



The Throne

By Malika Sharma

Part 1: Background Information

Many, many years ago the country of Genovia was ruled by the humble and honest King Richard. He was a loyal ruler who listened to his citizens and implemented many new laws to help the lives of many. He had been ruling Genovia, with his beautiful wife, four sons, and three daughters by his side, for over 20 years. He has done a lot for his country, but along the way he has also made a few enemies, one of which was the ruler of a nearby country called Runeba. Recently, a war has erupted between Genovia and Runeba. King Richard has been busy at work fighting in the war. Unfortunately, during the midst of the war, 58-year-old King Richard was shot by the soldiers of Runeba and died on the spot. As sad as this was for the country of Genovia and the royal family, there was no time waste. It was now time for King Richard's eldest son, Prince Julian, to fill his father's shoes and win the war.

Prince Julian's inevitable reign of Genovia was a highly popular topic ever since the day he was born 29 years ago. His ultimate succession to his father's throne had been something he had always been told ever since he could walk. He was treated as a king before he was even commemorated. So, the citizens of Genovia were very excited to see Prince Julian grow up to be a promising first heir to his father's throne. Prince Julian was kind, charming, and level-headed; all traits that make up a good ruler.

The demise of King Richard took the country by storm. His assassination by the neighboring country shook the entire country and the royal family. Fortunately, in a time of need, although emotionally shaken, Prince Julian, now King Julian, shook himself off and rose to the occasion to fight for his country. He eventually won the war, brought peace back to his land, and made his country proud.

As time went on, the people of Genovia grew very fond of King Julian and started to look at the future of the country with great optimism. However, only six months into his reign of Genovia, King Julian was found dead at night in his bed by his butler. This shocking turn of events led the country into absolute mayhem. Brawls flooded the streets. Crowds of people formed in remembrance of the young late king. Chaos struck in the royal palace.

As per the post-mortem report, King Julian mysteriously died presumably due to cardiac arrest because he suffered from Guillain-Barre syndrome (GBS), a rare disorder where the body's immune system damages nerve [3]. This information, however, did not sit well with the citizens of Genovia, one of them being you. You, alongside the millions of citizens of Genovia, found it

hard to believe that King Julian died by cardiac arrest via GBS because had the king or his doctor known about the illness prior, they would have both started treatment as soon as possible. Also, King Julian seemed like a healthy young man who was relatively very active and had no genetic disposition to GBS. It was odd for this to come out of nowhere.

While some people accepted the fact that King Julian died by cardiac arrest, you, a professional lawyer and bioinformatist, believed otherwise. You made it your mission to get to the bottom of this case and find the true reason of King Julian's death.

Part 2: What we know so far

You know so far that King Julian was not taking any treatment for his supposed GBS and that he did not have a genetic disposition to any heart diseases that could have led to the cardiac arrest. You also know that King Julian was an active young male who had regular doctor check-ups, thus ruling out that any health issue had to play with the death of King Julian.

Knowing that King Richard, King Julian's father, had a few enemies, you realized that there is a good chance that King Julian did too. So, you start to take a deep dive into the life of King Julian, the people around him, and their possible motives to kill King Julian.

King Julian had six younger siblings, all who grew up in the palace of Genovia, with a lovely staff consisting of a secretary, assistant, maid, butler, chauffeur, and cook, all who also lived in the palace. Knowing this information, you believe that searching everyone's living quarters will be the best place to start to get evidence for the case. As you make your way through the rooms, you notice each person has a small vial of canned food hidden in random places of their rooms. Whether it be on the nightstand, or in the closet, or in the vents, each room of a sibling or staff member has a small vial of old canned food in them. Upon searching the first few rooms, you think that maybe everyone likes always having a small snack in their rooms, but after finding a vial in all twelve rooms, you become suspicious. You decided to take a sample from each vial from each person's room to see what is inside all of them. You find out that each vial consisted of either fruits, green beans, seafood, spinach, tuna, mushrooms, beets, fish, or sausage. You find this to be a rather random and interesting array of food to keep in your bedroom, so your suspicion grew.

Part 3: Plan of Attack

Because you are fully confused with the vials, for a moment, you push the vials of food to the side and start to put your focus back on the body of the late King Julian. You decide to take a sample of his blood and run it through your bioinformatics tools to see if you can find anything interesting on the molecular level. After doing so you find out that King Julian's blood has traces of *Clostridium botulinum*. *Clostridium botulinum* is an anaerobic gram-positive spore-forming rod and is the most common cause implicated in reversible flaccid paralysis [1]. Other clostridial bacteria can also produce the toxin leading to botulism, a rare but serious illness caused by a toxin that attacks the body's nerves and causes difficulty breathing, muscle paralysis, and even death [1]. Despite its potential fatality and rarity, the illness can masquerade as other illnesses

making diagnosis difficult. Botulism can result from the consumption of foods contaminated with preformed botulinum toxin. The most common source is canned food [6].

