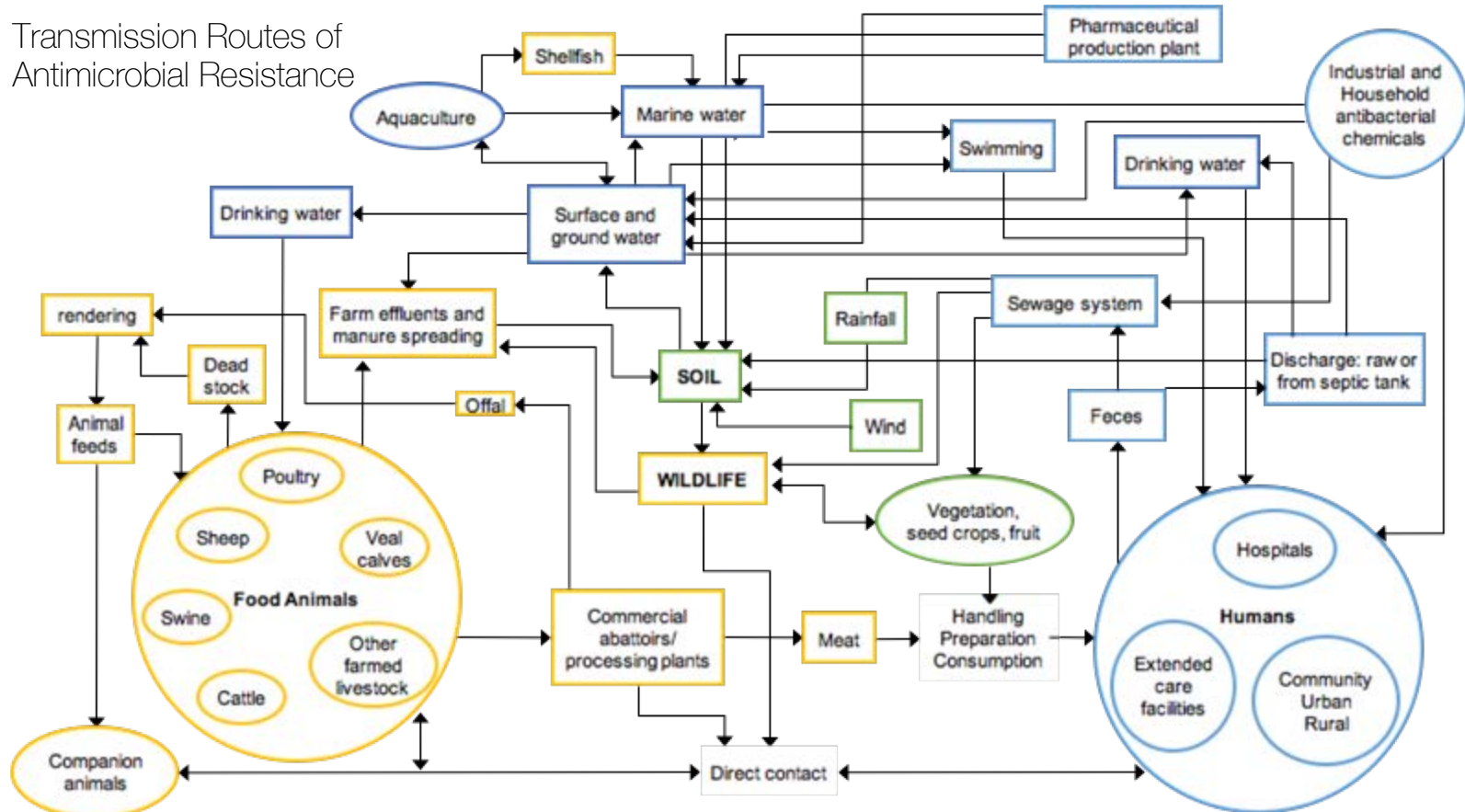


Prevalence Of Antimicrobial Resistance Genes In Nepal Using A One Health Approach

Cristin Young, Dibesh Kharmacharya, Manisha Bista, Ajay Sharma, Tracey Goldstein, Simon Anthony, Duncan Temple-Lang, Jonna Mazet, Woutrina Smith, and Christine Kreuder Johnson

