**TITLE:**

**Molecular Detection of *E. coli* Virulence Factors in Isolates from Pigs: Protocol for a Systematic Review of Comparative Studies.**

**REGISTRATION:**

This protocol will be made available online at Systematic Reviews for Animals and Food (SYREAF; [www.syreaf.org](http://www.syreaf.org)).

**REPORTING GUIDELINES:**

This protocol is reported using the items (headings) recommended in the PRISMA-P guidelines (Moher et al., 2015).

**AUTHORS AND CONTRIBUTIONS:**

**Elisa De Conti** ([deconti@iastate.edu](mailto:deconti@iastate.edu)) conceived the idea and developed the protocol.

**Brad Kuennen** ([kuennen@iastate.edu](mailto:kuennen@iastate.edu) developed the protocol.

**Rodrigo Paiva** ([paiva@iastate.edu](mailto:paiva@iastate.edu)) will contribute to the execution of the project.

**Giovani Trevisan** ([trevisan@iastate.edu](mailto:trevisan@iastate.edu)) conceived the idea and secured the funding.

**Daniel Linhares** ([linhares@iastate.edu](mailto:linhares@iastate.edu)) conceived the idea and secured the funding.

**Annette M. O’Connor** ([oconn445@msu.edu](mailto:oconn445@msu.edu)) provided critique and refinement of the protocol.

**Marcelo Almeida\*** ([malmeida@iastate.edu](mailto:malmeida@iastate.edu)) conceived the idea and developed the protocol.

\*Contact: 1856 Christensen Dr., Room 1912-1 Vet Med Annex. Ames, IA 50011-1134. Office Phone: (515)294-7385.

**AMENDMENTS:**

In the event that changes need to be made to the protocol, a dated amendment will be made available with a description and reason for the modification.

**SUPPORT: SOURCE, SPONSOR, AND ROLE OF FUNDER:**

The funders had no involvement in the study's design, data collection, analysis, or interpretation, nor in the manuscript's writing or the decision to publish the findings. The authors declare no conflicts of interest.

1. **INTRODUCTION** 
   1. ***RATIONALE***

*Escherichia* (*E.*) *coli* is a gram-negative bacterium that can be isolated from healthy and sick pigs (Chapman et al., 2006). This bacterium can cause a range of diseases in pigs, with diarrhea (post-weaning and neonatal) and edema disease being the most relevant clinical presentations (Fairbrother and Nadeau, 2019). Recent advances in understanding how *E. coli* causes disease have led to a better classification of pathogenic strains. The *E. coli* colonies can be tested by Polymerase Chain Reaction (PCR) for genes coding for bacterial traits involved in the pathogenesis of disease (e.g., F18, K88, AIDA, STX2e, EAST1), referred to as virulence factors (Casey and Bosworth, 2009). The combination of virulence factors detected in an isolate (i.e., virotype) determines its classification into a specific pathotype, defining the disease causation mechanism (Fairbrother and Nadeau, 2019). For example, the detection of the virulence factors F18 and STX2e in an isolate defines its virotype as F18;STX2e, which is classified as a Shiga toxin‐producing *E. coli* (STEC) pathotype.

The *E. coli* characterization based on the presence of virulence factors permits much more accurate diagnostic strategies and epidemiologic understanding. Data from the Iowa State University Veterinary Diagnostic Laboratory shows an increase in the number of cases of post-weaning colibacillosis, with F18 fimbria being the predominant fimbrial-adhesin detected (Paiva et al., 2024). A recent case-control study found that healthy pigs were 6.7 times more likely to shed *E. coli* carrying the EAE gene compared to pigs with diarrhea (Lin et al., 2024). Molecular detection of virulence factors has significantly contributed to a better understanding of *E. coli*'s pathogenic nature. Comparative studies involving pigs with and without clinical signs are particularly valuable, as they help understand the epidemiological significance of virulence factors molecular detection. The escalating challenge in swine production caused by *E. coli* requires a deeper understanding of the virulence factors potentially pathogenic to pigs. A systematic review of the literature can provide a comprehensive overview of the virulence genes identified in *E. coli* isolates from pigs, offering insights into their relevance and contributing to a better understanding of *E. coli* epidemiology.

