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Study of ESBL-producing *E. coli* in hospitalised horses

EMMA WINWARD

SUPERVISORS: DR GINA PINCHBECK & PROF NICOLA WILLIAMS

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INTRODUCTION: AMR

- One of the greatest challenges in both human and veterinary medicine. Multidrug resistance (MDR) is defined as resistance to 3 or more classes of antimicrobial.
- Resistance may evolve via random mutation or horizontal transfer of mobile genetic elements
- Use of antimicrobials creates a selection pressure for promotion and dissemination of various resistance mechanisms
- **Acquired resistance** can act by:
  - Decreasing drug concentration (↓permeability or upregulating efflux pump)
  - Modification of target site via mutation or protection
  - Inactivation of antimicrobial via **hydrolysis** or **modification**

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INTRODUCTION: *E. COLI*

- Gram negative bacteria belonging to family *Enterobacteriaceae* found commonly as a commensal of the gastrointestinal tract in humans and animals
- Some strains are pathogenic, causing either gastrointestinal or extra-intestinal disease, although commensal strains pose a threat to immunocompromised individuals
- As part of normal GI microflora, inevitably exposed to any antimicrobials administered for clinical infections, thus driving selection pressure for resistance
- Recombination and horizontal gene transfer lead to high genetic diversity

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INTRODUCTION: AMR IN *E. COLI*

- Wide range of chromosomal and plasmid-mediated resistance to various classes of antimicrobials reported
- β-lactams are a widely used class of antimicrobials including **penicillins, cephalosporins, monobactams** and **carbapemens**
- **β-lactamase production** is a type of resistance that hydrolyses the beta-lactam ring inactivating the antimicrobial. Resistance to penicillins, oxyimino-cephalosporins and monobactams
- Genes for β-lactamase production are located on plasmids

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INTRODUCTION: AMR IN *E. COLI*

- Most commonly, **extended-spectrum β-lactamase** (ESBL) producing *E. coli* contain TEM, SHV and OXA enzymes

- In 1988, ESBL-producing *E. coli* were discovered which contain **CTX-M enzymes**. These are encoded by ***bla*** genes located on plasmids and are intrinsically resistant to **cephalosporins**

- Since then, over **160 CTX-M enzymes** have been identified across multiple bacterial genera (including *Escherichia, Klebsiella* and *Enterobacter*)

- **5 clusters**: CTX-M1, CTX-M2, CTX-M8, CTX-M9 and CTX-M25
  - Based on amino acid sequence

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INTRODUCTION: ESBL-PRODUCING *E. COLI*

- **CTX-M-15** (belonging to **Group 1**) is the most prevalent ESBL enzyme isolated in human clinical infections, with MDR *E. coli* clone O25:H4-ST131 showing global dissemination

- **CTX-M-1** (Group 1) and **CTX-M-14** (Group 9) predominate in horses and livestock faeces in the UK

- Maddox *et. al* (2009) identified ESBL-producing *E.* coli in **27.3%** of faecal samples from hospitalised horses, 16.8% of which were **Group 1** positive. At the time they were assumed to be CTX-M-1

- More recent work has identified **CTX-M-15** in equine faecal samples after testing positive as Group 1

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PROJECT AIMS

- Investigate the prevalence of *bla*$_{\text{CTX-M-1}}$ and *bla*$_{\text{CTX-M-15}}$ genes in previously identified Group 1 positive *E. coli* isolates from hospitalised horses during two different time periods.

- Compare collections of Group 1 positive *bla*$_{\text{CTX-M}}$ *E. coli* isolates from two cohorts of horses at the same hospital and investigate changes in genotype and epidemiology.

- Investigate the mobility of ESBL genes via conjugation experiments and replicon typing to determine any patterns in ESBL and AMR transfer.