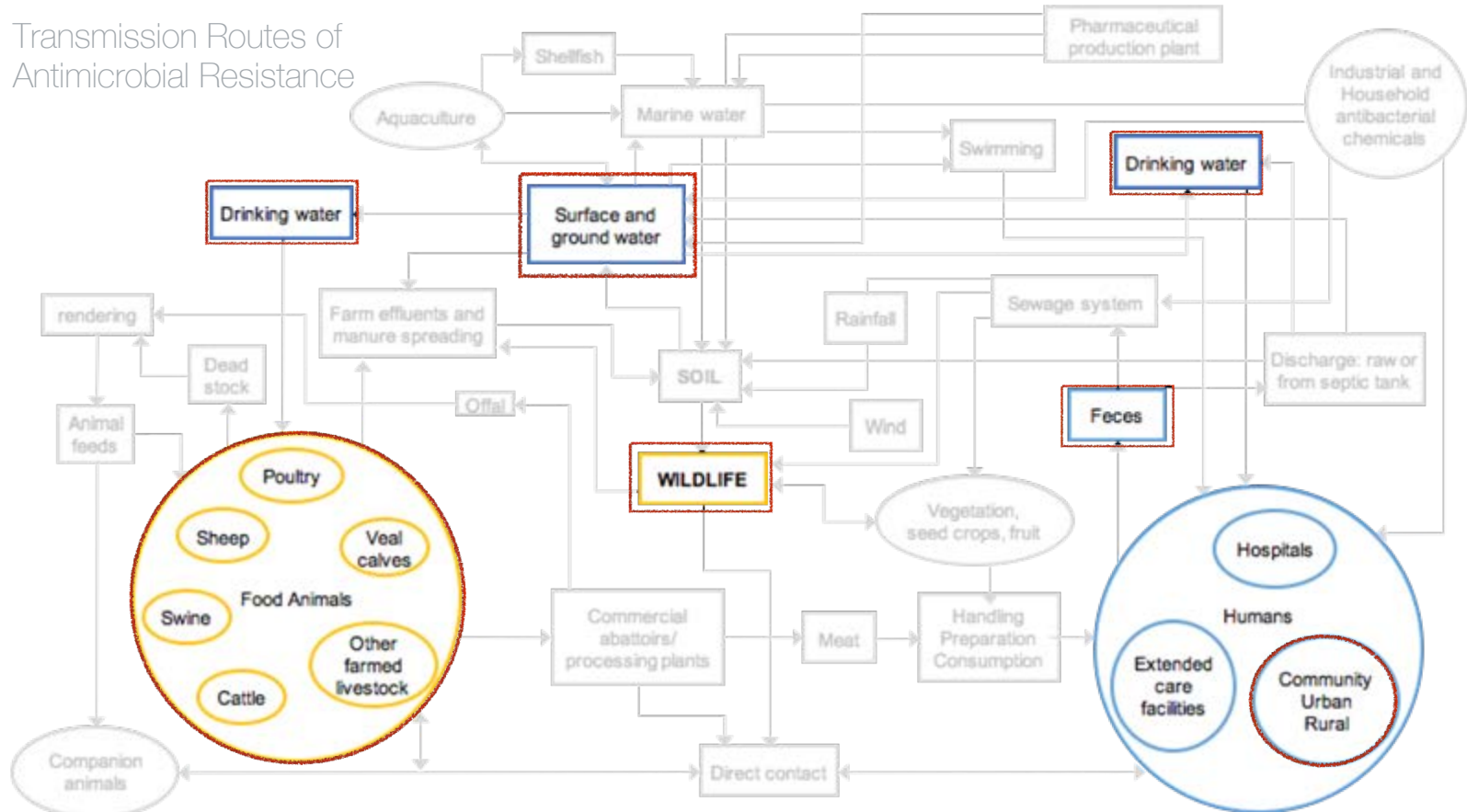


Transmission Routes of Antimicrobial Resistance



Linton AH. (1977). Antibiotic resistance: the present situation reviewed. Vet Rec. Apr 23;100(17):354-60.

Transmission Routes of Antimicrobial Resistance

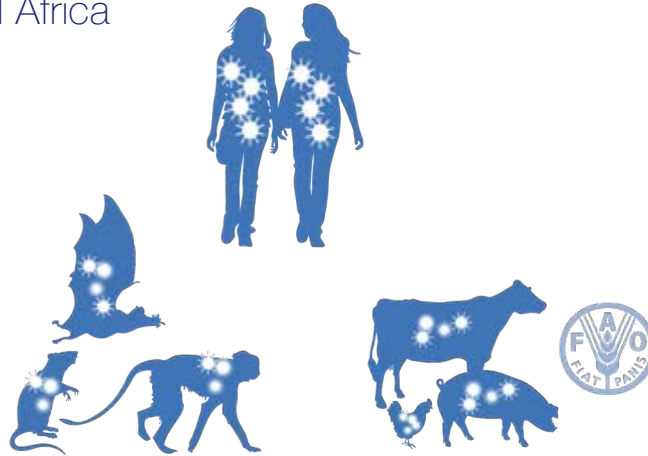


Linton AH. (1977). Antibiotic resistance: the present situation reviewed. Vet Rec. Apr 23;100(17):354-60.

