Development of a Carbapenem-Resistant Enterobacterales Patient Surveillance and Response Program

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Introduction and Rationale

- Carbapenem-resistant Enterobacterales (CRE) are a group of bacterial species (order Enterobacterales; EB) that are resistant to carbapenems, an antimicrobial of last resort.
- CRE can cause clinical disease, or can colonize the GI without signs.
- CRE subclinical carriage may complicate early detection and response, necessitating an active surveillance component.
- Resistance to carbapenems within the EB can be broadly classified as carbapenemase-producing EB (CPE), or non-carbapenemase producing (Fig 1).
- EB acquire carbapenemase genes via uptake of transferable plasmids, which often carry resistance genes for multiple antimicrobials. This leads to carbapenem and multi-drug resistance in these isolates. This renders plasmid acquisition of highest importance amongst carbapenem resistance mechanisms.
- CRE is of increasing concern as outbreaks in human and veterinary medicine rise.
- There have not yet been reports of CRE in Canadian veterinary hospitals or patients.
- Rising veterinary cases worldwide indicates a need for developing surveillance and outbreak prevention strategies.

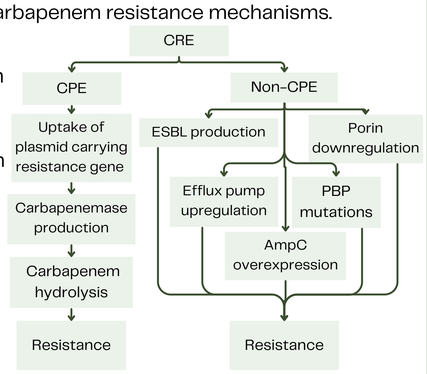

Fig 1: CRE Resistance Mechanisms. ESBL: Extended-spectrum beta-lactamase. PBP: Penicillin-binding proteins. AmpC: group of chromosomally-encoded beta-lactamase genes.

Aims

- Develop a model CRE surveillance plan for veterinary facilities.
- Pilot a surveillance system for detecting fecal carriage of CRE in Atlantic Veterinary College (AVC)-Veterinary Teaching Hospital (VTH) patients.
- Determine a period prevalence for CRE fecal carriage in AVC-VTH patients.

Hypothesis

- CRE is absent from, or occurring in low prevalence, within patients of AVC VTH.

Materials and Methods

- Fresh, naturally voided feces from equine, canine, and feline patients from 09 June 2025 to 19 July 2025, collected and analysed to determine CRE and CPE presence (Fig 2).
- Excluded from the study were any patients currently diagnosed, or suspected to have infectious GI disease.
- Patient demographic and history data were collected for each patient enrolled in the study. Descriptive statistics were calculated for key variables. Prevalence was calculated at the sample-level by animal species and clustering was not accounted for at this time. Relative risk (with 95% CI) was calculated to assess for associations between key predictor variables and outcomes (EB growth on selective agar plates, CRE, CPE).
- Well-characterized CPE outbreak strains were acquired to be used as positive controls.
- Positive and negative quality control strains were tested weekly on selective culture media and each time confirmatory assays were performed.

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Materials and Methods (cont)

