

Use of Whole Genome Sequencing to Track *E. coli* strains causing Colibacillosis in Saskatchewan Broiler Chickens



2022 Poultry Service Industry Workshop

By

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Outline

- Introduction - Colibacillosis
- Rationale of study
- Objectives of study
- Methodology
- Results
- Future directions
- Summary
- Acknowledgments

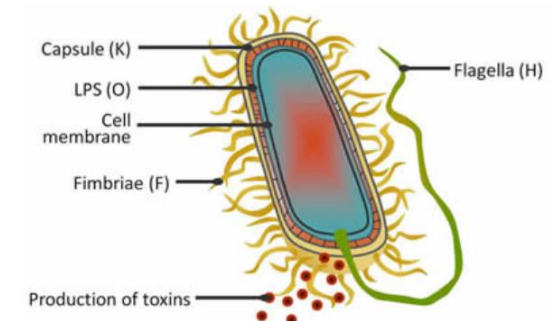
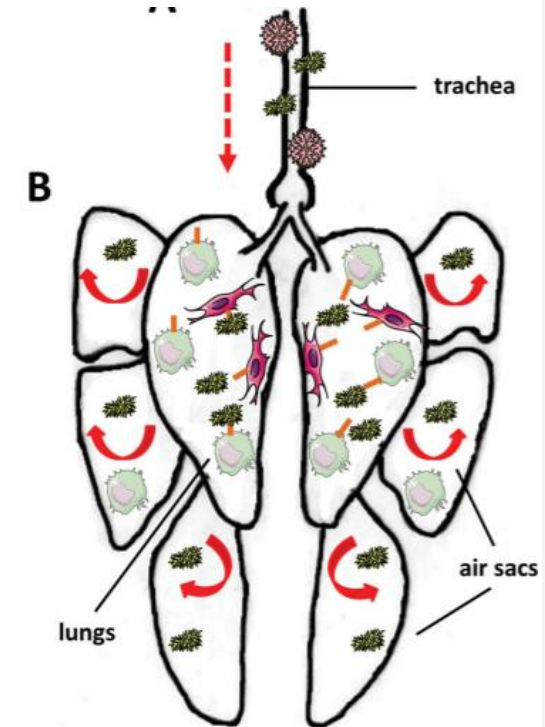
Introduction - Colibacillosis

- Broad term for localized and/or systemic infections in birds caused by *E. coli*
- Disease causing strain – Avian Pathogenic *E. coli* (APEC)
- Manifests in diverse ways – Typically presence of lesions in organs like liver, spleen and heart
- Challenging – Occurs alongside with other bacterial infection
- Diagnosis ? Culture and screen for Phenotypes to confirm that they are APECs



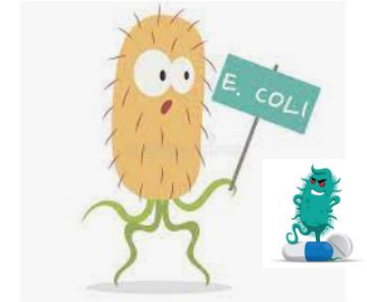
APECs – Still many black holes

- The most common virulence factors in APECs are rarely all present in the same isolate – APECs constitute heterogeneous group and are diverse
- APECs can colonize the GIT and respiratory tracts of chickens without causing disease – translocate to extra-intestinal sites in presence of stressors
- Route of Infection remains Unclear



Why Do We Care?

- #1 cause of mortality and morbidity in poultry
- Economic losses ~\$100,000,000 annually as a result of cost associated with treatment of disease, poor yield, condemnation of carcasses at slaughter
- Possible zoonotic transfer of APECs to humans via food chain to cause human extraintestinal infections like UTI
- Increased AMR among APECs
- Currently no comparative genomics on APECs and source tracking done in Saskatchewan