PREDICT Project

- USAID Emerging Pandemic Threats program
- One health surveillance strategy for emerging zoonotic viruses in 30 countries in Asia and Africa

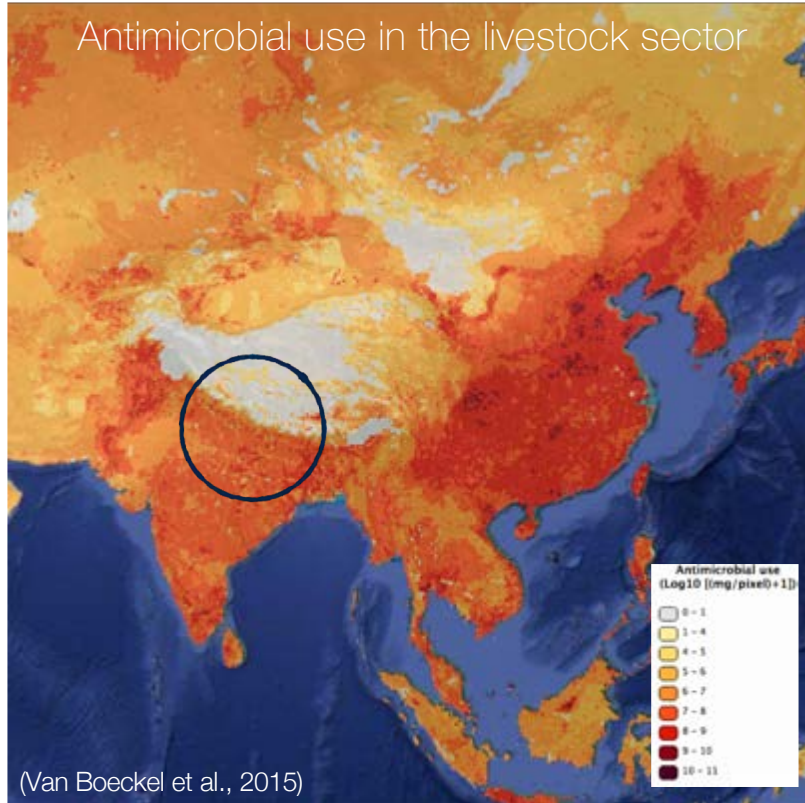
Concurrent
sampling of
wildlife, livestock,
and people



Identify cross-species transmission and spillover



Antimicrobial use in the livestock sector



(Van Boeckel et al., 2015)

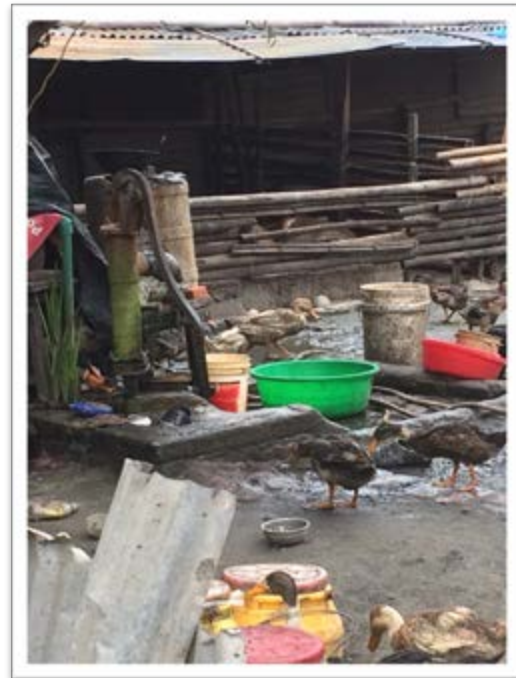
Why Nepal?

- Widespread use in humans and animals
- Inappropriate and frequent prescribing
- High prevalence of resistance