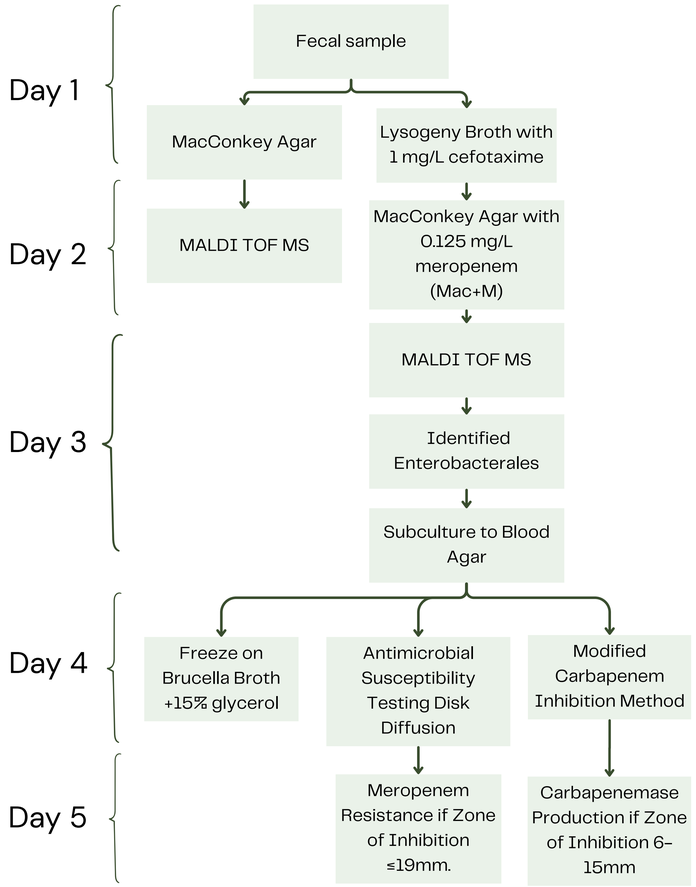


Fig 2: Sample Processing Methodology. Antimicrobial Susceptibility Testing, conducted as per CLSI standardized Disk Diffusion protocol. Modified Carbapenem Inhibition Method, conducted as per CLSI standards.

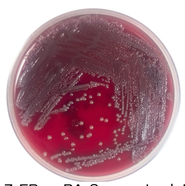


Fig 3: EB on BA. Source: Leah MacIsaac

- Enterobacterales Morphology on Blood Agar

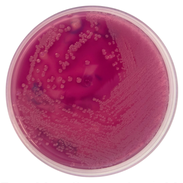


Fig 4: EB on Mac. Source: Leah MacIsaac

- Enterobacterales Morphology on MacConkey Agar

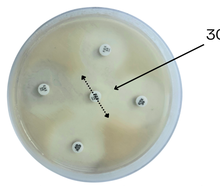


Fig 5: EB AST on MHA. Source: Leah MacIsaac

- Antimicrobial Susceptibility Testing: Negative Control.

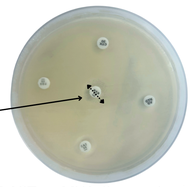


Fig 6: EB AST on MHA. Source: Leah MacIsaac

- Antimicrobial Susceptibility Testing: Positive Control.

Measurement of diameter of meropenem (center disk) zone of inhibition to determine susceptibility, (Fig 5. and 6.).

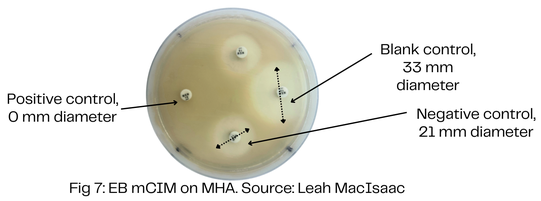


Fig 7: EB mCIM on MHA. Source: Leah MacIsaac

- Modified Carbapenem Inhibition Method: Positive and Negative Controls

Measurement of diameter of meropenem zone of inhibition to determine carbapenemase production, (Fig 7).

References

1.Anderson *et al.* 2020. *Can. J. Vet. Res.* 2.Cole *et al.* 2024. *J Vet Diagn Invest.* 3.Destefano *et al.* 2025. *J Am Vet Med Assoc.* 4.Dietrich *et al.* 2024. *Zoonoses and Public Health* 5.Holzman et al. 2025. *J Am Vet Med Assoc.* 6.Scarpellini *et al.* 2025. *Transbound Emerg Dis.* 7.Sobkowich *et al.* 2024. *J Vet Intern Med.*

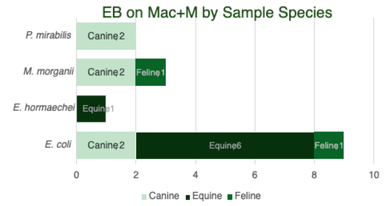
Results

Sample Demographics of EB Growth on Mac+M

- A total of 51 samples were collected from 45 unique patients; 29 samples were collected from 25 canines, 19 samples were collected from 17 equines, 3 samples were collected from 3 felines.
- 12/51 samples grew EB on Mac+M:

  Sample-level **prevalence** for reduced susceptibility to carbapenems for all animal species: **23.5%** (95% CI [12.8-37.5]).

  5/29 (17.2%) canine samples grew EB on Mac+M.
  6/19 (31.6%) equine samples grew EB on Mac+M.
  1/3 (33.3%) feline samples grew EB on Mac+M.
- MacConkey agar without antimicrobials (Mac), was used as a negative control for growth on selective plates of the same sample. All samples had EB growth on Mac, regardless of EB growth on Mac+M, serving as a baseline.



