Practice

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New E.Coli strain, carrying Shiga toxin genes and antibiotic resistance genes.

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Abstract

In April 2011, in Germany, E.coli outbreak have occurred. It was causing hemolytic uremic syndrome (HUS), kidney failure and death. Bean sprouts, tainted by the new E.Coli strain were the reason. This novel strain had derived from enteroaggregative E. coli (EAEC) 55989 strain, harboring the pAA plasmid, which contains aggregative adherence fimbria (AAF) genes allowing bacteria to stick to cells in the intestine. Also it carries Shiga toxin genes (stxA and stxB) from Shigella dysenteriae and multiple antibiotic resistance genes (β-lactamases, macrolides, aminoglycoside, etc.).

Keywords: E.Coli strain, shiga toxin, antibiotic resistance, hemolytic uremic syndrome (HUS)

Introduction

Hemolytic–uremic syndrome (HUS) is a group of blood disorders characterized by low red blood cells, acute kidney failure, and low platelets. Initial symptoms typically include bloody diarrhea, fever, vomiting, and weakness. Kidney problems and low platelets then occur as the diarrhea progresses. S. pneumoniae, Shigella, Salmonella, and certain medications can cause it, as well as the novel E.Coli strain which was studied in this project. The underlying mechanism typically involves the production of Shiga toxin by the bacteria. (Cody and Dixon 2019)
Shigella grows well in most of the same culture media as E. coli, as they are from the same family (Enterobacteriaceae). Some genes can transfer between their genomes, so Shiga toxin genes were obtained by E.Coli from Shigella. (Payne 2019)
Also the strain obtained antibiotic resistance via horizontal gene transfer(HGT). HGT is the primary mechanism for the spread of antibiotic resistance in bacteria. It often involves temperate bacteriophages and plasmids. Genes can be transferred to another species of bacteria through various mechanisms of HGT such as transformation, transduction and conjugation. Conjugation in E. coli, requires stable and extended contact between a donor and a recipient strain, is DNase resistant, and the transferred DNA is incorporated into the recipient chromosome by homologous recombination. (Gray *et al.* 2013)
Since bacterial genomes undergo rearrangements, various regions can be rearranged, even between two closely related bacterial species. (**?**) Reference-guided de novo assembly approach improves genome reconstruction for related species. It identifies structural variants and complex rearrangements, such as deletions, inversions, or translocations. That is why it was important to assemble the new strain genome sequence de novo.

Materials and methods

Sequencing results of the strain were obtained from NCBI Sequence Read Archive (SRA). The data is published there with labels SRR292678, SRR292862, SRR292770 respectively. Quality control of reads was performed with FastQC utility (Andrew 2019), FastQC showed that sequences are good in quality.
The read correction and assembly were performed by assembler SPAdes (Alekseyev 2011). QUAST (Gurevich 2013) was used to evaluate the overall quality of an assembly. Assemble with SRR292678 as a paired ends, SRR292862 and SRR292770 as a mate pairs was made, according to QUAST: N50 = 335515 with 105 contigs.
Prokka was used for the annotation. This tool identifies the coordinates of putative genes within contigs and then uses BLAST (Boratyn *et al.* 2019) for similarity-based annotation using all proteins from sequenced bacterial genomes in the RefSeq database.
Using 16S ribosomal RNA sequence we ran NCBI BLAST (Sayers *et al.* 2022) to find the closest relatives of our novel strain. It appeared to be the HEp-2 adherent E. coli (EAEC). To locate 16S rRNA in the assembled E. coli strain genome Barrnap (Seemann and Booth 2013) was used.
EAEC can't cause HUS though, so Mauve (Sczyrba *et al.* 2017) was used to perform the search of another virulence factor - Shiga toxin genes (stxA and stxB) from Shigella dysenteriae. Also various antibiotic resistance genes were found via ResFinder (Florensa *et al.* 2022). Strain is resistant to beta-lactam, sulfamethoxazole, trimethoprim, tetracycline, streptomycin, etc. These genes were obtained via HGT.

