Hemolytic uremic syndrome associated with *E. coli*

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Abstract

Hemolytic uremic syndrome is a group of blood disorders characterized by low concentration of erythrocytes, acute kidney failure, and low concentration of platelets. Initial symptoms typically include bloody diarrhea, fever, vomiting, and weakness. This disorders associated with high risk of death. In this particular case, E. coli provides non-common extrachromosomal genetic elements, which, at the end-point provides new extracellular toxin.

Purpose

Estimate pathogenic mechanism that involves E.coli strain and responsible for hemolytic uremic syndrome.

Tasks

Investigate genome sequence; define E.coli strain; build genome map of toxic E.coli; find genes that make this strain of E.coli distinct; propose mechanism of obtaining those genes;

Results

Pathogenic mechanism involves phage transduction, which prodvids Shiga toxin gene, and antibiotic resistance gene in particular for beta-lactam antibiotics.

Most strains of *E. coli* are not harmful and are part of the healthful bacterial flora in the human gut. E. coli is a Gram-negative, facultative anaerobic, coliform bacterium of the genus Eschericha [1]. The relationship between E. coli and the host should be defined as commensalism, in which one of the two organisms benefits from the interaction between them, whereas the other is neither notably harmed nor helped [2]. Horewer, some E.Coli strains also known as a widespread pathogen that kills 2 millions people per year [3]. This diversity relied on the ability to transfer DNA via bacterial conjugation or transduction, which allows genetic material to spread horizontally through an existing population [4]. Transduction caused by bacteriophage, which transfers a viral gene from one bacterium to another during his life cycle. This is possibly cause bacteriophage transmit his own genome into bacterial genome and replicates it as a plasmid or during site-specific recombination inserts his genome into host DNA. Thats how non phathogenic E.Coli could obtain toxins like Shiga toxin. Shiga toxins are a family of related toxins with two major groups, Stx1 and Stx2, expressed by genes considered to be part of the genome of lambdoid prophages [5]. The toxin is effective against small blood vessels, such as found in the digestive tract, lungs, and especially kidneys, where the toxin affects on the vascular endothelium of the glomerulus. The purpose of the study was estimation of the pathogenic mechanism that involves E.coli strain and responsible for hemolytic uremic syndrome.

Materials and methods.

Three libraries of the sequencing data of the isolate from the girl in Hamburg, how suffered from Hemolytic uremic syndrome: SRR292678 - paired end [6], SRR292862 – mate pair[7], SRR292770 – mate pair[8]. For quality and basic statistics of reads we used FastQC [9]. For genome assembling processe we used Spades assembler[10] with -1, -2 keys. We considered N50 as quality metric for this analysis. For gene prediction and anotation we used Prokka [11] with --centre X key. To achieve information about closest relatives of the pathogenic strain we used approach, that treats 16S ribosomal RNA as marker for closeness because of its high importance and evolutionarily conservancy. For 16S rRna location estimation and prediction we used Barrnap[12]. For nucleotide blasting we used 1900/01/01:2011/01/01[PDAT] as “Entrez Query” for filtering for the genomes which are presented at the beginnig of 2011. We

treated closest relative as a reference genome [13]. For genome comparison we used Mauve [14] with GUI interface.

Results

Assembling analysis was performed with QUAST v 5.0.2, statistics shown in the Table 1.

Table 1 – QUAST statistics

Statistics	SRR292678-contigs	SRR292678-scaffolds	Three libraries - contigs	Three libraries - scaffolds
N50	105346	105346	335515	2815616
Number of contigs	519	501	369	327

Result of assembled genome annotation is summarized in Table 2.

Prokka annotation statistics

Genome element	Quantity
Contigs	205
Bases	5424432
CDS	5064
gene	5144
tRNA	79
tmRNA	1

We have discovered 6 matches of 16S rRNA.

Table 3 – 16S rRNA location and size

Node ID	Start	End	Strand
565907	111955	113492	+
565849	46	1583	-
565899	43835	45372	+
565903	36481	38018	+
565879	42579	44116	-
565845	46	1583	-

Reference search

For the following analysis we chose first sequence from the table. Search of reference 16S rRNA was executed using BLAST and RefSeq database. The closest relative genome was *. coli 55989* (NC_011748.1).

When exploring alignment we found StxA and StxB genes, coding the subunits of Shiga toxins, nearby genes are marked as phage. We also tested the resistance of the strains in ResFinder and found out, that *E. coli X* is resistant to sulphonamide, trimethoprim, tetracycline, aminoglycoside and beta-lactam antibiotics, while reference strain is only tetracycline resistant. Next, we checked for the presence of a beta-lactamase gene in our strain and found several genes, one of which is located between phage genes and mobile elements.

Discussion

Thus *E. coli X* strain become pathogenic as it acquired genes encoding subunits of Stx2 shiga toxin, which causes HUC. These genes are surrounded by phage genes, so we can safely assume that they got there as a result of phage transduction. Also, our strain is resistant to several types of antibiotics, in contrast to the reference, and we found gene encoding beta-lactamase, which destroys beta-lactam antibiotics. Fragments of the phage genome are also located near the beta-lactamase gene. All of it raises a big evidence of phage transduction.

Patients infected with this strain can be treated with nitroimidazole, fosfomycin, fusidicacid, rifampicin, phenicol, glycopeptide, oxazolidinone, colistin or quinolone according to ResFinder results.

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