- Bacterial species identified on Mac+M varied by animal species, (Fig 8).
- ***Escherichia coli*** accounted for **9/15 (60%)** of EB species isolated from Mac+M, with most coming from **Equine** samples, **6 of 9 (66.7%).**

Fig. 8: EB on Mac+M by Sample Species. Contribution by animal species to total of each EB identified on Mac+M.

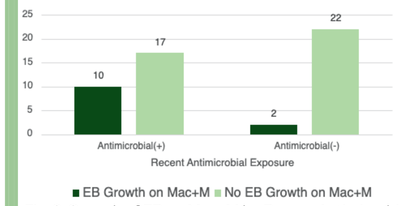
Phenotypic Confirmation for Carbapenem Resistance and Carbapenemase Production

Animal Species	Bacterial Species of Isolate	Number	Meropenem Resistance	mCIM Testing	Result
Equine	*E. coli*	6	0	0 positive	0 CRE or CPE
	E. hormaechei	1	0	0 positive	0 CRE or CPE
Canine	*E. coli*	2	0	0 positive	0 CRE or CPE
	M. morganii	2	1	0 positive	1 CRE, no CPE
	P. mirabilis	2	0	0 positive	0 CRE or CPE
Feline	*E. coli*	1	0	0 positive	0 CRE or CPE
	M. morganii	1	0	0 positive	0 CRE or CPE

Table 1. EB isolated from Mac+M phenotypic characterization. Antimicrobial Susceptibility Testing, conducted as per CLSI standardized Disk Diffusion protocol. Modified Carbapenem Inhibition Method, conducted as per CLSI standards.

- **Antimicrobial Susceptibility Testing**
  One *Morganella morganii* isolate showed resistance to meropenem.
  Sample-level **prevalence for CRE**: 1/51, **1.96%** (95% CI [0.04-10.3%]).
- **Modified Carbapenem Inhibition Method**
  The one *M. morganii* isolate that did show resistance to meropenem on AST, was shown to **not be a CPE** as tested negative by mCIM.
  Sample-level **prevalence for CPE:** 0/51, **0%** (97.5% CI [0.0-7.0%]).

Risk Factor Analysis of EB Isolated on Mac+M



- Recent **antimicrobial exposure** (within previous 30 days) was a **risk factor for EB isolated on Mac+M [RR=4.4, (37% vs 8%)** 95% CI (1.1-18.3)]; Fig 9.
- This trend was maintained when stratifying by animal species.

Fig. 9: Growth of EB on Mac+M by Recent Antimicrobial Exposure. Antimicrobial(+): Antimicrobial exposure within the last 30 days and/or at time of sample collection. Antimicrobial(-): No recorded antimicrobial exposure in the last 30 days.

Discussion

CRE Prevalence

One *M. morganii* isolate showed resistance to meropenem, but was negative for carbapenemase production. Resistance in EB can be via other mechanisms than carbapenemase production, (Fig 1), as with this isolate.
The growth of the other non-CRE EB isolates on Mac+M can be explained by the low concentration of meropenem in the Mac+M agar plates, allowing for growth of EB species of reduced susceptibility, but not full resistance to meropenem.

Pilot Surveillance System and CRE Response Plan: A valuable and effective protocol for CRE screening

Benefits:

- No advanced techniques or expertise required.
- Most consumable materials are commonly found in bacteriology laboratories.
- More cost effective than other CRE screening kits/protocols.

Drawbacks:

- Turnaround time, minimum 5 days, between sample collection and phenotypic results (Fig. 2).
- Requires acquisition of control strains for in-house AST and mCIM, otherwise would have to rely on referring out to specialty labs.

Conclusion and Future Directions

- The fecal carriage of EB with reduced susceptibility to carbapenems in veterinary patients highlights the importance of implementing CRE identification and prevention strategies in veterinary hospitals.
- To-date, we have not detected CPE in our canine, feline, or equine patient populations, confirming our study hypothesis. Given the small sample size, further sample testing is needed to confidently determine CPE absence. This work serves as an important foundation for future CRE/CPE surveillance work in our region.
- We look to further investigate these isolates with reduced meropenem susceptibility by determining the minimal inhibitory concentration to meropenem, as well as genotypic assays to determine which mechanisms of resistance are present.