Jadibuti
Kathmandu, Nepal





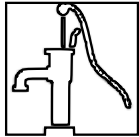


STUDY DESIGN

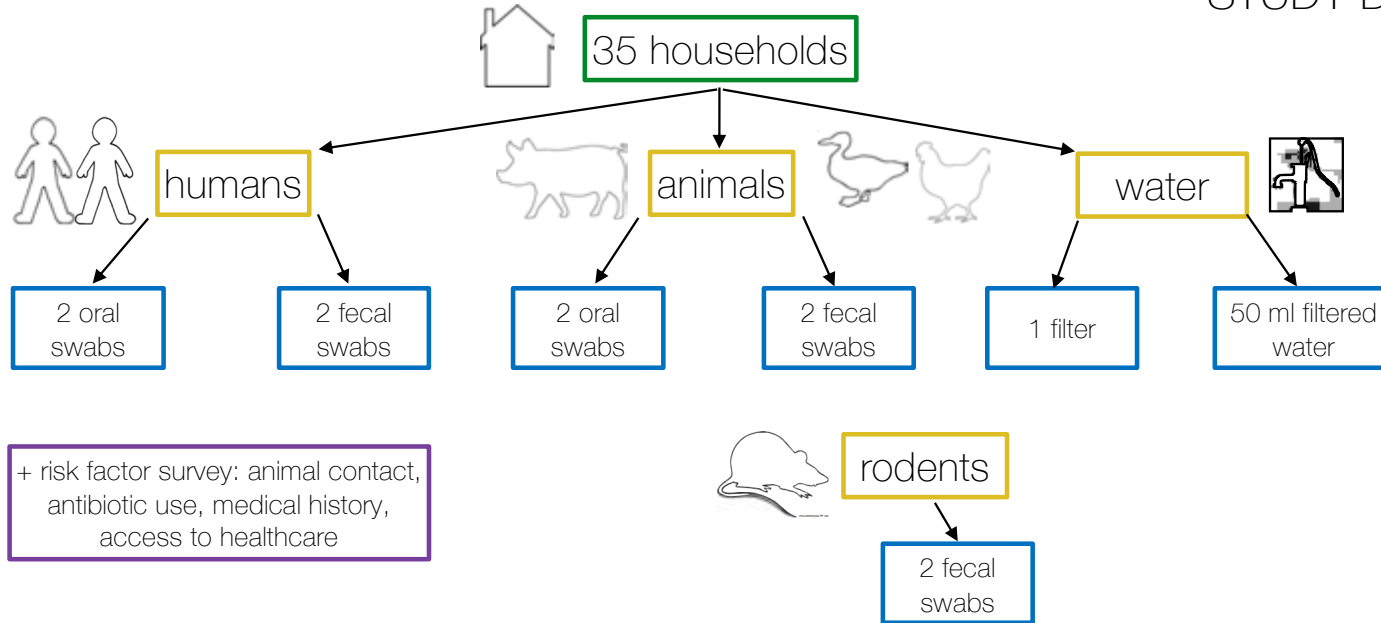
Objectives:

- Characterize resistome of community
- Examine highest concentrations of genes

-
- A diagram showing a water pump on the left with a hose leading to a series of animals. The animals are arranged in a line: a chicken, a cow, a pig, and a mouse. Arrows indicate the flow of water from the pump to each animal. The pump is labeled 'Pump' and the hose is labeled 'Hose'.



STUDY DESIGN



Total humans
n=67

Total animals
n=77

Total water
n=17

Laboratory Analysis

QIAGEN microbial DNA qPCR array for 87 resistance genes

- Aminoglycoside, β -lactam, erythromycin, fluoroquinolone, macrolide-lincosamide-streptogramin B, tetracycline, vancomycin, and multidrug resistance classification groups
- Ct < 34 : positive
- Ct 34-37 : inconclusive
- Ct > 37 : negative
 - For analysis, coded inconclusive as negative