Colibacillosis Diagnosis by PEX

1 Producers within 2 hours of Saskatoon submit dead broilers to Poultry Extension Team (PEX)

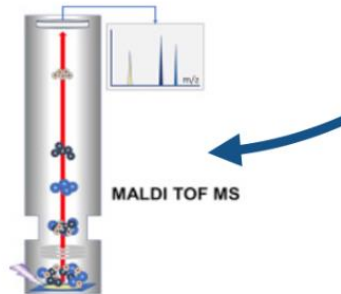


2 PEX performs necropsy on dead broilers to make preliminary Colibacillosis diagnosis



Poultry Extension Team

3 Prairie Diagnostic Services grows bacterial cultures from samples of the heart, liver, and spleen



4 Colibacillosis diagnosis is confirmed via MALDI-TOF MS

5 PEX submits pathology report to the producer.

No further characterization of E. coli

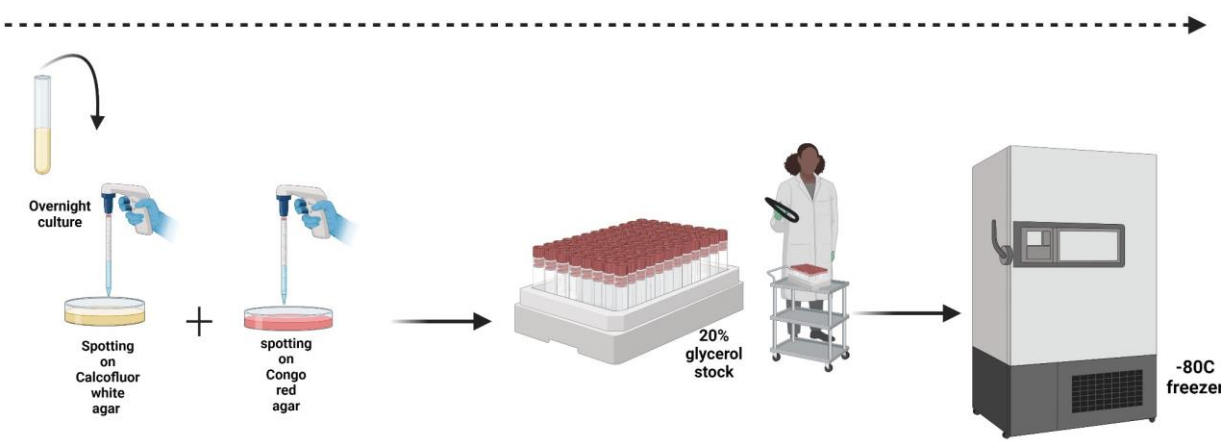
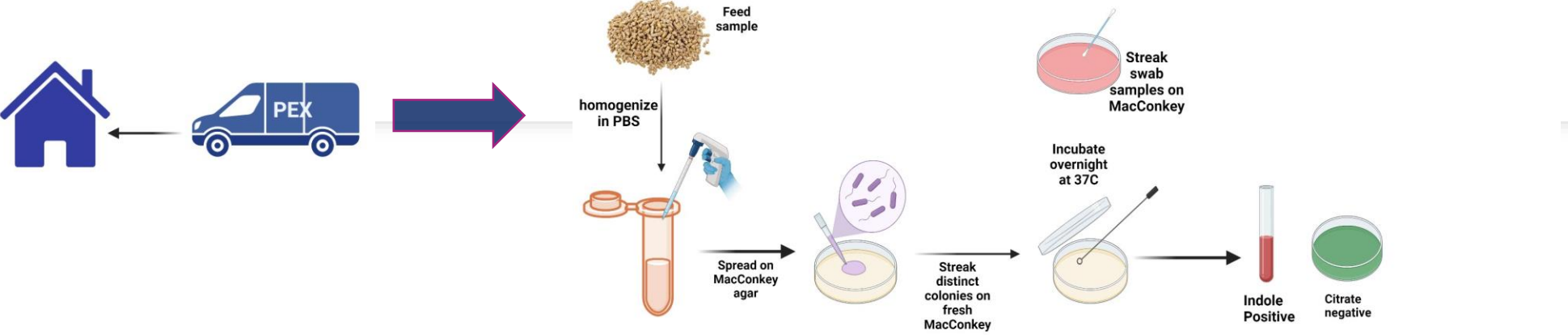
ISDC:
Producers submitted broilers from 3 barns at 20 days of age with history of late mortality and high condemnations. Mortality charts emailed to vet with daily numbers.

