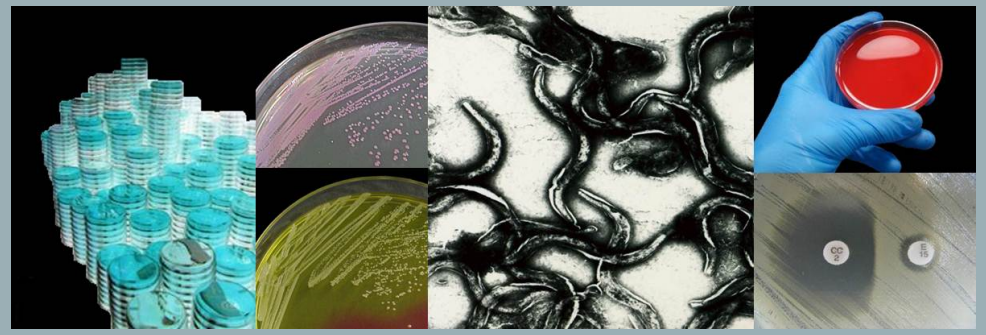
ANTIMICROBIAL STEWARDSHIP: GETTING THE MOST OUT OF CULTURE AND SUSCEPTIBILITY TESTS

INSIGHTS FROM A MICROBIOLOGIST



Joe Rubin, DVM, PhD
Associate Professor
Department of Veterinary Microbiology
University of Saskatchewan

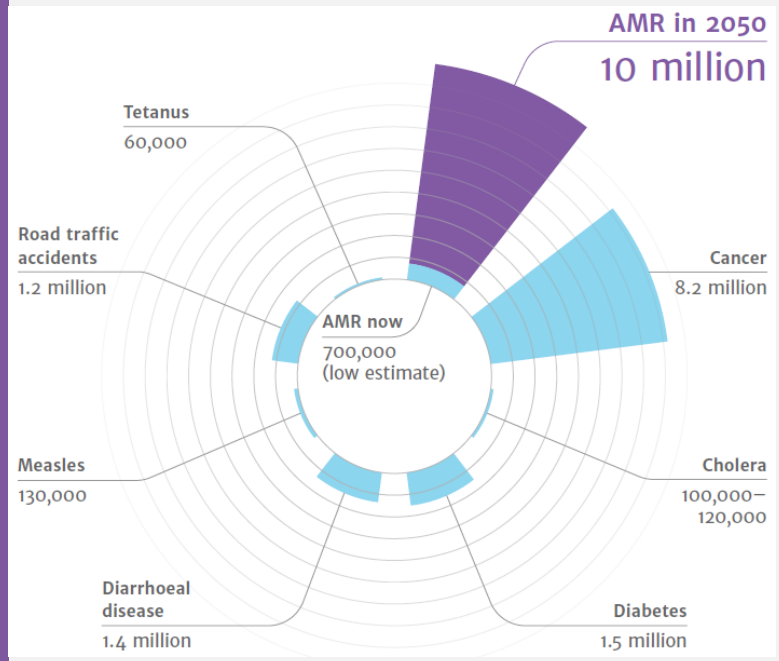
DISCLOSURES

- Received research grants from
  - Zoetis
  - Elanco/Novartis
- I am a microbiologist and not a practitioner!

OBJECTIVES

- To give an overview of the scope of the problem of AMR
- To inspire the intent to change/improve/reevaluate prescribing practices
- To provide tools to use antimicrobials more effectively
  - Antimicrobial mechanisms of action and resistance
  - Introduction to intrinsic resistance
  - Overview of key emerging resistance in veterinary medicine

THE POST-ANTIBIOTIC ERA



Estimated Attributable Deaths in 2050

https://amr-review.org/

CURRENT THREATS

Urgent Threats

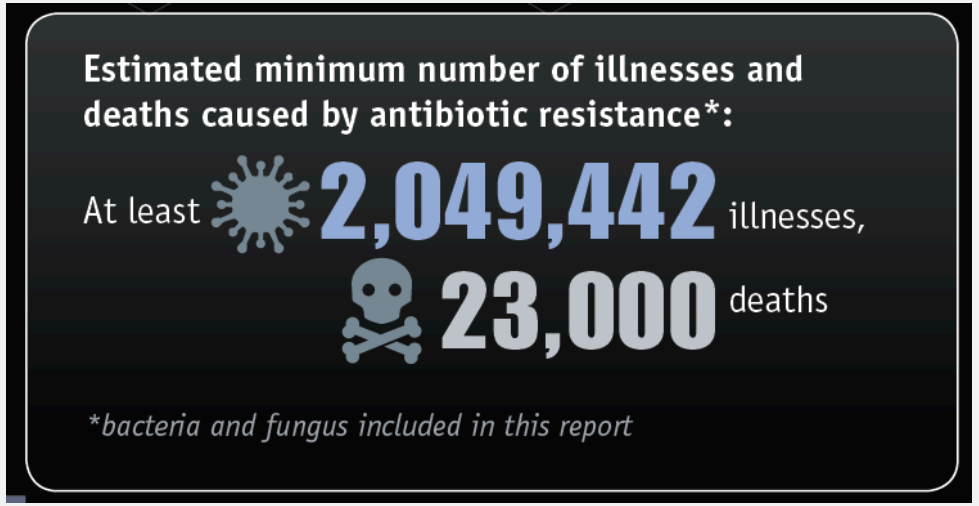
- *Clostridium difficile*
- Carbapenem-resistant Enterobacteriaceae (CRE)
- Drug-resistant *Neisseria gonorrhoeae*

Serious Threats

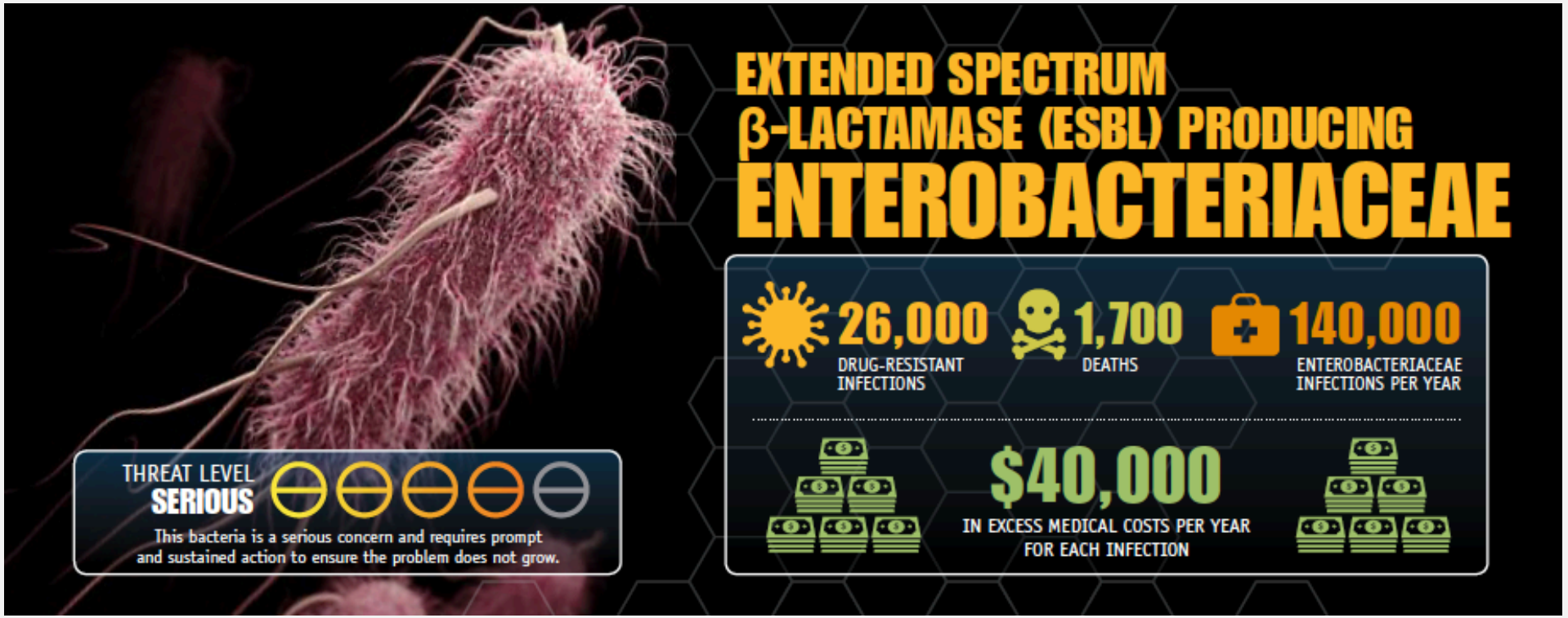
- Multidrug-resistant *Acinetobacter*
- Drug-resistant *Campylobacter*
- Fluconazole-resistant *Candida* (a fungus)
- Extended spectrum β-lactamase producing Enterobacteriaceae (ESBLs)
- Vancomycin-resistant *Enterococcus* (VRE)
- Multidrug-resistant *Pseudomonas aeruginosa*
- Drug-resistant Non-typhoidal *Salmonella*
- Drug-resistant *Salmonella* Typhi
- Drug-resistant *Shigella*
- Methicillin-resistant *Staphylococcus aureus* (MRSA)
- Drug-resistant *Streptococcus pneumoniae*
- Drug-resistant tuberculosis

Concerning Threats

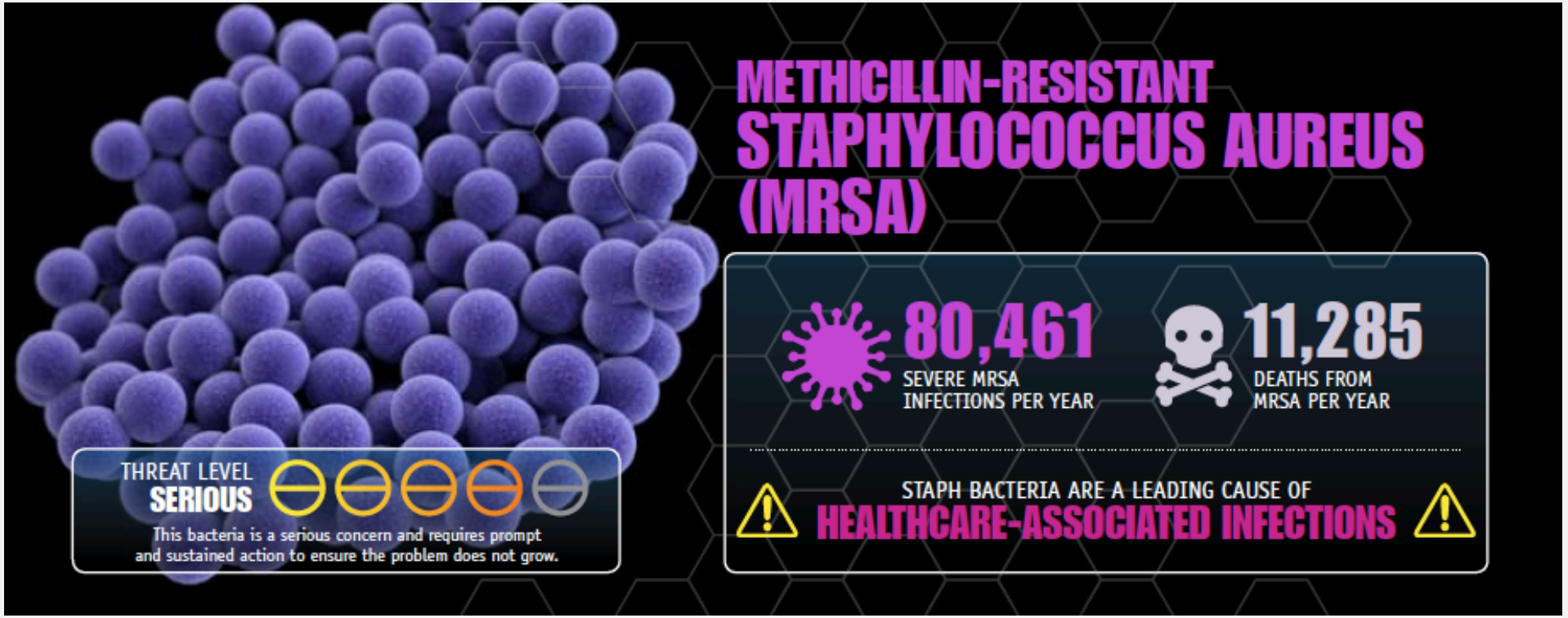
- Vancomycin-resistant *Staphylococcus aureus* (VRSA)
- Erythromycin-resistant Group A *Streptococcus*
- Clindamycin-resistant Group B *Streptococcus*