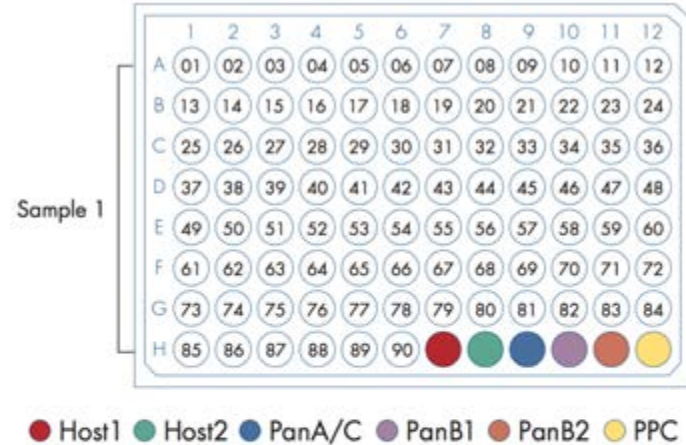
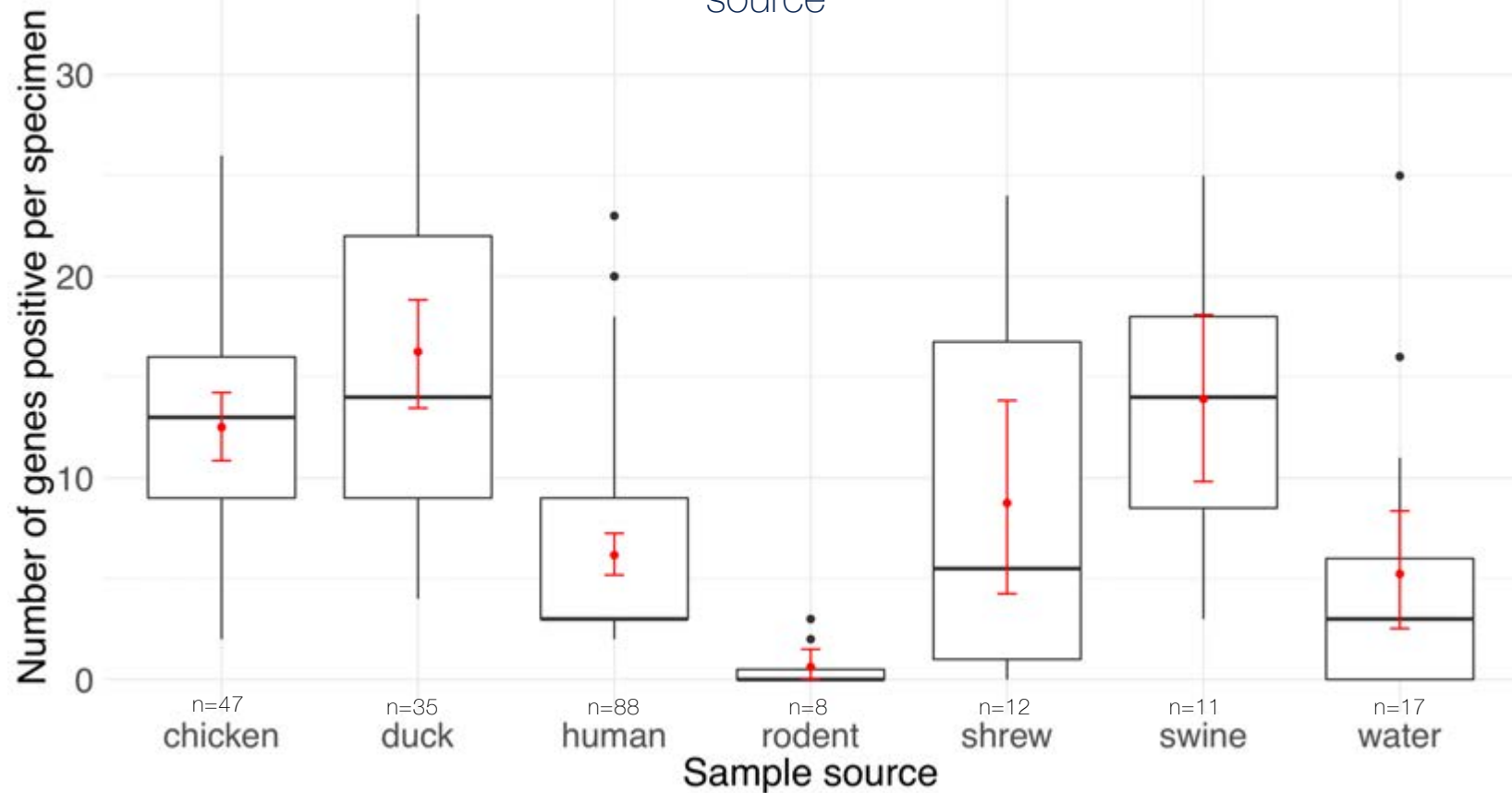