The fact that the most common source of botulism is canned food raises a suspicion in your head since you found many vials of canned food in everyone's room in the royal palace. Now you decide to use your bioinformatics tools, at hand, and take an even closer look at a molecular level of the foods in the vials and compare them to the blood sample of the late King Julian (that shows the prevalence of *Clostridium botulinum*) to see if any connections can possibly be made.

You produce the following data using National Library of Health NCBI:

Step 1: Run King Julian's blood sample to see what it contains using NCBI:

KT036213.1 *Clostridium botulinum* strain E-RUSS 23S rRNA (uracil-5-)-methyltransferase (rumA) gene, partial cds

Step 2: Run all the vial samples (12) from everyone's room to see what it contains using NCBI and fill in the table with the name of organism (answers are filled in in red).

Table 1: Accession numbers of all vials of canned food in the rooms of the palace.

Sibling #1	CP070943.1	<i>Clostridium botulinum</i> strain ZJK-9 chromosome, complete genome
Sibling #2	AP024849.1	<i>Clostridium gelidum</i> DNA, complete genome
Sibling #3	CP004121.1	<i>Clostridium saccharoperbutylacetonicum</i> N1-4(HMT), complete genome
Sibling #4	CP004121.1	<i>Clostridium saccharoperbutylacetonicum</i> N1-4(HMT), complete genome
Sibling #5	CP016091.1	<i>Clostridium saccharobutylicum</i> strain NCP 258 chromosome, complete genome
Sibling #6	CP073279.1	<i>Clostridium beijerinckii</i> strain CBEI chromosome, complete genome
Wife	CP016786.1	<i>Clostridium isatidis</i> strain DSM 15098, complete genome
Maid	LN908213.1	<i>Clostridium beijerinckii</i> isolate C. beijerinckii DSM 6423 genome assembly, chromosome: I
Butler	CP016086.1	<i>Clostridium saccharobutylicum</i> strain NCP 200 chromosome, complete genome
Chauffeur	CP018624.1	<i>Clostridium chauvoei</i> strain DSM 7528 chromosome, complete genome
Secretary	CP043998.1	<i>Clostridium diolis</i> strain DSM 15410 chromosome, complete genome
Assistant	CP053893.1	<i>Clostridium beijerinckii</i> strain ASCUSDY20 chromosome, complete genome
Cook	CP016087.1	<i>Clostridium saccharoperbutylacetonicum</i> strain N1-504, complete sequence

Step 3: Now all you have to do is use the bioinformatics tools to deduce who has a vial of canned food that has the closest strain of *Clostridium botulinum* to the one that was found in King Julian’s bloodstream.

Step 4: You started by going to the NCBI home page (<http://www.ncbi.nlm.nih.gov/>), and clicked on the “nucleotide” link to search for the nucleotide sequences, as seen in the red box.

The screenshot shows the NCBI homepage with a dark blue header. The header includes the NIH logo, the text "National Library of Medicine National Center for Biotechnology Information", and a "Log in" button. Below the header is a search bar with a dropdown menu set to "All Databases" and a "Search" button. On the left is a vertical navigation menu with categories like "NCBI Home", "Resource List (A-Z)", "All Resources", "Chemicals & Bioassays", "Data & Software", "DNA & RNA", "Domains & Structures", "Genes & Expression", "Genetics & Medicine", "Genomes & Maps", "Homology", "Literature", "Proteins", "Sequence Analysis", "Taxonomy", "Training & Tutorials", and "Variation". The main content area is titled "Welcome to NCBI" and contains a paragraph about the center's mission, followed by six action cards: "Submit" (Deposit data or manuscripts into NCBI databases), "Download" (Transfer NCBI data to your computer), "Learn" (Find help documents, attend a class or watch a tutorial), "Develop" (Use NCBI APIs and code libraries to build applications), "Analyze" (Identify an NCBI tool for your data analysis task), and "Research" (Explore NCBI research and collaborative projects). Below these is a "COVID-19 Information" section with links to CDC, NIH, SARS-CoV-2 data, HHS, and Spanish resources. On the right is a "Popular Resources" section with links to PubMed, Bookshelf, PubMed Central, BLAST, Nucleotide (highlighted with a red box), Gene, SNP, Gene, Protein, and PubChem. Below that is an "NCBI News & Blog" section with several news items, including "Streamlining Access to SRA COVID-19 Datasets on the Cloud" (09 Mar 2023), "To make it easier for you to find and access Sequence Read Archive (SRA)", "3+ Ways NCBI is Enhancing the SRA Database" (08 Mar 2023), "Do you submit or access Sequence Read Archive (SRA) data? In an ongoing effort", "New & Improved NCBI Datasets Genome and Assembly Pages" (07 Mar 2023), and "Legacy pages will be redirected effective June 2023. In June 2023, NCBI's".