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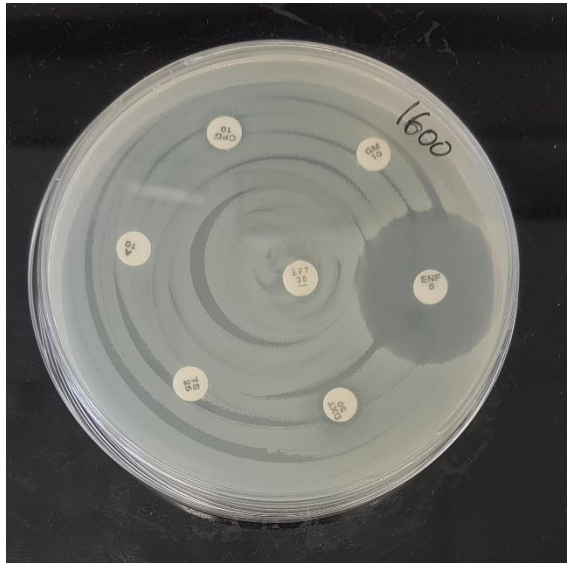
METHODS: ESBL ISOLATION

- Faecal samples were collected daily from inpatients a busy equine hospital in between 2007 and 2009, and again between 2016 and 2017.

- Samples inoculated onto Harlequin agar and Harlequin agar + cefotaxime and incubated at 37°C to select for ESBL-producing *E. coli*

- Antibiotic susceptibility tests were performed using seven clinically relevant antibiotics



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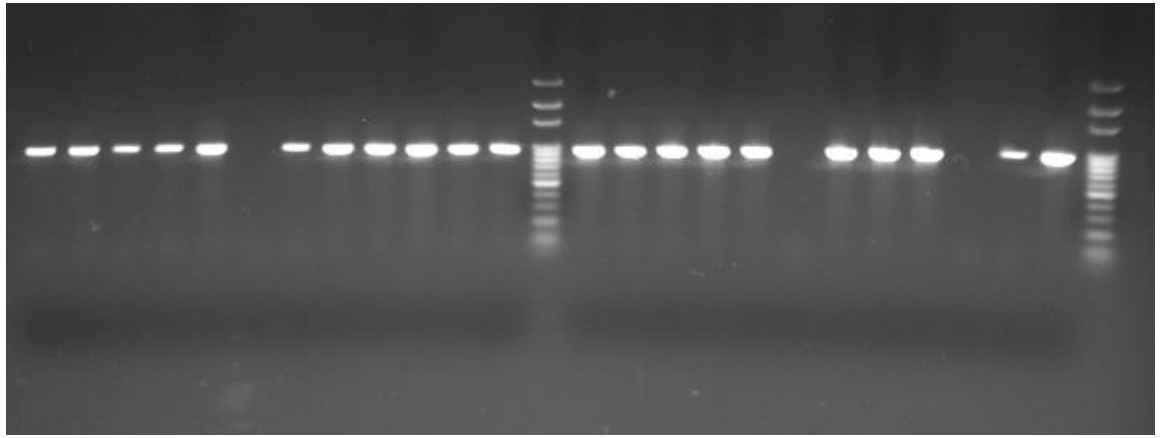
METHODS: ESBL ISOLATION & PCR

- ESBL-producers confirmed via double disc diffusion test and then subject to PCR assays for relevant ESBL genes (*bla*$_{\text{CTX-M}}$ Universal & CTX-M Group assays) and confirmed as *E. coli* (*uidA* PCR)