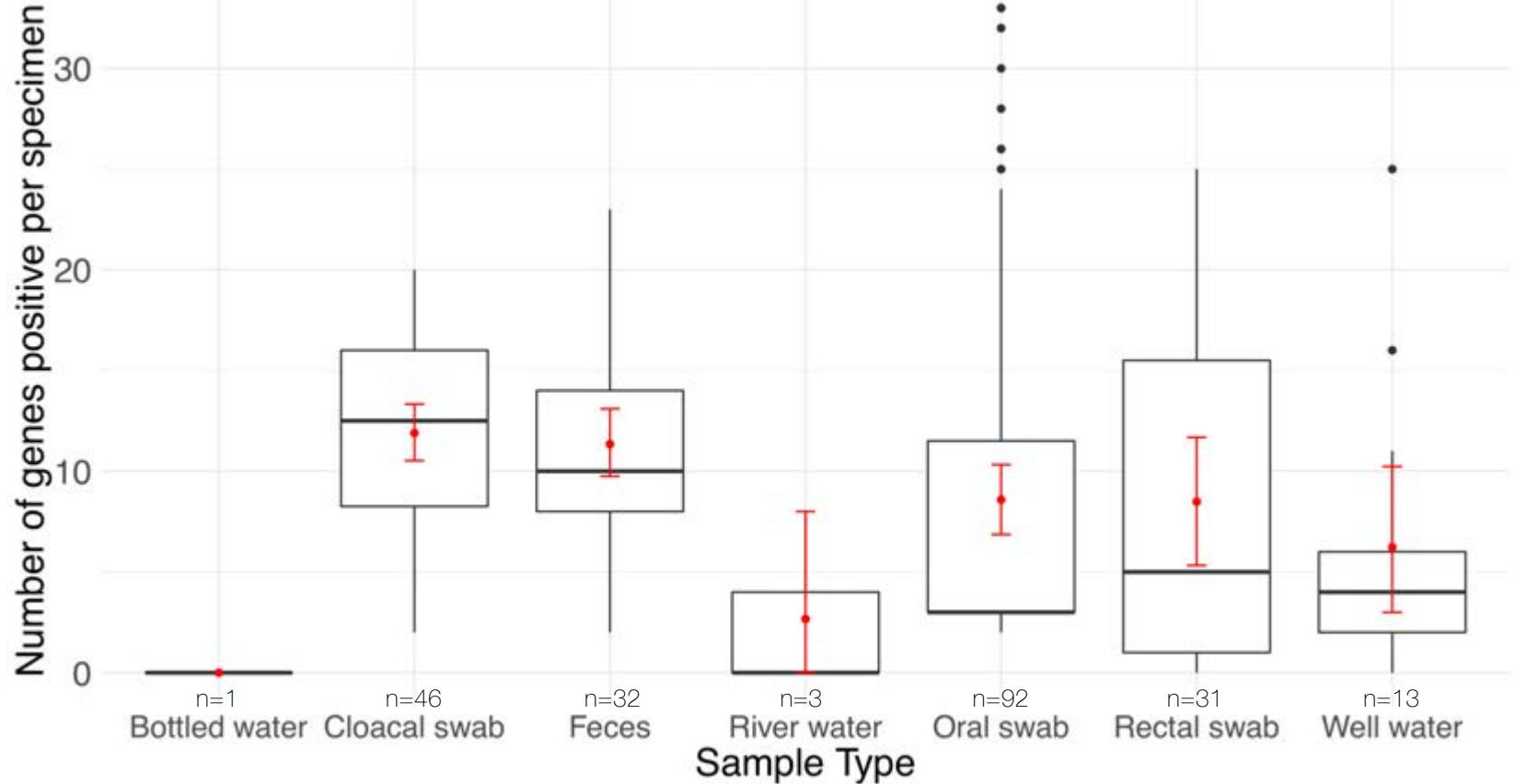
Figure 1. Sample Microbial DNA qPCR Array plate. This array plate format tests one sample with 90 microbial assays. Each well contains one unique test. Also included are controls for host DNA, pan-fungal DNA, pan-bacterial DNA and a positive PCR control.

<https://qiagen.com/us/shop/pcr/primer-sets/microbial-dna-qpcr-arrays/?catno=BAID-1901Z#resources>

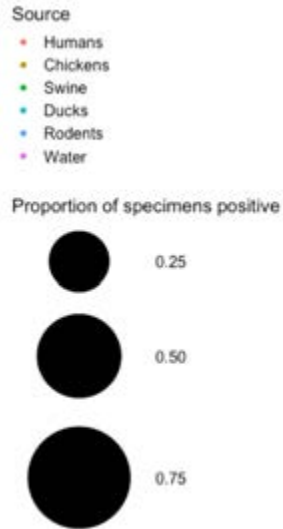
Number of antimicrobial resistance genes positive per specimen by sample source



