



The Tricycle Project: WHO Integrated Global Surveillance on ESBL Producing *E. coli* using “One Health” Approach

**Shivaramu Keelara¹, DVM, PhD, Paula J. Fedorka-Cray¹, PhD,
Megan Jacob¹, PhD and Jorge Matheu², MS,**

¹North Carolina State University, College of Veterinary Medicine, Raleigh, NC

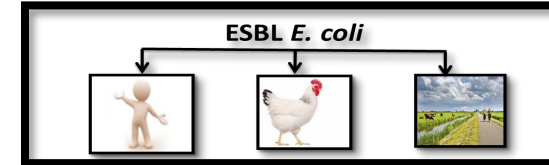
²World Health Organization, Geneva, Switzerland



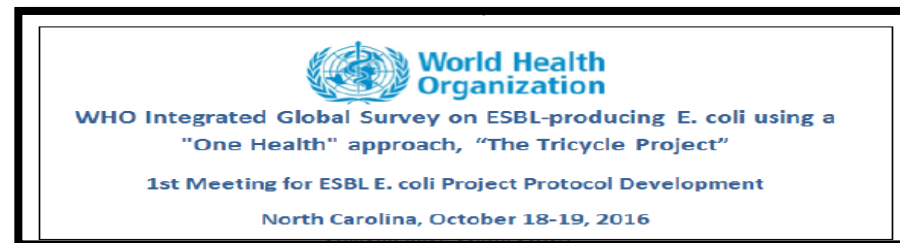
The ESBL *E. coli* Tricycle Project



- WHO integrated global surveillance on ESBL-producing *E. coli* using “One Health” approach -Humans, Animals and the Environment

