Characterization of Antimicrobial Resistant *Escherichia coli* in Irish Harbor Seals (*Phoca vitulina*) and their Enclosures

Nicole Cady[1], Ana Pereira do Vale[2,3], Barbara Byrne[1], Gerald Barry[2]

[1]School of Veterinary Medicine, University of California, Davis, CA; [2]School of Veterinary Medicine, University College Dublin, Ireland; [3]Institute of Technology, Sligo, Ireland

INTRODUCTION

- **Antimicrobial resistant (AMR)** bacteria are a critical threat to human and animal health[2,3].
- Marine environments are often sinks for agricultural, hospital, and sewage waste, all potential sources of antimicrobial resistance. Thus monitoring them is a critical tool in assessing this emerging problem[5].
- **Marine mammals may serve as sentinels** for marine environmental health due to their position at the top of the marine food chain and long lifespans[1,4,6].
- This study investigated *E. coli* from wild harbor seals and their enclosures at a rehabilitation center. **We hypothesized that Extended-spectrum beta-lactamase (ESBL) *E. coli* would be present in the feces of the seals, but not their cleaned enclosures prior to seal admittance.**

OBJECTIVES

- **Screen for ESBL *E. coli*** in fecal and environmental samples.
- **Perform susceptibility testing** on all *E. coli* isolates and determine Multidrug Resistant (MDR) status. MDR is defined as being resistant to at least 3 classes of antimicrobials.
- **Investigate presence of beta-lactamase genes** via PCR.

METHODS

Collect fecal and environmental samples

Plate on TBX and TBX + Cefotaxime
(to detect *E. coli* and ESBL *E. coli*)

Isolate and Purify E. coli isolates

Susceptibility Testing
(VITEK2)

PCR
(Presence of beta-lactamase genes)

ACKNOWLEDGEMENTS

Thank you to Seal Rescue Ireland, the UC Davis STAR Program, the UC Davis Global Programs Office, and University College Dublin.

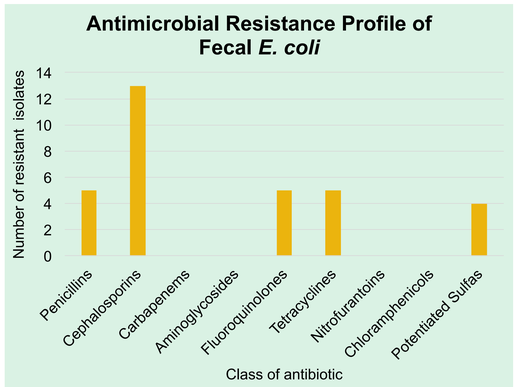

Fig. 1: Number of fecal *E. coli* isolates resistant to each class of antibiotics (n=13 isolates). Isolates may be resistant to multiple drugs within each class.

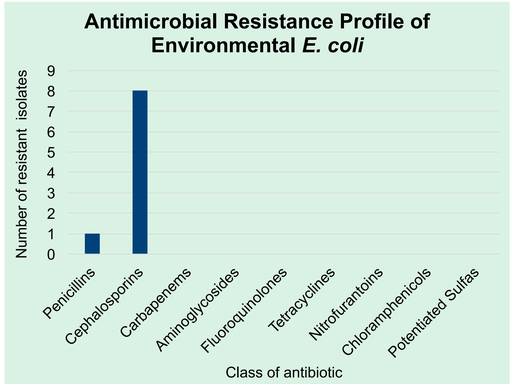

Fig. 2: Number of environmental *E. coli* isolates resistant to each class of antibiotics (n=8 isolates). Isolates may be resistant to multiple drugs within each class.

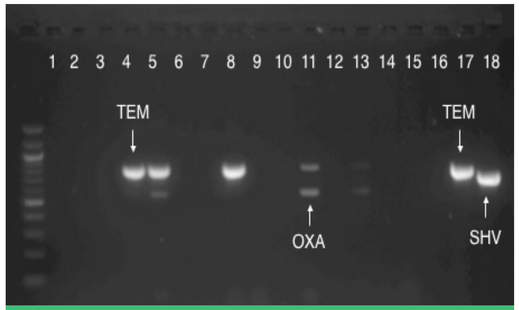

Fig. 3: Multiplex PCR for beta-lactamase detection (OXA, TEM, and SHV variants). See Table 1 for lane identification.