* 1. ***OBJECTIVES***

This study will summarize peer-reviewed literature on the *E. coli* virulence factors reported by PCR-based assays from bacterial isolates obtained from pigs, whether clinically ill or apparently healthy. Population: *E. coli* colonies isolated from clinically ill or apparently healthy pigs.

Exposure: *E. coli* further characterization of the virulence factors by PCR-based methods.

Outcome: Virulence factors identified in pigs with and without clinical signs. Depending upon the availability of data, the outcome will also be reported by key variables, including the type of study (experimental or observational), the age of the pigs, farm type, specimen from which the isolate was obtained, geographic location from where samples were obtained, and PCR methodology.

1. **METHODS** 
   1. ***ELIGIBILITY CRITERIA***

Peer-reviewed manuscripts will be included if they report *E. coli* virulence factors detected by PCR in bacterial isolates obtained from pig samples with known age or production status. The study population must have tested pigs with and without clinical signs. Eligible studies must be published in English. Literature reviews will be excluded.

* 1. ***INFORMATION SOURCES***

Literature searches will be conducted in the following article indexes and databases: PubMed and CAB Abstracts. PubMed will be accessed through the native interface (https://pubmed.ncbi.nlm.nih.gov ). CAB Abstracts will be searched using the Web of Science platform provided by the Iowa State University Library.

* 1. ***SEARCH STRATEGY***

The search strategy followed the PRISMA 2020 guideline (Page et al., 2021) and is presented in Tables 1 to 2. The search terms aimed to embrace a comprehensive number of studies on virulence factors detected by molecular methods in *E. coli* isolates from pigs.

Search strings were developed for the major topics of **swine** and ***E. coli*** **virulence factors.** The terms for *E. coli* virulence factors will be split into two separate search strings, one for *E. coli* (including terms for diarrhea and illnesses caused by *E. coli*) and the other for virulence and pathogenicity. The terms to be used for the **swine** search string come from the Animal Search Hedges (<https://osf.io/jy2hb/files/osfstorage/67a5428be407580bddaf4680>) developed by the Search Strategies Working Group of the Animal and Veterinary Information Specialists caucus of the Medical Library Association. The three search strings will be combined for the final search string.

Table 1. Search strategy in PUBMED https://pubmed.ncbi.nlm.nih.gov )

|  |  |
| --- | --- |
| **Groupings** | **Search terms** |
| #1 | (Sus scrofa[MeSH Terms] OR "Sus scrofa"[Title/Abstract] OR "S scrofa"[Title/Abstract] OR swine[Title/Abstract] OR piggery[Title/Abstract] OR pig[Title/Abstract] OR pigs[Title/Abstract] OR hog[Title/Abstract] OR hogs[Title/Abstract] OR porcine[Title/Abstract] OR sow[Title/Abstract] OR sows[Title/Abstract] OR boar[Title/Abstract] OR boars[Title/Abstract] OR piglet\*[Title/Abstract] OR gilt[Title/Abstract] OR gilts[Title/Abstract] OR barrow\*[Title/Abstract]) NOT (Guinea pigs[MeSH Terms] OR guinea[Title/Abstract] OR "pig a assay\*"[Title/Abstract] OR "wild boar\*"[Title/Abstract] OR "wart hog\*"[Title/Abstract] OR "ground hog\*"[Title/Abstract] OR wild[Title/Abstract] OR feral[Title/Abstract]) |
| #2 | "Escherichia coli"[MeSH Terms] OR "Escherichia coli"[Title/Abstract] OR "E coli"[Title/Abstract] OR "Neonatal Diarrhea"[Title/Abstract:~3] OR "Post-weaning Diarrhea"[Title/Abstract] OR "edema disease of swine"[MeSH Terms] OR "Edema Disease"[Title/Abstract] OR "diarrhea/veterinary"[MeSH Terms] OR "ETEC"[Title/Abstract] OR "EPEC"[Title/Abstract] OR "STEC"[Title/Abstract] OR "VTEC"[Title/Abstract] OR "ExPEC"[Title/Abstract] |
| #3 | "virulence factors"[MeSH Terms] OR "virulence"[MeSH Terms] OR "escherichia coli/pathogenicity"[MeSH Terms] OR "escherichia coli/virology"[MeSH Terms] OR "coli pathogenicity"[Title/Abstract:~5] OR "coli virology"[Title/Abstract:~5] OR "pathogen\* factor\*"[Title/Abstract] OR "virulence factors"[Title/Abstract:~3] OR "virulence factor"[Title/Abstract:~3] OR "virulence gene"[Title/Abstract:~3] OR "virulence genes"[Title/Abstract:~3] OR "pathogenicity factors"[Title/Abstract:~3] OR "pathogenic factors"[Title/Abstract:~3] |
| Complete search string | #1 AND #2 AND #3 |