Number of antimicrobial resistance genes positive per specimen by sample type



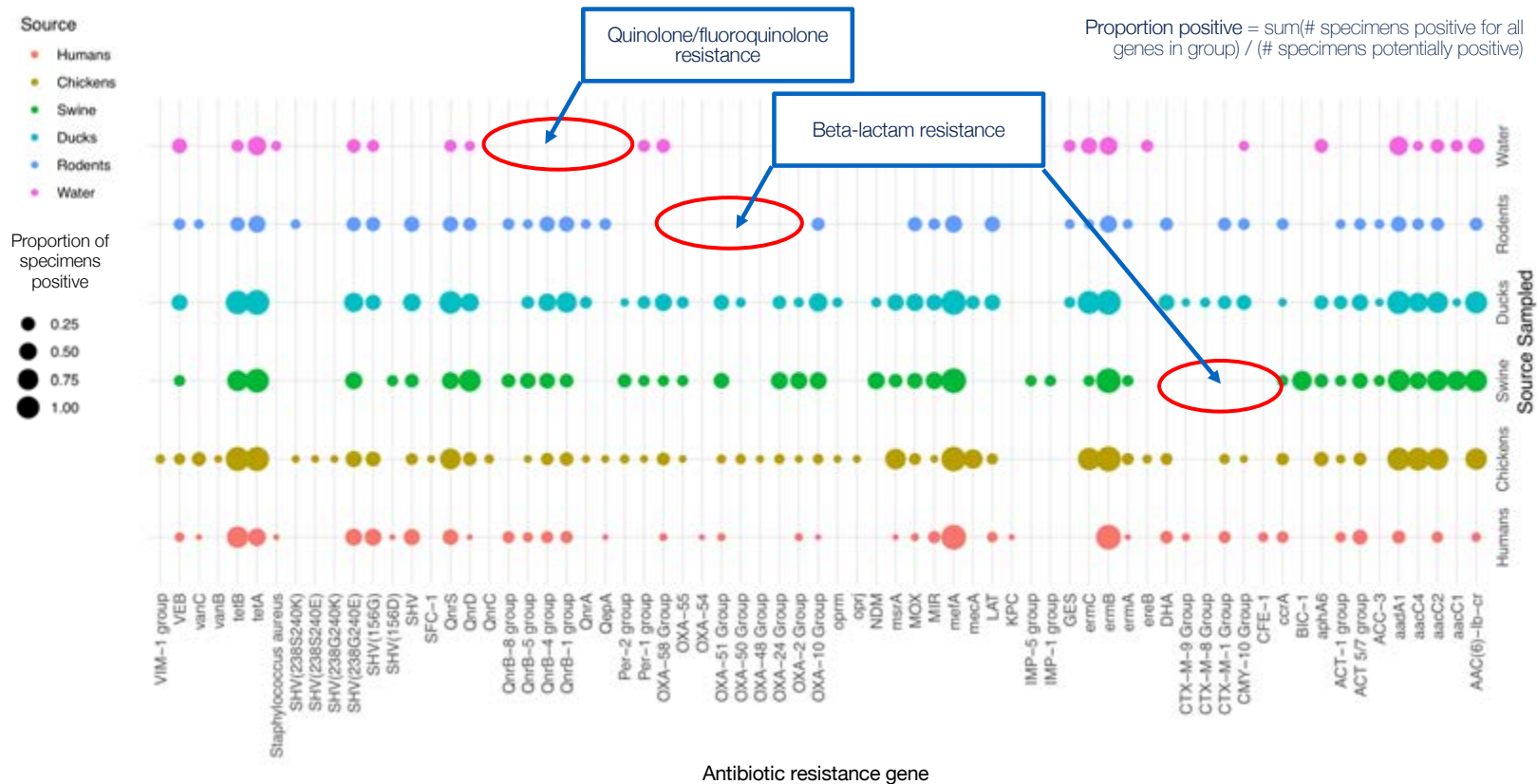
Proportion of samples positive by source sampled and antibiotic resistance classification group



Proportion positive = $\frac{\text{sum}(\# \text{ specimens positive for all genes in group})}{(\# \text{ specimens potentially positive} * \text{number of genes in group})}$



Proportion of samples positive by source sampled and antibiotic resistance genes

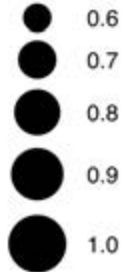


Proportion of samples positive (>0.50) by source sampled and antibiotic resistance genes