Lane	Contents	Lane	Contents
1	Fecal Isolate	10	Environmental Isolate
2	Fecal Isolate	11	Environmental Isolate
3	Fecal Isolate	12	Environmental Isolate
4	Fecal Isolate	13	Environmental Isolate
5	Fecal Isolate	14	Environmental Isolate
6	Fecal Isolate	15	Environmental Isolate
7	Fecal Isolate	16	Negative control
8	Environmental Isolate	17	Positive TEM-2 control
9	Environmental Isolate	18	Positive SHV-2 control

Table 1: Legend of PCR lane contents displayed in Fig. 3.

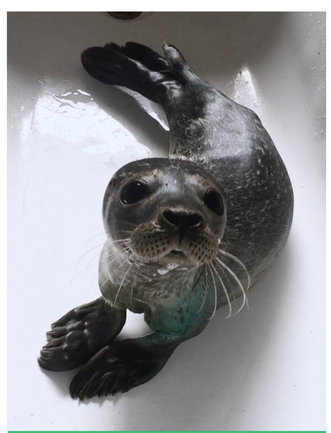

Fig. 4: Harbor seal (*Phoca vitulina*) at Seal Rescue Ireland.

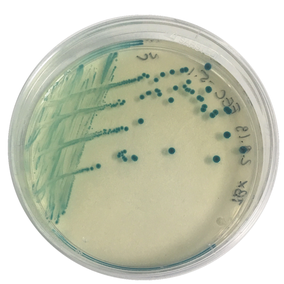

Fig. 5: Appearance of *E. coli* colonies on TBX medium.

Fig. 6: Locations of seal strandings prior to admittance into Seal Rescue Ireland.

RESULTS

Susceptibility testing (Fig. 1 and 2):
- No *E. coli* grew on TBX supplemented with cefotaxime
- All isolates were resistant to cephalexin
- 3 of 6 seals had MDR *E. coli* in their feces
- No environmental isolates were MDR

Beta-lactamase genes (Figure 3):
- 2/7 fecal isolates contained at least one beta-lactamase gene
  - PCR still pending for remaining fecal 6 isolates
- 3/8 environmental isolates contained at least one beta-lactamase gene

DISCUSSION

- **No *E. coli* displayed an ESBL phenotype** (did not grow on media supplemented with cefotaxime). This finding has public health implications as ESBL presence in wild seals would signify a threat to the clinical efficacy of important antibiotics.
- All isolates, both fecal and environmental, were resistant to cephalexin. This is interesting as cephalexin is not an antibiotic typically administered to the seals at this facility.
- **Multi-drug resistant *E. coli* was present in feces of the seals, but not their enclosures.** This suggests they did not acquire MDR *E. coli* from their enclosures at the rehabilitation center.
- This pilot study will ideally lead to future research employing direct sampling of seals in their natural environment.
- Number of seals sampled for feces was low (n=6) due to low rate of seal admittance into the rehabilitation center during this time, making statistical conclusions difficult.

REFERENCES

1. Bossart GD. Marine mammals as sentinel species for oceans and human health. Vet Pathol. 2011;48(3):676-690
2. Gibbons JF, Boland F, Egan J, Fanning S, Markey BK, Leonard FC. Antimicrobial resistance of faecal Escherichia coli isolates from pig farms with different durations of in-feed antimicrobial use. Zoonoses and Public Health. 2016;63:241-250.
3. Guenther S, Ewers C, Wieler LH. Extended-Spectrum Beta-Lactamases Producing *E. coli* in Wildlife, yet Another Form of Environmental Pollution? Frontiers in Microbiology. 2011;2:246. doi:10.3389/fmicb.2011.00246.
4. Stoddard RA, Atwill ER, Gulland FMD, et al. Risk factors for infection with pathogenic and antimicrobial-resistant fecal bacteria in northern elephant seals in California. Pub Health Rep. 2008;123:360-370.
5. Taylor NGH, Verner-Jeffreys DW, Baker-Austin C. Aquatic systems: maintaining, mixing and mobilising antimicrobial resistance?. Trends Ecol Evol. 2011;26(6):278-284.
6. Wallace CC, Yund PO, Ford TE, Matassa KA, Bass AL. Increase in antimicrobial resistance in bacteria isolated from stranded marine mammals of the northwest atlantic. EcoHealth. 2013;10(3):201-210.