Step 5: You then searched for the accession number KT036213.1 and hit the “Search” button. This accession number represents the strain of bacteria found in King Julian’s bloodstream. This then brought you to the page for the “*Clostridium botulinum* strain E-RUSS 23S rRNA (uracil-5-)-methyltransferase (rumA) gene, partial cds” as seen below. <https://www.ncbi.nlm.nih.gov/nucleotide/KT036213.1>

The screenshot shows the NCBI Nucleotide database search results page. At the top, there is a navigation bar with the NIH logo and the text "National Library of Medicine National Center for Biotechnology Information". A search bar contains the accession number "KT036213.1" and a "Search" button. Below the search bar, there is a section titled "Nucleotide" with a description: "The Nucleotide database is a collection of sequences from several sources, including GenBank, RefSeq, TPA and PDB. Genome, gene and transcript sequence data provide the foundation for biomedical research and discovery." Below this, there are three columns of links: "Using Nucleotide" (Quick Start Guide, FAQ, Help, GenBank FTP, RefSeq FTP), "Nucleotide Tools" (Submit to GenBank, LinkOut, E-Utilities, BLAST, Batch Entrez), and "Other Resources" (GenBank Home, RefSeq Home, Gene Home, SRA Home, INSDC). At the bottom, there is a "FOLLOW NCBI" section with social media icons for Twitter, Facebook, LinkedIn, and RSS.

Step 6: Once the results loaded onto the page, you were then able to click the “FASTA” button at the top left of the page, as seen in the red box below. This opened a new window with the FASTA sequence which allowed you to build a tree with a Bioinformatic program.

Clostridium botulinum strain E-RUSS 23S rRNA (uracil-5-)-methyltransferase (rumA) gene, partial cds

GenBank: KT036213.1

FASTA [Graphics](#)

[Go to:](#)

LOCUS KT036213 663 bp DNA linear BCT 14-JUL-2015

DEFINITION Clostridium botulinum strain E-RUSS 23S rRNA (uracil-5-)-methyltransferase (rumA) gene, partial cds.

ACCESSION KT036213

VERSION KT036213.1

KEYWORDS .

SOURCE Clostridium botulinum

ORGANISM [Clostridium botulinum](#)
Bacteria; Bacillota; Clostridia; Eubacteriales; Clostridiaceae; Clostridium.

REFERENCE 1 (bases 1 to 663)

AUTHORS Weedmark,K.A., Mabon,P., Hayden,K.L., Lambert,D., Van Domselaar,G., Austin,J.W. and Corbett,C.R.

TITLE Phylogenomic profiling of Clostridium botulinum Group II isolates using whole genome sequence data

JOURNAL Appl. Environ. Microbiol. (2015) In press

PUBMED [26116673](#)

REMARK Publication Status: Available-Online prior to print

REFERENCE 2 (bases 1 to 663)

AUTHORS Weedmark,K.A., Mabon,P., Hayden,K.L., Lambert,D., Van Domselaar,G., Austin,J.W. and Corbett,C.R.

TITLE Direct Submission

JOURNAL Submitted (09-JUN-2015) National Microbiology Laboratory, Public Health Agency of Canada, 1015 Arlington Street, Winnipeg, Manitoba R3E3R2, Canada

COMMENT ##Assembly-Data-START##
Assembly Method : srst2 v. 2.1
Sequencing Technology : Illumina
##Assembly-Data-END##

FEATURES

source Location/Qualifiers

1..663
/organism="Clostridium botulinum"
/mol_type="genomic DNA"
/strain="E-RUSS"
/serotype="monovalent E"
/serovar="E1"
/db_xref="taxon:1491"
/note="non-proteolytic;
group: II"

[gene](#) <1..663
/gene="rumA"

[CDS](#) <1..663
/gene="rumA"
/note="RumA"
/codon_start=3
/transl_table=11
/product="23S rRNA (uracil-5-)-methyltransferase"
/protein_id="AKP63097.1"
/translation="EKQLQVLEDEIKDLFKEADVTTGEFLGVLGSSEQWEYRNKMEFT
FGDEEKGDLSIGMHMRGKSGFIMTVDHCKIVDEYRKIIRLTADYFGKQDLPYYRVM
KREGYLRHLVIRKAQNTGEILVNLVTTQIDFDLSEYVELLKSQDYKGLVSIHTEN
NSFSDAUIPEKINVLVYGRDYIQEKLLGLFKISPFFQTNTKGAESLYSLVRDFMGS
SE"

ORIGIN

```

1 atgaaaagca gttacaagtt ttagaagatg aaataaaaga ttattttaa gaagctgatg
61 taactactgg agagttttta ggagtacttg gaagctcaga acaatgggaa tatagaaata
121 agatggaatt tacatttggg gatgaagaaa aaggtggaga tctttcaatt ggaatgcata
181 tgagaggtaa atcttttggg ataatgcagc ttgatcattg taagatagtt gatgaagact
241 atagaaaaat aataagatta actgcagatt attttgaaa acaagattta ccatattatc
301 gagttatgaa aagagaaggg tatttaagac atcttgaat aagaaaagct caaatacag
361 gtgaaatatt agtaaatcct gttacaacta ctcaaataga ttttgattg agtgaatag
421 ttgaattact aaaatctcaa gactataagg gtacattagt atcaatacta catactgaaa
481 ataattcatt ctgagatgca gtaataccag aaaagataaa tgtattatat ggaagagatt
541 atatacaaga aaaattatta ggacttaatt ttaaaatttc accatttca tttttcaaa
601 ctaacacaaa aggtgcagaa agcctttatt cattagttag agattttatg ggaagttcag
661 aaa

