschar. PLoS ONE 11: e0156987. doi: 10.1371/journal.pone.0156987.

14. Hoffmaster AR, Hill KK, Gee JE, Marston CK, De BK, Popovic T, Sue D, Wilkins PP, Avashia SB, Drumgoole R, Helma CH, Ticknor LO, Okinaka RT, Jackson PJ (2006) Characterization of *Bacillus cereus* isolates associated with fatal pneumonias: strains are closely related to *Bacillus anthracis* and harbor *B. anthracis* virulence genes. J Clin Microbiol 44: 3352-3360. doi: 10.1128/JCM.00561-06.
15. Avashia SB, Riggins WS, Lindley C, Hoffmaster A, Drumgoole R, Nekomoto T, Jackson PJ, Hill KK, Williams K, Lehman L, Libal MC, Wilkins PP, Alexander J, Tvaryanas A, Betz T (2007) Fatal pneumonia among metal workers due to inhalation exposure to *Bacillus cereus* containing *Bacillus anthracis* toxin genes. Clin Infect Dis 44: 414-416. doi: 10.1086/510429.
16. Wright AM, Beres SB, Consamus EN, Long SW, Flores AR, Barrios R, Richter GS, Oh SY, Garufi G, Maier H, Drews AL, Stockbauer KE, Cernoch P, Schneewind O, Olsen RJ, Musser JM (2011) Rapidly progressive, fatal, inhalation anthrax-like infection in a human: case report, pathogen genome sequencing, pathology, and coordinated response. Arch Pathol Lab Med 135: 1447-1459. doi: 10.5858/2011-0362-SAIR.1.
17. Cachat E, Barker M, Read TD, Priest FG (2008) A *Bacillus thuringiensis* strain producing a polyglutamate capsule resembling that of *Bacillus anthracis*. FEMS Microbiol Lett 285: 220-226. doi: 10.1111/j.1574-6968.2008.01231.x.
18. Bamidele TA, Odumosu BT, Adenola PT, Ameh J, Anejo-Okopi J, Musa AZ, Osoba B, Ezechi OC, Salako BL (2023) Microbial contents and antibiotics susceptibilities from hand washing stations during COVID-19 pandemic in Lagos, Nigeria. Adv Infect Dis 13: 54-65. doi: 10.4236/aid.2023.131007.
19. Wijnands LM, Dufrenne JB, Rombouts FM, Veld PH, van Leusden FM (2006) Prevalence of potentially pathogenic *Bacillus cereus* in food commodities in The Netherlands. J Food Prot 69: 2587-2594. doi: 10.4315/0362-028X-69.11.2587.
20. Fricker M, Messelhäußer U, Busch U, Scherer S, Ehling-Schulz M (2007) Diagnostic real-time PCR assays for the detection of emetic *Bacillus cereus* strains in foods and recent food-borne outbreaks. Appl Environ Microbiol 73: 1892-1898. doi: 10.1128/AEM.02219-06.
21. Oguntoyinbo FA, Huch M, Cho GS, Schillinger U, Holzapfel WH, Sanni AI, Franz CM (2010) Diversity of *Bacillus* species isolated from okpehe, a traditional fermented soup condiment from Nigeria. J Food Protec 73: 870-878. doi: 10.4315/0362-028X-73.5.870.
22. Turenne CY, Snyder JW, Alexander DC (2015) *Bacillus* and other aerobic endospore-forming bacteria. In Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, Warnock DW editors. Manual of Clinical Microbiology. Sterling: ASM Press. 441-461. doi: 10.1128/9781555817381.ch26.
23. Salgado JRS, Rabinovitch L, dos S Gomes MF, Allil RC, Werneck MM, Rodrigues RB, Picao RC, de Oliveira Luz FB, Vivoni AM (2020) Detection of *Bacillus anthracis* and *Bacillus anthracis*-like spores in soil from state of Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz 115: e200370. doi: 10.1590/0074-02760200370.