Requests: Pathology (FINAL) x 1, Culture(s) (FINAL) x 1, Kirby Bauer - Bacteriology - Necropsy (Poultry - 1 ATB) (FINAL) x 1

Bacteriology - Final

Requested NA	Received 03-Jan-2020	Tested 03-Jan-2020	Completed 05-Jan-2020		
Culture(s)					
Sample	Target	Type	Result	Units	Comment
Spleen	Escherichia coli		4+		
	Salmonella species		Not isolated		
Bone Marrow	Escherichia coli		4+		
Swab-Hook	Escherichia coli		4+		

Sample processing - Pipeline

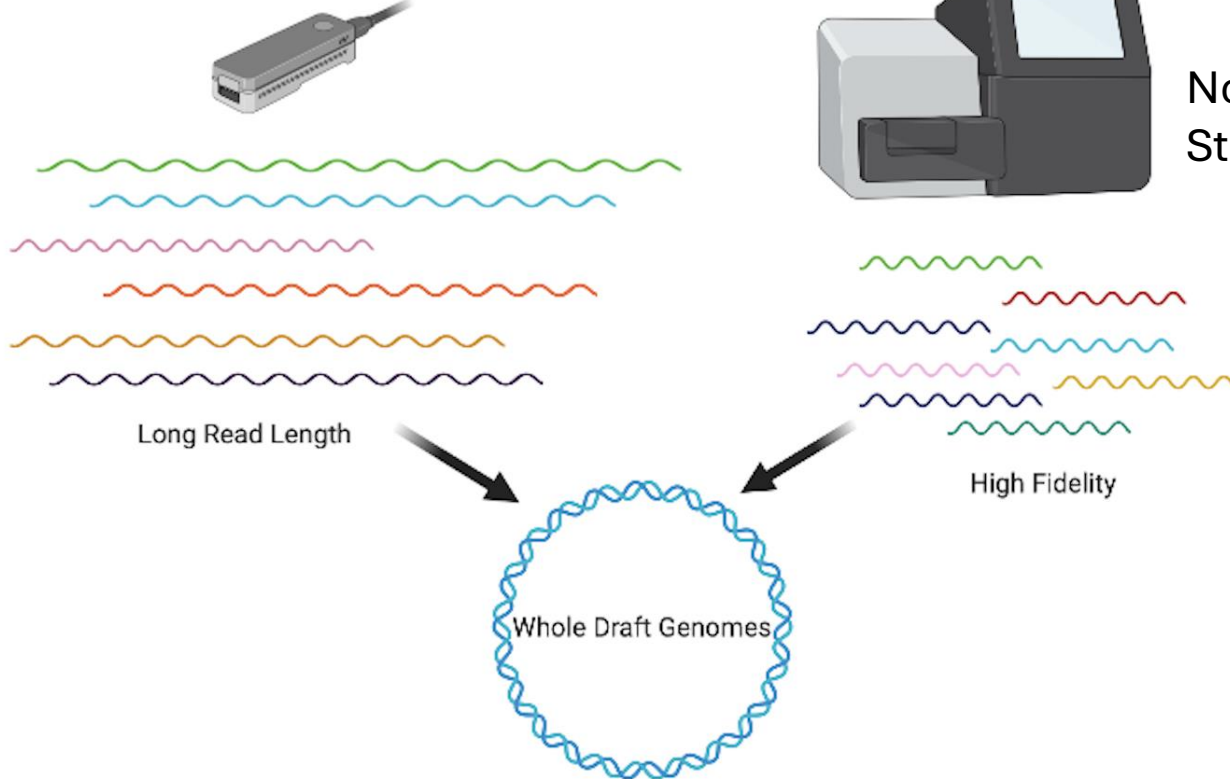


AMR testing

Whole Genome Sequencing

- Illumina (short) and Nanopore (long) sequencing for all *E. coli*
- Accurate gene order and sequence
- Track AMR and virulence genes