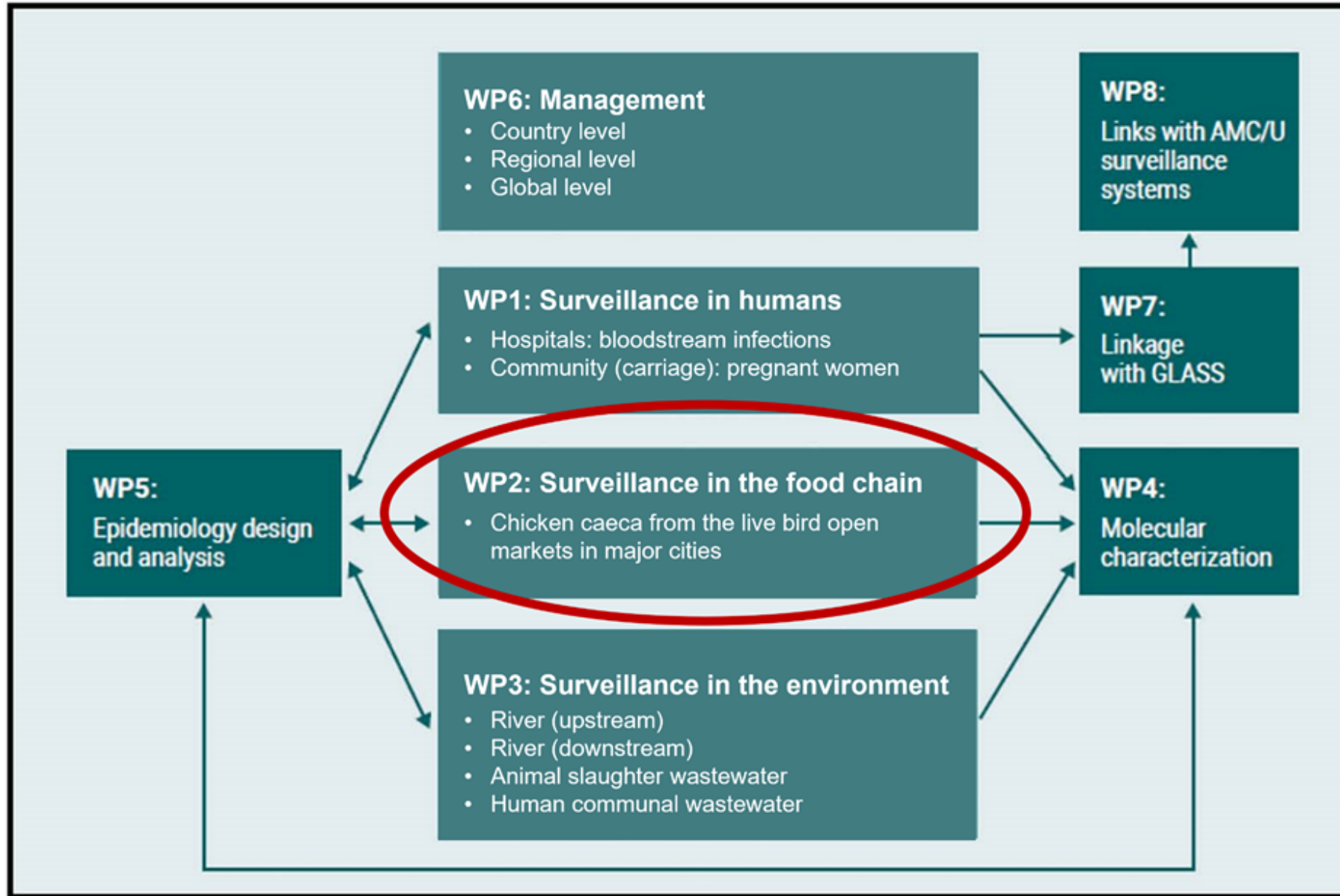


- Establish a simple and standardized methodology to isolate and monitor ESBL producing *E. coli*
- Compare the prevalence of ESBL *E. coli* at regional, national and global levels and develop intervention strategies
- WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) experts- conceptualized an idea to address the knowledge gap





The ESBL E. coli Tricycle -Structure





CVM-NCSU: WHO Collaborating Centre for Global One Health and Antimicrobial Resistance Initiatives



Our role: Implementation of the ESBL-producing *E. coli* global surveillance in Member States

- Development of protocol for isolation of ESBL *E. coli* from animals
- Supported procurement of essential laboratory supplies
 - Cameroon, Ghana, Indonesia, Malaysia, Pakistan, Sudan and Zimbabwe
- Facilitate workshops to train participants from human, animal and the environmental sectors
 - Quality control; Isolation and identification of ESBL *E. coli*
 - Antimicrobial susceptibility testing and data interpretation
- Implementation and laboratory capacity assessment visits



Optimization of ESBL *E. coli* Isolation Protocol



- Developed countries - automated equipment, molecular inference, and specialized chromogenic media
- Developing low- and middle-income countries (LMIC) need reliable, readily available, and cost-effective solutions
- **MacConkey agar** - reasonable cost and availability, familiarity in human and veterinary clinical settings, and relatively simple selectivity and interpretation
- Cefotaxime and Ceftriaxone at 2 & 4 µg/ml concentration

> J Clin Microbiol. 2020 Aug 24;58(9):e01039-19. doi: 10.1128/JCM.01039-19. Print 2020 Aug 24.

Optimizing a Screening Protocol for Potential Extended-Spectrum β -Lactamase *Escherichia coli* on MacConkey Agar for Use in a Global Surveillance Program

Megan E Jacob ^{# 1}, Shivaramu Keelara ^{# 1}, Awa Aidara-Kane ², Jorge R Matheu Alvarez ², Paula J Fedorka-Cray ³