24. Klee SR, Brzuszkiewicz EB, Nattermann H, Bruggemann H, Dupke S, Wollher A, Franz T, Pauli G, Appel B, Liebl W, Couacy-Hymann E, Boesch C, Meyer FD, Leendertz FH, Ellerbrok H, Gottschalk G, Grunow R, Liesegang H (2010) The genome of a *Bacillus* isolate causing anthrax in chimpanzees combines chromosomal properties of *B. cereus* with *B. anthracis* virulence plasmids. PloS One 5: e10986. doi: 10.1371/journal.pone.0010986.
25. Ticknor LO, Kolsto AB, Hill K, Keim P, Laker MT, Tonks M, Jackson PJ (2001) Fluorescent amplified fragment length polymorphism analysis of Norweigian *Bacillus cereus* and *Bacillus thuringiensis* soil isolates. Appl Environ Microbiol 67: 4863-4873. doi: 10.1128/AEM.67.10.4863-4873.2001.
26. Antonation KS, Gr¨ utzmacher K, Dupke S, Mabon P, Zimmermann F, Lankester F, Peller T, Feistner A, Todd A, Herbinger I, de Nys HM, Muyembe-Tamfun JJ, Karhemere S, Wittig RM, Couacy-Hymann E, Grunow R, Calvigna-Spencer S, Corbett CR, Klee SR, Leendertz FH (2016) *Bacillus cereus* biovar anthracis causing anthrax in sub-Saharan Africa-chromosomal monophyly and broad geographic distribution. PLOS Negl Trop Dis 10: e0004923. doi: 10.1371/journal.pntd.0004923.
27. Hoffmann C, Zimmermann F, Biek R, Kuehl H, Nowak K, Mundry R, Agbor A, Angedakin S, Arandjelovic M, Blankenburg A, Brazolla G, Corogenes K, Couacy-Hymann E, Deschner T, Dieguez P, Dierks K, Düx A, Dupke S, Eshuis H, Formenty P, Yuh YG, Goedmakers A, Gogarten JF, Granjon AC, McGraw S, Grunow R, Hart J, Jones S, Junker J, Kiang J, Langergraber K, Lapuente J, Lee K, Leendertz SA, Léguillon F, Leinert V, Löhrich T, Marrocoli S, Mätz-Rensing K, Meier A, Merkel K, Metzger S, Murai M, Niedorf S, De Nys H, Sachse A, Schijndel J, Thiesen U, Ton E, Wu D, Wieler LH, Boesch C, Klee SR, Wittig RM, Calvignac-Spencer S, Leendertz FH (2017) Persistent anthrax as a major driver of wildlife mortality in a tropical rainforest. Nature 548: 82-86. doi: 10.1038/nature23309.
28. City population (nd) Nigeria: Metro Lagos. Available: https://www.citypopulation.de/en/nigeria/metrolagos/. Accessed: 16 January 2023.
29. Thijs S, Op De Beeck M, Beckers B, Truyens S, Stevens V, Van Hamme JD, Weyens N, Vangronsveld J (2017) Comparative evaluation of four bacteria-specific primer pairs for 16S rRNA gene surveys. Front Microbiol 8: 494. doi: 10.3389/fmicb.2017.00494.
30. Ogawa H, Fujikura D, Ohnuma M, Ohnishi N, Hang'ombe BM, Mimuro H, Ezaki T, Mweene AS, Higashi H (2015) A novel multiplex PCR discriminates *Bacillus anthracis* and its genetically related strains from other *Bacillus cereus* group species. PloS One 10: e0122004. doi: 10.1371/journal.pone.0122004.

Corresponding author

Tajudeen Akanji Bamidele, MSc. PhD
Chief Research Fellow,
6 Edmund Crescent
Yaba-Lagos, Nigeria.
Tel: +234 803 857 8093
Email: deletaju@yahoo.co.uk

Conflict of interests: No conflict of interests is declared.