BROAD SPECTRUM B-LACTAMASES

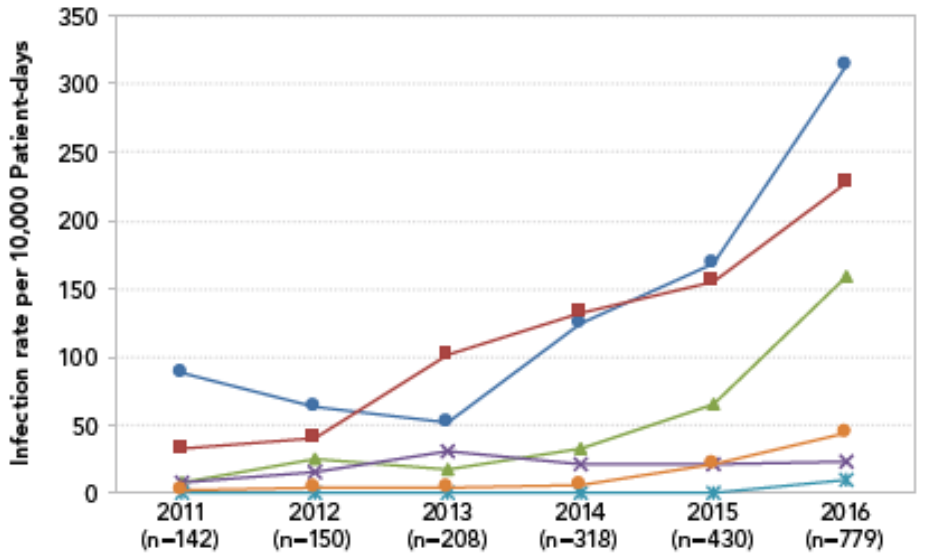


METHICILLIN RESISTANT STAPH AUREUS



EMERGING RESISTANCE IN CANADA

FIGURE 15: Count of CPE isolates by resistance gene, 2011-2016



	2011 (n=142)	2012 (n=150)	2013 (n=208)	2014 (n=318)	2015 (n=430)	2016 (n=779)
KPC	89	63	53	125	168	314
NDM	33	40	101	132	155	227
OXA-48-like	9	26	18	33	65	160
SME	8	17	31	22	21	24
OXA-48/NDM	0	0	0	0	0	160
Other	3	4	5	6	21	24

CANADIAN ANTIMICROBIAL RESISTANCE SURVEILLANCE SYSTEM

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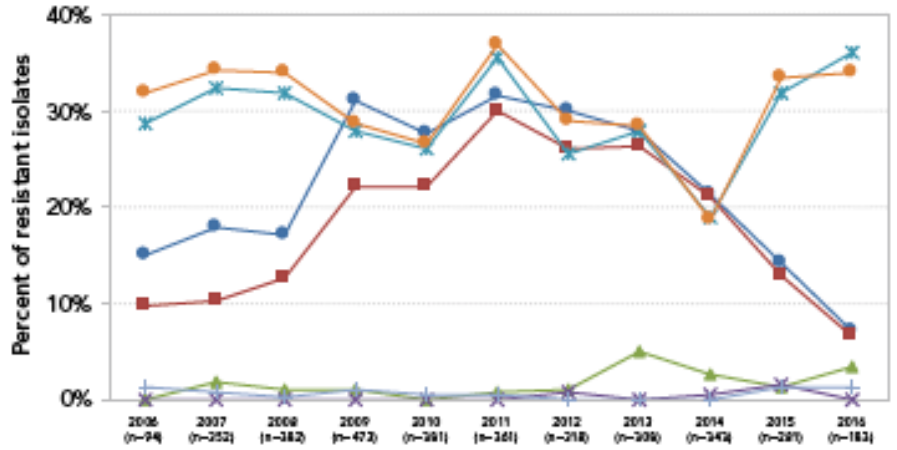
CHANGING RESISTANCE?

FIGURE 38: Resistance to selected antimicrobials among *Salmonella* isolates from chicken meat samples collected at retail stores, 2006-2016



	2006 (n=94)	2007 (n=252)	2008 (n=382)	2009 (n=472)	2010 (n=391)	2011 (n=361)	2012 (n=318)	2013 (n=309)	2014 (n=343)	2015 (n=297)	2016 (n=183)
Ampicillin	14.9%	17.9%	17.0%	31.1%	27.6%	31.6%	29.9%	27.9%	21.3%	14.2%	7.1%
Ceftriaxone	9.6%	10.2%	12.6%	22.0%	22.0%	29.9%	26.1%	26.3%	21.0%	12.8%	6.6%
Gentamicin	0.0%	1.7%	1.0%	0.8%	0.0%	0.6%	0.9%	4.9%	2.6%	1.1%	3.3%
Nalidixic acid	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.6%	0.0%	0.3%	1.4%	0.0%
Streptomycin	28.7%	32.4%	31.7%	27.9%	26.0%	35.5%	25.5%	27.9%	19.0%	31.7%	36.1%
Tetracycline	31.9%	34.1%	34.0%	28.8%	26.5%	36.8%	28.9%	28.2%	18.7%	33.5%	33.9%
Trimethoprim-sulfamethoxazole	1.1%	0.6%	0.3%	0.8%	0.5%	0.3%	0.0%	0.0%	0.0%	1.1%	1.1%

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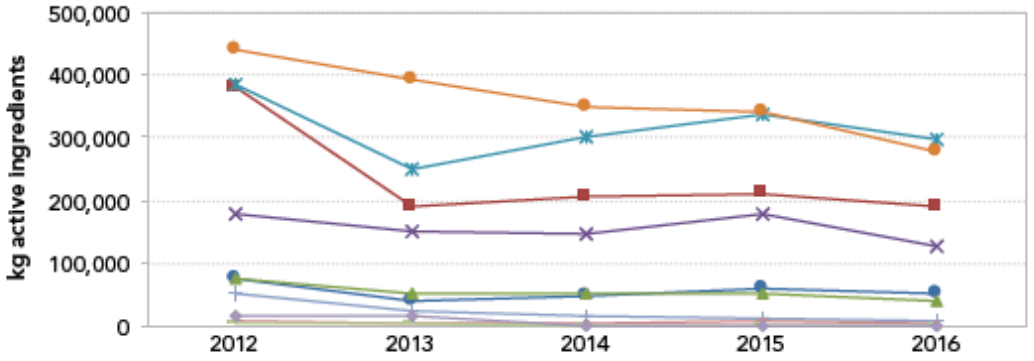
ANTIMICROBIAL USE ANIMALS

FIGURE 51: Quantity of medically important antimicrobials (kilograms) distributed for sale for use in animals, by province, 2012-2016



	2012	2013	2014	2015	2016
BC	74,376	38,680	47,351	60,067	52,738
AB	381,193	189,245	206,573	210,475	189,870
SK	77,971	50,961	50,333	50,708	39,976
MB	178,577	151,675	147,088	179,660	128,715
ON	386,917	249,621	303,240	336,049	296,916
QC	440,364	392,312	348,387	343,544	276,971
NB	50,797	23,850	14,696	11,092	8,066
NS	7,959	6,172	5,782	6,780	4,463
PE	3,781	4,164	1,134	1,147	1,378
NL	17,322	15,023	1,883	1,740	1,357

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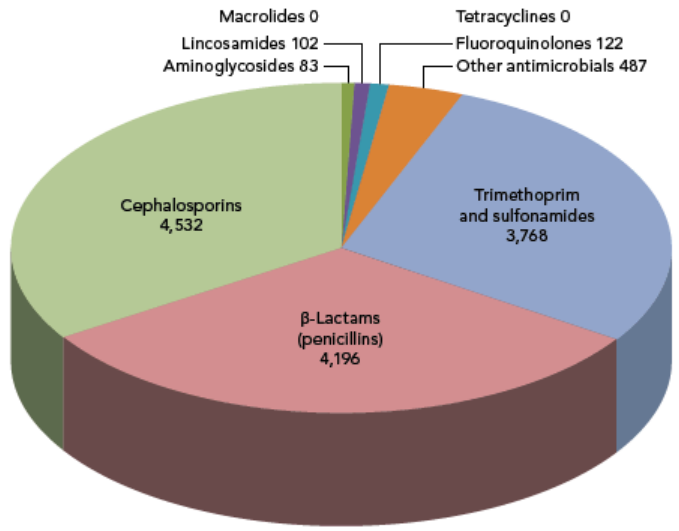
ANTIMICROBIAL USE COMPANION ANIMALS

Antimicrobial use in companion animals

In 2016, the predominant classes of antimicrobials used in companion animals were cephalosporins, β-lactams, and trimethoprim-sulfas (Figure 54). All three of these classes are antimicrobials of high importance to humans according to the classification system of the Veterinary Drugs Directorate, Health Canada[15].

FIGURE 54: Relative quantities of antimicrobial classes distributed for use in companion animals (percentages based on kg active ingredient), 2016.



NOTE: Data Sources: Canadian Animal Health Institute. Antimicrobial sales were assigned to animal type according to label claim and in the situation where mixed species was indicated on the label, the manufacturer assigned the kg to either "Companion animal" or "Production animal". Values do not include antimicrobials imported under the "own use" provision or imported as active pharmaceutical ingredients used in compounding. "Other antimicrobials" for 2016 included: avilamycin, bacitracins, bambermycin, chloramphenicol, chlorhexidine gluconate, florfenicol, fusidic acid, nitarsone, nitrofurantoin, nitrofurazone, novobiocin, polymixin, tiamulin, and virginiamycin.