Optimization of ESBL *E. coli* Isolation Protocol

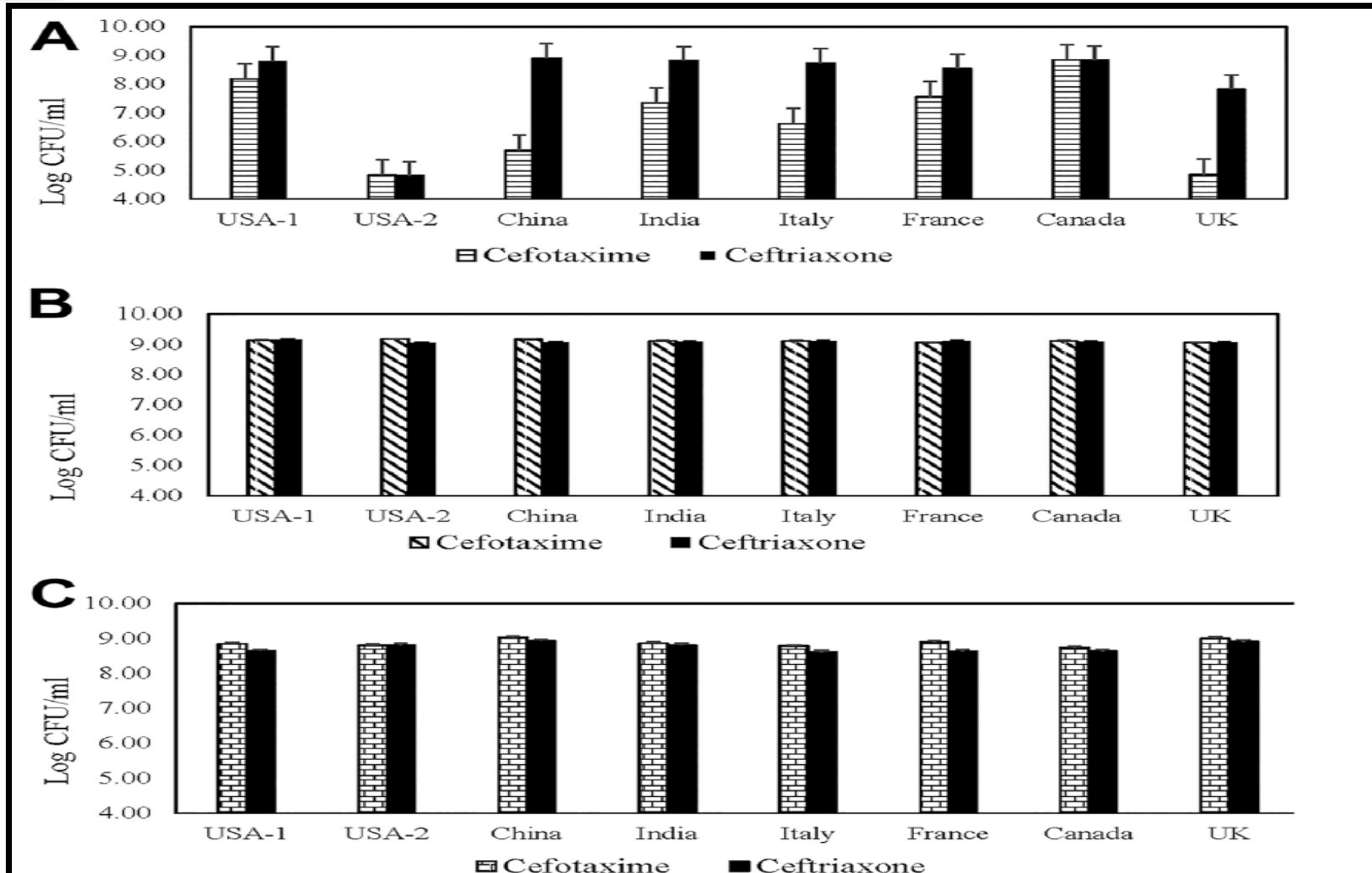


FIG 1 Concentration recovered and standard errors for *E. coli* 13457 (A), *E. coli* 10455 (B), and *Klebsiella pneumoniae* 11073 (C) on MacConkey agar from various manufacturers, representing seven countries, supplemented with 4 μ g/ml cefotaxime or 4 μ g/ml ceftriaxone.



