RESEARCH NOTE

Pangenome guided pharmacophore modelling of enterohemorrhagic *Escherichia coli* *sdiA* [version 1; peer review: 1 approved with reservations, 1 not approved]

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Abstract

Enterohemorrhagic *Escherichia coli* (EHEC) continues to be a significant public health risk. With the onset of next generation sequencing, whole genome sequences are a potential resource for predictive modelling of the different regulatory mechanism of pathogens, particularly quorum sensing. We used a pangenome approach to determine EHEC genome clustering, determine the synonymous and nonsynonymous mutations across the EHEC *sdiA* and modelled the associated amino acid changes. Across the EHEC population, nonsynonymous variants are notably absent in ligand binding site for quorum sensing, indicating that population wide conservation of *sdiA* ligand site can be targeted for potential prophylactic purposes. Applying pathotype-wide pangenomics as a guide for determining evolution of pharmacophore sites is a potential approach in drug discovery.

Keywords

pangenome, pharmacophore, EHEC, *Escherichia coli*

Open Peer Review

Approval Status

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(revision)		
01 Sep 2020		view
version 2		
(revision)	view	view
01 Oct 2019		
version 1		
09 Jan 2019	view	view

1. **Kerry K. Cooper** , University of Arizona, Tucson, USA
2. **Olivier Tenaillon**, French Institute of Health and Medical Research (INSERM), Paris, France

Any reports and responses or comments on the article can be found at the end of the article.

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Author roles: Bandoy DD: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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Introduction

One of the more prominent strain of *Escherichia coli* is the enterohemorrhagic (EHEC) pathotype associated with global outbreaks of bloody diarrhea and hemolytic uremic syndrome¹. EHEC is one of the classical examples of one health disease as the interface of animal health spills into human health. Within the cattle reservoir, *sdiA* gene is required by *E. coli* to survive the acidic rumen environment. SdiA is used by *E. coli* to sense acyl homoserine in a quorum sensing system². However, it is considered as an orphan as the cognate acyl homoserine synthase is absent, and hence *sdiA* is considered an environmental sensor to sense the nearby microbial community. SdiA is stabilized by acyl homoserine lactone and acts as transcription factor glutamate decarboxylase needed for survival in the acidic environment. Hence blocking the ability of EHEC to survive the acidic ruminal environment is a proposed mechanism to control shedding in the cattle reservoir.

Whole genome sequencing of bacterial pathogens, particularly EHEC, is quickly transforming the workflows of epidemiological investigations. However, most bioinformatic pipelines used in clinical investigation use data reduction of genomes and artificially reducing diversity due to comparison of a limited number of housekeeping genes³. While wgMLST attempts to increase the number of genes, the assignment of a single reference genome appears to be inadequate in light of the pangenome. Various studies have shown that a significant number of genes that are present to the entire universe of genes within a species is missed for variant calling if only a single reference gene is used⁴. In this study we applied multi-scale approach to generate genome wide clustering using pangenome phylogeny using genome wide gene presence absence variation (PAV). We used this genome wide clusters as guide in generating the pharmacophore of *sdiA* as potential design strategy to control shedding by reducing the acid survival in the rumen.

We applied the concept of the pangenome, which represents the entirety of the genes that are present within a species, to a pathotype level. The EHEC pangenome represents the combination of genes seen in the EHEC pathotype. While the pangenome of *E. coli* was published in 2008 contained 8 genomes, we generated an updated EHEC pangenome with 153 genomes. The pangenome enables clustering of isolates using gene presence and absence. We used the genome wide comparison to generate clusters and genomic clade specific pharmacophore model of *sdiA*. This strategy enables to capture the pangenome wide variation of *sdiA* and ensures all conserved variants are targeted by the drug discovery pipeline enabling a pangenome to pharmacophore approach.

Methods

EHEC population

Whole genome sequences with the associated EHEC metadata was downloaded from Patric Database 3.5.28 using the keyword search for *E. coli* as organism and EHEC as the pathotype within the *E. coli* species^{5,6}. This resulted to 196 results and 152 of which are closed genome and draft genomes while the remaining sequences are EHEC associated plasmids (Underlying data: Metadata from Patric Database of EHEC *E. coli* pangenome⁷).

EHEC pangenome

The genomes were annotated with Prokka 1.13.3 as per published protocol⁸. Gff files were extracted as input for the pangenome pipeline Roary 3.11.2 using the following parameters for not splitting paralogs (roary -s -p 32 *.gff) and the resulting presence absence matrix (Underlying data: EHEC *E. coli* pangenome presence absence matrix⁷) together with the accessory genome phylogeny visualized in Phandango 1.3.0 and is represented as Figure 1B^{9,10}. Each blue bar represents an individual gene and solid blue blocks represent gene clusters. The accessory genome-based phylogeny newick file was visualized using iTol 4.3¹¹⁻¹³.

Allelic variant calling

Snippy variant calling pipeline 4.3.5 was used to determine the synonymous and nonsynonymous protein mutations using *sdiA* of *Escherichia coli* O157:H7 str. Sakai as reference. The -contigs option was added to the standard commandline (snippy -outdir -ref *sdiA_sakai.gbk*). The resulting individual variants of *sdiA* was merged into EHEC *E. coli* *sdiA* variant calling data (Underlying data⁷).