```

Customize view

Analyze this sequence

Run BLAST

Pick Primers

Highlight Sequence Features

Find in this Sequence

Related information

Protein

PubMed

Taxonomy

Full text in PMC

Recent activity

[Turn Off](#) [Clear](#)

Clostridium botulinum strain E-RUSS 23S rRNA (uracil-5-)-methyltransferase (rumA) gene, partial cds Nucleotide

Vibrio cholerae strain Amazonia isolate 3E99 Nucleotide

Clostridium botulinum 14860 (0) Nucleotide

Clostridium botulinum BKT015925 plasmid Nucleotide

Clostridium Botulinum - StatPearls

[See more...](#)

Step 7: After the sequence had loaded, you navigated to the “BLAST” button on the right and run the blast which brought you to the selection page of the strains found in the vials of canned food in the thirteen rooms.

An official website of the United States government [Here's how you know](#)

NIH National Library of Medicine
National Center for Biotechnology Information Log in

Nucleotide Advanced Help

FASTA Send to: Change region shown Customize view

Clostridium botulinum strain E-RUSS 23S rRNA (uracil-5-)-methyltransferase (rumA) gene, partial cds

GenBank: KT036213.1 [GenBank](#) [Graphics](#)

>KT036213.1 Clostridium botulinum strain E-RUSS 23S rRNA (uracil-5-)-methyltransferase (rumA) gene, partial cds
ATGAAAAGCAGTTACAAGTTTTAGAAGATGAAATAAAAGATTTATTTAAAGAAGCTGATGTAAGTACTGG
AGAGTTTTAGGAGTACTTGGAAAGCTCAGAACATGGGAAATATAGAAATAAGATGGAAATTTACATTTGGT
GATGAAGAAAAGGTTGGAGATCTTCAATTGGAATGCATATGAGAGGTAATCTTTTGGAAATAAGACAG
TTGATCATTGTAAGATAGTTGATGAAGACTATAGAAAAAATAAAGATTAAGTAACTGCAGATATTTTGGAAA
ACAAGATTTACCATATTATCGAGTTATGAAAAGAGAAGGGTATTTAAGACATCTTGTAAATAAGAAAAGCT
CAAAAACAGGTGAAATATTAGTAAATCTTGTACAACTACTCAAATAGATTTTGATTTGAGTGAATATG
TTGAATTAATAAAATCTCAAGACTATAAGGGTACATTAGTATCAATACTACATACTGAAAAATAATCATT
CTCAGATGCAGTAATACCAGAAAAGATAAATGTATTATGGAAGAGATTATATACAAGAAAAATTATTA
GGACTTAATTTAAAATTTCAACATTTCAATTTTTCAAACAAACAAAAGGTGCAGAAAAGCCTTTATT
CATTAGTTAGAGATTTTATGGGAAGTTCAGAAA

Analyze this sequence Run BLAST Pick Primers

Related information Protein PubMed Taxonomy Full text in PMC

Recent activity Turn Off Clear

- Clostridium botulinum strain E-RUSS 23S rRNA (uracil-5-)-methyltransferase (r Nucleotide
- Vibrio cholerae strain Amazonia isolate 3509 pathogenicity island VPI-2, complet. Nucleotide
- Clostridium botulinum 14860 (0) Nucleotide
- Clostridium botulinum BKT015925 plasmid p1BKT015925, complete sequence Nucleotide

Step 8: Once the nucleotide blast page had opened, under “Choose Search Set” you selected “Standard databases” for the database and “Nucleotide collection from the database” dropdown menu. Under “Program Selection”, you selected the button to optimize for “Somewhat similar sequences”. Then you clicked the BLAST button.

Standard Nucleotide BLAST

blastn blastp blastx tblastn tblastx

BLASTN programs search nucleotide databases using a nucleotide query. more... Reset page Bookmark

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) [Clear](#) Query subrange [?](#)

KT036213.1 From To

Or, upload file Choose File no file selected [?](#)

Job Title

Enter a descriptive title for your BLAST search [?](#)

Align two or more sequences [?](#)

Choose Search Set

Database Standard databases (nr etc.): rRNA/ITS databases Genomic + transcript databases Betacoronavirus

Nucleotide collection (nr/nt) [?](#)

Organism Optional Enter organism name or id—completions will be suggested exclude [Add organism](#)

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown [?](#)

Exclude Optional Models (XM/XP) Uncultured/environmental sample sequences

Limit to Optional Sequences from type material

Entrez Query Optional [YouTube](#) [Create custom database](#)

Enter an Entrez query to limit search [?](#)

Program Selection

Optimize for

Highly similar sequences (megablast)

More dissimilar sequences (discontiguous megablast)

Somewhat similar sequences (blastn)

Choose a BLAST algorithm [?](#)

BLAST Search database Nucleotide collection (nr/nt) using Blastn (Optimize for somewhat similar sequences)

Show results in a new window

Step 9: You then selected the strains belonging to the vials (as answered in Table 1) and ran the “Distance Tree of Results” to obtain the phylogenetic tree.