Optimization of ESBL *E. coli* Isolation Protocol

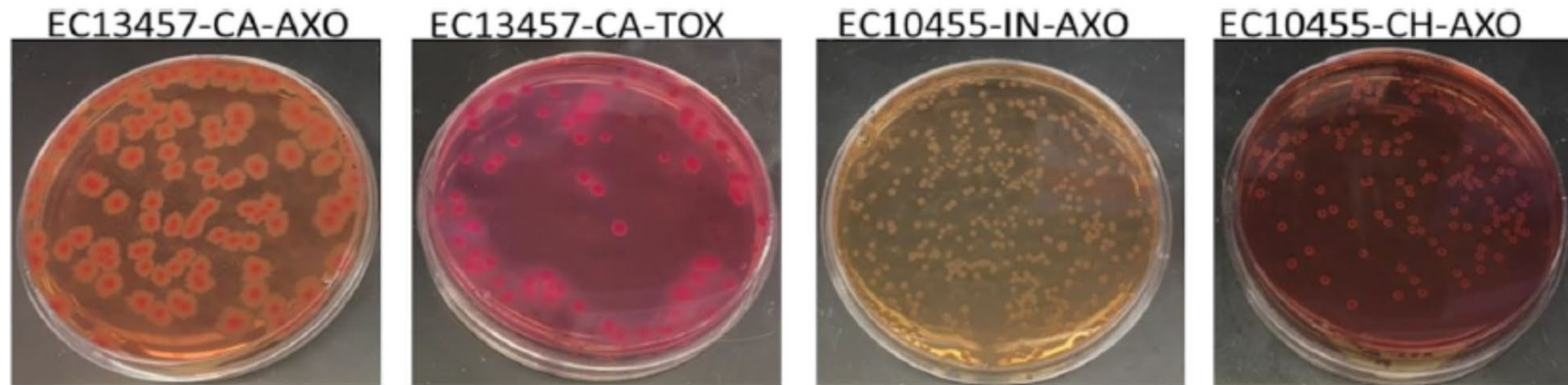


FIG 2 Phenotypic appearance of pure cultures of *Escherichia coli* (EC) 13457 and *E. coli* 10455 on MacConkey agar manufactured in Canada (CA), India (IN), and China (CH) supplemented with 4 μ g/ml either cefotaxime (TOX) or ceftriaxone (AXO).

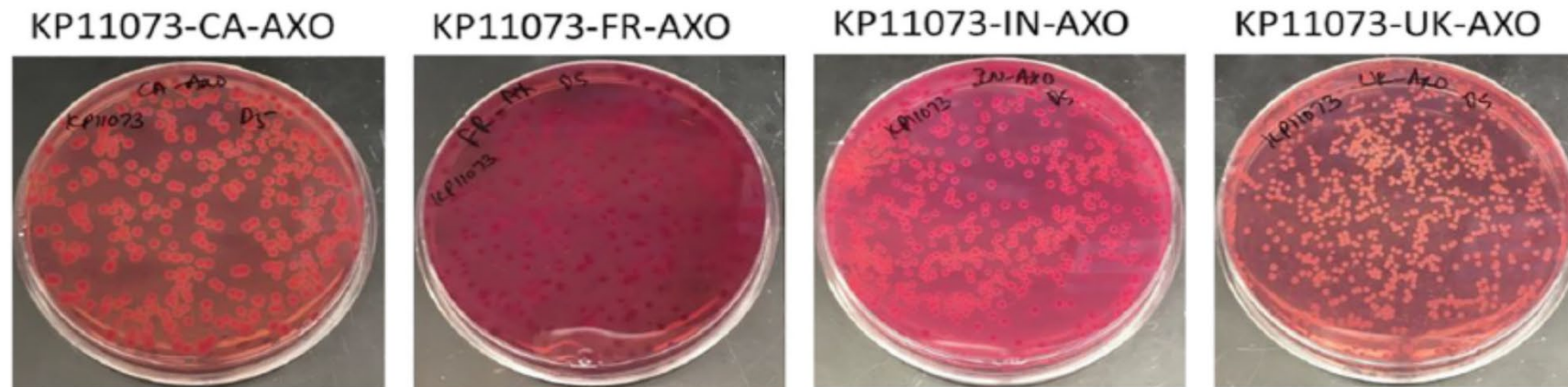


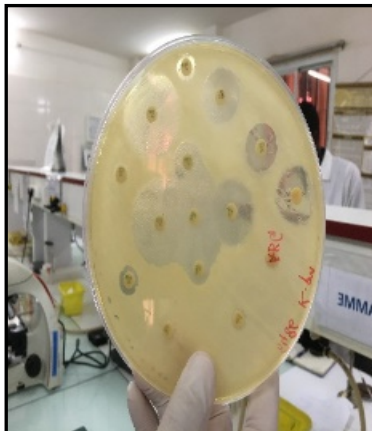
FIG 3 Phenotypic appearance of pure cultures of *Klebsiella pneumoniae* (KP) on MacConkey agar manufactured in Canada (CA), India (IN), France (FR), and the United Kingdom (UK) supplemented with 4 μ g/ml ceftriaxone (AXO).