Results

The de novo assembly was made via SPAdes in the paired-end mode, providing paired reads of E. coli X. from the library

Table 1 Antibiotic resistance

Antimicrobial	**Class**	**Genetic background**	
aztreonam	beta-lactam	blaCTX-M-15	(blaCTX-M-15$_A$Y044436)
ceftazidime	beta-lactam	blaCTX-M-15	(blaCTX-M-15$_A$Y044436)
cefotaxime	beta-lactam	blaCTX-M-15	(blaCTX-M-15$_A$Y044436)
ceftriaxone	beta-lactam	blaCTX-M-15	(blaCTX-M-15$_A$Y044436)
cephalothin	beta-lactam	blaTEM-1B	(blaTEM-1B$_A$Y458016)
cefepime	beta-lactam	blaTEM-1B	(blaCTX-M-15$_A$Y044436)
ampicillin	beta-lactam	blaTEM-1B	(blaTEM-1B$_A$Y458016)
		blaCTX-M-15	(blaCTX-M-15$_A$Y044436)
ticarcillin	beta-lactam	blaTEM-1B	(blaTEM-1B$_A$Y458016)
		blaCTX-M-15	(blaCTX-M-15$_A$Y044436)
piperacillin	beta-lactam	blaTEM-1B	(blaTEM-1B$_A$Y458016)
		blaCTX-M-15	(blaCTX-M-15$_A$Y044436)
amoxicillin	beta-lactam	blaTEM-1B	(blaTEM-1B$_A$Y458016)
		blaCTX-M-15	(blaCTX-M-15$_A$Y044436)
sulfamethoxazole	folate pathway antagonist	sul1	(sul1$_U$12338)
		sul2	
trimethoprim	folate pathway antagonist	dfrA7	(dfrA7$_A$B161450)
tetracycline	tetracycline	tet(A)	(tet(A)$_A$J517790)
doxycycline	tetracycline	tet(A)	(tet(A)$_A$J517790)
streptomycin	aminoglycoside	aph(3")-Ib	(aph(3")-Ib$_A$F321551)
		aph(6)-Id	(aph(6)-Id$_M$28829)

SRR292678. After checking the quality of this assemble with QUAST it was decided to work with more libraries in order to improve the quality of the assemble (N50 = 111860, 210 contigs). Assemble with SRR292678 as a paired ends, SRR292862 and SRR292770 as a mate pairs was made, and the result appeared to be much better according to QUAST: N50 = 335515 with 105 contigs. Contigs became longer which improved our assembly. For the annotation the variant with threee libraries was used.
80 tRNAs, 0 rRNAs, 1 CRISPR, 5064 CDS, 2923 Unique gene codes were found in the genome. A few 16S rRNA coding genes were found, all are the same 1537 nucleotide length.
After running BLAST on 16S rRNA the relative E.Coli strains were found on NCBI. The most similar strain appeared to be enteroaggregative E. coli (EAEC) strain. EAEC strain didn't cause HUS though, so there is an additional factor, causing internal bleeding. StxA and stxB genes were found in the genome. They are responsible for creating A and B subunits of Shiga toxin. The sequence matches Shigella dysenteriae genes according to Mauve (which uses BLAST for checking this information).
The strain gained resistance to multiple antibiotics (see table 1) according to ResFinder.

Discussion

Our novel E.coli strain has some enteroaggregative E. coli 55989 (EAEC or EAggEC) properties, and it seems like it was derived from EAEC. It was checked using 16S rRNA sequence, as this sequense is one of the most conservative sections in bacterial genomes. Other sites can be changed or transferred easily. HEp-2 adherent E. coli (EAEC) usually carries adherent (LA), aggregative adherent (AA), or diffuse adherent (DA) patterns. (Mossoro *et al.* 2022) Most probably these virulent factors are present in the new strain as well.
The new strain is antibiotic resistant as well. That might've contribute to the spread of the disease and complicate the treatment process. There are possible antibioticts we can use against this strain though, for instance, rifampicin or vancomycin. At the same time it is important to comply with treatment and use antibiotics correctly.
Resistance to β-lactam antibiotics is caused by bla_1 and bla_2 genes. They have a β-lactamase activity, so they degrade almost every antibiotic compound of this class.(Chen and Succi 2003)
Antibiotic resistant genes could get into genome gradually, from different strains. Such a resistance could be gained by a strain, which was developing in the antibiotic presence, it could be human or farm animals' guts. Feces from contaminated organisms could get into soil as a fertilizer or simply as waste. Bean sprouts usually don't contact with soil, but they are grown in unsterile and humid environment, E.coli could easily get there from unwashed hands and multiply. That is only one of possible scenario.
Shigella grows well in most of the same culture media as E. coli,

as they are from the same family (Enterobacteriaceae). (Shelley 2019) Most probably it happened inside some organism, where both pathogenic bacterias were present. Some genes can transfer between their genomes, so Shiga toxin genes were obtained by E.Coli from Shigella.

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