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ANTIMICROBIAL USE COMPANION ANIMALS

- Large study out of UK
  - 216 practices
  - Included data from >400,000 dogs and >200,000 cats
    - Beta-lactams most commonly used
      - Amox + clav in dogs
      - 3rd Generation cephalosporins in cats


The Veterinary Journal 224 (2017) 18–24

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Original article

Patterns of antimicrobial agent prescription in a sentinel population of canine and feline veterinary practices in the United Kingdom

D.A. Singleton[a,*,1], F. Sánchez-Vizcaíno[a,b], S. Dawson[c], P.H. Jones[a], P.J.M. Noble[c], G.L. Pinchbeck[a], N.J. Williams[a], A.D. Radford[a]

HOW CANADA'S AMU COMPARES

FIGURE 57: J01 Antimicrobial consumption (DDDs per 1,000 inhabitant-days), Canada (CA) and Europe (EU)

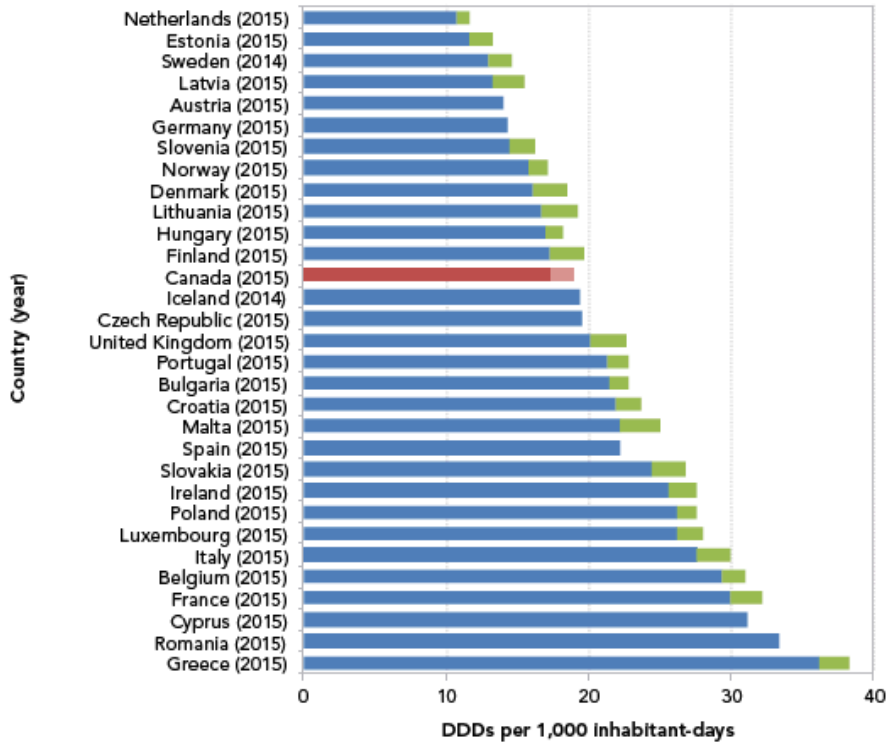
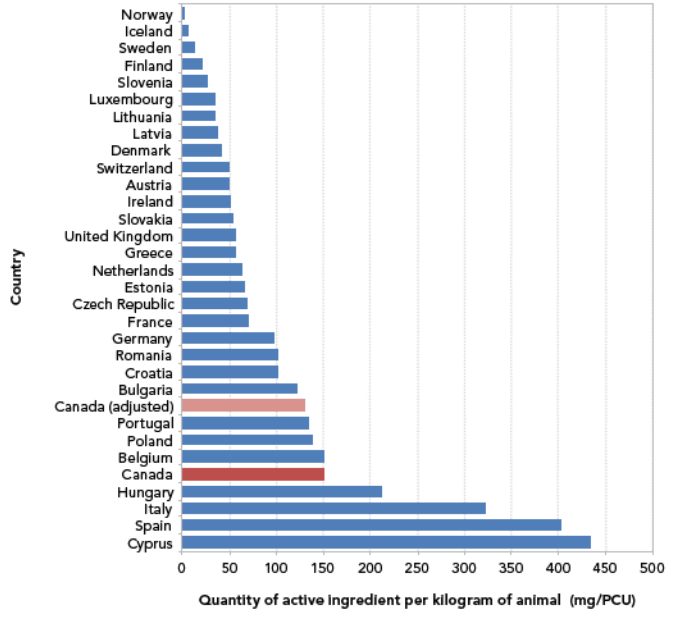


FIGURE 58: Sales of antimicrobials (adjusted by populations and weights) for Canada (2016) and countries participating in the European Surveillance of Veterinary Antimicrobial Consumption (2015)



NOTE: Data sources: Canadian Animal Health Institute, Statistics Canada, Agriculture and Agri-Food Canada, Equine Canada, European Surveillance of Veterinary Antimicrobial Consumption (ESVAC). PCU = population correction unit. The Canadian data used for live horses were from 2010 and fish from 2015; more recent data were unavailable. For the Canadian data, values do not include antimicrobials imported under the 'own use' provision or imported as active pharmaceutical ingredients used in compounding. The PCU denominator was harmonized to the greatest extent possible with ESVAC[47]. ESVAC denominator does not include beef cows, whereas in Canada beef cows are a significant population and are included. The ESVAC approach excludes companion animal data.

CANADIAN ANTIMICROBIAL RESISTANCE SURVEILLANCE SYSTEM

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STEWARDSHIP – WHAT IS IT?

"The term "antimicrobial stewardship" is used to describe the multifaceted and dynamic approaches required to sustain the clinical efficacy of antimicrobials by optimizing drug use, choice, dosing, duration, and route of administration, while minimizing the emergence of resistance and other adverse effects."

Antimicrobial Stewardship in Small Animal Veterinary Practice: From Theory to Practice

Luca Guardabassi, DVM, PhD[a,*], John F. Prescott, VetMB, DVM, PhD[b]

Vet Clin Small Anim 45 (2015) 361–376
http://dx.doi.org/10.1016/j.cvsm.2014.11.005 vetsmall.theclinics.com
0195-5616/15/$ – see front matter © 2015 Elsevier Inc. All rights reserved.

STEWARDSHIP – WHAT IS IT?

"…a coherent set of actions which promote using antimicrobials responsibly… translated into context-specific and time-specific actions."

Clinical Microbiology and Infection 23 (2017) 793–798

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Review

What is antimicrobial stewardship?

O.J. Dyar [1,*], B. Huttner [2], J. Schouten [3], C. Pulcini [4], on behalf of ESGAP (ESCMID Study Group for Antimicrobial stewardshiP)

STEWARDSHIP – WHAT IS IT?

- Active stewardship – changing behaviors
- Greatest impact on antimicrobial use
  - Specialist consultation on patient management (ID specialists, pharmacists)
  - Laboratory reports
    - Nudging
    - Suppressing
    - Framing
  - Active monitoring of antimicrobial usage (at an institutional level)
  - Audit and feedback

STEWARDSHIP – WHAT IS IT?

- Passive stewardship – providing knowledge
- Less effective
  - Prudent use guidelines
  - Educational opportunities (CE like today!)

The Veterinary Journal 247 (2019) 8–25

Contents lists available at ScienceDirect

The Veterinary Journal

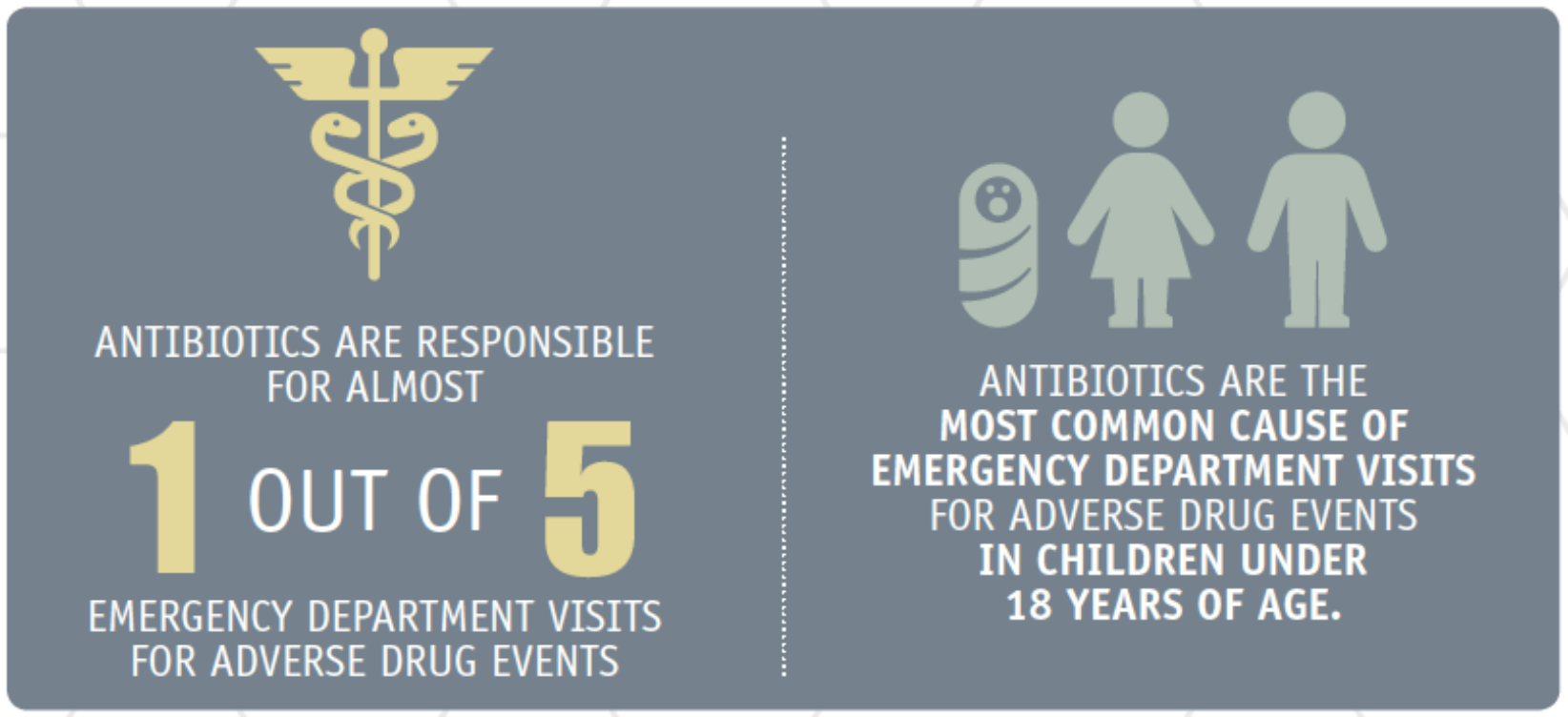
journal homepage: www.elsevier.com/locate/tvjl

International Society for Companion Animal Infectious Diseases (ISCAID) guidelines for the diagnosis and management of bacterial urinary tract infections in dogs and cats

J. Scott Weese[a,*], Joseph Blondeau[b,c], Dawn Boothe[d], Luca G. Guardabassi[e,f], Nigel Gumley[g], Mark Papich[h], Lisbeth Rem Jessen[i], Michael Lappin[j], Shelley Rankin[k], Jodi L. Westropp[l], Jane Sykes[l]

CVMA GUIDELINES FOR VETERINARY ANTIMICROBIAL USE

PROXIMATE RISKS OF ANTIMICROBIALS ADVERSE DRUG EVENTS



ADVERSE DRUG EVENTS

- 20% of hospitalized patients given antimicrobials had ADE
  - 19% of ADE occurred in patients not needing antimicrobials

JAMA Internal Medicine | Original Investigation

Association of Adverse Events With Antibiotic Use in Hospitalized Patients

Pranita D. Tamma, MD, MHS; Edina Avdic, PharmD, MBA; David X. Li, BS; Kathryn Dzintars, PharmD; Sara E. Cosgrove, MD, MS

JAMA Intern Med. 2017;177(9):1308-1315. doi:10.1001/jamainternmed.2017.1938
Published online June 12, 2017.

ADVERSE DRUG EVENTS

"… ADEs are common among inpatients receiving antibiotics, some of which may be avoidable with more judicious use of antibiotics."

"…antibiotic-associated ADEs may not be recognized by clinicians because ADEs have varied manifestations…"

JAMA Internal Medicine | Original Investigation

Association of Adverse Events With Antibiotic Use in Hospitalized Patients

Pranita D. Tamma, MD, MHS; Edina Avdic, PharmD, MBA; David X. Li, BS; Kathryn Dzintars, PharmD; Sara E. Cosgrove, MD, MS

JAMA Intern Med. 2017;177(9):1308-1315. doi:10.1001/jamainternmed.2017.1938
Published online June 12, 2017.

ADVERSE DRUG EVENTS

- >140,000 annual emergency department visits in the United States for antibiotic associated ADE

Although the risk of an ED visit for an antibiotic-associated adverse event is small for an individual patient, when antibiotics are commonly prescribed for indications for which they have no benefit, the burden of preventable adverse events in the population is great.