Job Title
KT036213:Clostridium botulinum strain E-RUSS...

RID
[OY1KJXD9013](#) Search expires on 03-14 19:18 pm
[Download All](#) ▾

Program
 BLASTN ⓘ [Citation](#) ▾

Database
 nt [See details](#) ▾

Query ID
[KT036213.1](#)

Description
 Clostridium botulinum strain E-RUSS 23S rRNA (uracil-⁵- ...

Molecule type
 nucleic acid

Query Length
 663

Other reports
[Distance tree of results](#) [MSA viewer](#) ⓘ

Filter Results

Organism *only top 20 will appear*

Type common name, binomial, taxid or group name

+ [Add organism](#)

Percent Identity to E value to Query Coverage to

[Filter](#) [Reset](#)

Descriptions Graphic Summary Alignments Taxonomy

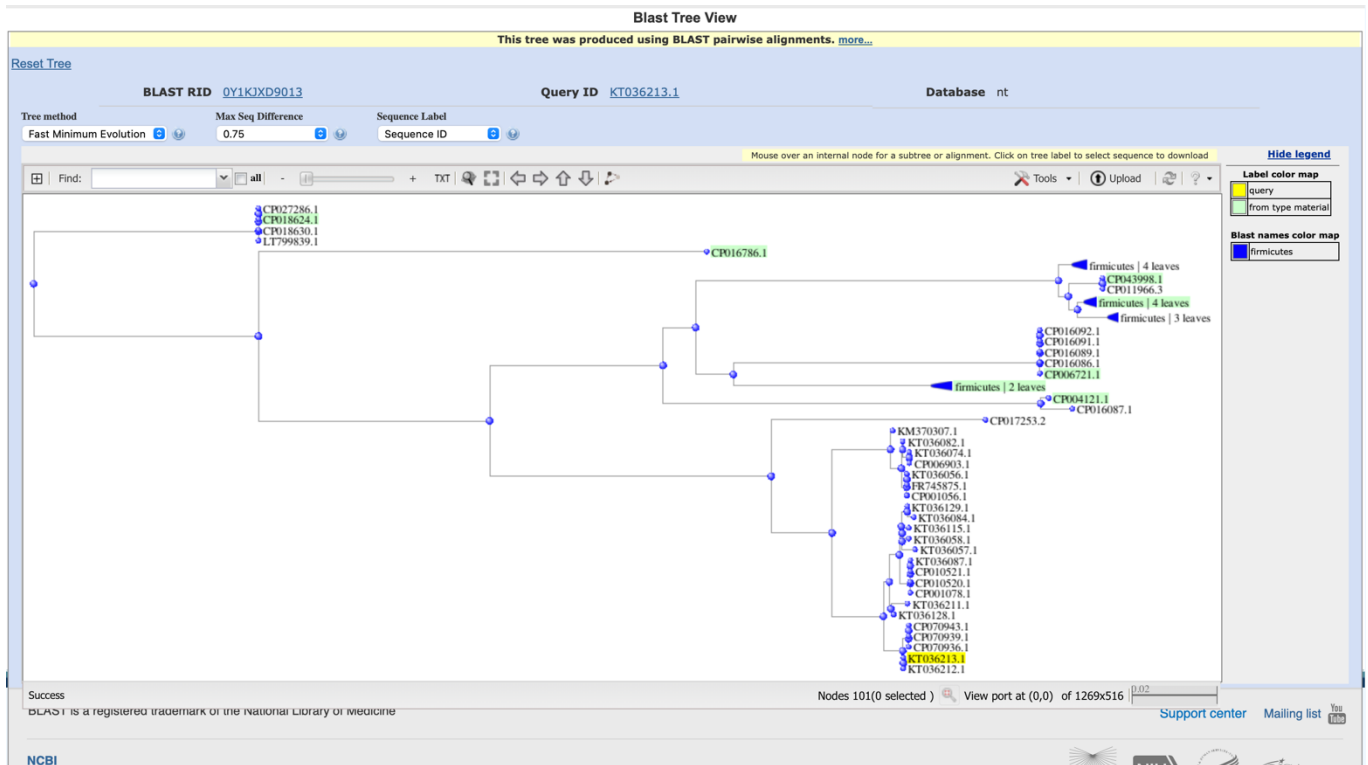
Sequences producing significant alignments Download ▾ Select columns ▾ Show 50 ▾ ⓘ

select all 14 sequences selected [GenBank](#) [Graphics](#) [Distance tree of results](#) [MSA Viewer](#)