NCSU-WHO Collaborating Center Activities



- Utrecht, Netherland (July 31–August 4, 2017)
 - Indonesia, Malaysia, Ghana, Pakistan and Srilanka
- Jakarta, Indonesia (November 2-11, 2017)
 - Indonesia, India and Malaysia
- Johannesburg, South Africa (October 15-19, 2018)
 - South Africa, Lesotho, Botswana, Zimbabwe and Eswatini
- Amman, Jordan (January 20-28, 2018)
 - Jordan, Egypt, Morocco, Sudan and Iran
- Implementation and laboratory capacity assessment visits (Ghana, Senegal, Indonesia, and Malaysia)





Tricycle Project Implementation



Region	Countries Implemented
AFRO	Ghana, Senegal, Madagascar
EMRO	Pakistan, Jordan
SEARO	Indonesia, India, Nepal
WPRO	Malaysia

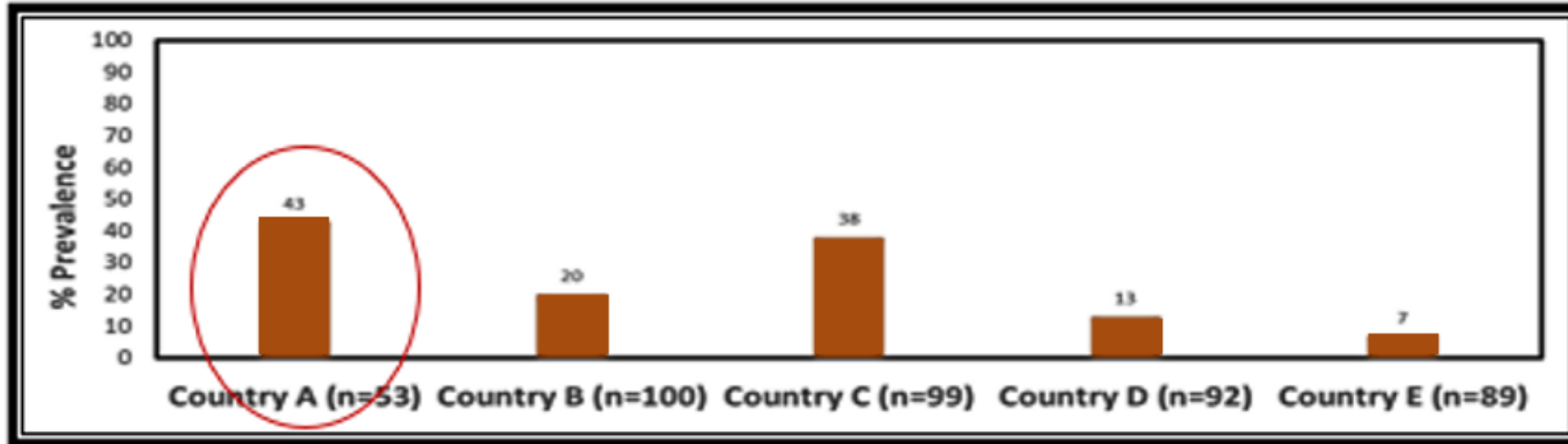
Region	Countries joining in 2021-2022
AFRO	Zimbabwe, Cameroon, Zambia, Morocco, Nigeria, Burkina Faso
EMRO	Morocco, Iran and Sudan
SEARO	Bhutan



