MBio 555 lab, Scientific article summarization

Genomic analysis of *Shigella* isolates from Lebanon reveals marked genetic diversity and antimicrobial resistance

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Introduction (Sarah Albogami)

- *Shigella*, a Gram- negative bacterium from the family *Enterobacteriaceae*, and it is a major cause of diarrheal diseases.
- *Shigella* is mostly transmitted through contaminated food, water or person- to- person contact.
- It can cause mild diarrhoea, severe dysentery with bloody stools and potentially fatal dehydration.
- The genus *Shigella* encompasses four *serogroups: S. dysenteriae, S. boydii, S. flexneri* and *S. sonnei.*
- Due to the increasing frequency of antimicrobial resistance (AMR) in *Shigella* strains, the World Health Organisation (WHO) decided to classify *Shigella* as a priority pathogen for which new antimicrobial drugs are urgently required.
- In Lebanon, the rates of *Shigella* infections are unclear due to various reasons. Therefore, this study will perform phenotypic and in-depth genomic analyses of *Shigella* isolates collected in North Lebanon to provide baseline information on the distribution of *Shigella* serotypes and antimicrobial resistance determinants in the country.

Methodology (Sarah and Buthainah)

Overview (Sarah Albogami)

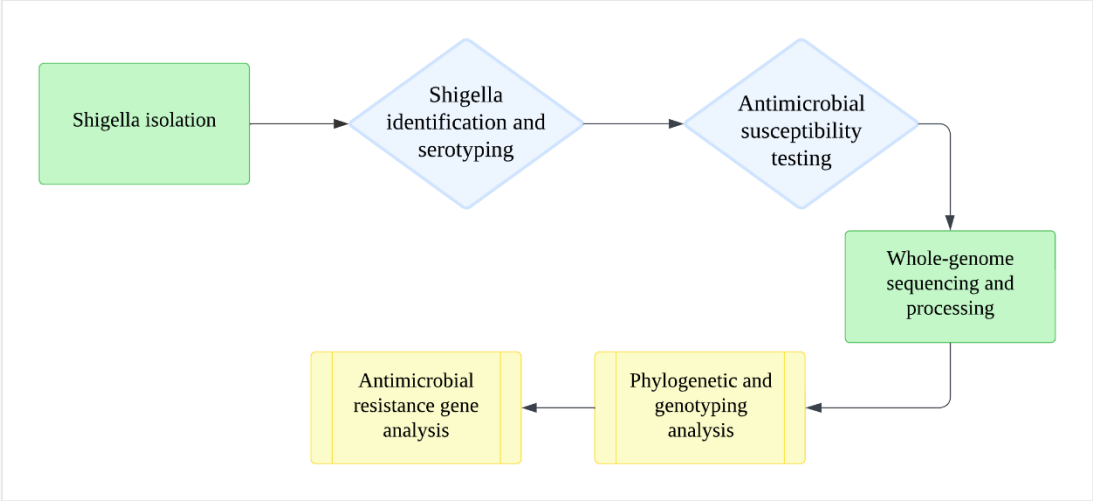

Figure 1. illustration of methodology workflow

Shigella isolates (Buthainah Alsughair)

- Bacillary dysentery patients' stool samples were collected from different hospitals
- Isolates were identified using biochemical tests (API 20E strips or RapID ONE system)

Figure 1 RapID ONE system

Figure 2 API 20 E strips

Shigella identification and serotyping (Sarah Albogami)

- Biochemical tests were performed for the isolates, such as lactose, motility, glycerol, glucose (acid and gas), and indole.
- Serotyping was performed with slide agglutination assays.

Antimicrobial susceptibility testing (Buthainah Alsughair)

1) Antimicrobial susceptibility testing (AST) was performed using the **Disc diffusion method** on **Mueller- Hinton (MH)** agar on 16 different antibiotics.