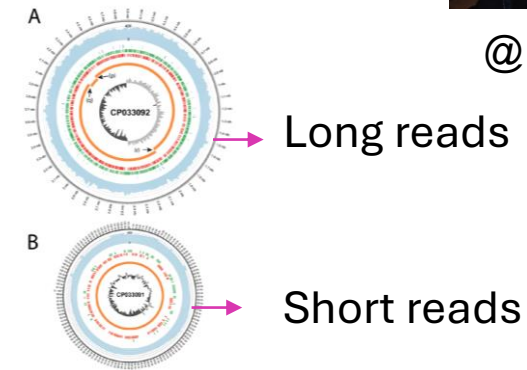
In-house



Novogene –
Stanford, CA



@ VIDO, 2021/2022

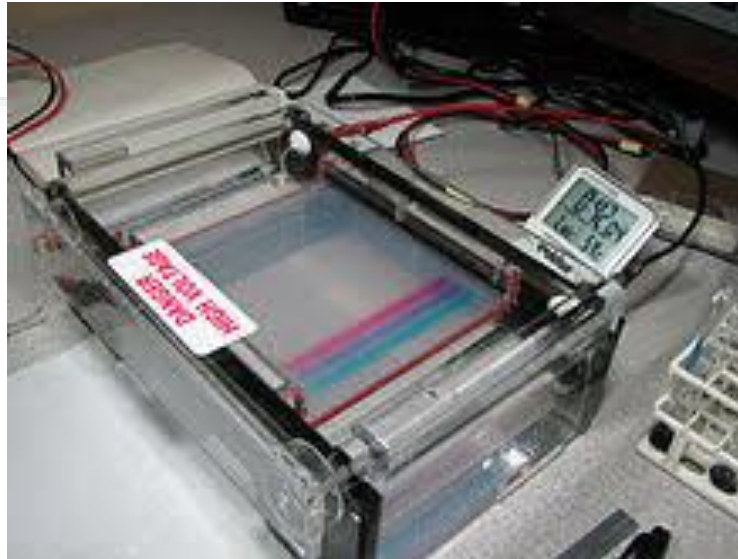


Quality of DNA and Library Preps



Nanodrop

- Fluorometer
- Spectrophotometer



Agarose Gel

- To assess the size of DNA fragment

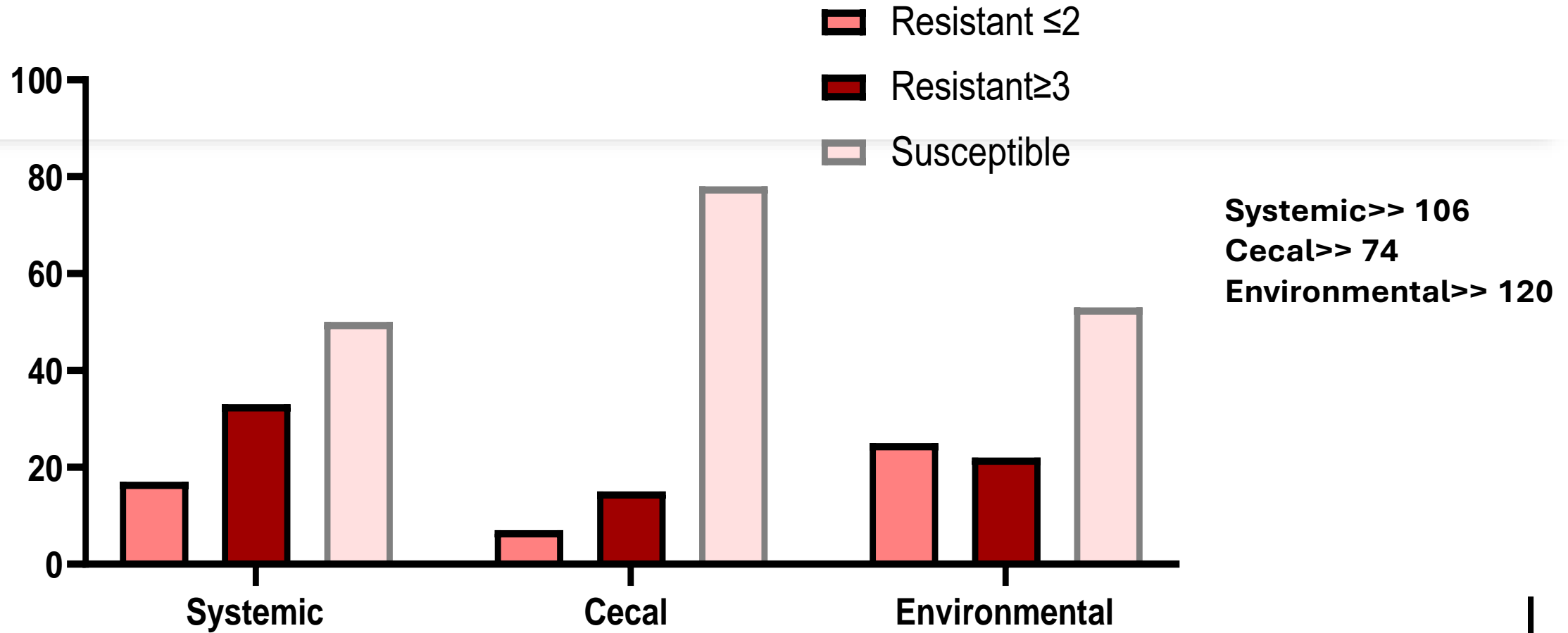


Agilent Bioanalyzer

- To assess the size and quality of DNA over a range of sizes and concentrations

RESULTS SO FAR.....