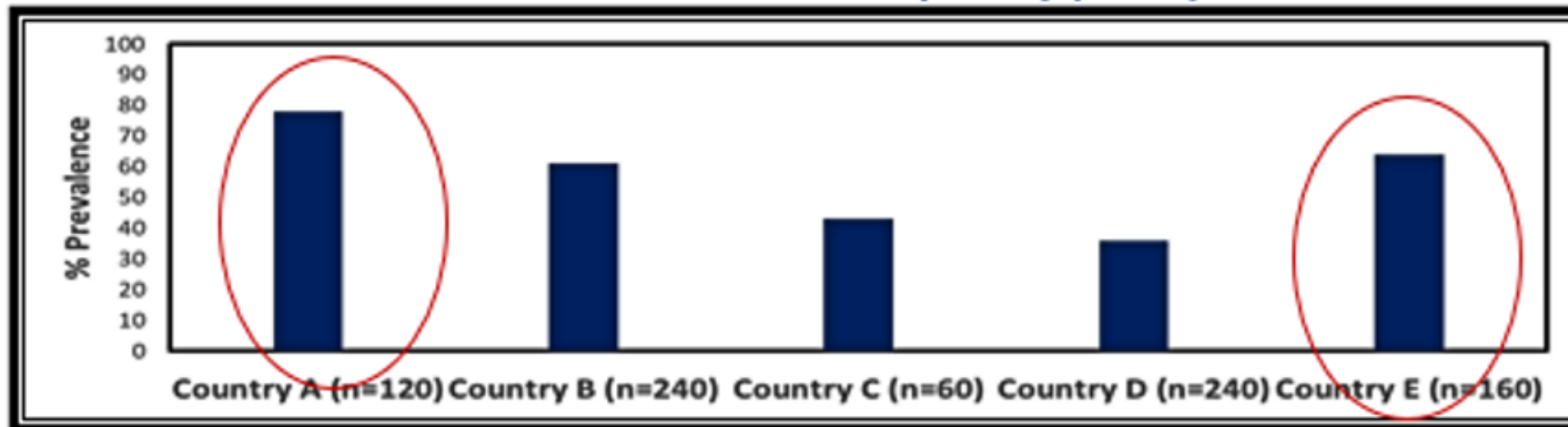
Community and Food Chain Results



Prevalence of ESBL *E. coli* in pregnant woman (Feces)