Emergency Department Visits for Antibiotic-Associated Adverse Events

Nadine Shehab, Priti R. Patel, Arjun Srinivasan, and Daniel S. Budnitz

Division of Healthcare Quality Promotion, National Center for Detection, Preparedness, and Control of Infectious Diseases, Coordinating Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia

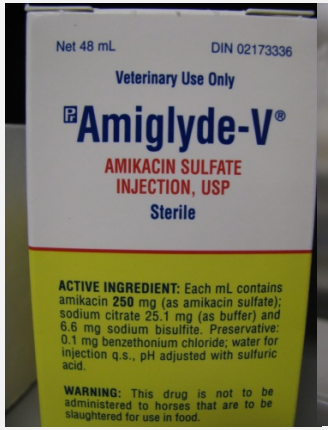
Clinical Infectious Diseases 2008; 47:735–43

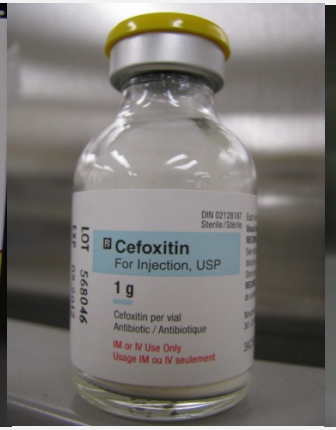
WHAT STEWARDSHIP MEANS TO ME:

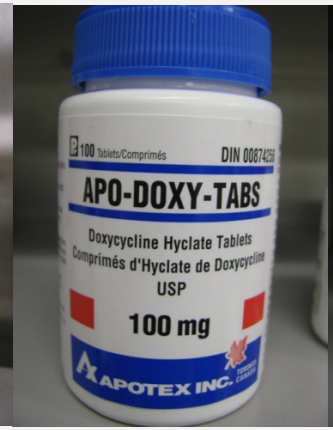
1. Thinking
2. Utilizing your knowledge of:
   - Drug mechanisms of action (spectrum of activity)
   - Mechanisms of resistance
   - Intrinsic resistance
3. Using a diagnostic lab, asking questions when you need more information
4. Being nimble and adapting to emerging resistance
5. Lifelong learning

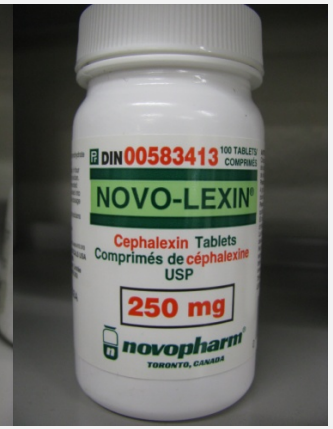
MECHANISMS OF ACTION

- Cell Wall
  - β-lactams
- Protein Synthesis
  - Tetracyclines, macrolides (MLSBK), aminoglycosides, chloramphenicol
- DNA Metabolism
  - Fluoroquinolones, metronidazole,
- Anti-metabolites
  - Folate synthesis inhibitors (sulfas)









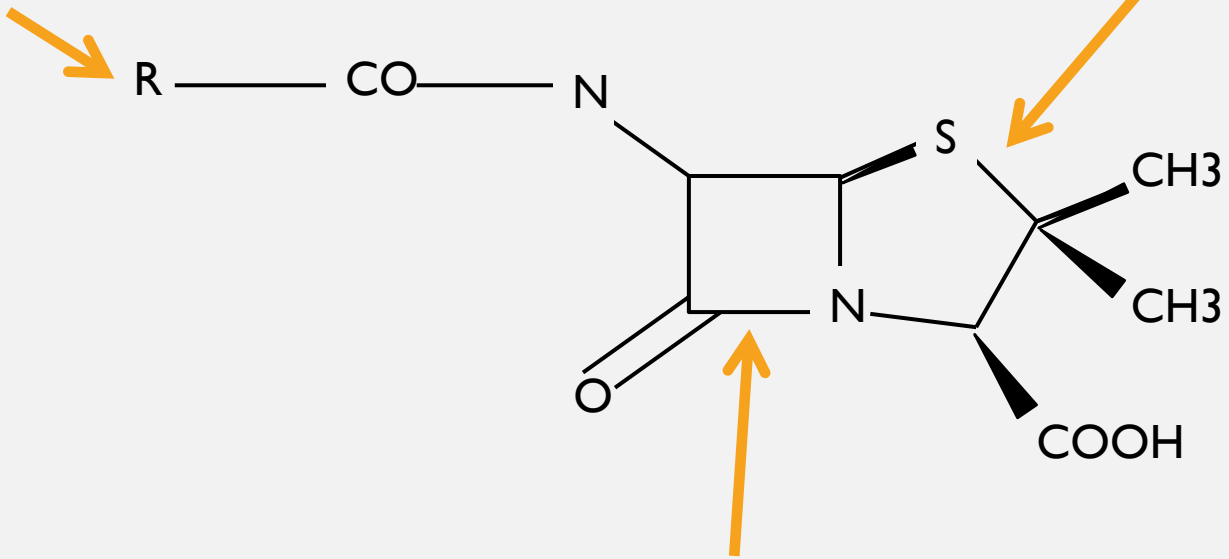
B-LACTAMS

- Inhibit cell wall synthesis
  - Bind to penicillin binding proteins
  - Prevent final stage of peptidoglycan synthesis
- Bacteriocidal
- Super family of antimicrobials
  - Penicillins
  - Cephalosporins
  - Carbapenems
  - β-lactamase inhibitors (clavulanic acid, sulbactam)

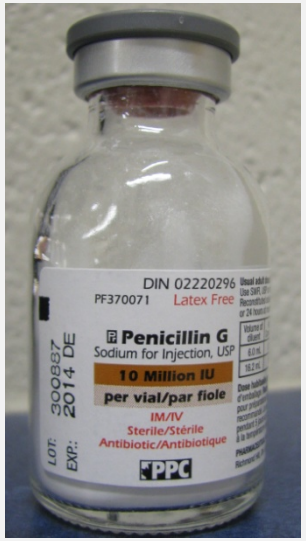
B-LACTAMS

Variable side chain yields different penicillins

5-membered penicillin ring



4-membered β-lactam ring



B-LACTAMS
PENICILLINS

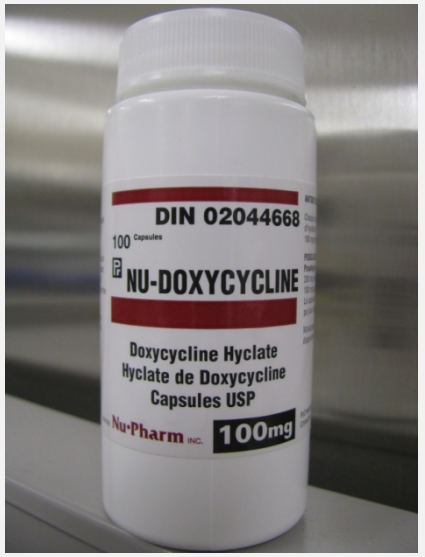
Group	Examples	Antimicrobial Spectrum
Benzyl penicillins	penicillin G	Gm +
Orally absorbed benzyl penicillins	penicillin V	Gm +
Anti-staphylococcal penicillins	cloxacillin, oxacillin	Staphylococci
Extended-spectrum penicillins	ampicillin, amoxicillin	Gm + and -, but not β-lactamase stable
Anti-pseudomonal penicillins	piperacillin	Gm – (less Gm +)
β-lactamase resistant penicillins	temocillin	

B-LACTAMS
CEPHALOSPORINS

Generation	Examples	Antimicrobial Spectrum
1st	cephalothin, cefazolin, cephalexin	Staphylococci, susceptible Enterobacteriaceae
2nd	cefuroxime	Enterobacteriaceae, anaerobes
3rd	cefevocin, ceftiofur, cefpodoxime, ceftriaxone	β-lactamase producing Enterobacteriaceae
4th	cefepime, cefpirome	Gram negatives, non-fermenters

TETRACYCLINES

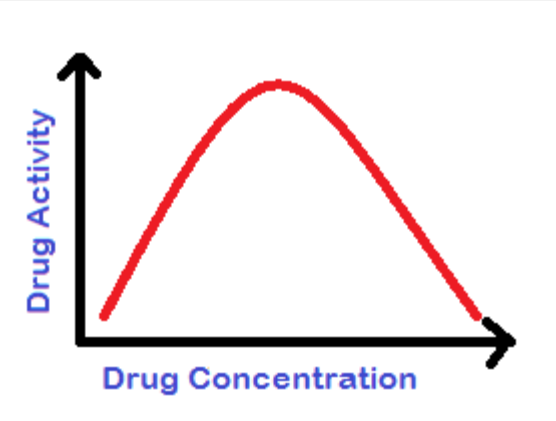
- Protein synthesis inhibitors
  - Bind to the 30S ribosomal subunit
  - Bacteriostatic
- Oxytetracycline, doxycycline, minocycline
  - Increasing lipophilicity
- Broad spectrum
  - Gram positives and negatives, intracellular parasites *Rickettsia, Ehrlichia*
  - When you think 'weird' organisms, think tetracyclines!



FLUOROQUINOLONES

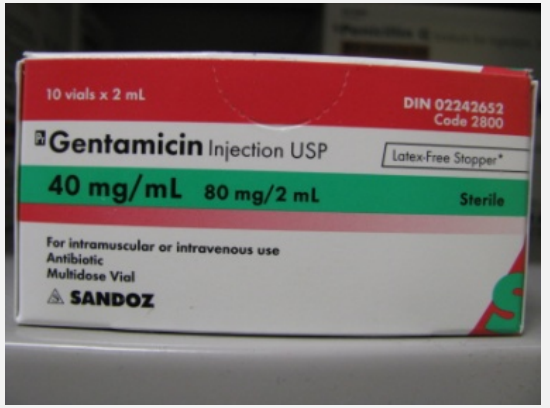
The "Goldilocks" Zone

- Interfere with DNA metabolism
  - Gyrases and topoisomerases which supercoil DNA
- Concentration dependent
  - Biphasic (less active at very low and very high concentrations)
- Naladixic acid – limited spectrum (Gram negative)
- Ciprofloxacin/enrofloxacin – broad spectrum (Gram positive and negative, intracellular pathogens)



AMINOGLYCOSIDES

- Protein synthesis inhibitors +
  - Also effects: electron transport chain, DNA metabolism, cell membrane structure
- Concentration dependent
- Some of the best anti-Gram negative drugs
  - Enterobacteriaceae, *P. aeruginosa*
- Anti-staphylococcal activity (important for MRSP)
- NO anaerobic activity – oxygen dependent uptake of drug by cell



MLS_BK

- Super-family of antimicrobials
  - Macrolides, lincosamides, streptogramins and ketolides
- Protein synthesis inhibitors
- Bacteriostatic
- Good activity against Gram positives, some Gram negatives well (*Brucella*, *Campylobacter* spp.,) and anaerobes.
- Generally poor activity against Enterobacteriaceae and non-fermenters (*P. aeruginosa*).

MLS_BK

Class	Examples	Spectrum of Activity
Macrolides	Erythromycin, tylosin	Gm +, some Gm – (*Haemophilus, Moraxella, Pasteurella* spp., and *Bordatella* spp. The 'odd ones' *Legionella pneumophila*, *Chlamydophila psittaci, Leptospira, Treponema pallidum, Mycoplasma*. Anaerobes – better against Gm + anaerobes than Gm - anaerobes
Lincosamides	Clindamycin, lincomycin	Gm +, anaerobes and the "odd ones" – see macrolides
Streptogramin B	Virginiamycin, quniupristin-dalfopristin	Gm + cocci and bacilli, Gm –ve cocci, *Moraxella, Bordatella,* intracellular organisms (*Chlamydia, Rickettsia, Mycobacterium tuberculosis*), anaerobes
Ketolides	Telithromycin, clarithromycin	Encompasses the spectrum of the macrolides and has better Gram + coverage.
Azalides	Azithromycin	Similar spectrum of activity as the macrolides but with better Gram negative activity.