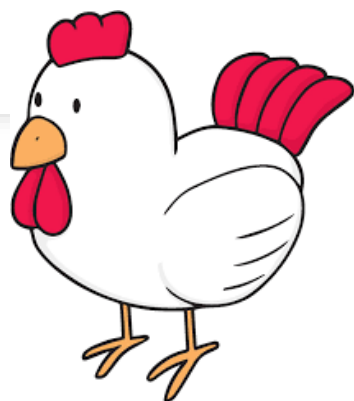
AMR Profile



Where we are at – Bigger Picture.....



Systemic (Diseased)
530 isolated
106 sequenced



Cecal (Healthy)
149 isolated
74 sequenced



Environmental (Farm sites)
216 isolated
120 sequenced



Are there alternatives to use of antibiotics??



British Poultry Science

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Antimicrobial peptides as an alternative to relieve antimicrobial growth promoters in poultry

N. Nazeer, S. Uribe-Diaz, J. C. Rodriguez-Lecompte & M. Ahmed

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Review > Br Poult Sci. 2021 Oct;62(5):672-685. doi: [10.1080/00071668.2021.1919993](https://doi.org/10.1080/00071668.2021.1919993).

Epub 2021 May 14.

Antimicrobial peptides as an alternative to relieve antimicrobial growth promoters in poultry

N Nazeer¹, S Uribe-Diaz^{1,2}, J C Rodriguez-Lecompte², M Ahmed¹

Affiliations + expand

PMID: 33908289 DOI: [10.1080/00071668.2021.1919993](https://doi.org/10.1080/00071668.2021.1919993)

Abstract

1. This review describes different classes of antimicrobial peptides (AMP) found in the gastrointestinal (GI) tract of avian species, and their antimicrobial and immunomodulatory activities. The potential benefits of synthetic AMP in poultry production are examined, in the context of the use of AMP as alternatives to antimicrobial growth promoters (AGP). 2. Since the mid-1950s, antibiotic growth promoters (AGP) have been used in feed at low prophylactic doses to modulate the homeostasis of intestinal microbiota, decreasing the risk of intestinal dysbacteriosis and the growth of pathogens



ELSEVIER

Poultry Science

Volume 99, Issue 12, December 2020, Pages 6481-6492



Immunology, Health and Disease

Effects of antibacterial peptide combinations on growth performance, intestinal health, and immune function of broiler chickens

Zi Xie^{*,†,‡,1}, Qi Qi Zhao^{†,‡,1}, Huan Wang^{†,‡,1}, Lijun Wen[§], Wei Li[§], Xinheng Zhang^{†,‡}, Wencheng Lin^{†,‡}, Hongxin Li^{†,‡}, Qingmei Xie^{*,†,‡}, Yan Wang^{*,‡}

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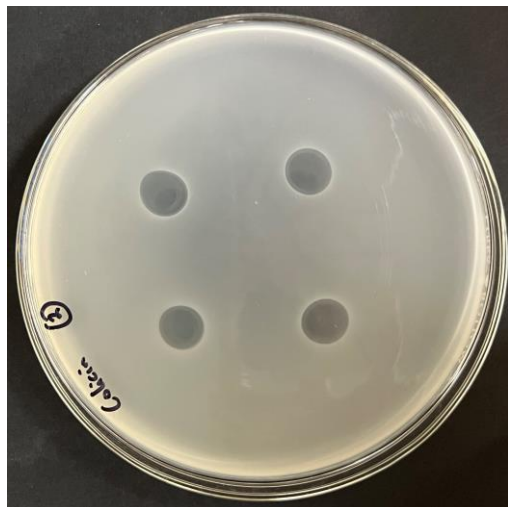


OPEN Effects of cLFchimera peptide on intestinal morphology, integrity, microbiota, and immune cells in broiler chickens challenged with necrotic enteritis

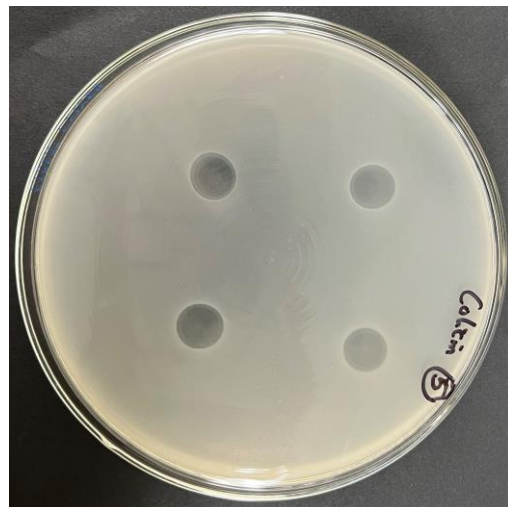
Ali Daneshmand¹, Hassan Kermanshahi², Mohammad Hadi Sekhavati³, Ali Javadmanesh, Monireh Ahmadian, Marzieh Alizadeh & Ahmed Aldawoodi