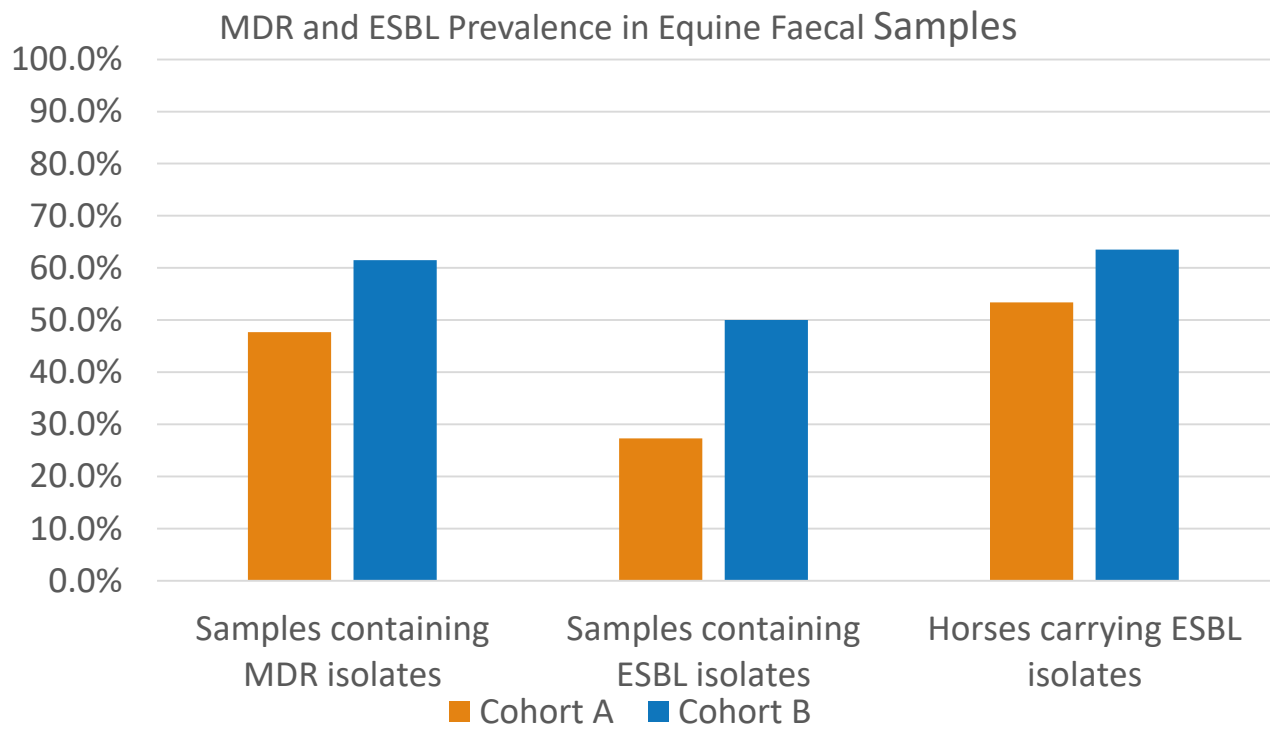
- *bla*$_{\text{CTX-M}}$ Group 1 positive isolates underwent real-time PCR to differentiate between *bla*$_{\text{CTX-M-1}}$ and *bla*$_{\text{CTX-M-15}}$ via melt curve analysis.



RESULTS: COMPARING TWO COHORTS

- **Cohort A**: 457 faecal samples from 103 horses staying at a busy referral hospital 2007-2009
  - **MDR** *E. coli* identified in **47.7%** of samples
  - **ESBL-producing** *E. coli* identified from **27.3%** of samples from **53.4% of horses**

- **Cohort B**: 314 faecal samples from 74 horses staying at the same hospital 2016/17
  - **MDR** *E. coli* identified in **61.5%** of samples
  - **ESBL-producing** *E. coli* identified from **50.0%** of samples from **63.5% of horses**



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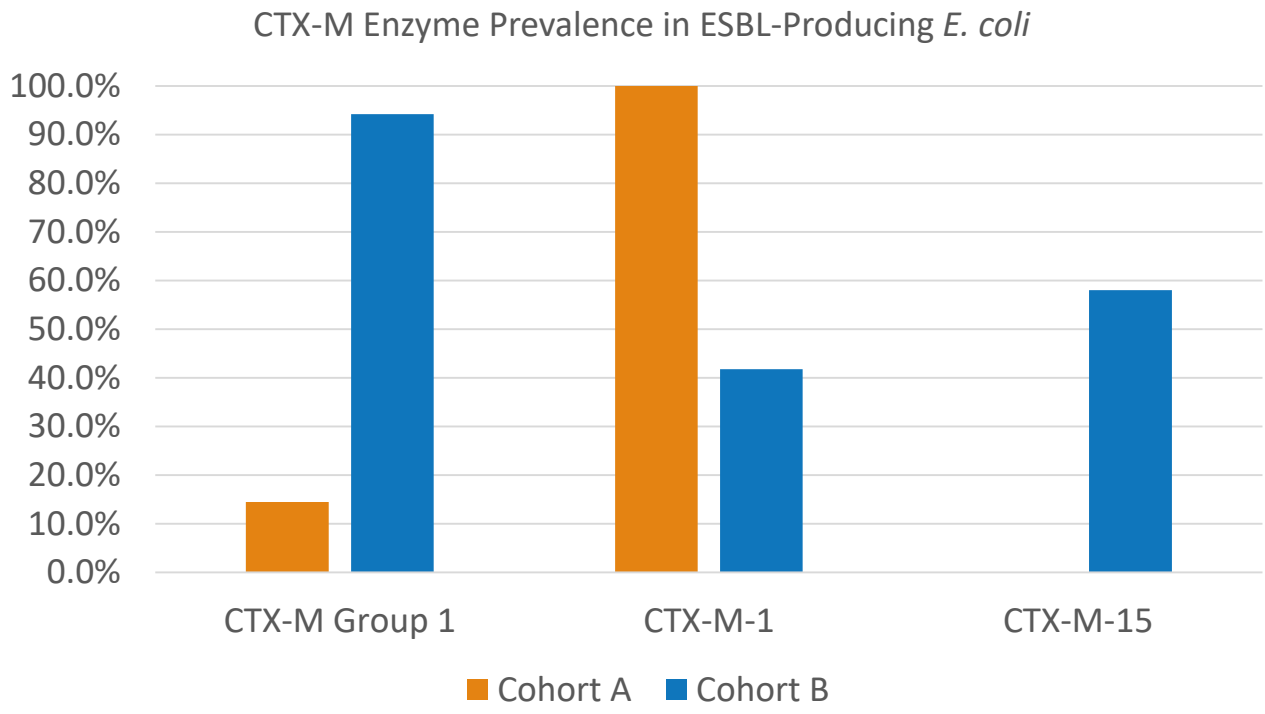
RESULTS: ESBL GENES