Table 2. Search strategy for CAB Abstracts (Web of Science)

|  |  |
| --- | --- |
| **Groupings** | **Search terms** |
| #1 | (DE=(pigs) OR TS=("Sus scrofa" OR "S scrofa" OR swine OR piggery OR pig OR pigs OR hog OR hogs OR porcine OR sow OR sows OR boar OR boars OR piglet\* OR gilt OR gilts OR barrow\*)) NOT TS=("Guinea pigs" OR guinea OR "pig a assay\*" OR "wild boar\*" OR "wart hog\*" OR "ground hog\*" OR wild OR feral) |
| #2 | (DE = (Escherichia coli OR Escherichia coli infections OR oedema OR diarrhoea) OR TS = (“Escherichia coli” OR "E. coli" OR "ETEC" OR "EPEC" OR "STEC" OR VTEC" OR ExPEC" OR diarrhoea OR diarrhea OR edema OR oedema)) |
| #3 | DE=(virulence factors OR virulence) OR TS=(virulence OR (pathogen\* NEAR/3 factor\*) OR (virulence NEAR/3 (factor\* OR gene\*)) OR (coli NEAR/3 (pathogeni\* OR virolog\*))) |
| Complete search string | #1 AND #2 AND #3 |

* 1. ***STUDY RECORDS*** 
     1. *Data management:* Article information will be exported from the chosen databases and imported into Zotero. Team members will conduct an initial deduplication of the articles using Zotero. The resulting collection of article citation information will be imported into the DistillerSR platform (<https://www.distillersr.com/>), which will be used by team members to select and review articles.
     2. *Selection process:* Two reviewers (EDC/RP) will go through the Title/Abstract and full-text articles. Study selection has two levels: a first level based on assessing information in titles and abstracts, and a second level based on assessing information from the full text of studies.

In the first level, the reviewers will independently evaluate the **Title/Abstract** for relevance using the following screening question:

* Does the study report *E. coli* virulence factors detected by PCR in bacterial isolates from pigs with and without clinical signs?
  1. Yes – include for full-text evaluation.
  2. No – exclude with no further review.

In the second level, for all studies that are retained after Title/Abstract screening, eligibility will be assessed through **full-text** screening.

1. Is the whole text written in English?
   1. Yes – proceed to Q2.
   2. No – exclude with no further review.
2. Does the study population include pigs?
   1. Yes – proceed to Q3.
   2. No – exclude with no further review.
3. Did the study include pigs with and without clinical signs?
   1. Yes – proceed to Q4.
   2. No – exclude with no further review.
4. Did the study describe the age and/or production stage of the pigs?
   1. Yes – proceed to Q5.
   2. No – exclude with no further review.
5. Were *E. coli* virulence factors tested by PCR-based assays from isolates?

a. Yes – proceed to Q6.

b. No – exclude with no further review

1. Can the data (virulence factors) be extracted from both groups (clinical and without clinical signs)?
   1. Yes – proceed to data extraction.
   2. No – exclude with no further review.

The full-text screening form will be pre-tested on four references by all reviewers.

* + 1. *Data collection process:* The information will be extracted in an Excel spreadsheet by (EDC) and reviewed/supervised by (MA).
  1. ***DATA ITEMS EXTRACTED***

Qualitative data related to the research question will be extracted from the selected articles. The variables and their definitions are explained in Table 4.

