

Targeting post-weaning diarrhea in piglets: Bacteriophage isolation against pathogenic *Escherichia coli*

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Introduction

Major cause of economic losses in **pig production**

Mainly caused by pathogenic ***Escherichia coli*** (especially **ETEC**)

Increasing **antimicrobial resistance** limits treatment options

Bacteriophages represent a promising alternative to antibiotics

F4 • F18 fimbriae
STa • STb • LT enterotoxins

ETEC: enterotoxigenic *Escherichia coli*

Objectives

Characterize the genomic diversity of weaning-associated porcine *E. coli* circulating in Belgium

Isolate bacteriophages targeting pathogenic porcine *E. coli*

Materials and methods

Bacterial isolate collection

- 198 isolates
- Belgian weaned pigs, 2021–2026

172 intestinal isolates

Phage isolation process

- 10 independent phage isolation experiments
- Total of **74 wastewater samples** (including repeated sampling from some locations) collected across **Flanders, Wallonia, and Brussels** were processed
- 8 porcine *E. coli* strains were tested as bacterial hosts

Sites: Awans, Fontin, Sclessin, Grosses-Battes, Oreye, Brussels Sewer museum, Asse, Deurne, Landen, Ouwegem, Geetbets

Host strains genomic characterization

- **Nanopore sequencing**
- Assembly: **Flye 2.9.6 + Medaka 1.4.4**
- Annotation: **Bakta 1.9.4**
- Virulence genes: **ABRicate 1.4.0**
- MLST: **PubMLST**
- Serotyping: **ECTyper 2.0.0**

Results

PATHOTYPE DISTRIBUTION

ETEC most frequent (29,7%). Atypical/hybrid profiles: 32.6%

*Other: ExPEC (2.9%) + EPEC (0.6%) + Atypical ETEC/STEC pAA+ (0.6%) = 7 isolates (4.1%).

GENOMIC DIVERSITY

81
O:H serotypes

50
MLST sequence types

FIMBRIAL PROFILES

Fimbrial type distribution. F18 was the most prevalent (40.7%). 34.3% of isolates lacked any classical fimbria.

Host strains genomic characterization and phage isolation

A8

O-serogroup: **O9**

SEROTYPE: **--:H7** FIMBRIA: **-**

PATHOTYPE: **Unclassified**

VIRULENCE GENES: **FimH, FimA, AIDA-I**

Excluded — not weaned piglets

7 phages isolated

A13

O-serogroup: **O9**

SEROTYPE: **O9:H25** FIMBRIA: **-**

PATHOTYPE: **Intestinal ExPEC-associated (AIDA+)**

VIRULENCE GENES: **iucD, iucC, iutA, AIDA-I**

Excluded — not weaned piglets

3 phages isolated

Plaque morphology of *E. coli* bacteriophages after the second replating

A14

O-serogroup: **-**

SEROTYPE: **--:H38** FIMBRIA: **F18**

PATHOTYPE: **STEC**

VIRULENCE GENES: **Stx2e, AIDA-I**

Excluded — weaning timing unsure

4 phages isolated

A12

O-serogroup: **O9**

SEROTYPE: **O9:H19** FIMBRIA: **-**

PATHOTYPE: **Intestinal ExPEC-associated (AIDA+)**

VIRULENCE GENES: **iucD, iucC, iutA, AIDA-I, papC**

1 phage isolated

The phage targeting strain A12 was selected for further characterization based on the clinical metadata of its host strain (enteritis)

Conclusion and perspectives

- ❖ **15 bacteriophages** were isolated from **Belgian wastewater**, targeting **porcine pathogenic *E. coli*** strains
- ❖ Whole-genome sequencing revealed genetically diverse bacterial host strains, highlighting the need for broad phage coverage
- ❖ Further phage isolation will be based on target strains selected based on both **genomic features** (virulence genes, fimbrial types, serogroups, MLST) and **clinical metadata** (anatomical site of isolation and disease context)
- ❖ These results constitute the first step toward phage therapy against PWD in Belgian pig production
- ❖ **Further characterization of the selected phage isolated against strain A12, including host range, cycle type, and genomic analysis, will be essential to evaluate its suitability for future therapeutic applications**

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