- **Cohort A**: 131 non-duplicate ESBL isolates
  - **16.8%** *bla*$_{\text{CTX-M}}$ Group 1 positive
  - Of which, **all** identified as *bla*$_{\text{CTX-M-1}}$

- **Cohort B**: 189 non-duplicate ESBL isolates
  - **94.2%** *bla*$_{\text{CTX-M}}$ Group 1 positive
  - **66 samples** *bla*$_{\text{CTX-M-1}}$ (21%)
  - **91 samples** *bla*$_{\text{CTX-M-15}}$ (58%)



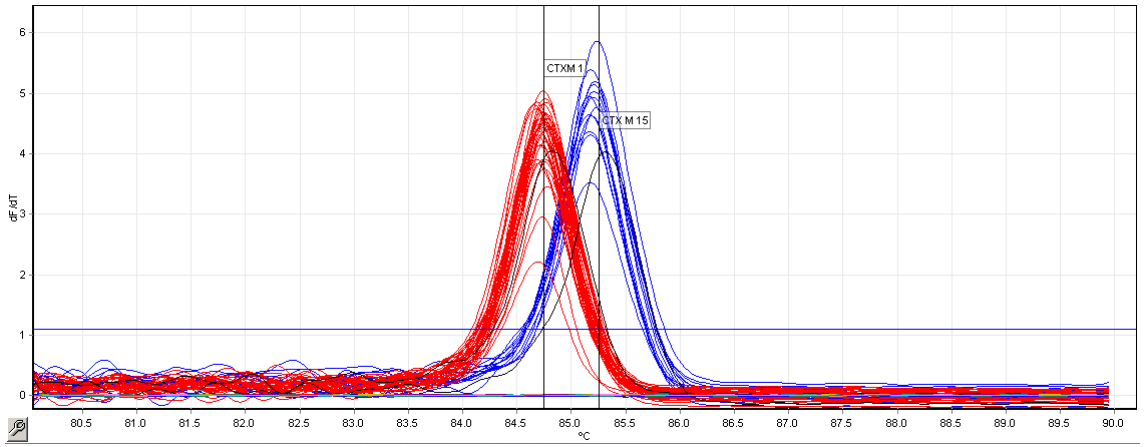
DISCUSSION: COMPARING COHORTS

- Similar proportions of **horses** carrying ESBL-producing *E. coli* within the same hospital. However a higher rate of faecal shedding and more non-duplicate ESBL-producing isolates in 2016/17

- Cohort B had a much higher proportion of $bla_{\text{CTX-M}}$ Group 1 positive isolates
  - **14.5**% vs **94.2**%

- Cohort B had a higher proportion of $bla_{\text{CTX-M-15}}$ positive isolates than $bla_{\text{CTX-M-1}}$ positive
  - Cohort A had **no** $bla_{\text{CTX-M-15}}$ positive isolates.