Table 3: Variables extracted from the articles, description, and possible simplifications.

|  |  |
| --- | --- |
| **Variable** | **Description of items** |
| **General study characteristics** | |
| Study location | Country and region where the study was conducted. |
| Year of publication | Year of publication or year of proceeding. |
| Country | The country where the samples were collected. |
| **Detailed study characteristics** | |
| Time-range of sampling | The period of time when the samples were collected (e.g., a study that gathered laboratory results from 2020 to 2022). |
| Production phase and/or age of the animals | Production phase from where the samples were collected (i.e., breeding herd, nursery, finishing) or the age of the animals. |
| Specimen and/or sample type | The specimen (e.g., feces, small intestines) or sample type (e.g., swab) that was submitted to bacterial isolation. |
| Virulence factors | Virulence factors detected by molecular methods in *E. coli* isolates, including EAST1, LT, Sta, STb, Stx1, Stx2, Stx2e, F18, F41, K88 (F4), K99 (F5), 987P (F6), AIDA, EAEA, and PAA. If other genes are reported, they will be specified. When a gene name is provided (e.g., "*FedA*"), the outcome will be standardized according to fimbriae/toxin nomenclature (e.g., "*FedA*" will be reported as "F18"). |
| Clinical presentation | Describe the clinical presentation of the pigs from which the isolates came (e.g., diarrhea, edema disease, neurological disease, or apparently healthy). |
| Sample size | The number of animals sampled and the number of isolates tested. |

* 1. ***OUTCOMES AND PRIORITIZATION***

The primary and preferred outcome of data extraction will be the prevalence (r/n) of *E. coli* virulence factors reported based on PCR in each group of pigs (health and not health). If the prevalence data are not reported, summary data such as prevalence ratio, odds ratio (adjusted or unadjusted) will be extracted. This will be reported by the clinical presentation that the pigs (or pig population) had when the samples were collected. The other items that are described in the detailed study characteristics (section 2.5) will be reported as available.

* 1. ***DATA SYNTHESIS***

Information will be extracted on a spreadsheet in Excel. The results will be synthesized descriptively.

* 1. ***CONFIDENCE IN CUMULATIVE EVIDENCE AND RISK OF BIAS IN INDIVIDUAL STUDIES***

Not applicable to this review.

**REFERENCES**

Casey, T.A., Bosworth, B.T., 2009. Design and evaluation of a multiplex polymerase chain reaction assay for the simultaneous identification of genes for nine different virulence factors associated with Escherichia coli that cause diarrhea and edema disease in swine. Journal of veterinary diagnostic investigation 21, 25–30.

Fairbrother, J.M., Nadeau, É., 2019. Colibacillosis. Diseases of swine 807–834.

Lin, J., Schut, C., Fairbrother, J.M., Lillie, B.N., Friendship, R., Farzan, V., 2024. Characterizing *E coli* isolates contributing to postweaning diarrhea in pigs in Ontario, Canada: A case-control study, in: AASV Annual Meeting. Presented at the AASV Annual Meeting, American Association of Swine Veterinarians. https://doi.org/10.54846/am2024/22

Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., Shekelle, P., Stewart, L.A., 2015. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev 4, 1. https://doi.org/10.1186/2046-4053-4-1

Page, M.J., McKenzie, J.E., Bossuyt, P.M., Boutron, I., Hoffmann, T.C., Mulrow, C.D., Shamseer, L., Tetzlaff, J.M., Akl, E.A., Brennan, S.E., Chou, R., Glanville, J., Grimshaw, J.M., Hróbjartsson, A., Lalu, M.M., Li, T., Loder, E.W., Mayo-Wilson, E., McDonald, S., McGuinness, L.A., Stewart, L.A., Thomas, J., Tricco, A.C., Welch, V.A., Whiting, P., Moher, D., 2021. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ n71. https://doi.org/10.1136/bmj.n71

Paiva, R.C., Burrough, E., Piñeyro, P., Almeida, M., 2024. Characterization of hemolytic *E coli* and antimicrobial susceptibility from ISU-VDL submitted cases from 2010 to 2022, in: AASV Annual Meeting. Presented at the AASV Annual Meeting, American Association of Swine Veterinarians. https://doi.org/10.54846/am2024/14