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/>	Clostridium botulinum strain BFLY-1 23S rRNA (uracil-5-)-met...	Clostridiu...	1196	1196	100%	0.0	100.00%	663	KT036212.1
<input checked="" type="checkbox"/>	Clostridium botulinum strain ZJK-9 chromosome, complete g...	Clostridiu...	1192	1192	100%	0.0	99.85%	3780374	CP070943.1
<input type="checkbox"/>	Clostridium botulinum strain ZJK-8 chromosome, complete g...	Clostridiu...	1192	1192	100%	0.0	99.85%	3821175	CP070939.1
<input type="checkbox"/>	Clostridium botulinum strain ZJK-3 chromosome, complete g...	Clostridiu...	1192	1192	100%	0.0	99.85%	3780354	CP070936.1
<input type="checkbox"/>	Clostridium botulinum strain NCTC_11219 23S rRNA (uracil-...	Clostridiu...	1174	1174	100%	0.0	99.25%	663	KT036128.1
<input type="checkbox"/>	Clostridium botulinum strain 211_VH_Dolman 23S rRNA (ura...	Clostridiu...	1169	1169	100%	0.0	99.10%	663	KT036211.1
<input type="checkbox"/>	Clostridium botulinum strain F9508EPB 23S rRNA (uracil-5-);...	Clostridiu...	1165	1165	100%	0.0	98.94%	663	KT036087.1
<input type="checkbox"/>	Clostridium botulinum strain NCTC 8550, complete genome	Clostridiu...	1165	1165	100%	0.0	98.94%	3611898	CP010521.1
<input type="checkbox"/>	Clostridium botulinum strain NCTC 8266, complete genome	Clostridiu...	1165	1165	100%	0.0	98.94%	3611897	CP010520.1
<input type="checkbox"/>	Clostridium botulinum E3 str. Alaska E43, complete genome	Clostridiu...	1165	1165	100%	0.0	98.94%	3659644	CP001078.1
<input type="checkbox"/>	Clostridium botulinum strain BE9708E1 23S rRNA (uracil-5-);...	Clostridiu...	1160	1160	100%	0.0	98.79%	663	KT036129.1
<input type="checkbox"/>	Clostridium botulinum strain FE0201E1BC 23S rRNA (uracil-...	Clostridiu...	1160	1160	100%	0.0	98.79%	663	KT036115.1
<input type="checkbox"/>	Clostridium botulinum strain FWSK02-02E1 23S rRNA (uracil...	Clostridiu...	1160	1160	100%	0.0	98.79%	663	KT036058.1
<input type="checkbox"/>	Clostridium botulinum strain FWSK02-05E1 23S rRNA (uracil...	Clostridiu...	1156	1156	100%	0.0	98.64%	663	KT036084.1
<input type="checkbox"/>	Clostridium botulinum strain E1_Dolman 23S rRNA (uracil-5-);...	Clostridiu...	1156	1156	100%	0.0	98.64%	663	KT036057.1
<input type="checkbox"/>	Clostridium botulinum strain 84-10 RNA methyltransferase ge...	Clostridiu...	1106	1106	100%	0.0	96.98%	663	KM370307.1
<input type="checkbox"/>	Clostridium botulinum strain BFLY-2 23S rRNA (uracil-5-)-met...	Clostridiu...	1097	1097	100%	0.0	96.68%	663	KT036082.1