Three hundred and sixty 1-day-old male broiler chicks were randomly allocated to 4 treatments of 6 replicates to evaluate the effects of cLFchimera, a recombinant antimicrobial peptide (AMP), on gut health attributes of broiler chickens under necrotic enteritis (NE) challenge. Treatments were as follows: (T1) unchallenged group fed with corn-soybean meal (CSM) without NE challenge and additives (NC); (T2) group fed with CSM and challenged with NE without any additives (PC); (T3) PC group supplemented with 20 mg cLFchimera/kg diet (AMP); (T4) PC group supplemented with 45 mg antibiotic (bacitracin methylene disalicylate)/kg diet (antibiotic). Birds were sampled for villi morphology, ileal microbiota, and jejunal gene expression of cytokines, tight junction proteins, and mucin. Results showed that AMP ameliorated NE-related intestinal lesions, reduced mortality, and rehabilitated jejunal villi morphology in NE challenged birds. While the antibiotic non-selectively reduced the count of bacteria, AMP restored microbial balance in the ileum of challenged birds. cLFchimera regulated the expression of cytokines, junctional proteins, and mucin transcripts in the jejunum of NE challenged birds. In conclusion, cLFchimera can be a reliable candidate to substitute growth promoter antibiotics, while more research is required to unveil the exact mode of action of this synthetic peptide.

a



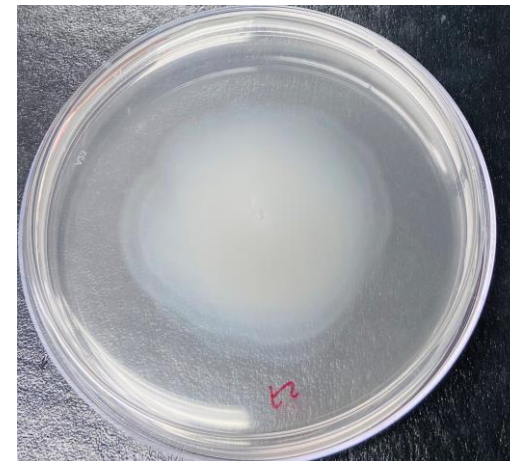
b



c



d



Colicin Production (a-c)

- Peptides and proteinous antibiotics produced and released by some strains of *E. coli* to reduce competition and kills very closely related strains

Swimming Motility (d)

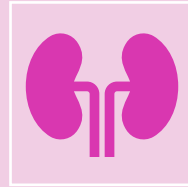
- Flagella mediated movement, helps bacteria in migration and colonization of host

Future Directions

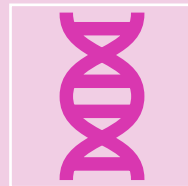


Dr. Sanderson

10/6/2022



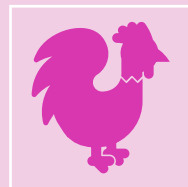
Set up pipeline for further Phenotypic Characterization – Colicin Production, Serum Resistance, Aerobactin production (with Dr Wolfgang) – September 2022



Genome analysis and comparison (Dr. Haley Sanderson)

Genome comparisons – Environmental vs. disease vs. cecal

Plasmid recovery and analysis (Sanderson, Nnajide et al.,)



Chicken Infection Experiments – Maybe Alternatives to antibiotics Antibiofilm?? Anti-adherence???

Chinenye Nnajide

Summary

Antibiotic-resistance

- Systemic > Environmental > Cecal
- A lot of diversity and no absolute patterns

Whole genome sequencing

- 120 Environmental, 106 systemic, 74 cecal>>>(300)
- Hope to be completed with finished genomes by November

Preliminary Virulence, AMR gene and Plasmid screen

- Systemic > Cecal24 strains (Plasmid paper)
- Repeat for our big project

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Poultry Extension (U of S)

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- Tennille Knezacek
- Nikki Storbakken

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- Dr. Yejun Wang (China)
- Dr. Wolfgang Koester (VIDO)

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White's Lab - VIDO

Chinenye Nnajide