CHLORAMPHENICOL

- Banned in food animals!
- Idiosyncratic aplastic anemia associated in people
  - Rare (1-20,000-40,000)
- Protein synthesis inhibitor
  - Bacteriostatic
- Broad spectrum of activity
  - Gram positives and negatives
- Florfenicol is a veterinary drug related to chloramphenicol
  - Not associated with aplastic anemia

METRONIDAZOLE

- Banned in food animals!
  - Carcinogenic
- Damage DNA and interfere with repair mechanisms
  - Bacteriocidal
- Active against anaerobic bacteria
  - Gram positive and negative bacteria
  - Protozoans (*Tritrichomonas foetus, Giardia*)
- Drug that we don't know tons about vis-a-vis resistance
  - Avoid the temptation of "dog with diarrhea = metronidazole"

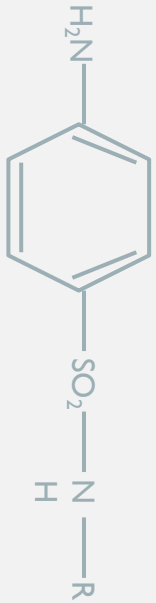
FOLATE SYNTHESIS INHIBITORS

- Sulfonamides and diaminopyrimidines (trimethoprim)
- Bacteriostatic
- Broad spectrum
  - Gram positive and negative
  - Protozoans and *Toxoplasma*

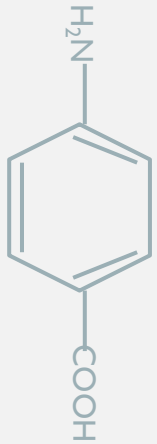
Folate Synthesis Pathway

Competitive Inhibition

Basic Sulfa



PABA



Dihydropteridine

Para-aminobenzoic acid (PABA)

Dihydropteroate synthetase

Dihydropteroate

L-glutamate

Dihydrofolate synthetase

Dihydrofolate

Enzymatic Inhibition

Dihydrofolate reductase

Tetrahydrofolate

Various Metabolic Products

DNA, RNA and Protein Synthesis

Diaminopyrimidines

GENERAL MECHANISMS OF RESISTANCE

1. Decreased permeability
2. Increased efflux
3. Enzymatic alteration of drug
4. Target modification
5. Alternate metabolic pathways

WHERE DOES RESISTANCE COME FROM?

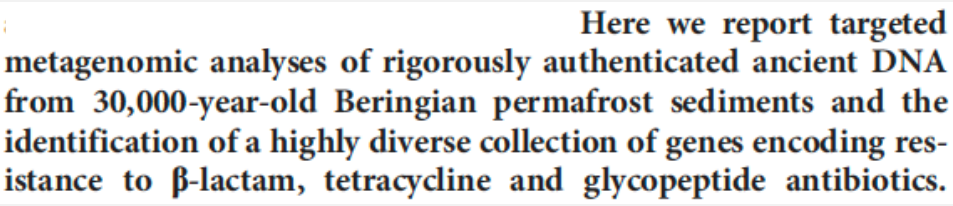
- Natural phenomenon!
  - Soil organisms survive in an environment that contains antimicrobial compounds
  - Enteric organisms need to survive in the presence of bile acids

LETTER

doi:10.1038/nature10388

Antibiotic resistance is ancient

Vanessa M. D'Costa[1,2]*, Christine E. King[3,4]*, Lindsay Kalan[1,2], Mariya Morar[1,2], Wilson W. L. Sung[4], Carsten Schwarz[3], Duane Froese[5], Grant Zazula[6], Fabrice Calmels[5], Regis Debruyne[7], G. Brian Golding[4], Hendrik N. Poinar[1,3,4] & Gerard D. Wright[1,2]



Here we report targeted metagenomic analyses of rigorously authenticated ancient DNA from 30,000-year-old Beringian permafrost sediments and the identification of a highly diverse collection of genes encoding resistance to β-lactam, tetracycline and glycopeptide antibiotics.

- Resistance to every drug that has, is or will be used in the future already exists
  - Drug resistance is often a byproduct of something else
- ANY/ALL drug use (appropriate or inappropriate) results in selection pressure
  - If you use a drug it better be worth it!

WORDS OF WISDOM FOR NEW TOOLS

"It is a neck-and-neck race in which many of us tend to underestimate the opponent. Staphylococci will not be defeated by the haphazard use of each new antibiotic. As new antibacterial agents are discovered, let us use them with discrimination."

Dr. Mary Barber - 1955

THE EVOLUTIONARY POWER OF BACTERIA

	Human Generations in our History as a Species	Bacterial Generations in the History of Antimicrobials
Time	2 Million Years	78 years
Generation Length	25 years	20 minutes
Generations in Period	80,000	2,049,840

BASIC DEFINITION – WHAT IS RESISTANCE?

- Resistance can be sub-divided into intrinsic and acquired
- Intrinsic resistance is constitutive for an organism
  - Natural "superbugs", it's just part of what they are

Pseudomonas aeruginosa =

BASIC DEFINITION – WHAT IS RESISTANCE?

- Resistance can be sub-divided into intrinsic and acquired
- Intrinsic resistance is constitutive for an organism
- Acquired resistance is not inherent to the organism, these bugs have something that makes them "super"

Staphylococcus aureus =

MECHANISMS OF RESISTANCE

- How do bacteria acquire resistance "genes"?
  - Mutation – single nucleotide polymorphisms
  - Conjugation – exchange between bacteria (mobile genetic elements)
  - Transduction - phages
  - Transformation – acquisition of exogenous DNA

MECHANISMS OF RESISTANCE B-LACTAMS

- Enzymatic inactivation
  - Primary mechanism of resistance among Enterobacteriaceae
  - β-lactamases
    - Great diversity of enzymes
- Altered binding sites
  - Streps, enterococci, methicillin-resistant Staph

MECHANISMS OF RESISTANCE TETRACYCLINES

- Efflux
  - Common in Gram positive and negative
    - Resistance not necessarily across class...
      - If you want to use a drug test it!
- Ribosomal protection
  - Very common
    - *S. pseudintermedius* (tetM)
  - Conformational change in tetracycline binding site on 30S subunit of ribosome
- Ribosomal mutations, enzymatic inactivation also occur

MECHANISMS OF RESISTANCE FLUOROQUINOLONES

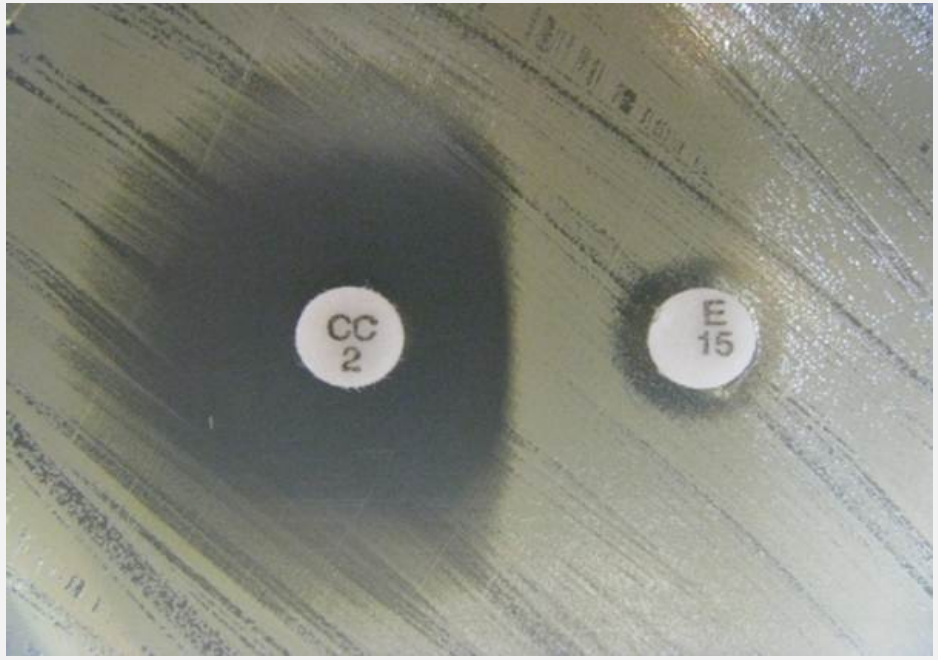
- Target mutations (Gram positive and negative)
  - *gyr*A and *par*C particularly
  - Step-wise resistance (MIC creep)
- Efflux
  - Multidrug resistance
- Plasmid mediated
  - *qnr* (target protection)
  - *qep* (efflux)
  - *aac*6-Ib-cr (enzymatic inactivation – cross resistance with aminoglycosides)

MECHANISMS OF RESISTANCE AMINOGLYCOSIDES

- Enzymatic inactivation
  - Aminoglycoside modifying enzymes
  - Most common mechanism of resistance
- Decreased permeability
  - Cross resistance to other antimicrobials

MECHANISMS OF RESISTANCE MLS_BK

- Target Modification
  - Ribosomal methylases
    - *erm* gene family
  - Be aware of inducible resistance
- Active Efflux
- Enzymatic Inactivation



Inducible clindamycin resistance in *S. aureus*

Detection requires specialized laboratory tests

MECHANISMS OF RESISTANCE FOLATE SYNTHESIS INHIBITORS

- Altered enzymes
  - *dfr* genes (trimethoprim resistance)
    - Gram positive and negative
  - *sul* genes (sulfa resistance)
    - Gram negative bacteria
    - Often found in multi-resistant bacteria, linkage to other resistance genes
- Hyper-production of PABA

INTRINSIC RESISTANCE

- A good grasp of normal allows lab data to be interpreted
  - What do all of those "R's" really mean?
- Intrinsic resistance is independent of antibiotic exposure
- "Wild-type" phenotype
- *Mycoplasma* spp. intrinsically resistant to penicillin
  - They lack a cell wall and therefore don't have the drug target

INTRINSIC RESISTANCE ENTEROBACTERIACEAE

- ALL Enterobacteriaceae intrinsically resistant to:
  - Benzylpenicillin (original penicillin)
  - Macrolides
  - Lincosamides (clindamycin)
- SPICE organisms:
  - *Serratia, Providencia, Proteus vulgaris* (indole positive), *Citrobacter* and *Enterobacter*
  - Resistant to many β-lactams including clavamox

Table 1. Intrinsic resistance in Enterobacteriaceae. Enterobacteriaceae are also intrinsically resistant to benzylpenicillin, glycopeptides, fusidic acid, macrolides (with some exceptions[1]), lincosamides, streptogramins, rifampicin, daptomycin and linezolid.

Rule no.	Organisms	Ampicillin	Amoxicilin-Clavulanic acid	Ampicillin-sulbactam	Ticarcillin	Cefazolin, Cefalotin Cefalexin, Cefadroxil	Cefoxitin[2]	Cefuroxime	Tetracyclines	Tigecycline	Polymyxin B, Colistin	Nitrofurantoin
1.1	*Citrobacter koseri,Citrobacter amalonaticus*[3]	R			R							
1.2	*Citrobacter freundii*[4]	R	R	R		R	R					
1.3	*Enterobacter cloacae* complex	R	R	R		R	R					
1.4	*Enterobacter aerogenes*	R	R	R		R	R					
1.5	*Escherichia hermannii*	R			R							
1.6	*Hafnia alvei*	R	R	R		R	R					
1.7	*Klebsiella pneumoniae*	R			R							
1.8	*Klebsiella oxytoca*	R			R							
1.9	*Morganella morganii*	R	R	R		R			R		R	R
1.10	*Proteus mirabilis*								R	R	R	R
1.11	*Proteus penneri*	R				R		R	R	R	R	R
1.12	*Proteus vulgaris*	R				R		R	R	R	R	R
1.13	*Providencia rettgeri*	R	R	R		R		R	R	R	R	R
1.14	*Providencia stuartii*	R	R	R		R		R	R	R	R	R
1.15	*Raoultella* spp.	R			R							
1.16	*Serratia marcescens*	R	R	R		R	R	R	R[5]		R	R
1.17	*Yersinia enterocolitica*	R	R	R	R	R	R					
1.18	*Yersinia pseudotuberculosis*										R	

R = resistant

[1] Azithromycin is effective *in vivo* for the treatment of typhoid fever and erythromycin may be used to treat travellers' diarrhoea.