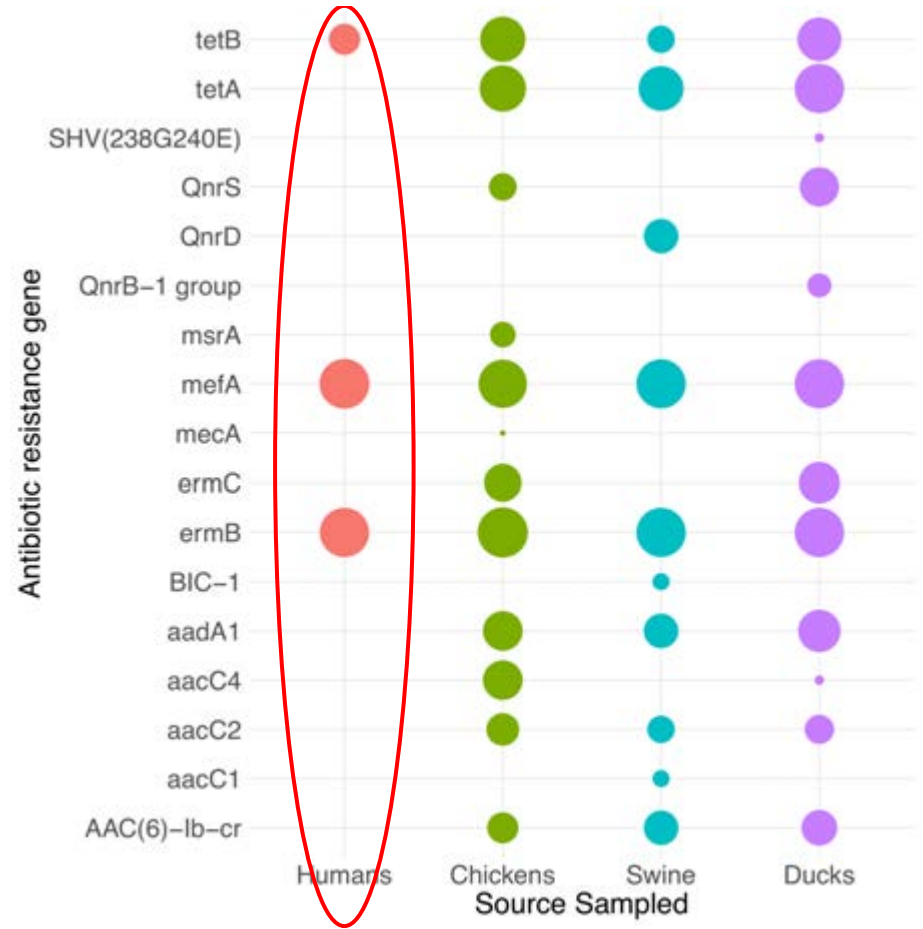
Source

- Humans
- Chickens
- Swine
- Ducks

Proportion of specimens positive



Proportion positive = $\frac{\text{sum}(\# \text{ specimens positive for all genes in group})}{\# \text{ specimens potentially positive}}$

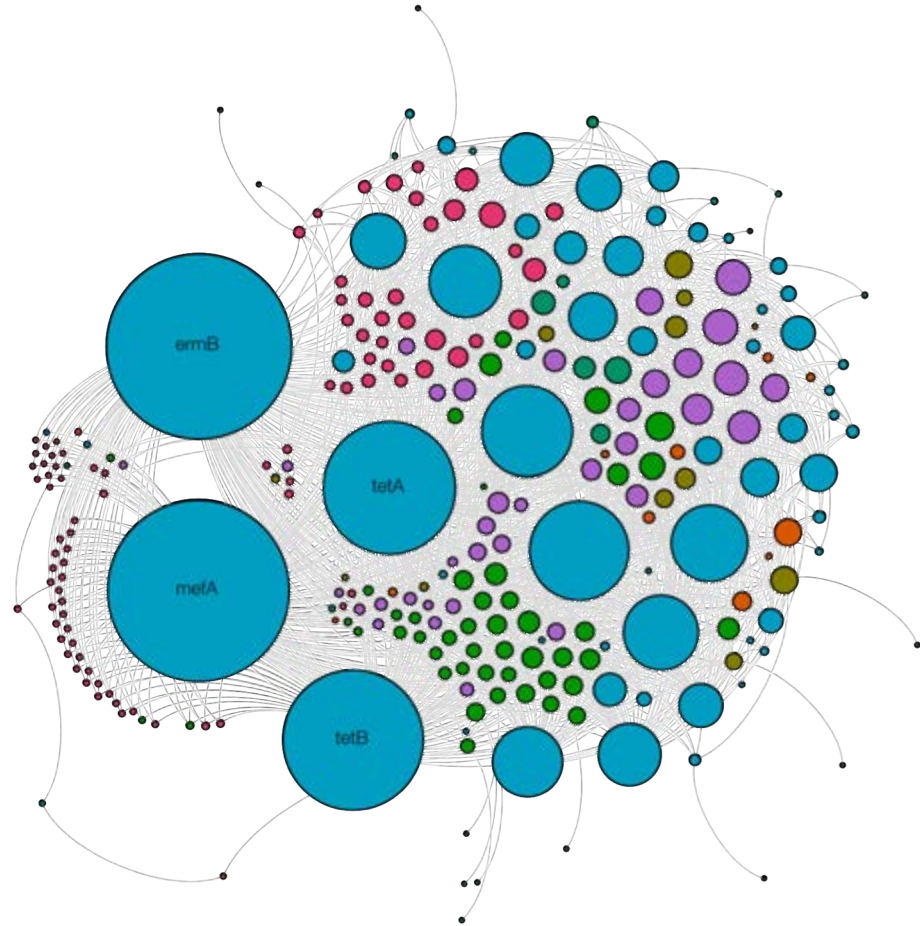


Antimicrobial resistance gene bipartite network analysis

Legend

- human
- gene
- chicken
- duck
- water
- shrew
- swine
- rodent

Note: color based on sources sampled
and size based on network degree





Conclusions

- *ermB*, *mefA*, *tetA*, *tetB* most commonly detected genes
 - Antibiotics associated with these genes widely used in Nepal (azithromycin, doxycycline)
- Prevalence patterns:
 - Gene prevalence varied markedly by species and sample type
 - Detection of same gene among different species was widespread
- Next steps:
 - Culture-based methods + metagenomics to characterize bacterial reservoirs
 - Increased wildlife and environmental sampling

Many thanks to:

- Center for Molecular Dynamics, Nepal lab and field teams
- One Health Institute lab team
- PREDICT project