<input type="checkbox"/>	Clostridium botulinum strain FWSK02-05E1 23S rRNA (uracil-5-...)	Clostridiu...	1156	1156	100%	0.0	98.64%	663	KT036084.1
<input type="checkbox"/>	Clostridium botulinum strain E1_Dolman 23S rRNA (uracil-5-)...	Clostridiu...	1156	1156	100%	0.0	98.64%	663	KT036057.1
<input type="checkbox"/>	Clostridium botulinum strain 84-10 RNA methyltransferase ge...	Clostridiu...	1106	1106	100%	0.0	96.98%	663	KM370307.1
<input type="checkbox"/>	Clostridium botulinum strain BFLY-2 23S rRNA (uracil-5-)-met...	Clostridiu...	1097	1097	100%	0.0	96.68%	663	KT036082.1
<input type="checkbox"/>	Clostridium botulinum strain Eklund_202F 23S rRNA (uracil-5-...)	Clostridiu...	1093	1093	100%	0.0	96.53%	663	KT036074.1
<input type="checkbox"/>	Clostridium botulinum strain Eklund_2B 23S rRNA (uracil-5-)-...	Clostridiu...	1093	1093	100%	0.0	96.53%	663	KT036056.1
<input type="checkbox"/>	Clostridium botulinum 202F, complete genome	Clostridiu...	1093	1093	100%	0.0	96.53%	3874462	CP006903.1
<input type="checkbox"/>	Clostridium botulinum B str. Eklund 17B(NRP), complete chro...	Clostridiu...	1093	1093	100%	0.0	96.53%	3781509	FR745875.1
<input type="checkbox"/>	Clostridium botulinum B str. Eklund 17B, complete genome	Clostridiu...	1093	1093	100%	0.0	96.53%	3800327	CP001056.1
<input type="checkbox"/>	Clostridium taeniosporum strain 1/k chromosome, complete g...	Clostridiu...	966	966	100%	0.0	92.31%	3264813	CP017253.2
<input checked="" type="checkbox"/>	Clostridium saccharoperbutylaceticum N1-4(HMT), comple...	Clostridiu...	628	628	98%	3e-175	81.32%	6530257	CP004121.1
<input checked="" type="checkbox"/>	Clostridium saccharoperbutylaceticum strain N1-504, com...	Clostridiu...	606	606	98%	4e-168	80.55%	6216458	CP016087.1
<input checked="" type="checkbox"/>	Clostridium beijerinckii strain ASCUSDY20 chromosome, co...	Clostridiu...	592	592	98%	2e-164	80.09%	5984367	CP053893.1
<input type="checkbox"/>	Clostridium beijerinckii strain ASCUSBR67 chromosome	Clostridiu...	592	592	98%	2e-164	80.09%	5966646	CP070895.1
<input type="checkbox"/>	Clostridium beijerinckii strain BAS/B3/I/124 chromosome, co...	Clostridiu...	588	588	98%	1e-162	79.94%	6123550	CP016090.1
<input type="checkbox"/>	Clostridium beijerinckii ATCC 35702, complete genome	Clostridiu...	588	588	98%	1e-162	79.94%	5999050	CP006777.1
<input type="checkbox"/>	Clostridium beijerinckii strain DSM 791 chromosome, comple...	Clostridiu...	588	588	98%	1e-162	79.94%	5876902	CP073653.1
<input checked="" type="checkbox"/>	Clostridium beijerinckii strain CBEI chromosome, complete g...	Clostridiu...	588	588	98%	1e-162	79.94%	6008343	CP073279.1
<input type="checkbox"/>	Clostridium beijerinckii NCIMB 8052, complete genome	Clostridiu...	588	588	98%	1e-162	79.94%	6000632	CP000721.1
<input checked="" type="checkbox"/>	Clostridium diolis strain DSM 15410 chromosome, complete ...	Clostridiu...	583	583	98%	1e-161	79.79%	5940808	CP043998.1
<input type="checkbox"/>	Clostridium beijerinckii NRRL B-598, complete genome	Clostridiu...	583	583	98%	1e-161	79.79%	6186993	CP011966.3
<input checked="" type="checkbox"/>	Clostridium beijerinckii isolate C. beijerinckii DSM 6423 geno...	Clostridiu...	579	579	98%	5e-160	79.63%	6383364	LN908213.1
<input type="checkbox"/>	Clostridium saccharobutylicum strain NCP 195 chromosome, ...	Clostridiu...	579	579	98%	5e-160	79.63%	5108176	CP016092.1
<input checked="" type="checkbox"/>	Clostridium saccharobutylicum strain NCP 258 chromosome, ...	Clostridiu...	579	579	98%	5e-160	79.63%	4950933	CP016091.1
<input type="checkbox"/>	Clostridium saccharobutylicum strain BAS/B3/SW/136 chrom...	Clostridiu...	579	579	98%	5e-160	79.63%	5108304	CP016089.1
<input checked="" type="checkbox"/>	Clostridium saccharobutylicum strain NCP 200 chromosome, ...	Clostridiu...	579	579	98%	5e-160	79.63%	5108287	CP016086.1
<input type="checkbox"/>	Clostridium beijerinckii strain CloBei18h chromosome, compl...	Clostridiu...	579	579	98%	5e-160	79.63%	6292543	CP107022.1
<input checked="" type="checkbox"/>	Clostridium saccharobutylicum DSM 13864, complete genome	Clostridiu...	579	579	98%	5e-160	79.63%	5107814	CP006721.1
<input type="checkbox"/>	Clostridium sp. MF28 chromosome	Clostridiu...	574	574	98%	6e-159	79.48%	6148198	CP014331.1
<input type="checkbox"/>	Clostridium beijerinckii strain NCIMB 14988 chromosome, co...	Clostridiu...	570	570	98%	3e-157	79.33%	6485394	CP010086.2
<input checked="" type="checkbox"/>	Clostridium gelidum DNA, complete genome	Clostridiu...	565	565	98%	3e-156	79.17%	6041432	AP024849.1
<input type="checkbox"/>	Clostridium beijerinckii isolate WB53 chromosome, complete ...	Clostridiu...	558	558	98%	5e-154	78.90%	4258077	CP029329.1
<input type="checkbox"/>	Clostridium chauvoei strain SBP 07/09 chromosome, complet...	Clostridiu...	542	542	98%	4e-149	78.59%	2883701	CP027286.1
<input checked="" type="checkbox"/>	Clostridium chauvoei strain DSM 7528 chromosome, complet...	Clostridiu...	542	542	98%	4e-149	78.59%	2872664	CP018624.1
<input type="checkbox"/>	Clostridium chauvoei strain 12S0467, complete genome	Clostridiu...	542	542	98%	4e-149	78.59%	2885628	CP018630.1
<input type="checkbox"/>	Clostridium chauvoei JF4335 genome assembly, chromosom...	Clostridiu...	542	542	98%	4e-149	78.59%	2887451	LT799839.1
<input checked="" type="checkbox"/>	Clostridium isatidis strain DSM 15098, complete genome	Clostridiu...	534	534	98%	2e-146	78.10%	2869927	CP016786.1

Step 10: You observed the phylogenetic tree created and analysed the results.