INTRINSIC RESISTANCE NON-FERMERTERS

Table 2. Intrinsic resistance in non-fermentative Gram-negative bacteria. Non-fermentative Gram-negative bacteria are also generally intrinsically resistant to benzylpenicillin, first and second generation cephalosporins, glycopeptides, fusidic acid, macrolides, lincosamides, streptogramins, rifampicin, daptomycin and linezolid

Rule no.	Organisms	Ampicillin	Amoxicillin-Clavulanic acid	Ampicillin-sulbactam	Ticarcillin	Ticarcillin-clavulanic acid	Piperacillin	Piperacillin-tazobactam	Cefazolin, Cefalothin Cefalexin, Cefadroxil	Cefotaxime	Ceftriaxone	Ceftazidime	Cefepime	Aztreonam	Ertapenem	Imipenem	Meropenem	Ciprofloxacin	Chloramphenicol	Aminoglycosides	Trimethoprim	Fosfomycin	Tetracyclines	Tigecycline	Polymyxin B/Colistin
2.1	*Acinetobacter baumannii*, *Acinetobacter pittii*, *Acinetobacter nosocomialis* and *Acinetobacter calcoaceticus* complex	R	R	Note[1]					R	R	R			R	R						R	R	R[2]	Note[2]	
2.2	*Achromobacter xylosoxydans*	R							R	R	R				R										
2.3	*Burkholderia cepacia* complex[3]	R	R	R	R	R	R	R	R	R	R			R	R			R	R	R[4]	R	R			R
2.4	*Elizabethkingia meningoseptica*	R	R	R	R	R	R		R	R	R	R	R	R	R	R	R								R
2.5	*Ochrobactrum anthropi*	R	R	R	R	R	R	R	R	R	R	R	R	R	R										
2.6	*Pseudomonas aeruginosa*	R	R	R					R	R	R				R				R	Note[5]	R		R	R	
2.7	*Stenotrophomonas maltophilia*	R	R	R	R		R	R	R	R	R			R	R	R	R			R[4]	R[6]	R	R[7]		

R = resistant

INTRINSIC RESISTANCE GRAM-POSITIVES

- *Enterococci* intrinsically resistant to many drugs
- Accurate speciation is important
  - *E. faecalis* intrinsically clindamycin resistant
  - *E. faecium* NOT intrinsically clindamycin resistant
- *Enterococcus* spp. don't tend to produce β-lactamases, amoxicillin + clavulanic acid does not offer advantage over amoxicillin

Table 4. Intrinsic resistance in Gram-positive bacteria. Gram-positive bacteria are also intrinsically resistant to aztreonam, temocillin, polymyxin B/colistin and nalidixic acid

Rule no.	Organisms	Fusidic acid	Ceftazidime	Cephalosporins (except ceftazidime)	Aminoglycosides	Macrolides	Clindamycin	Quinupristin-dalfopristin	Vancomycin	Teicoplanin	Fosfomycin	Novobiocin	Sulfonamides
4.1	*Staphylococcus saprophyticus*	R	R								R	R	
4.2	*Staphylococcus cohnii,*		R									R	
4.3	*Staphylococcus xylosus*		R									R	
4.4	*Staphylococcus capitis*		R								R		
4.5	Other coagulase-negative staphylococci and *Staphylococcus aureus*		R										
4.6	*Streptococcus* spp.	R	R		R[1]								
4.7	*Enterococcus faecalis*	R	R	R	R[1]	R	R	R					R
4.8	*Enterococcus gallinarum, Enterococcus casseliflavus*	R	R	R	R[1]	R	R	R	R				R
4.9	*Enterococcus faecium*	R	R	R	R[1,2]	R							R
4.10	*Corynebacterium* spp.										R		
4.11	*Listeria monocytogenes*		R	R									
4.12	*Leuconostoc* spp., *Pediococcus* spp.								R	R			
4.13	*Lactobacillus* spp. (*L. casei, L. casei* var. *rhamnosus*)								R	R			
4.14	*Clostridium ramosum, Clostridium innocuum*								R				

R = resistant

[1] Low-level resistance (LLR) to aminoglycosides. Combinations of aminoglycosides with cell wall inhibitors (penicillins and glycopeptides) are synergistic and bactericidal against isolates that are susceptible to cell wall inhibitors and do not display high-level resistance to aminoglycosides.

[2] In addition to LLR to aminoglycosides, *Enterococcus faecium* produces a chromosomal AAC(6')-I enzyme that is responsible for the loss of synergism between aminoglycosides (except gentamicin, amikacin and streptomycin) and penicillins or glycopeptides.

METHICILLIN RESISTANCE

EMERGENCE OF METHICILLIN RESISTANCE

- MRSA first identified in people in 1961
- In 1990s spread into the community
- In people associated with
  - Higher mortality and health care costs
- In dogs, the similar negative healthcare outcomes not demonstrated
- In Saskatoon, methicillin resistance first recognized in mid to late 2000s
  - Canine MRSA first recognized in 2006
  - Canine MRSP first recognized in 2008

EMERGENCE OF METHICILLIN RESISTANCE

- Unfortunately little BC specific data – 2015 report found 12.9% MR among dermatological isolates including BC

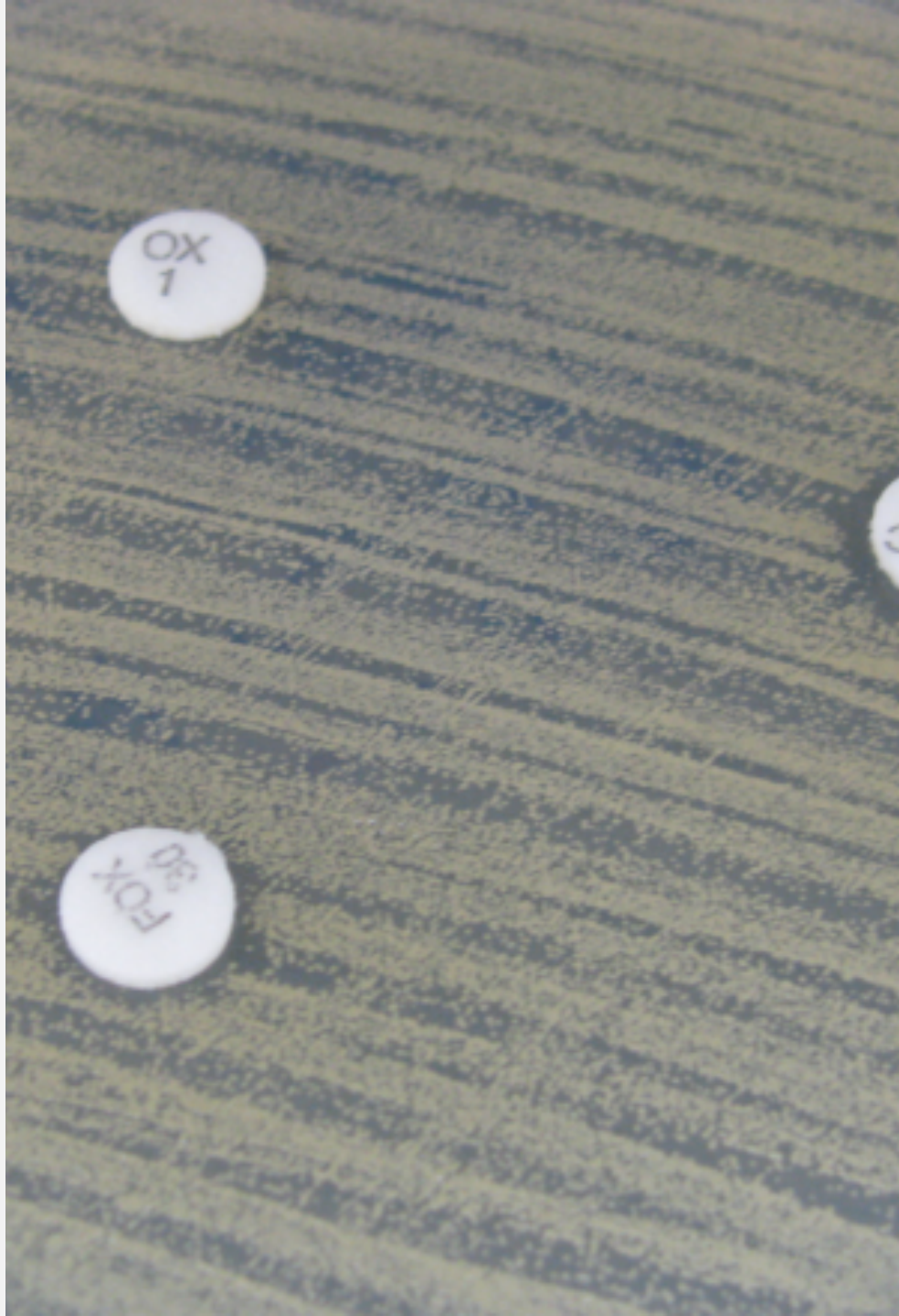
Brief Communication Communication brève

Prevalence of methicillin-resistant staphylococci in canine pyoderma cases in primary care veterinary practices in Canada: A preliminary study

Daniel Joffe, Fiona Goulding, Ken Langelier, Gabor Magyar, Les McCurdy, Moe Milstein, Kia Nielsen, Stephanie Villemaire

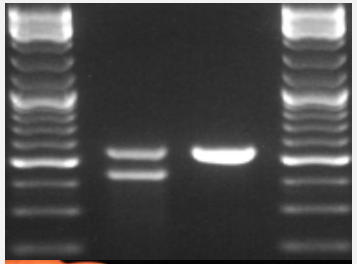
WHAT IS METHICILLIN RESISTANCE?

- More than just resistance to methicillin!
- Resistance to **ALL β-LACTAMS**
- *mec*A (*mec* family) gene
  - Codes altered penicillin binding protein (PBP2a)
    - Decreased binding affinity β-lactams drugs
    - Resistance to penicillins, cephalosporins and carbapenems
    - β-lactamase inhibitors won't help!
- Frequently multidrug resistant



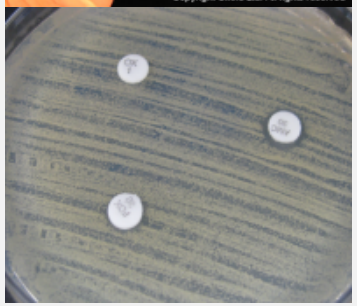
IDENTIFICATION OF METHICILLIN RESISTANCE

Test	*S. aureus*	*S. pseudintermedius*
mecA	Gold Standard	Gold Standard
PBP2a Latex Agglutination	+	+
Phenotypic Resistance	Cefoxitin or Oxacillin	**ONLY Oxacillin**



PCR Amplification of *mecA*

Agglutination of PBP2a



Phenotypic β-lactam resistance

THE CURRENT STATE OF MRSP…

1986-2000 Clinical (n=60)		2008 Colonized (n=153)	2014 Colonized (n=78)
Drug	% Resistant	% Resistant	% Resistant
Penicillin	7	40	73
Ampicillin	0	10	62
Oxacillin	0	0	9
Erythromycin	8	3	5
Clindamycin	13	3	5
Tetracycline	34	24	26
Trimethoprim/Sulfa	5	0	4
Gentamicin	0	0	1
Chloramphenicol	0	0	3

THE CURRENT STATE OF MRSP…

- Survey of diagnostic isolates from PDS – 2013-2015
  - Urinary and dermatological
- Overall dermatological isolates more resistant than urinary
  - 51 dermatological isolates, 6 MRSP (16%)
  - 50 urinary isolates, 1 MRSP (2%)
  - Macrolide and chloramphenicol resistance also more common among dermatological than urinary isolates

METHICILLIN RESISTANCE TAKE AWAYS

1. MR = resistance to **ALL** β-lactam drugs
2. Because MR is **NOT** due to the production of β-lactamases, drugs like amoxicillin + clavulanic acid are **NOT** helpful
3. Susceptibility profiles of *Staphylococci* are changing, and laboratory guidance is **VERY** important for aiding therapeutic selection
4. MR doesn't just affect companion animals, watch out for these bugs in livestock:
   - Mastitis in cattle
   - Bumble foot in chickens
   - *S. hyicus* greasy pig disease or MRSA skin infection in pigs

ESBLS AND CARBAPENEMASES

WHAT ARE ESBLS AND CARBAPENEMASES?