In silico *sdiA* protein modelling

SdiA genes were extracted from the pangenome output of Roary and protein in silico modelling performed using SWISS-MODEL¹⁴⁻¹⁸. *SdiA* protein sequences were used as targets to search for protein templates within the SWISS-MODEL library. Model selection was based on the template with the highest quality prediction by the target-template alignment.

Results and discussion

Three main genomic clades were generated using the pangenome PAV of genes (Figure 1A). Clade I includes the genomes isolated from the earliest reported outbreak in 1982 as well as the more recent cases. Clade II includes genomes from European (predominantly Czech and German) enterohemorrhagic *E. coli* isolates (EHEC) belonging to the O26 serotype, while Clade III includes cluster of cases associated with acute renal failure in children. Clade III is associated with hybrid pathotype of serogroup O80 has aside from Shiga toxin, an extra-intestinal virulence plasmid (pS88), is currently emerging in France. Specific gene clusters are found in cluster I and noticeably absent in cluster III, particularly prophages and associated genes (Underlying data: Table 2, Figure 1B). This indicates that specific genome wide gene presence and absence can be used for functional cluster instructive of the underlying epidemiological dynamics. The acquisition of genomic islands unique to individual isolates are well defined in the pangenome gene presence absence matrix (Figure 1B). The core genome is 2145 (Table 1) and total gene count within the EHEC pangenome is 17152, which is not that far off the total *E. coli* pangenome 22,000¹⁹. This enormous difference between the core gene and total gene highlights the variation between the different isolates, which can be strain specific and individual isolate specific as indicated by the pangenome data.

All of the examined genomes contain *sdiA* gene, which is contained within the 2145 core genome. Using *sdiA* of *Escherichia coli* O157:H7 str. Sakai for variant calling, we identified the non-synonymous mutations that are widely distributed across the

Figure 1A. EHEC *E. coli* pangene (152 genomes) accessory genome phylogeny showing three major clades.

Figure 1B. EHEC *E. coli* pangene (152 genomes) showing genomic diversity with the presence absence variation (PAV) matrix.

Table 1. EHEC *E coli* pangenome (152 genomes) metrics.

	Percentage Occurrence	Gene Number
Core genes	(99% <= strains <= 100%)	2145
Soft core genes	(95% <= strains < 99%)	1308
Shell genes	(15% <= strains < 95%)	3085
Cloud genes	(0% <= strains < 15%)	10614
Total genes	(0% <= strains <= 100%)	17152

EHEC isolates (Figure 3). 45.0% (49/109) of the nonsynonymous mutation is due to conversion of arginine to lysine at position 189 of sdiA (Figure 2A). This amino acid is located with the α -6 domain, adjacent to the amino acid clusters associated with sdiA dimerization. Previous protein modelling determined role of the guanidinium group of arginine which enables interactions in three different directions enabling a more complex electrostatic interaction versus lysine as well as the higher pKa value in arginine that can yield a more stable ionic interaction compared to lysine²⁰. The arginine to lysine mutations are found mostly in clade III isolates. The second ranked nonsynonymous

Figure 2. sdiA nonsynonymous variants protein modelling.

Figure 3. EHEC *E coli* sdiA allelic variants showing synonymous and nonsynonymous mutations.

mutation asparagine to serine at amino acid position 101 with 17.4% (19/109) which is located adjacent to η -4 phenylalanine which is associated with the ligand and distributed among the different clades (Figure 2B). β -5 domain alanine to threonine change at amino acid position 140 is the third ranked nonsynonymous mutation with 9.2 % (10/109) (Figure 2C). The fourth ranked nonsynonymous mutation is at amino acid position 138 with the conversion of cysteine to serine (Figure 2D) also within the β -5 domain. Both nonsynonymous variants of amino acid position 140 and 138 are found within clade II and III. None of the highly ranked nonsynonymous mutations impact the ligand interaction, indicating the conservation of the *sdhA* motif across the population in geographic and temporal distribution, which suggests the possibility of targeting *sdhA* for quorum sensing inhibition.

Conclusion

While EHEC pangenome is remarkably diverse, the allelic variants of *sdhA*, particularly nonsynonymous mutants, indicate the conservation of quorum sensing domain, indicating that targeting this structure can be effective across the different lineages of EHEC pathotype.

Data availability

All underlying and extended data available from Open Science Framework: Supplemental Data for Pangenome guided

pharmacophore modelling of enterohemorrhagic *Escherichia coli* *sdhA*, <https://doi.org/10.17605/OSF.IO/BNZ857>

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

Underlying data

Table 1 Metadata from Patric Database of EHEC *E. coli* pangenome

Table 2 EHEC *E. coli* pangenome presence absence matrix

Table 3 EHEC *E. coli* *sdhA* variant calling data

Extended data

SWISS-MODEL Homology Modelling Report available at [osf.io/bnz85](https://www.expasy.org/swiss/modelling).