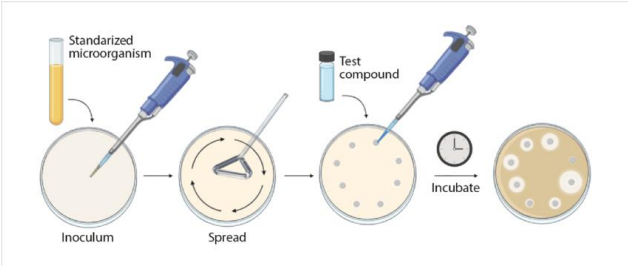

Figure 3disc diffusion method

2) AST results were interpreted according to the 2020 guidelines of the antibiogram committee of the French Society for Microbiology and the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

3) Minimum inhibitory concentrations (MICs) for some antibiotics were tested using E-strips.

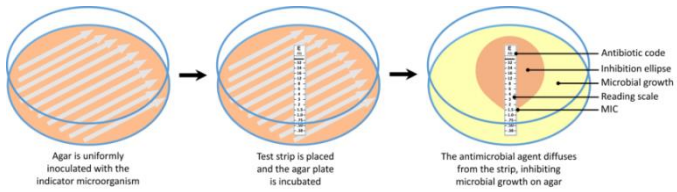

Figure 4 E-strips

Whole genome sequencing and processing (Sarah Albogami)

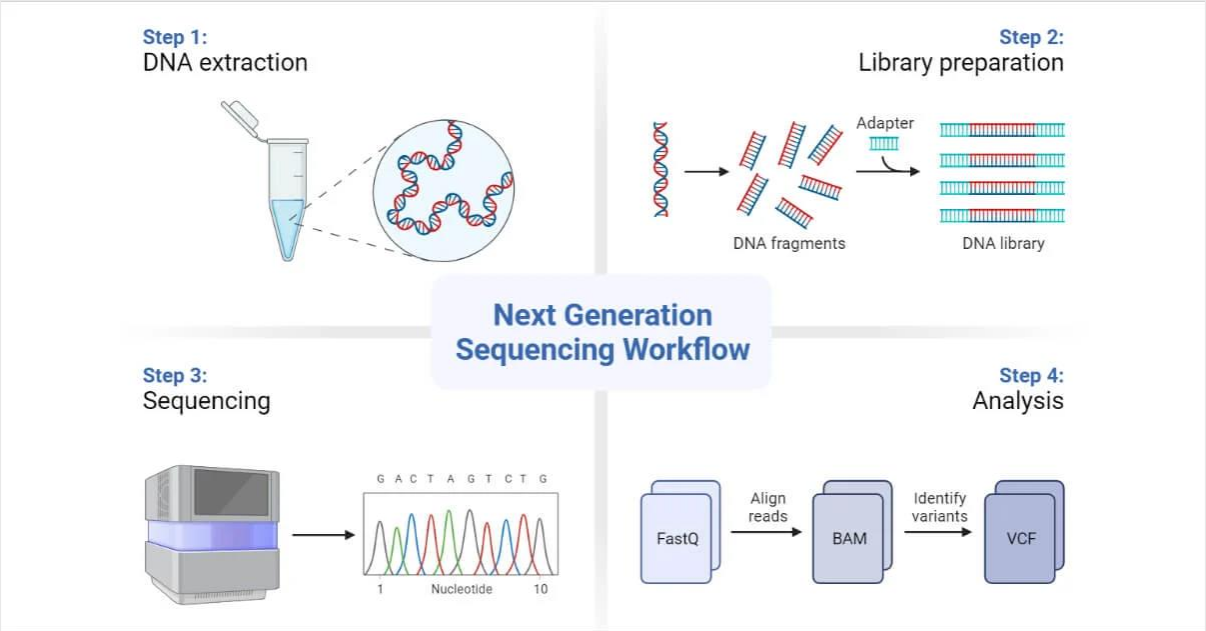

Figure 2. NGS workflow, (G, 2020)

1. DNA extraction from cultures.
2. Whole genome sequencing:
   2.1. Libraries were constructed to perform the sequencing.
   2.2.Then the reads were assembled and passed through quality control criteria.
3. ESBL-Producing *Shigella* Isolates Sequencing:
   3.1. Five *shigella* isolates were selected and sequenced.
      3.1.1. DNA was extracted from isolates.
      3.1.2. Libraries were prepared to perform the sequencing.
      3.1.3. Finally, the assembly of the genome sequences, then annotation of plasmids.