- Gram-negative problem
- These broad spectrum β-lactamases are going to be the "Next Big Thing" in the veterinary AMR world
  - There is a lack of awareness of these enzymes in the profession
  - We know remarkably little about the incidence of distribution of these resistance mechanisms in animals
- Often transmissible between bacteria
- These β-lactamases are emerging unnoticed in animals!

ESBL producing *E. coli* on CHROMagar ESBL

B-LACTAMASE CLASSES

Enzymes		Ambler Class	Examples	Spectrum of Resistance	Inhibitors
ESBLs Extended-spectrum β-lactamases		Class A	TEM (other than parent enzymes TEM-1, 2 and 13), SHV (other than parent enzyme SHV-1), CTX-M	Penicillins Cephalosporins Monobactams	Clavulanic acid Tazobactam Sulbactam
AmpC		Class C	CMY, FOX, ACT, MOX, ACC, DHA	Penicillins Cephalosporins Cephamycins Monobactams	Cloxacillin Boronic acid
Carbapenemases	**Metallo-β-lactamases (MBL)**	Class B	NDM, VIM, IMP	Penicillins Cephalosporins Cephamycins Carbapenems	EDTA and other metal chelators
	KPC type	Class A	KPC	Penicillins Cephalosporins Cephamycins Carbapenems	Clavulanic acid (weak inhibition) Tazobactam Boronic acid
	OXA type	Class D	OXA-48	Penicillins Carbapenems	NaCl

IDENTIFICATION AND IMPLICATIONS OF B-LACTAMASES

- The first think you'll see is β-lactam resistance
  - Diagnostic labs not doing genotyping routinely
- Will most likely affect your practice dealing with Enterobacteriaceae

Resistance Genes	Resistance Seen	Treatment Guidance
Narrow spectrum	Pen + 1GC	Potentiated Penicillin
ESBL	Pen + 1GC + 3GC	Non β-lactam
AmpC (CMY)	Pen + 1GC + 3GC + Amox/Clav + Cefoxitin	Non β-lactam
Carbapebemase	All β-lactams	Non β-lactam

Pen – penicillins (including amoxicillin and ampicillin), 1GC – first generation cephalosporins, 3GC – 3rd generation cephalosporins

CARBAPENEMASES

- Carbapenems are one of our last lines of defense!
  - Broad spectrum drugs
- Capable of degrading the vast majority of β-lactams
- Variety of enzymes with carbapenem degrading activity
  - Metallo-β-lactamases (NDM, VIM and IMP)
  - KPC type
- Distinct epidemiological characteristics

OUR WORST NIGHTMARE

NEW DELHI METALLO-B-LACTAMASE

- NDM-1
- First reported in 2008
  - 59 year old, male Swedish patient
  - Diabetic, had suffered multiple strokes
  - Decubital ulcers, UTI with ESBL producing *K. pneumoniae*
  - Rectal swab screening revealed carbapenem resistant *E. coli*
  - Recent history of hospitalization in India

NEW DELHI METALLO-B-LACTAMASE

- Dissemination from India, other endemic foci
  - Has been found on every continent except Antarctica
- Association with travel to Indian sub-continent
  - Pleasure and medical tourism
- Widely disseminated in India
  - Water
- Found in livestock in China

Travel-Related Carbapenemase-Producing Gram-Negative Bacteria in Alberta, Canada: the First 3 Years

Gisele Peirano,[a,b] Jasmine Ahmed-Bentley,[e,f] Jeff Fuller,[e,g] Joseph E. Rubin,[a,b,d] Johann D. D. Pitout[a,c]

Division of Microbiology, Calgary Laboratory Services,[a] and Departments of Pathology and Laboratory Medicine[b] and Microbiology, Immunology and Infectious Diseases,[c] University of Calgary, Calgary, Alberta, Canada; Department of Veterinary Microbiology, University of Saskatchewan, Saskatoon, Canada[d]; Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada[e]; DynaLIFE$_{DX}$, Edmonton, Alberta, Canada[f]; Provincial Laboratory for Public Health, Edmonton, Alberta, Canada[g]

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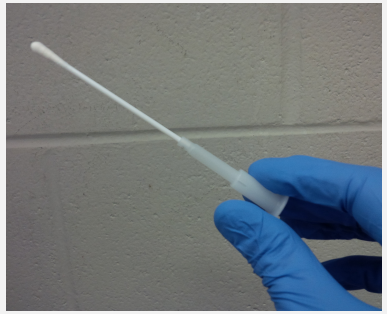
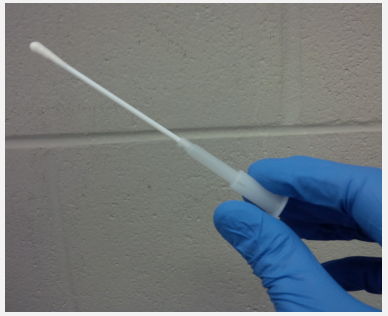
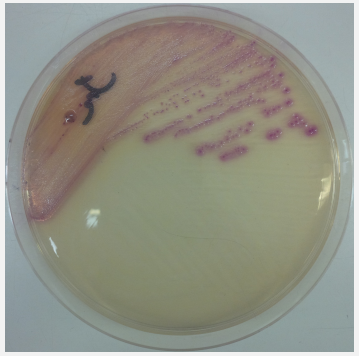
TRAVEL… MY FAVORITE ACTIVITY

A swab before the trip…





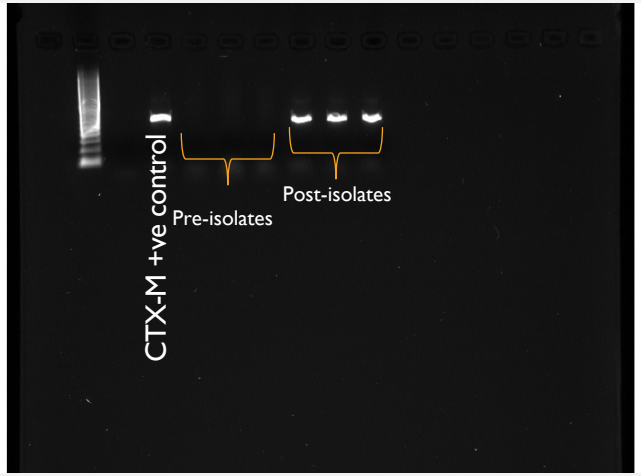
….and one on return



A little extra souvenir?

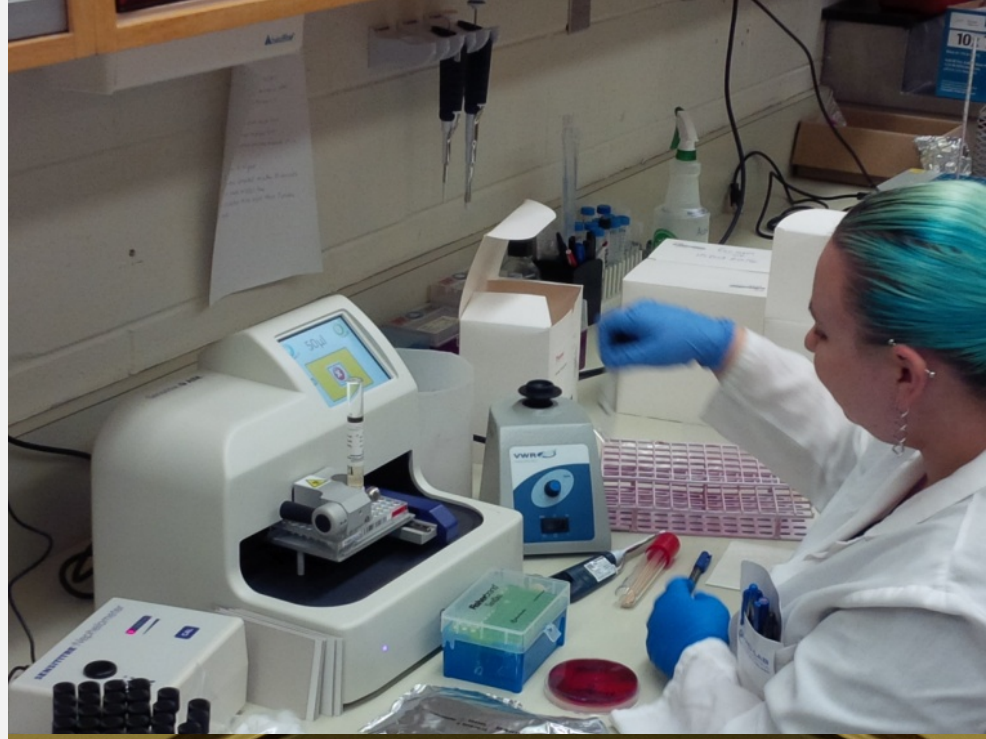
WHAT I PICKED UP…

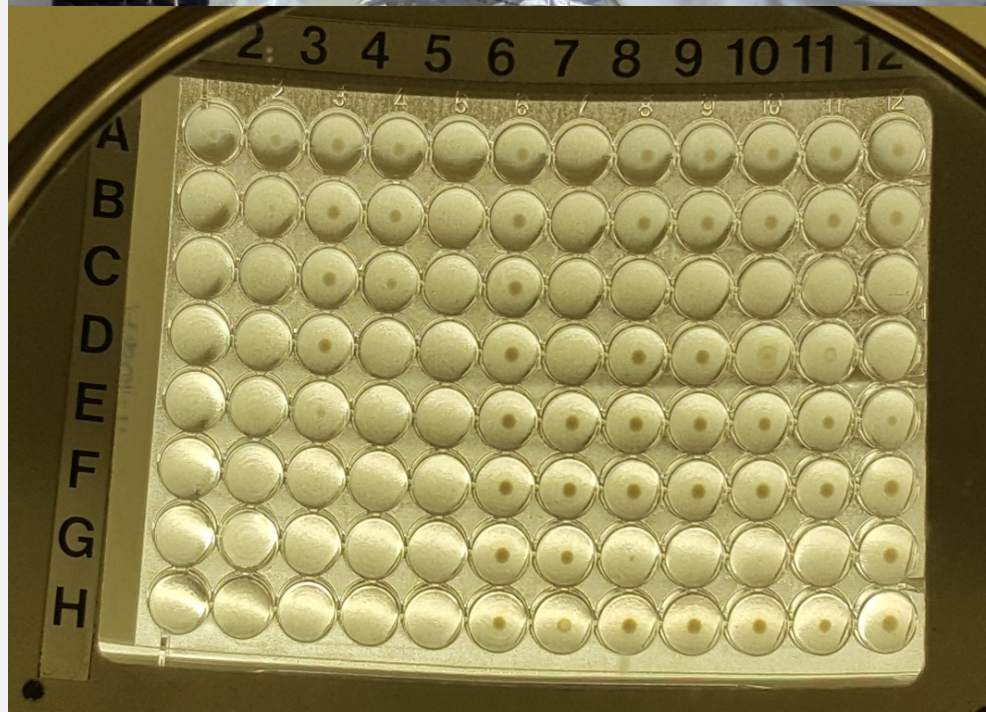
- Before leaving, colonized with *E. coli*
  - Resistant to tetracycline
  - Susceptible to all beta-lactams, fluoroquinolones, aminoglycosides, sulfonamides
- On return, *E. coli*
  - Resistant to ampicillin, ceftriaxone and ciprofloxacin
  - Susceptible to cefoxitin, amoxicillin + clavulanic acid and all other drugs