It can be seen in the tree that the yellow highlighted strain is the strain found in King Julian's bloodstream which contains *C. botulinum*. The closest branch in the tree to the yellow highlighted strain represents the sample that was closest to the strain found in King Julian's blood. Therefore, the strain closest to the yellow highlighted one is the vial from which King Julian was most likely infected from.

Part 4: The Culprit

Because the sample with the accession number CP070943.1 was closest to the strain found in King Julian's blood, by using the table you made from earlier, you can determine that the vial came from Sibling #1's room. In other words, King Julian's younger brother Prince Noah's room.

You then interrogate Prince Noah as to why he has the vial and what he did with it. Prince Noah confesses that with the help of the cook, he had been adding pieces of the fermented canned fish from his room into King Julian's food.

C. botulinum grows best under low-oxygen conditions and produces spores and toxins [4]. The toxin is most commonly formed when food is improperly processed (canned) at home. In this case, the fish was improperly canned at home. The toxins formed were then added to King Julian's food. The toxin can then attack the body's nerves and causes difficulty breathing, muscle paralysis, and even death, which lead to King Julian's death in his sleep [2]. By adding this toxin to King Julian's food and thus killing him would lead to the succession of Prince Noah to the

throne. His lifelong dream of getting out of his brother's shadow and into the limelight would happen. By killing his brother, he wanted power.

Part 5: Some Questions to Guide you

1. Draw a quick sketch of the resulting tree, and briefly describe it.
 - a. Answer can be found within the document
2. How does this tree support that Prince Noah was responsible for King Julian's death?
 - a. Answer can be found within the document
3. Why did the forensics team mistake botulism to Guillain-Barre syndrome (GBS)?
 - a. Both GBS and botulism are medical emergencies and can result in death from respiratory muscle paralysis or complications of dysautonomia. Botulism can cause death by paralyzing the muscles people use to breathe. Interruption of pulmonary gas exchange for > 5 minutes may irreversibly damage vital organs, especially the brain. Cardiac arrest almost always follows unless respiratory function is rapidly restored [5].

Part 6: Importance of Bioinformatics

From this case study, I hope you understood the value of bioinformatics and got to see a practical use of it. Bioinformatics is a multidisciplinary field that applies computational techniques and methods to solve problems related to biology, genetics, and medicine. The importance of bioinformatics lies in its ability to process and analyze vast amounts of biological data, which has become increasingly important as the amount of biological data being generated continues to grow exponentially.

Here are some ways in which bioinformatics is important:

- *Advancing medical research:* Bioinformatics plays a critical role in advancing medical research by helping researchers to better understand the genetic basis of diseases and to identify potential drug targets.
- *Improving agriculture:* Bioinformatics can be used to study the genetics of crops and livestock, helping to develop new varieties that are more resistant to disease and better suited to specific environments.
- *Enhancing drug discovery:* Bioinformatics can be used to identify potential drug targets and to predict how different drugs will interact with specific proteins and molecules in the body.
- *Understanding evolution:* Bioinformatics can be used to analyze genetic data from different species, providing insights into the evolutionary relationships between them.

References:

- [1] Centers for Disease Control and Prevention. (2021, June 1). *About botulism*. Centers for Disease Control and Prevention. Retrieved March 13, 2023, from <https://www.cdc.gov/botulism/general.html>
- [2] Centers for Disease Control and Prevention. (2022, June 8). *Botulism*. Centers for Disease Control and Prevention. Retrieved March 13, 2023, from <https://www.cdc.gov/botulism/index.html>
- [3] Centers for Disease Control and Prevention. (2023, February 6). *GBS (Guillain-Barré Syndrome) and vaccines*. Centers for Disease Control and Prevention. Retrieved March 13, 2023, from <https://www.cdc.gov/vaccinesafety/concerns/guillain-barre-syndrome.html>
- [4] *Food Safety and Inspection Service*. Clostridium botulinum & Botulism | Food Safety and Inspection Service. (n.d.). Retrieved March 13, 2023, from <https://www.fsis.usda.gov/food-safety/foodborne-illness-and-disease/pathogens/clostridium-botulinum>
- [5] Moll, V. (2023, February 14). *Overview of respiratory arrest - critical care medicine*. Merck Manuals Professional Edition. Retrieved March 13, 2023, from <https://www.merckmanuals.com/professional/critical-care-medicine/respiratory-arrest/overview-of-respiratory-arrest>
- [6] U.S. National Library of Medicine. (n.d.). *Home - books - NCBI*. National Center for Biotechnology Information. Retrieved March 13, 2023, from <https://www.ncbi.nlm.nih.gov/books>
- [7] Awasthi, A. (2023, February 16). *It's official, Pablo Neruda was murdered by Poison*. Firstpost. Retrieved March 13, 2023, from <https://www.firstpost.com/world/its-official-pablo-neruda-was-murdered-by-poison-12157542.html>