Grant information

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Reviewer Report 15 May 2019

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Olivier Tenaillon

IAME (Infection Antimicrobials Modelling Evolution), UMR 1137, French Institute of Health and Medical Research (INSERM), Paris, France

The present manuscript presents a state-of-the-art pan genome analysis of EHEC strains and a subsequent analysis of the variation in *sdiA*.

The analysis of *sdiA* could have been completed with simple KVs analysis and compared to that of the core genome. For now, there is no connection between the two analysis, the authors could have simply performed the analysis of *sdiA*.

Some mutants have frame shifts in the gene, this is not discussed.

I think the author could try to connect more the two parts of the analysis.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Microbial genomics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 15 May 2019

DJ Darwin Bandoy, University of the Philippines Los Baños, Los Baños, Philippines

I thank the reviewer for the effort in doing the review. I accept all the suggestions and will add the population genetic analysis in the next version of the paper.

Competing Interests: No competing interests were disclosed.

Reviewer Report 24 April 2019

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Kerry K. Cooper

School of Animal and Comparative Biomedical Sciences, University of Arizona, Tucson, AZ, USA

I would state the biggest issue with the manuscript is the work is not technically sound, because upon examining the metadata file from Patric, numerous strains included in the EHEC pangenome were in fact not EHEC strains. Many of the isolates are UPEC, STEC or EPEC strains that should not be included in the analysis and are resulting in potential errors in the results and conclusions of the manuscript. All O55 strains are EPECs, O104 from the Germany outbreak is a STEC not EHEC, O127 is an EPEC, and CFT073 is an UPEC strain, just as some examples.

Furthermore, the analysis also includes O157:H7 strains that are wild type and mutant strains, and the mutants should not be included in the analysis. Additionally, the authors mention three clades include O157 (Clade I), O26 (Clade II) and O80 (Clade III), however these clades are formed because the vast majority of the strains used in the analysis come from those three serotypes. The analysis is missing three serotypes from the "big 6" serotypes, including O45, O121 and O145, and only include one or two representatives of two of the other serotypes O111, O103. There are numerous genomes available for each of these serotypes through NCBI that should be included in this analysis. Particularly as the "big 6" serotypes represent >50% of the infections, and in the United States represent adulterants in ground beef or other meat products. Therefore, they are a

vital aspect for the development of pharmacophore modelling of *sdhA* to prevent colonization in cattle.

Additionally, several genomic studies by Ogura et al (2009)¹ and Cooper et al (2014)² have shown that many of these "big 6" serotypes arise along different evolutionary pathways or split from O157 at different time points thus acquire different genes. It would be vital to include these in analysis to see if these different pathways impacted the conservation of *sdhA*. The author should also provide a much cleaner version of the metadata as a separate tab in the spreadsheet that includes only those strains that were included in the analysis. Unfortunately, the above-mentioned issue means that all of the results in the manuscript are potentially erroneous and need to be completely re-done with the elimination of non-EHEC strains and the inclusion of additional "big 6" genomes to provide a scientifically sound analysis.

It would also be helpful to include in the methods section the date of the search, as the database is constantly changing making reproduction a little bit easier by other researchers. Upon the new analysis it would be helpful to include a brief table or statement of the serotype breakdown included in the EHEC pangenome that would also eliminate some of the above-mentioned issues and make it easier for readers to get a sense of those serotypes included in the analysis.

There are a number of grammatical errors or poor phrasing in the manuscript that should be reviewed and corrected. Such as there is only one author and no indication of other researchers on the manuscript, yet the manuscript keeps stating we instead of I.

Finally, there are several points made in the introduction and discussion that do not have references. For example, in the introduction the author mentions "pangenome of *E. coli* was published in 2008 contained 8 genomes" but does not reference the paper. Additionally, the author mentions "serogroup O80 has aside from Shiga toxin, an extra-intestinal virulence plasmid (pS88), is currently emerging in France" but does not reference anything indicating the emergence in France. I have also provided the citations for Ogura et al.¹ and Cooper et al.² for the author to review and potentially cite in the manuscript.

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Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: I am an expert in foodborne bacterial genomics, epidemiology and pathogenesis, particularly *E. coli*, *Salmonella*, *Campylobacter*, and *Listeria*.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 15 May 2019

DJ Darwin Bandoy, University of the Philippines Los Baños, Los Baños, Philippines

I appreciate the work of the reviewer for going through the metadata. The inclusion of non-EHEC is necessary as outgroup comparison group and does not debunk the validity of the analysis pipeline. In light of this clarification, since this is the sole reason for the non-approval, I appeal for an approval with reservations as the analysis is technically and scientifically sound.

I am in the process of including the big 6 serotypes in the analysis based on the reviewer's comments, as well as the additional references which are very constructive additions to the paper. Again, as the reviewer sees the value in redoing a more inclusive analysis of EHEC serotypes, this is another justification to approve with reservation the paper submitted.

I beg to disagree with the comment of using "inclusive we" in place of I as a grammatical error. The use of royal or inclusive we in lieu of I is a matter of preference. This is the only part of the review I do not agree with.

Competing Interests: No competing interests were disclosed.

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