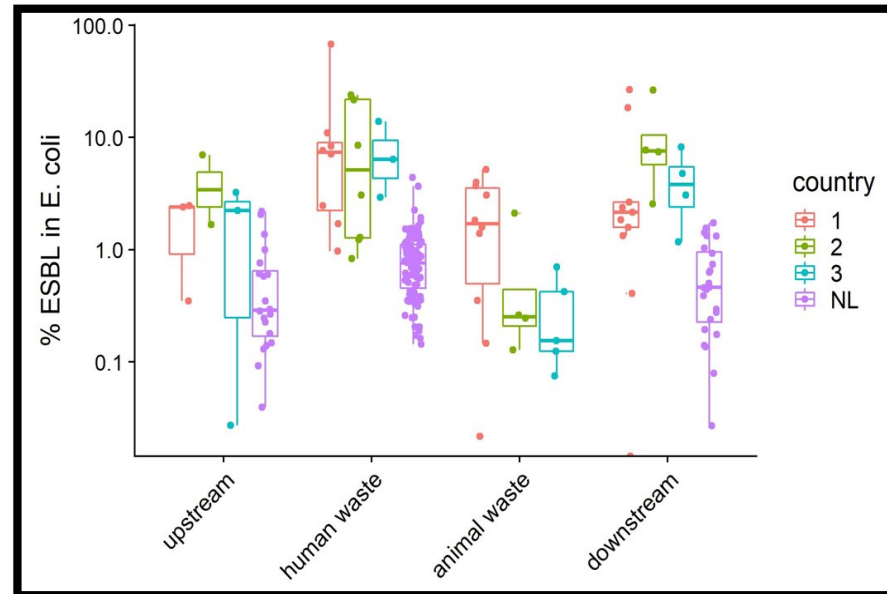
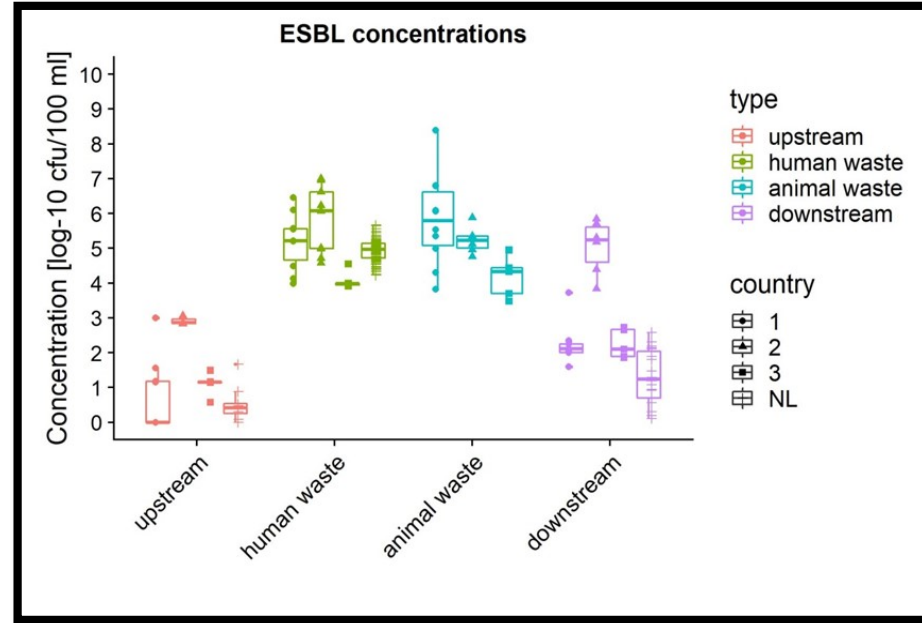
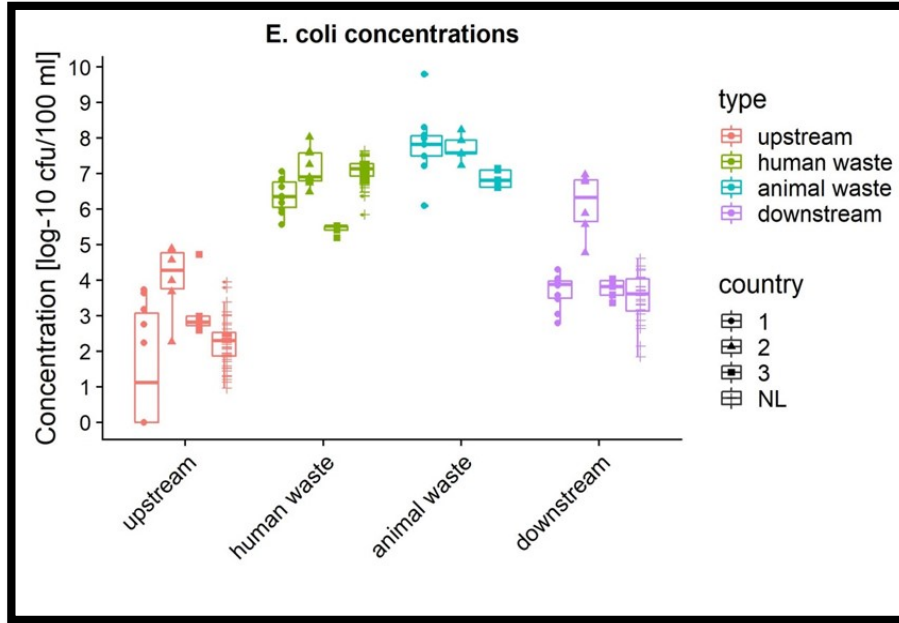


Prevalence of ESBL *E. coli* in poultry (Feces)





Environment Results





Summary and Future Work



- CVM-NCSU played a major role in optimization of ESBL protocol for animal samples and continues to support WHO Global Tricycle surveillance
- Successfully implemented Tricycle project in nine countries
 - Many more to add in the coming years
- The Tricycle project will enable the global community to establish a baseline surveillance system for AMR at the country level using a “One Health” approach
- The study results/data from this harmonized project can be compared at the global level
 - Global mitigation strategies to combat AMR can be planned
- Similar “One Health” approach can be extended to monitor other emerging infectious diseases and pathogens



Thank you and Questions?