- Higher carriage of MDR *E. coli* in Cohort B compared to Cohort A
  - **61.5%** vs **47.7%**



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DISCUSSION: COMPARING COHORTS

- Over an 8 year period the prevalence of *bla*$_{CTX-M}$ Group 1 positive ESBLs has increased significantly. Within this group, *bla*$_{CTX-M-15}$ have become more prevalent.
  - Further work should identify if these isolates are related to the human pandemic strain O25b:ST131

- The proportion of horses carrying ESBLs has remained steady, however a higher proportion of horses carry multi-drug resistant *E. coli*
  - Antibiotic use creates the selection pressure for drug resistance to develop
  - Direct and indirect spread is possible within an equine hospital

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INTRODUCTION: CONJUGATION

- **Conjugation** is a type of horizontal gene transfer that requires **direct contact** between bacteria, usually via a **pilus**

- **Plasmids** are self-replicating extra-chromosomal elements that encode various traits including antimicrobial and heavy metal resistance, virulence and environmental adaptations

- Plasmids can be classified by their replication into **Incompatibility** (Inc) **groups**, whereby two plasmids sharing common replication and partitioning elements cannot proliferate in the same cell line.

- There are **26 known** Inc groups in *Enterobacteriaceae* and particular plasmid types have been associated with virulence and/or antimicrobial resistance.

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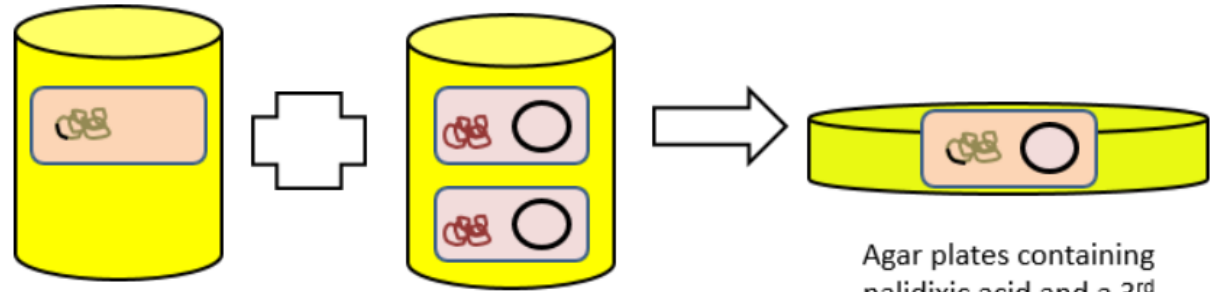
METHODS: GENE MOBILITY

- *bla*$_{CTX-M}$ Group 1 positive isolates from five equine hospitals were tested for susceptibility to nalidixic acid and streptomycin to pair them with suitable recipient strains

- Donor and recipient strains were inoculated in nutrient broth and incubated overnight, mixed together and incubated for an hour, and then plated onto Muller-Hinton agar containing cefotaxime and nalidixic acid/streptomycin



Binh CTT, et al (2007). FEMS.Microbiol.Ecol. 62:290-302

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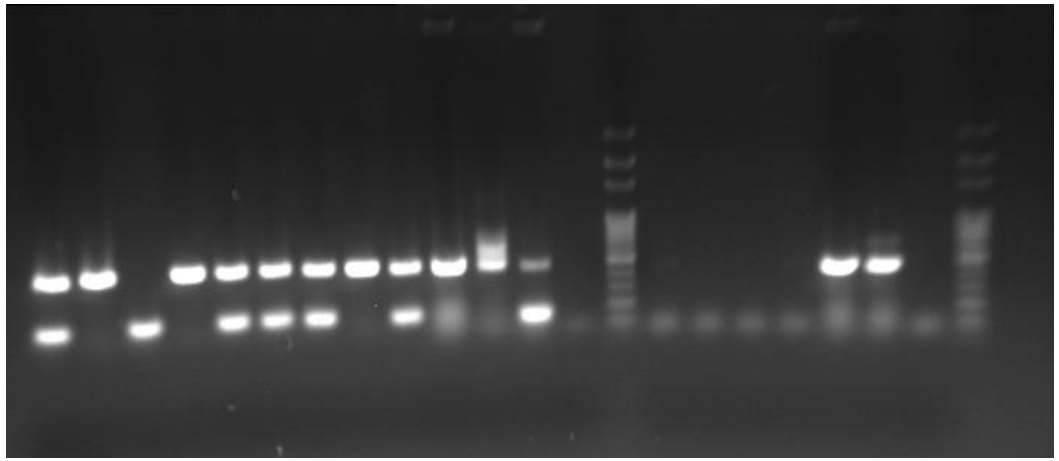
METHODS: GENE MOBILITY

- Antibiotic susceptibility tests were carried out on conjugated isolates to look for phenotypic transfer of resistance and a *bla*$_{CTX-M}$ Group 1 PCR assay used to confirm transfer of ESBL genes.