Phylogenetic and genotyping analysis (Buthainah Alsughair)

- Phylogenetic and genotyping analysis called (ShigaPass) was used for **serotype confirmation**.
- *Escherichia/Shigella* core-genome multilocus sequence typing (cgMLST) scheme **used to type all isolates** implemented in EnteroBase
- *S. sonnei* genotyping was performed with the hierarchical single- nucleotide variant-based genotyping scheme implemented in Mykrobe software
- The phylogenetic tree was generated with the EnteroBase

Antimicrobial resistance gene analysis (Sarah Albogami)

1. Antimicrobial resistance genes were identified.
2. Typing the plasmids.
3. Identifying the plasmids by comparing the sequence to the NCBI BLASTn nucleotide database.
4. Finally, Alignments of Plasmids and visualization.

Result and discussion (Sarah and Buthainah)

Shigella serotype distribution (Buthainah Alsughair)

1. *Shigella* serotyping was performed by slide agglutination and *in silico* with ShigaPass with no discrepancies observed between the results of the two methods.
2. *S. sonne*i was the most prevalent serogroup.
3. *S. boydii* is the most common serotype.
4. Serotyping results concluded the diversity of the *Shigella* serotypes in North Lebanon and demonstrated the predominance of *S. sonnei.*
5. The study had several limitations in:

- Sample collection.
- Shigellosis has general symptoms (some patients may have it but not diagnosed).
- Most clinical laboratories are not equipped to test for *shigella*.
- The study was in one region of the country.

Antimicrobial resistance (Sarah Albogami)

87% of isolates showed resistance to three or more classes of antimicrobial drugs, leading to their classification as multidrug-resistant (MDR).

- The highest AMR display was for trimethoprim, while the lowest was for ciprofloxacin.
- In total 19 antimicrobial drug resistance were identified.
- These results suggest that azithromycin remains effective in Lebanon.

Analysis of the AMR genes (Buthainah Alsughair)

- AMR genes identified include:

– Sulfonamides (*sul1* and *sul2*)
– Tetracycline (*teta* and *tetb*)
– Trimethoprim (*dfra1*, *dfra5* and *dfra14*)
– Phenicols (*cata1*).

- The most frequent were *strA*, *strB*, and *aadA1* in Aminoglycoside resistance.
- Quinolone resistance was high frequent.
- Ciprofloxacin- resistant isolates were rare.
- Ampicillin resistance was presented by the *bla*$_{OXA-1}$ and *blaTEM*$_{-1B}$ beta- lactamase genes

Charctrization of ESBL encoding plasmids (Sarah Albogami)

- ESBL genes were identified on the contig carrying the plasmid replicon genes, confirming that they were plasmid-borne.
- Two different Inc-type plasmids were associated with the ESBL genes: IncI1, and IncFIB.
- IncI1 and IncFIB plasmids was found in different *Shigella* serogroups (*S. sonnei*, *S. flexneri*, *and S. boydii*) from different countries.
- The presence of the same IncI1/IncFIB plasmids in different *Shigella* serogroups suggest that these plasmids have been successfully transmitted horizontally between different strains and even across continents.

Conclusion (Buthainah Alsughair)

- The study provides the serotypes of Shigella and the AMR determinants they carry.
- It revealed a high degree of genetic diversity of Shigella strains, with a marked prevalence of MDR isolates.

Reference

Yassine, I., Rafei, R., De La Gandara, M. P., Osman, M., Fabre, L., Dabboussi, F., Hamze, M., & Weill, F. (2023). Genomic analysis of Shigella isolates from Lebanon reveals marked genetic diversity and antimicrobial resistance. *Microbial Genomics*, *9*(12). https://doi.org/10.1099/mgen.0.001157

Figures

Figure 2 G, J. (2020, October 5). *NGS overview: from sample to sequencer to results*. iRepertoire. https://irepertoire.com/ngs-overview-from-sample-to-sequencer-to-results/