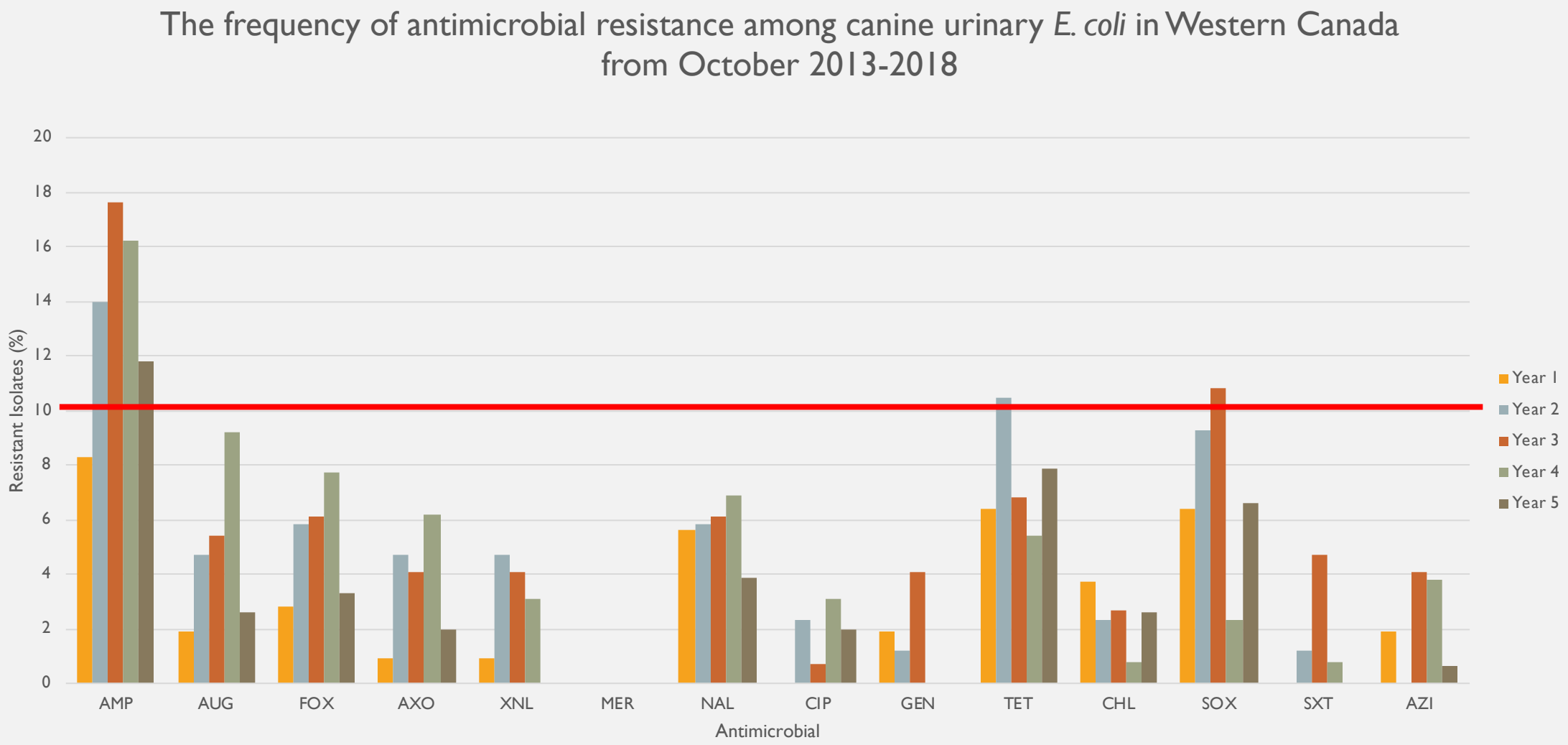


HOW COMMON ARE THESE ENZYMES IN OUR PATIENTS?

- Collecting canine urinary *E. coli* isolates
  - Starting in 2013 and continuing
- 625 Samples collected in first 5 years
- MICs determined by broth micro-dilution
- β-lactamases detected by PCR







Number of isolates (n=624) exhibiting resistance across five years of a canine *E. coli* resistance surveillance program. AMP- ampicillin, AUG- amoxicillin + clavulanate, FOX- cefoxitin, AXO- ceftriaxone, XNL-ceftiofur, MER- meropenem, NAL- nalidixic acid, CIP- ciprofloxacin, GEN- gentamicin, TET- tetracycline, CHL- chloramphenicol SOX- sulfisoxazole, SXT- trimethoprim/sulfamethoxazole, and AZI- azithromycin

EMERGENCE OF ESBL PRODUCING *E. COLI* IN CANINE UTIS

Table 1: Prevalence (%) of phenotypic and genotypic resistance among canine urinary *E. coli* (n=625) during a five year surveillance period

	Pan-susceptible	MDR	CTX-M	CMY-2
Year 1 (n=108)	78.7 (85)	4.6 (5)	0 (0)	0.93 (1)
Year 2 (n=87)	80.5 (70)	6.9 (6)	1.1 (1)	2.3 (2)
Year 3 (n=148)	75 (111)	6.1 (9)	1.4 (2)	2.0 (3)
Year 4 (n=130)	80.8 (105)	4.6 (6)	1.5 (2)	2.7 (4)
Year 5 (n=152)	83.5 (127)	5.3 (8)	0.66 (1)	0.66 (1)

EMERGENCE OF ESBL PRODUCING *E. COLI* IN CANINE UTIS

Canine	Urinary	Sporadic cystitis	**RECOMMENDED TREATMENT:** 1. Amoxicillin: 11-15 mg/kg PO q12h 2. Amoxicillin/clavulanic acid: 12.5-25 mg/kg PO q12h 3. Trimethoprim-sulfonamide (TMS): 15-30 mg/kg PO q12h Duration: 3-5d **ALTERNATIVE TREATMENT:** 4. Enrofloxacin: 10-20 mg/kg PO q24h 5. Marbofloxacin: 2.7-5.5 mg/kg PO q24h 6. Orbifloxacin: 2.5-7.5 mg/kg PO q24h 7. Pradofloxacin: 3-5 mg/kg PO q24h 8. Cefpodoxime: 3-5 mg/kg PO q24h 9. Cephalexin: 3-5 mg/kg PO q24h 10. Cefovecin: 3-5 mg/kg PO q24h	1. II 2. I 3. II 4. I 5. I 6. I 7. I 8. I 9. II 10. I	Benefit of amoxicillin/clavulanic acid over amoxicillin is unclear. NSAIDs should be considered to control cystitis, when appropriate for that patient (e.g. consider renal function). An initial course of NSAIDs without antimicrobials can be considered.

B-LACTAMASES TAKE AWAYS

- By-and-large canine UTIs can still be treated with 1st line therapies
- Broad spectrum β-lactamases are increasingly common in Gram-negatives
  - You're probably already dealing with them and don't even realize it!
  - Stay tuned, they're only going to become more common
- Multidrug resistance, and pan-resistance are still rare in veterinary contexts

SOMETIMES THINGS DON'T WORK AS EXPECTED…

Possible Reasons for Disagreement Between Test Results and Clinical Outcome			
	Factor	Positive Outcomes	Negative Outcomes
Patient/Disease Factors	Pharmacokinetic	High urine drug concentrations	Failure of drugs to penetrate sequestered sites (ex. CNS) Drug interactions decreasing absorption or increasing elimination
	Pharmacodynamic		Failure of aminoglycosides in acidic or anaerobic environments Failure of folate synthesis inhibitors in purulent environments (excessive PABA in environment)
	Disease/pathology	No infection Self-limiting infection	Predisposing disease or underlying pathology such as atopy, diabetes or neoplasia Indwelling medical device
	Therapeutic	Utilization of localized therapy, high concentrations overcoming low level resistance Off label use (dose, dosing frequency, route of administration)	Off label use (dose, dosing frequency, route of administration) Poor owner compliance
Organism/Test Factors	Resistance		Development of resistance in vivo
	Organism lifestyle		Biofilm formation Intracellular infections
	Organism Identification	Mis-identified organism False positive culture	Mis-identified organism Mixed infection
	Antimicrobial Susceptibility Test	Incorrectly performed or reported test	Incorrectly performed or reported test Inducible resistance

TAKE HOME MESSAGES
THE EASY AND OBVIOUS

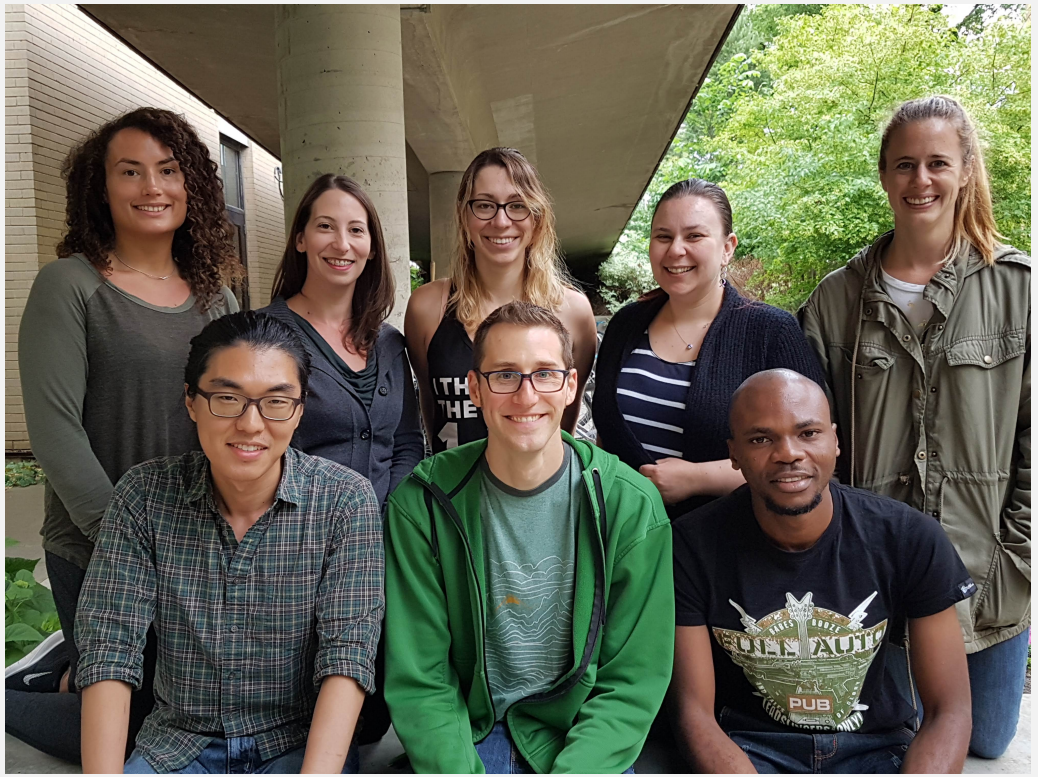
- Antimicrobial resistance is increasing
  - The post-antibiotic era is on it's way
- Treat documented (or at least infections w/ evidence!)
- Next time you think "… just in case" your next thought should be "…but what if?"
- Optimize drug/dose to infection
- Familiarize yourself with relevant guidelines (CVMA, ISCAID, industry recommendations)
- Susceptibility profiles are highly variable, laboratory guidance is **VERY** important for aiding therapeutic selection

TAKE HOME MESSAGES
THE HARDER ONES…

- Be aware of local susceptibility profiles
  - Use them to guide empiric therapy
- Don’t forget about intrinsic resistance

- Reflect on outcomes
  - Did you ‘cure’ that animal?

QUESTIONS?



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