- Isolates were subject to replicon typing to determine the type of plasmid encoding such resistance.



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RESULTS: AMR IN CONJUGANTS

- **159** isolates from 5 hospitals were selected for conjugation experiments. All were MDR ESBL-producing *E. coli* that were $bla_{CTX\text{-}M}$ Group 1 positive.

- Of the **130 isolates** successfully paired with a suitable recipient, 129 transferred resistance to the recipient strain, with **125** (96.9%) transferring $bla_{CTX\text{-}M}$ Group 1 genes

- Complete phenotypic AMR transfer was seen in **89.9%** of isolates (n=116)
  - **13** isolates did not transfer full phenotypic resistance
  - **8** did **not** transfer resistance to **enrofloxacin**
  - **6** did **not** transfer resistance to **doxycycline**, but 3 of these also lost resistance to enrofloxacin
  - **3** did **not** transfer resistance to more than one antimicrobial

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RESULTS: REPLICON TYPING

Premises/year	Total transconjugants	IncH	IncI	IncF	Notes
Hospital **1**, 2008	17	17	1	0	
Hospital **1**, 2016	47	8	1	33	5 unidentified
Hospital **2**, 2016	28	28	0	0	
Hospital **3**, 2016	7	7	1	0	Low prevalence of CTX-M-1
Hospital **4**, 2016	3	3	0	0	83.3% resistant to Nal & Strep
Hospital **5**, 2016	27	27	4	0	

RESULTS: REPLICON TYPING CLUSTER

- **33** isolates contained **IncF** plasmids, and a further **5** isolates could not identify a plasmid

- All of these are ***bla*$_{\text{CTX-M-15}}$ positive** isolates, whereas all the other isolates within that group and in other groups are *bla*$_{\text{CTX-M-1}}$ positive isolates

- This suggests that within hospital 1 in 2016 a particular plasmid containing multiple antimicrobial resistance genes (including ***bla*$_{\text{CTX-M-15}}$**) was circulating within the hospital
  - Further work including full sequencing required to confirm whether the same plasmid containing the same genes was being circulated

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DISCUSSION: GENETIC TRANSFERENCE

- High rates of AMR transfer support previous findings that *bla*$_{CTX-M}$ genes are located on plasmids
  - Slightly lower rate of fluoroquinolone resistance transfer agrees with studies showing this resistance is determined by a mixture of chromosomal and plasmid genes

- These plasmids containing AMR genes are readily able to replicate and disseminate themselves to other *E. coli* via conjugation

- As plasmids carry multiple resistance genes, this can lead to **co-selection** and therefore resistance to one class of antimicrobial can lead to selection for MDR plasmids or strains

- This high level of genetic transfer could explain why ESBL isolates and MDR *E. coli* prevalence have increased in the last 10 years

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CONCLUSIONS

- There is a **high prevalence** of **MDR** and **ESBL-producing *E. coli*** in horses staying in hospitals across the UK.

- **MDR** is encoded by a variety of genes located on **plasmids** that are mobile and apparently easily transferable via **conjugation** in *E. coli*

- Prevalence of **CTX-M-1** ESBL-producing *E. coli* appears to be increasing with time suggesting dissemination of this enzyme

- **CTX-M-15** producing *E. coli* have been identified in equine samples and further work is needed to determine whether these are related to the human pandemic strain.

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CONCLUSIONS

- Antimicrobial use creates the selection pressure for resistance gene promotion and dissemination – **good antimicrobial stewardship** prevents this selection

- **Infection control** should also take priority to prevent antimicrobial use and reduce dissemination of these plasmids within an equine hospital

- The risk of these MDR and ESBL-producing genes and their role in **clinical infections** needs further investigation
  - Studies are needed to ascertain presence of these genes in **pathogenic bacteria**
  - Currently studies underway looking at surgical site infections and clinical infections
  - Might be useful to look at the presence of these genes in **in-contact humans** to see if there is inter-species transmission and risk to public health

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